

Improving productivity and profitability in coconut farming

Edited by
George V. Thomas
V. Krishnakumar
B. Augustine Jerard

PROCEEDINGS
International Conference on Coconut Biodiversity for Prosperity
October 25-28, 2010



Central Plantation Crops Research Institute
(*Indian Council of Agricultural Research*)
Kasaragod- 671 124, Kerala, India



Citation: George V. Thomas, V. Krishnakumar and B. Augustine Jerard (Eds.) Improving productivity and profitability in coconut farming. *Proc. International Conference on Coconut Biodiversity for Prosperity*, Central Plantation Crops Research Institute, Kasaragod, Kerala, India 467 p.

Published by

George V. Thomas

Secretary

International Conference on Coconut Biodiversity for Prosperity &
Director, CPCRI

Proceedings of International **Conference on Coconut Biodiversity for Prosperity**,
Oct 25-28, 2010

Central Plantation Crops Research Institute, Kasaragod, Kerala

(Indian Council of Agricultural Research)

Kasaragod- 671 124, Kerala, India

Phone : 04994-232 893, 232 894, 232 895, 232 090

Fax : 91- 4994-232 322

E mail : cpcri@nic.in , cpcri@yahoo.com

Printed at

Print Express, Kaloor, Kochi- 682 017



डा. एस. अय्यप्पन
सचिव एवं महानिदेशक
Dr. S. AYYAPPAN
SECRETARY & DIRECTOR GENERAL

भारत सरकार
कृषि अनुसंधान और शिक्षा विभाग एवं
भारतीय कृषि अनुसंधान परिषद
कृषि मंत्रालय, कृषि भवन, नई दिल्ली 110 001

GOVERNMENT OF INDIA
DEPARTMENT OF AGRICULTURAL RESEARCH & EDUCATION
AND
INDIAN COUNCIL OF AGRICULTURAL RESEARCH
MINISTRY OF AGRICULTURE, KRISHI BHAVAN, NEW DELHI 110 001
Tel.: 23382629; 23386711 Fax: 91-11-23384773
E-mail: dg.icar@nic.in

FOREWORD

The coconut palm is referred to as “*Kalpavriksha*” or “Tree of Heaven” due to its innumerable uses for the human kind. Being a widely cultivated palm species in the tropics, it is important in providing food and livelihood security to several millions of people across the globe. Cultivation of this versatile multipurpose palm is important to the economy of several countries in the Asian and Pacific region. Besides forming a source of healthy food and nutritious health drink, it provides the best natural fibre, shelter and an aesthetic appeal to the landscape. The palm is also ecologically important in sustaining several fragile island ecosystems and is a crop of future in view of its carbon sequestration potential in the context of climate change. In the present global scenario, there are several challenges faced by the farmers and industry in production, processing and marketing. Technological advancements are vital to address these challenges and to make the crop more competitive. As a perennial tree crop, coconut facilitates growing many crops in the interspaces in diverse cropping systems, thereby helping in biodiversity conservation to a considerable extent.

The research on coconut in India was initiated as early as in 1916 with the establishment of Coconut Research Station at Kasaragod, Kerala. Coconut research was brought under the Indian Council of Agricultural Research during 1970 by establishment of Central Plantation Crops Research Institute (CPCRI) with head quarters at Kasaragod. The research programmes on coconut in State Agricultural Universities are coordinated through the All India Coordinated Research project on Palms at CPCRI. An International Conference on Coconut Biodiversity for Prosperity was organized by CPCRI, Kasaragod in association with many national and International scientific societies and organizations to deliberate upon the issues in coconut sector and share the experiences of global researchers. The conference brought together 250 delegates from 12 countries involved in coconut research and development.

This book is a compilation of the papers presented in technical sessions viz. Global and national scenario of coconut biodiversity, Conservation and utilization of genetic resources, Biotechnological tools in crop improvement, Resource management and sustainable crop production, Integrated management of pests and diseases, Post harvest technology and value addition, Technology transfer and Impact of developmental strategies. I am sure that this compilation will be of immense help to coconut researchers, extension personnel, policy planners, students and others who consider coconut as a crop that needs greater attention. I congratulate the editors for the tremendous work done to bring out this useful compilation for the benefit of all the stakeholders.

(S. Ayyappan)

Secretary, DARE and Director General, ICAR

PREFACE

The coconut palm with its multifaceted uses, is a source of livelihood to several millions of people in 93 countries, particularly in humid tropics in the Asian and Pacific Regions, wherein the crop has cultural and socio-economic significance. Nine decades of coconut research has resulted in development of technologies to enhance the productivity and profitability. In the present global scenario, farmers and industry across the coconut growing countries face innumerable challenges in production, processing and marketing. Important coconut diversity, the basis for sustainable coconut production, is under threat of genetic erosion. Technological innovations and research efforts are needed to make the crop more competitive in providing food, nutritional and health security, sustainable environment and above all the livelihood security to the growers.

In order to deliberate on the current status of technological development, identify the gaps, and formulate future policies and programmes giving thrust on coconut diversity and sustainable development, an “International Conference on Coconut Biodiversity for Prosperity” was organized by the Central Plantation Crops Research Institute, Indian Council of Agricultural Research, Indian Society for Plantation Crops along with the Bioversity International as well as other National and International agencies including Coconut Development Board, National Bank for Agriculture and Rural Development, National Horticultural Board, Confederation of Horticultural Association of India, Association for the Improvement in Production and Utilization of Banana and Asia-Pacific Coconut Community. The conference served as a common platform for different stakeholders to come together and deliberate upon the issues in coconut sector and share the experiences of global researchers. The conference had the participation of about 250 delegates from twelve countries involved in coconut research and development *viz.*, India, Brazil, France, United Kingdom, Australia, Sri Lanka, Philippines, Indonesia, Cote d’Ivoire, Mexico, USA and Nigeria.

This book is a compilation of the selected papers presented in various technical sessions *viz.* Global and national scenario of coconut biodiversity, Conservation and utilization of genetic resources, Biotechnological tools in crop improvement, Resource management and sustainable crop production, Integrated management of pests and diseases, Post harvest technology and value addition, Technology transfer and Impact of developmental strategies and other contributions from the invited experts. The publication of the book has become possible with the whole hearted support and cooperation from all the contributors. We also would appreciate Sri C.H. Amarnath, CPCRI for timely support in bringing out this compilation.

It is hoped that this book will be useful to all stakeholders involved in coconut research and development in formulating research priorities and policy planning aimed at higher productivity and profitability.

Editors

CONTENTS

No.	Title and Authors	Page no
1	Technological advancements in coconut research <i>H.P. Singh</i>	001-009
2	Current status of coconut diversity conservation and utilization in India <i>George V. Thomas, V. Niral and B. A. Jerard</i>	010-021
3	Genetic resources of coconut <i>V. Niral, K. Samsudeen and R. V. Nair</i>	022-028
4	Varietal improvement of coconut <i>R. V. Nair, K. Samsudeen, V. Niral and R. J. Thomas</i>	029-034
5	International coconut gene bank for South Asia-Conservation and characterization <i>Niral, V., Jerard, B. A., Samsudeen, K., Patil, D. V., Ananda, K. S., Nair, R. V. and George V. Thomas</i>	035-040
6	Achievements of breeding for resistance to root (wilt) disease in coconut <i>P. M. Jacob, Regi J. Thomas and R. V. Nair</i>	041-048
7	'Mohacha Naral-' A coconut with sweet endosperm from Maharashtra, India <i>K. Samsudeen, D. D. Nagwaker, Anitha Karun, V. Niral, B. A. Jerard, P. Ajith Kumar, K. Devadas and R. V. Nair</i>	049-052
8	Performance evaluation of coconut cultivars under rain fed conditions <i>Lissamma Joseph, S. Anitha and K. Aravindakshan</i>	053-054
9	Genetic diversity in coconut germplasm based on morphological descriptors <i>S. Geethanjali, M. Kumar, K. Rajamanickam and S. Arulraj</i>	055-062
10	<i>In vitro</i> approaches in coconut: an Indian perspective <i>Anitha Karun and M. K. Rajesh</i>	063-066
11	Genetic relatedness of coconut populations from Andaman and Nicobar Islands traced to the Indian sub continent using microsatellite markers <i>Deva Kumar, K., Jerard, B. A., Dhanapal, R., Damodaran, V., Niral, V. and Naganeeswaran, S.</i>	067-075
12	Effect of various auxins on high frequency callus induction from coconut plumule explants <i>Anitha Karun., Bhavyashree U., Lakshmi Jayaraj K., Fayas T. P., Rajamanickam R., Radha E. and Sajini K. K.</i>	076-085

13	Modified pregrowth desiccation for cryopreservation of coconut (<i>Cocos nucifera</i> L.) zygotic embryos <i>K. K. Sajini and Anitha Karun</i>	086-096
14	Alternate genetics and genomics of coconut <i>Arunachalam, V., Jerard, B.A., Manju, K.P. and Niral, V</i>	097-101
15	<i>In silico</i> prediction of properties of KNOX and BABY BOOM proteins in coconut <i>Nithya, S., Rajesh, M.K., Bobby Paul., Anitha Karun and George V. Thomas</i>	102-108
16	Bioinformatics approach towards mining of candidate genes for water stress in coconut <i>Bobby Paul, Rajesh, M. K and Naresh Kumar, S</i>	109-115
17	Differential expression of genes regulated in response to water stress in coconut <i>Bobby Paul, Rajesh M.K., Kiran A.G., George V. Thomas and Naresh Kumar S</i>	116-122
18	Improved production technologies for higher productivity <i>R. Dhanapal, P. Subramanian, H. P. Maheswarappa and P. Geetha</i>	123-137
19	Integrated nutrient management in ECT coconut through organics <i>S. Mohandas, P. S. Subramaniam, A. Gurusamy, C. Natarajan and Arulraj, S</i>	138-143
20	Nutrient management of hybrid (T x D) coconut with nitrogen substitution through organic manure <i>S. Mohandas, P. S. Subramaniam, A. Gurusamy, C. Natarajan and S. Arulraj</i>	144-151
21	Coconut based farming system <i>V. Krishnakumar</i>	152-161
22	Multispecies cropping system in coconut for East Coast region of Tamil Nadu <i>P. S. Subramaniam, S. Mohandas, R. Marimuthu, A. Gurusamy, C. Natarajan and S. Arulraj</i>	162-166
23	Second generation coconut based high density multispecies cropping system in Assam <i>J.C. Nath and K.K. Deka</i>	167-173
24	Intercropping with fruits and annual spices in coconut under Konkan condition of Maharashtra <i>D.D. Nagwekar, V.S. Desai, V.S. Sawant, P.M. Haldankar, S. Arulraj and B.B. Jadhav</i>	174-177
25	Prospects of intercropping medicinal and aromatic plants in coconut <i>S. Mohandas, P. S. Subramaniam, C. Natarajan and S. Arulraj</i>	178-180

26	Integration of meat goats on the productivity of coconut farm <i>S. Anitha, Lissamma Joseph and K. Aravindakshan</i>	181-182
27	Performance of elite cocoa clones intercropped in coconut <i>Elain Apshara, S</i>	183-187
28	Benefit cost ratio analysis of coconut based cropping systems <i>Girija Devi, L., Gladis, R. and Biju Joseph</i>	188-193
29	Improving use efficiency of water by integrating fish culture and irrigation in coconut based farming system-A case study <i>Mathew, A. C. and Manoj Samuel</i>	194-206
30	Effective utilization of bioresources for yield improvement in <i>Plumbago rosea</i> intercropped in coconut garden <i>Nihad, K</i>	207-210
31	An assessment of species structure and crop diversity in the homestead farms of Kerala, India <i>V. Krishnakumar, S. Rageena, Jacob John, K. Geetha and D. V. S. Reddy</i>	211-214
32	A holistic systems approach to identify sustainable perennial crop based systems-a case study <i>Jacob John, M. Joy, V. Geetha and U. Sheeba</i>	215-229
33	Organic farming as a viable resource management strategy for sustainability in coconut farming <i>George V. Thomas, V. Krishnakumar, P. Subramanian and Alka Gupta</i>	230-237
34	In search of bioinoculants for coconut palm <i>Sheeja Purushothaman, Sunny Thomas, Mathew George</i>	238-241
35	Integrated disease management in coconut <i>R. Chandra Mohanan and Merin Babu</i>	242-252
36	Current status of phytoplasma research <i>R. Manimekalai</i>	253-257
37	A fast and versatile molecular tool for detection of root (wilt) phytoplasma in coconut palms <i>R. Ramjagathesh, G. Karthikeyan, B. Meena, T. Raguchander and R. Samiyappan</i>	258-263
38	Effect of <i>Pseudomonas fluorescens</i> and <i>Trichoderma viride</i> in the management of basal stem rot disease in coconut <i>K. Rajappan, M. Surulirajan, C. Natarajan and S. Arulraj</i>	264-268
39	Biological management of basal stem rot disease of coconut <i>Rajendran, L., Karthikeyan, G., Raguchander, T and Samiyappan, R</i>	269-286
40	Collection of coconut diseases databases and bioinformatics resources in Tami Nadu region <i>B. Hari Prasath</i>	287-291

41	Comparative genome analysis of different strains of phytoplasma <i>R. Manimekalai, Lijin, K.G., Roshna, O.M. and Thomas, G.V</i>	292-297
42	Superoxide dismutase isozymes and their heat stability in coconut (<i>Cocos nucifera</i> L.) leaves <i>Mukesh Kumar, Sugatha P., Muralikrishana K. S., John Sunoj V.S., and Hebbur K. B.</i>	298-304
43	Comparison of superoxide dismutase, succinic dehydrogenase, exochitinase and 1, 4- β -glucanase activities between healthy and root (wilt) disease affected coconut cultivars – CGD and WCT <i>V.K.Chaturvedi, N. Srinivasan, M. Sasikala, P.M. Jacob, G. Rajeev, C. Rincy Susan, M. Monisha and M.S. Jisha</i>	305-310
44	Management strategies for root (wilt) disease of coconut <i>V.Krishnakumar</i>	311-324
45	Integrated pest management in coconut <i>P. Rajan, Chandrika Mohan and A. Josephraj Kumar</i>	325-334
46	Biological methods of pest management in coconut <i>Chandrika Mohan, A. Josephraj Kumar and P. Rajan</i>	335-347
47	Emerging pests of coconut in India <i>Rajan, P., Joseph Raj Kumar, A., Chandrika Mohan and Subaharan, K</i>	348-356
48	Biology of coreid bug, <i>Paradasynus rostratus</i> Dist. in different hosts <i>Ambily Paul and C. Nandakumar</i>	357-361
49	Natural enemy complex associated with coconut eriophyid mite, <i>Aceria guerreronis</i> Keifer <i>Chandrika Mohan, P. Rajan and C.P.R. Nair</i>	362-369
50	Survey and monitoring of pest problems of coconut in Konkan region of Maharashtra <i>S.S.Gurav, V.S.Desai, B.N.Bhangare, D.D.Nagwekar and A.L.Narangalkar</i>	370-374
51	Reaction of different coconut genotypes against Eriophyid mite, <i>Aceria guerreronis</i> Keifer in Konkan region of Maharashtra <i>S.S.Gurav, V.S.Desai, D.D.Nagwekar and A.L.Narangalkar</i>	375-377
52	Survey and monitoring of eriophyid mite, <i>Aceria guerreronis</i> Keifer of coconut in Konkan region of Maharashtra <i>S.S.Gurav, V.S.Desai, B.N.Bhangare, D.D.Nagwekar and A.L.Narangalkar</i>	378-380
53	Product diversification and value-addition of coconut - an overview <i>K Madhavan and T.Arumuganathan</i>	381-393
54	Machineries and tools for enhanced efficiency in palms <i>T.Vidhan Singh and A.C. Mathew</i>	394-398

55	Development and performance evaluation of Virgin Coconut Oil Cooker <i>A.C Mathew, K. Madhavan and T. Arumuganathan</i>	399-403
56	Livelihood security through coconut farming <i>K. V. Ahamed Bavappa and V. Krishnakumar</i>	404-410
57	Community based interventions in coconut sector for enhanced profitability <i>P.K. Thampan and Remany Gopalakrishnan</i>	411-419
58	Success stories of technology adoption in coconut <i>C. Thamban</i>	420-423
59	Mass production and use of biocontrol agents in the integrated management of coconut leaf rot disease – technology popularization through participatory programmes in disease affected regions <i>N. Srinivasan, R. Chandramohanan, R. Bharathi, N. S. Radhika and Shanty Issak</i>	424-431
60	Successful user friendly rodent management techniques in coconut plantations as a frontline research in Mathilakom Block Panchayath in Thrissur District <i>Jim Thomas, Mani Chellappan and Haseena Bhaskar</i>	432-435
61	Bio-control of rhinoceros beetle – Participatory adaptation of low input multiplication of <i>Metarhizium anisopliae</i> for bridging adoption gap <i>P. Anithakumari and Chandrika Mohan</i>	436-442
62	Extension approaches to enhance technology utilization and income generation in coconut farming <i>Thamban, C</i>	443-456
63	Impact of training programmes among farmers of Kasaragod district, Kerala on adoption of vermicomposting technology utilizing palm wastes <i>Rayudu, B. T., Leena, S., Manikandan, Sanal Kumar, R., Jayasree, M. P and Manoj Kumar, T. S</i>	457-462
64	Application of spatial technique in field experiments with coconut <i>Jose, C. T., Ananda, K. S and Muralidharan, K</i>	463-466

Technological advancements in coconut research

H.P. Singh

Formerly Deputy Director General (Horticulture)
Indian Council of Agricultural Research, New Delhi - 110 012, India

The coconut palm is of considerable global significance as a versatile tree crop providing livelihood security to several millions of people. Being grown globally in an area of 12.29 million hectares in 93 countries and producing 11.04 million metric tones of copra (on equivalent basis), the crop influences the economy of several countries. India, Indonesia and Philippines are the major producers of coconut in the world. Importance of this crop in providing livelihood security, improved nutrition, employment and income generation are the driving force for the renewed interest in the crop. True to the sobriquet 'Kalpavriksha', it has got a high potential for food, shelter, industrial, pharmaceutical and energy uses and for environmental protection. Coconut by itself is a food crop, amenable for the preparation of a variety of food products. Coconut oil with high lauric acid content of 50% is considered as one of the best oils and coconut meat as an ideal source of dietary fibre and the diverse food products that could be prepared from coconut indicate the importance of this crop. Considering the pivotal role of coconut, predominantly a small holder's crop, and coconut based farming system in ensuring livelihood security of millions of farmers, it is imperative that the yield potential of all the crops of the system is holistically exploited so as to maximize the per unit area production.

Research support for coconut in India

Need for coconut research was recognized as early as in 1916 by establishing four research stations by then government of Madras, at Kasaragod (red loamy soil) and Pilicode (laterite gravelly soil), and two centres at Nileswar (red sandy loam soil and coarse sandy soil) in northern Kerala. The Indian Central Coconut Committee was set up in 1945, under the "Indian Coconut Committee Act, 1944" to take measures for the improvement and development of coconut to serve the entire need of the country. In 1966, Indian Central Coconut Committee was abolished and the development of the crop was entrusted to the Directorate of Coconut Development, under the Ministry of Agriculture, Govt. of India and the research was entrusted to the Indian Council of Agricultural Research. In 1970, ICAR established Central Plantation Crops Research Institute by merging the stations at Kasaragod and Kayamkulam and the Central Arecanut Research Station at Vittal with the five Regional Stations. The Central Plantation Crops Research Institute is the national institute to conduct research on coconut and has Regional Stations at Kayamkulam, Vittal and Minicoy and Research Centres at Kidu, Mohitnagar and Kahikuchi. The Institute addresses the problems related to crop improvement, production technology including management of pests and diseases and also some aspects of post harvest handling. During 1970, the ICAR also sanctioned the All India Coordinated Coconut and Arecanut Improvement Project with its headquarters at Kasaragod. At present, the AICRP is implemented in 16 Centres located in nine State Agricultural Universities and one Central Institute. The research efforts have recorded impressive achievements in terms of developing new cultivars and production technologies, which when demonstrated in the field resulted in two-three fold increase in yield.

Biodiversity conservation and utilization

Collection, conservation and cataloguing of coconut germplasm have been accorded the topmost priority in coconut research. The first exotic collections were made way back in 1924 from Philippines, Malaysia, Fiji, Indonesia, Sri Lanka and Vietnam. During 1940, the first germplasm bank was conducted at the erstwhile Central Coconut Research Station, Kasaragod. The first indigenous survey for germplasm collection was started in 1958. Later on, in 1981, under the FAO/IBPGR funded expedition, 24 exotic collections were made from six Pacific Ocean Islands viz., Papua New Guinea, Fiji, American Samoa, Tonga and French Polynesia. At present the Institute maintains the largest coconut germplasm collection in the world which comprises of 398 accessions, of which 132 are exotic collections from 26 countries and 266 are indigenous collections. One of the important additions made recently to the germplasm is a rare accession “ThairuThenga” (similar to makapuno type) from Andaman Islands.

Breeding efforts at CPCRI and State Agricultural Universities have resulted in release of several varieties (Table 1) and hybrids (Table 2) suitable for different parts of the country.

Table 1. Coconut varieties released by CPCRI and other State Agricultural Universities for commercial cultivation

Name	Agency	Remarks	Area recommended
Chandrakalpa	CPCRI	Drought tolerant, high oil content-72%	Lakshadweep, Kerala, Tamil Nadu
Kerachandra	CPCRI	High yield	All regions
Kalpa Pratibha	CPCRI	High nut, oil yield, tender nut, Drought tolerant	Kerala, Tamil Nadu
Kalpa Mitra	CPCRI	High nut, oil yield, Drought tolerant	Kerala, West Bengal, Andhra Pradesh
Kalpa Dhenu	CPCRI	High nut, oil yield, Drought tolerant	Andaman, Kerala, Tamil Nadu
Chowghat Orange	CPCRI	Dwarf, Tender nut Dwarf	All regions
Gautamiganga	APHU	Dwarf, High yield, tender nut	Andhra Pradesh, Tamil Nadu
Kalparaksha	CPCRI	High nut, oil yield in RWD prevalent areas	Kerala
Kalpasree	CPCRI	Dwarf, superior oil, high yield in RWD prevalent areas	Kerala
Pratap	KKV,	High yield	Konkan region, Kerala Dapoli
VPM-3	TNAU	High yield, Drought tolerant	Tamil Nadu
ALR 1	TNAU	High yield	Tamil Nadu

Kamrupa	AAU	High yield	NE region- Assam
Kera Sagara	KAU	High yield	Kerala
Kera Bastar	AICRP Palms	High yield	Chattisgarh
Kera Keralam	AICRP Palms	High yield	Tamil Nadu, Kerala
Kalyani Coconut	BCKV	High yield	West Bengal
Kalpatharu	AICRP Palms	Drought tolerant, ball copra, high yield	Kerala, Karnataka, Tamil Nadu

Table 2. Coconut hybrids released by CPCRI and other State Agricultural Universities for commercial cultivation

Hybrids	Parentage	Agency	Copra yield (kg)	Area
Chandra Sankara	COD x WCT	CPCRI	25	Kerala, Karnataka, Tamil Nadu
Kera Sankara	WCT x COD	CPCRI	21	Kerala, Karnataka
Chandra Laksha	LCT x COD	CPCRI	21	Kerala, Karnataka
Laksha Ganga	LCT x GBGD	KAU	21	Kerala
Kera Ganga	WCT x GBGD	KAU	21	Kerala
Kera Sree	WCT x MYD	KAU	24	Kerala
Kera Sowbhagya	WCT x SSAT	KAU	25	Kerala
Ananda Ganga	ADOT x GBGD	KAU	21	Kerala
Godavari Ganga	ECT x GBGD	APAU	21	Andhra Pradesh
VHC-1	ECT x GD	TNAU	13	Tamil Nadu
VHC-2	ECT x MYD	TNAU	16	Tamil Nadu
VHC-3	ECT x OD	TNAU	25	Tamil Nadu
Konkan	GBGD x ECT	RCRS, Bhatye	22	Konkan
Kalpa Sankara	CGD x WCT	CPCRI	18	Kerala
Kalpa Samrudhi	MYD x WCT	CPCRI	25	Kerala, Assam

Biotechnological advancements

Efforts made to harness biotechnological tools have yielded fruitful results in addressing the problems in coconut. A protocol for aseptic collection of embryos in coconut, their

storage and successful culturing to develop plantlets has been standardized. Many exotic coconut accessions were collected using this technique from Indian Ocean Islands. Besides, plantlet development has been obtained from various explants of coconut (plumular and inflorescence tissue) in Y3 medium supplemented with polyamine and picloram in both dwarf and tall cultivars. The technique of plumular culture is being utilized for scaling up the planting material production in coconut and development of more uniform coconut populations.

Significant achievement has been made in establishing etiology of the root (wilt) disease, which is the most serious production constraint for coconut in India, by application of molecular techniques. PCR assay of DNA extracted from root (wilt) diseased palms with phytoplasma specific primers amplified expected bands and phytoplasmic nature of amplicons confirmed by sequencing. Phylogenetic analysis of root (wilt) phytoplasma by BLAST search revealed that the phytoplasma belong to 16Sr DNA XI group.

Technologies for production system management

Agronomic trials conducted during early forties and fifties helped to determine the optimum manurial and cultural requirements of coconut palm. The first systematic trial on cropping system was laid out in 1972 at CPCRI. Nursery management techniques such as selection of garden, mother palms and seed nuts, planting and maintaining the nursery and the technique for raising poly bag nursery were standardised. Square system of planting at a spacing of 7.5 x 7.5 m with a plant density of 175 palms/ha is recommended. Recommended dose of fertilizer for coconut palm is application of NPK @500:320:1200 g/palm/year in two split doses during May-June and August-September. Application of magnesium sulphate @ 500 g MgO/palm was found to be advantageous in areas where palms show yellowing of leaves.

Water management: Sprinkler irrigation or perfo-irrigation with 20 mm water was found to be the best suited to inter or mixed cropping systems where the entire surface requires wetting. Drip irrigation system was found to be more advantageous under littoral sandy soil than lateritic soil and irrigating palms with 66% Eo (32 l water/palm/day) from December to May is ideally suited for coconut resulting in 34 per cent saving of water. Fertigation studies in coconut have indicated that 50% NPK of the recommended dose of fertilizer through drip irrigation network has given the highest yield. A non-sequential sensor was designed at CPCRI.

Soil and moisture conservation: Moisture conservation methods such as mulching with coconut husk, coir dust, green leaves, dried coconut leaves, addition of organic manures or green manures, husk burial, inter cultivation, bunding, terracing etc., are found to be ideal. In sloppy terrains, trench filled with coconut husk, half moon bunds, staggered catch pit reinforced with pineapple and growing CO3 grass across the slope were found to be useful. The Institute has developed various bio-engineering measures to sustain crop production in rainfed areas of west coast region.

Coconut based cropping system: Various coconut based cropping system models involving different compatible crops like vegetables, tuber crops, flowering, medicinal and aromatic plants, fruits, spices etc in the interspaces of coconut palms have been developed. Application of 2/3 recommended dose of fertilizers for coconut along with organic matter recycling was found to be the most ideal nutrient management system under the High Density Multi Species

Cropping System. Mixed farming with various subsidiary enterprises including animal husbandry component was found to be quite advantageous.

Management of coconut gardens in coastal sandy soils: Coastal sandy soils are inherently poor in organic matter and soil nutrients as well as are poor in water holding capacity. CPCRI has developed technologies for overcoming these problems and could successfully cultivate coconut and other intercrops. Various intercrops such as banana (G9 variety), pineapple, ash gourd, Bhendi, Brinjal, Amaranth, Elephant foot yam and Hybrid Bajra Napier grass (CO 3) were grown with various soil moisture conservation measures. Burial of coconut husk in trenches or application of coir pith in trenches and planting intercrops was found to give better plant growth and higher yield. Among the different cropping systems tried, coconut + vegetable intercropping system recorded higher coconut productivity. The coconut based mixed farming system involving cultivation of fodder grass in the interspaces of coconut gardens and integration of animal enterprises involving dairy, poultry and pisciculture is in progress at CPCRI, Kasaragod.

Organic farming strategies for coconut: Technological advancements made in organic farming research resulted in the development of viable protocol for sustainable coconut production employing organic and bioinputs. Vermicomposting of coconut leaves using local strains of *Eudrilus* sp., production of vermiwash from coconut leaf vermicomposting system, utilization of legume- *Rhizobium* N₂ fixing systems, biofertilizers and eco-friendly pest and disease management techniques were found to be valuable inputs for organic farming in coconut.

Precision farming in coconut: In general, highly-weathered soils in the high rainfall zones commonly suffer from multiple nutrient deficiencies. The regression analysis of the soil available nutrients at different levels to coconut yield indicated existence of multiple nutrient limitations. Analysis for Multiple Nutrient Limitation Hypothesis of soil available nutrients and individual palm yield data in red sandy loam soil at CPCRI Kasaragod has indicated that increasing level of available K at the high level of total N had a linear increasing effect on coconut yield suggesting that N and K have a co-limiting role.

Technologies for diseases and pest management: Various diseases and pests affect growth and inflict considerable loss in coconut production. Central Plantation Crops Research Institute and various centres under AICRP on palms are working on their management and various technologies have been developed for tackling these problems.

Integrated disease management: The coconut root (wilt) disease is a non-lethal debilitating malady, caused by Phytoplasma that reduces the production potential of the palm. ELISA technique has been refined for the quick and easy detection of root (wilt) disease of coconut. Scanning electron microscopic (SEM) observation of root tissues of root (wilt) diseased palms and PCR with root (wilt) phytoplasma primers revealed presence of phytoplasma in the samples. Balanced application of fertilizers and growing green manure crops are the recommended management strategies for disease early gardens.

Bud rot caused by *Phytophthora palmivora* is another important disease of coconut and unless the management has not been adopted well in advance, under severe cases, can completely kill the palm. Pouring of 5 g mancozeb in 300 ml water coupled with placing of 2

nos. of mancozeb sachets (5 g mancozeb/sachet) in the innermost leaf axils around spindle leaf was found to be very effective in controlling disease.

Leaf rot disease in coconut is caused by a complex of fungal pathogens chiefly *Colletotrichum gloeosporioides*, *Exerohilium rostratum* and *Fusarium solani*. Integrated disease management strategy involves cutting of the rotten portions of the spindle leaf and pouring either Contaf-5EC @ 2 ml or Dithane-M45/Indofil-M45 @ 3 g dissolved in 300 ml water in the cavity around the base of spindle leaf. Field management of leaf rot disease through application of biological control agents especially consortium of bio agents (*P. fluorescens* and *B. subtilis*) along with phytosanitation was proved to be effective.

The Stem bleeding disease caused by *Thielaviopsis paradoxa* can also affect growth and yield of palms, if left unmanaged. Root feeding of 5% calixin or 5% Bavisitn at quarterly intervals is effective in the initial stage of the disease. In advanced stage, the affected bark should be chipped off till the healthy tissue is exposed and painted with 5% calixin (5 ml calixin in 95 ml water).

Integrated pest management: Rhinoceros beetle (*Oryctes rhinoceros*) is a major pest of coconut prevalent in all coconut growing areas. The IPM package for control of rhinoceros beetle includes extraction of the adult beetles using a beetle hook during the peak period of pest abundance (June-September) from the crown, treatment of all possible breeding sites of the insect with carbaryl 0.01% (50% WP) on w/w basis. Biological suppression of the pest can be carried out by releasing 10-15 beetles inoculated with *Oryctes* virus in one ha of garden and application of 5×10^{11} spores of *Metarhizium anisopliae* fungus/m³ area of the breeding site of the pest, during monsoon period. Prophylactic measure includes leaf axil filling of palms with 12.0 g naphthalene balls or 250 g each of neem cake/marotti cake covered with sand at 45 days interval. The conventionally used PVC traps for catching adult beetles of rhinoceros beetle were refined for better efficiency.

Red palm weevil (*Rhynchophorus ferrugineus*) is a serious pest causing mortality of palms. IPM package for red palm weevil includes periodical cleaning of palm crown to avoid decaying of organic debris, proper cutting, splitting and burning of red palm weevil dead palms, treating of any wounds on the palm with coal tar + 1% carbaryl stem injection. Trapping of floating population of the weevil can be done by setting up of pheromone traps. Synthesis of Ferrugineol, the aggregation pheromone, is being done at CPCRI. Towards understanding the neuro-sensory mechanism in red palm weevil, the protocol for estimation of red weevil antennal response to volatiles in electroantennography has been standardized. The contact established with the antennae provided a stable electrical response for over an hour with minimal background noise, which could aid in assessing the response of the antennae to an array of compounds.

Leaf eating caterpillar (*Opisina arenosella*) has sporadic occurrence and feeds on the under surface of the leaflets within silken galleries resulting in considerable reduction of photosynthetic area. The *Opisina* infested palms showed reduction in bunch and leaf production to the tune of 21.9% and 17.4%, resulting in a crop loss of 45.38% in terms of nut yield. An IPM method for controlling the pest includes cutting and burning of badly infested outer leaves/leaflets, spraying of 0.02% dichlorvos if pest is in active larval stage and release of

larval parasitoids *Goniozus nephantidis* @ 20.5%, pre-pupal parasitoids like *Elasmus nephantidis* @ 49.4% and *Brachymeria nosatoi* @ 31.9% respectively at fortnightly intervals depending on the larvae, pre-pupal and pupal population of *Opisina*.

Eriophyid mite (*Aceria guerreronis*) is the most widespread pest affecting coconut palm in about 30 countries of Tropical America, Africa and Asia. Management of this pest is possible by spraying of 0.004% azadirachtin or 2% neem oil, garlic and soap mixture during April-May, Oct.-Nov. and Dec.-Jan., in such a way that all mite infested palms in an area should be covered at the shortest possible interval coupled with integrated nutrient management to improve the health of palms. Root feeding with neem formulations containing azadirachtin 50,000 ppm (5%) at 7.5 ml or azadirachtin 10,000 (1%) ppm at 10 ml mixed with equal volume of water is also recommended for managing the pest. Spraying of talc based preparation of virulent strains of the acaropathogenic fungus *H. thompsonii* (CPCRI -19; CPCRI. 51(2)) @ three sprays at 45 days interval showed 56-88% reduction in eriophyid mite population.

Post harvest technology

Machineries

Coconut-dehusking machine: A manually operated coconut-dehusking machine was developed with an out turn of 110 nuts/hr. A power operated semi-automatic coconut dehusking machine was also developed, which can dehusk 500-600 nuts/hr.

Coconut dryers: Simple smoke-free collapsible copra dryers have been developed in which about 1000 coconuts/batch can be dried in 24 hrs. A solar-cum-electrical dryer with agricultural waste as third source of energy was also developed for coconut drying with a capacity of more than 3,000 nuts/batch.

Testa remover: A mechanical testa remover with a capacity to process 200 nuts per hour has been developed to achieve greater efficiency in removal of testa from kernel of mature coconut. As removal of kernel testa is the first step in production of value added products such as coconut chips, virgin coconut oil, it will greatly help to accelerate the process of product development.

Coconut slicing machine: For faster slicing of coconut kernel and to ensure uniform quality, a manually operated coconut slicing machine has been developed with a capacity to handle 50 coconuts per hour. The machine consists of a pair of stainless steel slicing blades fixed on a circular blade supporting disc, a feeder, an exit guide and an electric motor as a prime mover.

Coconut palm climbing device: A serious problem faced by coconut farmers in undertaking timely harvesting and other plant protection measures is the inherent difficulties involved in palm climbing and inadequate availability of skilled manpower. To address this problem, a simple coconut palm climbing device has been developed by the Institute. The user can fix the climbing device to his feet by wearing the shoes attached to the device and can move up and down the coconut tree as easily as he climbs a vertical ladder.

Technologies for value addition

Snow Ball Tender Nut: A technology for making snow ball tender nut (SBTN) from eight months old coconuts has been developed. Along with the process, a suitable machine has also been developed for making SBTN.

Coconut chips & vinegar: A technology for the production of sweet coconut chips has been developed, by the process of osmotic dehydration, with a shelf life of six months. A process for production of coconut chips with different flavours, medicated and spicy chips and instant coconut chips using microwave oven has also been developed. The technology for production of coconut vinegar from coconut water has been refined to produce better quality vinegar which will be more acceptable to the consumers.

Minimally processed coconut tender nut: For easy transportation and to provide the tender nut a uniform and appealing appearance many small scale entrepreneurs have started producing the minimally processed tender coconut. In order to make the dehusking process simpler and to avoid the risk involved in manual processing, a minimal processing machine has been fabricated to partially dehusk tender coconut. Using the two cutting blades specially fabricated for this purpose, tender coconut could be dehusked to any required shape.

Virgin Coconut Oil: CPCRI has standardized the technologies for virgin coconut oil production by fermentation, hot and cold processes. Lipid profile of virgin coconut oil made through various processes like hot process, fermentation and direct micro expeller were analyzed. It was found that all these oils were containing high amount of C-12 Lauric acid (more than 50 %) and it was followed by C-14 Myristic acid (20 %) and other free fatty acids.

Other value added products from coconut: *Atta* incorporated with defatted coconut meal (3:1) was used for making chapatti. Sensory evaluation of the chapatti showed high value in terms of colour, flavour, texture and overall acceptability. Nutritional evaluation of the fortified *atta* showed high fat content than commercially available *atta*. Value addition of tender coconut wine using tender nut (kernel & water) and other ingredients has great potential. Coconut water squash was prepared from matured coconut water and their nutritional, sensory and consumer acceptability were found to be good. Nutritional evaluation of the product showed high sodium and potassium content in the coconut water squash.

Thrust areas of future research

Molecular characterisation of genetic resources will provide the basic data for effective utilization of available genetic resources in breeding programmes. Identification of molecular markers associated with quantitative traits will accelerate the breeding programme. However, the long breeding cycle and the inherent zygozity hinder development of mapping populations for undertaking linkage mapping for identification of QTLs. The alternative approach to gene mapping (association or linkage disequilibrium mapping) using associations at the population level, therefore, offers an opportunity to identify associations between marker patterns and trait expressions. Since coconut is a perennial crop and can act as a large carbon sequestration sink, it is essential to understand more about the cause-effects of climate change on coconut plantations with respect to the physiological efficiency of carbon sequestration, growth and yield. Development of more sustainable cropping system models by including compatible high value low volume crops that will optimize the utilization of natural resources and readily adopted by farmers are necessary. Development of effective formulations of agriculturally important microorganisms *viz.*, nitrogen fixers, phosphate solubilizers, PGPRs, mycorrhizae and organic matter decomposers and their applications to meet nutritional requirement and to achieve plant growth promotion are also needed. Product diversification of coconut and development of value added products become very much essential to derive maximum

economic returns for the farmer and the industry. Developing economically and commercially viable processes for the production of virgin coconut oil and standardization of quality parameters will enable its large scale production and higher export. A holistic approach for pest management integrating IPM and INM practices with the involvement of highly efficient bioagents, botanical pesticides, pheromones and biofertilizers is imperative to achieve higher productivity. The need for evaluation of newer molecules for their pest control efficiency is being felt increasingly. Extension efforts are to be further strengthened so that the impact is trickled beyond the vast area/people interacted. Research-extension-farmer-market–development linkage is necessary to achieve faster dissemination of technologies. There is a need to utilize more efficiently the prowess of ICT tools including cyber extension programmes to develop linkages and faster sharing of information with and among the stake holders in this vital sector.

Current status of coconut diversity conservation and utilization in India

George V. Thomas, V. Niral and B. A. Jerard

Central Plantation Crops Research Institute

Kasaragod 671 124, Kerala, India

Coconut, *Cocos nucifera* L., a monoecious perennial monocotyledon, is placed in Arecaceae family (formerly Palmaceae) and the sub family Cocoideae. India with about 1.8 million hectares under coconut and an annual production of 15,730 million nuts is the second largest coconut producing country in the world. Coconut makes a contribution of approximately Rs. 8500 crores to the GDP and Rs. 1300 crores to the export earnings of the country and provides direct and indirect employment to more than 10 million people. The palm is cultivated in States and Union Territories in the country, but 90% of the cultivation is in four southern states of Kerala, Tamil Nadu, Karnataka and Andhra Pradesh. The palm provides livelihood security to millions of people, who depend on the crop for production, processing and trade. In India, the palm is revered as “Kalpavriksha” or Tree of Heaven due to its innumerable uses to the mankind. The palm has a recorded history of cultivation of 4000 years and is interwoven with the socio-economic and cultural heritage of the people of the region.

Coconut genetic resources

Genetic resources form the backbone for undertaking any crop improvement programme. Variability within the gene pool is essential for selection and hybridization for bringing about improvement in the targeted traits. Locating, maintaining and using genetic diversity of coconut presents substantial challenges; the wide dispersal of the species, the limited knowledge of the history of that dispersion and of the current extent and distribution of diversity. The large, recalcitrant seeds also add to the complexity of managing coconut germplasm. Coconut genetic diversity is desirable for long term crop improvement and reduction of vulnerability to various biotic/abiotic factors. Collection, conservation and cataloguing of coconut germplasm have been accorded the topmost priority in coconut research and the Central Plantation Crops Research Institute Kasaragod, is actively involved in the collection and conservation of coconut biodiversity in the field gene bank for utilization in the coconut improvement programme.

Coconut Germplasm Collection and Conservation in India

India has been in the forefront in coconut improvement researches and systematic coconut breeding was initiated in the country in 1916 with the establishment of the erstwhile Coconut Research Stations at Kasaragod and Nileshwar, now under Central Plantation Crops Research Institute (CPCRI) and Kerala Agricultural University, respectively. Collecting of coconut germplasm in India began in 1924 with introductions from the coconut growing countries viz. Philippines, Malaysia, Fiji, Indonesia, Sri Lanka and Vietnam. The germplasm collection was further intensified in 1952 and in 1958 the first indigenous germplasm survey and collection was started. The Central Plantation Crops Research Institute Kasaragod is actively involved in the collection and conservation of coconut biodiversity in the field gene bank for utilization in the coconut improvement programme. The institute has undertaken

exotic collections of coconut germplasm from Pacific and Indian Ocean Islands and from the South Asian countries of Sri Lanka and Bangladesh with funding from ADB/IPGRI. Further, with funding from the National Agricultural Technology Project of the Indian Council of Agricultural Research, extensive prospection and collection of indigenous coconut germplasm from different coconut growing regions of the country has been undertaken and distinct accessions have been conserved in the National Active Germplasm Site at the institute.

The Indian coconut population harbours a wide range of diversity. The islands of Lakshadweep and Andaman and Nicobar with their natural coconut populations, some of which have established with no human interference, are reservoirs of vast genetic diversity. Majority of the native populations in the traditional coconut growing zones, belong to the wild type with greater proportion of husk while some of the evolved types have lesser husk and more endosperm. Further, introgression between the *Niu Kafa* and *Niu Vai* types in the nature has resulted in the emergence of intermediate types.

CPCRI has collected a total of 271 indigenous coconut accessions, with about 238 tall and 33 dwarf accessions, from almost all coconut growing regions within the country. In addition to variability for morphological traits and tolerance/resistance to biotic/abiotic factors in the conserved germplasm, the variability collected from within the country also includes a few unique types such as Laccadive Mini Micro with very small fruits (c" 5 cm long), *Mohacha Narel* (sweet endosperm coconut with less fibre content), soft endosperm coconut, Thairu thengai (buttery endosperm coconut similar to Makapuno), Klapawangi (edible husk), pink coconut (pink husked type), aromatic coconut (aroma in tender nut water), horned coconut etc.

In addition to the indigenous coconut germplasm, the field gene bank at CPCRI has exotic coconut germplasm from 28 countries (Table1). Coconut genetic diversity collected from the South Pacific Ocean Islands is conserved at the World Coconut germplasm Centre (WCGC) located at Sipighat, Andamans. The WCGC was initially envisaged by CPCRI as an off-shore quarantine centre and work on monitoring and characterization of the exotic accessions was undertaken at the centre (Ratnambal *et al.*, 2003). WCGC is now maintained as a germplasm conservation centre by Central Agricultural Research Institute (CARI), Port Blair (Table 2). Further, selected coconut germplasm is also available at the centres under the All India Coordinated Project on Palms (AICRPP) (Table 3).

Table 1. Coconut germplasm inventory at CPCRI

Place of collection – State/ Country	Number of accessions	Number of dwarf accessions	Number of tall accessions
<i>Indigenous</i>	272	36	236
Kerala	35	2	33
Tamil Nadu	22	5	17
Karnataka	15	6	9
Andhra Pradesh	9	4	5
Goa	8	0	8
Gujarat	8	1	7
Orissa	14	0	14

West Bengal	12	1	11
Andaman & Nicobar Islands	88	9	79
Lakshadweep Islands	36	5	31
Meghalaya	1	1	0
Maharashtra	7	0	7
Assam	17	2	15
Exotic	130	22	108
Bangladesh	11	1	10
Kenya	1	0	1
French Polynesia	7	1	6
Indonesia	4	0	4
Sri Lanka	10	6	4
Nigeria	2	1	1
Fiji	9	2	7
Madagascar	4	0	4
Guam	3	0	3
Comoros	5	0	5
Zanzibar	1	0	1
Panama	1	0	1
Ivory coast	3	1	2
Malaysia	9	3	6
American Samoa	3	1	2
Mauritius	6	3	3
Papua New Guinea	8	1	7
Jamaica	2	0	2
Maldives	8	0	8
British Solomon Islands	3	0	3
New Caledonia	7	0	7
Philippines	7	0	7
Surinam	2	1	1
Trinidad and Tobago	1	0	1
Tonga	3	0	3
Vietnam	1	0	1
Seychelles	6	0	6
Reunion	3	1	2
Sub total			
Total	402	58	344

Table 2. Pacific coconut germplasm at World Coconut Germplasm Centre, CARI

Accession Name	International Nomenclature
Solomon Tall	Solomon Island Tall
Rennell Tall	Rennell Island Tall
Wanigata Tall	Fijian Tall Wanigata

Niu Drau	Fiji Niu Bulundrau Tall
Niu Leka Dwarf II	Niu Leka Dwarf II
Niu Balavu	Fijian Tall Taveuni
Tutiala Niu Tall	Samoan Tall
Niu Oma Dwarf	Samoan Yellow Dwarf
Tutiala Local Tall	Samoan Tall Alao
Tonga Niu Ui Tall	Tonga Tall Veitongo
Niu Taukave Tall	Tonga Tall Alaki
Niu Hako Tall	Tonga Tall Kalanga
Tahitian Tall	Tahitian Tall
Pao-Pao Tall	Moorea Tall Pao-Pao
Haepiti Tall	Moorea Tall Haapiti
Bora Bora Tall	Bora Bora Tall
Rangiroa Tall	Rangiroa Tall Avatoru
Hari Papua Dwarf	Rangiroa Red Dwarf
Rangiroa Tiputa Tall	Rangiroa Tall Tiputa
Kiriwana Tall	New Guinea Kiriwana Tall
Tall Muwa	Muwa Tall
Nikkore	New Guinea Orange Dwarf
Kavieng Tall	New Guinea Kavieng Tall
Natava Tall	Gazelle Peninsula Tall

Table 3. Coconut germplasm in AICRP (Palms) Centres

Accession Name	Accession Name	Accession Name
Aliyarnagar Tall	Etamozhi Tall	Mohachoa Narel
Andaman Dwarf	Federated Malay States Tall	Nadora Tall
Andaman Giant Tall	Fiji Tall	Narangi Tall
Andaman Ordinary Tall	Gangabondam Green Dwarf	New Guinea Tall
Arasampatti Tall	Goa Tall	Nicobar Tall
Arsikere Tall	Goja Tall	Nigerian Tall
Assam Green Tall	Gol Tall	Nu Fella Tall
Ayiramkachi Tall	Gonthembili Tall	Nu Guli Tall
Bana Tall	Guam Tall	Nu Wehung Tall
Banawali Green Long Tall	Hazari Tall	Philippines Lono Tall
Banawali Red Round Tall	Jamaica Tall	Philippines Ordinary Tall
Banawali Yellow Long Tall	Jamaican San Blas Tall	Pillalakodi Tall
Banawali Yellow Round Tall	Java Tall	Rajapalayam Tall
Benaulim/Banawali Green Round Tall	Jonnalarasi Tall	Rotuman Tall
British Solomon Islands Tall	Klapawangi Tall	Sakhi Gopal Tall
Calangute Tall	Kappadam Tall	San Ramon Tall
Car Nicobar Tall	Kenthali Orange Dwarf	Seychelles Tall
Ceylon Green Dwarf	Kenya Tall	St. Vincent Tall

Ceylon Orange Dwarf	Laccadive Micro Tall	Strait Settlement Green Tall
Ceylon Tall	Laccadive Ordinary Tall	Suryabana Tall
Chaka Tall	Sakhi Gopal Brown Tall	Siam Tall/Thailand Tall
Chowghat Green Dwarf	Orissa Giant Tall	Tinisira Tall
Chowghat Orange Dwarf	Mondouri Local Tall	Tiptur Tall
Cochin China Tall	Malayan Green Dwarf	Verrikobbari Tall
Dhanei Tall	Malayan Orange Dwarf	West Coast Tall
Dhila Tall	Malayan Yellow Dwarf	West Coast Tall Spicata
East Coast Tall	Mamuli Tall	Zanzibar Tall

India also hosts the International Coconut Gene bank - South Asia (ICG-SA), at the CPCRI Research Centre at Kidu, Karnataka. Presently 91 accessions, representing indigenous coconut germplasm as well as Indian accessions representing coconut ecotypes of Philippines, Malaysia, Pacific Ocean Islands, Africa and the Caribbean Region and germplasm collected under ADB funding from Sri Lanka, Bangladesh, Indian Ocean Islands of Mauritius, Madagascar and Seychelles, Comoros and Reunion, Maldives, are planted in the ICG-SA.

Characterization and evaluation of coconut in India

The collected germplasm is conserved in the national gene bank hosted at the institute and characterized using the IBPGR descriptor. Characterization of coconut genetic resources has resulted in the identification of some trait specific variability with reference to morphological traits (Ratnambal *et al.*, 2003; Arunachalam *et al.*, 2005), floral traits and pollen recovery (Niral *et al.*, 2008), fruit component traits (Rao and Pillai, 1984) and tender nut water quality (Dhamodaran *et al.*, 1993). Evaluation for yield has been an important component of the coconut improvement programme.

Fruit component analysis in the Indian germplasm collection, indicated wide variability for most of the traits studied. The copra content ranged from 340 g in San Ramon Tall (SNRT) to 70.4 g in Surinam Brown Dwarf (SUBD). High copra content of >300g was also recorded in the accessions, Malayan Tall (MLT) and Markham Valley Tall (MVT), while low copra content >100g was also recorded in Pattukottai Green Dwarf (CGD 01), Chowghat Green Dwarf (CGD) and the tall accessions Laccadive Micro Tall (LMT) and Nu Fella Tall (NUFT).

Copra is the major product of coconut in India, which is used for extraction of oil and edible purposes. Milling copra is used for extraction of oil, while edible grade of copra is used for consumption. Best quality edible copra is in the form of ball copra and about 45,000 t of ball copra is produced annually in the country. Traditionally, production of ball copra is limited to certain coconut tracts and all coconut varieties are not utilized for ball copra production. Since ball copra fetches a premium price in trade, a preliminary study was undertaken to assess the suitability of 17 coconut accessions for ball copra production. Spoilage of copra due to germination of nuts during storage was observed and the percentage of spoilage varied across accessions. Recovery of ball copra was highest in Laccadive Micro Tall (LMT) followed by Tiptur Tall (TPT) West Coast Tall (WCT) and Java Tall (JVT), indicating the superiority of these indigenous tall accessions for ball copra production (Niral *et al.*, 2010) (Table 4).

Table 4. Evaluation of coconut genetic resources for ball copra production

Accession	Dry fruit weight(g)	Husk (%)	Copra weight (g)	% spoilage of nuts in storage
Tiptur Tall (TPT)	564	50.52	160	3.73
Malayan Green Dwarf (MGD)	504	49.94	168	75.00
Andaman Ordinary Tall (ADOT)	687	47.48	210	44.44
Andaman Giant Tall (AGT)	801	53.33	201	55.56
Ayiramkachi Tall (AYKT)	380	47.97	129	20.00
Cochin China Tall (CCNT)	680	49.59	288	83.33
Fiji Tall (FJT)	665	48.02	202	41.67
Federated Malay States Tall (FMST)	767	52.80	183	68.75
Gangabondam Green Dwarf (GBGD)	534	44.27	205	46.67
Java Tall (JVT)	608	44.68	186	13.33
Lakshadweep Micro Tall (LMT)	455	47.88	129	0.00
Malayan Orange (Red) Dwarf (MOD)	502	49.08	149	54.55
Malayan Yellow Dwarf (MYD)	318	44.12	92	71.43
Philippines Ordinary Tall (PHOT)	713	47.95	194	70.59
Straits Settlement Apricot Tall (SSAT)	630	47.63	178	81.82
Straits Settlement Green Tall (SSGT)	820	46.67	175	57.14
Indian West Coast Tall (WCT)	536	43.72	181	9.09

In addition, selected coconut germplasm at the institute was studied for their fatty acid composition and variability for fatty acid composition of coconut oil was observed among accessions (Naresh Kumar *et al.*, 2000)(Table 5). Based on the characteristics of coconut oil, accessions suitable for various industrial applications were identified.

Table 5. Evaluation of coconut genetic resources for fatty acid composition

Characteristics of oil	Accession/Hybrid
High saturated fatty acids	ADOT, LCT, SSGT
High medium chain fatty acids	Lakshadweep Ordinary Tall (LCT), ADOT, COD (Chowghat Orange Dwarf) x WCT
High myristic acid concentration	Benaulim Tall (BENT), WAT, FMST, MYD x WCT, WCT x GBGD
High lauric acid concentration	ADOT, LCT, LCT x GBGD, LCT x COD
High lauric acid content, low saturated/unsaturated fatty acids ratio	LCT x GBGD, LCT x COD, COD x WCT
Low saturated/unsaturated fatty acids and high medium chain fatty acids	LCT x GBGD, COD x WCT, LCT x COD, WCT COD

Further, in light of the importance of coconut husk fibre in the coconut coir industry, a pilot study on the physio-chemical and structural characteristics of coconut fibres of nine accessions was undertaken by CIRCOT, Mumbai, in collaboration with CPCRI, Kasaragod (Hussain *et al.*, 2007). The study identified varieties suitable for manufacture of stronger yarns (Gangabondam Green Dwarf), dyeing (Tiptur Tall, East Coast Tall, Benaulim Tall) and production of Q-cellulose (East Coast Tall, Benaulim Tall, Lakshadweep Ordinary Tall, Lakshadweep Micro Tall, Tiptur Tall). The husk fibre of the variety Chowghat Orange Dwarf was found to be comparatively coarser among the varieties studied (Table 6).

Table 6. Physical and chemical characteristics of coconut husk fibre of selected coconut accessions and varieties

Variety	Dry husk wt. (g)	Dry fibre wt. (g)	Bit wt. (g)	% Long Fibre	% Medium Fibre	% Short Fibre	% Bits	Lignin (%)	Holocellulose (%)	Cellulose (%)
BENT	106	42	64	16.98	12.26	10.38	60.38	35.0	64.1	56.4
COD	198	42	156	7.58	8.08	5.56	78.79	30.4	60.4	52.1
ADOT	382	52	330	5.50	4.97	3.14	86.39	33.1	65.8	52.3
Chandra Sankara	150	36	114	11.33	4.67	8.00	76.00	32.5	65.2	59.7
ECT	202	64	138	8.91	11.88	10.89	68.32	33.3	60.5	61.3
LCT	459	133	326	23.09	3.27	2.61	71.02	33.1	65.8	59.1
Kera Sankara	133	33	100	9.77	8.27	6.77	75.19	31.8	60.1	62.3
GBGD	222	62	160	12.61	7.66	7.66	72.07	31.7	61.9	51.3
TPT	308	75	233	10.06	6.82	7.47	75.65	32.7	67.7	60.8
WCT	300	73	227	9.00	7.67	7.67	75.67	31.4	62.1	53.0

Studies at C.P.C.R.I revealed the possibility of identifying drought tolerant cultivars on the basis of accumulation of epicuticular wax on the leaf surface, low stomatal frequency and leaf water potential, the activity of enzymes like glutamate oxaloacetate transaminase and acid phosphatase. A few accessions, *viz.* West Coast Tall, Federated Malay States Tall, Java Tall and Andaman Giant Tall have been identified as moisture stress tolerant and are being utilized in the drought tolerance breeding programme (Kasturi Bai *et al.*, 2006).

Breeding for disease resistance, especially root (wilt) disease of coconut has resulted in identification of Chowghat Green Dwarf and Malayan Green Dwarf as resistant varieties (Jacob *et al.*, 1988; Nair *et al.*, 2004). Screening of 26 selected coconut germplasm against the stem bleeding pathogen, *Thielaviopsis paradoxa* (de Seynes) Hohnel, using petiole inoculation technique, indicated differential levels of susceptibility, with higher lesion size in Malayan Green Dwarf and lesser lesion size in Benaulim Tall (Ramanujam *et al.*, 1998). Screening of the germplasm for identification of accessions with Eriophyid mite resistance has also been undertaken and fruits of Chowghat Orange Dwarf were found to have minimum mite damage incidence while maximum mite damage were recorded in palms of Laccadive Ordinary Tall (CPCRI, 2005). Preliminary screening of cultivars/hybrids against leaf eating caterpillar (*Nephantis serinopa* Meyr.), red palm weevil (*Rhyncophorus ferrugineus*) and rhinoceros beetle (*Oryctes rhinoceros* Linn), indicated variations in susceptibility among accessions, though no

resistant variety was observed. Some distinct trait-specific accessions of coconut in the Indian germplasm collection are given in Table 7.

Table 7. Some distinct trait-specific accessions of coconut in the Indian germplasm collection

Trait	Accession
High female flower production	Spicata Tall, Laccadive Micro Tall, Ayiramkachi Tall
Large inflorescence	Borneo Tall
High copra content (>300 g)	San Ramon Tall, Malayan Tall, Markham Tall
Low copra content (<125 g)	Surinam Brown Dwarf, Chowghat Green Dwarf, Malayan Yellow Dwarf, Laccadive Micro Tall, Ayiramkachi Tall
High Oil content (>72%)	Laccadive Micro Tall
Dwarf with high copra content (>200 g)	Cameroon Red Dwarf, Niu Leka Green Dwarf
High copra/oil output copra/ha & >2.5 t oil/ha)	Fiji Longtowan Tall, Adirampatnam Tall, Cochin (>4 t
Ball copra production	China Tall, Java Tall, Philippines Ordinary Tall Laccadive Micro Tall, Tiptur Tall, WCT, Ayiramkachi Tall, Java Tall
Good quality tender nut water	Chowghat Orange Dwarf, Chowghat Green Dwarf, Malayan Yellow Dwarf, Malayan Orange Dwarf, Philippines Ordinary Tall, Malayan Green Dwarf, Gangabondam Green Dwarf, Cochin China Tall, Kulasekharam Green Dwarf
Drought tolerance	Andaman Giant Tall, West Coast Tall, Java Tall, Federated Malay States Tall
Root (wilt) disease resistance	Chowghat Green Dwarf, Malayan Green Dwarf
Leaf spot resistance	Tiptur Tall
Eriophyid mite resistance	Chowghat Orange Dwarf
Stem bleeding - less infection	Cochin China Tall, Gangabondam Green Dwarf, Laccadive Ordinary Tall, Benaulim Tall
Leaf blight - less infection	Malayan Yellow Dwarf, Chowghat Green Dwarf
Aroma in coconut water and meat	Klapawangi Tall
Sweet endosperm	Guhagar Sweet Tall
Soft endosperm	Thairu Thenga Tall
Soft husk	Kaitathali tall
Pink husk (tender fruits)	Guelle Rose Tall, West Coast Pink Tall

Coconut Descriptors have been developed and published for 74 conserved coconut germplasm (Ratnambal *et al.*, 1995, 2000) and a coconut germplasm database has been developed at the institute (Rajagopal *et al.*, 2005). Further, the evaluation and characterization data of these conserved accessions is available in the Coconut Genetic Resources Database

(CGRD). The institute has also contributed to the development of the World Catalogue of Conserved Coconut Germplasm brought out by COGENT/Bioversity International (Bourdeix *et al.*, 2010).

In addition to morphological descriptors, work on molecular characterization of conserved coconut germplasm is also undertaken at the institute. Characterization of coconut germplasm based on isozyme analysis (Parthasarathy *et al.*, 2004) and protein polymorphism (Geethalakshmi *et al.*, 2005) has been attempted. Chempakam and Ratnambal (1993) studied the variation in leaf polyphenols in coconut germplasm. Molecular characterization of the coconut germplasm in the country has been undertaken using a range of marker systems *viz.* RAPD (Upadhyay *et al.*, 2004), AFLP (Nagaraju *et al.*, 2003), DAF (Jayadev *et al.*, 2005), ISTR (Manimekalai, 2005), IISR (Manimekalai *et al.*, 2006) and SSR markers (Devakumar *et al.*, 2006) and presently molecular characterization of coconut germplasm at the institute is undertaken through SSR analysis. In addition to characterization of germplasm, identification of genes/alleles for desirable traits is envisaged in order to develop a list of trait specific donor parents. A database “COCOGEN” has been developed for microsatellite data. This excel based database has provisions to analyze 11 microsatellite parameters

Research on identification of markers associated with economically important traits and dwarf plant habit for marker assisted selection and also for selection of hybrid seedlings in the nursery are in progress. Application of these molecular technologies in coconut improvement will help hasten the breeding programme through marker assisted selection of superior genotypes in the juvenile phase and also help in reducing the time and space required for experimentation.

The coconut germplasm base available in the country has been utilized in the coconut improvement programme both at the institute, various coordinating centres under the All India Coordinated Project on Palms (AICRPP) and State Agricultural Universities. So far, 18 varieties and 15 hybrids have been released for cultivation in the various agro-ecological zones of the country.

***In situ* conservation in coconut**

In situ conservation has the distinct advantage of conserving already adapted germplasm that has naturally evolved in particular environments. *In situ*/on-farm conservation will not only conserve the original materials that have been sampled for *ex situ* conservation (through the field gene banks, pollen or *in vitro* methods) but also those that have not been collected so far. Together with *ex situ*, on-farm conservation helps to conserve and use a much larger genetic diversity of coconut. The strategic aim of this component of the strategy is to learn more in order to strike an appropriate balance between *in situ*/on farm and *ex situ* conservation. However, there is no sustainable mechanism to effectively monitor and document the effect of *in situ* conservation without a specific conservation project and allocated budget in national programmes.

CPCRI has undertaken studies on on-farm diversity in coconut through various farmer participatory approaches and by application of modern molecular tools for characterization of diversity (Thamban *et al.*, 2007; Rajesh *et al.*, 2008). Germplasm conservation and

characterization in coconut, as in most crops, is mainly undertaken in *ex-situ* gene banks. An alternative approach to coconut germplasm conservation and characterization is the *in situ* conservation strategy which can be easily applied in the case of coconut, since the coconut palm has a long economic life span of > 50 years. The *in situ* conservation approach can be effectively applied for conservation of popular ecotypes and utilized for *in situ* characterization as well as for participatory breeding approaches.

Work on *in situ* characterization of coconut ecotypes has been initiated at CPCRI and in-depth studies into local coconut ecotypes, *viz.* Kuttiadi, Bedakam, Annur, Komadan etc., is in progress to promote *in situ* conservation of genetic resources in farmer's fields.

Future thrust

At CPCRI, efforts to enrich the coconut gene pool with trait-specific germplasm and utilize the coconut germplasm for development of varieties suitable for different end uses and specific industrial uses are in progress. Work on identification and conservation of soft endosperm types (Thairu Thenga), genotypes resistant/tolerant to biotic/abiotic stresses, including rare and economically important variability are in progress. Further, farmer participatory on-farm conservation is also being considered as an alternative conservation strategy to enable farmer participatory plant breeding in coconut. Focused characterization of germplasm for identification of more trait specific accessions is in progress and is being strengthened through molecular/genomic approaches to enable identification of genes/alleles for desirable traits and development of donor lines for effective utilization of the available diversity in coconut improvement. Identification of accessions with quality fibres is envisaged to further promote the coir industry as well as benefit the coconut farmer to a greater extent. Similarly, to promote value addition and enhance income generation of coconut farmers, specific genotypes suitable for production of coconut chips, higher recovery of inflorescence sap, and preparation of shell products need to be identified for utilization in the breeding programme. Dual purpose varieties for tender nut and copra production are being developed. Further, it is envisaged to develop superior genetic stocks, having greater homogeneity with the application of plumule culture technology, using selected mother palms of promising accessions, for utilization in crop improvement programme

References

- Arunachalam, V., Jerard, B.A., Damodaran, V., Ratnambal, M.J. and Kumaran, P. M. 2005. Phenotypic diversity of foliar traits in coconut germplasm. *Genetic Resources and Crop Evolution* 52: 1031–1037.
- Bourdeix, R., Batugal, P., Oliver, J.T., George, M.L.C. Eds. 2010. *Catalogue of conserved coconut germplasm*. International Coconut Genetic Resources Network (COGENT), Bioversity International, Regional Office for Asia, the Pacific and Oceania, Serdang, Selangor Darul Ehsan, Malaysia. ISBN: 978-92-9043-831-1.
- Chempakam, B. and Ratnambal, M. J. 1993. Variation for leaf polyphenols in coconut cultivars. In: *Advances in Coconut Research and Development*. (Eds. Nair, M. K., Khan, H.H., Gopalasundaram, P. and Bhaskara Rao, E.V.V.), Oxford and IBH Publishing Company, New Delhi, India, pp. 51-53.
- CPCRI 2005. *Annual Report 2004-05*. Central Plantation Crops Research Institute., Kasaragod 671 124, Kerala, India,

- Devakumar, K; Jayadev, K; Rajesh, M.K; Chandrasekhar, A; Manimekalai, R; Kumaran, P.M; Parthasarathy, V.A. 2006. Assessment of the genetic diversity of Indian coconut accessions and their relationship to other cultivars, using microsatellite markers. *Plant Genetic Resources Newsletter* **145**: 38–45.
- Dhamodaran, S; Ratnambal, M.J; Chempakam, B; Pillai, R.V; Viraktamath, B.C. 1993. Evaluation of tendernut water in coconut cultivars. In: *Proceedings International symposium on Coconut Research and Development (ISOCRAD*II)*, 26-29 November 1991, CPCRI, Kasaragod, India, pp.123-128.
- Geethalakshmi, P, Niral, V. and Parthasarathy, V.A. 2005. Characterization of coconut germplasm based on protein polymorphism. *Indian Journal of Horticulture* **62**:118-121.
- Hussain, G.F.S., Nachane, R.P., Sheikh, A.J., Paralikar, K.M., Manisha, Vidhan Singh, T. and Thampi, C.J. 2007. Database on physico chemical and structural characteristics of coconut fibres, In: *Coconut for rural welfare* (eds. P.K. Thampan and K.I. Vasu), Proc. International Coconut Summit 2007, Kochi, India, pp. 213-243.
- Jacob PM, Nair RV, Rawther TSS. 1998. Varietal resistance. In: *Coconut root (wilt) disease* (Nampoothiri KUK, Koshy PK, editors). CPCRI, Kasaragod, India, pp. 97–104.
- Jayadev, K., Rajesh, M.K., Devakumar, K., Thomas, R.J., Nair, R.V. and Parthasarathy, V.A. 2005. DNA amplification fingerprinting in coconut: protocol optimization and analysis of resistance to root (wilt) disease. *CORD* **21**:39-49.
- Kasturi Bai, K.V; Rajagopal, V; Arunachalam, V. 2006. Assessment of diversity in coconut varieties for draught responsive physiological traits. *Journal of Plantation Crops* **34**: 118-120
- Manimekalai, R 2005. Informative Inter Simple Sequence Repeat (ISSR) primers for genetic analysis of coconut (*Cocos nucifera* L.) germplasm. *CORD* **21** (2): 45-50.
- Nagaraju, V., He, G., Parthasarathy, V.A. and Prakash, C.S. 2003. Fingerprinting of coconut (*Cocos nucifera* L.) accession using DNA markers. *Journal of Plantation Crops* **31**:8-13.
- Nair, R.V; Jacob, P.M; Ajith Kumar, R. 2004. Screening of coconut cultivars against root (wilt) disease. *Journal of Plantation Crops* **32** (1): 59-60.
- Naresh Kumar, S; Champakam, B; Rajagopal, V. 2000. Fatty acid composition of coconut oil among the cultivars - An insight into industrial application. *Indian Coconut J.* **31** (3): 25-28.
- Niral, V., Augustine Jerard, B., Samsudeen, K. and Nair, R.V. 2010. Evaluation of Coconut Genetic Resources for Ball Copra Production. In: *Abstracts National Conference on Horticulture Bio-Diversity for Livelihood, Economic Development and Health Care*, 29-31st May, UHS Bangalore, pp.30.
- Niral, V., Jerard, B. A., Kavitha, K.V., Samsudeen, K. and Nair, R.V. 2008. Variability and association among floral traits and pollen recovery in coconut (*Cocos nucifera* L.). *J. Plantn. Crops* **36** (3): 186-191.
- Parthasarathy, V.A., Geethalakshmi, P. and Niral, V. 2004. Analysis of coconut cultivars and hybrids using isozyme polymorphism. *Acta Botanica Croatic* **63**: 69-74.
- Rajagopal, V., Manimekalai, R., Devakumar, K., Rajesh, M.K., Anitha Karun, Niral, V., Murali Gopal, Shamina Aziz, Gunasekaran, M., Ramesh Kumar, M. and Chandrasekar, A. 2005. A database for coconut crop improvement. *Bioinformation* **1** (2): 75-77.

- Rajesh, M.K; Arunachalam, V; Nagarajan, P; Lebrun, P; Samsudeen, K; Thamban, C. 2008. Genetic survey of 10 Indian coconut landraces by simple sequence repeats (SSRs). *Scientia Horticulturae* **118**: 282–287.
- Ramanujam, B., Nambiar, K.K.N. and Ratnambal, M.J. 1998. Screening of coconut culttvars/ hybrids against *Thielaviopsis paradoxa* (de Seynes) Hohnel, using petiole inoculation technique. In: *Developments in Plantation Crops Research*, Proceedings PLACROSYM XII, 27-29 November, 1996, RRII, Kottayam, pp. 284-286.
- Rao, E.V.V.B; Vasudevan Pillai, R. 1984. Characterization of coconut germplasm based on fruit component. Analysis. In: *Proceedings, PLACROSYM*, (K.V.A. Bavappa and others Eds.), 15-18 Dec. 1982, CPCRI, Kasaragod, pp. 112-124.
- Ratnamabal, M.J., **Niral, V.**, Krishnan, M. and Ravi Kumar, N. 2000. *Coconut Descriptors, Part II*, CD-ROM, C.P.C.R.I., Kasaragod, Kerala, India.
- Ratnambal M.J., Nair M.K., Muralidharan K., Bhaskara Rao E.V.V., Pillai R.V. 1995. *Coconut Descriptors Part I*. C.P.C.R.I., Kasaragod, Kerala, India.
- Ratnambal, M. J., V. Arunachalam, B. Augustine Jerard, Saran Kumar Rizal and V. Damodaran. 2002. Biometric analysis of diversity in Pacific Ocean coconut populations. *CORD* **18** (2): 74-88.
- Thamban, C; Samsudeen, K; Sairam, C.V; Bosco, S.J.D; Rajesh, M.K; Arunachalam, V; Arulraj, S; Rajagopal, V. 2007. On farm diversity in coconut - Participatory analysis in two coconut communities. Central Plantation Crops Research Institute, Kasaragod 671 124, Kerala, India.
- Upadhyay A., Jayadev, K., Manimekalai, R. and Parthasarathy, V.A. 2004. Genetic relationship and diversity in Indian coconut accessions based on RAPD markers. *Scientia Horticulturae* **99**:353-362.

Genetic resources of coconut

V. Niral, K. Samsudeen and R.V. Nair

Central Plantation Crops Research Institute
Kasaragod 671 124, Kerala, India

Cocos nucifera L., a monoecious perennial monocotyledon, is placed in Arecaceae family (formerly Palmaceae) and the sub family Cocoideae which includes a total of 27 genera and 600 species. Before 1910 many South American palm genera were classified as *Cocos* but now *Cocos nucifera* is considered a monotypic species, despite the pantropical distribution of this palm.

The coconut palm, a native of the humid tropics, is cultivated in the tropical region between latitudes 22°N and 22°S and in a few places even outside the limits of the tropics. Though it is a palm of the coastal tropics, it grows in interior areas also, far away from the sea. The main coconut growing areas are located in Asia, Oceania, West Indies, Central and South America and West and East Africa.

Coconut is considered to be an ancient species with a long history of domestication and cultivation. It is presumed that the early humans while domesticating habitats in coastal areas started domesticating the coconut palms, resulting in the absence of wild forms in the present day coconut population. *Cocos nucifera* is hence regarded as a semi-domesticated species, a complex of local populations with all degrees of dependence upon man, from nil to complete.

Coconut diversity and classification

Attempts to classify coconut varieties have not benefited from the pan-tropical distribution of this monotypic species. The present levels of genetic diversity in coconut are the result of natural evolution and adaptation, as well as human involvement in the exploitation of the species.

The coconut palms are primarily classified based on the stature and breeding habit into two main categories *viz.*, Talls and Dwarfs. The tall type is primarily out-crossing while the dwarf type is mainly self pollinating (with a few exceptions). The tall cultivars are the most commonly cultivated for commercial production in all coconut growing regions of the world while the dwarf varieties are usually grown for ornamental and breeding purpose. One system of classification, based on the observation of several morphological traits, classified the known cultivars of coconut into *var. typica* (talls), *var. nana* (dwarfs) and *var. javanica* (intermediate) with two mutant varieties *var. androgena* (male coconut palm) and *var. spicata* (unbranched inflorescence). On the other hand, based mainly on the fruit characters and seed germination traits, the term *Niu kafa* and *Niu vai* are used to represent the wild and domesticated coconuts, respectively. With the use of molecular marker technologies, a number of marker types have been used in the study of coconut populations' worldwide. Based on the results of these studies, coconut populations have been classified into two major groups: the Pacific group with five sub-groups (South East Asia, Melanesia, Micronesia, Polynesia and the Pacific coast of Central and South America), and the Indo-Atlantic group. The Pacific group includes the domesticated coconut while the Indo-Atlantic group, includes *Niu kafa* coconut

types. However, no single method of classification can account for the variability observed in the coconut populations worldwide.

The diversity in coconut existing today is assumed to be the result of interaction between two factors: its natural distribution and reproductive biology, and human selection. Through the centuries, this selection has led to a reduction in coconut diversity: first, through selection of high yielding types most adapted to specific environments by local populations, leading to well-adapted ecotypes. Subsequently, with the development of organized coconut improvement researches, improved high yielding varieties utilizing a limited set of accessions and focussing on a limited number of essential characters with direct impact on yield, have been developed for commercial cultivation in the different coconut growing tracts. The replacement of old plantations with these improved varieties, has contributed to reduction of genetic diversity in coconut. Furthermore, genetic erosion in coconut has occurred through loss of natural habitat to various developmental activities initiated by mankind.

Locating, maintaining and using genetic diversity of coconut presents substantial challenges; the wide dispersal of the species, the limited knowledge of the history of that dispersion and of the current extent and distribution of diversity. The large, recalcitrant seeds also add to the complexity of managing coconut germplasm. Genetic diversity is desirable for crop improvement and reduction to vulnerability to important crop pest and pathogens and for extending the crop in non traditional areas.

Talls: Tall palms are referred to as *var. typica* and are the most commonly cultivated for commercial production in all coconut growing regions of the world. Talls are sturdy and may continue bearing up to an age of 80-100 years.

Dwarfs: Dwarf palms are referred to as *var. nana*. They are not grown on a commercial scale. They have a short productive life of about 40-50 years.

The basic characteristics of tall and dwarf coconut are listed in Table 1.

Table 1. Basic characteristics of Tall and Dwarf coconut

Trait	Tall	Dwarf
Stem circumference	Sturdy with bulbous base	Thin cylindrical stem
Initiation of flowering	Late (5-7 years)	Early (3-4 years)
Mode of pollination	Highly cross pollinated	Highly self pollinated
Colour of fruits and petioles	Generally mixtures of greens and browns	Either pure green, yellow, red (orange) or brown
Height increment per year	Greater than 50 cm	Lesser than 50 cm
Nut size	Very big to very small	Very small to medium
Phenotypic variation		
Within cultivar	High	Low
Between cultivars	High	High
Root distribution	Generally more dense and plentiful	Less dense and few
Leaf and bunch attachment	Very strong	Fragile

The tall and dwarf types have been utilized for development of hybrid varieties, combining the early flowering trait of dwarfs with the hardiness and high yielding character of tall parents and also exploitation of hybrid vigour. The details of important tall and dwarf coconut cultivars are given in Table 2.

Table 2. Important Tall and Dwarf coconut cultivars

Region	Important cultivars	
	Talls	Dwarfs
South East Asia	Malayan, Klapawangi, San Ramon, Laguna, Dalig, Makapuno, Bali, Tenga, Thai Tall	Aromatic Green, Nias Yellow, Nias Green, Nias Yellow, Bali Yellow, Coco Nino, Catigan, Tacunan, Malayan (Red, Yellow, Green) Dwarf
Central & South America, Atlantic Region	Surinam, Jamaica, Sanblas, Panama Tall	Surinam Brown, Brazilian Green, Malayan Dwarf
African Regions	East African, West African Tall	Cameroon Red, Pemba Dwarf
Pacific Ocean Islands	Markham, New Guinea, Karkar, Rotuma, Fiji, Samoan, Rangiroa, Lifou, Solomon, Rennell, Vanuata, Gazelle Peninsula Tall	Niu Leka, Hari Papua, Madang Brown, Vanuata Red, Malayan Dwarf
Indian Ocean Is.	Seychelles, Comoros, Coco Raisin, Coco Bleu, Sambava Tall	Pemba (Red, Yellow, Green) Dwarf
South Asia	Sri Lankan, Gontheimbili, Indian West Coast, Indian East Coast, Tiptur, Andaman Giant, Andaman Ordinary, Lakshadweep Ordinary, Lakshadweep Micro, Kaithathali, Kappadam, Benaulim Tall	King Coconut, Sri Lankan (Red, Yellow, Green), Chowghat (Red, Yellow, Green), Gangabondam Green Dwarf

Variability in coconut forms

Considerable diversity is observed in the size, shape and colour of fruits of coconut palm. The fruits can either be large, medium, small or very small in size (€ 5 cm long as in Laccadive Mini Micro). The colour of the fruits varies from yellow, shades of green and brown to red (orange). The variations in shapes of the coconut fruit are broadly classified as round, oblong or elliptic. Further, based on the equatorial view, the shape of coconut fruits can be classified as angled, round or flat based on the curvature of the fruit and the presence of ridges

on the fruit. Variations are recorded in shape of the nut inside the fruit and these are broadly categorized as round, oval and oblong.

In addition, certain variant forms of coconut palms, such as androgena (male coconut tree with inflorescences containing only male flowers) spicata (having unbranched inflorescence with few male flowers as in the Indian Spicata Tall, Standard Kudat Tall, Red Spicata) or plicata (with fused leaflets) are also observed in nature. Further, while the endosperm of normal coconuts are solid and firm, when mature, certain palms bear fruits with buttery (jelly-like) endosperm and are referred to as Thairu Thengai (India), Dikri Pol (Sri Lanka) or Makapuno (Philippines). Similarly, soft endosperm types, and sweet endosperm types with lesser fibre content in the endosperm are also reported in coconut, such as Philippines Lono and *Mohacho Narel* of India. Aromatic coconut, wherein the coconut endosperm and tender nut water have a mild, pleasant aroma are also found in nature, such as the Klapawangi Tall and Thailand Aromatic Green Dwarf. The fresh husk of coconut fruit is sweet and edible in certain coconut types (Kaithatali Tall) and has been used as an antidote for sea sickness since times immemorial. Thick-shelled coconuts have also been reported in almost all coconut growing countries (for example Pora Pol in Sri Lanka).

Coconut germplasm collection and conservation in India

India has been in the forefront in coconut improvement researches and systematic coconut breeding was initiated in the country in 1916 with the establishment of the erstwhile Coconut Research Stations at Kasaragod and Nileshwar, now under Central Plantation Crops Research Institute (CPCRI) and Kerala Agricultural University, respectively. Collecting of coconut germplasm in India began in 1924 with introductions from the coconut growing countries *viz.* Philippines, Malaysia, Fiji, Indonesia, Sri Lanka and Vietnam. The Central Plantation Crops Research Institute, Kasaragod hosts the national field gene bank for coconut and is actively involved in the collection and conservation of coconut biodiversity for utilization in the coconut improvement programme. The institute has undertaken exotic collections of coconut germplasm from Pacific and Indian Ocean Islands and from the South Asian countries of Sri Lanka and Bangladesh, followed by extensive prospection and collection of indigenous coconut germplasm from different coconut growing regions within the country (Table 3).

Table 3. Coconut germplasm collection at CPCRI

Place of collection	Number of accessions
<i>Indigenous</i>	266
Kerala	31
Tamil Nadu	22
Karnataka	14
Andhra Pradesh	10
Goa	8
Gujarat	8
Orissa	14
West Bengal	12
Andaman & Nicobar Is	87
Lakshadweep Islands	36

Meghalaya	1
Maharashtra	7
Assam	16
Exotic	132
South East Asia (Indonesia, Malaysia, Philippines, Vietnam)	20
Central & South America, Atlantic region (Jamaica, Panama, Surinam, Trinidad)	7
Africa (Ivory coast, Kenya, Nigeria, Zanzibar)	7
Pacific Ocean region (American Samoa, Fiji, French Polynesia, Guam, Lifou Islands, New Caledonia, New (Hebrides, Papua New Guinea, Solomon Islands, Tonga)	45
Indian Ocean region (Comoros, Maldives, Mauritius, Madagascar, Reunion, Seychelles)	38
South Asia (Bangladesh, Sri Lanka)	15
Total	398

The collected germplasm is conserved in the national gene bank hosted at the institute and characterized using the IBPGR descriptor, CPCRI has so far prepared descriptor for 74 coconut accessions. India also hosts the International Coconut Genebank - South Asia (ICG-SA), which has been established at CPCRI Research Centre, Kidu, Karnataka. Presently 91 accessions, representing indigenous as well as exotic coconut germplasm are planted in the ICG-SA.

Evaluation of coconut genetic resources has resulted in the identification of some trait specific variability with reference to morphological traits, fruit component traits, ball copra production, drought tolerance and insect/disease resistance. Evaluation for yield has also been an important component of the coconut improvement programme.

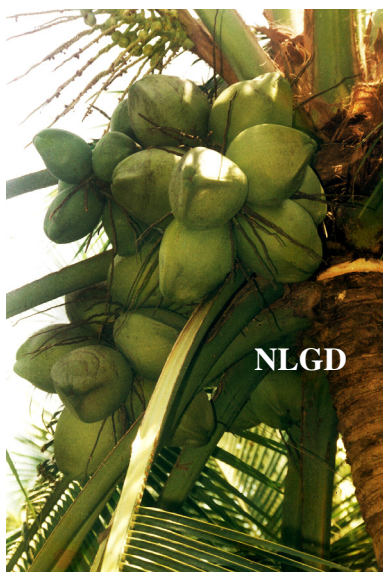
Details of some distinct trait-specific accessions of coconut are presented in Table 4.

Table 4. Some distinct trait-specific accessions of coconut	
Trait	Accession
High female flower production	Spicata Tall, Laccadive Micro Tall, Ayiramkachi Tall
Large inflorescence	Borneo Tall
High copra content (>300 g)	San Ramon Tall, Malayan Tall, Markham Tall
Low copra content (<125 g)	Surinam Brown Dwarf, Chowghat Green Dwarf, Malayan Yellow Dwarf, Laccadive Micro Tall, Ayiramkachi Tall
High Oil content (>72%)	Laccadive Micro Tall
Dwarf with high copra content (>200 g)	CRD, NLGD

High copra/oil output (>4 t copra/ha & >2.5 t oil/ha)	Fiji Longtowan Tall, Adirampatnam Tall, CCNT, JVT, PHOT
Ball copra production	LMT, TPT, WCT, AYKT, JVT
Good quality tender nut water	COD, MOD, PHOT, MGD, GBGD, CCNT
Drought tolerance	AGT, WCT, JVT, FMST
Root (wilt) disease resistance	CGD, MGD
Leaf spot resistance	TPT
Eriophyid mite resistance	COD
Stem bleeding - less infection	CCNT, GBGD, LCT
Leaf blight - less infection	MYD, CGD
Aroma in coconut water and meat	Klapawangi Tall
Sweet endosperm	<i>Mohacho Narel</i>
Soft husk	Kaitathali tall
Pink husk (tender fruits)	Guelle Rose Tall, West Coast Pink Tall



Coconut accessions suitable for ball copra production



Two superior dwarf accessions of coconut

The distinct and superior accessions are being utilized in the crop improvement programme for development of high yielding varieties and varieties suitable for specific end uses and for cultivation in the different agro-ecological zones of the country.

Future thrust

The gene pool maintained at the institute does not cover the entire variability available in the crop. In addition to high yielding genotypes and genotypes resistant/tolerant to biotic/abiotic stresses, certain rare and economically important variability like soft/jelly like endosperm, aromatic endosperm, sweet kernel and sweet husk in coconut are being introduced to the gene bank. Further, an in-depth study into the local ecotypes prevalent in the traditional coconut growing areas of the country is also in progress. Work has been initiated to identify varieties with higher rate of inflorescence sap production and also assess the fibre quality of different varieties and promising accessions for utilization in the coir industry. Similarly, specific genotypes suitable for production of coconut chips and preparation of shell products need to be identified for utilization in the breeding programs. In addition to characterization of germplasm, identification of genes/alleles for desirable traits is envisaged in order to develop a list of donor parents for desired traits. Further, it is envisaged to develop superior genetic stocks, having greater homogeneity, using selected mother palms of promising accessions, for utilization in crop improvement programme.

Varietal improvement of coconut

R.V. Nair, K. Samsudeen, V. Niral and R.J. Thomas

Central Plantation Crops Research Institute
Kasaragod-671 124, Kerala, India

Coconut, *Cocos nucifera* L., is a monotypic genus under *Cocos* with no known wild or domesticated relatives. It is a diploid species with a chromosome number $2n = 32$ and belongs to the family Arecaceae (earlier known as Palmae) under monocotyledons. Genetic resources essential for breeding programmes are mostly limited to cultivars found in the different geographical regions of the tropical world.

Coconut breeding is very tedious and time consuming. The main reasons for slow progress in coconut breeding work are long juvenile period, long interval between generations, heterozygous nature of the palm, lack of vegetative reproduction, long period of experimentation and the large area required for experiments. In spite of these difficulties, much work has been done overcoming such limitations and many improved varieties have been developed in the country.

Diversity and genetic resources: Arecaceae family to which coconut belongs is widely distributed throughout the world. Coconut can be broadly classified into two groups, the Talls and the Dwarfs. Talls are the commonly planted cultivars. Tall palms grow to a height of 20-30 m and are slow maturing, first flowering in 6-10 years after planting. They are sturdy and long-lived and may attain an age of 80-100 years. Talls are normally cross-pollinated and hence highly heterozygous and the population is heterogeneous. Tall palms are sometimes referred to as var. *typica*. The average copra content is usually above 150 g per nut and oil content varies from 66 to 70 per cent. The tall palms are hardy and adapted to different soil types and varying environmental conditions.

Dwarf palms are of shorter stature, 8-10 m high when 20 years old and flowers in 3 to 4 years after planting. They have a short productive life of 40 to 50 years. The dwarf palms are more homozygous than talls, due to high degree of self-pollination. Dwarf palms are sometimes referred to as var. *nana*. The fruits are medium to small and the colour of fruits may be green, yellow, orange (red) or brown. The palms have a tendency for irregular or alternate bearing. The average copra content in dwarf cultivars ranges from 90-120 g per nut and oil content is about 65 percent.

Breeding new varieties and hybrids: Varietal improvement of coconut mainly involves germplasm collection, evaluation and their utilization either by selection or through hybridization involving tall and dwarf varieties.

The organized coconut breeding was started for the first time in India in 1916 at the erstwhile Coconut Research Stations at Kasaragod and Nileshwar (Pilicode) of former Madras Presidency, now in Kerala. In India, coconut germplasm collection started in 1924 when exotic germplasm was introduced from Asian countries. Survey for collecting indigenous germplasm was started in 1958. Today, Central Plantation Crops Research Institute (CPCRI) holds the world's largest conserved coconut germplasm collection with 132 exotic and 266

indigenous collections (Table 1). The collection includes 342 tall types (109 exotic), two exotic semi tall types and 54 dwarf types (21 exotic). Germplasm collections are also being maintained at Coordinating centres in Tamil Nadu, Karnataka, Andhra Pradesh, Orissa, West Bengal, Assam and Maharashtra and also at Pilicode under Kerala Agricultural University. The coconut germplasm is being utilized in the ongoing coconut breeding programme. The major objectives of breeding coconut are improving the yield by increasing the size of the nut and per palm yield, improving the copra and oil content of nuts, production of short stature varieties and resistance to biotic and abiotic stresses.

Table 1. Coconut germplasm holding at CPCRI

Place of collection	Total	Tall	Semi tall	Dwarf
Indigenous	266	233	-	33
South East Asia	20	16	1	3
Central & South America, Atlantic Region	7	6	-	1
African Regions	7	4	1	2
Pacific Ocean Is.	45	39	-	6
Indian Ocean Is.	38	30	-	8
South Asia	15	14		1
Total	398	342	2	54

Selection: Traditional methods of mother palm selection followed by seedling selection continue to be widely practiced by coconut farmers.

Mother palm selection: Mother Palm selection is the most widely practiced method for coconut improvement in India. Palms yielding consistently 80 nuts and more with a copra out turn of 20 kg/palm/year are considered as mother palms. Studies have indicated that average number of female flowers, number of functional leaves in the crown, total leaf production upto three years after sowing and time taken for flowering are important components showing the largest direct effect on yield and thereby indicating their value in selection programme.

Seedling selection: Nursery selection of seedlings is based on characters which are correlated with high yield of adult palms, such as early sprouting, faster growth rate, early splitting of the unexpanded leaf into leaflets, seedling vigour in terms of girth at collar, height and number of leaves besides freedom from pests and diseases.

Effectiveness of mass selection through open pollination was limited. Therefore mother palms selected on the basis of the progeny testing for seedling characters have been advocated to give more scientific thrust to varietal improvement.

Evaluation of cultivars: Evaluation of local cultivars along with exotic cultivars in multi-location trials by CPCRI and other agencies has been a very successful method in coconut breeding and resulted in the development of many new varieties.

Exploitation of hybrid vigour: The discovery of hybrid vigour in coconut by Patel (1937) in a cross between West Coast Tall and Chowghat Green Dwarf crosses made in 1932 at Nileshtar Coconut Research Station was a significant landmark in the history of coconut improvement. This important finding paved the way for the successful breeding programmes in coconut not only in India but in other countries like the Philippines, Indonesia, Sri Lanka, Ivory Coast and Jamaica. Superior varieties selected after evaluation of germplasm have been utilized in crosses to develop high yielding hybrids. Generally two types of hybrids are produced between tall and dwarf varieties. They are D x T and T x D. Even though both types of hybrids are high yielding, D x T hybrids have distinct advantage over T x D hybrids as they could be produced on a large scale by regularly emasculating dwarf mother palms, permitting free natural crossing with pollen of tall palms standing nearby. Therefore in the recent years, production of D x T hybrids rather than T x D hybrids has been advocated because of the ease with which they can be produced.

These hybrids are early bearing and high yielding. They were also superior in terms of copra and oil yield per palm. More than 100 cross combinations involving tall and dwarf accessions were evaluated at CPCRI and other centres and many high yielding hybrids have been released for cultivation in India.

Subsequently, Tall x Tall crosses between unrelated lines of tall varieties are also carried out to develop improved varieties with high out turn of good quality copra and tolerance to biotic and abiotic stresses. Individual trees of high breeding value are identified and these genotypes are utilized for production of T x T hybrids. Though late in bearing, the yield potentials of T x T hybrids are good. In India T x T hybrids have not been released for commercial cultivation, so far. However, at CPCRI a few T x T hybrids are presently under evaluation.

Strategies to produce superior hybrids also include selection of parents for crossing on the basis of combining ability tests. Experience of countries like Ivory Coast in the production of MAWA hybrid point to the need for employing combining ability tests such as Line x Tester, diallele etc., for selection of parents for the crossing programme for the production of superior DxT and TxT hybrids with more homogenous performance. In India, diallele analysis involving 16 parental lines, indicated Ganga Bondam Green Dwarf (GBGD) and Laccadive Ordinary Tall (LCT) to be good general combiners and the combination LCT x GBGD as the combination with best specific combining ability.

Breeding for special characteristics

Disease resistance: Among the various diseases, root (wilt) is the most devastating disease of coconut in India. The characteristic symptom of the disease is flaccidity, yellowing and marginal necrosis of leaflets followed by a progressive decline in yield. The disease is caused by phytoplasma and transmitted by insect vectors. As the disease cannot be controlled by conventional plant protection measures, development of resistant varieties is the most ideal and practical method for the management of this disease. Major portions of southern districts of Kerala comprising Alappuzha, Pathanamthitta, Kottayam and Ernakulam districts are considered to be the hotspots of the disease where the disease is present in epidemic proportion. A comprehensive breeding programme for resistance to the disease was initiated in these districts during 1988.

The screening of the available germplasm during the early years of resistance breeding efforts has not yielded any resistant type. Hence hot-spot areas were surveyed and disease-free palms were identified. Healthy West Coast Tall (WCT) and Chowghat Green Dwarf palms (CGD) standing in the midst of root(wilt) diseased palms were utilized in the breeding programme. Kalparaksha (selection from Malayan Green Dwarf) was identified for its resistance to root (wilt) disease and high yield from a seed production plot of Coconut Development Farm in Neriamangalam (Ernakulam district). This variety was subsequently released for cultivation in the root (wilt) disease affected tracts due to its high yield and higher level of resistance to the disease. Resistance breeding programme involving disease-free WCT and CGD palms identified in farmer's plots located in hot spots of root (wilt) has also resulted in the release of two more varieties for cultivation in the root (wilt) prevalent areas viz., Kalpasree (selection from CGD) and Kalpa Sankara (hybrid between CGD and WCT).

Drought tolerance: Many parts of major coconut growing areas in India are periodically exposed to low rainfall and long dry period resulting in poor yield. Under these circumstances, evolving drought tolerant varieties/ hybrids is of great importance to increase coconut production in drought affected areas. Based on morphological and physiological studies, drought tolerant cultivars have been identified at the Institute, viz., Federated Malay States, Java Giant, Fiji, Laccadive Ordinary and Andaman Giant. These varieties are being utilized in the breeding programme. The high yielding hybrids Laccadive Ordinary Tall x Chowghat Orange Dwarf (COD) and Malayan Yellow Dwarf x West Coast Tall has been found to be drought tolerant.

Scope of biotechnology in coconut breeding: Advances in embryo culture, tissue culture and molecular biology are also being utilized to complement the breeding strategies adopted in coconut improvement.

Embryo culture and Tissue culture: At present, embryo culture has become an important tool for safe germplasm exchange as it reduces the cost of transportation and would meet the phytosanitary regulations. CPCRI has standardized a simple protocol for culturing coconut embryos and this has been utilized in the overseas germplasm expeditions. It is also useful in producing plantlets from special coconut types such as Mohacha Naral, Macapuno etc. where the endosperm is soft and jelly like and does not germinate under normal conditions.

Development of tissue culture techniques aimed at rapid multiplication of elite planting material can greatly save time, space and resources. Success in coconut micropropagation has been reported for regeneration of complete plantlets from plumular tissues of coconut. This is being utilized for scaling up the planting material production of released varieties of coconut, particularly the variety Kalparaksha which has been released for cultivation in the root (wilt) prevalent tracts of Kerala.

Molecular biology: DNA-based markers possess the potential to significantly increase the efficiency of coconut breeding programmes, especially in the areas of germplasm management, genotype identification and marker-assisted selection of economically important traits. Various molecular markers have been used for studying the genetic diversity in coconut germplasm viz, RAPD, RFLP and SSR. Presently at the institute, work on characterization of conserved coconut germplasm through SSR analysis is in progress. Further, work on identification of markers associated with economically important traits is in progress. Work on identification

of markers associated with dwarfness and for selection of hybrid seedlings in the nursery is also in progress. Application of these molecular technologies in coconut improvement will help hasten the breeding programme through marker assisted selection of superior genotypes in the juvenile phase and also help in reducing the time and space required for experimentation.

Future strategies

Though India has the largest collection of coconut germplasm, there is an urgent need to collect the germplasm from unexplored regions of the country and also special types such as aromatic coconut, makapuno etc. from abroad. Further selected germplasm and hybrids produced by crossing between selected cultivars need to be tested in multi location trials to assess the suitability of commercial cultivation in different agro-ecological zones of the country.

Superior accessions identified from germplasm collection need to be subjected to combining ability tests for selection of parents for the crossing programme. Superior hybrids for high yield, tender nut quality, and inflorescence sap (neera) etc. need to be identified and tested in multilocation trials. Improved varieties are to be developed for non traditional areas and regions experiencing drought, low temperature etc.

Because of the shortage of experienced climbers in many parts of the country, cultivation of the dwarf varieties are to be promoted. All the dwarf varieties available in the germplasm are to be evaluated and high yielding dwarfs are to be identified. Hybrids of Dwarf x Dwarf hybrids are also to be evaluated for plant height, high yield and quality including quality for tender nut. Development and release of varieties with resistance/tolerance to biotic and abiotic stress are also to be intensified.

Development of inbreds for production of hybrids has not received adequate attention of coconut breeders. About 40 S_2 generation palms of West Coast Tall are being maintained at Regional Agricultural Research Station, Pilicode under Kerala Agricultural University. These palms are presently being utilized for the production of third generation selves and crosses and to initiate a heterosis breeding programme.

For registration and protection of varieties and for complying with the regulations of PPV and FR Act, DUS standards for registration of varieties are to be developed. There is urgent need to develop a system to grant rights over the varieties developed and a system to get profits through licensing. Seed gardens are to be established to improve the availability of



Kalparaksha



Kalpa Sankara

quality planting materials of released varieties and hybrids in different coconut growing regions in the country.

Refinement of plumular technology for mass multiplication of genotypes is required. Tissue culture protocol using adult explants need to be standardized. *In vitro* consummation of coconut zygotic embryos and pollen has been standardized and needs further refinement. More emphasis should be given to the application of molecular markers for biotic and abiotic stresses and robust identification of hybrid seedlings in coconut nurseries. Studies on gene expression of water stress genes and somatic embryogenesis inducing genes should be strengthened.

International coconut gene bank for south Asia - conservation and characterization

Niral V., Augustine Jerard, B., Samsudeen, K., Patil, D.V., Ananda, K.S., Nair, R.V. and George, V. Thomas

Central Plantation Crops Research Institute
Kasaragod 671 124, Kerala, India

The International Coconut Gene bank - South Asia (ICG-SA), hosted by India, is located at the Central Plantation Crops Research Institute, Research Centre, Kidu, Karnataka, India. The ICG-SA is one of the five multi-site gene banks of coconut as identified by the COGENT Steering Committee with the rest being located in Indonesia (for Southeast and East Asia), Papua New Guinea (for the South Pacific), Cote d' Ivorie (for Africa and the Indian Ocean) and Brazil (for Latin America Caribbean).

International Coconut Gene bank

The International Coconut Gene banks (ICGs) are part of the International Network of *ex situ* collections, provided for in Article 7 of the International Undertaking on Plant Genetic Resources with the Food and Agriculture Organisation (FAO) of the United Nations acting as the trustee. With the entry into force of the International Treaty for Food and Agriculture, these *ex situ* collections are being brought under the aegis of the FAO Commission on Plant Genetic Resources for Food and Agriculture. The International Coconut Gene bank (ICG) has been established to enable the efficient conservation, evaluation and safe movement of coconut germplasm. The major mandates of the ICGs are:

- ☐ To conserve the nationally and regionally identified diversity.
- ☐ To conserve the internationally identified diversity.
- ☐ To further assess the diversity, evaluate the performance of the germplasm and distribute related information to COGENT member countries.
- ☐ To make available germplasm materials to interested COGENT member countries in accordance with agreed protocols.
- ☐ To conduct research and training in relation to the above.

To enable conservation of maximum crop diversity in the ICGs, criteria for identification of diversity to be conserved in the ICG have been formulated. In line with these guidelines, the ICGs are mandated to conserve:

- ☐ Major varieties
- ☐ Threatened varieties
- ☐ Varieties with special traits
- ☐ Varieties/diversity discovered out of explorations done by national programmes
- ☐ Duplicates (safety)

The optimum population size for coconut accessions being maintained in the ICGs is 90 palms. This requires a very vast area, approximately 0.5 hectares for each accession conserved in the gene bank.

International Coconut Gene bank for South Asia

The efforts for establishing the International Coconut Gene bank for South Asia were initiated during the year 1996 and this led to a spurt in germplasm prospection and collecting efforts, both within and outside the country. The MOA between GOI-FAO-IPGRI for hosting the ICG-SA was signed by the Director General, ICAR on behalf of the Government of India on 30.10.1998.

The International Coconut Gene bank - South Asia (ICG-SA), hosted by India, is located at the Central Plantation Crops Research Institute, Research Centre, Kidu, Karnataka, India. It is situated in Dakshina Kannada District of Karnataka which is about 90 km east of Mangalore city and about 100 km east of Kasaragod. The farm lies between 12.30°N latitude and 75.20E longitude, at an elevation of 291 m above mean sea level. The soil is mostly red laterite, slowly changing to alluvial laterite towards the riverbank.

Planting of coconut accessions in the ICG-SA was initiated in 1998. Presently 91 accessions are planted in the ICG-SA (Table 1). These represent indigenous coconut germplasm of the host country as well as Indian accessions representing coconut ecotypes of Philippines, Malaysia, Pacific Ocean Islands, Africa and the Caribbean Region. In addition, exotic coconut germplasm have been collected as zygotic embryos, with the financial assistance of IPGRI/ADB/COGENT, from the Indian Ocean Islands of Mauritius, Madagascar and Seychelles, Comoros and Reunion, Maldives and two ICG-SA member countries *viz.* Sri Lanka and Bangladesh and planted in the ICG-SA.

Table 1. Accessions conserved in the International Coconut Gene bank for South Asia

Sl. No.	Accession name	Source	Palms available
1	Agailjhara Tall	Bangladesh	9
2	Andaman Giant Tall	India	87
3	Andaman Ordinary Tall	India	90
4	Andaman Ranguchan Tall	India	71
5	Nicobar Tall Auckchung	India	19
6	Ayiramkachi Tall	India	86
7	Bagharpara Tall	Bangladesh	9
8	BARI Narikel- I	Bangladesh	11
9	BARI Narikel-II	Bangladesh	20
10	Benaulim Tall	India	90
11	Blanchissuse Tall	India	79
12	Borneo Tall	India	81
13	British Solomon Islands Tall	India	85

14	Calangute Tall	India	70
15	Cameroon Red Dwarf	India	83
16	Nicobar Tall Campbell Bay	India	9
17	Carnicobar Tall	India	81
18	Sri Lanka Tall	Sri Lanka	80
19	Chinashukania Orange Dwarf	Bangladesh	1
20	Chinashukania Tall	Bangladesh	4
21	Chowghat Green Dwarf	India	87
22	Cochin China Tall	India	85
23	Coco Fesse Tall	Reunion	2
24	Coco Bleu Tall	Seychelles	11
25	Coco Gra Tall	Seychelles	1
26	Coco LeHaut Tall	Seychelles	1
27	Coco LeRein Tall	Seychelles	1
28	Coco Raisin Tall	Seychelles	1
29	Comoros Brown Tall	Comoros	2
30	Comoros Green Tall	Comoros	2
31	Comoros Moheli Tall	Comoros	1
32	Comoros Red Tall	Comoros	2
33	Comoros Tall Madagascar	Madagascar	6
34	Comoros Yellow dwarf	Comoros	2
35	De La Reunion Tall	Reunion	2
36	Indian East Coast Tall	India	88
37	Malayan Tall FMS	India	43
38	Gon Thembili Tall II	Sri Lanka	9
39	Guelle Rose Tall	Mauritius	5
40	Hanimaadhoo Green Tall	Maldives	1
41	Hanimaadhoo Medium Round Tall	Maldives	1
42	Hanimaadhoo Oblong Semi Tall	Maldives	2
43	Hanimaadhoo Yellow Tall	Maldives	1
44	Panama Tall Jamaica	India	79
45	Java Tall	India	86
46	Kaadedhdhoo Oblong Tall	Maldives	1
47	Kaadedhdhoo Yellowish Green Tall	Maldives	1
48	Kappadam Tall	India	85
49	Nicobar Tall Katchal	India	51

50	Kayemkola Tall	Bangladesh	15
51	East African Tall Kenya	India	83
52	Khairtala Tall	Bangladesh	7
53	Nicobar Tall Kimios	India	4
54	Nicobar Tall Kimmai	India	17
55	King coconut	India	43
56	King Kumbra Tall	Maldives	5
57	Malayan Yellow Dwarf Kulasekharam	India	90
58	Malayan Green Dwarf Kulasekharam	India	85
59	Malayan Red Dwarf Kulasekharam	India	29
60	Laccadive Micro Tall	India	90
61	Lifou Tall	India	81
62	Malayan Green Dwarf	India	83
63	Malayan Red Dwarf	India	75
64	Malayan Yellow Dwarf	India	90
65	MAWA	India	14
66	Nadora Tall	India	79
67	Pemba Green Dwarf	Mauritius	1
68	Pemba Red Dwarf Mauritius	Mauritius	1
69	Pemba Red Tall	Mauritius	2
70	Philippines Lono Tall	India	88
71	Philippines Ordinary Tall	India	90
72	Pubail Tall	Bangladesh	3
73	Rupdia Tall	Bangladesh	16
74	Malayan SS Apricot Tall	India	87
75	Malayan SS Green Tall	India	86
76	Sambava Green Tall	Madagascar	7
77	Sambava Tall	Madagascar	7
78	San Ramon Tall	India	36
79	Indian West Coast Tall Spicata	India	75
80	Sri Lanka Green Dwarf II	Sri Lanka	9
81	Sri Lanka Red Dwarf II	Sri Lanka	15
82	Sri Lanka Yellow Dwarf II	Sri Lanka	9
83	Saint Vincent Tall	India	35
84	Standard Kudat Tall	India	82
85	Nicobar Tall Tamaloo	India	26

86	Tiptur Tall	India	90
87	Uzirpur Tall	Bangladesh	7
88	West African Tall	India	80
89	West African Tall Madagascar	Madagascar	3
90	Indian West Coast Tall	India	270
91	East African Tall Zanzibar	India	77

The germplasm plots are maintained under drip fertigation as per the recommended production technologies developed at the institute. In addition, the plots are monitored for pest and disease incidence and recommended integrated pest and diseases management packages are adopted for maintenance of field sanitation. Juvenile growth characters, onset of flowering and yield are recorded in the ICG-SA.

Among the 49 designated Indian accessions, in the ICG-SA, flowering has commenced in 41 accessions. In the first batch of exotic germplasm collected as embryos, from the Indian Ocean Islands of Madagascar, Mauritius and Seychelles, initiation of flowering was recorded in all accessions except Guelle Rose Tall and Coco Gra Tall. Among coconut germplasm obtained from Sri Lanka, Sri Lankan Green, Red and Yellow Dwarfs have entered the reproductive phase and among the coconut germplasm collected from Bangladesh, flowering initiation was recorded in the accessions, Kayemkola Tall, Rupdia Tall and BARI Narikel-I. Wide variation in time taken for flowering was observed between the accessions. Juvenile growth characters are being recorded in the ICG-SA and these also showed significant variations between the accessions. Tender nut studies and fruit component analysis are in progress in the ICG-SA.

Conclusion

The establishment of the ICG-SA under CPCRI has strengthened the pioneering role played by India in coconut research and improvement. The ICG-SA under CPCRI has made consistent efforts to conserve the major coconut diversity in the region. The broad areas under which research has to further intensified are:

- Prospecting in centres of diversity and strengthening the diversity in the ICG.
- Characterization and cataloguing of coconut diversity and developing repository of trait specific germplasm for coconut researchers
- Use of modern biotechnological tools for coconut genome sequencing for tackling production constraints in coconut and development of markers for marker assisted selection in coconut
- Intensive biotechnological research for enhancing efficiency of clonal propagation protocols
- Development of varieties for resistance/tolerance to biotic/abiotic stress, dwarf varieties and for different end uses
- Post harvest utilization/processing for enhancing livelihood options of coconut farmers

In addition, at the global level, there is a need for more stringent plant quarantine to support safe movement of germplasm and prevent accidental introduction of invasive pests and debilitating or lethal diseases. It is envisaged that the research in the ICG-SA is to be further strengthened by upgrading the ICG-SA as a Centre of excellence for coconut to promote greater interactions among the international coconut researchers and enable collaborative research to tackle the issues hampering coconut production.

Achievements of breeding for resistance to root (wilt) disease in coconut

P.M. Jacob, Regi J. Thomas and R.V. Nair

Central Plantation Crops Research Institute
Regional Station, Kayangulam - 690 533, Kerala, India

In India, coconut is grown in an area of 1.90 million hectares with an average productivity of 7,747 nuts/ha. The major coconut growing states in the country are Kerala, Tamil Nadu, Karnataka and Andhra Pradesh. However, the major chunk of total area (43%) under coconut is in Kerala state. In Tamil Nadu the productivity of coconut is 12,959 nuts/ha, whereas in Kerala the productivity is only 6,889 nuts/ha (about 39 nuts/palm/year). The low productivity in Kerala can be attributed to a great extent to pests and diseases affecting the palm. Root (wilt) is a very serious disease of coconut and is the major cause for low productivity of coconut in Kerala.

Investigations carried out at CPCRI, Regional Station, Kayangulam on the etiology of the disease, suggested the association of phytoplasma (Solomon *et al.*, 1983) transmitted through *Stephanitis typica* (Lace Bug) (Mathen *et al.*, 1990) and *Proutista moesta* (Plant hopper) (Anon., 1991). Because of the phytoplasmal etiology of the disease, it cannot be controlled by conventional plant protection measures. Hence, development of resistant varieties is considered to be the practical solution for the management of the disease. Breeding of disease resistant varieties is one of the focus areas of coconut research.

Search for resistance to root (wilt) disease

The search for resistance to coconut root (wilt) disease started with Butler (1908) who suggested that there could be resistance to coconut root (wilt) disease in the local cultivars grown in the diseased tract. Varghese (1934) initiated studies on identifying coconut genotypes resistant/tolerant to root (wilt) disease. However, genotypes resistant to root (wilt) disease could not be located.

Davis (1953) reported occurrence of high yielding palms among the heavily diseased palms. Studies also showed that progenies of palms from disease-free areas also develop the disease when planted in the diseased areas during the course of 15 years (Anon., 1966). Iyer *et al.*, (1979) identified nine elite West Coast Tall palms in the diseased tract and monitored them for yield and reaction to root (wilt) disease. All the open pollinated (OP) progenies of these elite palms planted during 1980 at CPCRI, Kayamkulam have contracted the disease. Rawther and Pillai (1972) studied the average yield in both healthy and diseased coconut palms and also disease incidence in Natural Cross Dwarf hybrids, Tall X Dwarf, Dwarf Orange, Dwarf Green and WCT. They found that healthy and diseased natural cross dwarf hybrids performed better compared to WCT with regard to disease resistance and yield performance. Ninan (1978) reported from a survey that Chowghat Green Dwarf (CGD) palms were tolerant to root (wilt) disease with 92.2 % palms free from the disease.

Attempts to screen coconut germplasm were made as early as 1961 at CPCRI, Regional Station, Kayamkulam. The cultivars tested were Andaman Ordinary, Andaman Giant, Cochin China, Ceylon Tall, Laccadive Dwarf, Laccadive Ordinary, New Guinea, Philippines, Strait Settlement Apricot, Strait Settlement Green, St. Vincent and Spicata. All these cultivars evaluated in the field developed typical symptoms of root (wilt) disease subsequently (Menon *et al.*, 1981).

Large-scale field experiments were undertaken during 1972 at CPCRI Kayamkulam and also in cultivator's garden in 63 villages of Alappuzha, Kollam and Kottayam districts. Open pollinated progenies of 36 cultivars and 43 hybrid combinations were planted in gardens with 40-70% incidence of root (wilt) disease. The experiment revealed that all the cultivars and hybrids combinations evaluated contracted the disease (Jacob *et al.*, 1998). Another field experiment, laid out in 1972, in cultivator's gardens with sandy loam soil indicated that COD x WCT gave an average yield of 80 nuts/palm/year with 50 % disease incidence followed by WCT x COD with 70.3 nuts compared to 70 nuts and 37.5 disease incidence in WCT (Jacob *et al.*, 1998).

Evaluation trail carried out with 21 cultivars and 15 hybrids along with WCT as control, planted at CPCRI, Kayamkulam during 1974 revealed that none of cultivars and hybrids was resistant to root (wilt) disease. Andaman Ordinary and Federated Malay States (FMS) recorded the lowest percentage of disease incidence (33.3%) with 18.4 and 18.7 nuts per palm per year whereas WCT recorded 61% disease incidence with 29 nuts/palm/year (Jacob *et al.*, 1998). Another trial involving 27 cultivars was laid out in cultivator's garden during 1982. Among the 27 cultivars tested, all except Kenthali have taken up the disease and the disease incidence ranged from 7.1% to 55.6%. The disease also affected the other hybrid combinations with disease incidence ranging from 12.5% to 66.7% at the fifth year of planting (Jacob *et al.*, 1998).

'Hot spot' breeding programme

During 1987 'hotspot breeding' programme was initiated based on the following observations (Nair *et al.*, 1996):

- In the disease endemic areas, in the midst of heavily disease-affected palms, disease-free and high yielding WCT palms were found. These disease-free palms were identified as the base material for the breeding programme.
- CGD is having higher level of resistance compared to other varieties.
- In the disease endemic areas, a number of disease-free and high yielding Chowghat Orange Dwarf (COD) palms were also found. These were also included in the programme to exploit the reported occurrence of high yield and tolerance to the disease in COD XWCT hybrids.

Artificial pollination of mother palms were carried out in farmers' plots with a view to produce artificially pollinated progenies which can be used either for developing a root (wilt) resistant / tolerant variety or for the production of parental materials for establishing seed gardens (Nair *et al.*, 2000). The following cross combinations were carried out:

1. WCT (self/ *inter se*) 2. CGD (self/ *inter se*) 3. COD (self/ *inter se*)
4. CGD x WCT 5. WCT x CGD

In the absence of an artificial screening technique for systematic evaluation of resistance, the seedlings produced were tested for resistance under natural conditions. Heavy incidence of root (wilt) disease was observed in the under planted seedlings. Observations on root (wilt) disease recorded twelve years after planting from various cross combinations of WCT (WCT *inter se* / self / mixed pollen) revealed that the disease incidence in the progenies varied from 55.0 % to 58.7 %, indicating that there was no significant variation in the susceptibility to the disease among the different cross combinations of WCT (Table 1) (Nair *et al.*, 2003). In the case of the open pollinated progenies of WCT there was 80 % disease incidence. This showed the superiority of the artificially pollinated seedlings (full sibs) over the open pollinated seedlings (half sibs) with regard to their resistance to the disease.

Table 1. Incidence of root (wilt) disease in various cross combination of West Coast Tall variety of coconut

Sl. No.	Cross combination	Year of Planting	No. of plants observed	September 2002	
				No. of plants diseased	Disease incidence(%)
1.	CGD x WCT	1991	31	20	64.5
2.	WCT (<i>inter se</i>)	1992	105	48	55.2
3.	WCT (self)	1992	37	21	55.3
4.	WCT(MP)	1992	118	63	53.4
5.	WCT (OP)	1992	20	14	70.0
	Total		311	166	59.68

CGD - Chowghat Green Dwarf

WCT - West Coast Tall

MP - Mixed Pollen

OP - Open Pollinated

Achievements of varietal evaluation trials including ‘hotspot breeding’

Systematic evaluation trials at CPCRI, Regional Station, Kayamkulam for developing varieties with resistance / tolerance to root (wilt) disease has led to the release of three coconut varieties for the root (wilt) disease prevalent tract. The details of the varieties released are listed below.

a) ‘Kalpasree’

In an intensive survey in large number of farmers’ plots in the ‘disease hot spots’ 200 Chowghat Green Dwarf (CGD) palms were observed for root (wilt) disease incidence. Studies revealed that 75 % of the observed palms were disease-free indicating that CGD is having higher level of resistance to root (wilt) disease when compared to other varieties. Besides, a screening trial involving ten varieties (Table 2) was also laid out at CPCRI, Kayamkulam in 1988. Observations recorded during 1994 based on percentage disease incidence revealed that CGD had the highest level of resistance (75%) followed by Philippines Lono, (70.8%) Zanzibar (70.8%) and FMS (66.7%) (Nair *et al.*, 2004). Accordingly, CGD was identified as the source of resistance for the breeding programme.

Table 2. Screening of coconut varieties for resistance to coconut root (wilt) disease

Sl. No.	Variety	Disease incidence (%)		
		Self	Inter-se	Average
01	CGD	25.0 (4.93)	25.0 (4.93)	25.0 ^a (4.93)
02	Philippines Lono	25.0 (4.93)	33.3 (5.58)	29.2 ^{ab} (5.25)
03	Zanzibar	33.3 (5.58)	25.0 (4.93)	29.2 ^{bc} (5.25)
04	FMS	25.0 (4.93)	41.7 (6.42)	33.3 ^{ab} (5.68)
05	WCT	41.7 (6.42)	33.3 (5.77)	37.5 ^{abc} (6.10)
06	Fiji Rotuma	58.3 (7.62)	41.7 (6.42)	50.0 ^{bc} (7.02)
07	SS Apricot	58.3 (7.62)	58.3 (7.45)	58.3 ^{bc} (7.53)
08	Karkar	50.0 (6.97)	66.7 (8.17)	58.3 ^{bc} (7.57)
09	Kappadam	58.3 (7.45)	75.0 (8.65)	66.7 ^{bcd} (8.05)
10	King Coconut	100.0 (10)	83.3 (9.08)	91.7 ^d (9.54)
General Mean		6.69		
C.V		19.06		
F Value		5.51		
C.D (P= 0.05)		1.85		

* Values in parentheses indicate square root transformed data

* Mean values followed by the same letter are not significantly different by ANOVA (p=0.05)

* Difference between self and *inter-se* is non- significant

Root (wilt) disease incidence and intensity of Chowghat Green Dwarf selection was further evaluated and compared with WCT in the disease prevalent tracts of Kerala. The studies confirmed the high yield and low incidence of root (wilt) disease in CGD (Tables 3 & 4) in hot spots of root (wilt) disease. Considering the high yield and low incidence of root

Table 3. Incidence and intensity of root (wilt) disease in Kalpasree and West Coast Tall variety of coconut in the disease prevalent tracts

Disease status	Farmer's plots				CDB Farm, Neriamangalam			
	Kalpasree		WCT		Kalpasree		WCT	
	No. of palms diseased	Disease incidence (%)	No. of palms diseased	Disease incidence (%)	No. of palms diseased	Disease incidence (%)	No. of diseased	Disease incidence (%)
a. Disease Early	61/551	11.07	23/664	3.46	52/52	19.9	01/100	1.0
b. Disease Middle	11/551	2.00	340/664	51.20	0/52	0.0	51/100	51.0
c. Disease Advanced	08/551	1.45	204/664	30.72	0/52	0.0	32/100	32.0
Total Disease incidence (a+b+c)	80/551	14.52	567/664	85.38	52/261	19.9	84/100	84.0

Table 4. Nut, Copra and Oil yield of Kalpasree in comparison to West Coast Tall variety at CPCRI, Regional Station, Kayamkulam

Variety	Nut/ palm/ year	Copra content (g/nut)	Copra palm (kg)	Palms/ ha	Copra (t/ha)	Oil content (%)	Oil (t/ha)
Kalpasree	90.0	96.3	8.667	236 (6.5x6.5 m spacing)	2.045	66.5	1.360
WCT	48.7	210.1	10.232	175 (7.5x7.5 m spacing)	1.791	67.0	1.200

(wilt) disease, selection made from Chowghat Green Dwarf was released by the name “Kalpasree” for cultivation in homesteads of the root (wilt) prevalent tract during the AICRP (Palms) workshop held during 2009 at Navsari, Gujarat (Arulraj, 2009).

b) ‘Kalpa Sankara’ hybrid

In order to develop a coconut hybrid with resistance to root (wilt) disease, a D x T hybrid produced by crossing between high yielding, disease-free CGD and WCT palms located in disease hot spots was evaluated for its performance in the disease prevalent tracts. Observations revealed that hybrids came to flowering in 30-40 months after planting and recorded a ten year average yield of 84 nuts/palm/year (Table 5). Observations on root (wilt) incidence, eighteen years after planting, indicated that 67.5 % of hybrids took up the disease (Nair *et al.*, 2006). It was concluded that CGD x WCT gave a high yield in spite of high disease incidence, which shows that the hybrid is tolerant to root (wilt) disease. Considering the performance of CGD x WCT hybrid in the diseased tract, this variety has been released for cultivation in the root (wilt) prevalent tracts by the name “Kalpa Sankara” during the AICRP (Palms) workshop held in 2009 at Navsari, Gujarat (Arulraj, 2009).

Table 5. Nut, Copra and Oil yield of Kalpa Sankara in comparison to its parents (Chowghat Green Dwarf and West Coast Tall)

Variety	Avg. nut/ yield/ palm	Copra content (g/nut)	No. of palms/ ha	Copra/ ha (tonnes)	Oil content (%)	Oil/ha (tonnes)
Kalpa Sankara	84.0	170	175	2.500	67.5	1.690
CGD	90.0	96.3	236	2.045	66.5	1.360
WCT	48.7	210.1	175 (6.5 m spacing)	1.791	67.0	1.200

c) ‘Kalparaksha’

Studies carried out during 2004 resulted in identification of another promising variety namely Malayan Green Dwarf as resistant to root (wilt) disease (Nair *et al.*, 2007). This observation was recorded from a seed production plot at Coconut Development Board Farm, Neriamangalam, planted with five dwarf varieties of coconut namely Malayan Green Dwarf

(MGD), Malayan Yellow Dwarf (MYD), Malayan Orange Dwarf (MOD), Chowghat Green Dwarf (CGD), and Chowghat Orange Dwarf (COD). The popular cultivated variety West Coast Tall (WCT) was treated as the control. WCT showed 84 % disease incidence indicating the availability of sufficient inoculum for evaluation of resistance of the dwarf varieties. With regard to resistance, CGD showed maximum resistance followed by MGD. Both CGD and MGD can be grouped as resistant on the basis of their percentage disease incidence.

Earlier studies in WCT clearly showed that the most susceptible stage of WCT palm to root (wilt) disease is the early juvenile phase of the palms *i.e.*, between 8 to 12 years after planting (Ramadasan *et al.*, 1971). Palms of different varieties included in this study had completed the most susceptible stage.

Studies on the intensity of root (wilt) disease among the varieties showed that the most of the diseased palms in Kalparaksha are in the early stage of the disease and progression of disease was slow in this variety (Table 6). Kalparaksha (MGD selection) gave the highest nut yield (64.5) followed by WCT (48.7). MGD out yielded WCT in terms of copra and oil yield mainly because of the higher nut yield and more bunches with nuts (Table 7). This high nut yield can again be attributed to its higher level of resistance to root (wilt) disease compared to WCT. Considering the high yield and resistance to root (wilt) disease, this MGD selection was released for cultivation in the root (wilt) prevalent tracts during the AICRP (Palms) workshop held during 2007 at Hyderabad, India (Nair *et al.*, 2009).

Table 6. Incidence and intensity of root (wilt) disease in dwarf coconut varieties at CDB Farm, Neriamangalam

Variety	Year of Planting	No. of palms	No. of diseased palms (2009)	Percent disease incidence (2009)	Disease intensity category					
					Disease Early		Disease Middle		Disease Advanced	
					No. of palms	Avg. disease index	No. of palms	Avg. disease index	No. of palms	Avg. disease index
Kalpasree	1993	261	52	19.92	52	10.46	0	0	-	-
Kalparaksha	1993	76	17	22.37	12	13.75	05	37.4	-	-
Malayan Yellow Dwarf	1993	201	62	30.85	52	10.27	10	27.5	-	-
Malayan Orange Dwarf	1993	110	71	64.55	62	14.45	06	30.83	03	55
West Coast Tall		100	84	84.00	01	20.00	51	40.39	32	62.5

Efforts for developing a resistant tall variety

Development of an improved WCT variety through recurrent selection is another promising approach to develop a resistant tall variety. A total of 30 S₁ WCT palms, produced by *inter se* mating/ selfing of mother palms in disease hot spots, are being selfed at CPCRI, Regional Station, Kayamkulam during 2009 for progeny evaluation and further selection to develop an improved resistant WCT variety.

Table 7. Nut yield of dwarf coconut varieties at CDB Farm, Neriamangalam

Year of planting: 1993-94

Varieties	Yield (nuts/palm/ year)	Copra content (g/nut)	Copra/ palm(Kg)	Copra/ ha (t/ha)	Oil/ha (t/ha)
Kalpasree	55	96.8	5.36	0.938	0.62
Kalparaksha	64.5	185.1	11.94	2.090	1.37
MYD	38	202.2	7.68	1.344	0.84
MOD	36	150.1	5.40	0.945	0.64
WCT	48.7	210.1	10.23	1.790	1.22

Large scale production of quality coconut seedlings:

In order to meet the acute shortage of quality planting materials, four seed gardens were established in the root (wilt) prevalent tract, by planting selfed or *inter se* progenies of selected WCT, CGD and COD mother palms. The details are as follows: Central Plantation Crop Research Institute, Kannara (1,190), Demonstration cum Seed Production Farm, Neriamangalam (647), District Agriculture Farm, Mavelikara (1,936) and State Coconut Nursery, Karunagappally (412)) spread over nearly 60 acres. These seed gardens, when they come to the full bearing stage, are expected to produce large quantity of disease resistant/tolerant seedlings. From the data so far collected, it appears that Kalparaksha, Kalpasree and Kalpa Sankara varieties of coconut have got a very important role to play in the management of root (wilt) disease. Control of root (wilt) disease, in the long run will most likely rely on cultivation of resistant varieties and integrated management practices.

References

- Anonymous, 1966. *Annual report for 1966*. Central Coconut Research Station Kayamkulam, Kerala, India, pp.64.
- Anonymous, 1991. *Annual report for 1990-91*. Central Plantation Crop Research Institute. Kasaragod, Kerala, India, pp. 154
- Arulraj, S. 2009. Proceedings & Recommendations of XIX Biennial Group Meeting of AICRP (Palms), October 12-14, 2009, Navsari Agricultural University pp 24 (mimeo).
- Butler, E. J. 1908. Report on Coconut Palm Disease in Travancore. *Bulletin No-9*. Imperial Agricultural Research Institute. Pusa. 1-23pp.
- Davis, T. A. 1953. Breeding in coconut for disease resistance. *Indian Coconut Journal* 6 (3): 95-100.
- Iyer, R.D., Rao, E. V. V. B. and Govindankutty, M. P. 1979. Super Yields in Coconut. *Indian Farming* 28 (10): 3-5.
- Jacob, P.M., Nair, R. V. and Rawther, T. S. S. 1998. Varietal resistance. In: *Coconut Root (Wilt) Disease*. (eds.) Nampoothiri, K. U. K. and Koshy, P. K. Central Plantation Crop Research Institute, Kasaragod. 97-104pp.

- Mathen, K., Rajan, P., Nair, C.P.R., Sasikala, M., Gunasekaran, M., Govindankutty, M.P.G. and Solomon, J.J. 1990. Transmission of root (wilt) disease to coconut seedlings through *Stephanitis typica* (Distant) (Heteroptera: Tingidae). *Tropical Agriculture* **67** (1) : 69-73
- Menon, K.S., Thomman, K.J. and Sukumaran, A.S. 1981. Varietal resistance studies. In: *Review of research on root (wilt) disease*, (eds.) Mathen, K., Govindankutty, M.P., Joseph, T. and Mathew, C. Central Plantation Crop Research Institute, Kayamkulam. 78-82.
- Nair, M.K., Koshy, P.K., Jacob, P.M., Nair, R.V., Rao, E.V.V.B., Nampoothiri, K.U.K. and Iyer, R.D. 1996. A root (wilt) disease resistant coconut hybrid and strategy for resistance breeding. *Indian Coconut Journal* **27**(1):2-5.
- Nair, R.V., Rajesh, M.G. and Jacob, P. M. 2000. Chowghat Green Dwarf - a suitable variety for the coconut root (wilt) diseased tract. *Indian Coconut Journal* **31**(3):55.
- Nair, R.V., Jacob, P.M., Rajesh, M.G. and Thomas, R. J. 2003. Incidence of root (wilt) disease in various cross combinations of West Coast Tall variety of coconut. *CORD* **19**(1): 45-50.
- Nair, R.V., Jacob, P.M. and Ajithkumar, R. 2004. Screening of coconut varieties against root (wilt) disease. *Journal of Plantation Crops* **32**(1): 50-51
- Nair, R.V., Jacob, P.M., Thomas, R.J., Pillai, S.P., and Mathews, C. 2006. Performance of CGD x WCT hybrid in the root prevalent tract. *Journal of Plantation Crops* **34** (1): 15-20.
- Nair, R.V., Thomas, R. J., Aravazhi, E., Sasikala, M and Nair, C.P.R. 2007. Malayan Green Dwarf, a root (wilt) resistant coconut variety with high yield suitable for the disease prevalent regions. *Proc. of 94th Indian Science Congress*, Annamalai University, Chidambaram, Tamil Nadu, 3-7th Jan 2007 pp 140.
- Nair, R.V., Thomas, R. J., Mathew, T., Nair, C.P.R., Niraj, V., Jerard, B.A., Samsudeen, K., Pillai, R.V., Sasikala, M., Koshy, P.K., Jacob, P.M., Thomas, G.V., Mathew, M. and Arulraj, S. 2009. Coconut variety Kalparaksha. *Indian J. Genetics and Plant Breeding* **69** (2): 167-168
- Ninan, C.A. 1978. Exploitation of dwarf germplasm in coconut breeding. *Indian Coconut Journal* **8**(11):1-4.
- Ramadasan, A., Shanta, P. and Lal, S.B. 1971. Resistance or susceptibility, age of bearing and yield in young coconut palms in relation to development of root (wilt) disease. *Indian Journal of Agricultural Science*. **41**: 1107-1109.
- Rawther, T.S.S. and Pillai, R.V. 1972. Note on field observations on the reaction of coconut varieties to root (wilt). *Indian Journal of Agricultural Sciences* **42**(8): 747-749.
- Solomon, J.J., Govindankutty, M.P. and Nieuhaus, F. 1983. Association of mycoplasma – like organisms with the coconut root (wilt) disease in India. *Z. Pflkrankh. Pflschutz*. **90**: 295-297
- Varghese, M. K. 1934. *Diseases of Coconut Palms*. Department of Agriculture & Fisheries, Travancore Government Press, Trivandrum pp 105.

'Mohacha Naral' - A coconut with sweet endosperm from Maharashtra, India

**K. Samsudeen, D.D. Nagwaker, Anitha Karun, V. Niral, B.A. Jerard,
P. Ajith Kumar, K. Devadas and R.V. Nair**
Central Plantation Crops Research Institute, Kasaragod

Coconut (*Cocos nucifera* L.) is predominantly grown in West and East coasts of India. Each and every part of coconut is useful to man making it tree of life. Endosperm is the most important part used by man. Besides culinary purposes, coconut endosperm is mostly used for extraction of coconut oil. Coconut oil is mainly used for culinary, confectionary and cosmetic purposes. The endosperm within a nut develops as liquid endosperm at early stages, where repeated free-nuclear divisions take place. As the nut matures, cell-wall formation occurs followed by the development of coconut meat. The endosperm is rich in proteins, amino acids, sugars, vitamins, minerals and growth factors. Endosperm is solid and white in colour. Certain coconut palms produce jelly like endosperm which is called *Makapuno* in Philippines has been commercially exploited for product diversification. *Kopyor*, the soft-endosperm coconut from Indonesia has long been used in food preparation. *Dikiri Pol* coconut with the soft, jelly-like endosperm was reported from the southern province of Sri Lanka. *Kelapa dadeh* is another Makapuno type reported from Malaysia. *Maphrao Kathi* from Thailand and *Niu Garuk* from Papua New Guinea are the other two of the type (Batugal, *et al.*, 2005). Similar types are sporadically reported from India. Another variant *MOHACHA NARAL* (Meaning sweet coconut in Marathi) with sweet and soft endosperm is now reported from Guhagar taluk of Ratnagiri district of Maharashtra state in India.

Ratnagiri is a coastal district in Maharashtra and Guhagar is a coastal tahsil in the district. Coconut population lines up the coast of Guhagar. Among many coconut varieties of the region Banvali is the well-known variety. The varieties are different in size of the fruit, shapes of fruit, in the coarseness of husk, the sweetness of water in the fruit etc. Another variety known as moha (Mohacha naral) is famous for its sweet endosperm but not grown on a large scale because of its lesser yield and lower oil contents. It was reported that assured supply of its seedling was also not possible in this variety (Arunchandra, 2006).

An exploration was conducted during November 2006 to identify and locate coconut palms producing fruits with sweet endosperm. Twenty nine palms of *MOHACHA NARAL* were located in Guhagar tahsil of Ratnagiri district (N17° 28' 55" to N17° 29' 50" and E73° 11' 03" to E73° 19' 50") and one palm was located near the Ratnagiri city (N16° 58' 15.3" and E73° 19' 50.4") (Table 1). These palms aged 40 to 80 years were found randomly distributed in the population. The palms were tall type with the stem height ranging from 7 m to 27 m. The palms had spherical crown with 30 leaves of 4.5m length. The trunk was stout with an average girth of 95cm and well paced leaf scars numbering 14/m at one meter from ground. The average length of inflorescence was 1.3m with 34 spikelets and 17 female flowers. The palms had 15 bunches and 10 nuts/bunch making it a good yielder (Table 2).

Table1. Location of coconut palms with sweet endosperm

Sl.No.	Name and address of farmers	Palm No.	latitude N	longitude E
1	Bhave Prakash Dattray, Opp. Pachayat samiti office, Guhagar, Ratnagiri	NSD2,NSD3	17°28' 57.3"	73°11' 21.3"
2	Bhave Rasmi Ravindra, Guhagar, Ratnagiri	NSD4	17°28' 57.3"	73°11' 24.7"
3	Parchure Srikrishna Nilakant, Guhagar, Ratnagiri	NSD5,NSD6, NSD7	17°28' 55.4"	73°11' 23.6"
4	Khare Vasudev Chintamani, Near KDC Bank, Guhagar, Ratnagiri	NSD8	17°28' 51.4"	73°11' 26.6"
5	Damle Pradeep Sadashiv, Hotel Suraj, Guhagar, Ratnagiri	NSD9,NSD10, NSD11	17°29' 02.5"	73°11' 20.2"
6	Kanitkar Sanjay Vinayak, Guhagar, Ratnagiri	NSD12	17°29' 01.3"	73°11' 18.9"
7	Dali Parhuram Krishnaji, Bazarpet, Guhagar, Ratnagiri	NSD13	17°29' 02.5"	73°11' 19.9"
8	Gadre Srikant Ganesh, Bazarpet, Guhagar, Ratnagiri	NSD14	17°29' 04.6"	73°11' 17.9"
9	Ahok Waman Athavale (new owner), Saraswati Nivas, Varsapat, Guhagar, Ratnagiri	NSD15	17°29' 09.0"	73°11' 19.3"
10	Vaze Shakuntala Kasinath, Varsapat, Guhagar, Ratnagiri	NSD16	17°29' 29.8"	73°11' 11.8"
11	Joshi Kesav Vaman, Varsapat, Guhagar, Ratnagiri	NSD17,NSD18	17°29' 31.0"	73°11' 09.7"
12	Khare Madhusudan Moreswar, Varsapat, Guhagar, Ratnagiri	NSD19,NSD20	17°29' 33.7"	73°11' 08.8"
13	Khare Sridhar Dattatre, Varsapat, Guhagar, Ratnagiri	NSD21,NSD22, NSD30	17°29' 46.2"	73°11' 07.7"
14	Joshi Vasant Parassuram, Varsapat, Guhagar, Ratnagiri	NSD23	17°29' 46.2"	73°11' 07.7"
15	Milinder Madhukar, Varsapat, Guhagar, Ratnagiri	NSD24	17°29' 46.4"	73°11' 07.1"
16	Dixit Govind Krishnaji, Varsapat, Guhagar, Ratnagiri	NSD25	17°29' 48.7"	73°11' 03.0"
17	Joshi Suyog Ramkrishna, Varsapat, Guhagar, Ratnagiri	NSD26	17°29' 48.7"	73°11' 03.0"
18	Damle Ravindra Narayan, Varsapat, Guhagar, Ratnagiri	NSD27	17°29' 51.8"	73°11' 07.6"
19	Jog Vijay Sakaram, Varsapat, Guhagar, Ratnagiri	NSD28	17°29' 51.8"	73°11' 07.6"
20	Koustabh Sadasiv Satte	NSD29		
21	Hemant Yadneswar Phatak, Phatakvadi, Someswar village, Ratnagiri	NSD1	16°58' 15.3"	73°19' 50.4"

Table 2. Palm characteristics

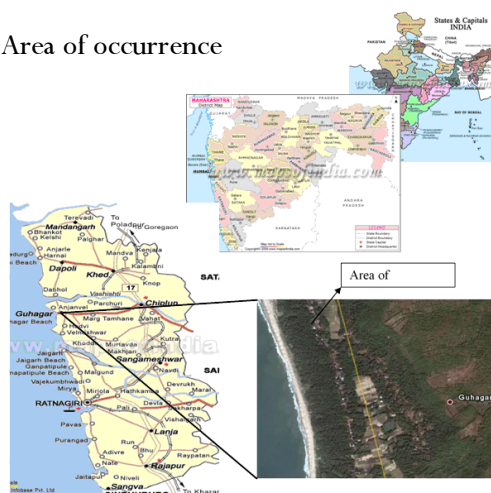
	Average	SD	Min.	Max.
Plant height (cm)	1733.86	460.05	692.00	2675.00
Girth (cm)	95.61	13.00	70.00	120.00
No. of leaves	30.18	2.68	26.00	35.00
Petiole length (cm)	104.46	20.41	85.00	170.00
Leaf length (cm)	358.18	40.79	292.00	432.00
Leaf scars/m	14.32	3.39	9.00	21.00
Inflorescence length (cm)	89.81	14.53	66.00	123.00
Inflorescence stalk length (cm)	41.41	6.42	30.00	56.00
No. of spikelet/Inflorescence	33.81	6.25	16.00	43.00
Female flowers/Inflorescence	16.81	13.38	7.00	81.00
No. of bunches	15.07	2.43	10.00	20.00
no. of nuts/bunch	10.11	2.97	4.00	17.00
Percentage of sweet endosperm nuts	46.00	17.53	10.00	77.00

All the fruits in a bunch were not of sweet endosperm type. The number of nuts with sweet endosperm per bunch varied from 10 to 77 percent in different palms. Forty six percent of all the nuts collected from 30 palms were of sweet endosperm type (Table 2). Classifying palms in to groups based on frequency of sweet endosperm, it was found that four palms had less than 25% sweet endosperm nuts, thirteen palms had 25 to 50% sweet endosperm nuts and eleven palms had more than 50% sweet endosperm nuts.

Fruit component traits of sweet and normal nuts of this population were studied. Sweet endosperm types had slightly less fruit and husked fruit weight compared to normal nuts. Husk thickness and weight were same in both the types. Shell thickness was similar in both, but the shell weight was more in normal types. Likewise, endosperm thickness was similar in both, but the endosperm weight was more in normal types. Copra weight and copra recovery was more in normal nuts compared to sweet endosperm types. Embryo weight of sweet endosperm nuts was significantly lower than normal endosperm (Table 3). Total soluble sugars were same in both type of nuts, but organoleptic test showed that water in sweet endosperm nuts was poor in taste.

Farmers of the area get a premium price for sweet endosperm nuts. It is mainly used for raw consumption. Frequency of sweet endosperm

Area of occurrence



nuts in consequent bunches and the seasonal variation in production of nuts with sweet kernel are to be further studied. Influence of pollen on the development of sweet endosperm also needs to be analysed. Seed nuts from identified palms were collected and a population was conserved at CPCRI, Kasaragod for further evaluation and possible utilization in the coconut improvement programme. Exploitation of sweet kernel trait will help in product diversification in coconut which will lead to profitability of coconut industry.

Table 3. Fruit characteristics

	Sweet		Normal	
	Mean	SD	Mean	SD
Fruit weight (g)	951.16	258.92	1000.04	244.23
Husk thickness (cm)	3.04	0.55	3.04	0.51
Husk (%)	53.50	7.45	54.19	6.83
Husked fruit weight (g)	417.83	89.96	439.45	87.81
Shell weight (g)	128.40	29.38	137.85	26.78
Shell thickness (cm)	0.38	0.08	0.36	0.08
Endosperm weight (g)	289.43	69.28	302.27	73.02
Endosperm thickness (cm)	1.31	0.14	1.33	0.14
Copra weight (g)	119.54	32.04	180.40	40.79
Oil (%)	36.72	7.68	57.2	12.50
Embryo weight (g)	0.06	0.01	0.11	0.03

References

- Arunchandra S. Pathak, editor. 2006. *Revised edition of Volume X of Original Gazetteer of the Bombay Presidency relating to Ratnagiri and Sawantwadi* (<http://ratnagiri.nic.in/Gazetter/GOM/home.html>)
- Batugal, P., Ramanatha Rao, V and Oliver, J. 2005. (Eds.). *Coconut Genetic Resources*. International Plant Genetic Resources Institute – Regional Office for Asia, the Pacific and Oceania (IPGRI-APO), Serdang, Selangor DE, Malaysia.

Performance evaluation of coconut cultivars under rain fed conditions

Lissamma Joseph, S. Anitha and K. Aravindakshan

Kerala Agricultural University, Vellanikkara, Thrissur -680656, Kerala

Coconut palm (*Cocos nucifera* L.) is one of the most versatile palms of the tropics. Genetic diversity existing in this monotypic species is unparalleled. The two major forms of coconut recognized in nature are the tall and dwarf types. Land races in each of these types have been identified which serve as the source for genetic improvements programmes. Over the years several high yielding varieties of coconut have been evolved through systematic selection and evaluation.

Though irrigation alone can double the yield of the coconut, shrinkage of water resources limit the percentage of irrigated coconut area to less than two. In this context identification of cultivars with high yield potential under rainfed situations assumes great significance.

Five tall cultivars *viz.*, Laccadive Ordinary, Komadan, WCT (Kuttiyadi), WCT (Kasaragod) and Tiptur Tall; two dwarf cultivars, Chowghat Green Dwarf and Ganga bondam were planted in the coconut farm attached to Campus Development, KAU, Vellanikkara during 1990-94 and 1997, respectively. Tall cultivars were spaced at 8 m x 8 m and dwarf cultivars at 5.5 m x 5.5 m. All the cultivars were grown rainfed with average management. For comparison of the performance of these cultivars, the number of nuts/palm/harvest was recorded from 2005 onwards. The mean nut yield/palm/year was assessed during the initial bearing period (10-14 years).

Yield data of the tall and dwarf cultivars are presented in Table 1. All the cultivars exhibited a progressive increase with respect to the nut yield/palm/year. Among the tall cultivars, WCT (Kasaragod) recorded the highest yield of 24 nuts/palm in 2005 followed by Komadan with 23 nuts, whereas, Lakshadweep Ordinary recorded only 10 nuts/palm. Within a five years span, Komadan recorded 77 nuts/palm/year followed by WCT (Kasaragod and Kuttiyadi) with 74 and 73 nuts/year respectively. Tiptur Tall showed yield increase from 19 to 66 nuts/year in the same period. In the present study, Komadan was found to show more nut production potential compared to other tall cultivars.

This is in conformity with the results of Manju *et al.* (1992) and Shylaraj *et al.* (1991) who reported superiority of Komadan for number of bunches per palm and annual nut yield/palm.

Among the dwarf cultivars, Chowghat Green Dwarf yielded maximum with 76 nuts/palm in 2006 which declined to 56 nuts/palm by 2009, whereas, for Gangabondam initial nut yield was 13 which increased to 24 nuts/palm by 2009. Nair *et al.* (2006) reported yield data of Chowghat Green Dwarf palms to be 55 nuts/palm/year from a study of 250 palms at CDB farm at Neriamangalam.

The present study gives an indication of the variability in yield potential within tall and dwarf cultivars of coconuts under rainfed conditions which can be taken as a basis for

further selection and evaluation. Solid conclusions can be derived after assessing the yield data and other yield contributing characters of these cultivars in their mature phase.

References

- Manju P., Gopimony R. and Saraswathy, P. 1992. Evolutionary status of the coconut type Komadan. *Abstracts of papers presented in Gregor Johan Mendel Birthday Lecture Series and Symposium International-1992* July 22, 23. Department of Botany, Univeristy of Calicut.
- Nair, R. V., Thomas, R. J. and Nair, S. A. 2006 Coconut varieties resistant to root (wilt) disease. *Indian Coconut Journal* **37**(6):2-4.
- Shylaraj, K.S., Bindu, M., Gopakumar, K. and Gopimony, R. 1991. Comparing Komadan coconut type with West coast tall. Coconut breeding and management. *Proceedings of the national Symposium on coconut breeding and management* held at the Kerala Agricultural University, Thrissur, India from 23rd and 26th November, 1988: 107-108.

Table 1. Average yield (nuts/palm) of coconut cultivars during the study period

Year	Laccadive Ordinary	Tiptur Tall	WCT (Kasaragod)	Komadan	WCT (Kuttiyadi)	Chowghat Green Dwarf	Ganga bondam
2005	50	19	24	23	20	-	-
2006	45	32	72	32	61	76	13
2007	40	19	41	29	13	78	76
2009	51	66	74	77	73	56	24

Genetic diversity in coconut germplasm based on morphological descriptors

S. Geethanjali, M. Kumar, K.Rajamanickam and S.Arulraj

Coconut Research Station, Tamil Nadu Agricultural University, Aliyarnagar-642101

Coconut palm (*Cocos nucifera* L.; $2n=32$) is an economically important crop grown over 80 countries in the tropics (Perera *et al.*, 2010). Coconut is grown in nearly two million hectares in India and is mainly cultivated by small scale and marginal farmers. In India, coconut plays a more prominent role in the social, economic and cultural life of the people. It provides livelihood directly and indirectly for many thousands of people in the three sectors: production, processing and marketing. It is one of the major sources of edible oils and fats to the Indian people.

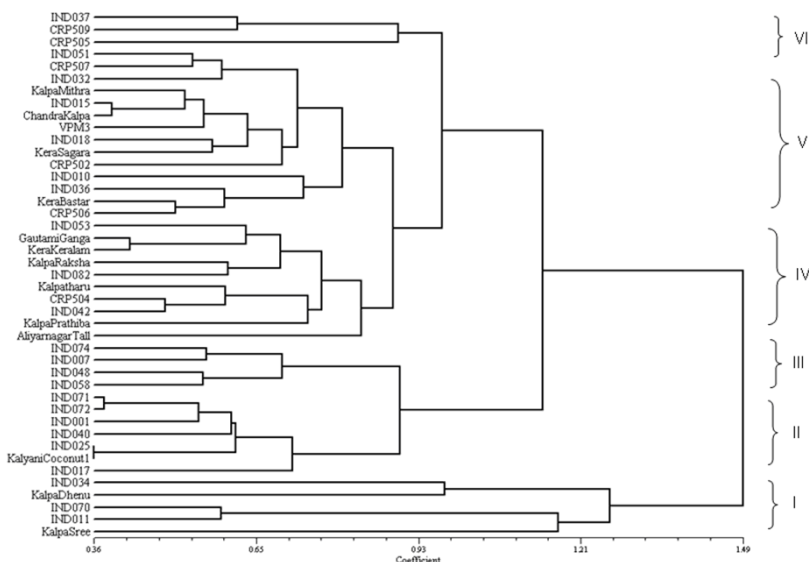
Coconut cultivars are classified into Talls, Dwarfs and intermediate types. The Tall types are most widely cultivated in India although Dwarf's occupy considerable area. A systematic collection and characterization of coconut germplasm collection started in India in 1949 (Narayana and John, 1949). Since then, several collections from different agro-ecological regions in India have been made, which include collections from Orissa (Panda, 1982), Andaman and Nicobar Islands (Balakrishnan and Nair, 1979) and Lakshadweep islands (Jacob and Krishnamoorthy, 1981). These collections are primarily characterized using morphological descriptors including fruit and leaf component characters (Harries, 1978; Raveendran *et al.*, 1987). Sangare *et al.* (1978) used floral biology and pollination behaviour to classify coconut populations. Ashburner *et al.* (1997) used fruit morphology to study genetic diversity in the South Pacific coconut populations. Arunachalam *et al.* (2005) studied genetic diversity of coconut populations using foliar traits such as petiole, leaf sheath and the extent of placation. Such knowledge on genetic diversity or relatedness of the accessions is important for effective germplasm conservation and exploitation in breeding programmes.

Tamil Nadu Agricultural University, Coimbatore maintains coconut germplasm in two of its research stations located at Aliyarnagar and Veppankulam. The collection includes indigenous and exotic accessions from different regions of the country and across the world. The objective of the study was to analyze the extent of genetic diversity present in the coconut germplasm collection at Aliyarnagar using morphological descriptors.

A subset of 43 accessions was used in this study. The details of the materials are included in Table 1. The morphological descriptors included were leaf production, number of functional leaves, girth at permanent mark (cm), height from base (cm), total leaf length (cm), petiole length (cm), leaflet length (cm), leaflet breadth (cm), number of leaflets, nut yield/palm/year, cumulative mean nut yield, weight of the nut (g), length of the nut (cm), breadth of the nut (cm), weight of the dehusked nut (g), weight of the split nut (g), weight of the kernel (g), thickness of the kernel (cm), volume of water (ml), copra yield / nut (g), and copra yield (kg)/palm/year. The data were analyzed by mean, range, standard deviation and co-efficient of variation. Standardization of data was performed in CropStat (IRRI, 2005). Manhattan dissimilarity co-efficients were calculated for the morphological variables. Cluster

analysis was carried out based on the Unweighted Pair Group Method with Arithmetic Averages (UPGMA) and Manhattan co-efficients using NTSYS (Rohlf, 1994) to depict genetic relationships among accessions.

The results of mean, range and SD of the traits are presented in Tables 1 and 2. Number of leaves ranged from 8.5 to 11.9 with the mean of 10.03. The number of functional leaves ranged from 23 to 38.5 with the mean of 31.5. The girth at permanent mark ranged from 61.3 to 144.5 with the mean of 107.5. Large variation was recorded for the height, which ranged from 293 cm to 1635.12 cm with the mean of 880.9 cm. Total leaf length ranged from 420 to 649 cm with the mean of 549.7 cm. Petiole length varied from 100 to 160 cm with the mean of 136.3 cm. Leaflet length ranged from 98.5 to 181.4 cm with the mean of 127.4 cm. Leaflet breadth ranged from 3.9 to 7 cm with the mean of 5.4 cm. Number of leaflets ranged from 85 to 127.2 with the mean of 111. Nut yield ranged from 32 to 135.3 per palm per year. Large variation was found in the weight of the nuts that ranged from 740 to 3838 g with the mean of 1523 g. The length and breadth of the nuts ranged from 23 to 40 cm and 14.25 to 26.75 cm, respectively. Weight of the dehusked nut ranged from 117.5 to 2240 g with the mean of 740.6 g. Weight of the split nut ranged from 310 to 1142 g with the mean of 548.9 g. Weight of the kernel ranged from 200 to 975 g with the mean of 353.4 g. Thickness of the kernel ranged from 1 to 1.3 cm. Volume of the water ranged from 50.5 to 790 ml with the mean of 193.9 ml. Copra yield per nut ranged from 115 to 350 g with the mean of 204.2 g. Copra yield per palm per year ranged from 4.8 to 30.1 kg with the mean of 15.85 kg.



The result of cluster analysis is shown in dendrogram (Fig.1). Manhattan genetic distance ranged from 0.36 to 1.49 for all genotypes. Six clusters were observed. Cluster I included five genotypes viz., IND034, Kalpa Dhenu, IND070, IND011 and Kalpasree. Cluster II included seven genotypes viz., IND071, IND072, IND001, IND040, IND025, Kalyani

Coconut 1 and IND017. Cluster III included four genotypes *viz.*, IND074, IND007, IND048 and IND058. Cluster IV included 10 genotypes *viz.*, IND053, Gautami Ganga, Kera Keralam, Kalparksha, IND082, Kalpatharu, CRP504, IND042, Kalpa Prathibha and Aliyarnagar Tall. Cluster V included 14 genotypes *viz.*, IND051, CRP507, IND032, Kalpamithra, IND015, Chandrakalpa, VPM3, IND018, Kerasagara, CRP502, IND010, IND036, Kerabastar and CRP506. Cluster VI included three genotypes *viz.*, IND037, CRP509 and CRP505.

The results clearly illustrate the extent of variability for the morphological as well as economically important traits present in a subset of 43 coconut accessions maintained at Aliyarnagar, TNAU, Coimbatore.

Traditionally, genetic diversity in a germplasm collection is assessed using morphological data (Rauf *et al.*, 2010). Standardization of data was required in order to remove the arbitrary variations caused by the different unit of measurements and to avoid over influence of any traits in forming clusters and make the traits contribute equally to the clustering of objects. Among several diversity indices, Manhattan co-efficients are preferred for agronomic traits. Manhattan genetic distances identify the same pair of accessions exhibiting minimum diversity but different pair of genotypes exhibiting maximum diversity (cropgenebank.sgrp.cgiar.org/index.php?). In this study, Manhattan genetic distance ranged from 0.36 to 1.49 suggesting high level of trait variability present in the collection.

The study revealed the presence of six clusters of genotypes. Cluster I comprised of genotypes with big sized nuts mostly originated from the Philippines, Andaman & Nicobar Islands, Papua New Guinea and Kerala. Cluster II included low yielding genotypes from Goa and Kerala producing about 40-50 nuts/palm/annum. Dwarf genotypes with orange and yellow coloured nuts suitable for tender nut purpose grouped together in cluster III. Cluster IV comprised predominantly of south Indian genotypes including Tamil Nadu, Karnataka and Kerala. This cluster consisted of potential yielders with more than 100 nuts/palm/year and many of these have been released as varieties for commercial cultivation such as Aliyarnagar Tall, Kalpa Prathibha, Kera Keralam, Gautami Ganga and Kalpatharu. Genotypes originating from the islands of Asia Pacific and Oceania region grouped together in cluster V. Cluster VI included genotypes from the east coast region of Tamil Nadu as well as a genotype from Zanzibar. These observations supported that morphological descriptors indicate geographical affinity of genotypes in the collection, which has been documented in various studies (Arunachalam *et al.*, 2005).

Morphological descriptors also provide clues about the economically important traits (Arunachalam *et al.*, 2005). For example, leaf cross sectional area in plants has strong association with wind tolerance. Width and thickness of petiole give the indirect estimate of the dry weight of the entire leaf in oil palm (Corley *et al.*, 1971), which in turn provides wind tolerance (Arunachalam *et al.*, 2005). Further evaluation of this germplasm collection for leaf sheath related traits would help in stress tolerance breeding efforts.

Assessment of genetic diversity based on morphological evaluation is often laborious and take years to draw a conclusion. The advent of molecular markers has led breeders to estimate genetic diversity more rapidly, which often found to corroborate diversity estimates based on morphological data (Rauf *et al.*, 2010). Molecular markers such as isozymes (Niral *et al.*, 2007), randomly amplified polymorphic DNA (RAPD) (Upadhyaya *et al.*, 2004), inter

simple sequence repeats (ISSR) (Manimekalai and Nagarajan, 2006), amplified fragment length polymorphism (AFLP) and simple sequence repeats (SSR) (Teulat *et al.*, 2000; Perera *et al.*, 2003; Devakumar *et al.*, 2006; Rajesh *et al.*, 2008a, Rajesh *et al.*, 2008b) were used to study genetic diversity in the coconut collection maintained at Central Plantation Crop Research Institute (CPCRI), India. Characterization of these accessions using molecular markers would further augment the genetic diversity information for the collection.

Thus, the results of this study would be useful (i) to select parental palms for hybridization programs based on diverse genotypic groups and (ii) to make critical decisions regarding the collection, exploration and introduction of new accessions from within India as well as exotic accessions for future breeding programs.

References

- Arunachalam, V., Jerard, B.A., Damodaran, V., Ratnambal, M.J. and Kumaran, P.M. 2005. Phenotypic diversity of foliar traits in coconut germplasm. *Genetic Resources and Crop Evolution* **52**:1031-1037.
- Ashburner, G.R., Thompson, W.K., Halloran, G.M. and Foale, M.A. 1997. Fruit component analysis of South Pacific coconut palm populations. *Genetic Resources Crop Evolution* **44**:327-355.
- Balakrishnan, N. P. and Nair, R. B. 1979. Wild populations of *Areca* and *Cocos* in Andaman and Nicobar islands. *Indian Journal of Forestry* **2**(4):350-363.
- Corley, R.H.V., Hardon J.J. and Tan, G.Y. 1971. Analysis of growth of Oil palm I. Estimation of growth parameters and application in plant breeding. *Euphytica* **20**:307-315.
- Devakumar, K., Jayadev, K., Rajesh, M.K., Chandrasekar, A., Manimekalai, R., Kumaran, P.M. and Parthasarathy, V.A. 2006. Assessment of the genetic diversity of Indian coconut accessions and their relationship to other cultivars, using microsatellite markers. *Plant Genetics Resources Newsletter* **145**:38-45.
- Harries, H. C. 1978. The evolution, dissemination and classification of *Cocos nucifera* L. *Botanical Review* **44**:265-319.
- IRRI, 2005. Cropstat version 7.2.3. International Rice Research Institute, Manila, Philippines. <http://archive.irri.org/science/software/cropstat.asp>
- Jacob, P. M. and Krishnamoorthy B. 1981. Coconut genotypes of Lakshadweep islands. Paper presented at the 4th PLACROYM conference, Mysore, India, 1981. PLACROSYM IV:3-8.
- Manimekalai, R. and Nagarajan, P. 2006. Assessing genetic relationships among coconut (*Cocos nucifera* L.) accessions using inter simple sequence repeat markers. *Scientia Horticulturae* **108**:49-54.
- Narayana, G.V. and John, C.M. 1949. Varieties and forms of coconut. *Madras Agricultural Journal* **36**:349-366.
- Niral V., Geethalakshmi, P. and Parthasarathy, V. A. 2007. Intrapopulation allelomorphism in tall and dwarf populations of the coconut. *Acta Bot. Croat.* **66**(1):35-42.
- Panda, K.C. 1982. Coconut types of Orissa. *Indian Coconut Journal* **12**(3):10-18.

Perera, L., Russell, J.R., Provan, J. and Powell, W. 2003. Studying genetic relationships among coconut varieties/populations using microsatellite markers. *Euphytica* **132**: 121–128.

Perera, L., Perera, S. A. C. N., Bandaranayake, C. K. and Harries, H. C. 2010. Coconut. In: *Oil crops Handbook of Plant Breeding*. (Eds. J. Vollmann, and I. Rajcan). Springer Science Business Media, LLC 2009. pp. 369-396.

Table 1. Variability for Height and leaf related traits in coconut accessions

	New accession number	Height from base (cm)	Leaf production	No of functional leaves	Girth at permanent mark (cm)	Total leaf length (cm)	Petiole length (cm)	Leaflet length (cm)	Leaflet breadth (cm)	No. of leaflets
Zanzibar	IND 037	1360.3	11.0	38.5	121.4	603.3	147.0	128.0	5.8	107.5
San Ramon	IND 034	1310.8	10.0	35.5	125.1	598.3	143.7	145.9	5.9	118.0
Gonthembili	IND 051	1390.1	9.5	32.0	106.3	562.4	148.6	134.0	6.0	121.0
Java	Kalpa	1414.0	10.0	33.5	142.4	569.2	140.0	122.0	5.5	120.0
	Mithra									
FMS	IND 010	1365.2	11.0	36.5	144.5	580.4	139.0	132.0	4.8	115.4
BSI	IND 036	1400.8	10.5	38.5	115.7	604.8	142.2	181.4	5.0	124.0
Kenthali	IND 074	845.0	9.0	28.0	80.2	460.0	100.0	106.4	3.9	99.2
St. Vincent	IND 053	1060.0	10.3	34.0	94.0	639.2	136.1	135.3	5.3	114.0
MGD	Kalpa Raksha	1630.7	10.0	33.5	115.0	530.0	129.0	123.0	4.4	101.0
Arasampatti Tall	Aliyarnagar Tall	1478.3	11.0	37.0	117.0	538.0	123.0	145.0	5.2	114.1
Tiptur tall	Kalpatharu	1080.4	10.0	32.1	115.0	560.2	135.1	125.8	5.6	121.0
ECT	CRP 509	1635.0	10.1	32.3	113.5	561.2	138.0	128.3	5.5	125.1
MOD	IND 048	730.5	8.5	26.0	72.1	500.0	120.0	125.0	4.0	97.0
MYD	IND 058	850.7	9.3	29.0	61.3	420.0	101.1	105.7	4.2	88.0
ADOT	IND 018	807.4	10.0	31.9	90.0	575.0	134.1	125.5	5.5	85.0
Calangute	IND 071	630.0	9.0	28.0	97.0	485.0	145.0	118.0	6.0	111.0
Nicobar	IND 070	925.6	10.0	30.0	124.2	547.1	130.0	130.0	7.0	97.0
Guam	IND 025	682.0	9.7	29.4	100.0	486.2	120.5	98.5	5.7	102.2
Ceylon Tall	IND 015	892.3	9.3	31.3	118.0	562.3	137.5	122.5	5.5	109.7
Nadora tall	IND 072	370.0	8.5	27.0	90.3	490.1	132.1	110.6	6.0	127.2
Spikelss	IND 040	540.7	11.5	32.2	94.0	465.0	120.0	117.0	5.8	109.0

A. Renguchan	IND 017	413.0	8.5	26.5	107.9	485.0	107.1	120.0	5.1	104.2
Jamaica	Kalyani	648.7	10.0	29.5	107.0	462.5	117.5	103.1	6.0	108.0
	Coconut 1									
Seychelles	Kera Sagara	790.7	9.5	33.5	93.0	575.0	150.0	137.1	5.4	121.0
LCOT	Chandra	801.2	10.3	32.5	113.0	585.1	124.8	127.0	5.9	121.9
	Kalpa									
Rajapalayam Tall	CRP 504	765.3	10.0	31.0	116.0	506.0	142.5	120.1	5.5	108.0
CGD	Kalpa Sree	638.4	9.1	26.0	89.8	540.1	136.8	114.0	5.0	116.0
Hazari	CRP 502	740.7	10.3	34.0	95.4	495.3	115.0	128.5	4.5	102.6
Ayiramkachi	IND 082	855.1	11.0	31.0	102.3	514.7	140.2	115.3	4.0	110.0
Andaman Giant	Kalpa	1031.7	11.0	32.0	123.5	590.4	156.0	130.0	5.3	114.1
	Dhenu									
Cochin China	Kalpa	960.7	10.3	34.5	113.5	615.3	148	132.5	6	125.4
	Prathiba									
GBD	Gautami	702.5	10.4	35.1	101.6	533	135	128.5	6.5	115
	Ganga									
San Blass	IND 032	700.0	9.0	34.0	97.7	590.0	115.0	134.0	6.0	116.0
WCT	Kera	570.4	10.3	33.0	95.5	560.0	145.0	138.0	6.0	101.5
	Keralam									
Etamozhi tall	CRP 505	780.5	11	34.0	133.0	649.0	157.0	142.0	6.0	111.0
Fiji	Kera Bastar	705.0	11.9	35.1	124.0	590.0	143.0	152.0	5.0	113.0
Thailand	CRP 506	585.2	10	35.2	110.0	612.5	158.0	143.0	5.2	112.5
COD	IND 007	293.0	10	24.0	70.0	480.0	125.3	117.0	4.7	100.5
New Guinea tall	IND 011	650.0	9.5	28.0	123.2	605.0	155.0	124.0	6.5	108.2
PHOT	VPM 3	702.0	9.5	25.0	117.0	560.4	160.0	120.0	6.0	118.0
Kappadam	IND 001	600.3	11	28.4	103.0	582.0	159.0	120.0	5.3	113.0
Siam	CRP 507	790.0	9.3	23.0	131.0	601.0	153.0	140.0	6.0	116.0
Kenya	IND 042	755.0	11	32.0	118.0	566.0	156.0	130.7	5.7	110.0
Range		293.0-1635.12	8.5-11.9	23.0-38.5	61.3-144.5	420.0-649.0	100.0-160.0	98.5-181.4	3.9-7.0	85.0-127.2
Mean		880.9	10.03	31.5	107.5	549.7	136.3	127.4	5.4	111.0
SD		336.9	0.81	3.8	18.1	53.6	15.7	14.5	0.7	9.6

Table 2. Variability for fruit related traits in coconut genotypes

Genotype	New accession number	Nut yield/palm/year	Cumulative nut yield	Weight of the nut (g)	Length of the nut (cm)	Breadth of the nut (cm)	Weight of the dehusked nut(g)	Weight of the split nut(g)	Weight of the Kernel (g)	Thickness of the kernel (cm)	Volume of water (ml)	Copra yield/ nut (g)	Copra yield (kg)/ palm/year
Zanzibar	IND 037	110.0	102.65	1962.50	26.00	22.50	987.50	705.00	400.00	1.20	225.10	260.00	28.60
San Ramon	IND 034	68.0	61.6	3837.50	31.75	26.75	2240.00	1142.50	975.00	1.30	790.00	250.00	17.00
Gonthembili	IND 051	59.1	61.4	1605.00	28.50	20.00	587.50	487.50	325.00	1.10	100.50	160.50	9.48
Java	Kalpa Mithra	83.0	86.45	1245.00	28.00	20.75	742.50	535.00	370.00	1.20	187.80	210.00	17.43
FMS	IND 010	105.4	100.7	1380.00	27.75	14.25	690.00	512.50	412.50	1.20	140.00	153.00	16.13
BSI	IND 036	88.0	89.1	1055.00	28.50	17.70	720.00	517.50	362.50	1.20	162.65	225.50	19.84
Kenthami	IND 074	48.0	46.9	1320.50	23.50	19.00	755.00	500.00	260.00	1.00	200.20	205.10	9.84
St. Vincent	IND 053	110.8	106.85	1737.50	28.20	21.00	750.00	535.00	357.50	1.10	162.80	127.60	14.14
MGD	Kalpa Raksha	112.3	113.2	1420.00	28.00	19.50	525.00	413.75	202.50	1.10	122.50	140.00	15.72
Arasampatti Tall	Aliyarnagar Tall	116.0	160.5	1135.00	23.50	17.00	532.50	405.00	262.50	1.10	78.50	115.00	18.40
Tiptur tall	Kalpitharu	135.3	130.5	740.00	23.00	16.50	350.00	310.00	200.00	1.20	50.50	147.50	19.96
ECT	CRP 509	120.1	118.7	1537.50	28.00	18.20	845.00	610.00	417.50	1.20	187.80	250.90	30.10
MOD	IND 048	75.0	72.5	1165.00	25.50	16.70	725.00	485.00	330.00	1.10	210.00	140.00	10.50
MYD	IND 058	78.0	75.1	925.00	24.00	15.70	540.00	382.50	270.00	1.10	110.00	150.00	11.70
ADOT	IND 018	83.7	81.4	1275.00	26.50	17.75	662.50	502.50	312.50	1.10	147.50	280.00	17.50
Calangute	IND 071	40.0	36.2	1447.50	28.00	21.20	670.00	520.00	320.00	1.10	142.50	185.00	7.40
Nicobar	IND 070	48.0	43.2	1880.00	34.30	24.30	1220.00	856.80	557.00	1.10	320.00	243.50	11.70
Guam	IND 025	50.8	53.65	1625.00	36.00	22.00	675.00	517.50	342.50	1.10	142.50	195.50	9.93
Ceylon Tall	IND 015	70.3	75.7	1557.50	29.00	18.75	777.50	552.50	352.50	1.10	145.00	230.50	16.20
Nadura tall	IND 072	38.7	36.5	2042.50	28.00	19.25	775.00	507.50	327.50	1.10	230.00	200.00	7.70
Spikelss	IND 040	45.0	45.3	1150.00	26.00	19.75	710.50	320.10	220.80	1.10	178.80	167.00	7.50
A. Renguchan	IND 017	32.0	30.7	1550.00	30.00	18.25	747.50	555.00	340.00	1.20	172.50	150.00	4.80

Jamaica	Kalyani Coconut 1	52.0	51.1	1625.00	27.00	21.50	777.50	600.00	340.00	1.10	185.00	230.00	11.90
Seychelles	Kera Sagara	89.3	90.1	1100.00	25.20	17.00	600.00	427.50	300.00	1.00	132.50	200.00	17.80
LCOT	Chandra	78.0	76.35	2200.00	28.75	19.20	767.50	522.50	312.50	1.10	202.50	210.00	16.30
Rajapalayam	Kalpa	84.5	79.6	902.50	23.25	17.20	430.00	357.50	267.50	1.20	85.00	180.00	15.21
Tall	CRP 504												
CGD	Kalpa Sree	80.2	81.5	3275.00	40.00	22.50	1075.00	725.00	347.50	1.10	310.00	295.00	23.60
Hazari	CRP 502	78.0	75.7	1485.00	30.25	22.70	665.00	505.00	322.50	1.10	162.50	210.00	16.38
Ayiramkachi	IND 082	130.0	129.5	1562.50	30.00	19.00	622.50	423.70	265.00	1.10	175.00	160.00	20.80
Andaman	Kalpa	65.4	63.5	3000.00	34.00	24.00	1585.00	1140.00	640.00	1.10	597.50	350.00	22.80
Giant	Dhenu												
Cochin	Kalpa	130	125.6	1492.50	29.25	18.75	495.00	407.50	235.00	1.30	97.50	190.00	24.70
China	Prathiba												
GBD	Gautami	110	113.4	1137.50	27.25	19.00	600.00	495.00	332.50	1.10	102.50	210.00	23.10
	Ganga												
San Blass	IND 032	60.0	54.6	1662.50	30.50	19.00	712.50	567.50	350.00	1.20	200.00	210.00	11.61
WCT	Kera	118.0	111.8	1132.50	27.20	21.50	445.00	365.00	220.00	1.10	158.00	210.00	24.15
	Keralam												
Etamozhi tall	CRP 505	95.0	91.6	1600.00	31.50	23.80	117.50	825.00	515.00	1.20	133.20	250.00	23.18
Fiji	Kera Bastar	67.2	69.7	1045.00	29.20	17.50	682.50	497.50	317.50	1.25	121.00	240.00	15.82
Thailand	CRP 506	80.0	78.1	1182.00	26.25	18.75	745.00	483.00	356.00	1.20	193.00	235.00	19.34
COD	IND 007	42.0	41.7	887.50	23.25	15.50	587.50	435.00	270.00	1.00	176.00	205.00	9.02
New Guinea	IND 011	44.0	40.7	2287.50	28.50	25.25	1040.50	907.50	577.50	1.10	495.00	240.00	9.60
tall													
PHOT	VPM 3	85.0	84.2	1157.50	26.00	18.50	852.50	602.50	405.00	1.10	190.00	225.00	18.65
Kappadam	IND 001	48.5	43.6	1165.00	28.00	19.50	667.50	502.50	310.00	1.10	132.50	195.00	7.82
Siam	CRP 507	62.0	61.7	1107.50	27.70	19.00	627.50	480.00	310.00	1.17	142.50	190.00	11.40
Kenya	IND 042	108.3	107.6	900.00	26.70	16.25	522.50	460.00	285.00	1.20	140.00	160.00	16.85
Range		32.0- 135.3	30.7- 160.5	740.0- 3838.0	23.0- 40.0	14.25- 26.75	117.5- 2240.0	310.0- 1142.0	200.0- 975.0	1.0- 1.3	50.5- 790.0	115.0- 350.0	4.8- 30.1
Mean		79.7	79.1	1523.00	28.18	19.59	740.60	548.90	353.40	1.13	193.90	204.20	15.85
SD		28.5	30.47	628.80	3.43	2.75	330.70	184.00	134.70	0.07	135.00	47.50	6.05

***In vitro* approaches in coconut: an Indian perspective**

Anitha Karun and M.K. Rajesh

Central Plantation Crops Research Institute, Kasaragod-671 124, Kerala, India

The productivity of coconut needs to be increased substantially to be competitive with other vegetable oils. The major coconut production problem includes lack of superior cultivars, low level of yield and productivity. The cloning of high yielding individuals could contribute to raising the productivity to the highest level possible. Commercial exploitation of coconut hybrids with high productivity has had limited success due to low seed multiplication coefficients, long juvenile period and lack of a repeatable protocol for cloning high yielding palms. Standardization of a viable protocol for clonal propagation through *in vitro* approaches opens up tremendous possibilities of meeting the requirements for quality planting materials and breaking down productivity barriers. *In vitro* culture of vegetative explants has been attempted at several coconut research institutes. Research on embryo culture for safe germplasm movement and cryopreservation has been undertaken at various laboratories.

Problems in genetic improvement of coconut includes long generation time, large phenotypic diversity, wide-spread distribution, different types of pollination, crossing properties, low multiplication rate, large recalcitrant seed and absence of vegetative propagation. Biotechnological options for varietal improvement under *in vitro* culture are:

- a) Embryo culture for safe movement of germplasm
- b) Tissue culture of elite genotypes
- c) Production of homozygous lines
- d) *In vitro* screening for stress tolerance
- e) Cryopreservation of coconut embryos and pollen
- f) Cloning and characterization of genes induced during somatic embryogenesis

Embryo culture for safe movement of germplasm: Coconut germplasm collection and storage are difficult due to the bulkiness of the nut, short dormancy of the seed and its water content. A simple protocol from field collection to till the field establishment was successfully standardized at CPCRI. This CPCRI protocol includes direct field collection of 9 to 11 month old coconut embryos, short term (active) storage *in vitro* retrieval, *ex vitro* and field establishment. The protocol for embryo culture developed by CPCRI has been successfully used for collection of both indigenous and exotic germplasm. The first set was the indigenous collection and transportation of 87 embryos of six Pacific Ocean accessions being maintained at the World Coconut Germplasm Center (CPCRI), Andaman Islands, India. Out of the 83 plantlets retrieved, twenty-five plantlets were field planted at International Coconut Gene bank for South Asia (ICG-SA), Kidu during 1996.

The protocol has also been used in eight exotic germplasm collection expeditions. The embryos were retrieved *in vitro* at Kasaragod and successfully transplanted to the field.

Forty-five accessions were collected from Mauritius (5 accessions), Madagascar (4 accessions), Seychelles (5 accessions), Reunion (3 accessions) Comoros (5 accessions), Maldives (8 accessions), Sri Lanka (4 accessions) and Bangladesh (11 accessions). A total of 503 plantlets have already been planted at ICG-SA. Some of these accessions have started flowering and yielding since 2004.

Mohacha Naral coconut, a variant of Benaulim Tall, is distributed in the Konkan districts of Ratnagiri and Guhagar of Maharashtra State. This accession is characterized by sweet and soft kernel with less fibre content. Embryo rescue is the only means to propagating this rare accession as the embryos are tiny and do not germinate normally. Identification of sweet kernel is only possible by breaking open the mature nuts. A total of 28 mother palms possessing nuts with sweet kernel have been identified in these areas from which one hundred and thirty one embryos were extracted and retrieved via CPCRI embryo culture protocol. The *in vitro* regenerated plantlets have been planted at Regional Coconut Research Centre (RCRS), Bhatye, Maharashtra.

Tissue culture of elite genotypes: Monocotyledons, especially with woody perennials, are very poor in their response to *in vitro* culture. Coconut is one of the most recalcitrant species to regenerate *in vitro*. The major problems encountered *in vitro* multiplication of coconut are intense browning of tissues, heterogeneity in tissue reaction, strong rooting ability and low embryogenesis and callogenesis and long response time.

More than a dozen laboratories in India were working on tissue culture during the beginning of 1980's. At present, coconut biotechnology research is done only at Central Plantation Crops Research Institute, Kasaragod. Coconut tissue culture works were initiated as early as 1977 at CPCRI, Kasaragod. Since then, various explants *viz.* inflorescence, leaf and endosperm were used to induce callus. A repeatable protocol for plantlet regeneration from plumular tissues was standardized at biotechnology section of CPCRI. A total of 13 plantlets could be retrieved from a single plumule and resulted plantlets were found to be uniform. Conversion of floral primordial to vegetative primordial at a very low frequency was achieved by prolonged incubation of inflorescence in auxin –cytokine combination media.

***In vitro* screening for stress tolerance:** Water stress in the culture system was progressively increased with each passage by incorporating polyethylene glycol (PEG –6000), mannitol and sodium chloride into culture medium. The effect of PEG, ABA, mannitol and proline on coconut maturation of zygotic embryo was reported. The studies on GA3 showed that, there was no interaction between the GA3 concentration and culture duration on shoot length. Growth of the shoots depended largely on the maturity of embryos. There is a significant interaction between maturity of the embryos and the ABA concentration for the initial shoot growth (60 days) and root development (120 days).

Cryopreservation: Coconut genetic resources are traditionally conserved *ex situ* as whole plants in field gene banks, but this requires large area. Being a recalcitrant crop, coconut is sensitive to desiccation and can thus be conserved for short periods only even in the optimal moisture condition. Cryopreservation is considered as safe and suitable for long-term conservation and enables to conserve large genetic variability. Pre-treatment of the embryo is very much essential before conserving the embryo in liquid nitrogen at –196 °C. These treatments include simple desiccation (using laminar air current and use of silica gel), the use

of high concentration of sucrose as osmotica, various cryoprotectants in various combinations (glycerol, propylene glycol, DMSO, sorbitol, formamide) and encapsulation with 3 % sodium alginate and dehydration techniques.

Mature embryos of Tall and Dwarf types of coconut could be cryopreserved after desiccation pretreatments and retrieved *in vitro*. The maximum retrieval of healthy plantlet could be obtained only from the embryos subjected to 18 hours silica gel and 24 hours laminar airflow desiccation pre treatment. Irreversible damage caused by desiccation to the death of the shoot meristem was noticed when the water content reduced to 20 %. At CPCRI, four different cryopreservation methods viz., air desiccation, pre growth desiccation, encapsulation-dehydration, and solution based vitrification were tested for the recovery of plantlets for field planting from cryopreserved embryos and the results revealed that methods such as pre growth desiccation with sucrose in combination with raffinose and solution based vitrification (PVS3) methods are very effective for cryostorage of coconut zygotic embryos. Plantlet retrieval in polybag was found to be 22.5% in PVS3 based method and 27.5% in pre growth desiccation procedures.

Cryopreservation of coconut pollen: Pollen cryopreservation is an additional technique for the conservation of genetic resources, which supplements seed or clonal preservation. Cryopreservation of coconut pollen is possible and viability maintained after the pollen was incubator-dried or cryopreserved. Though the germination percentage of cryopreserved pollen (38.54) was significantly lower than that of fresh pollen (46.65), more vigorous pollen tube growth was observed in the former. Significant palm-to-palm variation in germination and pollen tube growth was also noticed. Coconut pollen after cryogenic storage resulted in normal nut set under controlled pollination of WCT and COD palms at CPCRI. It was observed that coconut pollen retained the viability after 3 years of cryogenic storage as revealed by germination studies on artificial media.

Cloning and characterization of genes induced during somatic embryogenesis: Somatic embryogenesis involves differential gene expression and activation of numerous signal transduction pathways. Little is known about genes expressed during somatic embryogenesis in coconut. *SOMATIC EMBRYOGENESIS RECEPTOR KINASE (SERK)* genes play key roles during somatic embryogenesis in various plant species. Sequence encoding *SERK* were cloned from coconut using a degenerate primer-based PCR approach. Sequence analysis revealed high levels of similarity to other plant *SERKs*. Predicted amino acid sequences of *SERK* protein indicated presence of domains characteristic of *SERK* proteins from other species.

Coconut is considered as one of the recalcitrant species with regards to tissue culture. Ample efforts have been put into research on tissue culture in various laboratories in India and abroad. Studies are also strengthened to know the molecular aspects of somatic embryogenesis in coconut. The genes involved during somatic embryo formation such as *SERK*, *MAP Kinase*, *BABYBOOM* have been cloned and characterized and currently the gene expression studies are in progress at CPCRI.

References

Karun Anitha and Sajini, K.K. 1994. Short-term storage of coconut embryos in sterile water
Curr. Sci. 67 (2) pp 118-120.

- Karun Anitha, Parthasarathy V.A, Kumaran P.M, Iyer R.D. and Sajini, K.K. 2002 *Coconut Embryo Culture-Protocol for Germplasm Collection*. CPCRI Kasaragod, pp16.
- Karun Anitha, Sajini, K.K., Radha, E. and Rajesh M.K. 2007 Palm tissue and organ culture protocols . Technical bulletin no.51. CPCRI, Kasaragod.p.28.
- Karun Anitha,, Sajini, K.K., and Parthasarathy, V.A. 2005. Cryopreservation of mature zygotic embryos of coconut by desiccation methods. *CORD* 21(1): 13-19.
- Karun Anitha., Upadhyay, A., and Parthasarathy, V.A. 1998. Status of research on coconut embryo culture and acclimatization techniques in India. Coconut embryo culture *in vitro* culture proceedings of the first workshop on embryo culture held at Albay research Centre Philippines Oct 27-31 pp.29-36.
- Kovoor, A., 1981. Palm tissue culture. *FAO Plant Production and Protection Paper* (Italy) pp 30, 69.

Genetic relatedness of coconut populations from Andaman and Nicobar Islands traced to the Indian sub continent using microsatellite markers

Deva Kumar ,K., Jerard, B. A., Dhanapal, R., Damodaran, V., Niral, V and Naganeeswaran, S.

Central Plantation Crops Research Institute, Kasaragod - 671 124, Kerala, India

The Indian territory of Andaman and Nicobar group of islands is an archipelago of nearly 328 islands located in the Indian Ocean between South Asia and South East Asia. Of the 328 islands, humans inhabit only 128. The coconut is the predominant tree in many of these islands and exists as a natural forest. Coconut is grown in an area of 24,410 ha producing 85.44million nuts annually. The Andaman Islands are located close to the Southeast Asian mainland as a southward extension of the Irrawaddy delta of Burma. This island arc is created by the collision of the Indo-Australian Plate with Eurasia which lifted the Himalayas and most of the Indonesian islands, and created a long arc of the Andaman mountain chain and the farther southward extension of this forms the Nicobar Islands. The Nicobars, consisting of 19 islands are situated south of the neighboring Andaman Islands archipelago, and are located about 189 km northwest of the Indonesia island of Sumatra. At present, the coconut cultivation is confined to valleys and coastal areas in the Andaman Islands and in the coastal belts and beach forest in the Nicobar Islands. The major coconut producing areas are Car Nicobar, Nancowrie, Katchal, Campbell Bay and Andaman. The main varieties of coconut grown in these Islands are Andaman Ordinary, Andaman Giant, Katchal Tall and Andaman Micro. Some unclassified Tall varieties are also seen under cultivation.

The Andamans are high islands, and had few if any coconuts, so the early settlers were not cultivating coconuts when the Europeans arrived. The Nicobars are low lying, coral islands and probably had wild type coconuts before the first human settlement. Subsequently, the domestic type would have been introduced to both groups and the present day cultivated coconuts should have different (but introgressed) FCA results.

The Nicobar group of islands is one of the natural repository of coconut genetic diversity in the world. A wide range of variations in morphological traits were observed by coconut researchers. Coconut accession having fruits with modified perianths that extends as a hard structure resembling three short horns, palms producing beaked fruits /nuts and palms with persistent petiole and inflorescences (Rao and Sampath kumar, 1998) are found in Car Nicobar Islands. The Andaman coconut accessions have recorded encouraging levels of resistance (33% as compared to WCT and CGD-100%) to root (wilt) disease of southern Kerala (Jacob *et al.*, 1998), resistance to pestalotia leaf spot in West Bengal (Ray *et al.*, 1990) and Andaman giant accession showed tolerance to burrowing nematode (Sosamma *et al.*, 1988). Based on physiological parameters and yield performance studies the Andaman Ordinary was found to possess high relative drought tolerance. All these research information support the importance of coconut biodiversity in this region and its utility in the coconut breeding programmes.

A consultancy report on coconut collecting strategies (Bourdeix *et al.*, 1999) has identified these islands as one of the hotspot areas for collecting coconut diversity. As part of germplasm exploration and collection program, a total of 55 accessions has been collected and maintained at the genebank at Central Plantation Crops Research Institute, (CPCRI), Seed Farm at Kidu, Karnataka. In situ morphological studies and fruit nut characteristics were undertaken to study the coconut genetic diversity in these islands and has yielded a substantial amount of knowledge. The previous microsatellite studies on major coconut germplasm accessions around the world showed that the Andaman ordinary tall clustered with the South East Asian coconut accessions. (Teulat *et al.*, 2000; Devakumar *et al.*, 2005).

The origin of coconuts in the Nicobar Islands is considered a mystery and since time immemorial coconuts have been growing in these islands. Various folklore stories are circulated among the islanders to explain the origin and spread of these coconut forests. According to undated Nicobarese oral traditions, long time ago, a boatman laden with coconuts arrived in Nicobar. On seeing him breaking open a coconut and bringing out water from it, the islanders mistook him as a magician, killed him and buried all his belongings including coconuts brought by him in a remote part of the Nicobar Island. The buried coconuts gave rise to coconut trees which the islanders considered strange tree for a long time and the islanders were prohibited from going to that area. Some of the islanders developed some curious disease and were isolated from the healthy population by sending them to the magician's land. The diseased persons survived on the coconuts and from there most of whom returned healthy. The islanders realized the magical powers of coconut water and started using it as potable water in this dry land. The islanders also realized the mistake of killing a samaritan who has brought coconuts to their land, an annual festival is being organized every year to appease the magician's soul.

According to the theory on coconut origin and dissemination, the origin of coconut was in the south pacific islands and it slowly spread to South East Asia from where it was disseminated by ocean currents and human means. The Polynesians were a sea faring people who took coconuts during their long voyages as far as Africa and aiding the dissemination over long distances. Based on morphological and microsatellites analysis, the coconuts of the world have been identified into two groups, the South East Asian and South Pacific coconuts comprising the Dwarfs and the Indian Ocean coconuts from the India mainland, Srilanka, Africa and the Carribeans. The vedic records for coconut cultivation in the Indian mainland dates back to 2-4th century B.C. According to some authors, the Coconuts were introduced in India from Sri Lanka by a leper King of Kerala who patronized its cultivation because of its miraculous curing properties (Ref). Because of its geographic location as a long stretch of islands between the Indian subcontinent and the South East Asian and south pacific islands, the Andaman and nicobar group of islands might have played an important role as conduit in the dissemination of coconut cultivars cultivated in these two regions. The objective of this present study is to analyze and track the origin of Andaman and Nicobar coconuts and to ascertain the genetic relationship and dissemination of the two major coconut types from these islands.

The coconut accessions from Andaman and Nicobar group of islands were collected during a germplasm expedition conducted in the year 1999 and 2000. The seed

nuts were sown in the CPCRI seed garden nursery at Kidu, Karnataka, India and the leaf material from the germinated seedlings was used for DNA extraction (Table 1).

DNA was extracted from the spear leaf of the young seedlings following the procedure of Upadhyay *et al.* (1999), whereby 5 g of spear leaf tissue was ground in liquid nitrogen and transferred to extraction buffer containing 10% sodium dodecyl sulphate (SDS). The contents were boiled at 65°C, cooled and extracted with an equal volume of 24:1 chloroform: isoamyl alcohol mixture. The supernatant was transferred to a new tube and DNA was precipitated with 70% ethanol.

For microsatellite analysis, 12.5 ng of DNA template, 200 μ M deoxynucleotide triphosphate (dNTPS), 1 unit of *Taq* polymerase (Bangalore Genie, India) and 1 μ M of each primer were used as PCR reaction mixture. The polymerase chain reaction (PCR) conditions were identical to those of Perera *et al.* (2000). The amplified products were resolved in a 5% denaturing polyacrylamide gel and the bands were visualized by silver staining (Bassam and Caetano-Anollés, 1993). The microsatellite bands were scored manually and the alleles were sized with reference to a 30–330 bp ladder (Gibco Brl).

The calculation of genetic diversity values and construction of the unweighted pair group method with arithmetic mean (UPGMA) dendrogram using Nei's genetic distance (Nei *et al.*, 1983) was carried out using the POWERSSR v 1.2. Software (Liu, 2001). The PCA plot was constructed for all the accessions using Nei's genetic distance (1972) with gene frequency data using NTSYS pc v.2.02i software (Applied Biostatistics Inc, USA). The analysis of molecular variance was done using GENALEX software (Peakall and Smouse, 2001) with a significance setting permutation value of 999.

A total of 222 individual coconut palms representing thirty three accessions that are widely cultivated around the world and twenty six accessions from various regions of the Andaman and Nicobar Islands were studied for their genetic relationship and diversity. The observed heterozygosity was highest for Laccadive Ordinary Tall followed by the Guam Tall II. Similarly the observed gene diversity was highest for the LOT followed by the Guam Tall II. Both these are island coconut populations and belong to the niu kafa that were least subjected to human selection. Among the Andaman and Nicobar coconut populations, the Harminder Bay Giant Tall (HBGT) and the Andaman Yellow Tall (AYT) showed the maximum heterozygosity value of 0.63 each. The least observed heterozygosity was shown by the Andaman Ordinary Tall (ADOT). The two populations of East Africa, Zanzibar Tall (ZNT) and Kenya Tall (KNT) showed the maximum inbreeding coefficient. Probably, the coconut population of east Africa was generated from a small size of the introduced coconut palms and founder effect has led to inbreeding and hence there are fewer heterozygotes than predicted by the Hardy-Weinberg equilibrium (Table 1) The majority of the coconut populations of the Nicobar group of Islands such as Trinket Tall (TT), Katchal Green Dwarf (KGD), Beachdera Ordinary Tall (BOT), Andaman Yellow Tall (AYT), Trinket Papaya Tall (TPT) and Round Pointed Tall (RPT) had negative inbreeding coefficient that is characteristic of more heterozygotes than the expected and indicative of their high out crossing (Table 2).

The UPGMA dendrogram distinguished the accessions into two broad groups *i.e.* the Indian Ocean group and the South East Asia and Pacific group in accordance with

the earlier findings. The dwarfs clustered with the South East Asia and Pacific group based on their probable origin in this region of the world. The Andaman and Nicobar coconut accessions clustered as a separate sub group within the Indian Ocean group. The coconut populations from the Andaman group of Islands and Nicobar group of Islands formed separate clusters indicating their isolation from each other.

The Andaman and Nicobar group of islands are separated by 1200 kms of sea from the peninsular India and Sri Lanka that is considered as a second important centre of domestication of coconuts but only few hundred kms away from the SE Asian mainland where the coconuts are more diversified and are considered as the main centre of domestication of coconuts. The coconuts in SE Asia are characterized by more volume of nut water with thinner husks. On the other hand the Indian Ocean Coconuts are characterized by thick husk with low volume of nut water. These two independent domestication events must have occurred long time ago that these coconuts show variations morphologically and in physio-chemical properties located at the crossroads between these two centres of domestication and it is speculated that they might have played a transit role during the dissemination of coconuts in the Indian Ocean. Moreover, the Nicobar groups of Islands are located at the similar latitude to the Sri Lanka that might have acted as the entry point of the coconuts for the domestication in the South Asia.

The between population variation was found to be 33% while the within population variation was 67% in accordance with the cross pollinating nature of the crop. The percentage of variation explained by the first three PCA axes was 34.6%, 23.14% and 15.74% respectively.

A total of 51 alleles were detected by eight polymorphic microsatellite primers with an average of 6.3 allele/locus. The twelve Nicobar accessions grouped together as a separate cluster within the Indian sub continental coconut populations while the Andaman accessions were found interspersed among them. The results of our study are significant in defining the geographical boundary of the two genetically divergent coconut populations of the world. This study has implications in understanding the spread of coconuts in the Indian Ocean Islands and factors that led to the genetic divergence of this group of coconut populations from the South East Asian and Pacific populations.

Table1. List of Andaman and Nicobar coconut accessions used

S. No.	Accessions	Abbreviation	No. of samples	Observed Heterozygosity	Observed Gene Div	Inbreeding Coefficient
1	ANDAMAN: Dugong Creek Tall	DGT	4	0.44	0.39	0.01
2	Horned Cocos Tall	HCT	4	0.25	0.14	-0.2
3	South Bay I Tall	SBTI	4	0.49	0.48	0.06
4	South Bay II Tall	SBTII	4	0.34	0.33	0.07
5	Carbin Tall	CART	2	0.38	0.48	0.4

6	Carbin Brown Tall	CBT	4	0.41	0.45	0.1
7	Burmanella Green Tall	BGT	4	0.39	0.51	0.3
8	Brurmanella Brown Tall	BBT	4	0.38	0.43	0.2
9	Biolinton Green Tall	BLGT	4	0.27	0.37	0.27
10	Kodiaghat Brown Tall	KBT	4	0.38	0.48	0.51
11	Harminder Bay Giant Tall	HBGT	4	0.63	0.44	-0.14
12	Andaman Ordinary Tall	ADOT	4	0.09	0.33	0.54
13	Andaman Yellow Dwarf	AYD	4	0.13	0.06	0
NICOBAR:						
14	Nicobar Orange Dwarf	NOD	4	0.16	0.14	0.13
15	Konkana Tall (Oval)	KOT	4	0.38	0.39	0.09
16	Konkana Large Tall	KLT	2	0.19	0.31	0.33
17	Trinket Tall	TT	3	0.38	0.23	-0.04
18	Katchal Green Dwarf	KGD	4	0.47	0.41	-0.01
19	Beachdera Ordinary Tall	BOT	4	0.39	0.33	-0.02
20	Katchal Micro Tall	KMT	4	0.19	0.24	0.15
21	Andaman Yellow Tall	AYT	4	0.63	0.48	-0.04
22	Carnicobar Tall (Beak nuts)	NBT	4	0.38	0.5	0.35
23	Trinket Tall (Papaya shape)	TPT	3	0.13	0.06	-0.13
24	Carnicobar Long Tall	CLT	4	0.35	0.36	0.06
25	Round Pointed Tall	RPT	3	0.21	0.13	-0.14
26	Perka Green Tall	PGT		0.38	0.43	0.23

Table 2. List of coconut accessions from Indian and Pacific Ocean rim countries used

S. No.	Accessions	Abbreviation	No. of samples	Place of collection	Observed Heterozygosity	Observed Gene Div	Inbreeding Coefficient
1	Borneo Tall	BOR	4	Indonesia	0.15	0.33	0.43
2	Cochin China Tall	COC	4	Indonesia	0.25	0.23	0.08
3	Ceylon Tall	CT	4	Sri Lanka	0.35	0.28	-0.11
4	Fiji Tall	FJT	2	Fiji, Pacific islands	0.44	0.3	-0.13
5	Guam Tall I	GUBTI	2	Guam islands, Pacific islands	0.5	0.32	-0.26

6	Guam Tall II	GUBTII	4	Guam islands, Pacific islands	0.57	0.55	0.05
7	Java Tall	JVT	4	Indonesia	0.29	0.39	0.28
8	Kenya Tall	KNT	4	East Africa	0.28	0.37	0.48
9	Philippines Lono	P.Lono	4	Philippines	0.16	0.33	0.15
10	Philippines Ordinary Tall	PHOT	4	Philippines	0.38	0.36	0.19
11	Sanramon Tall	SNRT	4	Philippines	0.14	0.2	0.16
12	Straits Settlements Green	SSG	4	Malaysia	0.16	0.2	0.35
13	Federated Malayan States	FMS	4	Malaysia	0.3	0.43	0.29
14	Zanzibar Tall	ZNT	4	East Africa	0.22	0.36	0.57
15	Gonthembili Tall	GT	4	Sri Lanka	0.19	0.49	0.43
16	New Guinea Tall	NG	2	Papua New Guinea	0.16	0.35	0.29
17	Ayiramkachi Tall	AYRT	3	East Coast, India	0.19	0.32	0.1
18	East Coast Tall	ECT	4	East Coast, India	0.5	0.5	0.11
19	Gangapani Tall	GPNT	4	East Coast, India	0.42	0.34	-0.11
20	Kappadam Tall	KPDT	4	West Coast, India	0.48	0.43	-0.03
21	Laccadive Ordinary Tall	LCT	4	West Coast, India	0.61	0.59	0.11
22	Laccadive Micro Tall	LMT	3	West Coast, India	0.23	0.31	0.22
23	Orissa Giant Tall	OGT	4	East Coast, India	0.19	0.32	0.39
24	Orissa Tall	OT	3	East Coast, India	0.5	0.4	0.04
25	West Coast Tall	WCT	4	West Coast, India	0.43	0.35	0.02
26	Benalium Tall	BEN	4	West Coast, India	0.47	0.33	-0.02
27	Spicata Tall	WCT01	4	West Coast, India	0.15	0.39	0.43
28	Calangute Tall	CALT	4	West Coast, India	0.35	0.36	0.06

29	Malayan Green Dwarf	MGD	4	India	0.16	0.19	0.1
30	Malayan Orange Dwarf	MOD	4	Malaysia	0.09	0.11	0.06
31	Malayan Yellow Dwarf	MYD	4	Malaysia	0.09	0.18	0.25
32	Chowghat Orange Dwarf	COD	4	India	0	0	0
33	Chowghat Green Dwarf	CGD	4	India	0.03	0.03	0

Source	df	SS	MS	Est. Var.	%
Between Pops	58	242.152	4.175	0.719	33%
Within Pops	163	240.133	1.473	1.473	67%
Total	221	482.285		2.192	100%

Percentage of variation explained by the first 3 axes

Axis	1	2	3
%	34.60	23.14	15.74
Cum %	34.60	57.74	73.48

References

- Bourdeix R., Baudouin. L., Ollivier, J. and Labouisse, J.P.1999. Consultancy report on coconut collecting strategies: ADB-CGRNAP report, Montpellier, France, CIRAD-CP, 110 p.
- Dhanapal, R., Jerard, B. A., Damodaran, V. and Arunachalam, V. 2000. Status of Coconut Plantations in Andaman and Nicobar group of Islands. *Indian Coconut Journal*
- Pandey, C.B. Rai, R.B. Lalita Singh and Singh, A.K. 2007. Homegardens of Andaman and Nicobar, India. *Agricultural Systems* 92(1-3):1-22.
- Perera, L., Russel, J. R. Provan, J. Mc Nicol J. W. and Powell, W. 1998. Evaluating genetic relationships between indigenous Coconut (*Cocos nucifera* L.) accessions from Sri Lanka by means of AFLP profiling. *Theor. Appl. Genet.* 96: 545-550.
- Perera, L., Russel, J.R. Provan, J. and Powell, W. 2000. Use of microsatellite DNA markers to investigate the level of genetic diversity and population genetic structure of Coconut (*Cocos nucifera* L.). *Genome* 43:15-21.
- Rao and Sampath Kumar. 1998. Some botanical curiosities. *Current Science* 75 (2):91-92.

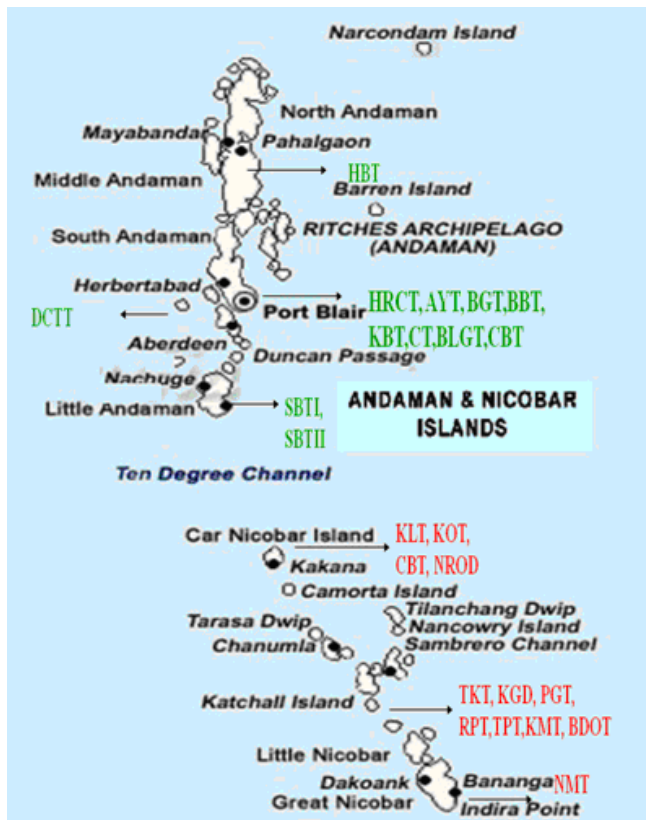
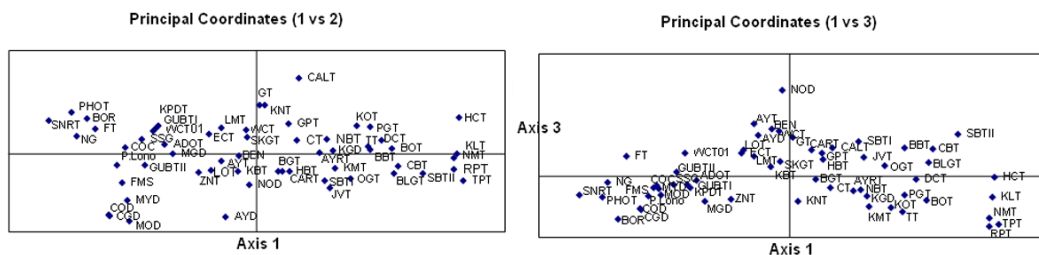
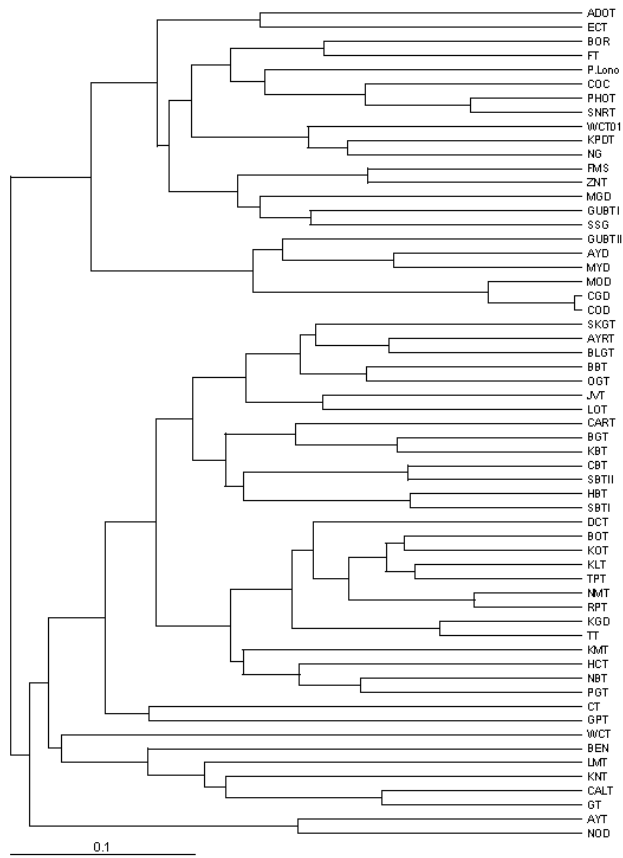


Fig. 1. Map showing the location of coconut accessions used for the microsatellite analysis in the Andaman and Nicobar Islands





Effect of various auxins on high frequency callus induction from coconut plumule explants

**Anitha Karun., Bhavyashree U., Lakshmi Jayaraj K., Fayas T.P.,
Rajamanickam R., Radha E. and Sajini K.K.**

Central Plantation Crops Research Institute, Kasaragod - 671124, Kerala, India

Coconut is a very important crop in tropical areas providing cash and subsistence to small holders. As every part of the tree is made into universally used products, It is popularly known as the “tree of life”. Most coconut grooves worldwide require replanting either because of senescence or because of loss due to diseases such as lethal yellowing in America (Arelano and Oropeza, 1995), the lethal diseases in Africa (Eden-Green, 1995), Cadang-cadang in Asia (Hanold and Randles, 1991) and *phytophthora*, which is widely spread (Schierer 1970; Joseph and Radha 1975; Franqueville *et al.*, 1991). India is the first country to start systematic research in coconut in the year 1919. Tissue culture of coconut has been carried out in several countries including UK (Wye College), France (IRHO/CIRAD), Philippines (UPLB and PCA), Srilanka (CRI) and Mexico (CICY). The *in-vitro* technique for mass production of clonal palms is most advanced with date palm (Fki *et al.*, 2002) and oil palm (Buffard-Morel *et al.*, 1992). In contrast coconut palm has remained largely recalcitrant as far as tissue culture is concerned (Ammirato, 1983).

Studies on callus, produced from embryos (Gupta *et al.*, 1984), immature leaves (Raju *et al.*, 1984; Buffard – Morel *et al.*, 1992) and inflorescences (Branton and Blake, 1984), indicate that regeneration is possible although rather difficult. Protocol for coconut micropropagation has been developed in various laboratories using different explant sources. (Branton and Blake, 1983a, 1986b; Buffard-Morel *et al.*, 1988; Karunaratne and periyapperuma, 1989; Shirke *et al.*, 1993; Verdeil *et al.*, 1994; Blake and Hornung, 1995; Hornung, 1995a; Karun *et al.*, 2007). However, most of the progress has been achieved using inflorescence explants. When cultured, these explants develop a partly dedifferentiated callus that has been reffered to as “calloid” by Brackpool *et al.* (1986). This is followed by the formation of somatic embryos, which subsequently germinate and eventually form clonal plantlets (Blake 1990; Verdeil *et al.*, 1994; Blake and Hornung, 1995). However, only limited success has been achieved and the protocols lack reliability. In order to achieve successful callus production in coconut explants, it is critical to define the most suitable combination of auxins and activated charcoal, two of the essential components in the callus induction medium. The beneficial effect of activated charcoal is attributed to its absorption of phenolics and other growth inhibitory substances. However, it also absorbs auxins present in the culture medium, leading to undefined conditions with regard to available auxin concentration in the medium (Ebert and Taylor, 1990; Verdeil and Buffard-Morel, 1995). The present paper reports the effect of different auxins on high frequency callus induction from coconut plumule explant.

The matured nuts (11-12 months old) were harvested from WCT (West Coast Tall) palms maintained at CPCRI Kasaragod. Embryos with endosperm were excised from the split opened nuts using a cork borer and placed in distilled water. The endosperms enclosing the embryos were sterilized with 0.01% mercuric chloride for 5 min. Rinsed three times

with sterile distilled water to remove the traces of HgCl_2 . Embryos were extracted from endosperm plugs, surface sterilized with 20% NaClO solution for 20 minutes and subsequently rinsed with sterile distilled water for 5-6 times.

Two methods were tried for plumule extraction from mature zygotic embryos.

- a) Surface sterilized embryos were aseptically inoculated into germination media (Y_3 with different concentrations of GA_3). After 10 days of germination plumules were scooped and inoculated into callus induction media.
- b) Plumules were directly extracted from the surface sterilized matured embryos in aseptical condition and inoculated in to the media containing different concentrations of callogenic chemicals.

Eeuwens (1976) Y_3 was used as the basal medium and it was supplemented with 3% (w/v) sucrose, 0.1% (w/v) activated charcoal. Different concentrations of each growth regulators were added to the media as described in the following experiments. After adjusting the pH to 5.8, the media was autoclaved at 121°C for 20 min. Different auxin concentrations used are:

Five concentrations of GA_3 (1mg, 2mg, 4mg, 8mg, and 10mg) supplemented with basal medium.

Four treatments that consisted of basal medium with 2, 4-D (2, 4-Dichlorophenoxyacetic acid) 75 μM (control), 100 μM , 150 μM and 200 μM .

Four treatments that consisted of basal medium with Picloram 4-amino3,5,6-trichloropicolinic acid) 75 μM , 100 μM , 150 μM and 200 μM .

Four treatments that consisted of basal medium with α -Naphthalene acetic acid 75 μM , 100 μM , 150 μM and 200 μM .

Four treatments that consisted of basal medium with Dicamba (3, 6-Dichloro-2-methoxy benzoic acid) 75 μM , 100 μM , 150 μM and 200 μM .

Four treatments that consisted of basal medium with 2, 4, 5- T (2,4,5-Trichlorophenoxy acetic acid) 75 μM , 100 μM , 150 μM and 200 μM .

Four treatments that consisted of basal medium with CPA (Chlorophenoxy acetic acid) 75 μM , 100 μM , 150 μM and 200 μM .

Two treatments as pulse treatment of 2, 4-Dichlorophenoxyacetic acid were given: (i) Constant concentration of 2, 4-D (200 μM) with different intervals of time. (ii) Different concentration of 2, 4-D with constant time (30 min.)

Plumules extracted from GA_3 treated matured embryos showed less callus initiation (28%) than the directly scooped plumules from fresh embryos. (Table1). Callus multiplication and embryogenic calli development was also found to be less in GA_3 treated embryos (60%) (Table1). For germination of embryo the least concentration of GA_3 (1mg/L) tried was found to be the best.

In the case of direct plumule extraction, 70 % of cultures responded for embryogenic calli after 30 days of culture incubation (Table 1). (Fig. 7). Embryogenic calli multiplication

was also observed to be higher (80%) after 120 days of culture incubation (Fig. 9). From this study it was revealed that scooping the plumule from the fresh embryo, was found to be the best for embryogenic calli initiation and embryogenic calli multiplication.

Table 1. Per cent embryogenic Callus induction and embryogenic calli multiplication from plumule extracted from GA₃ treated embryos and directly extracted plumule of matured embryo after 30 days and 120 days of culture incubation (DCI)

Methods of extraction of plumule	Callus initiation after 30 days (%)	Embryogenic calli multiplication after 120 days (%)
Plumule extracted from GA ₃ treated (after 5 days) germinated matured embryos	28	60
Direct plumule extraction from the matured nuts	70	80

The response of plumule explant for callus induction was studied in media containing a various concentration of 2,4-D (75µM (control), 100µM, 150 µM, 200 µM) and picloram (75µM (control), 100µM, 150 µM, and 200 µM). For each treatment 420 embryos were utilized. Early callus induction was observed, plumule inoculated into media containing 2,4-D. Higher percentage of callus initiation (68.3%) was observed at the concentration of 100µM 2, 4-D (Table 2) (Fig. 8). But subsequently the callus multiplication and embryogenic calli formation was found to be more in control media 2, 4-D 75µM (Table 2)(Fig. 7). Callogenesis was found to be very less in picloram containing media. The explants showed severe browning and direct shoot development from the plumule explants (Fig. 5). Picloram 100µM showed only 12% of callus initiation. 2, 4-D 75µM media was found to be the best for callus maintenance and multiplication.

Table 2. Effect of 2,4-Dichlorophenoxyacetic acid and 4-Amino3,5,6-Trichloropicolinic acid (Picloram) on callus initiation after 180 days of culture incubation (DCI)

Treatments		Browning (%)	Callus initiation (%)	Embryogenic calli (%)
2,4-D	75µM (Control)	35	56.6	45
	100µM	35	68.3	38
	150µM	39	50	20
	200µM	40	50	15
Picloram	75 µM(Control)	40	10	-
	200µM	45	8	-
	150µM	40	8	-
	100µM	40	12	-

Plumules were tested for callus induction in media containing a range of 2, 4, 5-T 75µM (Control), 100µM, 150µM, 200 µM and a range of Chlorophenoxy acetic acid 75µM(control), 100µM, 150µM, 200µM. 17.5% callus initiation was observed in medium containing 2, 4, 5- Trichlorophenoxy acetic acid 200 µM compared to other concentration (Fig. 6) (Table.3.) Where as among the various concentration of Chlorophenoxy acetic acid, maximum percentage of callus initiation of 10% was observed in 100µM (Fig. 4) (Table.3.).

Table 3. Effect of Chlorophenoxy acetic acid and 2, 4, 5-Trichlorophenoxy acetic acid on callus induction after 180days of culture incubation (DCI)

Treatments	Browning (%)	Callus initiation (%)	Embryogenic calli (%)
2,4,5-T 75µM (Control)	40	13	-
100µM	46	12	-
150µM	60	10	-
200µM	30	17.5	-
CPA 75 µM (Control)	65	7.2	-
200µM	45	6.5	-
150µM	35	9	-
100µM	30	10	-

Effect of α-Naphthalene acetic acid (NAA) and 3,6-Dichloro-2-methoxy benzoic acid (Dicamba): Plumules were tested for callus induction in media containing a range of α-Naphthalene acetic acid (NAA) 75µM(control), 100µM, 150 150µM, 200 µM and 3,6-Dichloro-2-methoxy benzoic acid (Dicamba) 75µM(control), 100µM, 150 µM, 200 µM. Maximum of only 6.2 percent cultures responded for callus initiation in medium supplemented with 100µM 6-Dichloro-2-methoxy benzoic acid compared to other concentrations tried. (Table 4). Similarly only 8 and 7.5 percent of the plumule could initiate callus in 100 and 150µM α-Naphthalene acetic acid, respectively (Table 4).

Table 4. Effect of α-Naphthalene acetic acid and 3,6-Dichloro-2-methoxy benzoic acid (Dicamba) after 180 days of culture incubation (DCI)

Treatments	Browning (%)	Callus initiation (%)
Dicamba 75µM (Control)	30	5.5
100µM	35	6.2
150µM	52	5
200µM	54	4.5
NAA 75µM (Control)	60	6
100µM	55	8
150µM	65	7.5
200 µM	50	5

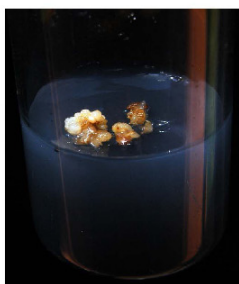


Fig.2.



Fig.3.



Fig.4.



Fig.5.



Fig.6.

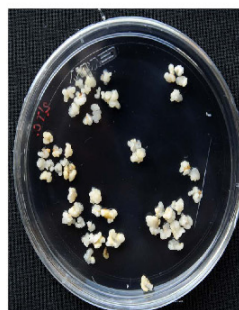


Fig.7.

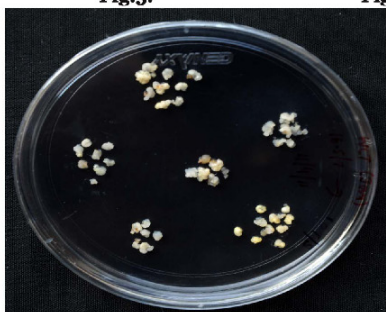


Fig.8.



Fig.9.

Plate-1: Fig.2. Callus induction observed after 60 days in culture media supplemented with dicamba (100µM). Fig.3. Callus induction observed after 60 days in culture media supplemented with NAA (100µM). Fig.4. Callus induction observed after 60 days in culture media supplemented with CPA (100µM). Fig.5. Callus induction observed after 60 days in culture media supplemented with CPA (100µM). Fig.6. Callus induction observed after 60 days in culture media supplemented with Picloram(100µM). Fig.7. Callus multiplication after 60 days in culture media supplemented with 2,4-D(75µM). Fig.8 calli initiation in 2,4-D (100µM) after 30 days. Fig.9. Embryogenic calli formation after 120 days in 2,4-D (75µM)

Pulse treatment of high concentration of 2, 4-D was also tested on embryos for callus induction. Constant concentration of 2, 4-D (200 µM) is tested in different time intervals (0, 5, 15, 30, 45, 60, 75, 90 min). Among the various time intervals the embryos treated with 2, 4-D (200 µM) for 75 min. has given good percentage of callusing (41%), compare to other time intervals (Table.5.). Different concentrations of 2, 4-D (100, 150, 200, 250, 300 µM) is also tested for a constant time (30 min.). The 2, 4-D at 200 µM only responded for callus induction after 2 months (25%)(Table 6).

Table 5. Per cent browning and callusing observed after pulse treatment of embryos in 2, 4-D 200 μ M solution in different time interval

Treatment	Time interval(min.)	Callusing (%)	Browning(%)
T1	0	10	36
T2	5	18	35
T3	15	21	5
T4	30	10	10
T5	45	26	33.3
T6	60	36	0
T7	75	41	59
T7	90	15	58

Table 6. Per cent browning and callusing observed after Pulse treatment of embryos in constant time (30 min) with different concentration of 2,4-D

Treatment	Concentration (μM)	Callusing(%)	Browning(%)
T1	100	0	0
T2	150	0	10
T3	200	25	10
T4	250	0	10
T5	300	0	60

All the hormones tried in various experiment were showed callus initiation. But 2, 4-D (2, 4-Dichlorophenoxyacetic acid) was found to be best auxin for callus initiation. The overall percentage of callus initiation was more in 2, 4-D, about 56.2% (Fig.1.). Among the 2,4-D concentration tried for callus multiplication and maintenance, 75 μ M 2, 4-D concentration was found to be best.

In coconut, exogenic auxins play a predominant role in the development of embryogenic structures. Coconut somatic embryogenesis usually occurs through a callus phase. Therefore, production of embryogenic callus is a prerequisite for successful plant regeneration. Among several other factors, the presence of an auxin in the medium is critical for successful callogenesis (Auge, 1995). The most commonly used auxin in coconut tissue culture media is 2, 4-D and its concentration depends on the explant and concentration of activated charcoal in the culture medium (Verdeil and Buffard-Morel, 1995). The present study describes the effect of various auxin on callus induction of Plumule explants. This source of juvenile tissue has been shown previously to respond well and more rapidly than immature inflorescence explants in terms of callus formation and the development of embryogenic capacity (Hornung, 1995b).

It has been found that Y₃ basal medium with GA₃ 1 mg/l (2.8 μ M) promotes the germination of embryo. Our result was compared with the study of effect of gibberellic acid

on the *in vitro* germination of coconut zygotic embryos (Pechy Aké *et al.*, 2004). They found 98% of germination in semisolid media with GA₃ 4.6 µM. The treatment with GA₃ promoted the germination rapidly; it concomitantly improved the synchrony of embryo germination, particularly in solid media.

Out of two types of plumule extraction, direct plumule extraction from fresh embryos were found to be more effective in callus initiation (70%) and formation of embryogenic calli (80%). Chan *et al.* (1998) also reported the direct isolation of plumule from fresh embryos resulted in 54.3% of embryogenic calli formation.

Callus formation in plumule explant was observed by using various auxin such as 2, 4, D, CPA, 2,4,5 T, Picloram, NAA and Dicamba. All the auxins showed callus initiation. However the early callus initiation and embryogenic calli formation was significantly higher with 2,4-D(58%). Other hormones such as Picloram showed 13%, CPA (12.5%), 2, 4, 5 T (17.5%), NAA (2.5%) and Dicamba (6.2%) callus formation. Among the various concentrations of 2, 4 D (75µM(control), 100 µM, 150 µM and 200 µM) studied, it was found that, 2, 4 D 100 µM, was the optimum concentration for callus initiation (about 68.3%) compared to other concentration. But callus multiplication and embryogenic calli formation was found to be more in control media 2,4-D 75µM. In case of Picloram and NAA, severe browning was observed and rate of callusing was very less and direct shoot development was noticed.

Chan *et al.* (1998) has also found the same concentration of 2, 4 D (100 µM) along with Y₃ media as an optimum concentration for callus induction from plumule explant of coconut and they got 60 % of callusing. Same study has conducted by Hornung (1995) by using MS medium supplemented with 400 µM of 2, 4-D and 75% percentage of callusing was reported. Fernando *et al.* (2004) have found 46.7% callusing in the plumule explant by using Y₃ media supplemented with 2, 4-D 100 µM.

Most of the developed calli showed meristematic centers, indicative of embryogenic capacity and of a multicellular pathway for embryo formation according to Verdeil and Buffard-Morel (1995). In addition, individualization of embryogenic cells was observed indicative of a unicellular pathway of embryo formation. A greater proportion of plumule explants developed into calli bearing embryogenic structures when the cultures were distributed and subculturing was practiced.

Two types of pulse treatments were tested on fresh embryo explants. In the first treatment 2, 4-D (200 µM) for 75 min. resulted in higher percentage of callusing (41%) compared to other time intervals. In the second treatment, among the various concentrations 2, 4-D 200 µM for a constant time of 30 min. resulted 25% of callus induction. In order to reduce oxidation and increase the callus induction rate, pulse treatments strategy was employed in other palm *in vitro* systems, such as *Euterpe* (Guerra and Handro, 1998). Fernando *et al.* (1995) reported the use of pulse treated IAA for the induction of root from immature embryo explant of coconut.

A pulse treatment step also enhanced Somatic embryogenesis in coffee (*Coffea arabica* L.; Quiroz-Figueroa *et al.*, 2002), orchard grass (*Dactylis glomerata* L.; Alexandrova and Conger, 2002) and kodo millet (Vikrant and Rashid, 2003). Nhut *et al.* (2000) observed that the pulse

treatment increased somatic embryogenesis induction in rice (*Oryza sativa* L.) and suggested that this step alters the endogenous hormonal balance. In addition, the pulse treatment step could stress the cells, triggering cell division (Feher *et al.* 2003). David and Prudence (1993) reported the effect of auxins pulse treatment for callus induction in leafy spurge. Vikrant and Rashid (2003) reported pulse treatment of 2, 4-D for somatic embryogenesis and shoot formation in *Paspalum scrobiculatum*.

From this study, it is revealed that, 2,4 D is the ideal auxin for callus initiation from coconut plumule explants. The critical step in coconut embryogenesis is the gradual drop in 2, 4-D concentration in relation to the initial level. If the drop in auxin level was too rapid, the maturation of the embryogenic structures usually led to incomplete or deviated forms (Buffard-Morel *et al.*, 1995). This may be one of the reasons for obtaining shoots with suppressed growth, fused shoots, haustorial tissues and roots.

References

- Alexandrova, K.S. and Conger, B.V. 2002. Isolation of two somatic embryogenesis-related genes from orchardgrass (*Dactylis glomerata*). *Plant Sci.* **162**(2):301–307
- Ammirato, P.V. 1983. Organizational events during somatic embryogenesis. In: Liss A.R. (ed). *Plant tissue and cell culture*, Macmillan Inc, New York. Pp 57-81.
- Arellano, J. and Oropeza, C. 1995. Lethal yellowing. In: Oropeza C., Howard F.W., Ashburner G.R. (eds) *Lethal yellowing research and practical aspects*. Kluwer, Dordrecht, pp.1-16.
- Auge, R. 1995. The physiological phenomena related to the realization of culture *in vitro*. In: Razdan, M. K. (Ed), *In vitro* culture and its application in horticulture, Science Publishers Inc., USA, p. 7-31.
- Blake, J. 1990. Coconut (*Cocos nucifera* L.) :micropropagation, In : Bajaj YPS (ed) *Biotechnology in agriculture and forestry*, vol 10. Legumes and oilseed crops I. Springer, Berlin Heidelberg New York, pp 538-554.
- Blake, J. and Hornung, R. 1995. Somatic embryogenesis in coconut. In: Jain S., Gupta P., Newton R. (eds) *Somatic embryogenesis in woody plants*, Kluwer, Dordrecht, pp 327-349.
- Brackpool, A.L., Branton, R.L. and Blake, J. 1986. Regeneration in palms. In: vasil I.K (ed) *Cell culture and somatic cell genetics in plants*, vol. 3 Academic press, New York, pp 207-222.
- Branton, R.L. and Blake, J. 1984. In: Pushparajah E., Chew Poh Soon. (eds). *Proc. Int. Cocoa/coconuts*. Kuala Lumpur. pp1-8
- Branton, R.L. and Blake, J. 1983a. A lovely clone of coconuts. *New Sci.* **98**: 554-557.
- Branton, R.L. and Blake, J. 1986b. *In vitro* plantlet development from colloid derived from immature inflorescence of *Cocos nucifera* L. In: Somers D. A., Gengenbach B.G., Biesboer D.O., Hackett W.P., Green C.E. (eds) *Abstracts of VI International Conference of plant tissue and cell culture*. University of Minnesota, Minneapolis, p-399.
- Buffard-Morel, J., Verdeil, J. L. and Pannetier, C. 1992. Embryogenese somatique du cocotier (*Cocos nucifera* L.) a partir d'explants foliaires: etude histologique. *Can J. Bot.* **70**:735-741.

- Buffard-Morel, J., Verdeil, J.L. and Pannetier, C. 1988. Vegetative propagation of coconut palm (*Cocos nucifera* L.) through somatic embryogenesis. In: Durand G., Bobichon L., Florent J. (eds) Proceedings of the 8th International Biotechnology Symposium, Paris. p-177.
- Chan, J. L., Saenz, L., Talavera, C., Hornung, R., Robert, C. and Oropeza, C. 1998. Regeneration of coconut (*Cocos nucifera* L.) from plumule explants through somatic embryogenesis. *Plant Cell Reports* **17**: 515-521.
- David, D.J. and Prudence, A.O. 1993. Organogenesis in Leafy Spurge (*Euphorbia esula* L.). In *vitro Cellular and Developmental Biology* **29**: 97-101
- Ebert, A. and Taylor, H.F. 1990. Assessment of the changes of 2,4-Dichlorophenoxyacetic acid concentrations in plant tissue culture media in the presence of activated charcoal. *Plant Cell Tissue Organ Cult.* **20**: 165-172.
- Eden-Green S. 1995. A brief history of Lethal yellowing research. In: Oropeza C., Howard F.W., Ashburner G.R. (eds) Lethal yellowing research and practical aspects. Kluwer, Dordrecht, pp. 17-33.
- Feher, A., Pasternak, T.P. and Dudits, D. 2003. Transition of somatic plant cells to an embryogenic state. *Plant Cell Tissue Organ Cult.* **74**(3): 201–228.
- Fernando, S. C. and Gamage, C. K. A. 1995. Clonal propagation of coconut: Improved culture conditions for rhizogenesis, *Cocos* **10**: 20-25.
- Fernando, S.C., Weerakoon, L.K. and Gunathilake. 2004. Micropropagation of coconut through plumule culture Coconut Research Institute Lunuwila Sri Lanka. *Cocos* **16**: 1-10.
- Franqueville H. de., Sangare, A., Le Saint, J.P., Taffin G, de., Pomier M. and Renard, J.L. 1991. Detection of *Phytophthora heveae* tolerance characters in coconut in Cote d' Ivoire. In: Silas E.G., Aravindak-Kerala Agricultural University. pp. 125-135.
- Guerra, M.P. and Handro, W. 1998. Somatic embryogenesis and plant regeneration in different organs of *Euterpe edulis* Mart. (*Palmae*): Control and structural features. *J. Plant Res.* **111**(1101): 65–71.
- Gupta, P.K., Kendurka, S.V., Kulkarni, V.M., Shirgurkar, M.V. and Mascarenhas, A.F. 1984. *Plant Cell Rep.* **3**: 222-225.
- Hanold, D. and Randles, J.W. 1991. Cadang-cadang disease and its viroid agent. *Plant Dis* **75**: 33-335.
- Hornung, R. 1995a. Initiation of callogenesis in coconut palm (*Cocos nucifera* L.). In: : Oropeza C., Howard F.W., Ashburner G.R. (eds) Lethal yellowing research and practical aspects. Kluwer, Dordrecht, pp 203-215.
- Hornung, R. 1995b. Micropropagation of *Cocos nucifera* L. from plumular tissue excised from mature zygotic embryo. *Plant Rech Dev* **2**: 38-41.
- Joseph, T. and Radha, K. 1975. Role of *Phytophthora palmivora* in bud rot of coconut. *Plant Dis Rep* **5**: 1014-1017.

- Karun Anitha., Sajini, K.K., Radha, E., and Rajesh, M.K. 2007. Palm tissue and organ culture protocol. Tech. Bulletin No. 51 CPCRI, pp-28.
- Karunaratne, S. and Periyapperuma, K. 1989. Culture of immature embryos of Coconut (*Cocos nucifera* L.): Callus proliferation and somatic embryogenesis. *Plant Sci.* **62**:247-253.
- Karunaratne, S., Kurukulaarachchi, C. and Gamage, C. 1985. A report on the culture of embryos of dwarf coconut, *Cocos nucifera* L. var. nana, *in vitro*. *Cocos* **3**:1-8.
- Nhut, D.T., Le, B.V. and Van, K. T.T. 2000. Somatic embryogenesis and direct shoot regeneration of rice (*Oryza sativa* L.) using thin cell layer culture of apical meristematic tissue. *J. Plant Physiol.* **157**(5):559-565.
- Pech y Aké A., Maust, B., Orozco-Segovia, A. and Oropeza, C. 2007. The effect of gibberellic acid on the *in vitro* germination of coconut zygotic embryos and their conversion into plantlets. *In Vitro Cell Dev. Biol-Plant* **43**:247-253
- Quiroz-Figueroa, F.R., Fuentes-Cerda, C.F.J., Rojas-Herrera, R. and Loyola-Vargas, V.M. 2002. Histological studies on the developmental stages and differentiation of two different somatic embryogenesis systems of *Coffea arabica*. *Plant Cell Rep.* **20**(12):1141-1149.
- Raju, C. R., Prakash Kumar, P. Chandra Mohanan, M. and Iyer, R. D. 1984. Coconut plantlets from leaf tissue cultures. *J. Plantn. Crops* **12**:5-81.
- Schierer, E. 1970. Enfermedades importantes del cocotero (*Cocos nucifera* L) en la Republica Dominicana. *Turrialba* **20**: 171-176.
- Shirke, S. V., Klenurkar, S.V., Apte, A.V., Phadke, C H., Nadgauda, R. S., Mascarenhas, A.F. .1993. Regeneration in coconut tissue culture. In: Nair MK, Khan NH, Gopalasundaram P, Bhaskara Rao E.V.V (eds) *Advances in coconut research and development*. Oxford and IBH, New Delhi, pp 597-604.
- Verdeil, J. L. and Buffard-Morel, J. 1995. Somatic embryogenesis in coconut (*Cocos nucifera* L.). In: Bajaj YPS (ed) *Biotechnology in agriculture and forestry*, vol. 30. Somatic embryogenesis and synthetic seeds I. Springer-Verlag, Berlin, pp 299-317.
- Verdeil, J.L., Huet, C., Grosdemange, R. and Buffard-Morel, J. 1994. Plant regeneration from cultured immature inflorescence of coconut (*Cocos nucifera* L.): evidence for somatic embryogenesis. *Plant Cell Rep.* **13**: 218-221.
- Vikrant and Rashid. 2003. Somatic embryogenesis and shoot formation following high 2,4-D pulse treatment of mature embryos of *Paspalum scrobiculatum*. *Biologia Plantarum* **46**(2): 297-300.

Modified pregrowth desiccation for cryopreservation of coconut (*Cocos nucifera* L.) zygotic embryos

K. K. Sajini and Anitha Karun

Central Plantation Crops Research Institute

Kasaragod-671124, Kerala, India

The conservation of recalcitrant seed species is gaining momentum in the context of climate change and outbreak of new and devastating diseases. The characteristics of recalcitrant seeds like coconut such as short viability period, lack of maturation drying, metabolic activeness, high water content at the time of shedding and bigger size (Chin and Roberts, 1980) make them unsuitable for storage under conventional regimes unlike orthodox seeds. Consequently, cryopreservation is the only available alternative approach for the long term conservation of coconut genetic resources. The efficacy of coconut embryo for cryostorage has been reported by different workers (Assy–Bah & Engelmann, 1992; Sajini *et al.*, 2006) with emphasis on the positive effect of sucrose preculture for plantlet retrieval after cryopreservation.

The prime pre requisite for post thaw survival in plant cryopreservation is the removal of water from the material to be cryopreserved which otherwise will result in intracellular ice formation and cellular damage upon exposure to liquid nitrogen (Dumet *et al.*, 1993). Hence, the induction of high level of desiccation tolerance in the tissue or organ to be preserve becomes vital in desiccation based cryopreservation procedure (Uragami *et al.*, 1990). Incubation with high concentrations of soluble sugars, especially sucrose (Hoekstra *et al.*, 2001) been reported to augment desiccation tolerance (Suzuki *et al.*, 1998, 2006) and membrane protection (Sopia and Samygin, 1993). Studies by different workers have shown that tolerance to dehydration during cryopreservation coincides with the freezing tolerance (Lagis and Steponkus, 1991; Zhang *et al.*, 2001). The onset of desiccation tolerance during orthodox seed development has been confirmed to increased levels of sucrose and raffinose family oligosaccharides (Chen and Burris, 1990). The cryoprotective effect of sucrose and raffinose has been reported in Puma rye (Koster and Lych, 1992) and in polyembryogenic cultures of oil palm (Dumet *et al.*, 1994). Raffinose was also found to be effective for the inhibition of sucrose crystallization that was found to occur in nearly dry state that limits its availability for membrane protection (Smythe, 1967). Even though sucrose has been reported as a good cryoprotectant for coconut zygotic embryos (Sajini *et al.*, 2006) its synergistic effect with other raffinose family oligosaccharides (RFOs) such as tri-saccharide raffinose has not been studied so far. The present study is focused on the effect of sucrose with raffinose in the preculture medium for the development of an effective cryopreservation protocol for coconut zygotic embryos.

The embryos extracted from the mature (11-12 month old) freshly harvested nuts from WCT (West Coast Tall) were utilized for the selection of best preculture treatment for cryopreservation. To validate the varietal response to the selected best procedure, embryos from two varieties *viz.*, Malayan Yellow Dwarf and Fiji Tall were utilized for evaluation of regrowth after cryopreservation.

The endosperm plug containing the embryo was scooped out from the soft eye of the split opened nut. The embryos extracted from it were surface sterilized with sodium

hypochlorite solution (6 % active chlorine) for 20 min. Thereafter the embryos were thoroughly rinsed in sterile water 4-5 times.

The preculture procedure comprised of two steps. In the first step the embryos were incubated in full strength Y3 solid medium containing 0.6M sucrose for one day. The sucrose treatment was prolonged for three days to study preculture duration on embryo retrieval after cryopreservation. This step gave mild osmotic stress to the embryos.

In the subsequent step (step 2) these embryos were cultured on full strength Y3 liquid medium containing 2 M sucrose alone or with 0.25 M raffinose for 24 hours in a conical flask under constant stirring (60 rpm). In other supplementary experiments the first step was avoided and the embryos were directly incubated either in Y3 liquid medium supplemented with 2 M sucrose or 0.25 M raffinose alone to study its individual effect.

Desiccation was performed by placing 20 embryos in an airtight glass bottle (250 ml) containing 50 g of activated silica gel for seven hours. Following desiccation, the embryos were then placed in 4.5 ml sterile cryovials (20 embryos per cryovial), and plunged directly in liquid nitrogen (+LN). The samples were stored in LN for minimum 24 hours. Each treatment contained 20 embryos and experiment was replicated thrice. Controls correspond to embryos subjected to all treatments except cryopreservation and absolute control to without any treatments. After removal from liquid nitrogen the embryos were rapidly thawed by plunging the cryovials in a water bath kept at 40°C for 2 min.

The total soluble sugar content of embryos after different preculture treatments was estimated using the method of Dubois *et al.* (1956).

The embryos after desiccation/thawing were cultured for one week in Y3 semi solid medium supplemented with 60 g/l sucrose and 1 g/l charcoal and later subcultured into the same basal medium combination with lower sucrose concentration (40 g/l). After 2-3 weeks, the embryos were cultured according to the protocol developed by Karun *et al.* (2002) until transfer of whole plants to pots. The percentage of embryos that showed any sign of growth including normal emergence of shoot or root pole, development of root pole only or callus formation after 40-50 days of culture initiation was assessed for survival. Germination/regrowth in embryos was estimated as number of normal plants developing from the embryos and expressed as a percentage. And plantlet recovery in pots as percentage of normal plantlets established in pots from control, desiccated or cryopreserved embryos. Embryo weight, shoot and root length were also recorded at 90, 180, 240 days after culture (DAC).

The optimal protocol followed for cryopreservation of embryos from Malayan Yellow Dwarf and Fiji Tall accessions consist of the following steps. The embryos were sequentially precultured in Y3 semi solid medium supplemented with 0.6 M sucrose for three days and Y3 liquid medium containing 2 M sucrose and 0.25 M raffinose for one day in a conical flask under constant stirring (60 rpm). Thereafter, the embryos were desiccated with activated silica gel for seven hours.

The moisture content (% of MC, fresh weight basis) of the embryo at different time intervals after air desiccation was measured by drying them in an oven at 103 °C for 24 h. Three samples of five embryos per treatment were utilized for moisture estimation.

The results were subjected to analysis of variance (ANOVA) and the means were compared by the Duncan's Multiple Range Test (DMRT) using SPSS 15.0. The percentage data was arcsine transformed prior to analysis where ever necessary.

The moisture content of the embryos progressively reduced after different preculture combinations and durations. Moisture content of fresh embryos was found to be 78.46 %. It reduced to 20% and 19.85%, respectively after direct preculture with 2 M sucrose for one day or two step preculture with sucrose alone and subsequent air desiccation for 7 h using silica gel. When sucrose and raffinose was used for two step preculture, the moisture reduction was found to be 12.49%. The effect of silica gel desiccation on moisture content and regrowth of embryos after two-step preculture with sucrose and raffinose is summarized in the Fig. 1. Germination of embryos was optimum (73%) after silica gel desiccation for 7 hours at optimum moisture content of 12.49 %. Further reduction of moisture content to 12.18 % the germination was reduced to 71 %.

The effect of the duration of first preculture step using 0.6 M sucrose for one day or three days and second preculture step with 2 M sucrose for 1 day, on survival and germination of embryos after desiccation or cryopreservation was investigated. The results shows that there were no significant differences in survival or germination of cryopreserved embryos precultured with 0.6 M sucrose for one day or three days. Omitting the first preculture step by direct incubation of embryos in 2 M sucrose for one day did not affect survival of embryos.

The combined effect of sucrose and raffinose for conferring desiccation and freezing tolerance was studied. For this, the embryos were first precultured in 0.6 M sucrose for 1 or 3 days and thereafter in 2 M sucrose and 0.25 M raffinose for one day or direct preculture with 0.25 M raffinose and air desiccated using silica gel for 7 hours. When the embryos were precultured directly in 0.25 M raffinose for one day, 90 % of the embryos survived after desiccation. Subsequently 72 % of embryos germinated and 35 % were pot established. But none of the embryos survived after cryopreservation. These results showed that even though raffinose could induce desiccation tolerance in embryos, it might not confer freezing tolerance.

The effect of sucrose and raffinose for improved germination after cryopreservation became manifested in embryos precultured for three days in the first step with 0.6 M sucrose followed by sucrose and raffinose in the second step (one day). Following this treatment significantly high ($p=0.004$) germination percentage of 72.78% was observed after desiccation or cryopreservation (Fig.2). Preculture with 0.6 M sucrose for one day and subsequent preculture with 2M sucrose and raffinose for one day was less effective in inducing freezing tolerance since the embryos from this treatment gave significantly low germination % (52.81) that was on par with the embryos directly precultured with 2 M sucrose alone (51.33%).

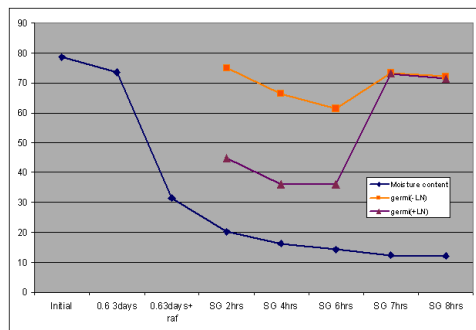


Fig. 1. Effect of two step preculture with sucrose and raffinose and subsequent air desiccation 7 h using silica gel or thereafter cryopreservation on moisture content, survival and germination of coconut zygotic embryos

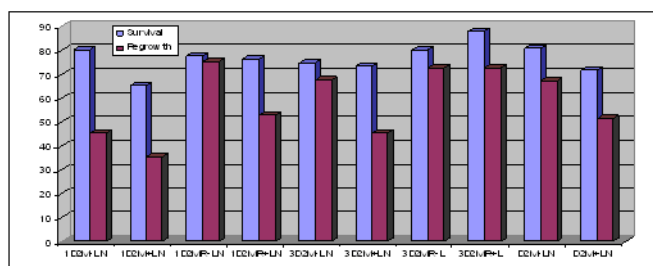


Fig.2. Percentage of survival and germination in coconut zygotic embryos subjected to two step preculture with sucrose alone or in combination with raffinose and thereafter desiccation with silica gel for 7h (-LN) or after cryopreservation (+LN). Preculture conditions are - Direct preculture with 2 M sucrose - D2M, 1 day 0.6 M sucrose + 1 day 2 M sucrose -1D2M, 1 day 0.6 M sucrose +1day 2M sucrose + 0.25 M raffinose-1D2MR, 3 days 0.6 M sucrose + 1 day 2 M sucrose 3D2M, 3 days 0.6 M sucrose +1day 2M sucrose + 0.25 M raffinose 3D2MR)

Soluble sugar accumulation in embryos was dependent on the duration of preculture with sucrose in the first step and the presence of sucrose and raffinose in the second step. When the embryos were directly precultured in Y3 medium with 2 M sucrose, the soluble sugar content increased to 11.85% from 10.11% that found in fresh embryos. Step wise increase of sugar in the preculture medium and its treatment duration (for 3 days) in the first step together with sucrose and raffinose in the second step significantly increased soluble sugar content of the embryo to 26.01%. High soluble sugar content in the embryo was found to be highly correlated with germination and negatively correlated with moisture content (Table 1). This is in agreement with high germination percentage observed in desiccated or cryopreserved embryos after three days preculture with 0.6 M sucrose in step 1 followed by sucrose and raffinose in the second step.

Table 1. Correlation of soluble sugar content of coconut zygotic embryos precultured with sucrose alone or in combination with raffinose on moisture content and germination after cryopreservation

Characters	Moisture content %	Regrowth
Soluble sugar %	-0.899**	0.790**
Moisture content%		-0.648*

** Significant at the 0.01 level (2-tailed); * Significant at the 0.05 level (2-tailed)

=Variation with regard to embryo weight after different periods of culture initiation was observed between treatments. Desiccation and freezing tolerance as manifested by embryo weight gain was significantly high ($p=0.002$) in embryos precultured for 1 day in 0.6M sucrose and thereafter in 2 M sucrose and raffinose for one day than all other treatments after ninety days of culture initiation. In subsequent subcultures (after 180 and 240 days), embryo weight significantly reduced to minimum in cryopreserved embryos directly precultured in 2 M sucrose medium for one day. Weight of embryos precultured for three days in 0.6 M sucrose and thereafter in sucrose and raffinose for one day was significantly high in cryopreserved embryos after 240 days of culture initiation.

Shoot growth was similar after cryopreservation in all the treatments after 90 and 180 days of culture initiation. But after 240 days, the cryopreserved embryos directly precultured in 2 M sucrose showed significantly high shoot length ($p=0.035$) than other treatments. Shoot growth was similar in cryopreserved embryos resulted from two step preculture with sucrose alone (one day in the first step) or sequentially with sucrose for 1-3 days and one day with sucrose and raffinose. Shoot growth was significantly low in cryopreserved embryos precultured with sucrose alone in the first (3 days) and second preculture step. There were no significant differences in root growth between treatments.

Flaccid embryos were observed in all the preculture combinations used for cryopreservation in the present study. The percentage of abnormality was highest in embryos treated with sucrose for one day in the first step followed by sucrose alone in the second step that consisted of malformed shoots (10%), flaccidity (6.66%), root without shoot development (3.3%), and embryos with flat plumular region (3.3%). Abnormality was comparatively less when embryos were precultured in 0.6 M sucrose for three days and sucrose and raffinose in the second step (8% flaccid embryos). The abnormal embryos subsequently died in the course of subculture.

There were no significant differences in percentage of normal plants established in pots following different preculture treatments (Table 2). But more number of vigorous plants within 10-11 months of culture initiation was obtained from embryos pretreated with 0.6 M sucrose for 3 days and thereafter in 2 M sucrose and 0.25 M raffinose for one day and cryopreserved. Abnormality was also very less in this treatment. The plants retrieved from the aforesaid treatment showed height variation from 9cm to 21cm with root length ranging from 2.5cm to 3.5cm. Whereas in control plants from embryos directly inoculated into the Y3 medium showed height variation from 15 to 25cm and root length of 3.5 to 5.5 cm.

Table 2. Effect of preculture on plantlet weight, shoot length, and pot survival (%) in embryos after desiccation (–LN) or cryopreservation (+LN) embryos. Means in a column followed by different letters imply significant differences according to DMRT($p<0.05$) For embryo weight and shoot length data was recorded after 240 days of culture initiation and for pot survival after 330 days

Preculture	Treatment	Embryo weight (g)	Shoot length (cm)	Pot survival (%)	Abnormality (%)
Dir 2M	-LN	1.12 abcd	1.63 ab	23.21	7
	+LN	0.682 d	1.29 abc	20.0	12
1D 2M	-LN	1.003abcd	1.27 abc	21.37	30
	+LN	0.811 cd	1.12 bc	17.96	23
1D 2MR	-LN	1.421 a	2.08 a	25.91	7
	+LN	0.803 cd	1.10 bc	19.87	20
3D 2M	-LN	1.285 abc	1.69 ab	29.67	22
	+LN	0.857 bcd	0.73 c	20.52	20
3D 2MR	-LN	1.292 abc	1.53 abc	30.43	8.33
	+LN	1.331 ab	1.17 bc	27.55	8.0

The best preculture protocol selected from modified pregrowth desiccation method was validated using embryos from Malayan Yellow Dwarf and Fiji Tall accessions. The embryos were sequentially precultured in Y3 medium supplemented with 0.6 M sucrose for three days, 2 M sucrose and 0.25 M raffinose for one day and air desiccated using silica gel for seven hours. In Malayan Yellow Dwarf and Fiji Tall accessions, there were no significant differences in survival and germination of embryos between desiccated, cryopreserved and absolute control embryos. But the number of plants established in pots was significantly high in absolute control plants without any treatments than that of cryopreserved or desiccated embryos. Survival was found to be 81% in Malayan Yellow Dwarf and 88% in Fiji tall embryos after desiccation. After cryopreservation 76.67 % survival was observed in Malayan Yellow Dwarf embryos and 85% in Fiji Tall embryos. Germination was found to be 68 % in Malayan Yellow Dwarf embryos after desiccation and after cryopreservation it reduced to 56.67 %. In Fiji Tall embryos 80 % embryos germinated after desiccation and 76 % after cryopreservation. In Fiji Tall accession growth was rather slow in desiccated or cryopreserved embryos than absolute control ones. The percentage of plantlets established in pots was found to be 28.33 in Malayan Yellow Dwarf and 23.33 in Fiji Tall accession after cryopreservation. The plants retrieved from desiccated or cryopreserved embryos from WCT, MYD and Fiji tall accessions at various developmental stages are shown in the Plate 1.

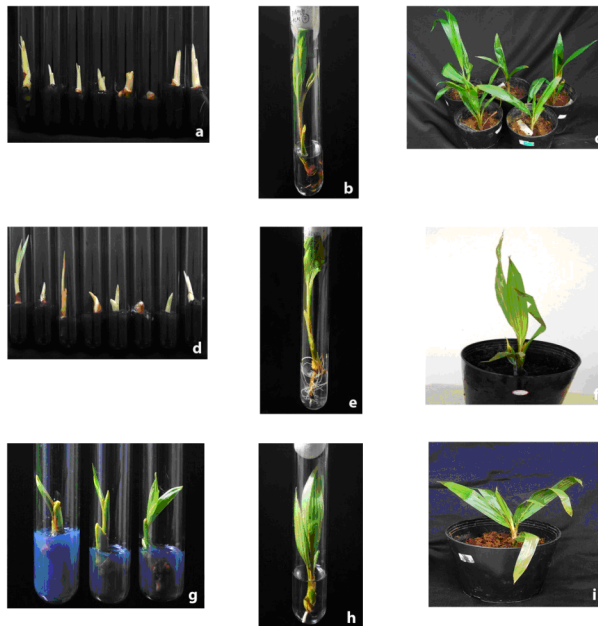


Plate 1. The developmental stages of cryopreserved embryos from WCT, MYD and Fiji tall nuts in modified pregrowth desiccation method (a) germination in embryos from WCT - 90 days after culture (DAC) (b) in liquid medium at 300 DAC (c) pot established plant at 340 DAC (d) germination in embryos from MYD at 90 DAC (e) in liquid medium at 300 DAC (f) pot established plant at 340 DAC (g) germination in embryos from Fiji tall at 90 DAC (h) in liquid medium at 300 DAC (i) pot established plant at 340 DAC

The phenotypic characters observed in plants retrieved from embryos after desiccation, cryopreservation or normal control plants from WCT, MYD and Fiji tall accessions are summarized in the Table 3. The plantlets derived from these accessions after desiccation or cryopreservation was found to be phenotypically similar to control plants ($P > 0.5$).

Table 3. Phenotypic characters observed in plants retrieved from desiccated, cryopreserved or embryos without any treatment from West Coast Tall, Malayan Yellow Dwarf and Fiji tall nuts. The data were evaluated 300 days after culture of embryos and analyzed statistically by univariate ANOVA

Accessions	No. of leaves	No. of roots	Leaf length	Root length	Plant height	Collar girth	Root volume
WCT +LN	2.6	1	20.1	2.4	28	2.96	2.48
WCT	2.75	1.25	19.5	7.37	31	3.55	3.5
MYD -LN	3	1.75	17.25	3.07	26.75	3.5	3.75
MYD +LN	4	1	18.75	4.0	25.92	3.8	3.25
MYD	3	1	17.5	3.16	24.33	3.36	3.3
Fiji Tall -LN	3	1.2	16.5	3.1	23.5	4	4.2
Fiji Tall +LN	3	1.7	15	3.56	20.1	3.5	3.8
Fiji tall	3	1.9	17.5	3.89	28.8	4.1	4.5

In any cryopreservation method, the explant is to be conditioned for high level of desiccation tolerance in order to bear the rigorous dehydration steps before immersion in liquid nitrogen. The most common method for inducing desiccation tolerance is by preculturing with high concentration of sucrose (Suzuki *et al.*, 2006). In view of the positive correlation of sucrose and raffinose in desiccation tolerance during development of orthodox seeds (Leprince *et al.*, 1990) and in embryos (Brenac *et al.*, 1997), its use in preculture treatments for cryopreservation studies in coconut zygotic embryos was investigated. Germination of embryos after desiccation or cryopreservation was highly positive with marked increase in soluble sugar content under optimum preculture conditions followed in the present study. The positive correlation of soluble sugar content in the embryo with germination in cryopreserved coconut zygotic embryo in the present study substantiates its cryoprotective effect as in the case of freeze resistant cell lines of *Eucalyptus gunnii* reported by Leborgne *et al.* (1995) who hypothesized the interaction of carbohydrates with cell membranes to the origin of cryoprotective effect. There are several reports that relate dehydration tolerance after sucrose pretreatment with accumulation of sucrose (Crowe and Crowe, 1992; Sun *et al.*, 1994; Sun and Leopold 1997). Suzuki *et al.* (2006) reported dehydration and freezing tolerance induced by two step preculturing with sucrose in gentian axillary buds, wherein the first preculture step resulted in increased soluble sugar content mainly of raffinose followed by sucrose. In the second preculture step this increase was mainly due to sucrose while raffinose content decreased to the level of control buds that was similar to molar ratio of sucrose to raffinose (8-6: 1) observed in desiccation tolerant orthodox seeds (Koster, 1991; Sun *et al.*, 1994). Contrary to this, desiccation tolerance induced in oil palm embryos by culture in the presence

of high sucrose was associated with its accumulation in the absence of oligosaccharide (Aberlenc-Bertossi *et al.*, 2003). In the present study, preculture of embryos with raffinose alone showed high desiccation tolerance but it does not confer freezing tolerance as none of the embryos survived when raffinose alone was used for preculture prior to cryopreservation. Similarly in protoplasts of desiccation tolerant pea seed embryos a mixture of sucrose and raffinose provided better protection during drying but raffinose alone was not effective (Xio and Koster, 2001). In coconut zygotic embryos also raffinose has no cryoprotective effect without sucrose in the preculture medium. The highest level of plantlet recovery observed in the case of preculture for three days in first step followed by one day in sucrose with raffinose implies the positive effect of sucrose and raffinose in the survival and germination of coconut embryos after cryopreservation. In studies conducted by Caffrey *et al.* (1998) using model membrane systems, it was concluded that the presence of raffinose can assist in the protection of membranes from desiccation damage by preventing the crystallizing tendency in sucrose at low moisture contents. Shahba *et al.* (2003) reported significant correlation between raffinose content with freezing tolerance in salt grass. The importance of sucrose in combination with raffinose in the formation of stable glasses at ambient temperatures has been reported in desiccation tolerant seeds (Koster, 1991). Sucrose is proposed to have important protective role during the early stage of drying (Sun *et al.*, 1994) whereas, raffinose, an inhibitor of sucrose crystallization (Caffrey *et al.*, 1988) appears to be important for survival during later stages of drying in seeds. In model membrane systems, vitrification of sucrose around fluid membrane phospholipids stabilized the lipid in the fluid state during desiccation (Koster *et al.*, 1994). Raffinose conferred significant cryoprotection and also was effective in reducing volume expansion – a unique attribute for being a good cryoprotectant, at low concentrations in asparagus nodal segments cryopreserved by slow freezing method (Suzuki *et al.*, 2005). Similarly Koster and Lynch (1992) reported cryoprotective effect of raffinose and sucrose, located in the extracellular compartment, on the plasma membrane of Puma rye. Shahba *et al.* (2003) reported significant correlation between raffinose concentration and freezing tolerance in salt grass. The concept that sucrose plays an important role in membrane protection at low hydration (Koster *et al.*, 2000; Bryant *et al.*, 2001) and raffinose for increasing its efficiency (Caffrey *et al.*, 1988) is supportive in the present study since germination and normal development of plants were noticed after preculture with sucrose and raffinose and subsequent cryopreservation at low hydration (12.49 % MC) in coconut zygotic embryos.

The reduced shoot growth in optimized cryopreservation protocol in the present study is consistent with the earlier reports by Assy Bah and Engelman (1992) and also Sisunder *et al.* (2010) in plants derived from cryopreserved embryos of coconut. The flaccid embryos might have resulted from osmotic stress leading to defective membrane permeability in embryos after cryopreservation.

In validation studies using modified pregrowth desiccation method, the cryopreserved embryos showed similarity in response between Malayan Yellow Dwarf and Fiji Tall accessions for survival, germination and *ex vitro* establishment in pots. But the percentage plants retrieved in pots was less compared to high survival and germination percentages of embryos. Comparatively more number of *ex vitro* retrieved plants, its phenotypic similarity with control plants and low rate of abnormality in this modified pregrowth desiccation method shows the efficacy of this method for cryopreservation of coconut zygotic embryos. But modification of

regrowth medium by supplementation with growth hormones like GA3 and other growth stimulants like glutathione may be looked into to get the full potential of the embryo for germination and development of vigorous plants for *ex vitro* establishment. Recent studies in coconut zygotic embryos by Lopez-Villalobos *et al.* (2011) have shown that addition of lauric acid into the culture medium at 60 or 75 days of culture initiation may mimic the supply of fatty acid *in situ* that utilizes non-esterified fatty acids mainly lauric acid from coconut endosperm in later stages of embryo development. Hence modification of nutrient supply *in vitro* by timed addition of lauric acid in combination with other growth regulators can be explored for the normal growth of *in vitro* retrieved plants after cryopreservation utilizing this method. Screening trials with more accessions is necessary to verify the varietal response to cryopreservation by this method. Moreover the effect of storage period on plantlet development after cryopreservation utilizing this method is to be verified in order to use this method for routine application for gene banking.

References

- Assy-Bah, B. and Engelmann, F. 1992. Cryopreservation of mature embryos of coconut (*Cocos nucifera* L.) *Cryo Letters* 13: 117-126.
- Aberlenc-Bertossi, F., Chabrilange, N., Corbineau, F. and Duval, Y. 2003. Acquisition of desiccation tolerance in developing oil palm (*Elaeis guineensis* Jacq.) embryos in planta and in vitro in relation to sugar content. *Seed Sc. Res.* 13: 179-186.
- Brenac, P., Horbowicz, M., Downer, S.M., Dickerman, A.M., Smith, M.E. and Obendorf, R.L. 1997. Raffinose accumulation related to desiccation tolerance during maize (*Zea mays* L.) seed development and maturation. *J. Pl. Physiol.* 150: 481-488.
- Bryant, G., Koster, K. L. and Wolfe, J. 2001. Membrane behavior in seeds and other systems at low water content: the various effects of solutes. *Seed Sc. Res.* 11: 17-25.
- Chin, H.F. and Roberts, E.H. 1980. Recalcitrant crop seeds. Kuala Lumpur, Malaysia: Tropical Press SDN, BDH.
- Crowe, J.H. and Crowe, L.M. 1992. Membrane integrity in anhydrobiotic organisms: towards a mechanism for stabilizing dry cells. In: *Water and Life* (Eds. Somero, G.N., Osmond, C.B. and Bolis, C.L.) pp 87-103. Springer-Verlag, Berlin. ISBN 3-540-54112-8.
- Chen, Y. and Burris, J.S. 1990. Role of carbohydrates in desiccation tolerance and membrane behaviour in maturing maize seed. *Crop Sci.* 30: 971-975.
- Caffrey, M., Fonseca, V. and Leopold, A.C. 1988. Lipid-sugar interactions: Relevance to anhydrous biology. *Plant Physiol.* 86: 754-758.
- Dumet, D., Engelmann, F., Chabrilange, N. and Duval, Y. 1993. Importance of sucrose for the acquisition of tolerance to desiccation and cryopreservation of oil palm somatic embryos. *Cryo Letters* 14: 243-250.
- Dumet, D., Engelmann, F., Chabrilange, N., Dussert, S. and Duval, Y. 1994. Effect of various sugars and polyols on the tolerance to desiccation and freezing of oil palm polyembryonic cultures. *Seed Sci. Res.* 4: 307-313.
- Hoekstra, F.A., Golovina, E.A., Tetters, F.A.A. and Wolkers, W.F. 2001. Induction of desiccation tolerance in plant somatic embryos: How exclusive is the protective role of sugars. *Cryobiology* 43: 140-150.

- Karun, A., Parthasarathy, V.A., Kumaran, P.M., Iyer, R.D., and Sajini, K.K. 2002. Coconut Embryo Culture Protocol for Germplasm Collection, Technical Bulletin No. 45, CPCRI, Kasaragod, pp. 1-16.
- Koster, K.L. 1991. Glass formation and desiccation tolerance in seeds. *Plant Physiol.* 96: 302-304.
- Koster, K.L. and Lynch, D.V. 1992. Solute accumulation and compartmentation during the cold acclimation of Puma rye. *Plant Physiol.* 98: 108-113.
- Koster, K. I., Webb, M.S., Bryant, G. and Lynch, D.V. 1994. Interactions between soluble sugars and POPC (1-palmitoyl-2-oleoyl-phosphatidylcholine) during dehydration : vitrification of sugars alters the phase behavior of the phospholipid. *Biochim. Biophys. Acta* 1193: 143-150.
- Koster, K. L., Lei, Y.P., Anderson, M., Marlin, S and Bryant, G. 2000. Effects of vitrified and nonvitrified sugars on phosphatidylcholine fluid-to-gel phase transitions. *Biophys. J.* 78: 1932-1946.
- Langis, R and Steponkus, P. L. 1991. Vitrification of isolated rye protoplasts: protection against dehydration injury by ethylene glycol. *Cryo Letters* 12: 107-112.
- LePrince, O., Bronchart, R. and Deltour, R. 1990. Changes in starch and soluble sugars in relation to the acquisition of desiccation tolerance during maturation of *Brassica campestris* seed. *Plant Cell Environ.* 13: 539-546.
- Leborgne, N., Teulieres, C., Travert, S., Rols, M.P., Teissie, J and Boudet, A.M. 1995. Introduction of specific carbohydrates into *Eucalyptus gunni* cells increases their freezing tolerance. *European J. Biochem.* 229: 710-717.
- Lopez-Villalobos, A., Dodds, P.F. and Hornung, R. 2011. Lauric acid improves the growth of zygotic coconut (*Cocos nucifera* L.) embryos in vitro. *Plant Cell Tiss. Organ Cult.* 106 : 317-327.
- Sajini, K.K., Anitha Karun, and Kumaran, P.M. 2006. Cryopreservation of coconut (*Cocos nucifera* L.) zygotic embryos after pregrowth desiccation. *J. Plantn. Crops* 34: 576-581.
- Sisunandar, Rival, A., Turquay, P., Samosir, Y. and Adkins, S.W. 2010. Cryopreservation of coconut (*Cocos nucifera* L.) zygotic embryos does not induce morphological, cytological or molecular changes in recovered seedlings. *Planta* 232: 435-447.
- Suzuki, M., Ishikawa, M. and Akihama, T. 1998. A novel preculture method for induction of desiccation tolerance in gentian axillary buds for cryopreservation . *Plant Sci.* 135: 69-76.
- Suzuki, M., Ishikawa, M., Okuda ,H., Noda, K., Kishimoto, T., Nakamura, T., Ogiwara I., Shimura, I. and Akihama, T. 2006. Physiological changes in Gentian axillary buds during two step preculturing with sucrose that conferred high levels of tolerance to desiccation and cryopreservation. *Ann. Bot.* 97: 1073-1081.
- Suzuki, T., Kami, D., Oosawa, K and McGann, L.E. 2005. Cryoprotection in plant tissues related to reduced volume expansion of cryoprotectant solution. *Cryo Letters* 26: 159-168.

- Shahba, M.A., Qian, Y.L., Hughes, H.G., Koski, A.J. and Christensen, D. 2003. Relationships of soluble carbohydrates and freeze tolerance in saltgrass. *Crop Sci.* 43: 2148-2153.
- Sun, W.Q., Irving, T.C. and Leopold, A.C. 1994 The role of sugar, vitrification and membrane phase transition in seed desiccation tolerance. *Physiol. Plant.* 90: 621-628.
- Sun, W.Q. and Leopold, A.C. 1997. Cytoplasmic vitrification of anhydrobiotic organisms. *Comp. Biochem. Physiol.* 117: 327-333.
- Sopia, N.F. and Samygin, G.A. 1993. Mechanism of the protective effect of plasmolyzing sucrose solutions during freezing of wheat cell suspension. *Fiziologiya Rastenii (Moscow)* 40: 636-641.
- Smythe, B.M. 1967 Sucrose crystal growth II. Rate of growth in the presence of impurities. *Aust. J. Chem.* 20: 1097-1114.
- Uragami, A., Sakai, A. and Nagai, M. 1990 Cryopreservation of dried axillary buds from plantlet of *Asparagus officinalis* L. grown in vitro. *Plant Cell Rep.* 9: 328-331.
- Xiao, L. and Koster, K.L. 2001. Desiccation tolerance of protoplasts isolated from pea embryos. *J. Exp. Bot.* 52: 2105-2114.
- Zhang, Y.X., Wang, J.H., Bian, H.W. and Zhu, M.Y. 2001. Pregrowth-desiccation: a simple and efficient procedure for the cryopreservation of rice (*Oryza sativa* L.) embryogenic suspension cells. *Cryo Letters* 22: 221-228.

Alternate genetics and genomics of coconut

V. Arunachalam, B.A. Jerard, K.P. Manju, V. Niral

Central Plantation Crops Research Institute

Kasaragod- 671124, Kerala, India

Coconut (*Cocos nucifera* L.) is an important palm of humid tropics providing multiple products for millions of people. Conventional genetics and genomics approaches to coconut research involve gene pool enrichment, selection, hybridization, exploitation of mutants such as macapuno and prepotency, etc. (review by Arunachalam and Rajesh, 2008). Coconut breeding is long process demanding for long periods of experiments and larger experimental areas. Coconut is a monoecious palm and produces branched inflorescences with protoandry. However mutants for branching of inflorescence and sex expression do occur. Spicata mutants display maximal female flowers and few male flowers on unbranched inflorescences. Androgena mutant is characteristic of maximum male flowers and few female flowers on highly branched (secondary and tertiary) inflorescences. Plicata is a mutant in which the leaflets remain fused, as seen in seedling leaves. It is also known as idolatrica in oil palm (Zeven, 1964) and is due to a single recessive gene. The spicata mutation of coconut, is a heterozygous state of a single dominant (Ss) gene (Ninan and Satyabalan, 1963) over the normal typica inflorescence (ss). Androgena palms have shown meiotic abnormalities such as aneuploidy ($2n-1$) in a few cells (Ninan *et al.*, 1960). We reported a single palm TT50 with two mutations on a single palm viz., (androgena and plicata) (Arunachalam *et al.*, 2001). Genomics research in coconut received much attention in recent years to understand genetic diversity and map the genes and traits of interest (review: Arunachalam, 2012).

Single Nucleotide Polymorphism (SNP) markers in WRKY transcription factor genes are employed to analyse the genetic diversity in coconut (Mauro *et al.*, 2006, 2007). However *in silico* mining of SNPs in palms (oil palm-Riju *et al.*, 2007) have great potential to develop many genic markers. Elite pedigree breeding and single plant selection are successful methods in genetic improvement of many crop plants. Hence, in this report we attempted the exploitation of single plant selection, single gene mutation, and single nucleotide mutation in coconut by:

1. Selfing of few single elite coconut palms with promising yields over three generations
2. Crossing of spicata and androgena+plicata mutants,
3. *In silico* mining of SNP/indels in nucleotide sequences of coconut

Elite coconut palms with very high nut yield (also known as super yielders) were identified by earlier coconut breeders at CPCRI Kasaragod. It includes few two hybrid progenies (WB 169, WB 179) WCT x COD (West Coast Tall x Chowghat Orange Dwarf) whose parent palms also were high yielders (Annual reports CPCRI). Open pollinated progenies of these palms were planted as trial in 29N block of the experimental farm at CPCRI, Kasaragod. We self pollinated the three such progenies (29N-210, 211, 217) of above mentioned hybrid progenies of WCT x COD. Details of the pedigree of the palms used in the study are given as

Fig. 1. The self pollinated progenies were planted in two batches at CPCRI, RC Kidu, Nettana Taluk South Canara District Karnataka. They were evaluated for morphometric traits and yield performance.

Hybridization was attempted between a spicata palm and TT50 palm (androgena and plicata mutations). Hybridization was attempted between single palms of COD and Philippines Laguna. A replicated trial was laid out with progenies of COD x Philippines Laguna, Spicata x androgena, selfed progenies of TT50 palm and Philippines Laguna at CPCRI, RC, Kidu Nettana Taluk South Canara District Karnataka. The plants were evaluated for morphometric traits and yield performance.

Twenty nucleotide sequences of 5 Kb size from coconut were retrieved from GenBank (<http://www.ncbi.nih.nlm.gov>) database and analyzed for SNPs and indels using AutoSNP software after clustering with CAP3 software.

Among 28 selfed progenies of elite pedigree, 24 are surviving now. Of these, six had flowered by 6th year of planting. One of them had yielded 60 nuts per palm in 6th year of planting (Table1). Iyer *et al.* (1979) identified super yielding palms in coconut. Our study confirmed the single plant selection offering promise in generating early high yielding progenies. Yield data in these progenies after ten years is necessary to draw conclusions. Performance of the selfed/open pollinated progenies of such single high yielders are essential. Such progenies have promise as genetic stocks for yield improvement in coconut.

Table 1. Morphometric traits and yield in progenies of elite pedigree at 6th year of planting

Traits	Mean	Range	SD
Plant Height (cm)	713	350-900	129
No. of leaves	14	6-18	2.9
Petiole length (cm)	134	110-160	13.8
Rachis length (cm)	503	300-600	61.7
No. of leaflets	97	44-130	14.5
Leaflet length (cm)	99	80-124	11.8
Breadth of leaflet (cm)	5.3	4.2-6.7	0.7
Girth (cm)	108	40-145	23.9
No. of inflorescence	8	2-11	3.6
No. of nuts/palm/year	16	6-40	14.6

Morphometric traits of progenies of spicata, spicata x androgena+plicata, dwarf x tall at 4th year of planting are given as Table 2. Dwarf x tall progenies recorded lower values for vegetative morphometric traits. Of the 19 surviving flowered palms of spicata x androgena progenies, four out of showed spicata trait and remaining 15 palms showed normal typical inflorescence. Open-pollinated progenies of spicata palm segregated in 1:1 ratio of spicata and normal palms (Ninan and Satyabalan, 1963). Spicata palm also has shown many meiotic irregularities and a high degree (12%) of pollen sterility (Ninan and Satyabalan, 1963). Floral biology of spicata palms has unique duration of male and female phases (Ratnambal *et al.*,

Table 2. Morphological traits in progenies of mutant palms at 4th year of planting

Characters	Spicata	Spicata x androgena	Dwarf x Tall
Plant Height (cm)	777	725	640
No. of leaves	14	13	12
Petiole length (cm)	140	153	107
Rachis length (cm)	526	550	446
No. of leaflets	95	102	81
Length of leaflet (cm)	111	95	93
Breadth of leaflet (cm)	5.4	5.9	5.1
Girth (cm)	108	80	76

2003). Out of the five inbred lines of Markham Valley Tall, two were mutants bearing inflorescences with secondary branching (Sugimura *et al.*, 1994). Secondary spikelets are associated with an increased number of male flowers in coconut.

Table 3. *In silico* SNP/indels in nucleotide sequences of coconut

Total sequences analyzed	Total SNPs	SNP consensus size	SNP frequency	Smallest contig	Total Transitions	Total transversions	Total indels
20	838	4999bp	16.76/100 bp	2 sequences	325	454	59

Table 4. Details of the contigs and SNPs in sequences of coconut

Contig	Number of sequences	Genes	SNPs / indels
Contig 1	3	Protein Kinase	11
Contig 2	2	ACP synthase isoform 1b mRNA, 3' UTR	0
Contig 3	2	trnQ-rps16 intergenic spacer, partial sequence; chloroplast	235
Contig 4	3	trnD gene, partial sequence; trnY and trnE genes, complete sequence; and trnE-trnT intergenic spacer; chloroplast	231
Contig 5	5	clone (tall) B1 phosphoribulokinase-like protein 2 (PRK) gene, exons 4, 5 and partial cds	238
Contig 6	3	(Eno) mRNA, partial cds	30
Contig 7	2	(Ecorep2) repetitive	93

SNP/indel analysis by bioinformatics methods in coconut revealed 325 transitions, 454 transversions and 59 indels in the twenty nucleotide sequences of coconut. One transition and one transversion in MAP kinase sequence discovered in the sequences are important being a disease responsive gene. We found a high density of 16.76/100 bp in the few sequences of coconut. In expressed sequences of oil palm, Riju *et al.*, (2007) found SNP/indels at a density of 1.36 /100 bp. Manju and Arunachalam (2011) found SNPs at a frequency of 2.84/100bp in the WRKY sequences of palms. However some of these SNPs might be false due to errors during process in thermal cycler and/or sequencing etc. We sequenced a SNP containing sequence in chloroplast trnQ-rps16 intergenic spacer region and blast analysis is given as Fig.2. SNPs from *in silico* analysis need to be confirmed by re-sequencing and single strand confirmation polymorphism analysis. SNP markers from WRKY genes (Mauro *et al.*, 2006, 2007) were found suitable in coconut for genetic diversity analysis.

We give new perspective to coconut breeders by using single plant selection among progenies of elite pedigree, hybridization among mutants and computational analysis of sequences for point mutations.

References

- Arunachalam, V. 2012. Coconut In: *Genomics of cultivated palms*. Elsevier Insights Series. Elsevier Inc., United Kingdom. pp.13-27.
- Arunachalam, V. and Rajesh, M.K. 2008. Breeding of coconut palm. CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources. CAB Reviews: (CABI Press) 3(053):1-12.
- Arunachalam, V. Jerard, B.A. Elangovan, M. Ratnambal, M.J. Dhanapal, R. Rizal, S.K. and Damodaran, V. 2001. Unexploited diversity in coconut palm (*Cocos nucifera* L.). *Plant Genetic Resources Newsletter* 127:39-43.
- Iyer, R.D., Bhaskara Rao, E.V.V. and Govindankutty, M.P. 1979. Super yielders in coconut. *Indian Farming* 1979;28:3-5.
- Mauro, H.M., Meerow, A.W., Borrone, J.W., Kuhn, D.N. and Schnell, R.J. 2006. Ten informative markers developed from WRKY sequences in coconut (*Cocos nucifera*). *Mol. Ecol. Notes*. 6:904-906.
- Mauro, H.M., Meerow, A.W., Borrone, J.W., Kuhn, D.N. and Schnell, R.J. 2007. Usefulness of WRKY gene-derived markers for assessing genetic population structure: an example with Florida coconut cultivars. *Sci. Hort.* 115:19-26.
- Manju, K.P. and Arunachalam, V. 2011. Bioinformatic prediction of SNP markers in WRKY sequences of palms. *Cord* (Asia Pacific Coconut Community). 27(2):17-25.
- Ninan, C.A. and Satyabalan, K. 1963. Cytogenetic studies in the genus *Cocos*. 2. Some observations on the 'spicata' character in coconuts. *Indian Coconut Journal* 16:109-14.
- Ninan, C.A., Pillai, R.V. and Josy Joseph. 1960. Cytogenetic studies in the genus *Cocos*. I. Chromosome number in *C. nucifera* L. vars. *Spicata* and *androgena*. *Indian Coconut J.* XIII (4):120-132.
- Ratnambal M.J. Arunachalam V and Krishnan M (2003) Floral biology of some coconut accessions. *Journal of Plantation Crops* 31(1): 14-22.

- Riju, A. Chandrasekhar, A. and Arunachalam, V.(2007) Mining for Single nucleotide polymorphisms and insertions/deletions in expressed sequence tag libraries of oil palm. *Bioinformation* 2(4): 128-131.
- Sugimura, Y., Rocat, D.A. Salud, C.D. and Kamata. N. (1994) An inflorescence mutant appeared in the inbred line of coconut “Markham” *Jpn. J. Trop. Agric.* 38(2):145-147.
- Zeven, A.C. 1964. *The Idolatraca*. *Baileya* 12:11.

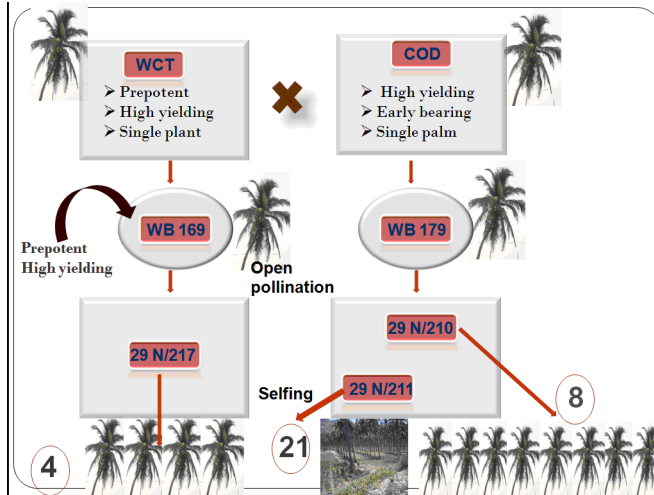


Fig.1. Pedigree details of the elite pedigree progenies

```

Identities = 579/582 (99%), Gaps = 0/582 (0%)
Strand=Plus/Minus

Query 46 AACATAAAATGTTAACGAGAACAACTCTTTGTTCTGAATCTAAGAATGATCTTTAGAA 105
Sbjct 582 AACATAAAATGTTAACGAGAACAACTCTTTGTTTGAATTTAAGAATGATTTTAGAA 523

Query 106 TCATATTTTTCTTTTCATTGGTTTCTCACAACGTAATCAAGTGTCAAATGTGGGTGGA 165
Sbjct 522 TCATATTTTTCTTTTCATTGGTTTCTCACAACGTAATCAAGTGTCAAATGTGGGTGGA 463

Query 166 AATAAATATCtcttttttttAATTCAAAAAGAAATCTGGGTTTATCATTACATTCAG 225
Sbjct 462 AATAAATATCtcttttttttAATTCAAAAAGAAATCTGGGTTTATCATTACATTCAG 403

Query 226 GATATGTTCTGACTACTCAATATTCAATTTAAAGTTAGTTACTTTTGAAACCTTATGTC 285
Sbjct 402 GATATGTTCTGACTACTCAATATTCAATTTAAAGTTAGTTACTTTTGAAACCTTATGTC 343

Query 286 CCATATCTATGAAATGTCAATTCTCACATGAAGCATTCTGAATCGAAATGTGAATGAAAG 345
Sbjct 342 CCATATCTATGAAATGTCAATTCTCACATGAAGCATTCTGAATCGAAATGTGAATGAAAG 283

Query 346 AAAGGCCCTCTTTATTCCTTTACCAAGGGTAAAAAGCCTAaaaaaaTAAATGGGGTATCC 405
Sbjct 282 AAAGGCCCTCTTTATTCCTTTACCAAGGGTAAAAAGCCTAaaaaaaTAAATGGGGTATCC 223

Query 406 CAATTCACAGTCTATGTGGAGACTAAAGAGTAGGGAGTTTGTGTTACTAATTCATCAC 465
Sbjct 222 CAATTCACAGTCTATGTGGAGACTAAAGAGTAGGGAGTTTGTGTTACTAATTCATCAC 163

Query 466 TGGTCTTAAAACTTCTAAAACTGGTGTGGGaaaaaaTAGTAAAAACGATCTGGACCCCA 525
Sbjct 162 TGGTCTTAAAACTTCTAAAACTGGTGTGGGAAAAAATAGTAAAAACGATCTGGACCCCA 103

Query 526 ttttttGATTTTATCTGGTTTCATCTTATATCTTATCCGGTTCAAATGAATAATTGTAA 585
Sbjct 102 TTTTGTGATTTTATCTGGTTTCATCTTATATCTTATCCGGTTCAAATGAATAATTGTAA 43

Query 586 ATCAATGTAATGTAGCGATTGGGGGGAATTCGTATATTGCA 627

```

Fig.2. SNP in *Cocos nucifera* chloroplast trnQ-rps16 intergenic spacer, complete sequence; and ribosomal protein S16 gene, partial cds

***In silico* prediction of properties of KNOX AND BABY BOOM proteins in coconut**

Nithya S., Rajesh M.K., Bobby Paul, Anitha Karun and George V.Thomas

Central Plantation Crops Research Institute
Kasaragod 671124, Kerala, India.

Plant regeneration through tissue culture is mainly obtained via organogenesis and somatic embryogenesis. Somatic embryogenesis is the process by which somatic cells develop into plants through characteristic morphological embryo stages generally encountered in zygotic embryogenesis. Somatic embryogenesis is a valid vegetative propagation procedure in terms of the multiplication rate and resulting rejuvenation. It is the only large-scale method for producing tropical plants from cross-fertilizing species and/or species with no natural vegetative propagation system (e.g. oil palm or coconut) (Dublin *et al.*, 1991). Somatic embryos are bipolar in organization with an integrated root and shoot axis arising in callus cultures. These somatic embryos show developmental sequences like pro-embryonal, globular, heart and torpedo stages. One of the most important achievements in the field of plant tissue culture is the discovery of somatic embryogenesis.

The coconut palm (*Cocos nucifera* L.) is a major agricultural crop in tropical areas. Coconut micropropagation that has been the subject of research for nearly five decades, as this species is highly recalcitrant to *in vitro* regeneration and success so far has only been achieved through somatic embryogenesis as the sole path for coconut regeneration (Sa'enz *et al.*, 2006). In coconut, somatic embryogenesis is a slow process (10 to 12 months to obtain embryos from somatic cells vs. 12 months for zygotic embryogenesis) (Verdeil *et al.*, 2001).

A number of genes positively influence the regenerative competence of plant cells for somatic embryogenesis has been identified. During somatic embryogenesis, biochemical and morphological changes occur, which is strongly related to alterations in gene expression patterns. Proteins known to be induced during somatic embryogenesis include KNOX and BABY BOOM. *BABY BOOM* (*BBM*) has been reported to be expressed preferentially in developing embryos and seeds. Ectopic expression of *BBM*, in *Arabidopsis* and *Brassica*, led to the spontaneous formation of somatic embryos and cotyledon-like structures on seedlings and also induced additional pleiotropic phenotypes, including neoplastic growth, hormone-free regeneration of explants, and alterations in leaf and flower morphology (Boutilier *et al.*, 2002). *KNOTTED*-like homeobox (*KNOX*), play important roles during different phases of plant growth and development, controlling cell specification and pattern formation (Chang *et al.*, 1998). The expression of the class I *KNOX* genes seems to play an important role during somatic embryogenesis in *Glycine max* (Ma *et al.*, 1994), *Picea abies* (Hjortswang *et al.*, 2004) and *Elaeis guineensis* (Jouannic *et al.*, 2007).

With the development of techniques in molecular biology that allow rapid identification, isolation, and sequencing of genes, we are now able to infer the sequences of many proteins. However, it is still a time-consuming task to obtain the three-dimensional structures of these proteins. A major goal of structural biology is to predict the three-dimensional structure from the sequence, a pursuit that has not yet been realized. Thus,

alternative strategies are being applied to develop models of protein structure when the constraints from X-ray diffraction or NMR are not yet available. One method that can be applied to generate reasonable models of protein structures is homology modeling. This procedure, also termed comparative modeling or knowledge-based modeling, develops a three-dimensional model from a protein sequence based on the structures of homologous proteins.

The present study was undertaken to characterize KNOX and BABY BOOM proteins in coconut using bioinformatic tools.

Sequences of coconut CnKNOX1 (GU937114), CNKNOX2 (accession number GU937115) and BABY BOOM (FJ606793) were retrieved from NCBI. BLAST searches of the Swiss-ProtTrEMBL and Uniprot-curated databases were performed using WU-BLAST2.0 algorithm in order to identify local alignments for the two proteins. Physicochemical properties of the selected proteins were determined using the PROTPARAM tools. The INTERPRO analysis, based on the PROSITE and Pfam databases, was done to identify sequence patterns associated with the protein family and to determine the modular architecture of these proteins. Hydropathy plots of the deduced proteins were determined using PEPWINDOW available at Mobyle server.

Secondary structure analysis was performed in PREDATOR available at NPS@ server (Network Protein Sequence Analysis). The two proteins were subjected to CATH (Class Architecture Topology and Homology) analysis to find the protein structure classification. Tertiary structure of the proteins was modeled by submitting the deduced amino acid sequences the Computational Biology Service Unit. The protein models were generated by aligning to the structural homologues in the fold recognition program of LOOPP v3.0 (Learning, Observing and Outputting Protein Patterns) server. The quality of the protein models was assessed using PROCHECK. The visual displays of the models were performed by SwissPDB viewer.

The WU-Blast analyses results of KNOX and BABY BOOM proteins are shown in the Tables 1 and 2. KNOX from coconut showed homology to KNOXs from *Elaeis guineensis*, *Ricinus communis*, *Populus tremula*, *Populus trichocarpa*, *Prunus dulcis* etc. BABY BOOM from coconut showed similarity to BABY BOOM proteins from *Phoradendron serotinum*, *Populus trichocarpa*, *Arabidopsis thaliana*, *Cycas revolute*, *Triticum aestivum* etc.

Table 1. WU-BLAST results for coconut KNOX protein

Species showing similarity to KNOX protein from coconut	Accession number	E-value	Identity
<i>Elaeis guineensis</i>	A5H237	1.2e-135	83
<i>Ricinus communis</i>	B9RXF1	7.6e-94	65
<i>Populus tremula</i>	Q5XLC9	1.8e-92	64
<i>Populus trichocarpa</i>	Q5UL96	4.8e-92	64
<i>Prunus dulcis</i>	A3QNN5	3.8e-91	63
<i>Solanum lycopersicum</i>	LET6	9.0e-90	61
<i>Medicago truncatula</i>	A5Y4G6	3.4e-87	57
<i>Helianthus annuus</i>	Q8LPN1	3.6e-86	63
<i>Brassica oleracea</i>	Q9M6D9	2.9e-84	65

Table 2. WU-BLAST results for coconut BABY BOOM protein

Species showing similarity to BABYBOOM protein from coconut	Accession number	E-value	Identity
<i>Phoradendron serotinum</i>	C9WI51	2.9e-17	93
<i>Populus trichocarpa</i>	B9GKV0	1.8e-16	93
<i>Arabidopsis thaliana</i>	Q1PFE1	3.4e-16	57
<i>Populus trichocarpa</i>	B9GW20	5.1e-16	85
<i>Cycas revolute</i>	Q2Z2G6	7.5e-16	87
<i>Arabidopsis lyrata</i>	D7KHN8	1.1e-15	84
<i>Brassica napus</i>	D6RT97	3.4e-15	78
<i>Triticum aestivum</i>	C1PH82	8.5e-15	81

The INTERPRO analysis of BABY BOOM showed that this protein belongs to AP2 family containing the AP2/ERF domain. This 60 amino acid residue domain can bind to DNA and is found in transcription factor proteins. These proteins are a plant-specific class of putative transcription factors that have been shown to regulate a wide range of developmental processes. BBM is likely to promote cell proliferation and morphogenesis during embryogenesis (Boutillier *et al.*, 2002). The INTERPRO analysis of KNOX showed that this protein contains two types of domains ELK domain containing the pattern ELKGQLLRKYSGYLSSLKQEF at position 215 – 23 (prosite id PS51213) and homeobox domain containing the consensus pattern [LIVMFYGY] - [ASLVR] - x(2) - [LIVMSTACN] - x - [LIVM] - {Y} - x(2) - {L} - [LIV] - [RKNQUESTAIY] - [LIVFSTNKH] - W - [FYVC] - x - [NDQTAH] - x(5) - [RKNAIMW] at position 235 - 298 (prosite id PS50071). KNOX1 plays a role in suppressing target gene expression. KNOX is involved in the control of shoot apical meristem (SAM) during embryogenesis (Smith *et al.*, 1995).

Secondary structure prediction of KNOX sequence showed 40.24% helical, 0.00% extended and 59.76% loops/other structures (Fig 1). Secondary structure prediction of BABY BOOM showed 20.90% helical, 4.52% extended and 74.58% loops/other structures (Fig. 2). Secondary structure result for KNOX protein sequence showed that coil region was more in number followed by helix.

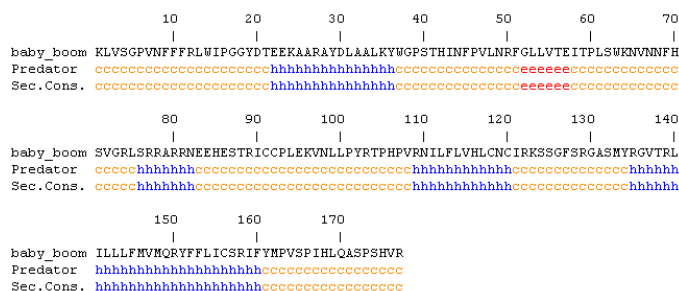


Fig.1. Predicted secondary structure result for BABY BOOM protein

	10	20	30	40	50	60	70
knox1x0	MEGRGGSGDNSGLMGGFGDGSGLSPLMIMPLMASRSVLPPTPHMSNNGLFLPPPPSNAGGEDYDDSIK						
Predator	cccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccc	hhhhhhhh	cccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccc	hhhh			
Sec.Cons.	cccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccc	hhhhhhhh	cccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccc	hhhh			
	80	90	100	110	120	130	140
knox1x0	AKIMAHPOQYPRLLSATVNCCHKVGAPPEVVARLEEACATSLMMGRASSSAAAGDGGSGGGGGEDPALDQFM						
Predator	hhhhcccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccc	hhhhhhhh	cccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccc	hhhhhhhh	cccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccc	hhhhhh	
Sec.Cons.	hhhhcccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccc	hhhhhhhh	cccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccc	hhhhhhhh	cccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccc	hhhhhh	
	150	160	170	180	190	200	210
knox1x0	EAYCEMLTKYEQLSKPFKEAMLFLSRIDAQFKSLSLSTPPPPQVYGEQLERNGSSEEEFGASGSYVDPO						
Predator	hhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhh	cccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccc	cccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccc	cccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccc	cccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccc	cccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccc	cccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccc
Sec.Cons.	hhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhh	cccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccc	cccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccc	cccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccc	cccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccc	cccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccc	cccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccc
	220	230	240	250	260	270	280
knox1x0	AEDRELKQQLLRKYSGYLSSLKQEFLLKKRKKGLPKPEARQQLDWWNRHYKWPYPSEAKLALAQSTGLD						
Predator	hhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhh	cccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccc	cccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccc	hhhhhhhhhh	cccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccc	hhhhhhhhhh	cccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccc
Sec.Cons.	hhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhh	cccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccc	cccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccc	hhhhhhhhhh	cccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccc	hhhhhhhhhh	cccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccc
	290	300	310	320	330		
knox1x0	QKQINNWFINQKRHRWKPSEEMQFVVMDDTAHPHYFMDNSLGNPFPLDCAPALL						
Predator	cccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccc	eeeeeeee	cccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccc	cccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccc	cccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccc	cccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccc	cccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccc
Sec.Cons.	cccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccc	eeeeeeee	cccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccc	cccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccc	cccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccc	cccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccc	cccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccc

Fig. 2. Predicted Secondary structure result for KNOX protein

Based on Kyte and Doolittle values, hydropathy analysis of KNOX proteins shows that majority of the residues falls under negative values and obviously the protein is hydrophilic in nature (Fig.3, 4, 5).

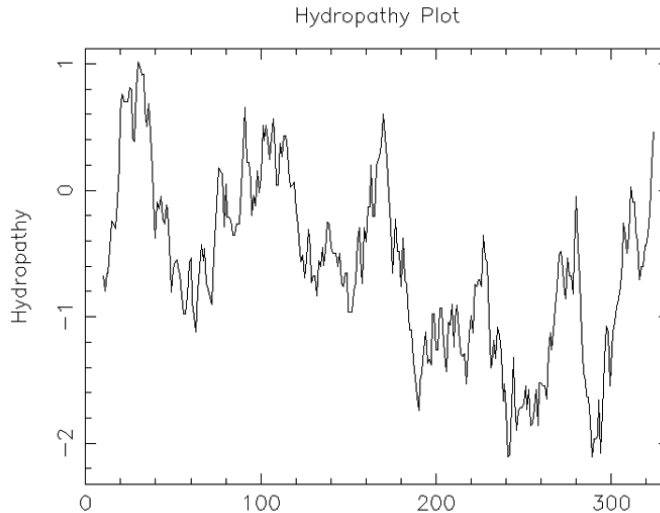


Fig. 3. Hydropathy plot for coconut KNOX1 protein

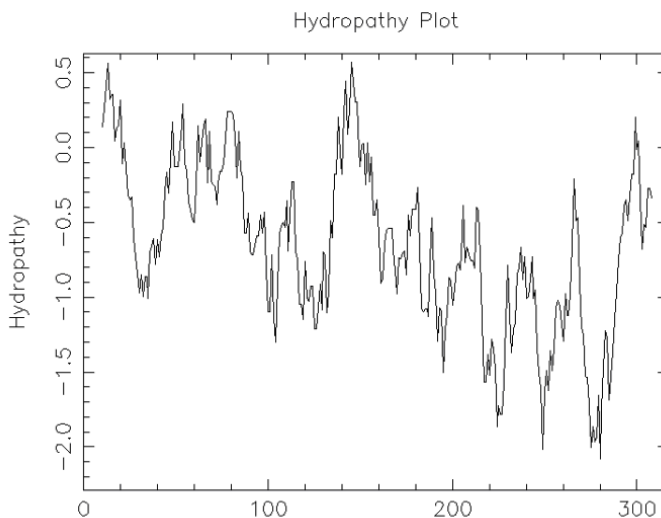


Fig.4. Hydropathy plot for coconut KNOX2 protein

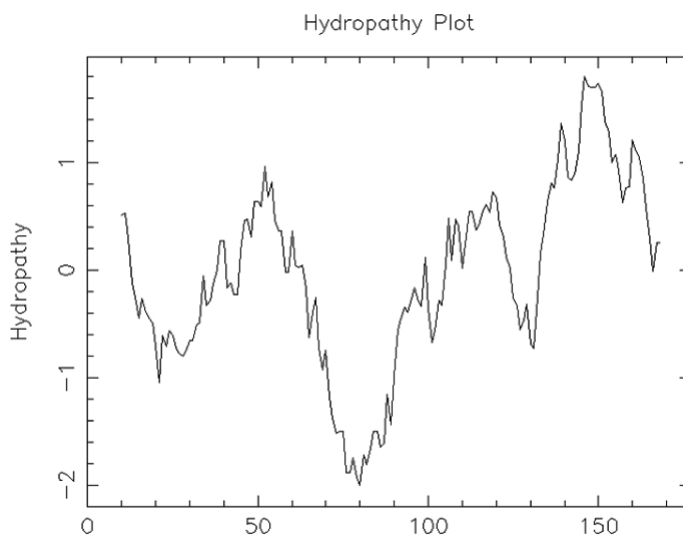


Fig. 5. Hydropathy plot for BABYBOOM protein

CATH analysis of KNOX protein showed that this protein belongs to mainly alpha-type class and the architecture is orthogonal bundle type. The BABYBOOM protein belongs to alpha beta type class and the Architecture is two layer sandwich. The protein models were generated by aligning to the structural homologues in the fold recognition program of LOOPP v3.0 (server). The quality of the protein models was assessed using PROCHECK. The visual displays of the models, performed by SwissPDB viewer, are given in Fig.6 and Fig.7.

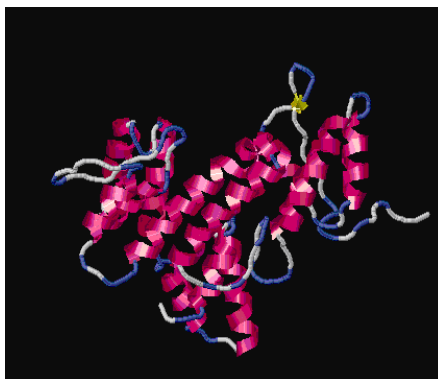


Fig.6. Predicted structure of coconut KNOX protein in LOOPP server

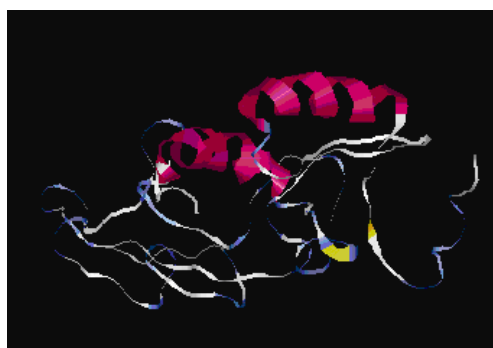


Fig.7. Predicted structure of coconut BABY BOOM protein in LOOPP server

Homology modeling plays a central role in determining protein structure in the structural genomics project. The importance of homology modeling has been steadily increasing because of the large gap that exists between the overwhelming number of available protein sequences and experimentally solved protein structures, and also, more importantly, because of the increasing reliability and accuracy of the method. In fact, a protein sequence with over 30% identity to a known structure can often be predicted with an accuracy equivalent to a low-resolution X-ray structure. The recent advances in homology modeling, especially in detecting distant homologues, aligning sequences with template structures, modeling of loops and side chains, as well as detecting errors in a model, have contributed to reliable prediction of protein structure, which was not possible even several years ago. The ongoing efforts in solving protein structures, which can be time-consuming and often difficult, will continue to spur the development of a host of new computational methods that can fill in the gap and further contribute to understanding the relationship between protein structure and function. The structures modeled in this study form the starting point for future docking studies to prediction potential ligands that can bind to coconut KNOX and BBM proteins as well as critical residues that stabilize ligands. KNOX proteins are extremely hydrophilic and the predominantly occurring secondary structure is helix. The *in silico* analysis of KNOX protein shows that this protein contains homeobox domain which is involved in transcriptional regulation of plant development. BBM proteins are also hydrophilic and the loop regions are

predominantly represented. These proteins contain AP2/ERF domain which is involved in the regulation of gene expression by stress factors and by components of stress signal transduction pathways.

Acknowledgments

The authors thank DBT (Distributed Information Sub-Centre) for financial support.

References

- Boutilier, K., Offringa, R., Sharma, V.K., Kieft, H., Ouellet, T., Zhang, L., Hattori, J., Liu, C.M., van Lammeren, A.A., Miki, B.L., Custers J.B., and van Lookeren Campagne, M.M. 2002. Ectopic expression of BABY BOOM triggers a conversion from vegetative to embryonic growth. *Plant Cell* **14** : 1737–1749.
- Dublin, P., Enjalric, F., Lardet, L., Carron, M.P., Trolinder, N. and Pannetier, C. 1991. Estate crops. pp. 337-361. In: *Micropropagation technology and application*. (Eds.) Debergh, P.C., and Zimmerman R.H. Kluwer Academic Publishers, Dordrecht.
- Chang, R.L., Gago, G.M., Palena, C.M. and Gonzalez, D.H. 1998. Homeoboxes in plant development. *Biochim. Biophys. Acta* **1442**: 1–19.
- Hjortswang, H.I., Filonova, L.H., Vahala, T. and von Arnold, S. 2002 Modified expression of the *Pal8* gene interferes with somatic embryo development in Norway spruce. *Plant Growth Regul.* **38**:75–82.
- Jouannic, S., Collin, M., Vidal, B., Verdeil, J.L. and Tregear, W. 2007. A class I KNOX gene from the palm species *Elaeis guineensis* (Arecaceae) is associated with meristem function and a distinct mode of leaf dissection. *New Phytol.* **174**:551–568.
- Ma, H., McMullen, M.D. and Finer, J.J. 1994. Identification of a homeobox containing gene with enhanced expression during soybean (*Glycine max* L.) somatic embryo development. *Plant Mol. Biol.* **24**:465–473.
- Sa'enz, L., Azpeitia, A., Chuc-Armendariz, B., Chan, J.L., Verdeil, J.L., Hoche, V. and Oropeza, C. 2006. Morphological and histological changes during somatic embryo formation from coconut plumule explant. *In Vitro Cell. Dev. Biol.* **42**:19–25.
- Smith, L.G., Jackson, D. and Hake, S. 1995. Expression of Knotted1 marks shoot meristem formation during maize embryogenesis. *Dev. Genet.* **16**: 344–348.
- Verdeil, J.L., Ocher, V.H., Huet, C., Grosdemange, F., Escoute, J., Ferrière, N. and Nicole, M. 2001 Ultra-structural changes in coconut calli associated with the acquisition of embryogenic competence. *Annals of Botany* **88**: 9-18.

Bioinformatics approach towards mining of candidate genes for water stress in coconut

Bobby Paul, Rajesh, M. K and Naresh Kumar, S

Central Plantation Crops Research Institute

Kasaragod, 671124, Kerala, India

Plants are subjected to various abiotic stresses due to unfavorable environmental changes that affect their growth, metabolism and yield. Water stress is one of the major abiotic stresses which limit the crop production in arid and semi arid tropics like India. Plant responses to water stress occur through changes in their morphological, biochemical and metabolic processes. The water stress-induced genes encoded proteins that play important roles in water stress response by conferring desiccation tolerance, protect cellular structures or are involved in the signal transduction pathway that leads to gene induction under these conditions. To develop crop plant with enhanced tolerance of water stress, a basic understanding of physiological, biochemical, and gene regulatory networks is essential.

Traditionally, the approach used to identify for genes underlying adaptive trait variation has been to map quantitative trait loci (QTLs), based on significant statistical associations between molecular markers and trait variation, either in known pedigrees or more rarely in undomesticated natural populations. In the past decade, functional genomic tools have dramatically changed the prospects to better understand the genetic basis of adaptation to water stress. These tools include a wide variety of techniques and engineering strategies that allow the identification of genes involved in molecular stress response. These studies on the molecular responses to water stress have revealed changes in gene expression in a wide variety of plants and a large number of drought-induced genes have been identified (Amuda and Balasubramani, 2011; Hussain *et al.*, 2011).

Coconut palm is one of the major perennial oil crops of the tropics providing the basis in many developing countries for food products as well as serving industrial purposes (Jones, 1991). The coconut palm experiences water stress when exposed to excess radiation $>265 \text{ Wm}^{-2}$, temperature $> 33^{\circ}\text{C}$ and vapor pressure deficit $>26 \text{ m bar}$ (Kasturibai *et al.*, 1988), aggravated by soil water deficit during the period. The duration of dry spell during initiation of inflorescence primordium, ovary development and button size nut stages, in that order, have greater influence on nut yield than other stages (Rajagopal *et al.*, 1996). The biochemical responses of coconut palm to drought stress include up regulation or synthesis of scavenging enzymes to maintain cell membrane integrity thus enabling cells to tolerate stress. Concentrations of leaf epicuticular wax, proline, reducing sugars and amino acids increased during summer and tall cultivars, *viz.*, WCT and LCT exhibited more accumulation of these compounds.

This resulted in wider adaptability of tall cultivars to different environments (Siju *et al.*, 2006). The metabolic status of the coconut palm gets affected during summer months when the available soil moisture falls below 20 mm during prolonged dry spell. Concomitant with the decrease in the leaf water status during drought stress, the activities of stress sensitive enzymes differ depending upon the nature and function of the enzyme in question. Drought

stress caused an increase in the activities of some of the stress sensitive enzymes, viz. peroxidase (PO), polyphenol oxidase (PPO), superoxide dismutase (SOD), acid phosphatase (APH) and L-aspartate: 2-oxoglutarate amino transferase (AAT) in adult palms, while activities of malic dehydrogenase (MDH) and nitrate reductase (NR) were decreased (Shivashankar *et al.*, 1991; Kasturi Bai *et al.*, 1996; Kasturi Bai *et al.*, 2003).

In coconut, development of molecular markers for water stress/drought tolerance is in its infancy. Efforts are on to link the water stress tolerance to molecular diversity to find putative molecular markers, which can be useful for marker-assisted selection (MAS). Even though lack of a viable regeneration technique is a bottleneck for genetic engineering of coconut palm, identification of molecular markers linked to water stress would enable large-scale rapid screening of coconut accessions to identify water-stress resistant palms. This will not only increase the efficiency for selection of parental material, but also will reduce the gestation period for breeding improved varieties with drought tolerance (Batugal, 1999).

There is little knowledge on the function of water-stress responsive genes in coconut. Therefore, a thorough study of the genes that are expressed during water stress conditions will provide knowledge about the molecular aspects of response of coconut to the water stress. Molecular markers developed from the expressed genes can be used in development of expressed sequence tag (EST) markers and hybridization based functional molecular markers for stress tolerance. Once the markers are established, they will be of prime importance to identify the parental material in breeding for water stress tolerance.

The aim of the present study was to identify genes expressed under water stress phase in coconut through reverse transcription RT-PCR coupled with a degenerate primer strategy.

Seed nut of the local coconut cultivar, West Coast Tall, were collected from source palms maintained at coconut germplasm field (CPCRI, Kasaragod) and sown in the nursery. Germinated seed-nuts were transplanted onto cement pots (50 x 70 cm) containing potting mixture soil (soil, sand, peat, 1:1:1 ratio) and were maintained in shaded condition. Seedlings were provided with recommended dosage fertilizer and irrigation.

Stress treatment

Two-year old seedlings were subjected to water-stress treatment. Ten seedlings were chosen as control (unstressed) and maintained under well-watered conditions and ten seedlings were subjected to water stress condition by withholding water. Leaf samples were collected from coconut seedlings 30 days after induction of water stress.

The nucleotide sequences of selected water stress inducing genes reported from other plant species, viz. *AP-2* (Apetala-2), *MAPK* (mitogen activated protein kinase), *NAC* (*NAM*, *ATAF*, and *CUC*), *WRKY* (WRKY transcription factor) and *14-3-3* (14-3-3 transcription factor) were retrieved from National Centre for Biotechnology Information (<http://www.ncbi.nlm.nih.gov/>).

Multiple sequence alignment of the complete coding sequences from different species was performed using online multiple sequence alignment tool, ClustalW. Conserved regions obtained from both 5' and 3' ends were chosen for designing forward and reverse primers respectively. Two sets of primers were designed for each of the gene from the conserved

regions of these sequences using FastPCR software. The qualities of the designed primers were validated using both PrimerBLAST and FastPCR. Primers were also designed for amplifying the house-keeping genes *actin* and *rbcL* to serve as housekeeping genes.

Leaf samples from control coconut seedlings and seedlings subjected to water-stress for 30 days were collected and RNA was isolated via RNeasy Plant Mini Kit (Qiagen) as per the manufacturer's instructions. On column, DNase I (Fermentas) treatment was given as per manufactures instructions to remove any DNA contamination from isolated RNA.

RT-PCR reactions were performed in 25 μ L volumes using the One-Step RT-PCR kit (Qiagen) as per the manufacturer's instructions. RT-PCR amplification was carried out in 5x Qiagen One-Step RT-PCR reaction buffer containing 0.6 μ M of each primer, 400 μ M dNTPs, and 5 Units Qiagen One-Step RT-PCR Enzyme Mix. Total RNA isolated from leaves of seedlings subjected to water stress and also control seedlings were used as templates to detect genes induced during water-stress. Reverse transcription was performed at 50°C for 30 min, and was immediately followed by a denaturation step at 95°C for 15 min, 30 cycles of 94°C for 30 seconds, annealing for 30 seconds at different temperatures based on the primers T_m , 72°C for 1 min and finally 72°C for 10 min. After amplification, the PCR products were resolved in 1.2% agarose gel using 1XTBE buffer, stained with ethidium bromide, visualized in gel documentation system and results documented. The sizes of the amplified fragments were estimated by comparison with a 1kb DNA ladder (Fermentas). For each primer pair, specific PCR conditions were determined, including annealing temperature and cycle's number that provided the sensitivity needed to detect the differences in expression. Actin and *rbcL* were used as constitutive controls in all experiments. The RT-PCR products were visualized by electrophoresis on 1.2% agarose gel.

PCR amplicons of expected sizes were excised from the agarose gel and purified using a gel cleanup kit. Cloning of the eluted fragments was carried out using the InsT/A clone™ PCR Product Cloning Kit (Fermentas). After transformation, the cells were finally plated on pre-warmed LB- agar plates [ampicillin (100 ppm) / IPTG (100 ppm) / X-gal (160 ppm)] and incubated overnight at 37°C. The recombinant clones were identified by blue/white colony selection. Colony PCR was carried out for direct analysis of the positive transformants. One colony was picked up and resuspended in 20 μ L of the PCR mixture. The reaction mixture was incubated for 5 min at 94°C to lyse the cells and inactivate the nucleases. PCR amplification was carried out and the PCR products visualized by agarose gel electrophoresis. Highly pure plasmid DNA was obtained by means of GenElute™ Plasmid Miniprep Kit (Sigma). PCR was carried out using the recombinant plasmid DNA as template. The PCR products were analyzed on 1.5% agarose gel. The plasmids harbouring the inserts were then sequenced. The sequencing was done from both ends using the specific primers using BigDye® Terminator v3.1 Sequencing Kit and analyzed on ABI PRISM® 377 Genetic Analyzer.

The vector contamination from the cloned sequence was removed with the help of VecScreen tool provided by NCBI. Sequence analysis was performed using the Basic Local Alignment Search Tool (BLAST) search program (Altschul *et al.*, 1990). Multiple sequence alignment was carried out using the CLUSTALW (Thompson *et al.*, 1994) with default parameters through EMBnet (<http://www.ch.embnet.org/software/ClustalW.html>). A phylogenetic analysis was carried out with homologous sequences publicly available for plants. A rooted neighbor-joining phylogenetic tree was generated using the MEGA software package version 3.0 (Kumar *et al.*, 2004). To determine relative level of support for the tree topology, bootstrap values were generated from 1000 replicates.

Environmental stresses represent limiting factors for plant productivity on the globe. Water stress is one of the important abiotic stresses, constraining global crop production and quality seriously and recent global climate change and increasingly erratic weather patterns in the future are likely to enhance this situation more seriously. Plant adaptation to water stress is regulated through multiple physiological mechanisms at the cellular, tissue, and whole-plant levels (Hazen *et al.*, 2005; Ito *et al.*, 2006; Yamaguchi-Shinozaki and Shinozaki, 2005). Identification and detailed analysis of a large number of candidate genes involved in water stress tolerance may enable to elucidate the molecular basis. Recent progress in molecular biology and bioinformatics has provided insight into plant gene regulatory network system, which is mainly composed of inducible-genes (environmental factors and developmental cues), their expression programming and regulatory elements (cis-element and trans-element), corresponding biochemical pathways and diverse signal factors.

The aim of our study was to investigate expression pattern of genes that are altered in response to water stress in coconut. Reverse-transcriptase PCR (RT-PCR), using designed degenerate primers, was used to study the expression patterns of the five well-documented genes (*MAPK*, *AP2*, *14-3-3*, *NAC* and *WRKY*) in coconut seedlings subjected to water stress.

A total of 10 pair of primers was designed for the five selected stress induced during water stress based on the conserved domain and optimum *in silico* amplification. The quality of the designed primers was checked with FastPCR. No secondary structure or dimer formation was noticed for the designed primers.

Gene expression analysis was carried out on RNA isolated from leaf samples of control seedlings and seedlings subjected to water stress. All the five genes studied, *viz.* *MAPK*, *AP2*, *WRKY*, *14-3-3* and *NAC*, were up-regulated under the 30-day water deficit stress regime when

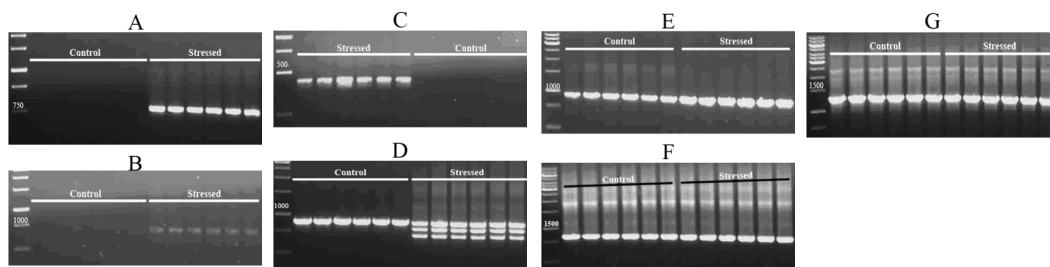


Fig. 1. Amplification of candidate genes for water stress in coconut. A: 14-3-3; B: AP2; C: MAPK; D: NAC; E: WRKY; F: Actin; G: *rbcL*.

compared to control seedlings. The two housekeeping genes, *viz.* *actin* and *rbcL* were amplified in control and treated seedlings in the same intensity (Fig. 1).

Amplicons of expected sizes were eluted, purified and cloned. Ligated and transformed cells were plated onto LB/x-gal/IPTG/ampicillin selective plates followed by the blue white screening. White colonies were transferred onto gridded selective plates. White colonies were screened for presence of insert using colony PCR.

The sequence analysis of candidate genes which are induced during water stress in coconut, detected by RT-PCR, showed significant homology to similar known genes. Analysis

of sequences in BLASTN and BLASTX search revealed an identity of 92-99%. The sequences obtained via RT-PCR amplification were deposited in NCBI.

Transcriptional elements are defined as the protein combining with the specialized DNA sequence of eukaryotic promoters or the protein having structural characteristics of known DNA-combining region, whose main function is to activate or suppress transcriptional effect of corresponding genes. It is a critical factor for the transduction of stress signal. The transcriptional elements would be synthesized under the stress, and it could deliver and amplify the signal to regulate the genes expression to change the physiological function performance.

Numerous studies have shown that MAPK cascades play crucial roles in plant responses to multiple stresses (Zhang and Klessig, 2001). Analyses of complete genome sequences in Arabidopsis and rice have revealed the existence of more than 20 MAPK genes in Arabidopsis (MAPK group, 2002; Asai *et al.*, 2002) and 17 MAPK genes in rice (Reyna and Yang, 2006), suggesting the complexity of MAPK cascades in plants. A large number of MAP kinases, induced in response to abiotic stresses, have been isolated and characterized from different plant species (Yu *et al.*, 2005; Wang *et al.*, 2007; Lizama-Uc *et al.*, 2007; Xu *et al.*, 2008). Several of these have been shown to be involved in the transmission of water stress signals.

AP2/EREBP transcription factors are implicated in plant development and hormone-dependent gene expression. AP2/ EREBP family proteins are unique to plants and share a highly conserved region of about 60 to 70 amino acids (the so-called AP2 domain) with no apparent similarity outside this domain. The AP2 DNA binding module includes two regions: a 20-amino acid long N-terminal stretch rich in basic and hydrophilic residues (YRG element), and a C-terminal sequence capable of forming an amphipathic K-helix (RAYD element). DNA binding is thought to depend on the YRG element, whereas the RAYD element could contribute to this interaction or mediate protein-protein associations. Several lines of evidence support induction of AP2 transcription factors after initiation of stress (Dietz *et al.*, 2010).

The WRKY class of zinc-finger proteins, similar to the ethylene-responsive-element binding factors (ERF), DNA binding one finger and basic domain leucine zipper families, is a family of proteins that contains many members that mediate plant stress responses (Eulgem *et al.*, 2000; Ulker and Somssich, 2004).

14-3-3 proteins function as regulators of a wide range of target proteins in all eukaryotes by effecting direct protein-protein interactions. Primarily, interactions between 14-3-3 proteins and their targets are mediated by phosphorylation at specific sites on the target protein. Hence, interactions with 14-3-3s are subject to environmental control through signalling pathways which impact on 14-3-3 binding sites. Because 14-3-3 proteins regulate the activities of many proteins involved in signal transduction, there are multiple levels at which 14-3-3 proteins may play roles in stress responses in higher plants.

NAC transcription factors have been shown to regulate a number of biological processes, including those which protect plants under water stress conditions (Olsen *et al.*, 2005; Demura *et al.*, 2010).

Our knowledge of the molecular mechanisms underlying the responses of coconut to environmental stresses such as drought is still rather limited, but an increasing number of genes have been identified in recent years that mediate those responses. Some of these genes

are induced by stress stimuli and encode products that confer tolerance to adverse conditions, whereas others encode upstream regulators that function within signalling pathways controlling the stress response. The challenge now is to understand the relative roles of various proteins in different pathways, their coordinated regulation and the interactions with other signalling elements. As we learn more about water stress induced genes and their regulation, it may be possible to design additional strategies that target specific aspects of plant tolerance to stress.

Acknowledgement: This work was supported by a grant from Department of Biotechnology, New Delhi, India.

References

- Altschul, S.F., Gish, W., Miller, W., Myers, E. W. and Lipman, D.J. 1990. Basic local alignment search tool. *J. Mol. Biol.* **215**: 403-410.
- Amudha, J and Balasubramani, G. 2011. Recent molecular advances to combat abiotic stress tolerance in crop plants. *Biotechnol. Mol. Biol. Rev.* **6**(2): 31-58.
- Asai, T., Tena, G., Plotnikova, J., Willmann, M.R., Chiu, W.L., Gomez-Gomez, L., Boller, T., Ausubel, F.M. and Sheen, J. 2002. MAP kinase signalling cascade in Arabidopsis innate immunity. *Nature* **415**: 977-983.
- Batugal, P.A. 1999. The role of international cooperation in the development of biotechnology in coconut. Pp.19-30. In: *Current Advances in Coconut Biotechnology*. (Eds.) Oropeza, C., Verdeil, J.L., Ashburner, G.R., Cardena, R. and Santamaria, J. M. Kluwer Academic Pub., The Netherlands.
- Demura, T. and Ye, Z.H. 2010, Regulation of plant biomass production. *Curr. Opin. Plant Biol.* **13**: 299–304.
- Dietz, K.J., Vogel, M. O. and Viehhauser, A. 2010. *AP2/EREBP* transcription factors are part of gene regulatory networks and integrate metabolic, hormonal and environmental signals in stress acclimation and retrograde signalling. *Protoplasma* **245**:3-14.
- Eulgem, T., Rushton, P.J., Robatzek, S. and Somssich, I.E. 2000. The WRKY superfamily of plant transcription factors. *Trends Plant Sci.* **5**:199-206.
- Hazen, S.P., Pathan, M.S., Sanchez, A., Baxter, I., Dunn, M., Estes, B., Chang, H.S., Zhu, T., Kreps, J.A. and Nguyen, H.T. 2005. Expression profiling of rice segregating for drought tolerance QTLs using a rice genome array. *Funct. Integr. Genom.* **5**:104-116.
- Hussain, S.S., Kayani, M.A and Amjad, M. 2011. Transcription Factors as Tools to Engineer Enhanced Drought Stress Tolerance in Plants. *Biotechnol. Prog.* **27**(2): 297-306.
- Ito, Y., Katsura, K., Maruyama, K., Taji, T., Kobayashi, M., Seki, M., Shinozaki, K., Yamaguchi-Shinozaki, K. 2006. Functional analysis of rice DREB1/CBF-type transcription factors involved in coldresponsive gene expression in transgenic rice. *Plant and Cell Physiol.* **47**: 141-153.
- Jones, L.H. 1991. Perennial vegetable oil crops. pp. 213–224. In: *Agricultural Biotechnology: Opportunities for International Development*. (Eds.) Persley, G.J. CAB International, Wallingford, UK.
- Kasturi Bai, K.V., Reddy, D.V.S., Ratnambal, M.J. and Laxman, R.H. 2003. Factors contributing to female flower production and button shedding in coconut (*Cocos nucifera* L.). *J. Plantn. Crops* **31**(2): 33-36.

- Kasturi Bai, K.V., Rajagopal, V., Chempakam, B. and Prabha, C.D. 1996. Assay of enzymes in coconut cultivars and hybrids under non-stress and stress conditions. *J. Plantn. Crops*. **24** (Supplement): 548-554.
- Kasturibai, K.V., Voleti, S.R. and Rajagopal, V. 1988. Water relations of coconut palms as influenced by environmental variables. *Agric. For. Meteorol.* **1** (43): 193-199.
- Kumar, S., Tamura, K., Nei, M. 2004. Mega 3: integrated software for molecular evolutionary genetics analysis and sequence alignment. *Brief. Bioinform.* **5**: 150-63.
- Lizama-Uc, G., Estrada-Mota, I.A., Caamal-Chan, M.G., Souza-Perera, R., Oropeza-Salin, C., Islas-Flores, I. and Zúñiga-Aguilar, J.J. 2007. Chitosan activates a MAP-kinase pathway and modifies abundance of defense-related transcripts in calli of *Cocos nucifera* L. *Physiol. Mol. Plant Pathol.* **70**: 130-41.
- MAPK Group. 2002. Mitogen-activated protein kinase cascades in plants: a new nomenclature. *Trends Plant Sci.* **7**: 301-308.
- Olsen, A.N., Ernst, H.A., Leggio, L.L. and Skriver, K. 2005, NAC transcription factors: structurally distinct, functionally diverse. *Trends Plant Sci.* **10**: 79-87.
- Rajagopal, V., Shivashankar, S., Mathew, J. 1996. Impact of dry spells on the ontogeny of coconut fruits and its relation to yield. *Plant. Rech. Dévelop.* **3**: 251-255.
- Reyna, N.S. and Yang, Y.N. 2006. Molecular analysis of the rice MAP kinase gene family in relation to *Magnaporthe grisea* infection. *Mol. Plant Microbe Interact.* **19**: 530-540.
- Shivashankar, S., Bai, K.V.K. and Rajagopal, V. 1991. Leaf water potential, stomatal resistance and activity of enzymes during the development of moisture stress in coconut palm. *Trop. Agric.* **68** (2): 106-110.
- Siju, T.T., Kumar, S. N., Cherian, V.K., Bai, K.V.K. and Rajagopal, V. 2006. Role of certain biochemical compounds in adaptation of coconut to different weather conditions- A study in two agro-climatic regions of India. *Indian J. Hort.* **63** (1): 1-7.
- Thompson, D., Higgins, D.G. and Gibson, T.J. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucl. Acids Res.* **22**: 4673-4680
- Ulker, B. and Somssich, I.E. 2004. WRKY transcription factors: From DNA binding towards biological function. *Curr. Opin. Plant Biol.* **7**:491-498.
- Wang, H., Ngwenyama, N., Liu, Y., Walker, J.C. and Zhang, S. 2007. Stomatal Development and Patterning Are Regulated by Environmentally Responsive Mitogen-Activated Protein **Kinases** in *Arabidopsis*. *The Plant Cell.* **19**: 63-73.
- Xu, H., Wang, X., Sun, X., Shi, Q., Yang, F. and Du, D. 2008. Molecular cloning and characterization of a cucumber MAP kinase gene in response to excess NO₃ and other abiotic stresses. *Scient. Hort.* **117**: 1-8.
- Yamaguchi-Shinozaki, K. and Shinozaki, K. 2005. Organization of cisacting regulatory elements in osmotic- and cold-stress-responsive promoters. *Trends Plant Sci.* **10**: 88-94.
- Yu, S.W., Zhang, L.D., Zuo, K.J., Tang, D.Q. and Tang, K.X. 2005. Isolation and characterization of an oilseed rape MAP kinase BnMPK3 involved in diverse environmental stresses. *Plant Sci.* **169**: 413-421.
- Zhang, S. and Klessig, D.F. 2001. MAPK cascades in plant defense signaling. *Trends Plant Sci.* **6**: 520-527.

Differential expression of genes regulated in response to water stress in coconut

Bobby Paul, Rajesh M.K., Kiran A.G., George V.Thomas and Naresh Kumar S

Central Plantation Crops Research Institute
Kasaragod, 671124, Kerala, India

Water stress is one of the most important abiotic stresses. It decreases the rate of photosynthesis and limits the productivity of crops worldwide, reducing yield up to 50% (Bray *et al.*, 2000). Water stress is a severe problem the effects of which exacerbated by global climate change. When plants are exposed to water stress, they respond through various cellular signal transduction pathways, which result in accumulation of certain differentially expressed gene products (Beck *et al.*, 2007). A numbers of genes that respond to water stress have recently been described (Amudha and Balasubramani, 2011; Hussain *et al.*, 2011). These genes are induced in response to water stress and the products of these genes function in response to stress and tolerance (Bray *et al.*, 2000; Shinozaki and Yamaguchi-Shinozaki, 2000). Transcriptome studies have helped to provide a better understanding of plant stress responses. Through these studies, numerous novel stress-responsive genes have been discovered. Overlaps of genes induced by various stress conditions suggest extensive cross-talk between the signaling pathways.

The photosynthetic rates, dry matter production and its partitioning are influenced in coconut palm by the soil water status (Naresh Kumar *et al.*, 2002). Various factors at the level of morphological, anatomical, physiological and biochemical level in coconut have been attributed for the water stress tolerance (Rajagopal *et al.*, 1990; Naresh Kumar *et al.*, 2003). Genetic determinants governing abiotic stress resistance have been reported in diverse plants (Kim *et al.*, 2000; Mahalingam *et al.*, 2003; Bray, 2004; Ameline-Torregrosa *et al.*, 2006) and detailed studies on gene expression patterns in relation to water stress have been conducted in number of other crops *viz.* rice, barley, sunflower and citrus (Ouvrard *et al.*, 1996; Sanchez-Ballesta *et al.*, 2004), but no such studies have been carried out so far in coconut. To identify and isolate cDNAs representing transcripts differentially expressed during water stress in coconut, *in vitro* grown coconut plantlets were treated with PEG 6000 to create stress conditions. We have then applied mRNA differential display technique to the comparison of PEG 6000-treated and untreated plantlets. Identification of water stress-related transcripts will enable a more in-depth understanding of the physiological events that occur during water stress conditions in coconut.

Embryo culture of *West Coast Tall* cultivar was initiated. The basal medium used for retrieval of the coconut embryos was Y3 medium supplemented with 30 g/L of sucrose. The embryos were sub-cultured to the same basal medium at monthly intervals. On germination of the embryos, 0.5 mg/L BAP and 0.5 mg/L NAA were supplemented in the medium for proper root and shoot development. When the plantlet reached the 1-2 leaflet stage, they were subcultured into Y3 liquid medium containing 2-4 mg/L NAA and 2 mg/L BAP.

The plantlets were subcultured into the same medium once every 40 days. Fully grown *in vitro* cultured WCT plantlets were subcultured into Y3 basal medium containing two levels of poly ethylene glycol (PEG, 6000 MW) *viz.* 20% and 30% w/v to induce water stress.

Leaf samples (approximately 100 mg) from two plantlets each were collected after 5 days and 10 days of PEG treatment (20% and 30%) and also control plantlets (without PEG) and total RNA was isolated via RNeasy Plant Mini Kit (QIAGEN). On column, DNase I (Fermentas) treatment was given to remove any DNA contamination from isolated RNA. Reaction was incubated at 37°C for 30 minutes followed by incubation at 65°C for 15 minutes to denature DNase I. After isolation, to check the integrity of RNA, the samples were run on a 1.5% agarose-formaldehyde gel. The concentration of total RNA in each sample was determined spectrophotometrically at 260 nm.

The RNA, isolated from control, 20% PEG treated and 30% PEG treated plantlets were subjected to three separate reverse transcription experiments, each with one of the three one-base-anchored oligo (dT) primers viz., H-T₁₁G, H-T₁₁A or H-T₁₁C. The 20 ml reaction volume contained 2 µL of total RNA, 1x RT buffer, 20 mM dNTPs, 1 U RNase inhibitor (Fermentas) and 0.2 µM one-base-anchored oligo-dT primer. The mixture was subjected to one cycle of PCR under the following conditions: 65°C for 5 minutes; 37°C for 60 minutes; 75°C for 5 minutes. The M-MuLV reverse transcriptase (Fermentas) 0.5 Unit was added 10 minutes after the reaction mixture reached 37°C.

The gene expression profile during water stress in coconut was carried out by differential display analysis according to Liang and Pardee (1992) with the modifications (Kavitha and Thomas, 2008). Differential display polymerized chain reaction was performed on the reverse transcribed products (first strand synthesized) using the corresponding oligo (dT) primers and random primers. The 20 mL reaction mixture contained 2 mL reverse transcription product, 1X PCR buffer, 20 mM dNTPs, 0.2 µM of one of the anchor primers, 0.2 µM of one of the random primers, 0.2 mL of 2000 Ci/mmol [γ-³²P] dATP and 0.05 Unit *Taq* DNA polymerase (Qiagen). The PCR was performed in Mastercycler[®] pro Thermal Cyclers (Eppendorf) and the conditions were set were as follows: 94°C for 30 seconds; 42°C for 60 seconds; 72°C for 60 seconds for 35 cycles followed by 72°C for 5 min.

The differential display analysis was carried out using three anchor primers HT₁₁G, HT₁₁C and HT11A which were used in first strand synthesis and made 75 combinations with seven 13-mer arbitrary primers from the RNAimage kit (HAP 01 - HAP 07; GenHunter) and 24 random decamer primers from kit B (OPB1-5, 9-10, 13-16 and 18) and kit F (OPF 1-12) (Operon technologies, Alameda, CA).

The PCR products were fractionated on a 6% polyacrylamide gel at 50 W for 2.5 hours. The gel was dried under vacuum, and was autoradiographed initially using Phosphor Imager (BioRad) to test the success of the experiment and subsequently by exposing an X-ray film over the gel in an X-ray cassette for approximately 72 hours at -70°C. While exposing, the gel and film were stapled together and a couple of punches were given at the edges, using a paper puncher to facilitate correct aligning of gel and the film for band excision. In addition, a cut was made at the right hand top corner using scissors. The X-ray film was developed and the autoradiogram was aligned to the gel and the differential bands were eluted.

The autoradiogram and the gel were aligned with the help of punch marks and the cut made at the right hand top corner and were stapled together. A couple of pricks were given to the autoradiogram at selected band positions so as to reach the pricks at the corresponding positions on the gel. The selected bands were excised using a sterile razor. The

gel pieces were transferred to sterile microfuge tubes and were re-swelled in 100 mL water for 10 minutes at room temperature. The swollen gel pieces were boiled for 15 minutes with the caps of the tube tightly closed and was centrifuged at 10000 g for 2 minutes (Rota 4R-V/F_M, Plasto Crafts) to collect the gel and paper debris. The supernatant was transferred to a new microfuge tube and the DNA was precipitated with double the volume of absolute alcohol in the presence of 60 mM sodium acetate and 100 mg/mL glycogen for 30 minutes at -70°C. The DNA was pelleted and rinsed with 85% ethanol and dissolved in 10 mL DEPC treated water. The cDNA fragments eluted from the gel were re-amplified following the same PCR conditions, which were used for the amplification of the reverse transcribed product.

Re-amplification PCR was performed on the differentially expressed fragments using the corresponding oligo-dT primers and random primers. The 20 mL reaction mix contained 2 mL eluted fragments, 2 mL of 10x PCR buffer, 2 mM dNTPs, 10 pM of one of the anchor primers, 10 pM of one of the random primers 1 Unit Taq DNA Polymerase (Qiagen). The PCR conditions were same as differential display PCR performed.

PCR products were eluted from the gel using gel extraction kit supplied by Qiagen as per the manufactures instructions. Cloning of the eluted fragments was carried out using the InsT/A clone™ PCR Product Cloning Kit (M/s MBI Fermentas Inc.) as per the manufacturer's instructions. After transformation, the cells were finally plated on pre-warmed LB- agar plates [ampicillin (100 ppm) / IPTG (100 ppm) / X-gal (160 ppm)] and incubated overnight at 37°C. The recombinant clones were identified by blue/white colony selection and plasmids were isolated. The recombinant plasmids were sent for sequencing.

The vector contamination from the cloned sequence was removed with the help of VECSCREEN facility found at NCBI. Poly A tail was removed by using EST trimmer software. Homology searches for emerged sequence were performed with local alignment search tool tBLASTX at the National Center for Biotechnology Information (NCBI). Sequences generated through DDRT-PCR analysis were submitted in dbEST, an EST database found at NCBI.

Total RNA isolated from the control and two level PEG treated embryo cultured plantlets resulted in RNA of high quality. RNA examined by electrophoresis on 1.5% agarose-formaldehyde gel showed a 28S rRNA band equal to or more abundant than the 18S rRNA band, indicating that little or no RNA degradation occurred during the extraction (Fig.1). RNA yields from two control plantlets and two lag phases (5 days and 10 days) and two concentration of PEG (20% and 30%) gave an average A_{260}/A_{280} ratio of 2.0414 . The quantification of RNA by spectrophotometer readings revealed that the isolated RNA was intact for further downstream applications.

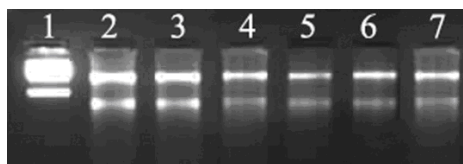


Fig.1. Total RNA isolation, lane 1: 1Kb DNA ladder, lanes 2 and 3: control (without PEG), lanes 4 and 5: 20% PEG treatment and lanes 6 and 7: 30%PEG treatment

Coconut cDNA was generated by three anchor primers HT11A, HT11C and HT11G by reverse transcription of isolated RNA utilizing MMLV-reverse transcriptase.

Differential display profiles of coconut plantlets grown under water stressed and unstressed conditions were generated by 75 primer combinations in six RNA samples, two from the unstressed two from 20% PEG treated (for five days and 10 days) and two from 30% PEG (for five days and 10 days).

Examination of the cDNA molecules produced from mRNA isolated from unstressed and stressed coconut seedlings showed a number of differences in gene expression. For the selection of differentially expressed, upregulated or downregulated fragments, the amplicons expressed during induced water stress were compared with amplicons expression in unstressed plantlets. Amplicons with above 150 bp length were only selected for comparative amplicon expression in unstressed and water stressed conditions in coconut plantlets. Hundred and twenty nine differential amplicon changes were observed in comparative study of unstressed and PEG induced water stressed plantlets and was finally processed from the initial screen. A total of 15 6% poly acrylamide gel was performed for separating 75 combinations of differentially display PCR products (Fig.2). The amplified fragments were eluted in final volume of 10 μ L sterile water and stored in -20°C for future re-amplification procedure.

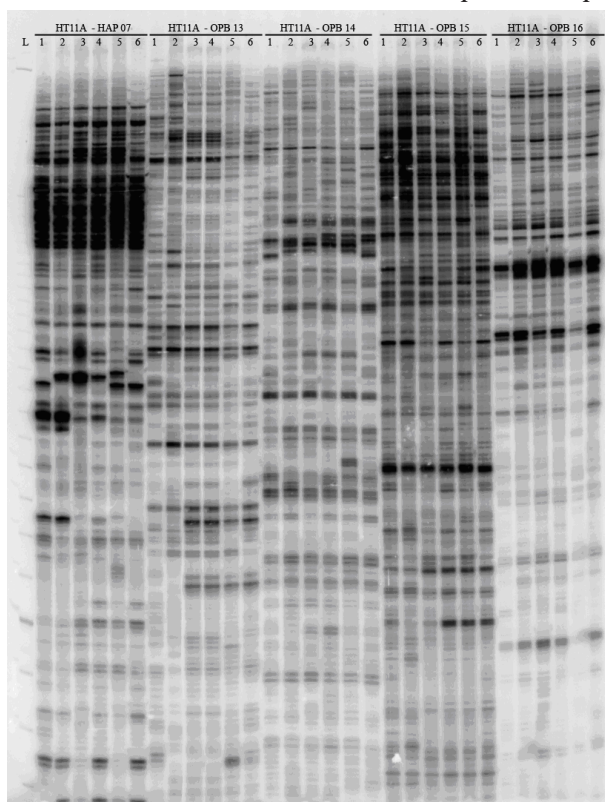


Fig.2. Polyacrylamide gel analysis of DDRT-PCR performed in coconut after water stress induction.

L: ladder; Lanes 1 and 2: control; Lane 3: 20% PEG treated (5 days); Lane 4: 20% PEG treated (10 days); Lane 5: 30% PEG treated (5 days);

Lane 6: 30% PEG treated (10 days)

Re-amplification profile of the eluted fragments showed that only 114 fragments were only reamplified. The differentially expressed fragments in low intensity (faint bands) were failed in re-amplification procedure. The reamplified products were checked in 1.2% agarose gel showed good in quality and quantity (Fig.3).

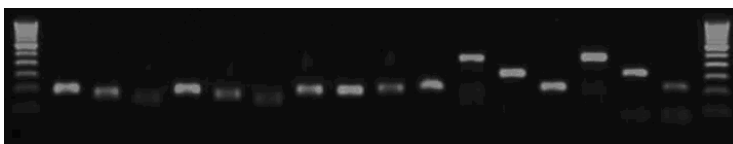


Fig. 3. Re-amplification of differentially expressed fragments

Ligated and transformed cells were plated onto LB/x-gal/IPTG/ampicillin selective plates followed by the blue white screening. White colonies were transferred onto gridded selective plates. A total of two hundred and twenty white colonies were screened using colony PCR.

The results obtained from the sequence comparison of the differentially expressed fragments in BLAST database revealed that several transcripts were significantly homologous to stress regulated genes/proteins isolated from other plant species. The highly similar sequence representing genes were selected and grouped based on their function. A total of 114 differentially expressed fragments were cloned and sequenced obtained were deposited in dbEST database. Low-quality sequence regions were trimmed and sequences less than 100 bp in length were excluded. The results obtained from the sequence comparison of the differentially expressed fragments in BLAST database revealed that several transcripts were significantly homologous to stress regulated genes/proteins isolated from other plant species. The highly similar sequence representing genes were selected and grouped into 11 groups based on their function and sub- cellular location. The hierarchies of the grouped transcripts are as follows: abiotic stress regulating genes (22%), enzymes from metabolic pathways (20%), DNA binding domains (11%), housekeeping gens (8%), protein family (7%), hypothetical proteins (7%), transcription factors (6%), contings (5%), chromosomal segments (5%), other clones (4%) and no similarity (4%).

Water availability is one of the principal physical factors which limit the primary productivity of plants. Water stress is one of the most critical environmental stresses reducing coconut yield in rain-fed areas and this concern has magnified the importance of research into response of coconut to water stress. In this regard, the search for genes and that can serve as both markers with which to better estimate the severity of water stress that the palms experience and as targets for breeding efforts to increase drought tolerance in coconut assumes significant importance.

Genomic approaches to analyzing the genetic responses occurring during water stress are providing information that will allow faster progress in this area. A host of water-stress

responsive genes have been identified in a variety of plant species (Amuda and Balasubramani, 2011; Hussain *et al.*, 2011). To obtain insight into the response of coconut palms to water stress, *in vitro* coconut plantlets were subject to progressively induced water stress by supplementation of PEG in the culture medium and the gene expression patterns were studied using DDRT-PCR.

Several methods, which range from withdrawal of water to plants to the use of chemicals such as polyethylene glycol, mannitol *etc.* have been employed to create water stress in plants. It has been reasonably well established that polyethylene glycol induced water stress mimics that caused by withdrawal of water from plants. Plants expose their root system to this solution and no other toxicities were observed at plant level following the addition of PEG-6000 (Emmerich and Hardegree, 1990).

These results demonstrate that the classical technique of differential display is able to identify a significant number of changes in gene expression in the coconut seedlings during the stages unstressed and PEG induced water stressed condition. One hundred and fourteen changes were finally processed from our scrutinized screening. The primer combinations used for PCR amplification would have amplified only around one third of the potential sequences. The amplicons for which we detected significant changes in gene expression showed significant sequence homology with known proteins or with functional motifs in other plant species.

Functional categories showed that the genes represented by those cDNAs had a wide range of roles in different cell activity, which indicated that maize seedlings responded to water stress in a global level. The largest set of genes was assigned to the series of metabolism category. Changes in primary metabolism are a general response to stress in plants (Ingram and Bartels, 1996). On the other hand, the precise functions for most of the genes in response to water stress remain unknown, which need detailed analysis for each of the genes.

This is the first report of identification transcriptional changes occurring in coconut in response to induced water stress. Using mRNA differential display analysis, we were able to identify cDNA clones with close homology to genes involved at different stages of abiotic stress tolerance in other plant species, including those which could be involved in water stress signaling pathway. The genes identified in responsive to water stress, are therefore good candidates for adaptive genes that might provide useful targets for breeding. It is important to get their corresponding full-length genes and analyze their precise functions during water stress of coconut palms. Information of such genes could contribute to bring us closer to our goal of understanding the molecular mechanism of water stress tolerance.

Acknowledgement

This work was supported by a grant from Department of Biotechnology, New Delhi, India.

References

- Ameline-Torregrosa, C., Dumas, B., Krajinski, F., Esquerre-Tugaye, M.T. and Jacquet, C. 2006. Transcriptomic approaches to unravel plant–pathogen interactions in legumes; *Euphytica* **147**: 25–36.
- Amudha, J and Balasubramani, G. 2011. Recent molecular advances to combat abiotic stress tolerance in crop plants. *Biotechnol. Mol. Biol. Rev.* **6**(2): 31-58.

- Bray, E.A. 2004. Genes commonly regulated by water-decit stress in *Arabidopsis thaliana*. *J. Exp. Bot.* **55**: 2331–2341.
- Bray, E.A. J., Bailey, S.E., Weretilnyk, E. 2000. Responses to abiotic stresses. pp1158-1176. In: *Biochemistry and Molecular Biology of Plants*. (Eds.) Buchanan, W., Gruissem, R., Jones. American Society of Plant Physiologists.
- Emmerich, W.E. and Hardegree., S.P. 1990. Polyethylene glycol solution contact effects on seed germination. *Agronomy Journal* **82**: 1103-7.
- Hussain, S.S., Kayani, M.A and Amjad, M. 2011. Transcription Factors as Tools to Engineer Enhanced Drought Stress Tolerance in Plants. *Biotechnol. Prog.* **27**(2): 297-306.
- Kavitha, P.G. and Thomas, G. 2008. Defence transcriptome profiling of *Zingiber zerumbet* (L.) Smith by mRNA differential display. *J. Biosci.* **33**(1): 81-90.
- Kim, C.Y., Lee, S.H., Park, H.C., Bae, C.G., Cheong, Y.H., Choi, Y.J., Han, C.D., Lee, S.Y, Lim, C.O. and Cho, M.J. 2000. Identification of rice blast fungal elicitor-responsive genes by differential display analysis. *Mol. Plant Microbe Interact.* **13**: 470–474.
- Liang, P. and Pardee, A.B. 1992. Differential display of eukaryotic messenger RNA by means of the polymerase chain reaction. *Science* **257**: 967–971.
- Mahalingam, R., Gomez-Buitrago, A., Eckardt, N., Shah, N., Guevara-Garcia, A., Day, P., Raina, R. and Fedoroff, N.V. 2003. Characterizing the stress/defense transcriptome of *Arabidopsis*. *Genome Biol.* **4**: R20.
- Naresh Kumar, S., Rajagopal, V., Laxman, R. H., Dhanapal, R.. and Maheswarappa, H.P. 2002. Photosynthetic characteristics and water relations in coconut palm under drip irrigation on sandy and laterite soils. Pp. 116-120. In: *Plantation Crops Research and development in the new millennium*. (Eds.) Rethinam, P., Khan, H.H., Reddy, V.M., Mandal, P.K. and Suresh, K. Coconut Development Board, Pub, Kochi.
- Naresh Kumar, S., Rajagopal, V., Siju, T.T., Vinu, K.C. 2003. Influence of soil moisture conservation on coconut (*Cocos nucifera* L.) under different agroclimatic conditions. Pp. 572. In: *Proceedings of the 2nd International Congress on Plant Physiology*. IARI, New Delhi, India.
- Ouvrard, O., Cellier, F., Ferrare, K., Tousch, D., Lamaze, T., Dupuis, J.M., Casse-Delbart, F. 1996. Identification and expression of water stress- and abscisic acid-regulated genes in a droughttolerant sunflower genotype. *Plant Mol. Biol.* **31**: 819–829.
- Rajagopal, V., Kasturi Bai, K.V. and Voleti, S.R. 1990. Screening of coconut genotypes for drought tolerance. *Oleagineux* **45** (5): 215-223.
- Sanchez-Ballesta, M.T., Rodrigo, M.J., Lafuente, M.T., Granell, A. and Zacarias, L. 2004. Dehydrin from *citrus*, which confers in vitro dehydration and freezing protection activity, is constitutive and highly expressed in the flavedo of fruit but responsive to cold and water stress in leaves. *J. Agric. Food Chem.* **52**: 1950-1957.
- Shinozaki, K. and Yamaguchi-Shinozaki, K. 2000. Molecular responses to dehydration and low temperature: differences and cross-talk between two stress signaling pathways. *Curr. Opin. Plant Biol.* **3**: 217-223.

Improved production technologies for higher productivity

R. Dhanapal, P. Subramanian, H. P. Maheswarappa and P. Geetha

Central Plantation Crops Research Institute

Kasaragod - 671124, Kerala, India

The Coconut Palm (*Cocos nucifera* Linn.) is supposed to be one of the five legendary Devavrikshas and is eulogized as Kalpavriksha - the all giving tree - in Indian classics. All parts of the palm are used in some way or other in the daily life of the people of the west coast; the traditional coconut growing area. Its fruit is called Lakshmi Phal and is used in social and religious functions in India irrespective of whether palm is locally grown or not.

India is one of the major coconut producing countries ranking second in production of coconut, holding a share of 26.65 per cent of the world production followed by Indonesia which accounts for 26.95 per cent share in the production. Coconut is an important crop of economic importance in India especially for States viz., Kerala, Tamil Nadu, Karnataka, Goa, Maharashtra, Andhra Pradesh and to a some extent other states like Orissa and West Bengal. It contributes 6 per cent to the national vegetable oil pool and about 10 million families depend on it for their livelihood. Unlike other crops coconut produce inflorescence throughout the year, thus availability of nutrient and moisture to coconut is very important throughout its life period for improved production.

In India, most of the acreage under coconut palm (90%) lies in the four southern states i.e., Kerala, Tamil Nadu, Karnataka, and Andhra Pradesh. Kerala ranks first in area (53.76%) and production (45%) followed by TN, Karnataka and Andhra Pradesh. The area, production and productivity of coconut in different states of India are given in Table 1.

Table 1. State wise area, production and productivity of coconut in India (2008-09)

States/Union Territories	Area ('000 Hectares)	Production (Million nuts)	Productivity (Nuts/ha)
Andhra Pradesh	104.00	970.00	9327
Assam	18.80	147.10	7824
Goa	25.61	128.18	5005
Gujarat	15.98	157.42	9851
Karnataka	419.00	2176.00	5193
Kerala	787.77	5802.00	7365
Maharashtra	21.00	175.10	8338
Nagaland	0.92	0.55	598
Orissa	51.00	275.80	5408
Tamil Nadu	389.60	5365.00	13771
Tripura	5.80	11.40	1966
West Bengal	28.60	355.50	12430

A & N Islands	21.69	82.00	3781
Lakshadweep	2.70	53.00	19630
Pondicherry	2.10	30.70	14619
All India	1894.57	15729.75	8303

Source: Directorate of Economics & Statistics, Ministry of Agriculture, Govt. of India.

From the table it is evident that 64 % of the coconut growing areas come under Kerala and Karnataka. However, the coconut productivity of these two states is lower than that of national average. Similarly, the other part of coconut growing region of India the productivity is not promising except the states like Tamil Nadu, West Bengal Lakshadweep and Pondicherry. Even the coconut productivity of these states are also lower, compared to research station yield (30625 nuts/ha/year). The low coconut productivity is mainly due to inadequate irrigation, manuring, and presence of senile and unproductive palms, low genetic potential of native palms, pests and diseases, and cultivation in marginal and sub-marginal lands. Added to this, the price fluctuation in coconut and labour availability for farm operations aggravates the conditions of the coconut farmer. Planting high yielding varieties and adopting modern production technologies can increase the productivity. Therefore to bring about sustainability in coconut farming, increase the productivity, crop intensification and crop and product diversification are important factors.

The coconut is essentially a crop of the lowland humid tropics, with 90% of production coming from the zone between 20° N and 20° 'S latitude. It has been successfully grown up to the elevation of over 1,000 m near the equator. The required average temperature is in the range 27°-32°C with a diurnal variation of not more than 7°C. Temperature less than 15°C results in abnormalities of the fruits. Annual rainfall requirements range between 1,000 mm and 2500 mm and sunshine is required preferably in excess of 2,000 hours, with 120 hr of sunshine per month. Ideal relative humidity is 80 to 90% and R.H below 60% results in stomatal closure that restricts transpiration. While humidity level close to saturation pre disposes the palm to a number of diseases.

Coconuts are grown under diverse soil conditions ranging from littoral sands to clayey soils, ill-drained low lying areas to well drained hill slopes, strongly acidic peaty soils to alkaline calcareous soils. The major coconut growing soils along the west coast are laterites, littoral sands and red sandy loams, while deltaic alluvium, red soils and littoral sand are important coconut soils along the east coast. Among these soils, the best soil is deltaic alluvial followed by red sandy loam. In Kerala where 50 % of the area of coconut is there, laterite is major soil group (65 %) in which coconut is grown. Laterites are in general poor in available nitrogen, phosphorus and potassium and are low in the bases. They have poor water-holding capacity, CEC and high P fixing capacity with low organic matter content. They are generally acidic with pH ranging from 4.5 to 6.2. Any soil if it is deep upto 1.0-1.5 meter, well drained, rich in organic matter that has good fertility and water holding capacity with a pH range of 5.0 to 8.5 (ideal pH is 5.5-7.5), for growing coconut.

Healthy and good quality planting material is the foundation of successful crop production. In coconut, seedling vigour is highly correlated with adult palm characters such

as early flowering, nut yield and copra production. If the seed nuts happen to be of poor quality, the new plantation will prove to be uneconomic, causing considerable loss of time and money to the grower. Coconut being a perennial crop, the performance of the new progeny can be judged only several years after plant-ing and continue as source of loss throughout its life period. Further, being a cross-fertilized, palm does not breed true which makes the selection of seed nuts and seedlings more important and difficult. Through a series of selections made at different stages, it is possible to obtain quality seed nuts and seedlings.

Seed gardens should have palms with a high proportion of heavy bearers. Garden should be free from the incidence of diseases and not prone to severe attacks of pests.

Palms should be regular bearers with annual yield of greater than 80 nuts and copra content not less than 150g/nut under rain fed condition (under irrigated conditions-120 nuts/year). Palms should have reached full bearing stages and have been giving consistently high yields for at least four years. Avoid very old palms of above 60 years age. Palms which produce barren nuts or those shedding large number of immature nuts should be discarded.

Generally the seed nuts are collected from January to May in the West Coast region when there is more nut and the seed nuts will have better germination. However, there is no harm in collection of seed nuts in other months where there is peak coconut yield in other parts of the region (Nelliat *et al.*, 1976). Only fully matured nuts i.e. about 12 months old should be harvested. Care should be taken not to damage the seed nuts while harvesting. Discard nuts which are too big or too small in the bunch and also the nuts of irregular shape and size.

Select well-drained, coarse-textured soil near dependable water source for irrigation. The seed nuts can be sown in flat beds if there is no drainage problem. The seeds are to be sown in raised beds if water stagnation is a problem during rainy season. The soil may be treated with chlordane 5% dust @ 120 kg/ha, or drenched with Chlorpyrifos in places where nursery is being raised for the first time as a precaution against white grubs and termites. Nursery can be raised either in the open with artificial shade or in gardens where the palms are tall and the ground is not completely shaded. The seed nuts should be planted in long and narrow beds at a spacing of 40 x 30cm during May-June, either vertically or horizontally in 20-25cm deep trenches. Advantage in vertical planting is less damage during transit. However, in delayed planting when the nut water goes down considerably it is good to go for horizontal sowing for better germination.

More vigorous seedlings can be obtained by this method. For this germinated seeds are transplanted in poly bags of size 60 x 40 cm with 8-10 holes at the bottom. The potting mixture is in the 2:1:1 ratio of topsoil, sand and compost mixture. The advantage of poly bag seedlings is that there is no transplanting shock and the seedlings are with better vigour. But the disadvantage is in transportation and increase the cost of the seedlings (Reddy *et al.*, 1996).

Mulching and shading should be done immediately after the monsoon ends, when the nursery is raised in the open space. Keep nursery free of weeds. Regular surveillance for any incidence of pests and disease should be done. Remove those nuts that have not sprouted even after five months of sowing.

Nursery requires light and frequent irrigation (once in 2 to 4 days). About 10 mm of water should be applied for every irrigation. In porous soils like sandy and laterite soil the frequency of irrigation should be more.

Every coconut nursery contains some plants which are deformed or whose development is stunted, which must be eliminated. Select seedlings, which have germinated early. From the one- year- old nursery, select vigorous seedlings having six to eight leaves and girth of 10cm at the collar. Early splitting of leaves is a good indicator of the rapid developers and early bearing. The recovery of good seedlings will be 60 to 65% of total seed nuts sown in spite of garden selection, palm selection and seed nut selection.

Most of the existing coconut plantations are planted in the square system at spacing of 7.5 m x 7.5 m. Adoption of hedge (rectangular) system of planting coconut with wider row spacing and rows oriented in east-west direction would increase light availability to intercrops (e.g. 9.0 m x 6.5 m = 170 palms/ha). This would facilitate growing annuals and perennials right from the time of planting coconuts. Triangular system of planting (equilateral triangle) accommodates 30 palms more than square system of planting; however, it interferes with the cultural operations and growing of intercrops.

The size of the pit for planting of seedlings is 1 m x 1m x 1m in normal soils. However, in soils with rocky or hard substrate: large pits (1.2 to 1.5 m³) are preferred. In coastal sandy soils the size of the pit is 0.75m x 0.75m x 0.75 m. After opening the pit, fill up the pit with top - soil to a height of 60 cm below ground level and plant seedlings inside the pit. If FYM or any compost available that can be mixed with top - soil and fill the pit to a height of 60 cm. After planting press the soil well so as to avoid water stagnation. In water logged soil mounds are made and seedlings are planted in the centre of the mound.

Planting is generally done during the beginning of south west monsoon season or north east monsoon season. In areas liable to water logging, planting can be taken up towards the end of monsoon.

Replanting or under planting becomes necessary when the yield become very low due to old age, long-term neglect and continuous exposure to adverse conditions. Generally under planting is practiced where old palms are removed in stages over a period of three to four years.

Mark the area to be planted. Remove very poor yielder and those very close to the planting pits. Plant the seedlings in the usual way. The other trees are removed @ one-third each year during 2nd, 3rd and 4th year after planting. If the existing garden is irregularly spaced, remove old palms within 1 m radial distance in first year, 2 m distance in second year, 3 m distance in third year and the rest in fourth year. Generally flowering is delayed a little in under planted palms.

Mulching should be done at the base with coconut husks, dry leaves, etc. Husk Burial in the planting pits is much favourable. Irrigation must be done during dry months at the rate of 45 litters once in 4 days. Pitcher irrigation may be adopted in water scarcity areas. Shading can be done during summer to protect the seedlings. Regular weed control should be done to avoid competition.

Coconut is a perennial crop that exports nutrients to the above ground parts continuously from a limited volume of soil throughout its existence. CPCRI has recommended that, once the seedlings are planted in the main field, about one-tenth of the adult dosage should be applied after three months, one third after two years of growth, two thirds after three years and full dosage from fourth year onwards. General requirement of fertilizer elements for palms yielding an average of 50 nuts palm⁻¹ year⁻¹ could be 500g N, 320 g P₂O₅ and 1200 g K₂O palm/year (Table 2).

Table 2. Fertilizer recommendation (g/tree)

Year	May-June			September-October		
	N	P ₂ O ₅	K ₂ O	N	P ₂ O ₅	K ₂ O
First year	—	—	—	50	40	135
Second year	50	40	135	110	80	270
Third year	110	80	270	220	160	540
Fourth year onwards	170	120	400	330	200	800

- * Powdered rock phosphate is the best and cheapest source for acid soils.
- * Phosphorus application can be skipped for a few years if soil available phosphorus is more than 20 ppm

Fertilizers should be applied under optimum soil moisture conditions. Fertilizer application is usually recommended in two splits a year, one third of the recommended dose must be spread around the palms within a radius of 1.8 m and forked in immediately after the pre monsoon showers. Remaining two third fertilizer doses should be applied in September when the monsoon rain recedes along with 30 kg of green matter or 25 kg of FYM. Fertigation is an efficient method of fertilizer application through drip system. Drip fertigation increases the fertilizer use efficiency, saving in fertilizer costs, reduced labour requirement, supplying nutrients according to crop demand during varying physiological stages, uniform, precise and direct application to root zone, correction of micronutrient deficiencies etc. Soluble fertilizers viz., urea, phosphoric acid and potassium chloride can be combined and supplied through drip irrigation. Results conducted at CPCRI revealed that the fertilizer application at the rate of 50% of the recommended dose of fertilizer through drip irrigation produced yield equivalent to 100% of the recommended dose of fertilizer applied both by conventional as well as through drip irrigation. Application of micro nutrients has good response for coconut. The requirement of micronutrient for coconut for different coconut growing area are Boron, copper, Zinc, Magnesium, Manganese and Fe. However it all depends on the area where coconut is grown. In the west coast region there is good response for Boron, Copper, Zinc, Mg. If deficiencies are noticed 50 g of borax, 20 gm of copper sulphate, 30 g of Zinc sulphate can be applied to the palms.

The nitrogen fixing potential of legumes - Rhizobium symbiosis can be exploited in the interspaces of coconut through intercropping of green manures, cover legumes and forage legumes. *Pueraria phaseoloides*, *Calapogonium muconoides* and *Mimosa invisa* can be grown as suitable green manure in coconut basin as well as in the interspaces. Cover cropping helps to prevent soil erosion, weed growth, add a large quantity of organic matter and form thick

mulch that improves soil fertility and water holding capacity. Legume cover crops also fix atmospheric nitrogen. When these legumes are grown in coconut basin (1.8 m radius), they contribute 15-30 kg of green matter which adds 150-200 g of N per basin, when incorporated after four months of sowing. Green manuring results in enhanced microbial activity and fertility of coconut soils (Thomas and Shantaram, 1984).

Generation of large quantities of nitrogen rich biomass is also possible through the cultivation of the fast growing leguminous crop, *Glyricidia* in the coconut plantations. It can also be grown in littoral sandy soils where no other green manure can establish. The planting density and pruning frequency to obtain higher biomass yield from *Glyricidia sepium* when grown as green manure crop in coconut plantation in littoral sandy soil was standardized. The treatment, three rows of *Glyricidia* in between two rows of coconut palms with three prunings per year (February, June and October) resulted in higher biomass yield of 7.9 t/ha/yr. Application of *Glyricidia* prunings from interspace of one hectare of coconut garden can meet a major portion of nitrogen (90%), part of phosphorus (25%) and potassium (15%) requirement of coconut palm. Growing *Glyricidia* in the interspaces of coconut did not affect the growth characters of coconut (Subramanian *et al.* 2000).

In the experiment on the effect of glyricidia green manuring on production of coconut and to find out the possibility of substituting inorganic nitrogen source through glyricidia green manure, it was observed that the treatment, where 50% N was substituted through green manure recorded highest nut yield and it was significantly superior to the treatments 100% N substituted through chemical fertilizers, 100% N substituted through green manure. The microbial load was higher in the treatments substituted with green manure. Green manuring had good impact on soil physico-chemical properties such as water holding capacity and bulk density etc. and treatments substituted with green manure recorded higher level of foliar NPK content (Subramanian *et al.* 2005).

The process of composting of organic matter using earthworms is vermicomposting and the end product is known as vermicompost. The steps involved in vermicomposting are: multiplication of earthworms, preparation of base materials for vermicomposting, introduction of earthworm, management of vermicomposting bed and separation of vermicompost from un-decomposed materials. The management of vermicomposting requires regular watering to keep the bed moist. Direct sunlight is harmful for worms and the bed should be mulched with dry grasses or moist gunny bags. It takes nearly 60 days to compost most of the materials. Harvesting of vermicompost requires separation of worms (adult and juveniles) and cocoons by sieving from the compost. The granular vermicastings so obtained can be dried and stored.

Coconut palm wastes being very hard are decomposed rather slowly and vermicomposting has more relevance. Ligno-cellulosic waste materials from coconut plantation can be converted into brown, non-odorous, granular vermicastings using earthworms. At CPCRI, a local earthworm closely related to the African night crawler (*Eudrilus* spp.) has been isolated and has been found to be effective in vermicomposting of coconut palm wastes (Prabhu *et al.* 1998 and Thomas *et al.* 2001)). *Eudrilus* spp. can be multiplied in a mixture of cow dung and decayed leaves in 1:1 ratio taken in cement tub, wooden box or plastic bucket with drainage facilities. Worms should be introduced at the rate of 50 numbers per 10 kg of organic wastes. Within 1-2 months, the earthworm multiplies 300 times, which can be used for large scale

composting. Organic wastes weathered in rains for 3-4 months can be directly used for vermicomposting. Composting can be done in cement tanks or pits of convenient size, with depth less than 1 meter dug in coconut plantation. Cow dung may be added to the wastes at the rate of 10% by weight and the heap should be watered and allowed to undergo preliminary decomposition for 1-2 weeks. Then earthworms may be added at the rate of 1kg per ton of organic wastes. The bed should be mulched and watered regularly to maintain sufficient moisture and the composting will be over in about 70 days. Watering should be avoided for one week before the removal of compost. Worms will move to deeper moist area and compost can be collected, sieved and dried. The recovery of compost could be as high as 70%. The waste material can be converted into brown, non-odourous, granular vermicastings. From one hectare coconut garden we get 4,000 kg of vermicompost from leaves alone. The final product will contain 1.2-1.8% Nitrogen, 0.1-0.2% Phosphorous and 0.2-0.4% Potassium. Depending upon the availability 20 to 25 kg of vermicopost can be applied to each palm.



Eudrilus spp (Local species)



Large scale Vermicomposting

Coir pith is yet another organic material resistant to decomposition, available in huge quantities from coir processing units and can improve soil physical properties and moisture holding capacity to a great extent. But this can be applied to soil only after composting and efforts were made to prepare vermicompost from this waste also using the local strain of *Eudrilus* sp. Coir pith alone was not able to support the growth of earthworms. Coir pith terated with lime and rock phosphate @ 0.5 % each and incubated for three weeks has to be mixed with cowdung @10 % and fresh vermicompost @10 %. This mixture has to be layered with uncut coconut leaves @ 20% to facilitate aeration in the bed. The earthworm *Eudrilus* sp. has to be introduced at the rate of 1000 numbers/ t of organic materials and the bed should be mulched and protected from direct sunlight. Moisture has to be maintained at 50 % by regular irrigation. Earthworms form burrows in the bed and vermicastings appear as surface casts. A granular vermicompost with 1.2% nitrogen and 16.7:1 C: N ratio can be obtained in two months. Preparation of vermicompost from coir pith on a large scale would bring back both economic and environmental benefits.

The worm - worked wastes are currently marketed in both solid and liquid forms in many countries. All the physiologically active water soluble components of vermicompost such as humic acids, plant growth regulators, amino acids, vitamins, micro nutrients and microbial cells are extracted in water and is known as vermiwash. The water soluble components from vermicompost may be collected by passing water slowly through the worm

beds or by simple suspension of vermicompost in water. This is used for foliar applications as such or after sufficiently diluting, based on the need. Vegetables and ornamental plants have been reported to respond very well to this treatment (Murali Gopal *et al.* 2010).

The root regions of coconut palm are inhabited by a number of free living and associative symbiotic nitrogen-fixing bacteria having nitrogenase activity. The association of N_2 -fixing *Azospirillum amazonense* with the roots of coconut palm has recently been confirmed. The other associative nitrogen fixers include *Azospirillum lipoferum*, *Azospirillum brasilense*, *Herbaspirillum frisingense*, *Bacillus* spp., *Burkholderia* spp., *Azoarcus* spp., *Arthrobacter* spp. and many more which are yet to be identified. Some of these are capable of fixing nitrogen even in the presence of nitrogen fertilizer. These organisms may have additive or synergistic effects on plant growth and health if they function in concert in the rhizosphere of coconut palms.

Irrigation methods commonly adopted in coconut gardens are flooding, basin irrigation, sprinkler or perfo-sprays and drip irrigation. In certain areas of Tamil Nadu, East and West Godavari Districts of Andhra Pradesh where adequate supply of water is available, coconut gardens are flood irrigated. There is considerable wastage of irrigation water under flood irrigation. In basin irrigation, water is applied in the basins of 1.8 to 2.0 m which is the active root zone of coconut. Irrigation channels are provided in between two rows and each basin is connected with the channel. In this method there will be some loss of water due to deep percolation, seepage and evaporation. However this loss is reduced when basins are irrigated through hose pipes. This is being advocated to reduce water loss in transit. Still, there is loss due to deep percolation and surface evaporation. CPCRI recommends application of 200 L. of water once in four days. For the WCT palms in red sandy loam soils on the west coast, perfo irrigation with 20 mm water, when cumulative pan evaporation was 20 mm found to be the best irrigation schedule.

Sprinkler irrigation or perfo sprays are most suited to inter or mixed cropping systems where the entire surface requires wetting. The quantity of water applied should be at least 75 per cent of open pan Evaporation (E_o). Drip irrigation is ideally suited for widely spaced crops like coconut as it saves water, energy and labour and the WUE is high. Based on a study conducted at CPCRI, it was concluded that yield of coconut with drip irrigation daily @ 66 % of the E_o from December to May was

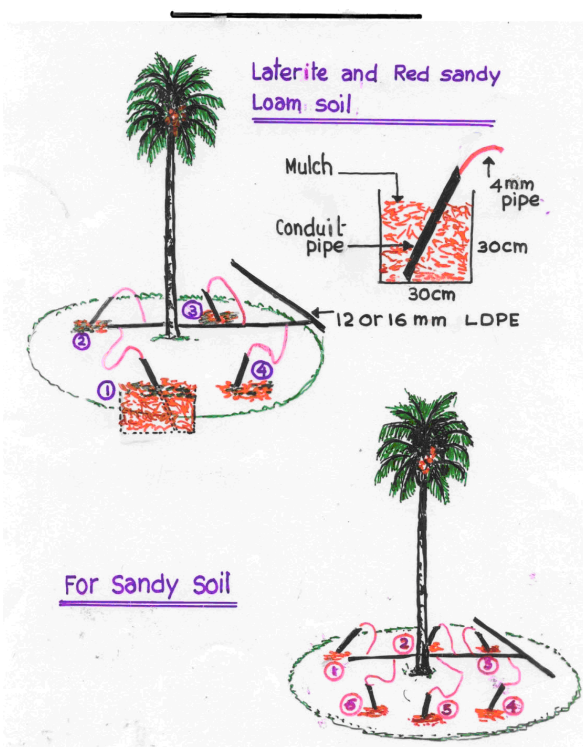


Fig 1. Drip irrigation layout in the coconut basin

adequate and comparable to basin irrigation @ 200 litres palm⁻¹ once in four days. Thus, there is 34 per cent saving of water in drip irrigation. This is applicable to varieties and hybrids and also in different soil types. The no. of dripping points should be six for sandy soils and four for other soil types (Dhanapal *et al.*, 1999) The rate of water application should be 2-3 litres hour⁻¹ emitter⁻¹ (Fig.1).

Coconut is mainly cultivated by small and marginal holdings (> 90 %) in major coconut producing countries. It has been well established in several coconuts growing countries that coconut as a mono crop is less productive and profitable. The interplay of various factors viz. limited size of holdings, number of trees, varied needs of the family, labour requirement for crop, fluctuating returns to farm families and easiness in marketing are some of the considerations for the grower to diversify his farm operations for getting higher returns by adopting intercropping, mixed cropping or introducing other enterprises like dairy, poultry etc. Moreover, under coconut based cropping system, the same land can be put to use to produce other crops so that the total factor productivity of the land is increased.

Intercropping refers to the practice of growing annuals/biennials in the interspaces of coconut. A large variety of crops have been found suitable for growing under irrigated and rainfed conditions

Experiments carried out during 1991 at Kasaragod have indicated vegetables like snake gourd, bottle gourd, amaranthus, coccinia, brinjal and bitter gourd as compatible crops with coconut. Intercropping with vegetables helped to generate additional employment to the tune of 215 to 365 mandays/ha/year. Among the different sequences tried snake gourd - ridge gourd - amaranthus was found to be the most remunerative one (Rs 22217/ha/year) followed by amaranthus - bottle gourd - brinjal (Rs 20920/ha/year)

The feasibility studies of intercropping coconut palm with ornamental, medicinal and aromatic crops in the interspace revealed that *Heliconia*, *Anthurium*, *Jasminum pubescence* and Marigold under ornamental crops and =Long pepper and Patchouli under medicinal crops were compatible as intercrops in coconut garden.

Growing of perennial crops in association with matured coconut palm is referred to as mixed cropping. A number of perennials like cocoa, clove, nutmeg, coffee, pepper, mulberry, jack, breadfruit, mango, sapota, papaya and timber yielding trees can be grown in association with coconut. Studies carried out at CPCRI, Kasaragod and elsewhere reveal that cocoa, pepper, clove and nutmeg are the most compatible crops with coconut and can be grown as mixed crops in the west coast region.

This refers to the cultivation of three or more crops having different morphological characteristics in the interspaces of coconut so as to intercept solar radiation at different levels and exploit different soil zones. The multi-storeyed cropping system was conceived and put to practice in 1970-71.

In the above system, pineapple performed well in the initial years. However, once cocoa had developed its full canopy, very little sunlight percolates down through its canopy to pineapple. Thus, pineapple could be grown only in the initial 5 or 6 years.

High-density multispecies cropping systems (HDMSCS) involve growing a large number of crops at very high plant population per unit area to meet the diverse needs of the

farmer such as food, fuel, timber, fodder and cash. They are ideally suited for smaller units of land and aim at maximum production per unit area of land, time and inputs with minimum or no deterioration of land. The salient features of the system are as follows:

- HDMSCS models consist of a large number of crop species with at very high plant density.
- It includes annuals, biennials and perennials
- The crops selected include cash crops, food crops and fodder crops.
- It includes large, medium, and small canopy crops arranged in a systematic way.
- The soil disturbance should be less, hence only slash weeding can be followed.
- The biomass (other than the economic part) is recycled within the system.
- The annual crops are removed as the canopy size of perennial crops increases.

In the coconut based high density multispecies cropping system, the coconut yield for the year 2002-03 ranged from 130 nuts/palm/year under no fertilizer control treatment to 143 nuts/palm/year at two third of the recommended fertilizer dose. The productivity of the palm declined with the reduction in the fertilizer levels beyond $1/3^{\text{rd}}$ of the recommended fertilizer treatment. The yield of the clove tree varied with the fertilizer treatments. The clove yield ranged from 0.246 kg/tree/year under no fertilizer control treatment to 1.44 kg/tree/year at full dose of the recommended fertilizer dose. The average weight of banana bunch was highest in the full recommended dose treatment (6.20 kg/bunch). The average weight of pineapple fruit was highest in the full recommended fertilizer dose treatment (528 g).

The highest coconut biomass was obtained at full dose treatment (23.51 t/ha) and the lowest of 19 t/ha in the control treatment. The total nutrient exhaust ranged from 130.45, 18.29 and 172.64 kg of N, P and K respectively per ha in the full dose to 97.11, 13.06 and 125.45 kg of N, P and K respectively per ha in the no fertilizer treatment plot. The extent of nutrient recycling in terms of leaflets, petioles, bunches, spathe and husk was highest in full dose treatment.

Mixed farming in coconut refers to the integration of other enterprises such as dairying, poultry and sericulture with coconut cultivation by raising fodder crops, mulberry etc in the interspaces. Coconut based mixed farming with dairying involves establishment of pastures in the interspaces of coconut, maintenance of milch animals on the fodder produced and recycling the cattle manure urine etc to the fodder crops and coconut. Studies have shown that some of the fodder grasses like. Hybrid bajra napier grass Co 3, hybrid Napier grass (*Pennisetum typhoides* ' *P. purpureum*), Sudan grass (*Sorghum sudanense*), Guinea grass (*Panicum maximum*), setaria, Rhodes grass (*Chloris gayana*) and legumes like *Stylosanthes*, lucerne (*Medicago sativa*) and berseem (*Trifolium alexandrinum*), come up very well in the partially shaded conditions of coconut plantations in humid tropics.

Mixed farming provides additional employment to the tune of 900 man days and ensures good returns without any yield decline in coconut. In the mixed farming in 1 ha. coconut garden at Kasaragod, comprising coconut, grass, dairy, poultry, sericulture and pisciculture, coconut, milk yield and broiler's sale accounted for 97% of the revenue generated

from the system. The net returns obtained were around 1.5 lakhs per annum. About 34 t of cow dung, 53.2 kg silkworm waste and 487 kg of poultry and quail manure obtained from the system were recycled in the coconut and grass plot, thereby improving the soil physico-chemical and biological properties of the soil.

In general, coconut comes up well in soft and easily broken laterites. However, the performance of coconut in hard, firm physically resistant laterite soil i.e. hard laterite out crop is not satisfactory and hence not recommended for highly profitable coconut cultivation. In these soils by not adopting proper technologies the growth of the coconut during initial period is good and in the later stage the plant shows yellowing symptoms resulting in poor yield. This is mainly due to poor root proliferation and poor nutrients absorption. To overcome this soil constraint, two things are to be concentrated one is the pit size and secondly, amending the soil with husk or coir pith. As 80 to 90 per cent of the root concentrates vertically to a depth of 1.2 m and horizontally to a radius of 2m from the bole of the palm, it is advisable to go for a pit size of 2 x 2x1.5m with application of husk (quantity 1/3 volume of the pit) i.e. after opening the pit, apply one or two layer of husk and then apply the soil which was dug out of the pit to a height of one foot then apply again one layer of husk. Like this the pit is filled up to 2/3 height. Then the seedlings can be planted in the pit (Fig. 2). While planting some organic manure need to be added. In hard laterite for proper root proliferation we dig the soil horizontally 2 m, otherwise in good soil the size of the pit for planting coconut is 1 x 1x1m. By adding the husk and breaking the hard rock the root can penetrate and multiply easily as they grow up resulting in good growth and yield.

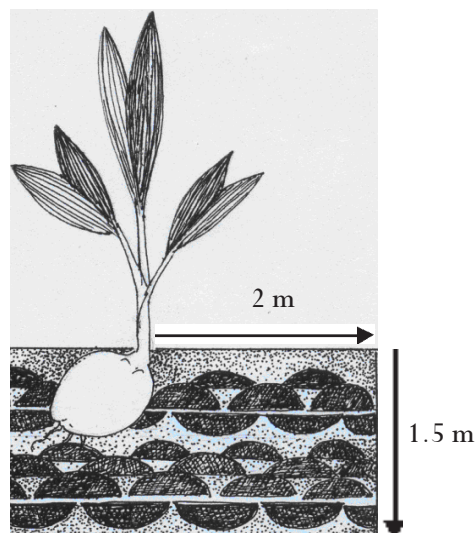


Fig. 2. Seedling planted in trenches of hard laterite soil filled with alternate layers of coconut husk and soil



Fig. 3 Performance of fodder grass with husk incorporation technology under coastal sandy soil

Bajra napier hybrid grass (Co 3) can be successfully grown as intercrop (Fig. 3) in coconut garden under coastal sandy soil by using husk burial/coir pith incorporation technology. From one hectare of coconut garden around 90 tonnes of green fodder/ha can be harvested which will be sufficient to maintain 9 cows. By adopting moisture conservation practices through husk/coir pith as amendments and growing grass in the inter-space the growth and yield of main crop (coconut) can be improved (Subramanian et al. 2009). By adopting this technology, an increase of 171 per cent in coconut yield (95 nuts/palm/year) was realized compared to the pre treatment yield (35 nuts/palm/year) in the coastal sandy soil.

Black soils are restricted in their occurrence to Chittoor taluk of Palakkad districts of Kerala. They are found to occur in patches and are considered as extension of the black cotton soils observed in the adjacent Coimbatore district of Tamil Nadu. Black soils are also seen in Andhra Pradesh and Karnataka where coconut is grown. These soils are dark, low in organic matter, calcareous, neutral to moderately alkaline (pH 7.0 to 8.5) and high in clay content and CEC. Hence they exhibit the characteristic cracking during dry periods. They are usually located in gently sloping to nearly level lands. The levels of potassium and calcium are moderate and those of nitrogen and phosphorus, low.

In a relatively small area of 1000 ha in Chittoor block, a highly dispersed soil termed as “Poonthalpadam” soil, is seen. This soil occurs as a slushy layer to a depth of about 0.5m to 1.5m. A bed of limestone is seen beneath the slushy layer. The physical properties like plasticity, cohesion, expansion and shrinkage are similar to those of the regular soils of the Deccan.

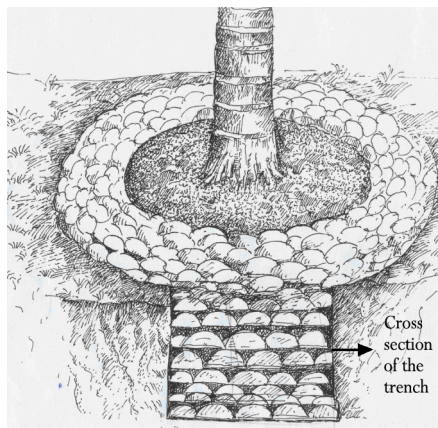


Fig. 4. Circular trench filled with coconut husk in heavy clay soil to a depth of 1m

Farmers who have not realized the difficulty of growing coconut in black cotton soil often suffer mental stress, in the future, after seeing the poor growth of coconut seedling planted in such soil. Due to various reasons like water logging in rainy season, poor aeration and hardness of the soil, the root proliferation is affected thus the growth of the seedlings remain stunted. In such cases, a circular trench can be opened leaving 100 cm from the bole of the palm and fill the trench with husk/coir pith. This trench can be left as such without covering with the soil (Fig. 4). Top layer of the husk can be arranged in convex fashion so that evaporation can be avoided. In the future irrigation and fertilizer can also be provided in the trench only.

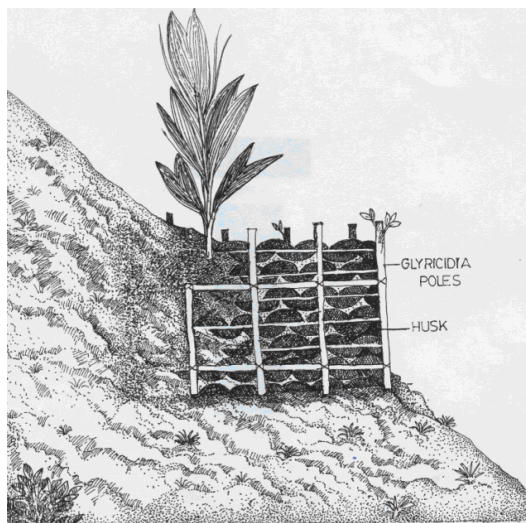


Fig. 5. Basin formation with husk and Glyricidia poles in > 14 % Sloppy terrain

About 80 to 90% of the coconut roots mine nutrients in the root zone of 2.m radius and up to 1.2 m depth. In sloppy terrain of more than 14% slope, it is difficult to get this basin area. Here again in the initial years of planting the seedling grow well and later it will get affected because of the poor anchorage and nutrient availability. In the later years not only the growth affects but also, it is difficult for us to go for different operation like harvesting, fertilizer application, irrigation, etc. Further there is lot of chance for soil and nutrient loss due to

erosion. Therefore, to overcome the problems to certain extent an artificial basin is created with glyricidia poles and filling the depth with husk. We use the Glyricidia poles since they pick up rooting and the poles would not get damaged even after few years till the basin gets established. Arrange the poles vertically and closely so that the husk can be held in the basin area (Fig. 5). As the years pass on and the eroded soil get collected in the basin area a perfect coconut basin will be ready after few years. The green biomass from the Glyricidia can be chopped and put in the basin area of the coconut seedling/ palm which adds green manure.

Conserving natural resources like water and soil is essential for sustaining the crop production in both high and low rainfall areas, particularly in sloppy areas, which are prone to soil erosion. The role played by the bio-engineering and agronomic measure in reducing the soil and nutrient loss and in conserving the runoff water is immense. Various conservation measures for sloppy areas tried at CPCRI which include half moon bund with planting pineapple on the bund, staggered catch pit with pineapple on the bund and contour trenches filled in with coconut husk and pineapple on the bund which are discussed in the following sub headings.

This measure is to be taken up where the land slope is high (7-14%). Trenches of 50 cm width x 50 cm depth and convenient length would be made in between two rows of coconut palms. These trenches would then be filled with coconut husk. Coconut husks need to be filled in layers with the bottom layers facing up and top layer facing down. A bund of 50 cm height and suitable width (50 cm) is made at the downstream using the excavated soil. Two layers of pineapple plants would be planted on the bund with a spacing of 20 cm plant to plant and 20 cm row to row (Fig. 6). Pineapple plants would stabilize the bund and provide additional income to the farmer. The runoff water from the upper side would be collected in the trenches. Soil particles would also get collected in the trench along with the runoff water. Coconut husk retains the moisture and makes it available for plants during summer months. The research conducted at CPCRI, Kidu revealed that husk filled in trench with pineapple border was observed to be the best in soil and water conservation measure and was observed to reduce 98 % of the soil loss (0.14 t/ha) compared to control (10.52 t/ha) in coconut plantation. Less run off was observed (0.17 %) compared to control (5.6 %). Similar results were obtained in terms of nutrient conservation also. Minimum loss of nutrient were observed in trench filled with coconut husk with 2 lines of pineapple border (1.28, 0.20 and 1.15 Kg N, P, K /ha/year, respectively) compared to control (105, 22 and 167 kg N, P, K/ha/year, respectively). It has very good effect on coconut yield also. The coconut yield was increased from the pretreatment yield of 35 nuts/ palm/year to 79 nuts / palm/year over a period of four years under rainfed situation.



Fig .6. Sloppy land with 7- 14% slope and soil and moisture conservation measures of contour trenches filled with husk with pineapple border

This measure is to be taken up where there is mild slope. Here a flat basin with a slight inward slope in the upstream is made by excavating soil from the upstream side and filling the excavated soil at the downstream side. After making the basin a bund of 50 cm height and 50 cm width is made at the downstream side of the coconut using the excavated soil. Two layers of pineapple plants would be planted with a spacing of 20 cm row to row and 20 cm plant to plant on the bund. The bund prevents runoff and water gets collected within the basin and percolates down. Pineapple would help to protect the bund and stabilize the same in addition to giving fruit yield. This measure results in less run off, soil erosion, soil and nutrient loss and resulting in 63 % increase in yield(70 nuts/palm/year) over pre treatment (43 nuts/palm/year).



Fig. 7. Staggered catch pit with pineapple border for a slope up to 7%

Catch pits can be constructed at all slopes ranging 1 to 7% to conserve soil and water. Though there are no standard dimensions for catch pits, we may go for catch pits of 1.5 m length x 0.5 m width x 0.5 m depth. A bund is to be made at the downstream using the excavated soil and pineapple plants planted on it. This pit also may or may not be filled with coconut husk (Fig. 7). If it is without husk, periodic measurement of the depth of the pit gives the amount of soil collected inside the pit, a direct measurement of soil erosion. Remarkable impact of catch pit on coconut in yield i.e.96 % in increase of coconut yield (49 nuts/palm/year) over pre treatment yield (25 nuts/palm/year) has been observed in the experiment conducted at CPCRI.

In this hour of high market price fluctuation, it is imperative to make coconut cultivation more profitable and viable. Hence, it is necessary to increase the productivity, with latest technologies developed which will support sustained higher yield. Moreover, emphasis should be given on product diversification. Adoption of improved technologies, is the possible way out to achieve higher productivity and higher income

References

- Dhanapal R., Subramanian, P. and Maheswarappa, H. P. 1999. Methods of Irrigation in Coconut Gardens. *Indian Coconut Journal* 29(11): 4-7.
- Murali Gopal, Alka Gupta, C. Palaniswami, R. Dhanapal and George V Thomas. 2010. Coconut leaf vermiwash: a bio liquid from coconut leaf vermicompost for improving coconut production capacities of soil. *Current Science* 98(9):1202-1210.
- Nelliath, E. V., Nair, P.K.R. and Kushawa B.L. 1976. Effect of month of harvest of seed nuts and fertilizer application to nursery on vigour and quality of seedlings. *J. Plantn. Crops* 4(1):185-189
- Prabhu, S. R., Subramanian, P., Biddappa, C. C. And Bopaiah, b. M. 1998. Prospects of improving coconut productivity through vermiculture technologies. *Indian Coconut Journal* 29(4): 79-84.

- Reddy, DVS., Ratnambal, R.J. and Gopalasundaram, P. 1996. Polybag nursery for production of vigorous seedlings. *Indian Coconut Journal* **27** (3); 5-7
- Subramanian, .P., Biddappa, C.C., Maheswarappa, H.P., Dhanapal, R., and Palaniswami, C. 2000. Feasibility studies of growing *Glyricidis sepium* as intercrop in coconut garden under littoral sandy soil. *J. Plantn. Crops* **28**(3):212-217.
- Subramanian.,P., Dhanapal., R., Sanil, P., Palaniswami, C., Sairam, C.V. and H.P. Maheswarappa 2005. Glyricidia (*Gliricidia sepium*) as green manure in improving soil fertility and productivity of coconut under coastal littoral sandy soil. *J. Plantn. Crops* **33**(3):185-189.
- Subramanian.,P., Dhanapal.,R., Palaniswami, C, and Joseph Sebastin 2007. Feasibility studies on growing hybrid bajra napier fodder grass as intercrop in coconut under coastal sandy soil . *J. Plantn. Crops* **35**(1):19-22
- Thomas, G.V. and M.V. Shantaram 1984. *In situ* cultivation and incorporation of green manure legumes in coconut basins; an approach to improve soil fertility and microbial activity. *Plant and Soils* **80**: 373-380
- Thomas, G.V., Prabhu, S.R., Subramanium, P. and Iyer, R. 2001. *Organic Farming Technologies in Coconut*. ATIC series Publication No. 4., Central Plantation Crop Research Institute, Kasaragod, Kerala.

Integrated nutrient management in ECT coconut through organics

S. Mohandas, P. S. Subramaniam, A. Gurusamy, C. Natarajan and P. Arulraj

Coconut Research Station, Tamil Nadu Agricultural University

Veppankulam – 614 906, Tamil Nadu

Adequate fertilization is vital to enhance the productivity and quality of coconut. Most coconut soils throughout the country are highly weathered and low in fertility. Soil fertility management is important in coconut plantations, since coconut is a perennial palm with a continuous productive life. Continuous use of fertilizers with total negligence of organic manure often leads to decline in nut yield and deterioration of soil health. Organic manures are thus indispensable in the fertilizer schedule of coconut for sustained production and preserving soil health. Neither organic manure nor fertilizer alone can satisfy the nutrient requirement of an exhaustive crop like coconut. Only a judicious use of organic manure and fertilizer will solve the twin problems of soil health and sustainable production. About 1 percent of coconut plots receive inorganic fertilizers and around 50 percent of the plots receive meager quantity of organic manure in the form of farmyard manure and green leaf manures (Mathew, 1976). In India, huge quantity of organic wastes is being generated annually. Coconut produces 11.2 mt of organic biomass which includes husk, dried leaves, coir dust etc. (Biddappa *et al.*, 1996). If part of this waste is recycled, at least 50 percent of coconut nutrition can be supplemented by the organic manure generated in coconut gardens. This will pave way for the safe disposal of organic wastes and prevent deterioration of soil health in coconut based cropping system. Coir pith, a lignocellulosic material, has wide C:N ratio from 112:1 (Nagarajan *et al.*, 1985) to 58:1 (Ravichandran, 1988) and the composting will reduce the C:N to the tune of 20:1 to 15:1. Organic farming in coconut includes use of green manure, green leaf manures, vermicompost, CCP, animal manures, rock phosphate etc., to supplement chemical fertilizers by way of waste recycling. The present investigation deals with substitution of 50 percent nitrogen for coconut through organic manure by way of waste recycling.

A field experiment was initiated during 2006 at Coconut Research Station, Veppankulam to study the scope for nitrogen substitution in coconut through organic manure (coconut waste recycling). The age of the palm was 36 years. There were five treatments and the experiment was conducted in randomized block design, replicated four times with six palms per treatment. The experimental site was located at 20 m above MSL, with an average mean annual rainfall of 1,125 mm. The experimental soil is sandy loam in texture with a pH of 7.01, EC 0.13 dSm⁻¹ and organic carbon content 0.23 per cent. The available NPK content of the experimental soil is 112, 10.5, 203 kg / ha respectively. The palms were supplied with 50 percent nitrogen through composted coir pith and remaining 50 percent nitrogen, phosphorus, potassium through fertilizers. The composted coir pith was applied to substitute 50 percent nitrogen, based on equal nutrient basis, out of the total recommendation of 560 g N / palm / year. The composted coir pith had a NPK content of 1.02: 0.60: 1.06 percent and C:N ratio of 24:1.

P and K were applied @ 320 and 1200 g / palm / year, respectively excluding the quantum supplied by the composted coir pith substitute to supply 50 per cent nitrogen. The

annual nut yield and copra yield and growth parameters were recorded and analyzed statistically. The total return and B:C ratio were also worked out. The soil NPK availability and index leaf NPK content were estimated at the end of each year and analyzed statistically.

Application of recommended NPK consistently produced the maximum number of functional leaves per palm. It recorded an increase from 21.2 to 30.7 (2006-'07) and 28.8 to 33.1 leaves / palm (2007-'08) (Table 1). With respect to treatments, the application of recommended NPK (560:320:1200 g/palm/year) recorded the highest number of functional leaves (30.7 for 2006-'07 and 33.1 for 2007-'08) and it was followed by the treatment receiving 50% N as CCP + 50% N, balance P and K as fertilizers (30.3 for 2006-'07 and 31.1 for 2007-'08). However, these two treatments are comparable with each other. The 100% N as CCP (T_3) and various combination of organics - T_5 (Neem cake, bone meal and wood ash) produced relatively lesser number of leaves / palm. Nitrogen is indispensable as a constituent of amino-acids, proteins and nucleic acids. Shortage of nitrogen makes itself felt throughout the coconut's physiology and provokes a substantial yield decline (Manciot *et al.*, 1980). Khan *et al.* (1990) based on the farmer's field experimentation reported that doubling the recommended dose in the first year followed by the recommended dose viz., 500, 320, 1200 N, P_2O_5 , K_2O respectively /palm/year helped in early enhancement of nut yield.

The number of female flowers produced due to the application of recommended NPK (T_2) ranged from 23.1 to 35.3 (2006-'07) and 24.3 to 36.8 / inflorescence (2007-'08) (Table 1). As in the case of functional leaf production, the highest (35.3 for 2006-'07 and 36.8 for 2007-'08) female flower production was registered due to the application of recommended NPK in combination with 50 kg of FYM /palm/year. However, it was at par with treatment that received 50% N as CCP 50%, remaining 50% N and P,K as fertilizers which registered a female flower production of 32.4 (2006-'07) and 34.1 (2007-'08) / inflorescence. While other two treatments viz., 100% N as CCP (T_3) and various organic combinations (T_5) registered comparable number of female flowers and they were at par with T_4 . The absolute control (T_1) registered the lowest female flower production.

Table 1. Effect of treatments on yield attributes of East Coast Tall (ECT) coconut

Treatments	No. of functional leaves/palm		No. of bunches/palm		No. of female flower/spathe	
	'06-'07	'07-'08	'06-'07	'07-'08	'06-'07	'07-'08
T_1 Control	21.2	28.8	11.3	12.9	23.1	24.3
T_2 NPK (560: 320:1200g/palm/ year)	30.7	33.1	12.6	14.3	35.3	36.8
T_3 - 100% N as CCP + balance PK as fertilizer	22.7	30.7	11.6	13.8	28.3	29.1
T_4 50% N as CCP + balance NPK as fertilizer	30.3	31.1	12.3	13.5	32.4	34.1

T ₅ - 10 kg Neem cake + 20 kg ash+2 kg bone meal+ 20kg wood ash	24.2	30.5	11.2	13.5	26.1	28.5
SEd	1.5	1.4	0.5	0.1	1.4	1.2
CD (P=0.05)	3.2	2.9	NS	NS	3.1	2.8

NS= Not significant

Nitrogen substitution through organic manure positively enhanced the soil available nitrogen besides, the phosphorus and potassium. However, the highest available NPK were recorded under the treatment T₂, which received the recommended NPK, which was significantly superior over other treatments (130 and 146 kg / ha in 2007 and 2008, respectively). The important point to be noted here is a significant reduction in soil NPK availability over the years under absolute control. Hence, it is obvious that the total omission of external inputs either organics are inorganics leads to rapid depletion of soil fertility and results in soil degradation. Khan (2004) brought that fertilizer application and INM practices are must for increasing and sustaining productivity of coconut garden.

As observed in soil status, the index leaf NPK content was also significantly altered due to different treatments. The highest leaf NPK (2.20, 0.24, 1.42 & 2.25, 0.26, 1.46 per cent respectively for 2007 and 2008) was observed due to the adoption of recommended package for coconut. This was significantly superior over other treatments. The immediate release and its subsequent absorption by the palms resulted in higher NPK in leaf under the NPK treatment (Sahoo *et al.*, 2004). The 50 percent N substitution through organics registered the next best values. As expected the absolute control recorded the lowest NPK content of leaf tissues.

The treatments registered a significant effect on nut yield of coconut. As in the case of other parameters, application of recommended NPK (T₂) registered the maximum nuts/palm/year which showed a increase from 108 (2000-'06) to 120/palm/year (2007-'08) and it was at par with treatment receiving 50% N as CCP remaining 50% N and P as fertilizer (T₄). The nut yield under T₄ was in the range of 95 (2005-06) to 116 (2007-08) / palm / year (Table 2). The nut yield recorded under the treatment receiving 100% N as CCP (T₃) was comparable with combination of organics (T₅). Analysis of mean nut yield (2000-06 to 2007-08) showed that application of recommended NPK (T₂) recorded the highest number of nuts (117/palm / year) which was followed by 50 per cent N as CCP remaining 50 per cent N and P,K as fertilizer with 111 nuts/palm/ year (T₄). Treatment receiving 100% N as CCP (T₃) and various organic input combinations (T₅) recorded 95 and 84 nuts /palm/year respectively. Rethinam *et al.* (1991) reported that integrated nutrient management increased the nut yield from 28 to 51 nuts/palm/year within three years.

Comparing the mean nut yield with pretreatment yield, all the treatments recorded higher number of nuts / palm with exception of absolute control (T₁), which recorded 66 nuts / palm / year (Table 3). The reason here for stagnation in nut yield may be due to poor nutrient supply in the complete negligence of nutrients. The enhanced nut yield registered in

Table 2. Effect of treatments on soil fertility and index leaf nutrient content

Treatments	Available NPK (kg ha ⁻¹)						Index leaf NPK content (%)					
	'06-'07			'07-'08			'06-'07			'07-'08		
	N	P	K	N	P	K	N	P	K	N	P	K
T ₁ Control	110	10.1	198	102	8.8	191	1.80	0.19	1.21	1.70	0.11	0.96
T ₂ NPK (560: 320: 1200g/palm/year)	130	15.8	236	146	17.1	242	2.20	0.24	1.42	2.25	0.26	1.46
T ₃ 100% N as CCP + balance PK as fertilizer	121	12.1	211	127	13.1	217	2.12	0.19	1.28	2.15	0.21	1.32
T ₄ 50% N as CCP + balance NPK as fertilizer	131	13.5	222	137	14.2	228	2.10	0.21	1.38	2.15	0.23	0.38
T ₅ 10 kg Neem cake + 20 kg ash + 2 kg bone meal + 20 kg wood ash	116	11.2	205	121	11.8	211	1.91	0.21	1.40	1.94	0.28	1.34
SEd	3	0.3	5	3	0.4	6	0.06	0.03	0.08	0.06	0.02	0.09
CD (P=0.05)	6	0.6	10	8	0.7	11	0.12	NS	NS	0.13	NS	NS

other treatments even within two years might be due to staggered and continuous supply of nutrients from the decomposing organics coupled with enhanced use efficiency of inorganics. Thus integrated nutrient management lays emphasis on improving and maintaining soil fertility for sustained productivity (Khan *et al.*, 2000). The coconut based cropping system offers excellent scope for INM practices in view of biomass generated in the system (Biddappa *et al.*, 1996). Hence, integrated nutrient management in fact is an integral part of the coconut gardens as it offers enormous scope for organic matter build up by way of waste recycling.

Table 3. Effect of treatments on nut yield of ECT coconut

Treatments	5 years average (2001-2006)	2006-07	2007-08	Mean (2006-08)
T ₁ Absolute control	69	63	68	66
T ₂ Rec NPK(560:320:1200g/palm)	108	114	120	117
T ₃ 100% N as CCP+balance PK as fertilizer	88	86	111	99
T ₄ 50% N as CCP+balance NPK as fertilizer	95	105	116	111
T ₅ 10kg Neem cake+20kg ash+2kg bone meal+20kg wood ash	80	84	101	93
SEd	6	4	5	-
CD (P=0.05)	12	10	11	-

The different treatments registered a significant influence on the copra yield of coconut. The highest copra yield of 14.3 kg / palm / year was recorded by the application of recommended NPK (T_2), which was comparable with treatment receiving 50 per cent N as CCP remaining 50 per cent N and P, K as fertilizers (13.2 kg / palm / year) in 2007-08. The rest of the treatments recorded a copra yield ranging from 8.2 to 4.2 kg / palm / year (Table 4). Price of coconut during 2006'-07 was Rs. 3.34 / nut and for 2007'-08 was Rs. 3.90/nut. Increase in copra production by the application of Composted Coir Pith was earlier reported by Venkitaswamy (2003) and Ghosh and Bandopadhyay *et al.* (2009). The treatment of observation was similar in 2007-08.

Table 4. Effect of treatments on coconut yield and economics

Treatments	No. of nuts/ palm / year		Copra yield mean/palm/		Total return/ ha(Rs)		B:C	
	06-07	07-08	06-07	07-08	06-07	07-08	06-07	07-08
T_1 Absolute control	63	68	8.2	8.5	30415	33,152	2.05	2.09
T_2 NPK (560:320:1200g/ palm/year)	114	120	14.3	15.6	59640	63000	2.13	2.17
T_3 100% N as CCP+ balance PK as fertilizer	86	111	10.8	13.9	45486	58,275	1.82	2.00
T_4 50% N as CCP+balance NPK as fertilizer	105	116	13.2	14.7	54861	60,900	2.04	2.03
T_5 10 kg Neem cake+20 kg ash+2 kg bone meal+ 20 kg wood ash	84	89	10.7	11.2	43,836	46,726	1.03	1.60
SEd	4.0	5.0	0.2	0.03	-	-	-	-
CD (P=0.05)	10.0	11.0	0.5	0.6	-	-	-	-

The cultivation cost and total returns were worked out for the nut yield obtained under different treatments for the year 2006-07 and 2007-08 (Table 4). Application of recommended NPK (T_2) showed the highest benefit cost ratio of 2.13 (2006-07) and this was followed by treatment receiving 50% N as CCP remaining 50% N,P and K as inorganic fertilizer (T_4) with a B: C of 2.04. Comparing the B: C of other treatments with absolute control, the B: C obtained for T_3 (1.82) and T_5 (1.03) were lower than control. The trend of observation was similar in 2007-08 as well. The cost of inputs *viz.*, composted coir pith, neem cake, bone meal and ash were the contributing factors for higher cost of cultivation under this treatment, which eventually reduced the B: C.

The application of recommended doses of NPK (T_2) and the treatment of 50 percent nitrogen substitution through composted coir pith (CCP) and supplying remaining 50 percent N, and P and K through fertilizers (T_4) recorded the comparable nut yield and the economics. Considering the addition of organic matter, the latter is effective in sustaining the nut yield and soil fertility in the long run and also higher economic return. The availability of organics and economic feasibility of converting them into manure in coconut gardens, out of total recommendation, 50 per cent nitrogen may be supplied through organics and remaining 50

percent N and P, K (excluding the quantum of P and K supplied by the organics) may be supplied through fertilizers.

References

- Bidappa, C.C., Upadhyay, A.K., Hegde, M. R. and Palaniswamy, C. 1996. Organic matter recycling in plantation crops. *J. Plantn. Crops* **24**(2): 71-85.
- Ghosh, D. K. and Bandopadhyay, A, 2009. Studies on the influence of integrated nutrient management on growth and yield of young coconut palms. *Indian coconut Journal* **LII** (1): 17-21.
- Khan, H. H., Biddappa, C.C. and Robert Cecil, S. 1990. Improving the coconut production: Future needs related to nutritional aspects. *Indian Coconut J.* pp 2-7.
- Khan, H. H., Upadhyay, A.K. and palaniswami, C. 2000. Integrated nutrient management of plantation crops. In *Plantation Crop Res. & Dev. Proc. Placrosym XIV*, CDB Kochi pp 9-22.
- Khan, H. H. 2004. Initiatives towards improving coconut productivity. *J. Plantn. Crops* **32** (Suppl.):173-185.
- Manciot, R., Ollagnier, M. and Ochs, R. 1980. Mineral nutrition and fertilization of the coconut around the world. *Part III. Oleagineux* **35**(1): 13-27.
- Mathew, J. 1976. Pattern of production and productivity of coconut in Kerala. In: *Proc. PLACROSYM*, I. (Ed.) V. NELLIAT *et al.*, I. S. P. C., Central Plantation Crops Research Institute, Kasaragod, Kerala, India. p: 531.
- Nagarajan, R., Manickam, T. S., Kothandaraman, G. V., Ramaswamy, K. and Palaniswamy, G. V. 1985. Manurial value of coir pith. *Madras Agric. J.* **72**: 533-535.
- Ravichandran, B. C. 1988. Evaluation of decomposed coir pith on the grain yield of maize. *M.Sc., (Agri) Thesis*, UAS, Bangalore.
- Rethinam, P., Antony, K. J. and Muralidharan, A. 1991. Management of coconut root (wilt) disease. Abstract of papers. *Second international symposium of coconut research and development*, CPCRI, Kasaragod, India p.60.
- Sahoo. S.C, Dillip K. Dora, Acharya, G.C. and Panda J. M. 2004. Influence of integrated nutrient management on the performance of coconut palm in littoral sand. *J. Plantn. Crops*. **32** (Suppl.): 224-228.
- Venkitaswamy, R. 2003. Integrated nutrient management in coconut with composted coir pith. *Madras Agric. J.* **90**: 54-56.

Nutrient management of hybrid (T x D) coconut with nitrogen substitution through organic manure

S. Mohandas, P. S. Subramaniam, A. Gurusamy, C. Natarajan and S. Arulraj

Coconut Research Station, Tamil Nadu Agricultural University

Veppankulam – 614 906, Tamil Nadu

Coconut (*Coconus nucifera* L.) is an important plantation crop mainly cultivated in coastal belts of India. In general, coconuts survive for more than 60 years and continue to yield under ideal management conditions throughout the year and hence, require a continuous and balanced supply of nutrients for higher productivity (Upadhyay *et al.*, 1998). Coconut palm absorbs large quantities of nutrients from the soil. A high proportion of nutrients are removed from the soil with the harvested produce and debris. Application of fertilizers containing NPK and Mg at recommended rates partially compensates the depletion (Nadheesha and Tennakoon, 2008). Fertilizer requirement in coconut is generally assessed through computing the annual harvest of the nutrients by the palm or by studying the yield response to graded levels of applied fertilizers (Wahid, 1984). The annual nutrient removal by the coconut palms through nuts, fronds, trunk, bunch, spathe etc., varied from 20 to 174 kg N, 2.5 to 20 kg P₂O₅ and 35 to 49 kg K₂O ha⁻¹ (Ouverier and Ochs, 1978). The removed nutrients are to be replenished by one way or other for the sustainable coconut production. Hence, the nutrient management plays a key role in determining the productivity of palms.

Either under nourishment/lack of application of fertilizers or organics to coconut / total dependence on chemical fertilizers alone often leads to poor productivity and leads to soil health deterioration. Hence, for sustaining the palm productivity and health of coconut soil, a judicious combination of organic manures and fertilizer nutrients are essential. Coconut based cropping system offers enormous scope for nitrogen substitution through organic manure by way of *in-situ* waste recycling through vermicomposting. The present experiment aims to supplement the chemical fertilizers with organic manures through vermicomposting / composted coir pith in coconut gardens. This may pave way for the gradual replacement of chemical fertilizers in coconut based cropping system.

A field experiment was laid out at Coconut Research Station, Veppankulam in order to assess the nutrient requirement of hybrid coconut (VHC 2) with 50 percent N substitution through organic manures. The experimental site is located at 20 m above MSL, the average mean annual rainfall is 1,125 mm. The experimental soil is sandy loam in texture with a pH of 7.1, EC 0.16 and organic carbon content of 0.20 percent. The available NPK content of the experimental soil is 118, 11.5 and 136 kg/ha respectively. The palms were 35 years old and treatments consisted of each three levels of N (0, 500 and 1000 g/palm/year), P (0, 250 and 500 g/ palm/year) and K (0, 1000 and 2000 g/palm/year) and thus there were 27 treatment combinations. Three palms were selected for each treatment and the experiment was conducted in a 3³ non-replicated confounded factorial design.

Based on the NPK content of vermicompost (1.4% N, 0.14% P and 0.29% K) and the composted coir pith (1.02: 0.60: 1.06 % and C: N ratio of 24: 1), the required quantity of vermicompost or composted coir pith equivalent to 50% N for each treatment along with P,

K fertilizers (excluding the quantum P, K supplied by the vermicompost / composted coir pith CCP) were applied as per treatments. The fertilizers and organic manure (vermicompost/ CCP) were applied in two equal splits, first during February and second during September of every year. The yield attributes *viz.*, number of functional leaves produced, number of bunches produced, nut setting per cent, and nut yield for every treatments at the end of year were recorded. Besides, the soil available NPK and index leaf NPK content were estimated at the end of each year.

The number of functional leaves recorded was significantly influenced by the NPK applied at graded levels (Tables 1 and 3). The number of functional leaves and bunches produced were in the range of 26 to 34 and 11 to 13 /palm, respectively among the NPK levels. The number of buttons produced was between 20.0 and 35.0 / bunch. The levels of fertilizers did not have much effect on the growth characters of adult palms (Reddy *et al.*, 2002). Among the various NPK combinations, an NPK level of 1000: 500: 2000 g/palm/year registered the highest mean annual nut yield of 158, 176 and 182 nuts/palm/year during 2006-07, 2007-08 and 2008-09 respectively. However, it was comparable with a NPK level of 1000:250:2000 g/palm/year (156, 172 and 178 nuts/palm/year), here out of 1000 g of N, 500 g was supplied through composted coir pith or vermicompost based on availability. With regard to N, K, for every incremental addition of each nutrient, there observed a corresponding increase in nut yield, whereas in case of P, the increase in nut yield was observed only up to 250 g/palm/year. Incremental addition of nitrogen, from 0 to 1000 g / palm /year, the increase in nut yield was from 146 to 158 nuts/palm/year (2008-09). For P level from 0 to 250 g/palm/year, the yield increase was from 144 to 156 nuts/palm/year and for K level from 0 to 2000 g/palm/year, the increase was from 136 to 165 nuts/palm/year (2008-09). Though there was some numerical variation among the various NPK levels, the trend of observation was similar in all the years of experimentation with respect to yield and yield attributes of coconut. The different varieties of coconut gave varying yield responses to fertilizer application (Hameed Khan *et al.*, 1986). The overall performance of the palm could be improved by the application of NPK fertilizers at recommended level (Sudhakara and Nambiar, 1991). Rethinam *et al.* (1991) reported that integrated nutrient management increased the nut yield from 28 to 51 nuts/palm/year within three years. Integrated nutrient management is a handy tool for productivity increase and its sustenance (Hameed Khan, 2004).

The soil available NPK was appreciably increased for every incremental addition of respective nutrients (Tables 2 and 3). Among the different levels of NPK, the highest soil available NPK was registered at their highest level tried i.e. 1000: 500: 2000 g of NPK/palm/year, though the economic nut yield was obtained at 1000: 250: 2000 g NPK/palm/year. This fact was true throughout the period of experimentation. The results on soil available NPK revealed that all the treatments recorded low status of available N, however there was some numerical difference between the treatments. Treatment combinations *viz.*, T₁ to T₉ was found to have available nitrogen in the range of 101 to 117 kg ha⁻¹ (2008-09) wherein no N was applied. Among the treatments, from T₁₀ to T₁₈ that received 500 g N/palm/year registered the available N in the range of 142 to 152 kg ha⁻¹ (2008-09). The higher available N was observed for the application of nitrogen at it's highest level i.e., 1000 g/palm/year (178 to 211 kg N ha⁻¹ in 2008-09). Increasing the P level from 0 to 500 g/palm/year, progressively enhanced the P availability in soil. The available P content of 11.7, 19.0 and 22.7 k ha⁻¹ was

recorded for P, P₂ and P₅ levels, respectively (2008-09). Similar increase in K level from 1000 to 2000 g/palm/year, enhanced the soil available K content from medium to high level (130 to 259 kg ha⁻¹ 2008-09). The effect of treatments on soil fertility status was almost similar in all the years of experimentation. Organic farming in coconut includes use of green manure, green leaf manures, vermicompost, CCP, animal manures, rock phosphate etc., to supplement the chemical fertilizers by way of waste recycling. Integrated nutrient management lays emphasis on improving and maintaining soil fertility for sustained productivity (Hameed Khan *et al.*, 2000).

The index leaf nutrient (NPK) content was significantly influenced due to added levels of NPK as observed in other parameters (Table 3). Here again, the highest leaf NPK content was observed due to the application of NPK at their highest level *viz.*, 1000, 500, 2000 g NPK, respectively per palm per year. Enhanced nutrient release at the highest level of NPK and its subsequent absorption by the palm ultimately resulted in higher NPK in the index leaf, which may helps in better photosynthesis and leads to better palm productivity. At the highest level of NPK i.e., 1000: 500: 2000 g/palm/year respectively. The index leaf NPK content were also highest i.e., 1.63, 1.62 and 1.65 percent. This proved the beneficial effect of INM enhanced the leaf nutrient content. Similar, observation was earlier made by Ghosh and Bandopadhyay (2009).

For hybrid coconut, a fertilizer level of 1000:250:2000 g NPK/palm/year along with 50 kg of organic manures was found to be economical for achieving higher nut yield and sustaining the soil fertility, out of which 50 per cent nitrogen was supplied through organic manure (composted coir pith / vermicompost). Considering consistent nut yield and sustainable soil fertility, 50 percent of recommended nitrogen may be supplied through organic manure of either vermicompost or composted coir pith and the remaining 50 per cent nitrogen may be applied as fertilizer. While, the soil available NPK and index leaf NPK content was highest at 1000:500:2000 g NPK/palm/year. The organic manure that carries 50 percent nitrogen can satisfy the organic matter requirement of organically poor coconut soils to some extent. This may pave way for the gradual replacement of chemical fertilizer in coconut based cropping system by way of *in-situ* waste recycling.

References

- Ghosh. D.K. and Bandopadhyay. A. 2009. Studies on the influence of integrated nutrient management on growth and yield of young coconut palm. *Indian Coconut J.* **LII (1)**: 17-21.
- Hameed Khan, H., Gopalasundaram, P., Joshi, O.P. and Nelliath, E.V. 1986. Effect of NPK fertilization on the mineral nutrition and yield of coconut genotypes. *Fert. Res.* **10(2)**: 185-190.
- Hameed Khan, H., Upadhyay, A.K. and Palaniswami, C. 2000. Integrated nutrient management of plantation crops. In *plantation crop Res. & Dev. Proc. Placrosym XIV*, CDB Kochi pp 9-22.
- Hameed Khan, H. 2004. Initiatives towards improving coconut productivity. *J. Plant. Crops* **32** (Suppl.):173-185.
- Nadheesha, M.K.F. and Tennakoon, A. 2008. Removal of micronutrients from high and moderate yielding coconut plantations in Sri Lanka. pp. 164-169. In: *Proceedings of Second Symposium on Plantation Crop Research*. Held at Colombo, Sri Lanka 16th -17th October 2008.

Ouvrier, M. and Ochs, R. 1978. Mineral exportation of the hybrid coconut. PB 121. *Oleagineux* 33: 437-443.

Rethinam, P., Antony, K. J. and Muralidharan, A. 1991. Management of coconut root (wilt) disease. Abstract of papers. p.60. In: *Second International Symposium of Coconut Research and Development*, CPCRI, Kasaragod, India.

Sudhakara, K. and Nambiar, P.K.N. 1991. Evaluation of fertilizer recommendations for coconut under farmers' field conditions. *Indian Coconut J.* 4-5.??

Upadhyay, A.K., Srinivasa Reddy, D.V. and Biddappa, C.C. 1998. Organic farming technology for coconut. *Indian coconut J.* 24: 74-78.

Wahid, P.A. 1984. Diagnosis and correction of nutrient deficiencies in coconut palm. *J. Plant. Crops* 12 (2): 98-111.

Table 1. Effect of NPK on yield attributes and nut yield of hybrid (T x D) coconut t

Treatments	No. functional leaves/palm/year			No. bunches/palm/year			Mean annual nut / palm / year		
	06-07	07-08	08-09	06-07	07-08	08-09	06-07	07-08	08-09
T ₁ N ₀ P ₀ K ₀	26.0	25.0	24.2	12	12	11	116	106	102
T ₂ N ₀ P ₀ K ₁₀₀₀	31.6	31.3	30.8	11	12	12	130	148	152
T ₃ N ₀ P ₀ K ₂₀₀₀	32.0	35.0	33.1	12	14	13	142	156	159
T ₄ N ₀ P ₂₅₀ K ₀	32.5	30.0	30.5	11	15	12	138	145	141
T ₅ N ₀ P ₂₅₀ K ₁₀₀₀	32.0	31.0	31.2	12	14	13	140	150	158
T ₆ N ₀ P ₂₅₀ K ₂₀₀₀	30.6	26.0	33.5	12	13	12	142	150	152
T ₇ N ₀ P ₅₀₀ K ₀	30.8	31.0	31.5	11	12	12	132	140	140
T ₈ N ₀ P ₅₀₀ K ₁₀₀₀	31.0	32.0	33.2	11	11	13	135	144	145
T ₉ N ₀ P ₅₀₀ K ₂₀₀₀	32.0	34.0	34.2	13	12	13	133	154	158
T ₁₀ N ₅₀₀ P ₀ K ₀	28.1	24.0	31.0	10	13	12	132	131	132
T ₁₁ N ₅₀₀ P ₀ K ₁₀₀₀	30.9	31.2	32.5	12	13	13	138	138	148
T ₁₂ N ₅₀₀ P ₀ K ₂₀₀₀	32.0	30.6	33.2	12	12	12	145	146	156
T ₁₃ N ₅₀₀ P ₂₅₀ K ₀	30.0	29.2	31.0	13	12	13	138	142	146
T ₁₄ N ₅₀₀ P ₂₅₀ K ₁₀₀₀	32.0	32.0	32.5	13	13	13	147	155	155
T ₁₅ N ₅₀₀ P ₂₅₀ K ₂₀₀₀	33.0	33.2	33.6	13	12	13	150	160	162
T ₁₆ N ₅₀₀ P ₅₀₀ K ₀	30.0	33.0	31.8	12	14	12	130	141	142
T ₁₇ N ₅₀₀ P ₅₀₀ K ₁₀₀₀	33.0	34.4	34.2	12	13	13	135	156	158
T ₁₈ N ₅₀₀ P ₅₀₀ K ₂₀₀₀	33.5	33.8	35.0	13	13	13	139	164	166
T ₁₉ N ₁₀₀₀ P ₀ K ₀	28.0	30.0	30.0	12	13	11	125	136	135
T ₂₀ N ₁₀₀₀ P ₀ K ₁₀₀₀	30.0	29.8	31.2	12	13	12	138	142	156

T ₂₁ N ₁₀₀₀ P ₀ K ₂₀₀₀	30.0	30.6	32.0	12	12	12	140	150	160
T ₂₂ N ₁₀₀₀ P ₂₅₀ K ₀	30.5	31.2	32.5	12	12	12	136	140	142
T ₂₃ N ₁₀₀₀ P ₂₅₀ K ₁₀₀₀	32.0	32.1	33.2	12	14	13.0	150	158	160
T ₂₄ N ₁₀₀₀ P ₂₅₀ K ₂₀₀₀	34.0	30.5	38.2	13	12	13.0	156	172	178
T ₂₅ N ₁₀₀₀ P ₅₀₀ K ₀	30.1	30.6	32.8	10	11	12.0	134	148	142
T ₂₆ N ₁₀₀₀ P ₅₀₀ K ₁₀₀₀	33.0	31.2	33.2	11	12	12.0	146	164	168
T ₂₇ N ₁₀₀₀ P ₅₀₀ K ₂₀₀₀	34.0	31.2	36.0	13	14	13.0	158	176	182
SE / Plot	0.93	0.90	0.96	0.36	0.39	0.41	12.03	13.58	15.46
Gen. Mean	32.71	31.70	33.08	12.28	12.38	14.38	148.2	153.5	156.8
CV (%)	2.23	2.20	2.40	2.91	3.06	3.43	9.54	8.85	11.23
CD(P=0.05) for NPK	1.07	1.06	1.08	0.42	0.44	0.46	13.89	15.69	17.52

Table 2. Effect of NPK on soil fertility (kg ha⁻¹)

Treatments	Available N			Available P			Available K		
	06-07	07-08	08-09	06-07	07-08	08-09	06-07	07-08	08-09
T ₁ N ₀ P ₀ K ₀	116	110	101	11.0	10.2	10.0	130	125	122
T ₂ N ₀ P ₀ K ₁₀₀₀	118	112	111	12.0	11.1	11.6	170	176	178
T ₃ N ₀ P ₀ K ₂₀₀₀	113	117	112	11.3	12.2	12.5	251	262	262
T ₄ N ₀ P ₂₅₀ K ₀	101	110	110	16.1	18.6	18.8	160	130	126
T ₅ N ₀ P ₂₅₀ K ₁₀₀₀	105	118	102	17.1	19.0	19.1	252	180	176
T ₆ N ₀ P ₂₅₀ K ₂₀₀₀	115	106	117	17.9	19.8	19.5	160	255	258
T ₇ N ₀ P ₅₀₀ K ₀	102	108	107	18.5	22.2	22.5	123	132	128
T ₈ N ₀ P ₅₀₀ K ₁₀₀₀	123	101	108	20.0	22.0	23.0	175	178	178
T ₉ N ₀ P ₅₀₀ K ₂₀₀₀	120	112	113	18.0	23.5	23.5	234	242	250
T ₁₀ N ₅₀₀ P ₀ K ₀	143	148	148	20.1	11.0	11.0	126	136	130
T ₁₁ N ₅₀₀ P ₀ K ₁₀₀₀	152	155	152	13.0	11.6	11.5	210	181	182
T ₁₂ N ₅₀₀ P ₀ K ₂₀₀₀	154	161	150	11.3	12.0	12.0	226	248	256
T ₁₃ N ₅₀₀ P ₂₅₀ K ₀	148	150	145	15.4	18.5	19.0	187	132	131
T ₁₄ N ₅₀₀ P ₂₅₀ K ₁₀₀₀	155	162	142	14.4	18.2	18.5	215	178	180
T ₁₅ N ₅₀₀ P ₂₅₀ K ₂₀₀₀	144	160	150	15.9	17.6	20.0	230	252	262
T ₁₆ N ₅₀₀ P ₅₀₀ K ₀	150	154	152	17.2	22.1	23.6	172	130	136
T ₁₇ N ₅₀₀ P ₅₀₀ K ₁₀₀₀	143	162	146	19.2	21.5	22.5	187	182	182
T ₁₈ N ₅₀₀ P ₅₀₀ K ₂₀₀₀	148	168	142	19.9	20.5	23.0	244	261	263
T ₁₉ N ₁₀₀₀ P ₀ K ₀	160	178	178	13.0	11.0	11.8	168	138	132
T ₂₀ N ₁₀₀₀ P ₀ K ₁₀₀₀	164	181	181	12.9	10.5	12.0	187	190	178

T ₂₁ N ₁₀₀₀ P ₀ K ₂₀₀₀	167	175	192	13.0	11.2	12.4	250	258	266
T ₂₂ N ₁₀₀₀ P ₂₅₀ K ₀	167	192	199	16.6	18.0	18.0	168	130	130
T ₂₃ N ₁₀₀₀ P ₂₅₀ K ₁₀₀₀	168	190	201	17.6	19.0	19.1	218	178	176
T ₂₄ N ₁₀₀₀ P ₂₅₀ K ₂₀₀₀	162	188	211	18.1	19.8	19.2	255	252	256
T ₂₅ N ₁₀₀₀ P ₅₀₀ K ₀	175	190	201	19.1	21.5	21.2	192	132	132
T ₂₆ N ₁₀₀₀ P ₅₀₀ K ₁₀₀₀	158	170	208	23.1	22.2	22.0	230	176	180
T ₂₇ N ₁₀₀₀ P ₅₀₀ K ₂₀₀₀	165	188	206	22.2	21.6	22.6	243	246	258
SE / Plot	17.26	19.20	21.22	0.38	0.31	0.34	18.1	19.6	17.1
Gen. Mean	161.2	171.8	178.2	14.2	13.2	15.2	201.2	208.5	211.2
CV (%)	14.81	16.41	18.72	12.1	12.8	13.6	10.86	10.16	10.46
CD(P=0.05) for NPK	22.52	26.53	26.45	0.88	0.80	0.91	16.12	17.62	18.15

Table 3. Effect of NPK on leaf nutrient content (percent)

Treatments	Nitrogen			Phosphorus			Potassium		
	06-07	07-08	08-09	06-07	07-08	08-09	06-07	07-08	08-09
T ₁ N ₀ P ₀ K ₀	0.91	0.90	0.94	0.13	0.14	0.13	0.81	0.85	0.84
T ₂ N ₀ P ₀ K ₁₀₀₀	1.20	1.21	1.26	0.12	0.11	0.12	0.91	0.94	0.92
T ₃ N ₀ P ₀ K ₂₀₀₀	1.52	1.50	1.58	0.12	0.13	0.14	0.86	0.86	0.87
T ₄ N ₀ P ₂₅₀ K ₀	1.17	1.12	1.14	0.15	0.16	0.17	0.88	0.87	0.83
T ₅ N ₀ P ₂₅₀ K ₁₀₀₀	1.23	1.21	1.31	0.16	0.18	0.18	0.91	0.96	0.95
T ₆ N ₀ P ₂₅₀ K ₂₀₀₀	1.61	1.51	1.41	0.13	0.16	0.17	0.92	0.95	0.94
T ₇ N ₀ P ₅₀₀ K ₀	0.86	0.80	0.86	0.17	0.17	0.17	0.86	0.88	0.89
T ₈ N ₀ P ₅₀₀ K ₁₀₀₀	1.21	1.11	1.14	0.18	0.19	0.18	0.90	0.94	0.96
T ₉ N ₀ P ₅₀₀ K ₂₀₀₀	1.61	1.51	1.60	0.19	0.19	0.19	0.91	0.91	0.93
T ₁₀ N ₅₀₀ P ₀ K ₀	0.28	0.29	0.29	0.15	0.17	0.18	0.92	0.96	0.95
T ₁₁ N ₅₀₀ P ₀ K ₁₀₀₀	1.26	1.16	1.19	0.13	0.14	0.15	0.96	0.96	0.97
T ₁₂ N ₅₀₀ P ₀ K ₂₀₀₀	1.58	1.52	1.42	0.12	0.10	0.13	0.92	0.90	0.89
T ₁₃ N ₅₀₀ P ₂₅₀ K ₀	1.10	1.13	1.18	0.13	0.12	0.11	0.89	0.91	0.97
T ₁₄ N ₅₀₀ P ₂₅₀ K ₁₀₀₀	1.36	1.31	1.41	0.16	0.18	0.16	0.98	0.96	0.98
T ₁₅ N ₅₀₀ P ₂₅₀ K ₂₀₀₀	1.62	1.53	1.56	0.15	0.18	0.19	0.95	0.98	0.94
T ₁₆ N ₅₀₀ P ₅₀₀ K ₀	1.03	1.01	1.13	1.16	0.19	1.19	0.91	0.94	0.93
T ₁₇ N ₅₀₀ P ₅₀₀ K ₁₀₀₀	1.20	1.01	1.21	0.19	0.18	0.16	0.98	0.96	0.97
T ₁₈ N ₅₀₀ P ₅₀₀ K ₂₀₀₀	1.56	1.46	1.45	0.21	0.24	0.23	0.97	0.98	0.87
T ₁₉ N ₁₀₀₀ P ₀ K ₀	0.98	0.94	0.98	0.12	0.13	0.15	0.99	0.91	0.92

T ₂₀ N ₁₀₀₀ P ₀ K ₁₀₀₀	1.26	1.16	1.26	0.13	0.14	0.16	1.10	1.11	1.13
T ₂₁ N ₁₀₀₀ P ₀ K ₂₀₀₀	1.48	1.38	1.39	0.15	0.16	0.18	1.16	1.12	1.15
T ₂₂ N ₁₀₀₀ P ₂₅₀ K ₀	1.16	1.18	1.28	0.16	0.17	0.17	1.18	1.16	1.19
T ₂₃ N ₁₀₀₀ P ₂₅₀ K ₁₀₀₀	1.28	1.22	1.26	0.17	0.18	0.17	1.20	1.21	1.20
T ₂₄ N ₁₀₀₀ P ₂₅₀ K ₂₀₀₀	1.60	1.56	1.66	0.19	0.17	0.18	1.20	1.24	1.25
T ₂₅ N ₁₀₀₀ P ₅₀₀ K ₀	1.00	1.06	1.07	0.18	0.17	0.19	1.18	1.06	1.08
T ₂₆ N ₁₀₀₀ P ₅₀₀ K ₁₀₀₀	1.31	1.38	1.39	0.20	0.23	0.24	1.21	1.24	1.23
T ₂₇ N ₁₀₀₀ P ₅₀₀ K ₂₀₀₀	1.63	1.62	1.65	0.19	0.18	0.19	1.20	1.24	1.22
SE / Plot	0.15	0.18	0.19	0.01	0.01	0.02	0.04	0.05	0.06
Gen. Mean	1.26	1.21	1.28	0.16	0.16	0.18	0.99	1.01	1.08
CV (%)	12.20	11.20	13.20	9.15	9.14	9.26	4.00	4.06	4.10
CD(P=0.05) for NPK	0.18	0.17	0.19	0.02	0.02	0.03	0.05	0.06	0.08

Table 4. Effect of NPK levels on yield and yield attributes of coconut and soil fertility

Level of NPK	No. of functional leaves/palm	No. of bunches/palm/year	No. of buttons/bunch	Nut yield palm/year	Available nutrients (kg ha ⁻¹)
2006-07					
N ₀	30.9	11.7	23.2	134	1123
N ₅₀₀	31.3	12.2	26.6	139	149
N ₁₀₀₀	31.3	12.9	27.7	143	165
P ₀	29.8	11.7	22.9	134	13.0
P ₂₅₀	31.8	12.3	27.5	144	16.6
P ₅₀₀	31.9	11.8	26.9	137	19.7
K ₀	29.6	11.4	24.9	131	158
K ₁₀₀₀	31.7	11.8	25.5	140	205
K ₂₀₀₀	32.3	12.6	26.9	145	243
2007-08					
N ₀	30.6	12.8	23.8	146	110
N ₅₀₀	31.1	12.8	23.6	149	157
N ₁₀₀₀	31.8	12.6	25.9	155	184
P ₀	29.7	12.7	23.5	142	11.2
P ₂₅₀	30.6	13.0	28.2	153	18.6
P ₅₀₀	32.2	12.4	25.7	153	21.9
K ₀	29.3	12.7	24.9	136	132
K ₁₀₀₀	31.6	12.8	26.0	153	180

K ₂₀₀₀ 2008-09	31.6	12.7	25.7	161	253
N ₀	31.4	12.3	23.6	146	109
N ₅₀₀	32.8	12.6	25.9	152	148
N ₁₀₀₀	32.9	12.2	29.4	158	198
P ₀	30.9	12.0	24.0	144	11.7
P ₂₅₀	32.6	12.7	27.3	156	19.0
P ₅₀₀	33.5	12.6	27.4	156	22.7
K ₀	30.6	11.9	24.4	136	130
K ₁₀₀₀	32.4	12.7	26.4	156	179
K ₂₀₀₀	34.0	12.7	28.0	165	259

Coconut based farming system

V. Krishnakumar

Central Plantation Crops Research Institute
Regional station, Kayamkulam-690 533, Kerala, India

The coconut palm is a versatile tree crop and no other tree crop grown can match coconut palm in its versatility. It is one of the most important food and cash crops grown in tropical belt of the world. It is widely adapted to the environments and climatic conditions of many producing countries, notably occupying over 20 % of the available arable land in most of these countries. It is a major contributor to the economies of many Asian and Pacific Coconut Community member countries or APCC as well as other producing countries. Coconut is a traditional plantation crop of India, assuming the status of a high value commercial crop with an annual production of 14743.56 million nuts (2007-08). In India, most of the cultivated area under coconut palm (90 %) lies in the four southern states i.e., Kerala, Tamil Nadu, Karnataka, and Andhra Pradesh. Kerala ranks first in area (43.0 %) and production (38.0 %) followed by Tamil Nadu, Karnataka and Andhra Pradesh. The palm is amenable to both plantation and homestead management and it can be either a major crop or a minor one in a homestead garden of mixed crops. It can adapt to the divergent farming situations and management practices that are prevalent in the different agro-climatic regions. Coconut is mostly a crop of small farmers in India, being grown as a component crop of the homestead farm, where the average size of a holding is about 0.2 ha. More than 90 % of the five million coconut holdings in the country are less than one ha in size. The income derived from such small and fragmented holdings is not adequate enough to meet the economic requirements of small families. The total farm productivity is found to be lower than the potential under mono cropping in coconut. Moreover, the farmers are under employed engaged only for 100 and 120 man-days under rainfed and irrigated systems. Coconut gardens provide excellent opportunities to exploit the inter-space potential for maximizing returns per unit area. Coconut based cropping/farming systems, involving cultivation of compatible crops in the interspaces of coconut and integration with other enterprises like dairying offer considerable scope for increasing production and productivity per unit area, time and inputs by more efficient utilization of resources like sunlight, soil, water and labour.

- I. Inter cropping: It is the practice of growing seasonal, annual and semi- perennial crops in association with coconut in young (0- 5 years) and in mature stand simultaneously on the same area of land in the same year. Among seasonal crops, pulses and cereals, tubers and yams, ginger, turmeric, chillies and vegetables are more common. Banana, pineapple, passion fruit and papaya are the popular semi-perennials. Economic returns are high with banana and pineapple. Vegetables are mostly grown for household consumption.
- II. Mixed cropping: It is a farming system in which perennial crops and multipurpose trees are grown together in mature standoff coconut of 20-25 years and above. Mixed crops are cocoa, coffee, clove, cinnamon, nutmeg etc., and pasture and fodder for live stock.

- III. Multiple cropping: It is an intensive farming system, in which a mixture of compatible crops, either annual/perennial or annual/semi-perennial or annual/semi-perennial/perennial is grown together with coconut. This system is particularly popular among small holders due to scarce land resource. The production data from such system indicates improvement in coconut yield as a result of the benefits accrued from this system of cropping. The benefit cost ratio of such system will also be high.
- IV. Sequential cropping: It is the time dependent form of multiple cropping, where growing two or more crops in sequence on the same area of land in the same year is practiced. There is no intercrop competition and farmers manage only one crop at a time on the same area of land..
- V. Mixed farming: This is a farming system where livestock, mainly cattle and small ruminants such as goats form the major component in the production system. Fodder crops such as Hybrid Napier, Stylosanthes, fodder cowpea etc. can be raised as intercrops in coconut garden. At present cultivation of Hybrid Bajra Napier grass (CO-3) is becoming popular among the coconut farmers adopting mixed farming. Integrating coconut cultivation with rearing milch animals and raising fodder crops ensures additional income and employment to the farmers. They also provide organic manures for application to coconut and other crops. Installation of biogas plant gives light energy for farm family and slurry for use in the cropping system. Azolla, a floating fern can also be raised in small pits lined with polythene sheets in coconut gardens adopting mixed farming to supplement the feed requirement of dairy animals.
- VI. Multi-storeyed cropping: This is used to refer the multispecies crop combination involving annuals and perennials with an existing stand of a perennial. In the case of multi-storeyed cropping, the crops selected should have small, medium and large canopy architecture and are to be planted in a systematic manner to exploit space both in the vertical and horizontal dimensions. For example in a multi-storey cropping system of coconut, pepper/cocoa, cinnamon and pineapple, the air space is utilized vertically at four levels: the coconut crown occupying the highest level, at the lower level pepper has its canopy from 2-8 m on the coconut trunk, the canopy of cocoa and cinnamon is pruned and maintained at about 3.5 m above the ground level and pineapple occupies the lowest level near the ground. All these crops are so spaced horizontally that the solar energy incident on the plantation is intercepted at different canopy heights and utilized more efficiently by the foliage of these crops occupying different floors of the system. The root system of all these crops will also have different foraging zones and thus help in mining nutrients from different soil layers.
- VII. High density multispecies cropping system (HDMSCS): It is the simultaneous cultivation of large number of species (annual, biennial and perennial) of crops having canopies of various heights (small, medium and large) and rooting pattern in the same field at higher plant density. This system ensures effective utilization of natural resources such as land, water, light and space and provides biomass for recycling and maximum returns per unit area. It will not only ensure regular

economic returns to the farmers but also provides more employment opportunities for the farm family. The most profitable HDMSCS with coconut as main crops would be inclusion of black pepper, cocoa, pineapple, banana and various vegetable crops as component crops with canopy at different heights. In the system, black pepper is allowed to trail on coconut trees, cocoa seedlings are planted between rows of coconut and pineapple and banana suckers and vegetables planted between rows of coconut and cocoa. These crops will also supply large quantities of recyclable biomass and proper utilization of which can supplement the nutrient requirement to a considerable extent.

Coconut based cropping/farming system

Coconut-based farming systems (CBFS): Here the integration of complementary enterprises in coconut farming (e.g. inter cropping, livestock integration, processing of coconut products (by-products and intercrops and marketing) to increase productivity per unit area, increase employment opportunities and provide a buffer against low and fluctuating coconut prices are aimed at. CBFS aims at intensive cropping through suitable crop diversification in the inter space available in coconut gardens to increase the per palm productivity as well as productivity of unit holding in a system approach. Here all the available farm resources like soil and water/rainfall resource, farm labour, other agricultural inputs etc., are utilized to the maximum potential for economic production i.e., both nuts, food (both crops and livestock) and non-food agricultural products from the farm. In CBFS, all management practices and components of production systems should achieve higher productivity, profitability and sustainability of the existing stands of coconut palms to maximize the economic yields. The rationale for adoption of farming systems in coconut plantations is to promote effective use of land and other resources aimed at increasing the productivity using minimum external inputs without deleting the natural resources. The success of **CBFS** depends on the environmental (e.g. rainfall, soil); economical (e.g. marketability, profitability); and sociological (e.g. use of family labour) factors.

Suitability of coconuts for inter cropping

For any inter/mixed cropping system to be successful by providing economic yields, the crops selected and planted should receive at least a minimum amount of sunlight for growth and yield. This is possible in a coconut garden due to the characteristic growth nature of coconut palms, i.e., tall unbranched tree, with a terminal crown of leaves, growing to a height of about 20-30 m and with a life span of 80-100 years.

Inter cropping of coconut is a way of increasing productivity by planting interspaces of a coconut garden. Thus, crop production per unit area is usually increased by using the same piece of land with no other crop or land use being changed. This simultaneous growing of several crops in coconut areas offers several advantages over mono cropping, such as increased land utilization, increased productivity per unit area due to yield of intercrops and increased coconut production, more efficient use of farm labour, and increased overall farm income.

Bio-physical basis of intercropping

Crop morphology and planting system: In coconut monoculture, where the planting distance adopted is generally in the range 7 m × 7 m to 10 m × 10 m for tall cultivars, as little as 25 %

of the land only may be effectively utilized. The morphological features of the coconut plants indicate that the palms may occupy less than 30–40 % of the available air space between canopy and ground during the major part of their life span. The wide spacing intended to meet the resource requirement of trees at maturity, and the sub optimal utilization of site resources by palms at maturity make this crop particularly suitable for poly culture systems.

Plant spacing and light transmission: The unique architecture of the coconut fronds permits a larger part of the solar energy to penetrate below the canopy. The extent of sunlight reaching the ground varies with the age of coconut tree, number of functional leaves in the crown, distance between trees and geometry of planting. It starts with a high light transmission (80 %) at initial year of planting, 50 % at five years and lowest at 10-15 years; and a clear consistent increase starting 20 years. By the time the palms reach 40 years old, light transmission increases by 50 % due to taller trunks (15-20 m) and drooping leaves. The nature of the canopy of coconut fronds and the proportion of incoming solar radiation reaching the ground permits on average some 56 % of solar radiation for intercrops.

Root system: The active root system of the coconut is found normally to cluster within 2 m from the base of the trunk and vertically between 30-130 cm depth. The upper 30 cm of soil has very limited functional roots. Coconuts are spaced 7-10 m apart to suit the large canopy of the crop as well as to minimize the overlapping of palm leaves of adjacent trees. These account for the significant under-utilization of the soil resource in a coconut mono cropped area.

Based on the growth habit of the palm and the amount of light transmitted through its canopy, the life span of coconut palm could be divided into three distinct phases.

1. Planting till full development of canopy (about 5- 8 years): A major portion of the solar energy is not being intercepted by coconut leaves and hence, good transmission initially. As the palm canopy develops with age, the percentage of utilization increases progressively during the pre-bearing period, with a corresponding decrease in transmission of light making it suitable for growing annuals/biennials as intercrops.
2. Juvenile palms (8 to 25 years): As the palms grow with production of more leaves, maximum ground coverage takes place and hence, moderate to poor light availability. Not congenial for multiple cropping. However, shade loving/tolerant intercrops could be cultivated.
3. Mature palms (more than 25 years): Increase in trunk height and reduction in crown size takes place. Light transmission increasing with age and the apparent ground coverage by the coconut canopy decreases progressively. Hence, ideal for raising annual and / or perennial crops as multiple and multi-storeyed cropping models.

In any cropping system model, careful attention should be given for management of different crops in order to exploit their genetic potential and available resources. The success or failure of a CBFS is often decided by the choice of the system components. Compatible crops should be identified based on the reactions to intensity of sunlight/shade available in the coconut gardens (Table 1).

Table 1. Classification of crops based on tolerance to sunlight/shade for intercropping (Vikraman Nair *et al.*, 2003)

Sl. no.	Parameter	Effect on production/remarks	Example of crops
1	Non-tolerant to even slight shade	Drastic decline in yield even under slight shade. Cannot be grown successfully as intercrop	Rice, Bhendi, sweet potato, leguminous plants, ground nut
2	Non-tolerant to shade	Yield decline increases with intensity of shade. Can be grown in border areas where more sunlight is available	Coleus, Brinjal, Tomato, Chilli, Dioscorea, Nendran banana
3	Tolerant to shade	Slight reduction in yield due to shade. Can be grown successfully when 50 % sunlight is available	Colocasia, Banana varieties other than nendran
4	Shade loving	Yield increases to some extent with increase in shade. More ideal as intercrop than sole crop	Amorphophallus, Ginger, Turmeric, Arrow root, Kacholam

Relevance of coconut based farming systems

An agronomically desirable cropping system should ensure that all the components of production are utilized to the optimal level ensuring that the long term production capability of the system as a whole is not affected in any manner. Study of the response of component crops and the environment of the farming system under a given situation using various parameters is of utmost importance for understanding the dynamics of the systems and making mid-term corrections for their improvement.

The practice of CBFS can provide:

Social benefits: As was mentioned elsewhere coconut is the main component crop of the homestead farms. Coconut functions as the main source of income for the farmer. Thus any social benefit of the CBFS should reflect on the well being of farm families. This is possible through food sufficiency culminating on food and nutritional security. With CBFS, the same coconut growing area can be used to produce various crops(such as given in table 2 and below), which can satisfy varied needs of the coconut farmers.

- 1) leafy and fruit vegetables (as rich sources of vitamins, minerals and dietary fibres);
- 2) legumes (as source of protein and vegetable fats and oils);
- 3) fruit crops (as rich sources of vitamins and minerals, and carbohydrates);
- 4) root crops (as sources of carbohydrates and minerals);
- 5) cereals (as source of carbohydrates, protein, fats and oils);
- 6) spice crops (food flavouring, and vitamins and minerals);
- 7) coffee and cocoa (beverage and stimulants);
- 8) wood, timber and miscellaneous trees (housing materials, pulp and paper and fire wood).

Ecological benefits: Compared to the ecological conditions of the long-term mono cropping, lands put under CBCS are more favourable and stable for intensive and sustainable agricultural production. This is mainly possible as a result of more efficient utilization of the land resources due to 1) maximization of solar energy capture and utilization; 2) optimization of soil moisture use and conservation capabilities; 3) enhancement of soil fertility and microbial population build up due to higher biomass recycling over a period of time; and 4) minimal soil erosion and nutrients losses chiefly due to effective and efficient crop canopies and root systems.

Soil and water conservation: By keeping the coconut garden covered through crops grown in the lower strata, the direct impact of rainfall and the separation of soil particles under the coconut environment can be minimized. The presence of undergrowth vegetation in coconut plantations minimizes soil and water loss through surface runoff. The micro climate in coconut garden also allows other crops to grow favourably in the inter spaces of palms. An adequate ground cover can also increase rainwater infiltration and storage, eventually increasing water supply of the entire area. Ground covering through suitable intercrops can also reduce weed growth and thereby loss of nutrients and soil moisture.

Farm/enterprise diversification: In coconut mono-cropping system, only about 25% of the soil mass is normally utilized by the crop, the remaining 75% serves as the land resource or habitat for intercrops or other compatible farm operations in the production system. Quite apart from diversified production and acting as an insurance against crop failures, a well maintained CBFS will definitely ensure biodiversity conservation in managed ecosystems, which has become a vital issue in the present day context. Adoption of mixed farming by integration of animal component can also enhance farm diversity. The ecological value of the opportunity provided by coconut to grow an intercrop or several intercrops in mixed and multi-storey CBFS is the mean value of the products of intercrops and other components that could stabilize economic incomes of farmers. It could also be possible to considerably enhance employment generation from farm diversification.

Soil fertility build up: In CBFS with inclusion of crops and other animal husbandry components, there is lot of opportunity for organic recycling. Nutrient removal through harvested produce could be compensated by nutrient input through manure, fertilizer, recycled crop residues and tree nutrient cycling processes. The tree components by virtue of their deep roots intercept, absorb and recycle nutrients that would have been otherwise lost by leaching. Furthermore, there is possibility that the dense canopy cover provided by various trees/crops help to conserve the nutrient pool without considerable loss through mineralization that often takes place in an open field.

Factors deciding success of cropping system

Selection of suitable component crops: CBFS are very complex with a very sophisticated structure and components of various types (crops, animal husbandry etc.). Farmers who manage the farming system are guided mostly by their own perceptions and convictions about species selection, admixture and management (mostly family needs) so that each holding is a specialized entity itself. It is a judicious combination of both management skill and input use efficiency that decides the sustainable development of the farms.

The choice of intercrop generally depends on factors such as age of the palms, local needs, profitability and agro ecological situations. The success of a cropping system under

coconuts depends on the selection of compatible crop combinations in which each crop will exploit a distinct and different zone of atmosphere and soil so that competition for moisture, nutrients, space and solar radiation are kept to the minimum. They should have different canopy architecture and rooting pattern. Differential root activity under crop mixing not only enables better utilisation of the native and added nutrients but also prevents loss of nutrients from leaching and mobility. In most cases failure of inter/mixed cropping is due to the wrong choice of the crop(s). A large variety of crops belonging to the groups of vegetables, fruit crops, tuber crops, spices and condiments/tree spices, medicinal and aromatic crops, leguminous crops, flowering crops, oilseeds, millets (for low rainfall area), fodder crops etc., have been found suitable for growing as inter/mixed crop under irrigated and rainfed conditions in coconut garden.

Climatic factors: Rainfall and temperature are the most important factors affecting the growth of crop plants. While temperature may be uniform over a large area, rainfall could vary considerably. Coconut may grow well with at least 1,000 mm of evenly distributed annual rainfall. However, the ideal range is 1,500-2,500 mm/year. Annual, biennial, and perennial intercrops requiring less than 1,500 mm rainfall/year would grow well under coconut. Higher amounts of rainfall (e.g. over 3500 mm annually or 300 mm/month) could still be favourable to coconut and its intercrops if the soil is well-drained. Rainfall pattern and distribution are also important in determining the crops to be grown. Early-maturing crops are suited for areas with short rainy season. Drought-tolerant crops can be planted where water availability is limited. Vegetables like cowpea and radish could also be harvested in the middle of the wet season. Irrigation should be provided wherever possible for successful multiple cropping system.

Available market of intercrops: Small -scale inter cropping in coconut gardens (mostly in homestead farms) is meant for home consumption. However, if the intercrops are raised for sale, the assurance of a market is of primary importance. If possible, a marketing agreement should be made especially for crops which are highly perishable and have limited marketability.

Availability of quality planting materials: To increase the profitability of intercrops, varieties of selected crop species planted should be high yielding even under reduced sunlight and the microclimatic conditions prevailing in the coconut gardens. The success of an intercropping venture depends much on the availability of high-quality planting materials. If the planting materials used are of poor quality, no matter how good the crop management practises are adopted or how high the inputs are used, this will result in low production, higher production cost and marginal income.

Available post harvest equipment and facilities: Fruits and vegetables, which are perishable intercrops, are to be marketed immediately after harvest to attract better prices or else cold storage facilities should be available. For crops such as cereals, grain legumes, beverages and spices, drying and storage facilities are required to minimize post harvest losses.

Viable technologies of growing intercrops: As productivity and competitiveness of any cropping system depends highly on the right integration of most ideal crops, use of good-quality planting materials, manures and fertilizers, water and crop management including pest and disease management practices, it is necessary to apply the most appropriate technologies at all times to achieve maximum economic returns (i.e. the highest yield at the least cost per unit product). Adoption of farm mechanization, wherever possible will also enhance labour efficiency in the CBFS.

Availability of operating capital (cash or credit): In an agricultural production system, apart from availability of resources such as land and labour; adequate capital to meet the operational expenses on purchase of seeds and other planting materials, fertilizers and manures, timely land preparation and cultivation, irrigation and post harvest processing etc., should be available. Intercropping requires higher investment costs in order to increase farm productivity.

Economics of coconut based farming systems

Adoption of any cropping system by the farming community will ultimately be decided by its economic advantage. The relationship between the main crop and intercrops (and between intercrops) may be complimentary, supplementary or competitive (or positive, negative and independent). The most economically rewarding crop combination will be different, which depends on social, environmental, and economic factors, as well as crop varieties. Much higher employment potential exists for coconut based multistoried and mixed farming systems and it should be relatively more profitable to integrate a number of subsidiary crops and animal components with coconut rather than raising it as a mono crop. A large number of annual and perennial crops have been introduced for feasibility studies and to evolve different crop combinations with coconut. Cultivation of annual crops especially vegetables and spices in different seasons can ensure regular income generation. Perennial crops such as clove, nutmeg, cinnamon, pepper, cocoa etc. require meticulous care during the early stages of their establishment, whereas annuals could be grown as rainfed crops with relatively lesser ease of management. Once they attain bearing stage, these cash crops will provide income through the sale of additional products. Furthermore, coconut based mixed farming system provides considerable safeguard to the smallholder's returns, against the failure of coconut crop as well as the fluctuating prices of nuts and copra. The level of profitability also depends on the degree of intensification, suitability of the crops to the farm environment, level of investment and management. The scope and advantages for mixed farming in coconut garden involving cultivation of shade tolerant fodder crops in the inter spaces of coconut and integrating animal enterprises and recycling the by-products obtained is quite high in CBFS. In models where dairy is a component of the farming system, the relative profitability will be more due to direct as well as indirect benefits. In farms where cows are reared, the farmers could be able to meet about 50 to 75% of organic manure requirement of the farm, thereby considerably reducing the cost of cultivation.

Constraints in successful adoption of CBFS

The constraints in coconut farming are found to be multi-dimensional. The growing diminution in the size of the operational holdings and the scattered nature of holdings make coconut farming a non-remunerative vocation. Land reforms and subsequent conferment of land rights on agricultural workers, urbanization, proliferation of nuclear families and conversion of agricultural land for non-agricultural purposes have been instrumental for the fragmentation of agricultural holdings. In spite of research efforts over the last two decades or more leading to development of location specific and ideal CBFS models; various technical, economic and social constraints have led to relatively slow adoption of systematic inter/mixed cropping practices in coconut growing areas.

1. **Low price of agricultural produce.** Recently there has been a price crash for many of the agricultural produce commonly raised in the CBFS as black pepper etc. Even though production by individual farmer could be enhanced, direct marketing by the farmer himself often ends up in failure leading to realization of low price for commodities, but middle men will be playing their role in deciding what price the farmer should get at the production front.
2. **Lack of agricultural labour and high wage rate.** Though family labour could be put into use for some of the unskilled farm operations, timely availability of skilled labour for harvesting crops such as coconut, black pepper and arecanut is a problem and costly and this adversely affects success of management of CBFS.

Future thrust for research and development

1. Results of most of the studies related to inter/mixed cropping in coconut are based from mature coconut stands. Hence, studies are to be conducted to analyse the impact of intercropping and subsequent mixed or HDMSCS from the initial years of panting of coconut plants. Through such an approach, it could be possible to study the long term effect in soil fertility, change in rhizosphere micro climate, allelopathic effects etc. It should also address the Carbon sequestration potential and microbial dynamics of CBFS.
2. Future research and development efforts should be initiated to understand and improve the existing status of the CBFS under different agro-climatic and socio- economic environments of various coconut growing states. Restructuring and refinement of the existing farms are necessary to make reap the potential to the maximum extent. Several options of technical interventions by adopting a farmer participatory approach can achieve conversion of the existing farms into viable operational models.
3. The main bottleneck in the adoption of optimum management practices in CBFS is that the small farmers are burdened with heavy costs of production as well as in the marketing of their produces. To reduce the cost of cultivation, to provide timely credit, to build up marketable surpluses and to integrate the farmer with the market, it is necessary to cluster the farmers in economically viable groups and establish vertical linkages with the processors and retailers. Since a large majority of the coconut farms are less than one hectare in extent, in developing the inter/mixed cropping system, the feasibility and economics including employment opportunities that may be generated and credit requirements for such intensive cropping systems and the size of the holdings should be carefully taken into account. Of the in initial establishment cost, the investment on the intercrops alone becomes recoverable during the course of the year, as the response to inputs in mature coconut accrues only from the third year. This would mean that credit facilities are necessary for successful adoption of this intensive cropping programmes and realization of enhanced agricultural production.
4. Quantification of biomass production potential under varying CBFS models and its overall efficiency in attaining sustainability is necessary. Soil moisture relationship, nutrient cycling capacity and energy balance studies through simulation models in

CBFS are to be investigated. Such systems should also study the soil and plant health management possibilities through organic recycling by vermicomposting and vermiwash production.

Intensification and diversification of small, marginal and sub marginal farms are critical for realizing income and livelihood to coconut farmers. A technical change would be essential to shift from pursuing an exploitative mono cropping or specialized farming system to more diversified farming system with complimentary allied sector activities such as dairy, poultry etc. Adoption of any particular agricultural production system by the farming community will be ultimately determined by factors such as sustainability of the production system, social security and economic benefits. The main consideration for CBFS is basically economics, as against total crop loss and fluctuations in market prices. CBFS have been found to bestow social, cultural and environmental benefits to the coconut farming community. The socioeconomic benefits become more pronounced in the case of small farmers who dominate the coconut community. A rational cropping pattern would look for inclusion of a variety of crops than a single crop so that the cultivation expenses and labour employment are well distributed throughout the year and farmer gets income also accordingly. It should be borne in mind that all the component crops should be given due attention with respect to their requirement of manuring, irrigation, pest and disease management etc., to obtain the best results from CBFS. Crop rotation should also be followed wherever possible with a view to produce diversified commodities for food and nutritional security and to protect the soil from deterioration of fertility.

References

- Magat, S.S. 1998. Intercropping and toddy/sugar production in coconut-based farming systems: Possible integration with germplasm conservation and varietal improvement pp. 57-82. In: Promoting multipurpose uses and competitiveness of the coconut (P.A. Batugal, R. Rao and C. Bong, eds). Proceedings, COGENT/IPGRI, Chumpon, Thailand.
- Nair, P.K.R. 1979. Intensive Multiple Copping with Coconuts in India. Berlin and Hamburg: Verlag Paul Parrey. 135p.
- Rethinam, P and Sivaraman, K. 2008. Poly culture in coconut for humid tropics and coastal plains. Natural Resources India Foundation. 387p.
- Vikraman Nair, R., Pusphakumari, R., Rejeena, S., and Venugopal, V. K. 2003. *Ihtirimanni ninnu othiripanam* (Malayalam) *Kerala Karshakan* 48(11):24-28.

Multispecies cropping system in coconut for East Coast region of Tamil Nadu

**P. S. Subramaniam, S. Mohandas, R. Marimuthu, A. Gurusamy,
C. Natarajan and S. Arulraj**

Coconut Research Station, Tamil Nadu Agricultural University
Veppankulam – 614 906, Tamil Nadu

About 80 percent of the coconut holdings in India are either small or marginal and the average holding size is 0.22 ha. The income derived from such small holdings is quite insufficient to sustain even small families. In this scenario, intercropping in coconut is the best alternative to boost the income of the coconut growers from a unit holding by using horizontal and vertical space more efficiently. Coconut offers scope for introducing a wide range of commercial crops in the inter-space for maximizing productivity per unit area of land and unit time.

Coconut not fully utilizes the basic natural resources like soil and sunlight available in the garden owing to their unique physical structure. The active root zone of coconut is confined to 25 percent of the available land area and hence remaining area could be profitability utilizing for intercrops. In the present context of shrinking valuable agricultural land due to urbanization and industrialization, enhancing unit income from unit holding in unit time is the need of the hour. Raising high value commercial crops as intercrop in coconut can help in sustaining the income of coconut growers.

The main emphasis in planning coconut based cropping system model are the soil, climate, irrigation source, age of palm, labour, capital and market risk. These criteria are to be strictly adhered to while selecting the intercrops. The subsidiary crops should be selected in accordance with their shade tolerance and solar radiation requirement. Besides, they should have a shorter life span than the main crop. The systems like intercropping, mixed cropping, multistoried cropping, high density cropping are the different systems that can be adopted in a coconut garden. Intercropping in coconut with annual and perennial crops can be chosen in such away that it should not compete with coconut (main crop). To assess the performance different fruits, vegetables and spices in coconut, a field experiment was conducted at Coconut Research Station, Veppankulam between 2003 and 2009. The experimental soil is sandy loam with the pH of 7.7 and EC of 4.1. The soil was low, medium and high in its NPK status. The average rainfall was 1120 mm distributed over 52 rainy days. The experiment was carried out in 27 years old ECT palm spaced at 7.5m x 7.5 m. The whole cropping system was irrigated based on the field capacity of the soil. The required quantities of nutrients for coconut as well as for the intercrops were applied as per the recommendations. All the intercrops were raised in the inter space available (50%) in between two rows of coconut palms in all the years (2003-2009). The plot size was 18 cents per model and area covered either side of the 5 coconut palms. The need based plant production measures were adopted. The nut yield was recorded to assess the effect of intercropping system on coconut. The intercrops yield were recorded and economics were worked out and also the nut equivalent ratio of intercropping systems. The soils of pre and post experiment were analyzed for its fertility and microbial load. The cultural requirements of the cropping system are presented in Table 1.

Table 1. Cultural requirements of intercropping system in coconut

Models		Variety	Cropping period	Spacing	Population (nos./ha)
I	Coconut mono crop	ECT (coconut)	-	7.5 mx7.5 m	175
II	Coconut + black pepper + Banana	Robusta (banana)Panniyur 1(black pepper)	July-May	2.0 mx2.0 m	1050
III	Coconut + black pepper +banana + bhendi/brinjal	Arka Anamica(bhendi) PLR 1(brinjal)	Jan. - May June- Nov	45 cmx30 cm	37000
IV	Coconut + black pepper + banana + elephant foot yam/ greens	Sri Padma(elephant foot yam) Local(greens)	May- March	75 cmx75 cm	9000
V	Coconut + black pepper + banana + turmeric/greens	BSR- 2(turmeric)	June-March	50 cmx15 cm	66000
VI	Coconut +black pepper + banana + pineapple	Mauritius (pineapple)	June-June	90 cmx60 cm	9250
VII	Coconut + black pepper + banana + tapioca	Local (tapioca)	May-March	75 cmx75 cm	9000
VIII	Coconut + black pepper + banana + mango ginger	Local (mango ginger)	June-March	50 cmx15 cm	66000

Based on the economic yield of each intercrop in a model and cost of cultivation, economics were worked out for different models in accordance with prevailing market prices (Table 2). Among these, model IV (coconut + black pepper + banana + elephant foot yam + greens) recorded the maximum net return of Rs. 57,577 with the B: C ratio of 2.16 followed by model II (banana + black pepper), which recorded the net return of Rs. 45,557 with the B: C ratio of 2.08. According to the soil, irrigation and scope for marketing, the coconut farmers can chose any one of the above model of their convenience for enhancing his farm revenue.

Table 2. Economics of intercropping system in coconut

Model	Intercrop	Average yield (kg ha ⁻¹)	Incom (Rs.)	Cost of cultivation (Rs.)	Net return (Rs.)	B:C ratio (Rs.)
I	Monocrop					
II	Banana + black pepper	14520 43.75	87587	42000	45557	2.08

III	Banana+	43.75	65507	40176	25331	1.63
	Black pepper+	5270				
	Bhendi	4500				
IV	Black pepper +	43.75	107167	49590	57577	2.16
	Banana+	6100				
	Elephant foot yam+	8360				
	Greens	650				
V	Black pepper +	43.75	71222	46458	24764	1.53
	Banana +	5660				
	Turmeric +	5250				
	Greens	540				
VI	Black pepper +	43.75	57937	30970	26577	1.87
	Banana +	5450				
	Pineapple	3100				
VII	Black pepper +	43.75	56657	31300	25357	1.81
	Banana+	5520				
	Tapioca	7700				
VIII	Black pepper +	4375	65440	32500	32940	2.01
	banana +	5240				
	Mango ginger	8500				

The nut equivalent yield (NEY) for the different intercropping systems were worked out based on the component crops yield and average nut yield of the experimental area. The nut equivalent yield for the coconut based cropping system as a whole was 53,124 (Table 3).

Table 3. Nut equivalent yield for the coconut based cropping system

Component crop	Inter crop yield (kg 0.5 ha ⁻¹)	Unit price (Rs. kg ⁻¹)	% area of Inter crop	Inter crop yield (kg ha ⁻¹)	Gross return (Rs.)	NEY ha ⁻¹	NEY s for the system
Banana	18417	5	13.5	4972.59	24862.95	4972.59	53124
Bhendi	4062	3	13.5	1096.74	3290.22	658.04	
Elephant foot Yam	10172	12	13.5	2746.44	32957.28	6591.45	
Turmeric	7748	12	13.5	2091.96	25103.52	5020.70	
Pine apple	6100	7	13.5	1647	11529	2305.8	
Pepper	612.5	100	13.5	165.38	16538	3307.6	
Tapioca	8977	3	13.5	2423.79	7271.37	1454.27	
Mango ginger	10500	10	13.5	2835	28350	5670	
Coconut nut yield						29980.45	
						23143.75	

The influence different intercropping models on the main crop *viz.*, coconut was assessed. The increase in cumulative nut yield was 24.28 percent in model IV (coconut + black pepper + banana + elephant foot yam + greens) followed by model III (Coconut + Banana + black pepper + bhendi), where the percent increase in nut yield was 23.07 while in mono cropping (Model I), it was only of 1.1 percent (Table 4 and Fig.1.)

Table 4. Influence of intercropping system on coconut yield (palm/year)

Model	Pre-exptl. nut yield (98-03)	Nut yield						Cumulative mean nut yield (03-09)	%Increase over pre-exptl. nut yield
		03-04	04-05	05-06	06-07	07-08	08-09		
I	88	84	96	91	93	109	61	89	1.10
II	98	97	105	108	121	162	75	111	13.26
III	91	95	111	97	93	137	141	112	23.07
IV	70	74	86	75	77	128	92	87	24.28
V	69	60	68	75	87	85	82	76	10.14
VI	96	90	113	102	107	129	91	105	9.37
VII	59	55	62	62	69	105	81	72	22.0
VIII	120	140	147	126	121	203	81	136	13.33

The soil fertility status *viz.*, NPK availability in soil were analyzed after the experimental period (6 years) and compared with the pre experimental fertility status. (Table5). The numerical increase in all the three major nutrients was observed over the pre-treatment observation. The available NPK status was higher (281, 8.1, 139 kg ha⁻¹) in model III (coconut + banana + black pepper + bhendi). With regard to soil microbial

load, the model IV (coconut + black pepper + banana + elephant foot yam + greens) recorded the maximum fungal and bacterial population of 3.33 and 12.30 x 10⁵ cfu / g dry soil, respectively. In model I (coconut monocrop), the fungi population was the lowest (1.66 x 10⁵ cfu / g dry soil) and the model II (coconut + banana + black pepper) recorded the lowest bacterial population (1.66 x 10⁵ cfu / g dry soil).

Influence of CBMSCS models on the nut yield of coconut

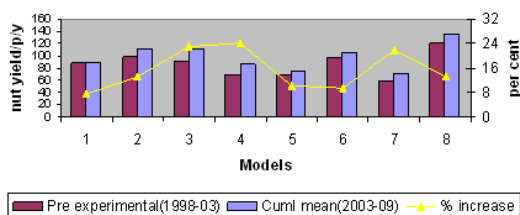


Fig.1. Influence of intercropping system on coconut yield

Table 5. The soil health under coconut based cropping system

Available nutrients (kg ha ⁻¹)							Population (cfu x 10 ⁵ g ⁻¹ dry soil)	
Model	Nitrogen		Phosphorus		Potassium			
	Pre-treatment	2009	Pre-treatment	2009	Pre-treatment	2009	Fungi	Bacteria
I	260	263	7.3	7.7	121	128	1.66	2.33
II	252	265	6.8	7.9	115	131	2.00	1.66
III	270	281	7.2	8.1	124	139	2.33	8.00
IV	243	261	6.3	7.2	116	138	3.33	12.30
V	263	277	6.4	7.5	128	137	2.66	2.66
VI	253	268	6.9	7.7	131	138	2.00	5.33
VII	265	278	7.2	7.8	127	135	2.00	2.33
VIII	267	275	7.1	7.6	131	138	3.00	7.00

Among the different models consisting of various intercrops evaluated in coconut from 2003-2009, the model IV comprising of coconut, black pepper, banana, elephant foot yam and coriander recorded consistently higher BC ratio followed by the model II (coconut + Banana + black pepper). With regard to soil health, model III (coconut +banana + black pepper + bhendi) recorded the higher soil available NPK and model IV (coconut +black pepper + banana + elephant foot yam + coriander) recorded the highest fungal and bacterial population. By considering the economic advantage, the field feasibility, marketability and economic viability, the model IV (coconut +black pepper + banana + elephant foot yam + coriander) is the best suited intercropping system for coconut garden for the east coast region of Tamil Nadu.

References

- Marimuthu, R. 2005. Multispecies cropping system in coconut garden, *Madras Agric. J.* **92**(7-9):404-406.
- Nair, M. K. and Gopalakrishnan, P. 1990. Coconut based farm family models in India. pp.360-389. In: *Proceedings of the 27th COCOTECH meeting on Coconut Based Farming Systems*. Manila, Philippine, June 25-09 1990.

Second generation coconut based high density multispecies cropping system in Assam

J. C. Nath and K. K. Deka

Horticultural Research Station, Assam Agricultural University
Kahikuchi, Guwahati-781 017, Assam, India

Coconut (*Cocos nucifera* L.) is one of the important plantation crops in Assam and is associated with the religious and cultural life of Assamese people. However, its cultivation is still confined to the homestead gardens. The venetian structure and orientation of adult coconut canopy permits about 55 per cent active radiation to penetrate down (Nelliat, 1979) and the gardens with palms spaced at 7.5 m x 7.5 m could be used effectively by growing different annuals and perennials to generate additional income per unit area. The performance of plantation based high density multispecies cropping system (HDMSCS) was reported by many workers (Nelliat *et al.*, 1979; Bavappa *et al.*, 1986; Rethinam, 1990 and Nath *et al.*, 2008). These systems utilize the resources like land, sunshine, moisture etc. to the maximum extent possible and would add a lot of biomass to the soil in the form of leaf shedding, pruning etc. resulting in addition of nutrients to the system. Though coconut is a non-traditional crop for the north-eastern part of the country, the crop is gaining popularity due to its easy adaptability, wide range of uses and higher economic returns. However, the performance of any specific crop combination under coconut garden has not been studied thoroughly for this region and there is a need to evolve more productive and remunerative coconut based cropping system under Assam condition.

The study was conducted during 2003-04 to 2007-08 at Horticultural Research Station of Assam Agricultural University, Kahikuchi under AICRP on Palms in a twenty eight years old coconut garden (var. Assam Green Tall) spaced at 7.5 m x 7.5 m. The experiment with six treatments viz., T₁ - Coconut + black pepper + ginger, T₂ - Coconut + black pepper + turmeric, T₃ - Coconut + black pepper + brinjal, T₄ - Coconut + black pepper + banana, T₅ - Coconut + black pepper + pineapple and T₆ - Coconut + black pepper (Control) was laid out in RBD with four replications. Intercrops were grown in the interspaces of coconut leaving 2 m radius around the coconut palm basin. The area occupied by the intercrops was 84 per cent of the area of the plot. Recommended cultivation practices were followed for both main crop as well as intercrops. Cultural operations and plant protection measures were undertaken as per need. Observations on yield of the base crop as well as intercrops, inputs used and economic returns were recorded and analyzed.

Soil samples were collected one m away from coconut base at 0-25 cm, 25-50 cm and 50-100 cm depths and leaf samples of coconut were collected from the index leaf (14th leaf) after completion of the experiment. The soil samples were analyzed for pH, OC, N, P₂O₅ and K₂O and leaf samples for NPK content following standard analytical procedures (Jackson, 1967). The serial dilution plating method was followed to enumerate the microbial population. The enumeration of culturable bacteria, fungi and actinomycetes was done using soil extract agar, Martin's rose Bengal agar and Kuster's agar, respectively (Allen, 1957) and the results were expressed as colony forming units (Cfu) per gram dry soil.

Data presented in Table 1 reveal that the mean nut yield of the main crop (coconut) over a period of five years was found to be the highest with intercropping of coconut + pepper + turmeric (15,085 nuts/ha) followed by coconut + pepper + banana (13,475 nuts/ha) and the lowest of 8,750 nuts/ha was obtained under control (coconut + pepper). The mean data on yield of intercrops recorded over five years of experimentation (Table 1) showed that on an average ginger produced 92.35 q/ha/year, turmeric: 177.52 q/ha/year, brinjal: 89.14 q/ha/year, banana: 173.63 q/ha/year, pineapple: 143.76 q/ha/year. The pepper

Table1. Yield of base crop and intercrops under coconut based cropping system

Crop components/ treatments	Yield/ha (Coconut in no. of nuts/ha, intercrops in q/ha)					Average of 5 years
	2003-04	2004-05	2005-06	2006-07	2007-08	
T ₁ = Coconut+ Pepper+ Ginger	9,625 1.10 84.67	12,075 1.31 87.66	12,600 1.57 103.83	13,825 1.40 99.96	14,875 1.68 90.62	12,600 1.41 92.35
T ₂ = Coconut+ Pepper+ Turmeric	11,900 1.28 130.00	14,000 1.52 163.11	150,50 1.84 202.50	17,150 1.90 192.60	17,325 1.80 199.40	15,085 1.67 177.52
T ₃ = Coconut+ Pepper+ Brinjal	10,500 1.02 75.32	11,900 1.26 81.16	12,600 1.40 94.15	13,650 1.44 94.41	16,100 1.89 100.64	12,950 1.40 89.14
T ₄ = Coconut+ Pepper+ Banana	11,375 1.00 155.84	12,425 1.05 158.44	13825 1.30 178.57	14,350 1.22 184.41	14,500 1.78 190.90	13,475 1.27 173.63
T ₅ = Coconut+ Pepper+ Pineapple	9,625 1.08 130.51	10,500 1.52 133.11	11,375 1.41 152.60	12,250 1.37 140.90	13,125 2.04 161.68	11,375 1.48 143.76
T ₆ = Coconut+ Pepper (Control)	8,400 1.01	8,575 1.05	8,925 1.07	9,100 1.00	8,750 1.14	8,750 1.05

production was in the range of 1.40 to 1.67 q/ha/year under different intercrops. The mean yield of intercrops and the yield in the whole system was converted into coconut equivalent yield and presented in Table 2. The total coconut equivalent yield in the intercropping system was significantly higher in coconut + pepper + turmeric (42610 nuts/ha/year) followed by coconut + pepper + ginger (33326 nuts/ha/year), coconut + pepper + brinjal (33018 nuts/ha/year) and the lowest coconut equivalent yield of 10430 nuts/ha/year) were recorded in control (coconut + pepper)

The mean pre-experimental nut yield/palm/year and during the experimental period from 2003 to 2008 revealed that the yield gradually increased over the period after planting of intercrops irrespective of different cropping system (Table 3). The maximum increase (68.62%)

Table 2. Coconut equivalent yield and their economics in intercropping system

Crop components/ treatments	Yield/ha (Coconut: No. of nuts/ha Intercrops: q/ha)	Total coconut equivalent yield (No. of nuts/ha)	Gross return (Rs./ha)	Net return (Rs./ha)	Benefit cost ratio
T ₁ = Coconut+ Pepper+ Ginger	12,600 1.41 92.35 92.35	33,326	1,66,630	1,02,130	1.58
T ₂ = Coconut+ Pepper+ Turmeric	15,085 1.67 177.52	42,610	2,13,048	1,46,549	2.20
T ₃ = Coconut+ Pepper+ Brinjal	12,950 1.40 89.14	33,018	1,65,090	99,590	1.52
T ₄ = Coconut+ Pepper+ Banana	13,475 1.27 173.63	32,870	1,64,350	98,850	1.50
T ₅ = Coconut+ Pepper+ Pineapple	11,375 1.48 143.76	28,120	1,40,600	76,600	1.20
T ₆ = Coconut+ Pepper (Control)	8,750 1.05	10,430	52,150	27,150	1.08
CD (P = 0.05)		8,024			

Table 3. Pre-experimental nut yield of coconut and per cent increase in nut yield over pre-experimental yield

Treatments	Pre- experimental yield (Nuts/ palm/year)	Nut yield after after 5 years (Av. of 5 years) 2003 to 2008	Per cent increase in nut yield
T ₁ = Coconut+Pepper+Ginger	48	72	50.00
T ₂ = Coconut+Pepper+Turmeric	51	86	68.62
T ₃ = Coconut+Pepper+Brinjal	52	74	42.30
T ₄ = Coconut+Pepper+Banana	55	77	40.00
T ₅ = Coconut+Pepper+Pineapple	48	65	35.41
T ₆ = Coconut+Pepper(Control)	46	50	8.69

of nut yield was recorded in coconut + pepper + turmeric followed by coconut + pepper + ginger (50.0 %), while only 8.69 % increase was observed in control (coconut + pepper). The increase in nut yield is due to the beneficial effect of intercrops which include improvement in soil fertility, increased microbial activities, higher interception of sun light, better micro-climate, reduced weed growth etc. Similar results of increase in nut yield of coconut due to impact of intercropping were reported by Maheswarappa *et al.* (1998), Singh *et al.* (2002), Ghosh and Hore (2007) and Nath *et al.* (2008).

The data presented in Tables 4 and 5 revealed the nutritional status of the soil as a function of different intercropping system. The nutrient content was higher in the surface soil as compared to subsurface soil in all the intercropping systems. The soil pH, organic carbon, available N, P and K content of soil were higher in coconut + pepper + turmeric followed by coconut + pepper + ginger, whereas, it was the lowest in control plots (coconut + pepper). The increase in nutrient status of soil with intercropping is attributed to the addition and recycling of organic matter and the manures and fertilizers applied to intercrops. The NPK content in the index leaf of coconut was also significantly higher with intercropping compared to control (coconut + pepper) (Table 6). However, the differences due to treatments were significant with respect to N and K only. The beneficial effect of intercropping may be due to improvement in soil physical, chemical and biological environment which favoured the higher nutrient uptake from the soil (Maheswarappa *et al.*, 1998).

Table 4. Soil nutrient status pH, Organic carbon(%) and available N (kg/ha)

Treatments	pH		Organic carbon (%)		Available N	
	0-25 cm	25-50 cm	0-25 cm	25-50 cm	0-25 cm	25-50 cm
T ₁ = Coconut+ Pepper+Ginger	5.2	5.0	0.89	0.66	297.0	265.4
T ₂ = Coconut+ Pepper+Turmeric	5.5	5.1	0.95	0.70	305.0	285.4
T ₃ = Coconut+ Pepper+Brinjal	5.3	5.0	0.86	0.64	295.0	275.6
T ₃ = Coconut+ Pepper+Banana	5.2	5.0	0.88	0.67	283.6	255.0
T ₅ = Coconut+ Pepper+Pineapple	5.0	4.8	0.82	0.54	277.0	247.2
T ₆ = Coconut+Pepper (Control)	5.0	4.7	0.77	0.51	268.4	225.6
CD (P=0.05)	0.02	NS	0.07	NS	15.27	7.06

Significant differences in microbial counts with respect to bacteria, fungi and actinomycetes were observed due to different intercropping in the basin of coconut garden (Table 5). In general, the population of bacteria, fungi and actinomycetes were considerably more under different intercropping system than the control plot. Among the different

Table 5. Soil nutrient status

Treatments	Available P ₂ O ₅ (kg/ha)		Available K ₂ O (kg/ha)		Microbial population (0-25 cm depth)		
	0-25 cm	25-50 cm	0-25 cm	25-50 cm	Bacteria (10 ⁵ CFU/ g soil)	Fungi (10 ⁴ CFU/ g soil)	Actinomycetes (10 ³ CFU/ g soil)
T ₁ = Coconut+ Pepper+ Ginger	24.0	20.5	241.5	191.0	112.0	52.0	30.6
T ₂ = Coconut+ Pepper+ Turmeric	26.9	22.0	244.2	197.0	128.3	59.5	33.4
T ₃ = Coconut+ Pepper+ Brinjal	22.3	20.4	224.6	204.3	98.6	51.0	28.0
T ₄ = Coconut+ Pepper+ Banana	21.0	19.4	236.3	210.0	104.0	48.1	27.6
T ₅ = Coconut+ Pepper+ Pineapple	20.1	18.8	215.0	198.0	96.0	45.0	21.6
T ₆ = Coconut+ Pepper (Control)	19.5	18.0	196.2	164.0	88.6	25.6	18.5
CD (P=0.05)	1.17	1.02	17.5	11.3	8.25	6.66	2.37

Table 6 . Leaf nutrient status (14th leaf) of coconut in intercropping system

Treatments	N(%)	P(%)	(K%)
T ₁ = Coconut+Pepper+Ginger	2.08	0.15	1.8
T ₂ = Coconut+Pepper+Turmeric	2.24	0.17	2.01
T ₃ = Coconut+Pepper+Brinjal	2.15	0.16	1.99
T ₄ = Coconut+Pepper+Banana	2.19	0.17	2.16
T ₅ = Coconut+Pepper+Pineapple	2.08	0.15	1.95
T ₆ = Coconut+Pepper(Control)	1.71	0.12	1.44
CD (P=0.05)	0.12	NS	0.11

intercropping systems, the maximum population of bacteria, fungi and actinomycetes were observed in coconut + pepper + turmeric cropping system followed by coconut + pepper + ginger and lowest population of these microflora were recorded in control plot (coconut + pepper). It is possibly due to the organic matter recycling in the form of vermicompost would be helpful in proliferation of the microbial activity as vermicompost prepared from plantation wastes was rich in nutrient content and microbial population (Chowdappa *et al.*, 1999). Besides, organic carbon input from crop roots, rhizosphere products and crop residues can have a large effect on soil microbial biomass and its activity, which in turn, affect the ability of soil to supply nutrients to plants through soil organic matter turnover (Bonde and Roswall, 1987).

Intercropping with coconut increased the net returns and benefit cost ratio of the system as compared to control (Table 2). The highest net return and benefit cost ratio of Rs.1, 46, 549/ha and 2.20, respectively were recorded in coconut + pepper + turmeric cropping system followed by coconut + pepper + ginger (Rs.1, 02, 130/ha and 1.58) whereas, the lowest net return of Rs. 27, 150/ha with a benefit cost ratio of 1.08 were obtained in control plot (coconut + pepper).

Acknowledgement: The financial assistance and facilities provided by AICRP on Palms to conduct this study is greatly acknowledged. The authors are thankful to Dr. S. Arulraj, Project Coordinator (Palms), CPCRI, Kasaragod for his constant encouragement and support.

References

- Allen, O.N. 1957. *Experiments in soil bacteriology*. pp. 117. Burgess Publication Co. Minneapolis Minn., U.S.A
- Bavappa, K.V.A, Kailasam, C., Khader, K.B.A., Biddappa, C.C., Khan, H.Hameed, Kasturi Bai, K.V., Ramadasan, A., Sundararaju, P., Bopaiah, B.M., Thomas, George, V., Misra, L.P., Balasimha, D., Bhat, N.T., and Bhat, K.Sharma. 1986. Coconut and arecanut based high density multispecies cropping system. *J. Plantn. Crops* **14**:74-87.
- Bonde, T.A. and Roswall, T. 1987. Seasonal variation of potentially mineralizable nitrogen in four cropping system. *Soil Sci Soc America J.* **51**: 1508-1514.
- Chowdappa, P., Biddappa, C.C. and Sujatha, S. 1999. Efficient recycling of organic wastes in arecanut (*Areca catechu* L.) and cocoa (*Theobroma cacao* L.) plantations through vermicomposting. *Indian J Agric Sci.* **69**: 563-56.
- Ghosh, D.K. and Hore, J.K., 2007. Effect of coconut based cropping system on yield of coconut and nutrient status of leaf and soil. *J. Plantn. Crops* **35**: 76-79.
- Jackson, M.L., 1967. *Soil Chemical Analysis*, Prentice Hall of India (P) Ltd., New Delhi
- Maheswarappa, H.P., and Hedge, M.R., Dhanapal, R. and Sunny, A.F. 1998. Mixed farming in coconut garden- Its impact on soil physical, chemical and biological properties, coconut nutrition and yield. *J. Plantn. Crops* **26**:139-143.
- Nath, J.C., Saud, B.K., Chowdhury, D., Deka, K.K. and Sarma, U.J. 2008. Coconut based high density multispecies cropping system in Assam. *J. Plantn. Crops* **36**:98-102.
- Nelliath, E.V. 1979. Prospect of multiple cropping. In: *Multiple cropping in coconut and arecanut garden*. (Eds.) Nelliath E.V and Bhatt K.S. CPCRI, Kasaragod, Kerala. *Tech. Bull.* **3**:6-23.

- Nelliat, E.V., Gopalasundaram, P., Varghese, P.T. and Sivaraman, K. 1979. Mixed cropping in coconut. pp. 28-34. In: Multiple cropping in coconut and arecanut garden. (Eds.) Nelliat E.V. and Bhatt K.S. CPCRI, Kasaragod, Kerala. *Tech. Bull.* 3.
- Rethinam, P. 1990. Cropping system involving plantation crops. In: *Plantation based multiple cropping system* (Eds.) Bandopadhyay, A.K., Michael, Raj. S., Gangwar, B. and Dagar, J. C. ICAR, Port Blair.
- Singh, S.P., Manjunath, B.L., Hameed Khan, H. and Shalini, Bhanu. 2002. Coconut based high density cropping in Goa. *Indian Coconut J.* **33**: 9-12.

Intercropping with fruits and annual spices in coconut under Konkan condition of Maharashtra

D.D. Nagwekar, V.S. Desai, V.S. Sawant, P.M. Haldankar, S. Arulraj and B.B. Jadhav

Regional Coconut Research Station, Bhatye, Ratnagiri
Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli, Maharashtra, India

The coconut is grown as an irrigated horticultural crop in Konkan region of Maharashtra at a spacing from 6 to 8 m in a square or triangular fashion. The effective root zone of the adult palm is confined laterally within a radius of 1.8m around the base of the palm and about 86 percent of the roots are found between 30 to 120 cm depth from surface (Kushwah, 1973) which provide ample scope for inter planting. Therefore 75 percent of the land in coconut plantation can be available for cultivation of different crops as intercrops. The studies conducted in Konkan region showed that the possibility of growing vegetables and spices (Patil *et al.*, 1992; Nageaker *et al.*, 1997; Nagwekar *et al.*, 2002). The current study was undertaken to develop an appropriate cropping system with fruit crops and annual spices compatible with the coconut, under Konkan condition of Maharashtra.

An investigation was undertaken during 2004-05 to 2008-09 at Regional Coconut Research Station Bhatye, Ratnagiri with five intercrops *viz.* banana, pineapple, ginger, turmeric and tapioca. The experiment was laid out in RBD with four replications and six palms per plot. All the crops were planted 2 m away from the bole on either sides of 47 year old coconut palms planted at a distance of 7.5m x 7.5m in costal sandy soil. Turmeric and ginger were planted on raised beds, Tapioca was planted on mound, banana planted at a distance of 1.8 m x 1.8 m, whereas, pineapple was planted in trenches. The recommended cultivation practices were followed for both main and intercrops. The control block was kept clean and no intercrop was grown in this block. The yield date of each intercrop was recorded after the harvest. The yield data of coconut recorded at quarterly interval was analyzed as per Panse and Sukhatme (1975). The gross returns in Rs. / ha for main and intercrop was worked out by considering the prevailing rates in the local market of Ratnagiri. The cost of production was calculated by considering actual input cost required for each crop. The net returns were calculated as the difference between gross returns and cost of production. The cost benefit ratio was also calculated.

The yield data of coconut before planting (2000-04 four years mean) and after planting (2005-09 for five years mean) per palm and per ha are presented in Table 1. It was revealed that there was no statically significant difference in yield of coconut before and after planting the intercrops. The yield of coconut was in the range of 9,625 to 16,275 nuts/ha before planting intercrops, where as it was in the range of 14,875 to 17,500 nuts/ha after planting the intercrops.

The percent increase in yield of coconut varied after planting the intercrops when compared with yield before planting the intercrops. The highest increase in nut yield was

recorded in coconut + turmeric block (56%) followed by coconut + pineapple (44%), coconut + ginger (33%) and coconut + tapioca (29%) which were statically on par with each other and significantly superior over control *i.e* coconut alone (4%) and coconut + banana model (8%). Similar results of increase in nut yield of coconut due to intercropping system was reported by Nagwekar *et al.* (2002) and Basavaraju *et al.* (2008).

Table 1. Yield of coconut under coconut based cropping system

Sl. No.	Treatment (Model)	Pre treatment period coconut yield 2000-04		Post treatment period coconut yield 2004-09		Percent increase over pre treatment yield
		No. of nuts/ palms	No. of nuts/ ha	No. of nuts/ palms	No. of nuts/ ha	
1.	C+Banana	93	16,275	100	17,500	08
2.	C+Pineapple	59	10,325	85	14,875	44
3.	C+Ginger	73	12,775	97	16,975	33
4.	C+Turmeric	55	9,625	86	15,050	56
5.	C+Tapioca	77	13,475	99	17,325	29
6.	Coconut alone	80	14,000	89	14,525	4
	SE $\pm$	11.07	---	10.4	---	9.18
	C.D(P=0.05)	N.S	---	N.S	---	19.00

NS= Not significant

The yield data of intercrops recorded for five years of experiment period are presented in Table 2. The mean yield data of five years indicated that on an average turmeric produced 12,200 kg raw rhizomes /ha/ year, banana produced 9,800 kg fruits/ha / year, tapioca 97,00 kg tubers/ ha / year, ginger 54,00 kg rhizomes/ha/ year and pineapple 5,390 kg fruits/ha/ year. Further the mean yield of different intercrops converted into coconut equivalent yield revealed that coconut equivalent yield was maximum in turmeric intercropping (24,000 nuts/ha/ year) followed by ginger (22,500 nut/ha/ year), banana (16,333 nuts/ha/ year), pineapple (53,920 nut/ha/ year) and tapioca (4,850 nuts/ha/ year). The mean coconut equivalent yield in intercropping system was higher in coconut + ginger (39,475 nuts/ha/year) followed by coconut + turmeric (39,050 nuts/ha/year), coconut + banana (33,833 nuts/ha/year), coconut + tapioca (22,175 nut/ha/year) and coconut + pineapple (20,265 nuts/ ha/year). However, coconut alone recorded lowest coconut equivalent yield of 14,525 nuts/ha/ year.

Table 2. Coconut equivalent yield in coconut based cropping system 2004 -09 (Mean of 5 years)

Sl. No.	Treatment (Model)	Intercrop yield (kg/ha)	Coconut equivalent yield in coconut based cropping system (No. of nuts / ha)		
			Coconut	Intercrop	Total
1.	C + Banana	9,800	17,500	16,333	33,833
2.	C + Pineapple	5,390	14,875	5,390	20,265

3.	C + Ginger	5,400	16,975	22,500	39,475
4.	C + Turmeric	12,200	15,050	24,000	39,050
5.	C + Tapioca	9,700	17,325	4,850	22,175
6.	Coconut alone		14,525	-	14,528

The data presented regarding economics of coconut based intercropping in Table 3 revealed that the highest net return per ha was recorded by coconut + turmeric (Rs. 1,29,450/- ha/year) followed by coconut + banana (Rs. 1,00,300/- ha/ year), coconut + ginger (Rs. 84,700/- ha/year) coconut + tapioca (Rs. 57,350/- ha/year) and coconut + pineapple (Rs.48,230/- ha/year). The minimum net return per hectare was recorded form the coconut alone (Rs. 28040/- ha/ year)The B.C. ratio was the highest with banana intercropping (2.02) followed by coconut + turmeric (1.83), coconut + tapioca (1.75), coconut + pineapple (1.65) and coconut + ginger (1.56). However, the lowest B.C ratio was observed in coconut alone (1.47).

Table 3. Economic of coconut based intercropping system

Sl. No	Treatment (Model)	Cost of cultivation (Rs/ha)	Gross return (Rs/ha)	Net return (Rs/ha)	Net return of intercrops (Rs/ha)	B:C ratio
1	Coconut+Banana	1,00,300	2,03,000	1,00,300	53,300	2.02
2	Coconut+Pineapple	73,260	1,21,490	48,230	16,980	1.65
3	Coconut+Ginger	1,52,150	2,36,850	84,700	40,850	1.56
4	Coconut+Turmeric	1,56,050	2,85,500	1,29,450	97,150	1.83
5	Coconut+Tapioca	75,700	1,33,050	57,350	11,400	1.75
6	Coconut	58,000	87,150	28,040	-	1.47

Market rate for Coconut and Intercrops

1) Coconut	Rs. 6/nut	2) Ginger	Rs. 25/kg	3) Banana	Rs. 10/kg
4) Pineapple	Rs. 5/ kg	5) Turmeric	Rs. 16/ kg	6) Tapioca	Rs. 3/kg

Considering the performance of different intercrops and market demand, the different intercrops viz., turmeric, banana, pineapple and tapioca are recommended in coconut plantation for Konkan region of Maharashtra.

References

- Basavaraju, T., B., Hanumanthappa, M., Nagaraja Kusagur and Boraiah, B.2008. Coconut based cropping systems for maidan tract of Karnataka, AICRP on palms, Agricultural Research Station, Arsikere-571 103, Karnataka. *J. Plantn Crops* **36** (3): 290-295.
- Kushwah, B.L., Nelliath, E. V., Markose, V.T and Sunny, A.F. 1973. Rooting pattern of Coconut *Indian J. Agron.* **18**: 139 -143.
- Nagwekar, D.D., Haldankar, P.M., Desai, A.G., Gunjate, R.T and Rajput, J.C.1977.Performance of rainfed vegetables as intercrops in coconut plantation. *J. Plantn Crops* **25**(2): 209-211.

- Nagwekar, D.D., Desai, A.G., Joshi, G.D, Magdum, M. B. and Khan, H. H. 2000. Performance of spice crop as intercrops in Coconut Plantation under Konkan Condition. Regional Coconut Research station, Bhatye. Ratnagiri. Konkan Krishi Vidyapeeth In: *Plantation Crops Research and Development in the Millennium*. pp.333-335.
- Panse, V.G. and Sukhatme, P.V. 1978. *Statistical methods for Agricultural workers*. ICAR. New Delhi, pp. 153-165
- Patil, J.L., Haldankar P.M., Jama- Dagani, B.M. and Salvi, M. J. 1992 Intercropping of vegetables in coconut. *J. Paltn. Crops* **20**(2):167-169.

Prospects of intercropping medicinal and aromatic plants in coconut

S. Mohandas, P. S. Subramaniam, C. Natarajan and S. Arulraj

Coconut Research Station, Tamil Nadu Agricultural University
Veppankulam – 614 906, Tamil Nadu, India

Coconut (*Cocos nucifera* Lin.) is a perennial palm and survives for more than 60 years. Nearly 60 percent of space in 7.5 x 7.5 m spaced adult coconut gardens and 40 per cent of sunlight are left unutilized, which provides ample scope for raising soil, climate and market specific intercrops profitably with synergistic effect on coconut (Nelliath, 1979). This provides additional revenue to the coconut farmers. Tuber crops, fruits crops, rhizomes, cereals, pulses and vegetables can be grown well as intercrop in coconut garden (Rethinam, 2001). Intercropping of citronella increased the land use efficiency from 21-46 percent with additional yield of main crop (Prakash Roa *et al.*, 1988). The possible intercrops in coconut garden include perennial, biennial and seasonal including medicinal and aromatic plants. In the present scenario of fluctuation in coconut price and high wage rate for labourers, the pure crop of coconut is no more economical. Hence, intercropping in coconut garden becomes indispensable for augmenting the income of the coconut farmers. The coconut based cropping system involves cultivation of compatible crops in the inter space of coconut leading to considerable increase in the production and productivity by increasing the cropping intensity and by the efficient utilization of sunlight, soil, water and labour. The World Health Organization compiled 20,000 medicinal plants used in different parts of the globe. Among these, over one hundred herbal botanicals are reported to have consistently larger market and are traded in major drug markets in the world. Baby *et al.* (2005) reported that ginger, sitharathai, turmeric, kasthurimanjal and patchouli are the medicinal plants suited for intercultivation in coconut gardens of Kerala. In this juncture, the present investigation was carried out to evaluate the performance of medicinal and aromatic plants as intercrops in adult coconut garden for augmenting the income of coconut farmers.

A field experiment was carried out between 2006 to 2008 in 36 years old ECT coconut garden at Coconut Research Station, Veppankulam to study the feasibility of raising soil and climate specific herbal plants as intercrop in coconut. The herbal plants chosen for this experiment were three medicinal plants *viz.*, Sitharathai (*Alpinia galanga*), Chotrukathalai (*Aloe vera*) and Tulsi (*Ocimum sanctum*) and two aromatic plants *viz.*, Lemon grass (*Cymbopogon flexuosus*) and Patchouli (*Pogestemon patchouli*). The experimental soil was sandy loam in texture with NPK availability of 199, 4.9, 79 kg ha⁻¹ respectively and pH, 7.03, EC 0.13 with a organic carbon of 0.22 percent. The experiment was conducted in Randomized Block Design, replicated five times with a plot size of 15 x 4 m (1.5 per cent) in between four coconut palms leaving 60 cm from the base of palms. The intercrop received only organic manures *viz.*, FYM@25 t ha⁻¹ and other intercultural operations as per the package for herbal plants. No serious pest and disease was noticed in the experimental crops. For minor seasonal pests, neem oil was sprayed based on need. The yield of economic parts of each herbal plant was recorded after processing and finally the economics of intercropping of herbal plants in coconut garden were worked out.

The selected medicinal and aromatic plants performed well and recorded better yield as intercrop in adult coconut garden. Sitharathai recorded a yield of 3,260 kg of dry tuber ha⁻¹. *Aloe vera* and Tulsi recorded 12,750 kg of fresh leaf ha⁻¹ and 16,000 kg of fresh leaf ha⁻¹ respectively. In case of lemon grass, the yield was 7,342 kg of dried leaf ha⁻¹ and for Patchouli, it was 1,240 kg of dried leaves ha⁻¹.

Table 1. Yield of herbal plants (economic parts) as intercrop in coconut garden (Mean of five plots of two years) (2006-07 & 2007-08)

Scientific Name	Duration	Yield (kg ha ⁻¹)
<i>Alpina galanga</i>	18 months	3,260
<i>Aloe vera</i>	6-7 months	12,750
<i>Ocimum sanctom</i>	1 year	16,000
<i>Cymbopogan flexuosus</i>	4 years	7,342
<i>Pogestemon patchouli</i>	3 years	1,240

The selected medicinal and aromatic plants recorded better net return and B: C ratio as intercrop in coconut garden. Sintharathai recorded a net return of Rs. 89,898 ha⁻¹ and a B: C of 1:3.2. *Aloe vera* and Tulsi recorded net returns of Rs. 1,41,058, 43,280 and B: C of 1:3.8 and 1:3.0 respectively. With respect to aromatic plants, lemon grass recorded a net return of Rs. 16,085 ha⁻¹ and B: C of 1:2.1 and *Patchouli* recorded a net return of Rs. 19,010 and B: C of 1:2.1. Maheswari *et al.* (1985) have brought out that growing of *ravolfia* as intercrop gave extra income in coconut groves. Maheswari *et al.* (1995) reported profitable cultivation of Patchouli in irrigated coconut garden of Kerala, wherein the shade intensity was between 25-50%. In addition, intercropping herbal plants in coconut enhanced the mean annual nut yield to the tune of 18 per cent (145 nuts/ palm / year) over that of pure coconut (123 nuts / palm / year).

Table 2. Economics of intercropping medicinal and aromatic plants in coconut garden

Sl. No.	Particulars	Yield (kg ha ⁻¹)	Gross return (Rs.)	Cost of cultivation (Rs.)	Net return (Rs.)	B:C
1.	Sitharathai	3,260	1,30,400	40,502	89,898	1: 3.2
2.	Chothukathalai	12,750	1,91,250	50,192	1,41,058	1: 3.8
3.	Tulsi	16,000	64,000	20,720	43,280	1: 3.0
4.	Lemon grass	7,342	30,000	13,915	16,085	1: 2.1
5.	Patchouli	1,240	35,750	16,740	19,010	1: 2.1

Based on the performance and economics of the chosen herbal plants, all the selected plants *viz.*, Sitharathai, Chothukathalai, Tulsi, Lemon grass and Patchouli were found to be suitable intercrops in adult coconut garden. Based on the marketability and soil suitability, coconut farmers can choose any of them and grow profitability as intercrop in coconut garden to get additional revenue besides enhancing the nut yield of coconut.

Acknowledgment: The authors greatly acknowledge the AICRP on Palms for the financial support to carry out this study.

References

- Baby, P.S., Gracy Mathew., Joy, P.P. and Samuel Mathew. 2005. Aromatic and medicinal plants research station. *Indian Coconut J.* **XXXVI** : 12-18.
- Maheswari, S.K., Dhantonde, B.N., Yadav, S. and Gangrade, S. K. 1985. Intercropping of *Rauvolfia serpentina* for higher monetary return. *Indian J. Agric. Sci.* **58**: 487-88.
- Maheswari, S.K., Sharma, R.K. and Gangarade, S.K. 1995. Studies on spatial arrangements in Palmarosa-Pigeon pea intercropping in black cotton soil. *Agron. J.* **92**: 812-818.
- Nelliat, E.V. 1979. Prospects of multiple cropping in coconut based farming system –The Indian experience. *Indian Coconut J.* **32**: 3-11.
- Prakash Rao, E. V. S., Singh, M. and Ganesh RAO, R.S. 1988. Intercropping studies in Java citronella (*Cymbopogon winterianus*). *Field Crop Res. (Admestrum)* **18**: 279-286.
- Rethinam, P. 2001. Research output and farmers adoption of technology on coconut based farming system –The Indian experience. *Indian Coconut J.* **32**: 3-11.

Integration of meat goats on the productivity of coconut farm

S. Anitha, Lissamma Joseph and K. Aravindakshan

Kerala Agricultural University
Vellanikkara, Thrissur -680656, Kerala, India

In India, coconut is being cultivated for the past over 4,000 years. Coconut is the most important crop of Kerala occupying the largest area among crops (8 lakh ha) grown extensively in every homesteads. Despite the enormous potential of the crop, coconut farmers are suffering due to decreasing farm productivity and income. Planting of high yielding varieties and better management practices seldom result in sustainability in productivity of coconut. Integrated farming is a viable option to increase the farm productivity and income. Integrated farming combines crop production with livestock. Salam and Sreekumar (1990) reported that mixed farming is an excellent way to achieve the sustainability of coconut farms. The live stock enterprise is complementary to crop production program so as to provide a balanced and productive system of farming (Liyanage and Fernando, 1991). In this study, integration of meat goat with coconut was conducted to study its suitability, profitability and sustainability of the system.

An unreplicated study on integration of meat goats with coconut was conducted in a 10 year old coconut plantation (8 ha area) at Coconut Development Farm, Campus Development of the Kerala Agricultural University from 2005-2009. Planting was done at a spacing of 8 m x 8 m in a square pattern (156 palms ha⁻¹). Palms are kept under average management under rain fed condition. Interspaces of palms are available for natural grazing. A unit of 10 goats (Alpine x Malabari x Boer) for meat purpose was introduced ha⁻¹ of coconut farm utilizing the natural feeds available in the farm. Observation on the yield from coconut palms and goats were taken and economics worked out based on the prevailing market rate.

Data on the yield and economics were given in Table 1. Result of the study revealed that there was a progressive increase in coconut production from 24 nuts palm⁻¹ during 2005 to 42 nuts palm⁻¹ during 2009. Anithakumari (2009) reported that due to integrated farming, yield of coconut increased from 33 nuts year⁻¹ to 53 nut year⁻¹ after 3 years of management practice. Salam and Sreekumar (1990) opined that yield increase due mixed farming was mainly by ensuring a prolonged soil health due to synergic interaction between the crop and animal component. Income from coconut increased from Rs.11,364 ha⁻¹ year⁻¹ to Rs. 19,920 ha⁻¹ year⁻¹ after a period of five years.

Considering all the expenses involved, the net income from goats increased from Rs. 2,788 ha⁻¹ during 2005 to Rs. 6,138 ha⁻¹ during 2009 by the sale of goat meat @ Rs. 100 kg⁻¹ live weight. Integration of meat goats in coconut increased the total profitability of the farm from Rs. 14,152 ha⁻¹ year⁻¹ to Rs. 26,058 ha⁻¹ year⁻¹ during the study period. Gunathilake and Joseph (2009) reported 3.5 fold increase in income from coconut plantation due to integrated farming. In this study the live stock component helped to augment the farm income by the sale of meat. Integration of coconut and meat goat in this model will provide all the advantage inherent in a mixed farming system. There is efficient utilization of farm resources and efficient biological

recycling by the synergistic interaction of crop and animal component. In this study the animals are allowed to graze the inter spaces of coconut. Grazing of animals prevent the wild growth in the interspaces of coconut thereby get a natural weed control. The goat droppings added to the field while grazing are excellent organic resources to maintain the soil health. The study indicated that integration of meat goats with coconut is agronomically productive, economically efficient and environmentally safe and a sustainable model suitable for small scale coconut farmers

References

- Anithakumari, P. 2008. Integrated farming of coconut based homesteads in root (wilt) affected area- The impact of extension interventions. *Indian Coconut Journal* **51** (3): 6-11.
- Gunathilake, H. A. J. and Joseph, P. G. 2009. Sustainable development and application of bio-energy in coconut plantations. *Indian Coconut Journal* **52** (2): 7-13.
- Liyanage, M. de. S. and Fernando, D. N. S. 1991. Role of legume based pasture, tree fodder and paddy straw in coconut/ cattle farming system in Sri Lanka. pp.56-64. In: International seminar on Livestock and Feed Development in the tropics, 21-25 October, 1991, Malony Indonesia:
- Salam, M. A. and Sreekumar, D. 1990. Coconut based mixed farming to sustain productivity. *Indian Coconut Journal* **20**(10): 3-5.

Table 1. Yield and economics of integration of meat goat with coconut

Particulars	2005	2006	2007	2008	2009
Coconut yield (Nuts palm ⁻¹ yr ⁻¹)	24	31	27	29	42
Coconut yield (Nuts ha ⁻¹ yr ⁻¹)	3,788	4,852	4,262	4,655	6,640
Income from coconut* (Rs. ha ⁻¹ yr ⁻¹)	11,364	14,556	12,786	13,965	19,920
Sale of goat meat (kg yr ⁻¹)	223	288	539	555	497
Net income from goat**(Rs. yr ⁻¹)	22,300	28,800	53,900	55,500	49,700
Net income from goat (Rs. ha ⁻¹ yr ⁻¹)	2,788	3,600	6,738	6,938	6,138
Total profitability (Rs. ha ⁻¹ yr ⁻¹)	14,152	18,156	19,524	20,903	26,058

* Price of coconut (average) – Rs. 3/nut, **Price of Live weight of goat - Rs. 100 kg⁻¹

Performance of elite cocoa clones intercropped in coconut

S. Elain Apshara

Central Plantation Crops Research Institute
Regional Station, Vittal 574243, Karnataka, India

Traditionally cocoa is a forest crop grown under the trees and so it needs shade of more than 50 percent for its better development and yield in their initial years of growth. Multipurpose trees like Coconut (Nelliat *et al.*, 1974; Bavappa *et al.*, 1986; George Thomas *et al.*, 2010) Arecanut (Shama Bhat and Bavappa, 1972; Abdul Khader *et al.*, 1984; Sujatha *et al.*, 2011), Rubber (Blencowe, 1971) and Oil Palm are used as shade trees in Malaysia, India, Papua New Guinea and Brazil for cocoa. Coconut price fluctuations in Malaysia, Sri Lanka and Indonesia identified cocoa as compatible and profitable intercrop (Rector *et al.*, 1972; 1982; Liyange *et al.*, 1986; Mialet *et al.*, 2001). Growth and yield of coconuts is not adversely affected by cocoa and establishment of cocoa caused suppression of weeds. Cocoa Research Institute of Ghana compared the merits of four cocoa-coconut intercropping systems and suggested that properly arranged high density cocoa under widely spaced coconuts can be a profitable intercrop system for adoption by cocoa farmers in Ghana (Opoku and Osei, 1997; Osei-Bonsu *et al.*, 2002). In Sri Lanka, coconut productivity increased considerably with agroforestry interventions with cocoa (Pushpakumara *et al.*, 2011).

In India, state of Andhra Pradesh stands first in cocoa area with 16,969 ha (2009-2010) followed by Kerala and Tamil Nadu and the major producer is Kerala with 6,344 MT (DCCD, 2011). All these gardens are under coconut. The rising demand in chocolate industry has encouraged farmers to raise their cocoa plantings. At CPCRI, during 1971 to 1978 research was conducted on single and double hedge system of planting cocoa in coconut gardens which was found to be highly beneficial and remunerative (Nelliat, 1978). Similarly Kerala Agriculture University also recommended cocoa under coconut (Vikraman Nair *et al.*, 2000). But there was not much work on evaluation on cocoa varieties in the cropping systems. Elite clones identified as good performers by CPCRI were from hybrids of progeny and clonal trials conducted under Arecanut (Vivek Bhat, 1999). So this study was conducted to assess the growth and yield performance of elite cocoa clones under coconut, to identify best clones suitable for coconut canopy and to compare the performance between mono cropped and intercropped coconut.

Nine elite clones were selected from earlier progeny trials, multiplied vegetatively and assembled between two rows of coconut cultivar Laccadive Ordinary Tall spaced at 7.5 m x 7.5 m, in a double row system of planting with a distance of 2.7 m x 2.7 m between cocoa trees. Experiment was replicated thrice with 6 trees per block with randomized block design. Fertilizers and irrigation schedules were practiced as per the recommendations to both the crops. Cocoa clones were evaluated for their height, girth, height at first branching, number of branches and canopy volume. The canopy volume was measured considering the canopy surface as cone shaped, using the formula $\frac{1}{3}\pi r^2 h$, whereas $r = \frac{EW + NS}{4}$ and $l = \sqrt{r^2 + h^2}$, h = canopy height. Number of pods yielded by individual trees of each clone during each harvest was compiled for five years. Individual pod weight (g) and number of bold beans per pod were measured. Beans were extracted, kept for fermentation, dried and observed for single dry bean weight, shell and fat contents. Total dry bean yield per tree per year was also compiled. Data analysed with WINSTAT software package.

The growth performance of cocoa clones grown under coconut garden was assessed and given in Table 1. Height of clones ranged from 2.85 to 3.67 m. Girth is the maximum (41.50 cm) in the clone VTLC-1 followed by VTLC-22. First branching heights differed from 49.13 to 77.57 cm) among the clones and number of branches ranged from 8.93 to 12.56. Spread of the canopy is the highest in VTLC-22 with 18.74 m³ and lowest (10.83 m³) in VTLC-17. The clone VTLC-22 had the maximum height, branch numbers, canopy volume and exhibited high vigour as well. VTLC-17 was considerably with reduced growth habits. Similar trend was observed in these clones under arecanut also (Elain Apshara *et al.*, 2008).

Table 1. Growth performance of elite clones under coconut

S.No.	Clones	Height (m)	Girth (cm)	HAFB (cm)	Branch No.	Canopy volume (m ³)
1	VTLC-1	3.48	41.50	55.57	12.10	17.77
2	VTLC-7	3.30	38.03	77.57	11.62	17.28
3	VTLC-8	2.98	36.20	49.33	10.27	14.78
4	VTLC-9	3.24	36.43	72.47	11.67	15.47
5	VTLC-17	2.85	39.76	72.67	8.93	10.83
6	VTLC-18	3.32	39.60	50.27	9.19	16.87
7	VTLC-22	3.67	39.97	63.86	12.56	18.74
8	VTLC-23	3.22	33.57	49.13	12.09	15.04
9	VTLC-1	2.88	32.20	57.20	11.07	11.43
	SEd	0.17	1.85	4.53	1.24	1.51
	CD	0.36	3.93	9.59	2.64	3.21

Pod yield was compiled for six years showed significant difference among clones (Table 2). In the initial years the lowest yield of 13.2 pods per tree per year was recorded and in the last year an increased yield of 39.1 pods per tree per year was recorded. There was a gradual increase in yield year after year. In few clones stable performance was observed. From

Table 2. Pod yield of elite clones under coconut

Sl. No.	Clones	Years					Pooled	Mean
		2006	2007	2008	2009	2010		
1	VTLC-1	16.6	25.0	28.7	28.6	39.1	138.0	27.6
2	VTLC-7	13.2	20.8	23.8	25.3	20.5	103.6	20.7
3	VTLC-8	17.1	20.6	26.2	23.2	38.2	125.3	25.1
4	VTLC-9	15.3	23.0	24.2	32.3	34.1	128.9	25.8
5	VTLC-17	15.2	28.5	32.1	28.1	30.3	134.2	26.8
6	VTLC-18	13.9	23.2	21.8	28.9	33.6	121.4	24.3
7	VTLC-22	16.6	31.3	32.0	33.4	37.8	151.1	30.2
8	VTLC-23	14.8	26.3	26.3	23.8	30.2	121.4	24.3
9	VTLC-1	14.0	21.7	22.2	24.4	29.9	112.2	22.4
	SEd	2.15	1.22	1.27	1.91	1.20		
	CD(P=0.05)	4.56	2.58	2.70	4.05	2.55		

the pooled and mean data it was clear that VTLC-22 yielded high (30.2 pods/tree/year) followed by VTLC-1 (27.6 pods/tree/year). Compared to arecanut canopy under coconut the yield was less invariably, that might be due to the damage caused by fallen coconut fronds in the early years of plant growth. Though the pod yield was less, the individual pod and bean characters were up to the mark and suitable for processing industry.

Pod and bean characters were given in Table 3. Pod weight among clones ranged from 235.41 to 485.16 grams. Six clones weighed more than 350 grams which is favourable. Except VTLC-9 all clones recorded more number of beans (>35). Single dry bean weights of 1 gram and above recorded in VTLC-22, VTLC-1, VTLC-23 and VTLC-9 and ultimately the high dry bean yields. In the industrial point of view all clones come under the favourable range of 10-15%. The clones VTLC-22 and VTLC-1 had >50% fat content.

Table 3. Pod and bean characters of elite clones under coconut

Sl. No.	Clones	Pod weight (g)	Bean no.	Single bean weight(g)	Dry bean yield (kg)	Shell (%)	Fat (%)
1	VTLC-1	386.04	41.7	1.07	1.26	10.6	52.7
2	VTLC-7	348.82	40.5	0.97	1.00	10.6	44.1
3	VTLC-8	282.62	40.8	0.93	0.87	12.0	41.6
4	VTLC-9	417.11	31.8	1.00	1.03	13.7	45.5
5	VTLC-17	283.46	39.8	0.85	0.95	13.8	40.4
6	VTLC-18	235.41	36.1	0.81	0.97	13.4	38.0
7	VTLC-22	455.60	41.5	1.13	1.42	11.1	52.1
8	VTLC-23	485.16	40.4	1.06	1.03	12.5	41.9
9	VTLC-1	350.52	36.7	0.88	0.79	14.2	37.7
	SEd	64.0	1.87	0.06	0.10	1.12	2.67
	CD(P=0.05)	135.7	2.09	0.12	0.21	2.37	5.67

Yield of main crop coconut was compared for five years before and after planting of cocoa. There was a considerable change in the yield level of coconut as well. Before planting of cocoa the yield ranged from 31.4 to 36.2 nuts per palm per year whereas after cocoa cultivation it increased to 58 to 65.5 nuts per palm per year (Table 4). This corroborated the results of earlier workers who have reported increase in coconut yield intercropped with cocoa both in single hedge and double hedge mixed cropping systems when compared with monocropped

Table 4. Yield performance of coconut

Before cocoa		After cocoa	
Year	Nuts/ Palm	Year	Nuts/ Palm
1996	31.4	2006	58.0
1997	33.2	2007	61.4
1998	35.3	2008	63.5
1999	36.2	2009	65.5
2000	34.5	2010	62.7

coconut (Nair *et al.*, 1975; Nair and Balakrishnan, 1977; Varghese *et al.*, 1978; Nair and Varghese, 1979).

From this study it was concluded that the clones VTLC-22 and VTLC-1 involving crosses of NA-33 x ICS-89 and II-67 x NC-29/66 performed the best with high vigor and yield under coconut. They had required canopy, more beans, with less shelling percentage and more fat content. Compared to monocropped palms, coconut intercropped with cocoa gave increased nut yields, healthy appearance and less weeds. It is proved that cocoa had a positive effect on micro and macro environment of coconut garden, utilised space, shade and light effectively and helped in maintaining the sustainability of both the crops.

References

- Abdul Khader, K.B., Rajamony, L. and Balakrishnan, P.C. 1984. Effect of raising cocoa as a mixed crop in adult coconut plantations. pp. 367-374. In: *PLACROSYMVI*, Oxford and IBH Publishing Co. Pvt. Ltd.
- Bavappa, K.V.A., Abdul Khader, K.B., Biddappa, C.C., Khan, H.H., Kasturi Bai, K.V., Ramadasan, A., Sundararaju, P., Bopaiah, B.M., Thomas, G.V., Misra, L.P., Balasimha, D., Bhat, N.T. and Shama Bhat, K. 1986. Coconut and arecanut based high density multi-species cropping systems. *J. Plantn. Crops* **14(2)**: 74-87.
- Blencowe, J.W. 1971. Cocoa and coconut in Malaya. pp. 16-19. In: *Annual Report*, Selangor Planters Association, Malaysia.
- DCCD, 2011. www.dccd.gov.in.
- Elain Apshara, S., Vivek Bhat, R. and Nair, R.V. 2008. Comparative studies on elite cocoa progenies in their initial years of growth. *Journal of Plantation Crops* **36 (1)**: 38- 44.
- George Thomas, V., Krishnakumar, V., Maheswarappa, H.P. and Palaniswami, C. 2010. *Coconut Based Cropping/Farming Systems*. 231 pp. Central Plantation Crops Research Institute, Kasaragod.
- Liyanage, M.De.S., Tejawani, K.G. and Nair, P.K.R. 1986. Intercropping under coconut in Sri Lanka. *Cocos* **4**: 23-34.
- Mialet, S.I., Bonneau, X., Mouchet, S. and Kitu, W.T. 2001. Growth and yield of coconut-cocoa intercrops. *Experimental Agriculture* **37(2)**: 195-210.
- Nair, P.K.R. and Varghese, P.T. 1979. Recent advances in the management of coconut-based agro-ecosystems on the west coast of India. pp. 569-580. In: *Tropical Ecology and Development Part I* (Ed.) Furtado, J. I., Int. Soc. for Tropical Ecology, Kuala Lumpur, Malaysia.
- Nair, P.K.R. and Balakrishnan, T.K. 1977. Ecoclimate of a coconut+cocoa crop combination in the West Coast of India. *Agricultural Meteorology* **18**: 455-462.
- Nair, P.K.R., Varma, R., Nelliath, E.V. and Bavappa, K.V.A. 1975. Beneficial effects of crop combination of coconut and cocoa. *Indian Journal of Agricultural Sciences* **45(3)**: 165-171.
- Nelliath, E.V. 1978. Multiple cropping in plantation crops. pp. 451-452. In: *Proceedings of Plantation Crops Symposium (PLACROSYM I)*, CPCRI, Kasaragod.
- Nelliath, E.V., Bavappa, K.V.A. and Nair, P.K.R. 1974. Multi-storeyed cropping - A new dimension in multiple cropping for coconut plantations. *World Crops* **26 (6)**: 262-266.

- Opoku-Ameyaw, K. and Osei-Bonsu, K. 1997. Growth resources study in the cocoa x coconut inter- cropping trial. 53 pp. In: *Annual Report*, Cocoa Research Institute, Ghana.
- Osei-Bonsu, K., Opoku-Ameyaw, K., Amoah, F.M. and Oppong, F.K. 2002. Cocoa-coconut intercropping in Ghana: agronomic and economic perspectives. *Agroforestry Systems* **55(1)**: 1-8.
- Pushpakumara, D.K.N.G., Gunasena, H.P.M., Gunathelake, H.A.J. and Singh, V.P. 2011. *Increasing Coconut and Productivity through Agroforestry Interventions*. 200 pp. Coconut Research Institute of Sri Lanka.
- Rector, D.H., Leach, J.R. and Lim, K.P. 1972. Cost of production and expected return from cocoa planted under coconuts. pp. 251- 268. In: *Cocoa and Coconuts in Malaysia* (Eds.) Wastie, R.L. and Earp, D.A., Incorporated Society of Planters, Kuala Lumpur, Malaysia.
- Shama Bhat, K. and Bavappa, K.V.A 1972. Cocoa under palms. pp. 116- 121. In: *Cocoa and Coconuts in Malaysia* (Eds.) Wastie, R. L. and Earp, D. A., Incorporated Society of Planters, Kuala Lumpur, Malaysia.
- Sujatha, S., Ravi Bhat, Balasimha, D. and Elain Apshara, S. 2011. Arecanut based inter/ mixed cropping system. pp. 6-26. In: *Arecanut based cropping/ farming system*. [Eds.] Thomas, G.V., Krishnakumar, V., Maheshwarappa, H.P., Ravi Bhat and Balasimha, D., Central Plantation Crops Research Institute, Kasaragod.
- Varghese, P.T., Nelliath, E.V. and Balakrishna, T.K. 1978. Beneficial interactions of coconut-cocoa, crop combination. pp. 383- 392. In: *PLACROSYMI*. (Eds.) Nelliath, E.V., Ranganathan, V., Vishveswara, S., Potti, S.N. and Zachariah, P.K., Placrosym standing committee, Kasaragod, India.
- Vikraman Nair, R., Mallika, V.K., Prasannakumari Amma, S., Koshy Abraham and Balasubramanian, P.P. 2000. Cocoa cultivation- science and techniques. 82 pp. Directorate of Cashewnut and Cocoa Development, Govt. of India, Ministry of Agriculture, Cochin.
- Vivek Bhat, R. 1999. Cocoa germplasm, characterization, conservation and utilization. pp. 77-85. In: *Improvement of plantation crops*. (Eds.) Ratnambal, M.J., Kumaran, P.M., Muralidharan, K., Nirali, V. and Arunachalam, V., CPCRI, Kasaragod.

Benefit cost ratio analysis of coconut based cropping systems

L.Girijadevi, R.Gladis and Biju Joseph

College of Agriculture, Kerala Agricultural University
Vellayani, Trivandrum, Kerala, India

Coconut is a crop of small and marginal farmers since 98 % of about 5 million coconut holdings in the country are less than 2 ha in extend. In Kerala the palm is an essential component in the homestead system of farming. Coconut a perennial crop which is committed to the land for decades utilizes the natural resources like light, water and nutrients only to a very limited extent due to the peculiarities of its rooting pattern and canopy structure. The rooting pattern of coconut is such that only 25 % of land area is effectively utilized. A spacing of 7.5 meter in the square system is recommended for coconut (175 palms / Ha) for optimum production. As coconut canopy's space utilization is very low, plenty of sunlight infiltrates and fall on the ground unutilized. Therefore, there is scope for exploiting the unutilized natural resources in a coconut garden so as to enhance the income of coconut farmer by way of introducing different cropping systems. Such study was conducted at Vellayani, Kerala to find out the most economic cropping system in coconut garden.

The field experiment was conducted at the Instructional farm, College of Agriculture, Vellayani during the year 2004 – 05. The soil of the experimental site is red loam. The experimental area was lying under robusta banana intercropped with other annual crops during the previous years.

The treatments consisted of ten cropping systems in coconut garden in the following crop combinations.

- T1 – 12 banana + 20 elephant foot yam + 72 vegetable cowpea as border around the plot (banana is planted at a spacing of 2 m apart and in the interspaces elephant foot yam is planted at 90 cm spacing).
- T2 – 20 cassava + 20 elephant foot yam + 72 vegetable cowpea (cassava is planted at 90 cm spacing and elephant foot yam also at 90 cm spacing).
- T3 – 12 banana plants + 200 ginger plants + 72 vegetable cowpea (banana – 2 m spacing, ginger 25 X 25 cm spacing in four inner corners of 2 m beds).
- T4 – 10 cassava + 10 elephant foot yam + 200 ginger plants + 72 vegetable cowpea (cassava 90 X 90 cm, elephant foot yam 90 X 90 cm, ginger 25 X 25 cm).
- T5 – 12 banana + 200 turmeric plants + 72 vegetable cowpea (banana 2 X 2 m, turmeric 25 X 25 cm in four inner corners of 2 m beds).
- T6 – 10 cassava + 10 elephant foot yam + 200 turmeric plants + 72 vegetable cowpea (cassava 90 X 90 cm, elephant foot yam 90 X 90 cm, turmeric 25 X 25 cm in beds).
- T7 – 12 banana + 20 cassava + 72 vegetable cowpea (banana 2 X 2 m, cassava 90 X 90 cm).
- T8 – 12 banana + 10 cassava + 10 elephant foot yam + 72 vegetable cowpea (banana 2 X 2 m, elephant foot yam 90 X 90 cm, cassava 90 X 90 cm).

T9 – 12 banana + 10 cassava + 100 ginger + 72 vegetable cowpea (banana 2 X 2 m, cassava 90 X 90 cm, ginger 25 X 25 cm, in four corners of 1 meter beds).

T10 – 12 banana + 10 cassava + 100 turmeric + 72 vegetable cowpea (banana 2 X 2 m, cassava 90 X 90 cm, turmeric 25 X 25 cm in the four corners of 1 meter beds).

Sole crops of banana, cassava, elephant foot yam, ginger, turmeric and vegetable cowpea were raised separately and simultaneously in open.

The experiment was conducted in RBD having three replications. The fertilizers were applied as per package of practices recommendations of Kerala Agricultural University for the respective crops. The population of individual crops in each cropping system compared to sole cropping is given in Table 1.

Table 1. Population of coconut and intercrops in different treatments (ha⁻¹)

Treatment	Gross area basis						
	Coconut	Banana	Elephant foot yam	Ginger	Turmeric	Cassava	Vegetable cowpea
T1	175	2132	3555	-	-	-	12800
T2	175	-	3555	-	-	3555	12800
T3	175	2132	-	35555	-	-	12800
T4	175	-	1777	35555	-	1777	12800
T5	175	2132	-	-	35555	-	12800
T6	175	-	1777	-	35555	1777	12800
T7	175	2132	-	-	-	3555	12800
T8	175	2132	1777	-	-	1777	12800
T9	175	2132	-	17777	-	1777	12800
T10	175	2132	-	-	17777	1777	12800
Sole crop	175	2500	12345	100000	100000	12345	111110

The yield data of all the crops tried in various cropping systems were statistically analysed and the results obtained are presented in Table 2. Economics of different cropping systems were worked out (Table 3) and the benefit cost ratio was calculated by dividing the gross income by the gross expenditure. The net returns were computed as the difference between the gross income and gross expenditure.

There was significant difference between cropping systems and between cropping system and sole cropping with respect to the bunch yield in banana. The highest per plant yield was observed in sole cropped banana (10.12 kg plant⁻¹), followed by banana in cropping system T3 (9.14 kg plant⁻¹), T1 (8.51 kg plant⁻¹) and T10 (8.35 kg plant⁻¹) respectively but not significantly different from each other. The highest bunch yield in banana could be attributed to the highest incident radiation in sole crop banana when compared to banana in cropping system. The highest incident photosynthetically active radiation (PAR) was in sole crop banana followed by banana in cropping system T3 (1220 $\mu\text{Em}^{-2}\text{s}^{-1}$ and 1039.43 $\mu\text{Em}^{-2}\text{s}^{-1}$ respectively). The lowest incident PAR was in banana in cropping system T9 and the lowest bunch yield was

also in the same cropping system. Banana being a sun loving crop, the leaves have large amount of Calvin cycle enzymes per unit leaf which might have enhanced the photosynthetic capacity of the banana crop and enhanced the bunch yield. The lowest incident PAR under shade might have caused stress on the plant, as irradiance limits photosynthesis and thus net carbon gain and dry matter accumulation (Lambers *et al.*, 1998). In this particular study since the intercrops were planted in a planned manner without causing any mutual shade may be one of the reasons for the low decrease in incident PAR and hence the less reduction in bunch yield in cropping systems in coconut garden when compared to open.

In case of ginger, though it is a shade loving crop, the rhizome yield was found lowest in coconut garden. This might be due to the increased shade in cropping systems in coconut garden compared to sole cropping. In ginger various studies indicated that the rhizome yield increased up to 25 per cent shade and there after declines. In this particular study, where ginger was a companion crop with nendran banana, cassava and elephant foot yam in coconut garden the shade may be more than 25 per cent resulting in decline in rhizome yield. The photosynthetic efficiencies were found to increase up to 25 per cent shade. This indicated that at 25 per cent shade the photosynthetic area was utilized efficiently. At shade levels above this, the leaf area was found to increase, which might have resulted in mutual shading. Under such situation the rate of dark respiration also increases (Bjorkman, 1981), leading to reduced photosynthetic efficiency under 50 and 75 per cent shade. In ginger Aclan and Ouisumbing (1976) found that yield under full sunlight was just as high as those obtained under 25 and 50 per cent light attenuation.

In elephant foot yam the sole cropping recorded the highest corm yield of 4.35 kg plant⁻¹ and was on par with the corm yield in cropping system T2, T6 and T4, where as it was significantly superior to the corm yield in cropping systems T8 and T1. The lowest corm yield was observed in cropping system T1 (2.85 kg plant⁻¹). The maximum yield was obtained under full sunlight and only marginal reduction in yield was noticed in cropping systems T2, T3 and T4. Prameela (1990) noticed that colocasia gave maximum tuber yield under 25 per cent shade followed by a reduction in yield with further increase in shade intensity. In cropping system T8 and T1 one of the companion crop was nendran banana and which was a tall growing crop in the combination might have produced more than 25 per cent shade resulting in yield reduction in elephant foot yam in these cropping systems. From the study it can be inferred that elephant foot yam is a shade tolerant crop and can be grown in association with another tuber crop cassava which forms a best companion crop for getting good yield. Togari (1950) reported that in shade, cambial activity and tuberization were suppressed in sweet potato tubers. Studies conducted in four sweet potato cultivars showed that fresh weight of tuber was not affected in 25 per cent shade but in 50 per cent shade, values were noticeably lower, in 73 per cent shade tuberization was almost completely suppressed in all cultivars. The responses were resulted from slower tuber growth in 50 per cent shade and from delayed tuber initiation and slower tuber growth in 73 per cent shade (Laura *et al.*, 1986).

The tuber yield in cassava was not found affected by cropping systems and sole cropping. In turmeric also there was no significant difference between cropping systems and between cropping systems and sole cropping.

The fresh pod weight in vegetable cowpea was found significantly influenced by cropping systems and sole cropping and the highest fresh pod yield was recorded in cropping

system T3 (152.1 g plant⁻¹) followed by sole cropping (148.7 g plant⁻¹) and cropping system T7 (132.68 g plant⁻¹), T5 (129.1 g plant⁻¹), T2 (87.5 g plant⁻¹), T4 (86.9g plant⁻¹) and T6 (82g plant⁻¹) and these were on par with each other. The lowest pod yield was in cropping system T9 (50.5g plant⁻¹) and was on par with the yield in cropping systems T8, T10, T1, T6 and T2. Since vegetable cowpea was planted as border row around all the other crops and away from coconut crown portion, the PAR incidence was high in all the cropping systems. Also vegetable cowpea is a sun loving crop and this might be the reason for on par yield of vegetable cowpea in cropping systems and sole cropping.

Economic analysis of the study revealed the superiority of cropping systems by producing a benefit cost ratio of more than 1 (Table3). The highest benefit cost ratio of 1.74 was in cropping system T4 involving cassava, elephant foot yam, ginger and vegetable cowpea in coconut garden. The net income was the highest in cropping system T3 involving banana, ginger and vegetable cowpea. When cassava was included in the system, the net income decreased due to the low value of the crop (T4) when compared to the cropping system (T3). But when we take into consideration the food value/ calorie value or energy value of the crop it is cassava the important. Similar results were obtained by Ravindran (1997) and Girijadevi and Wahab (2007). Cropping systems involving banana and cassava produced the lowest benefit cost ratio of 1.02, their net income were also the lowest among the systems (T7 and T9 respectively). Thus from the study it can be concluded that banana, ginger and elephant foot yam constitute ideal companion crops in coconut garden and thrive well as intercrops in coconut garden. The banana-cassava combination was not found remunerative with respect to the net income from the system as banana is high expensive crop and cassava is a low value crop.

Table 2. Economic yield of banana, cassava, elephant foot yam, ginger, turmeric and vegetable cowpea as influenced by cropping systems and sole cropping (kg / plant)

Treatment	Bunch yield of banana	Corm yield of elephant foot yam	Rhizome yield of ginger	Rhizome yield of turmeric	Tuber yield of cassava	Fresh pod yield of cowpea
T1	8.5 ^{ABC}	2.8 ^C	-	-	-	0.076 ^{BC}
T2	-	4.1 ^{AD}	-	-	2.9 ^A	0.088 ^{ABC}
T3	9.1 ^{AB}	-	0.16 ^B	-	-	0.152 ^A
T4	-	3.5 ^{ABC}	0.11 ^C	-	3.4 ^A	0.087 ^{ABC}
T5	7.9 ^{BC}	-	-	0.08 ^A	-	0.129 ^{AB}
T6	-	3.7 ^{ABC}	-	0.08 ^A	2.8 ^A	0.082 ^{ABC}
T7	7.7 ^{BC}	-	-	-	2.1 ^A	0.132 ^{AB}
T8	7.7 ^{BC}	3.2 ^{AC}	-	-	2.3 ^A	0.055 ^C
T9	7.1 ^C	-	0.13 ^C	-	1.8 ^A	0.051 ^C
T10	8.4 ^{ABC}	-	-	0.11 ^A	2.1 ^A	0.055 ^C
Sole crop	10.12 ^A	4.3 ^A	0.19 ^A	0.12 ^A	3.8 ^A	0.149 ^{AB}

Table 3. Economics of different cropping systems in coconut garden
(Rs. Gross ha⁻¹)

Cropping Inter system	Expenditure (Rs)	Produce (t/ha)	Value of individual crop (Rs.)	Gross income (Rs.)	Gross expenditure (Rs.)	Net income (Rs.)	BC ratio
T1 B (12)	1,90000	18.12	2,19464	3,08896	2,69000	39896	1.15
EFY (20)	54000	9.95	79632				
VC (72)	25000	0.98	9800				
T2 C (20)	25000	10.31	41240	1,69080	104000	65080	1.63
EFY (20)	54000	14.58	1,16640				
VC (72)	25000	1.12	11200				
T3 B (12)	1,90000	19.40	2,32812	3,66088	2,45000	1,21088	1.49
G (200)	30000	5.69	1,13776				
VC (72)	25000	1.95	19500				
T4 C (10)	12500	6.04	24160	1,63240	94000	69240	1.74
EFY (10)	27000	6.22	49760				
G (200)	30000	3.91	78220				
VC (72)	25000	1.11	11100				
T5 B (12)	1,90000	16.84	2,02116	2,35680	2,30000	5680	1.02
T (200)	15000	2.84	17064				
VC (72)	25000	1.65	1650				
T6 C (10)	12500	4.98	19920	1,00124	79500	20624	1.26
EFY (10)	27000	6.58	52640				
T (200)	15000	2.84	17064				
VC (72)	25000	1.05	10500				
T7 B (12)	1,90000	16.42	1,96992	2,43872	2,40000	3872	1.02
C (20)	25000	7.47	29880				
VC (72)	25000	1.70	17000				
T8 B (12)	1,90000	16.42	1,96992	2,65872	2,54500	11372	1.04
C (10)	12500	4.09	16360				
EFY (10)	27000	5.69	45520				
VC (72)	25000	0.70	7000				
T9 B (12)	1,90000	15.12	1,81644	2,47164	2,42500	4664	1.02
C (10)	12500	3.20	12800				
G (100)	15000	2.31	46220				
VC (72)	25000	0.65	6500				
T10 B (12)	1,90000	17.91	2,14896	2,48546	2,35000	13546	1.06
C (10)	12500	3.73	14920				
T (100)	7500	0.11	11730				
VC (72)	25000	0.70	7000				

The performance of different crops in suitable combination and population in coconut garden is economic than sole cropping of coconut. Inclusion of high value crops like ginger and banana increased the net income and inclusion of cassava, elephant foot yam and ginger

increased the BC ratio. It was also observed that combination of banana and cassava in coconut garden is inferior to all other combinations with respect to net income and BC ratio.

References

- Aclan, F. and Quisumbing, E.C. 1976. Fertilizer requirement, mulch and light attenuation on the yield and quality of ginger. *Phil. Agricst.*, 60: 183 – 191.
- Bjorkmam, O. 1981. Response to different quantum flux densities. *Encyclopedia of Plant Physiology* (eds. Lange, O.L., Noble, P.S., Osmund, C.B. and Ziegler, H.), Springer – Verlag, Berlin. P. 57 – 107.
- Girijadevi, L. and Wahab, K. 2007. Economic analysis of coconut based cropping systems. *J. Plantn. Crops* 35 (2): 82-83.
- Lambers, H., Chaplin, F. and Pens, T.L. 1998. *Plant Physiological Ecology*. Springer – Verlag, New York. P. 540.
- Laura Roberts, B., Nkrumah, L.B., Ferguson, T.V. and Wilson, L. 1986. Response of four sweet potato cultivars to levels of shade. *Trop. Agric.* 63: 265.
- Prameela, P. 1990. Screening different morphotypes of colocasia (*Colocasia esculenta*) for shade tolerance. M.Sc. (Ag.) thesis, Kerala Agricultural University, Thrissur. P. 62.
- Ravindran, C. S. 1997. Nutrient-moisture-light interactions in a coconut based cropping system. PhD thesis, KAU, Thrissur, Kerala.
- Togari, Y. 1950. A study in tuberous root formation in sweet potatoes. *Bull. Agric. Extension Studies* 3:1-96.

Improving use efficiency of water by integrating fish culture and irrigation in coconut based farming system - A case study

A.C. Mathew and Manoj P. Samuel

Central Plantation Crops Research Institute
Kasaragod - 671 124, Kerala, India

Kerala is probably one among the few states in India blessed with monsoon twice a year. But all this does not result in the perennial availability of water in the state. The only way to overcome the problem of water scarcity is to harvest the rain water. Since the State receives an average annual rainfall of 3000 mm, these copious natural showers are enough to satisfy the ever increasing water needs for domestic, irrigation and industrial needs. The effective development of water resources, which are distributed unevenly both spatially and temporally, and its efficient and judicious management holds the key to solve the paradoxical problem of frequent occurrence of floods and droughts in the region.

The development of water sources must be within the capacity of nature to replenish and to sustain. If this is not done, costly mistakes can occur with serious consequences. The application of innovative technologies and the improvement of indigenous ones should therefore be included in management of the water sources to ensure sustainability and to safeguard the sources against pollution. In view of all these considerations, now there is a paradigm shift among scientists, policy makers and farmers to develop and adopt low cost sustainable rain water harvesting technologies.

Being a high rainfall region, Kerala has great potential to harvest rainwater by various means. Though many such technically feasible water harvesting technologies are available, economical non viability of the same prevents many farmers from adopting these techniques. In this context, a field study was conducted in the Western Ghat region of Northern Kerala to develop a suitable rainwater harvesting system adaptable to hilly terrains and to test its efficacy for improving the water use efficiency by multiple use of the water. The cost-benefit analysis of the water harvesting system was also carried out to find out its affordability to farmers.

The universal value of perennial water collected on-farm through in situ water harvesting measures to enhance productivity and reduce drought related loss has been generally underestimated. Water bodies, both community and household, have traditionally served a variety of functions in rural areas including livestock and fish production. Maintaining or enhancing the multiple uses of water bodies may be the cornerstone of wider adoption of fish production (Khan 2010). Water bodies, whether farm ponds or small dams, have important roles in nutrient conservation and concentration in addition to their primary function of water storage for irrigation. Irrigation of high-value vegetable and fruit crops planted around farm ponds have greater impacts on household food security and income along with the fish produced. mathew

The raising of livestock in association with coconut is a well established practice in Kerala State. The rationale for this practice is that same land could be simultaneously and profitably utilised to produce different crops and livestock enterprises, so that the productivity of the land is increased. The practice minimizes resource demand and encourages efficient

resource use, especially in the low levels of resource endowments. However, fish culture in coconut garden is not very common mainly because of the lack of perennial water source. The universal value of perennial water on-farm to enhance productivity and to reduce drought related loss has been greatly underestimated. Improving the use efficiency of harvested water is the best solution to make it more economic. Rainwater is presently considered as an important input factor for healthy and productive watershed ecosystems and irrigated agriculture (Cortesi et al., 2009). Though many such technically feasible water harvesting technologies are available, economical non viability of the same prevents many farmers from adopting these techniques.

In the present study, apart from developing a cost-effective rainwater harvesting system, various means of improving the use efficiency of water such as (i) improving the water application efficiency of coconut and component crops in the cropping units (ii) developing multiple water use systems (iii) conjunctive use of multiple water sources are analyzed.

The study was conducted in the agricultural field of an innovative farmer, located in North-Eastern part of Kerala State (India) that receives an average annual rainfall of 3500 mm. In spite of getting high amount of rainfall, the locality experiences severe scarcity of water during summer months (Rajagopal, et al., 2004). This is mainly because of the uneven rainfall distribution pattern and huge runoff due to inclined topography. The rainfall data of the locality, depicted in the Fig. 1 shows the skewed distribution pattern of the much centralized rainfall. Most of the rainwater has been lost as surface runoff due to undulating topography and high intensity downpour. These factors coupled with low water holding capacity of the lateritic soil prevailing in this region results in moisture stress after the withdrawal of monsoon.

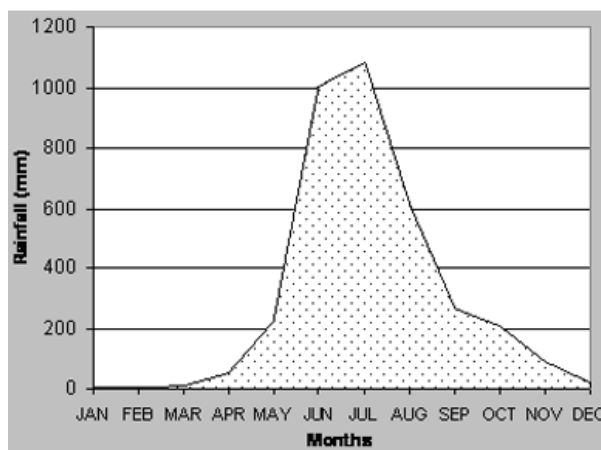


Fig. 1. Rainfall distribution pattern of Kasaragod District, Kerala (India)

Though coconut was the main crop in the 2.0 ha farm, other high value crops like vanilla (*Vanilla fragrans*), arecanut (*Areca catechu*), pepper (*Piper nigrum*), and vegetables were also being cultivated in the interspaces of coconut. A natural spring that yields water up to December and an open well that provides water up to March were the major water sources and the garden was irrigated manually using a hose pipe. In absence of a sustainable source to fetch water for life saving irrigation to the crops during summer months (March-May), severe crop losses were observed during the drought period and it was estimated that the crop loss occurred to vanilla alone was about 1 quintal of green vanilla beans. The approximate loss calculated as per the local rates prevailed during the period under study (on an average @ Rs. 100/kg.) was around Rs. 1.5 lakhs.

The region loses the lion share of the rainwater through runoff and in this context, the rainwater harvesting assumes great significance. It can be implemented as a viable alternative

to conventional water supply schemes considering the fact that any land anywhere can be used to harvest rainwater. Rainwater harvesting is in reality extending the fruits of the monsoon based on the principle of catching the water where it falls. It is considered to be an ideal solution for water problem where there is inadequate groundwater supply or where surface resources are either not available or insufficient. The Millennium Development Goals of the United Nations (UN MDG 2009) stresses on rainwater harvesting as an effective measure to ensure environmental sustainability. Previous studies showed that subsistence agriculture in hilly region could be successfully transformed into a profit earning enterprise by tapping and utilizing rainwater (Singh *et al.*, 2006). Venketaswarulu (2001) suggested that farm ponds in watershed areas helps to harvest both surface and sub-surface runoff from upper reaches. Rainwater harvesting can be instrumental to decentralized water supplies and local food security. When rainwater harvesting at the household or community level enables rainfed farms to access a source of supplementary irrigation, the economic security also improves. Jugale (2004) reported that adoption of rainwater harvesting technologies in drought prone areas of Maharashtra helped in enhancing the growth of animal husbandry, farm employment, improving the standard of living, income and output levels of farmers other than ensuring perennial availability of water.

Rainwater harvesting, irrespective of the technology used, essentially means harvesting and storing water in days of abundance, for use in lean days. In this context, it was decided to tackle the problem by constructing a large scale rain water harvesting tank. The direct rainwater, roof water or runoff can be harvested using eco-friendly low-cost technologies such as *uv* resistant plastic lined ponds, ferro-cement tanks etc. and used for multiple purposes (Anon., 2004). In the present case, a *uv* resistant plastic lined rainwater harvesting tank was planned and designed.

The rainwater harvesting tank was constructed during the months of September-November 2004 at the agricultural farm under study, which is located at Rajapuram Village in Kasaragod District (Kerala) at 12° 42' 0 N *latitude* and 75° 4' 0 E *longitude*. As the first step, a demand and supply analysis of the on-farm water availability and irrigation requirement was carried out and based on it the optimum size and dimensions of the tank were finalized. The tank was constructed by digging out soil by means of earth moving machinery and the sides were stabilized subsequently by way of stone pitching. The tank is further lined with cross laminated multi-layered *uv* resistant plastic sheets for large scale harvesting of rainwater and runoff and to hinder seepage losses.

The water requirement for two hectares of the cultivated land was calculated based on the water requirement of crops grown as shown below:

Daily irrigation requirement for 140 coconut palms @ 40 litre/day/palm	= 5,600 litre
Daily irrigation requirement for 260 arecanut palms @ 20 litre/day/palm	= 5,200 litre
Daily irrigation requirement for vanilla garden through 30 emitters @ 70 litre/emitter/day	= 2,100 litre
Therefore, total daily irrigation requirement	= 12,900 litre

As a normal practice, the irrigation is commenced in the month of January and continues it till the onset of monsoon during the month of June. The yearly irrigation requirement was calculated assuming that the crops are irrigated daily for about 150 days annually.

Hence, the annual water requirement = $12,900 \times 150 = 19,35,000$ litre = $1,935 \text{ m}^3$

Taking into consideration of getting an average amount of 323 mm of rainfall during the lean period under consideration in approximately 5 to 10 rainy days, which amounts to about 9 % of the total, the annual water requirement can be reduced to $1,806 \text{ m}^3$. The figure was arrived by reducing the water requirements for 10 rainy days, for which irrigation is not required, from the total annual water requirement.

It was estimated from the demand analysis that a tank which can hold around 1,800 cubic meter of water was required for providing irrigation during the summer season. It was approximated that the contribution of rainwater to the pond from summer showers would approximately compensate the losses due direct evaporation from the pond during the period under consideration.

A trapezoidal shaped storage tank with following specifications was constructed by excavating soil and dumping the excavated soil at the four sides of the tank (Fig. 2, 3).

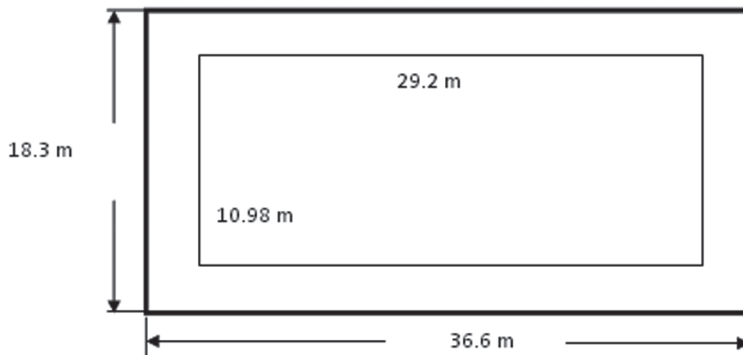


Fig. 2. Plan of the Trapezoidal water harvesting tank

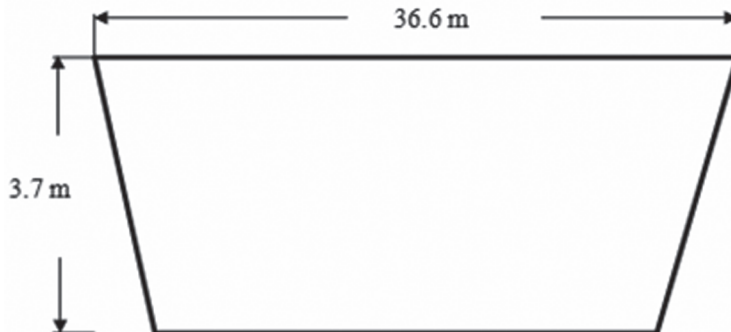


Fig. 3. Side View of the water harvesting tank

A trench of 50cm depth and 50cm width was taken all around the pond to anchor the sheet.

Top length (L_t) = 36.60 m

Top width (W_t) = 18.30 m

Depth (D) = 4.00 m

Free board = 0.30 m

Bottom length (L_b) = 29.20 m

Bottom width (W_b) = 10.98 m

Side slope(z) = 1:1

The wetted perimeter $P = W_b + 2D (\sqrt{1 + \cot^2 \theta})$

i.e., $P = 10.98 + 2 \times 3.7 (0.785 + \cot 45) = 24.19 \text{ m}$

The Volume of the tank is calculated using the following formula:

i.e., Carrying capacity of trapezoidal tank $V = L_b W_b D + (L_t + W_t)zD^2 + 4/3 z^2 D^3$

i.e. $V = (29.20 \times 10.98 \times 3.70) + (29.20 + 10.98)1 \times 3.70^2 + 4/3 \times 1^2 \times 3.70^3$

= 1803.88 m³

That means the tank can hold 1.8 million litres of water. However, it was observed that a considerable amount of water was lost by deep percolation and seepage because of the porous lateritic soil. The seepage and percolation losses were hindered by lining the tank using a 200 GSM UV resistant cross laminated polyethylene film commonly known as *Silpaulin*.

The sheet was made into the shape of the pond with exact dimensions by the process of thermal welding. After the thermal welding, the welded multi-layered plastic sheets, which were transformed into a single unit with the similar shape and size of the pond, was inserted into the pond. All the four sides of the sheet were buried in the trench taken on the side bund and was riveted at the corners with iron pegs.

The trench was then refilled with soil (Fig 4). Two sides of the tank that were above the ground surface were further stabilized with rubble pitching (Fig 5) and vegetative fencing. Subsequently the pond was completely covered with fishing net/ shade net to prevent birds from catching fishes and also to prevent the dried leaves falling from the nearby trees.

The inflow to the pond is from two different sources - a natural seasonal spring flowing down from the hilltop (Fig 6) and the direct precipitation. The average rate of flow of the spring was measured as 3 m³/hr and it was observed that in normal rainfall years the spring will last only for 6 months.



Fig 4. An overview of the pond covered with fishing net/shade net



Fig.5. Side of the pond stabilized with rubble pitching



Fig.6. Inflow to the Silpolin lined spring fed pond

Both the inflow to the pond and outflow from the pond were observed to study the water balance of the plastic lined dugout pond.

Storage of water in the pond + spill over water = (Inflow to the pond as direct precipitation/ annum + Contribution to the pond by the natural spring) - (Evaporation losses+ Irrigation requirement)

Inflow to the pond as direct precipitation/ annum = Surface area of the pond x annual rainfall = $36.6 \text{ m} \times 18.3 \text{ m} \times 3.5 \text{ m} = 2,344.23 \text{ m}^3$

Contribution to the pond by the natural spring = $180 \text{ days} \times 3 \text{ m}^3/\text{hr} \times 24 \text{ hr} = 12,960 \text{ m}^3$

Total annual inflow to the pond = $15,304.23 \text{ m}^3$

Evaporation losses @ 120 cm annually = 803.736 m^3

Annual irrigation requirement = $1,935 \text{ m}^3$

Hence, Storage of water in the pond + spill over water = $12,565.494 \text{ m}^3$

Since the Pond can store only $1,803.88 \text{ m}^3$ of water, the rest $10,761.614 \text{ m}^3$ of water was allowed to spill over from the pond. A major portion of the spilled over water had been diverted for groundwater recharge through contour trenches.

The study was based on two objectives: (1) development of low-cost water harvesting system and (2) improving the farming system

1) Development of Water Harvesting System

It was observed that the construction of a dugout pond and lining it with strong and durable cross laminated plastic sheets for harvesting rain and/or spring water has the following advantages:

- (i) Effective storage of harvested water by hindering seepage losses
- (ii) Low capital investment per litre of collected water

Effective storage of harvested water by preventing seepage losses

The seepage studies in a nearby farm pond with similar soil characteristics were carried out for analyzing the effect of lining. Daily observations on water depth, evaporation and rainfall were taken in respect of water saving efficiency. The storage behavior of the pond showed an average rate of seepage/ percolation loss up to $0.11 \text{ m}^3 / \text{m}^2$ wetted perimeter/day. Therefore, the total annual seepage loss expected from the constructed pond = $24.19 \text{ m}^2 \times 0.11 \times 365 = 971.23 \text{ m}^3$. Since the dugout-cum-embankment type unlined ponds had high rate of seepage and percolation, they could not hold water during the crucial dry season. However, the seepage/ percolation from the tank after lining was observed to be negligible. The storage hydrographs of the unlined and lined ponds (Fig. 7) clearly shows the increase in water saving efficiency of the pond after lining in terms of both quantity and duration of storage.

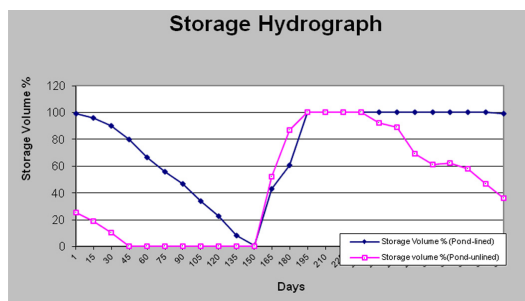


Fig. 7. Storage Hydrograph of UV resistant plastic lined pond

Low investment per litre of harvested water

Cost of construction of the water harvesting structure including excavation, rubble pitching, lining and other finishing works was Rs. 180686.00 as per the rates prevailing during the season under study. In terms of cost per m of storage capacity this would be Rs. 98.50 (Table 1).

Table 1. Financial analysis of costs involved in construction of plastic lined pond (as per the prevailing rates during the year 2004; Exchange rate: 1US \$= 44.95 INR)

Sl. No.	Items	Quantity	Rate per unit(Rs.)	Amount (Rs.)	Remarks
1.	Earth work in excavation of pond				
	a) Ordinary soil of pond bed	1105.68 m ³	39 per m ³	43,121.50	Rate fixed by committee CPWD rate
	b) hard rock of pond bed	698.2 m ³	70 per m ³	48,874.00	
2.	Smoothing and dressing of soil bed	672.412 m ²	3.50 / m ²	2,353.44	
3.	Fine sand cushioning	96.18 m ³	286 / m ³	27,508.85	
4.	UV resistant thermal welded 200 GSM (Silpaulin) plastic sheet	672.412 m ²	65 / m ²	43,706.78	
	HDPE Sheet in bottom for extra protection	320.616 m ²	35 / m ²	11,221.56	
5.	Laying and fixing of Silpaulin film	6 man days	Rs 150 per man day	900.00	As per Govt approved rates
6.	Miscellaneous expenditure			3000.00	
	Total amount			Rs.180686.13 say Rs.180686.00	
	Cost per m ³ of storage capacity of the pond			Rs. 98.50	

It was observed that the cost/litre of collected rain water/spring water in *uv* resistant plastic sheets was significantly less compared to other methods as shown in Table.2.

Table 2. Cost per unit volume of harvested water for different water harvesting structures

(Exchange rate in 2004: 1US \$ = 44.95 INR)

Sl. No.	Material	Cost/litre in Rs.
1.	Concrete	4.00 – 5.00
2.	Brick masonry	3.00 - 4.00
3.	Ferro-cement	1.50 - 2.00
4.	Fibre-glass	4.00- 5.00
5.	Clay (partial seepage)	0.05-0.10
6.	<i>uv</i> resistant plastic (<i>silpaulin</i>)	0.095-0.20

Moreover, the studies suggested that these technologies are sustainable, locally adoptable, cost-effective, applicable and affordable to the farmers. Saha et al., (2007) reported that lining of the water harvesting tank with 250 μ m low density polyethylene (LDPE) black agri-film involved an expenditure of ₹140 m⁻³ during the first year, which came down to 46 m⁻³ of stored water during the third year. However, the material used *i.e.* LDPE black agri-film is non *uv* resistant, which restrict its durability under the tropical humid climate. Whereas it is reported that the multi layered plastic sheets like *silpaulin* or nylon are cross laminated in structure and *uv* resistant in nature and therefore having high durability and strength (Samuel and Mathew, 2008).

2) Improving the Farming system

The development of a technically feasible perennial water source also made feasible the multiple uses of water other than irrigation and in turn helped to enhance the use efficiency of harvested water. Subsequently an innovative farming system had been developed by integrating three aspects viz., (i) Improving the use efficiency of harvested water, (ii) Developing multiple water use systems, and (iii) Cultivating high value crops using the harvested water.

Improving the productivity of coconut and component crops

Basin irrigation using a hosepipe connected to a centrifugal pump driven by a diesel or electric engine is the common method employed by farmers for irrigating coconut and other component crops. The basin irrigation requires more water and labour compared to micro-irrigation. After developing a permanent and continuous water source in the form of a water-harvesting tank, a drip irrigation system was installed in the farm mainly for crops like coconut and arecanut. It was observed that 40% of irrigation water could be saved by using the new system of irrigation.

While providing irrigation, vanilla (orchid species) stem and leaves also should get wet; this would in turn enhance the plant growth and yield. Therefore, irrigation was given to vanilla plants using micro jets attached to the lateral, which was laid at a height of 2 m above ground level by means of iron wires. The fine spray of water coming from the micro jets not

only wet the leaves and stem, but also created a microclimate favorable to vanilla. Gravitational force was employed to direct water from the tank, which was located at a higher elevation, for irrigating the palms through drip system, while a 2 HP electric centrifugal pump was used for the micro-jets as it requires higher head to produce fine spray. The water spilling over the tank was directed to contour trenches for groundwater recharging.

Creation of *in-situ* structures for this purpose are well demonstrated under undulating topography and steep hill sides of other high rainfall zones of India by many non-government development organizations (Rao, 2010; Kote and Wagle, 2010). Verma et al. (2003) reported increase of field crop productivity from 62.42% in case of paddy to 250.26% in linseed due to irrigation with harvested rain water along with application of farm yard manure to crops in a hill farming system.

Developing multiple water use systems

Intensive freshwater aquaculture in the storage tank was a relatively simple component the farmer integrated with irrigation to make it a multiple water use system. The aquaculture unit required substantial volume of water but actually consumed very little of that volume. By linking enterprises, the farmer could use the output water from the aquaculture directly as irrigation water. The nutrients (particularly N and P) in the output water were effectively utilised by the irrigated crop, partially substituting for the costly chemical fertilisers. In contrast, if each of the above enterprises were operated in isolation, the total volume of water extracted from the natural environment would be much higher, and the first enterprise (aquaculture) would require a separate strategy for disposal of the biological waste.

The African catfish (*Clarias gariepinus*) was grown in the constructed tank. This particular fish was selected mainly because it takes air from atmosphere. Since there was facility neither to circulate water nor to provide aeration, the water gets dirty very fast. This particular variety of fish was found to be very adaptable under these circumstances. Direct selling strategy was adopted for marketing the harvested fishes and was found to be very effective.

Nutrient rich water from the fishpond was used to irrigate the crops through gravity operated micro irrigation system. The fertilizer use could also be reduced by applying nutrient rich water from fishpond for irrigation.

Cultivating high value crops

In order to make the irrigated agriculture a profitable venture, high value crops that grow better under filtered sunlight were introduced. After introduction of continuous irrigation, more intercrops such as pepper, beetle vine and banana were established in the garden. Moreover, *Salvinia cuculata*, an aquatic weed plant, was introduced as a cover crop in the tank to control evaporation. This when harvested was utilized to provide mulch for coconut and arecanut palms.

The study revealed that the innovative approach to harvest and store rainwater in large quantity and its subsequent use for irrigation has the following visible impacts.

Increase in production and productivity

The area under Vanilla cultivation could be increased to 1 ha area from 0.4 ha due to assured irrigation water supply during summer months. The availability of water throughout

the year helped to provide assured irrigation in the entire garden through micro irrigation and to maintain a better microclimate.

The observations showed that the average productivity of coconut palm had been increased from 50 coconuts/ palm to 90 coconuts/ palm over a period of five years. During the same period Arecanut yield was increased from 1.6kg dried nuts/ palm to 2.2 kg dried nuts/ palm.

The increase in production of coconut palms could be attributed to the newly introduced soil and water management practices such as mulching the palm basins with dried *Salvinia* and consistent irrigation to the palms using the nutrient rich water from the tank.

The fish culture was adopted in the pond as a subsidiary enterprise by introducing 6,000 fingerlings of the variety African catfish. An amount of Rs.41,500.00 (Table.3) was spent annually towards the capital and maintenance costs, whereas the returns through the sale of fishes fetched an earning of Rs.90,000.00 per year.

It has been unequivocally accepted that soil mulching has several advantages like increase in moisture retention and improvement in other soil conditions. Water hyacinth and *salvinia* are considered better mulches among various organic mulches like leaf mould, rice husk, saw dust etc. (Mc Farland *et al.*, 2004). In the present case study, the dried *Salvinia* plants, which were grown as cover crop in the pond, mixed with fish waste and fish feeds served as an effective mulch and manure.

Previous studies suggested that ornamental fish culture in large tanks are financially and economically viable and investment friendly (Subhasis Mandal *et al.*, 2007). In the present study, it has been estimated that a sum of Rs.2,48,386 was required as capital cost to establish a uv resistant plastic lined water harvesting tank integrated with micro-irrigation system and fishery unit (Table 3). The major cost component in initial investment was construction and lining of tank and installation of micro-irrigation system (Table 3).

The major variable cost components were cost of labour, feed, fingerlings, plant/ fish protection, polythene bags and charges for electricity and maintenance and other miscellaneous costs. For the first year, as depicted in Table.3, it was estimated to be Rs.41,500, but in the subsequent years it was found to be a little less as the amount spend on purchase of fingerlings was less.

Based on the estimated annual costs and returns, the Benefit-Cost ratio was found to be 1.69, which is well acceptable. Other financial viability criteria (IRR and NPV) were also found favourable for investment on plastic lined water harvesting tank integrated with micro-irrigation system and fish farming (Table 4). The analysis indicated that establishment of such an integrated system was not only financially viable but a highly attractive proposition also for the low cost harvesting and effective use of rainwater/runoff. Some assumptions were also made in consultation with fishery scientists and the farmer.

Table 3. Details of fixed and variable costs and returns of the integrated water harvesting system (Exchange rate in 2006: 1US \$= 44.95 INR)

Sl.No.	Particulars	Amount (Rs.)
	Expenditure incurred (2006-07 prices in Kerala)	
	I. Initial Investment (fixed costs)	
1.	Rental value of land (670 sq.m @ Rs. 10/sq.m)	6,700.00
2.	Initial investment for constructing RWH tank	1,80,686.00
3.	Initial investment for installing micro-sprinkler system	37,000.00
4.	Electric Pump set (2 HP)	8,000.00
5.	Balance	7,000.00
6.	Net, bucket	4,000.00
7.	Glass wares	5,000.00
	Total	2,48,386.00
	II. Annual Costs (variable costs)	
	(as per 2006-07 prices in Kerala)	
1.	Cost of Brood fish	6000.00
2.	Feed	16000.00
3.	Chemicals, medicine, polythene, bags	3000.00
4.	Electricity and maintenance	3000.00
5.	Labour charges (Rs. 125 / man-day)	12500.00
6.	Miscellaneous	1000.00
	Total	41,500.00
	III. Returns	
1.	Sale of fish	90,000.00
2.	Reduction in labour charges (used for irrigation/fetching water(per annum) = 200 man days/year @ Rs. 125/manday)	25,000.00
3.	Reduction in crop (Vanilla) loss (previously 100 kg / year lost due to water @ Rs. 200/kg)	20,000.00
4.	Enhanced coconut yield from 50 to 90 nuts per palm for 140 coconut palms @ Rs.6/nut)	33,600.00
5.	Enhanced yield of 260 arecanut palms from 1.6 kg dried nuts/ palm to 2.2 kg dried nuts/ palm @ Rs.70/ kg)	10,920.00
6.	Reduction in fertilizer application (previously 1 kg/plant organic fertilizer was applied. This reduced to 0.5 kg/plant. Supplemented by additional application of <i>salvinia</i> enriched with fish meal)	4,500.00
	Total	1,84,020.00

Income from component crops such as banana, beetle vine and vegetables were not included since the same was not cultivated systematically and the produce was mainly consumed by the farmer.

Table 4. Financial viability of establishing plastic lined water harvesting tank

(Exchange rate in 2006: 1US \$ = 44.95 INR)

Sl.No.	Investment criteria	Value
1.	Net present value (NPV, in INR)	3,12,462.85
2.	Internal rate of Return (IRR, in %)	42
3.	Benefit-Cost ratio(BCR)	1.69

The farmers and local communities identified rainwater harvesting as a workable technology option for providing a dependable source of drinking as well as irrigation water and also for preserving the vital ground water reserves. More farmers need to be educated further on the problems crippling the conventional sources and the need for the “shift”.

In the present paper, the Rain Water Harvesting initiative of an innovative farmer in N-E Kerala was studied. The filed experience in mitigating the ill effects of drought and providing security against future droughts were brought together apart from exploring out new avenues for profitable agriculture through integrated fish farming. The financial analysis indicated that such an integrated system is not only financially and economically viable to the farmer but lead them to a sustainable farming approach. Based on the estimated annual costs and returns, all the financial viability criteria (IRR, NPV and BCR) were found favourable for investment on plastic lined water harvesting tank integrated with micro-irrigation system and fish farming. The approach to harvest rainfall in bulk quantity, as explained in this paper, for drought proofing with generation of additional income through integrated farming could be considered as a model and more such type of locally adaptable and sustainable on-farm technologies should be identified and explored.

References

- Anonymous. 2004. *Hand Book on Rainwater Harvesting*. Kerala Rural water Supply and Sanitation Agency, Thiruvananthapuram, Kerala, India, 26-30.
- Jugale.V.B. 2004. “Impact of Rain Harvesting Techniques in Dry Zone of Sangli District.” *Water Resources Development and Management*. (H. Chandrasekharan, R.K. Sharma and K.V. Sundaram, Eds.), Mittal Publications, New Delhi, India, 287-304.
- Khan, M. A. 2010. *Enhancing Water Productivity through Multiple Uses of Water in Indo- Gangetic Basin*. Innovation and Sustainable Development in Agriculture and Food- ISDA 2010, Montpellier, France June 28-30, 2010.
- Kote, R.C., Wagle, S. M., 2010. Integrated small scale water resources management in Maharashtra. *Leisa India*, 12(3): 30-31.
- Luisa Cortesi and Eklavya Prasad. 2009. “Rainwater harvesting for management of watershed ecosystems.” 2009. *Rainwater Harvesting: A Lifeline for Human Well-being*. Jennie Barron, Ed., A Report prepared for UNEP by Stockholm Environment Institute, Stockholm, 14-22.
- Mc Farland, D. G., Nelson, L. S., Grodowitz, M. J., Smart, R. M., and Owens, C.S. 2004. “*Salvinia molesta* D. S. Mitchell (Giant Salvinia) in the United States: A Review of Species

- Ecology and Approaches to Management". *Aquatic Plant Control Research Program*. U.S. Army Engineer Research and Development Center, Lewisville.
- Rajagopal, V., Arulraj, S., Rayudu, B.T., Manoj P. Samuel. 2004. *Watershed Based Approach for Sustainable Development*. Kasaragod District Panchayat and Central Plantation Crops Research Institute (ICAR), Kasaragod, Kerala, India, 65-72.
- Rao, K. R. 2010. Oases of productivity – A case of small scale watershed development. *Leisa India*, 12(3):6-7.
- Saha, R., Ghosh, P. K., Mishra, V. K., Bujarbaruah, K. M. 2007. Low-cost micro-rainwater harvesting technology (*Jalkund*) for new livelihood of rural hill farmers. *Current Science* 92(9):1258-1265.
- Samuel, M. P., Mathew, A.C.2008. Rejuvenation of water bodies by adopting rainwater harvesting and groundwater recharging practices in catchment area-a case study. In: Sengutha, M., Dalwani, R. (Eds.), *Proceedings of TAAL 2007: The 12th World lake Conference*, 12th World lake Conference, Rajasthan, India, pp.766-776.
- Singh, R.K., Lama, T.D., Saikia, U.S., Satapathy, K.K. 2006. "conomics of Rainwater Harvesting and Recycling for Winter Vegetable Production in Mid Hills of Meghalaya. *J. Agricultural Engineering* 143(2):33-36.
- Subhasis Mandal., Mohapatra, B.K., Tripathy, A.K., Med Ram Verma., Dutta, K.K., and Ngachan, S.V. 2007. Agribusiness Opportunities for Ornamental Fisheries in North- Eastern region of India. *Agricultural Economics Research Review* 20 (Conf.Issue), 471-488.
- United Nations Millennium Development Goal Indicators (UN MDG). 2009. *Official web site for monitoring MDG indicators*, <<http://unstats.un.org/unsd/mdg/Default.aspx>> (January27, 2009).
- Venkateswarlu, J. 2001, . *Technical Manual on Watershed Management-I: Resource Conservation*. National Institute of Agricultural Extension Management, Hyderabad, India.
- Verma, A. K., Singh, V. B., Singh, V. P. 2003. Water management for sustainable hill farming system: A case study, in: Bhatt, B.P., Bujarbaruah, K. M., Sharama, Y. P., Patiram. (Eds.), *Approaches for Increasing Agricultural Productivity in Hill and Mountain Ecosystem*. ICAR Research Complex for NEH Region, Barapani, India. pp. 87-94.

Effective utilization of bioresources for yield improvement in *Plumbago rosea* intercropped in coconut garden

K. Nihad, P. C. Jessykutty and P. Sivaprasad

Central Plantation Crops Research Institute
Regional Station, Kayamkulam-690 533, Kerala, India

Plumbago rosea L. (Family: Plumbaginaceae), known as Rosy flowered lead wort or fire plant in English, is an important medicinal plant, the root of which is used in more than 100 ayurvedic formulations such as *Chitrakasava*, *Koduvelisudhachoorana* etc. The roots are useful against fever, cough, ring worms, leucoderma, dyspepsia, skin diseases, scabies and anaemia. Eventhough the crop is cultivated in isolated pockets of the state; the domestic production is quite insufficient to meet the ever increasing demand. In Kerala, there is ample scope for introducing it as an intercrop in coconut and rubber plantations. *Plumbago rosea* has already been identified as a potential intercrop in coconut plantations. Agro-technology for the cultivation of *Plumbago rosea* as an intercrop in coconut garden has been developed which includes the use of both organic and chemical fertilizers (KAU, 2007). But, growing medicinal plants using chemical fertilizers and pesticides may alter their active ingredients and cause deterioration in their quality. In India, the single most important factor, which is standing in the way of wider acceptance of drugs of plant origin, is the non-availability of quality raw material. The quantity and quality of crude drug can be enhanced by growing under organic condition, but high cost of organic manures is the limiting factor. Hence, a study was taken up to identify the locally available and cheap bioresources that can enhance the yield of *Plumbago rosea*.

The study was carried out in the instructional farm, College of Agriculture Vellayani Kerala, which is situated at 8° 30' North latitude and 76° 54' East longitude, at an altitude of 29 m above MSL, during 2004-05. The crop was raised as an intercrop in coconut garden with palms above forty years of age spaced at 7.5m. The shade intensity of the field was estimated as 25 per cent using Model LI-250 Light meter. The climate of the experimental site is humid tropical. The mean annual rainfall during the period was 140mm. The mean annual maximum and minimum temperatures were 31.77 and 21.9° C. The pH of the soil was 5.2 and was low in available N and K but high in available P as per the soil sample analysis.

The treatments were formulated based on the Package of Practices (POP) Recommendations of Kerala Agricultural University (KAU, 2007). Three organic manures (Farm yard manure, Neem Cake and Vermicompost) and three microbial inoculants (Arbuscular Micorrhizal Fungi, *Azospirillum* and Phosphobacteria) were used in the experiment. The nutrient requirement of the crop (50:50:50 kg NPK ha⁻¹) based on nitrogen equivalents were calculated and supplied to the plants as 100%, 75% and 50% of recommended dose of nitrogen (RDN) through organic manures and additional P & K as rock phophosphate and wood ash respectively.

The treatments supplying 75% and 50% of the nitrogen requirement were supplemented with equal quantity of microbial inoculants mixture consisting of AMF (*Glomus* spp.), *Azospirillum* and Phosphobacteria (*Bacillus megatherium*). AMF was well mixed with top

soil @ 200 g m⁻², *Azospirillum* and phosphate solublizers (*B. megatherium*) applied as soil drench (each @ 2 kg ha⁻¹). Full dose of organic manures and microbial inoculants as per the treatments were applied as basal dose before planting. In the control plot (T₁) fertilizers (50:50:50 kg NPK ha⁻¹) and FYM (10 t ha⁻¹) were applied as per POP recommendations of KAU.

The analytical report of the major nutrients contained in the organic manures used for the experiment is given in Table I. Farmyard manure was applied @ 10 t ha⁻¹ uniformly to all the plots. Quantity of neem cake and vermicompost was so fixed as to supply 25 per cent of the N requirement of the respective treatment and the remaining quantity through FYM. Additional quantity of P and K requirement was supplied as rock phosphate and wood ash. Nutrients were applied on N equivalent basis. For the control treatment, urea (46 % N), rock phosphate (16 % P₂O₅) and muriate of potash (60 % K₂O) were used as chemical sources of nitrogen, phosphorus and potassium respectively. Commercial inoculum of AMF containing a mixture of *Glomus fasciculatum*, *Glomus etunicatum* and *Glomus* sp. maintained in pots using sorghum as host was used. The inoculum consisted of root bits, mycelial fragments, and vermiculite-perlite substrate carrying chlamydospores of AMF. The vermiculite-perlite inoculum containing 400 propagules per gram was used for the study. It was well mixed with top soil @ 200 g m⁻². *Azospirillum* and phosphate solublizers (*Bacillus megatherium*) applied as soil drench (2 kg ha⁻¹ in 60 litre water).

Table 1. NPK content of organic manures

Sl. No.	Organic manures	Nutrient content (%)		
		N	P	K
1	FYM	0.9	0.4	1.2
2	Vermicompost	0.6	0.5	1.2
3	Neem cake	1.7	0.2	2.0

The experiment was laid out in simple RBD with three replications as per the treatment combinations given in Table 2. Rooted cuttings of *P. rosea* were planted in raised beds of 2.5 m x 0.75 m at 50 x 15 cm spacing.

Treatment combination with FYM + Neemcake (50 % N) + microbial inoculants (T₇) recorded the highest root fresh weight, root dry weight and root yield/ha (Table 3). This may be due to the increased nitrogen use efficiency (NUE) as well as agronomic use efficiency of neem cake (Kumar and Ali, 2003) along with microbial inoculants. The treatments supplied with 75% and 50% N through FYM along with microbial inoculants also recorded higher root yield. Use of microbial inoculants as a cost effective supplement was reported by Pandey and Kumar (2002). The effect of microbial inoculants in enhancing yield was reported in many plants such as turmeric (Subramanian *et al.*, 2003), periwinkle (Gupta *et al.*, 2003) and ginger (Sreekala, 2004). These plants also recorded higher leaf count and chlorophyll content which might result in higher photosynthetic efficiency and thus total dry matter production. The higher root length and root girth of these plants also attributed to the higher yield. The plants were analyzed for NPK uptake. The highest N, P and K uptake was also recorded in the treatment combination with FYM + NC (50 % N) + microbial inoculants. Vermicompost supplied plants gave better performance in initial months but the growth rate and yield decreased in later phase.

The study revealed that treatment supplying 50 % RDN through Farm Yard Manure and Neem Cake along with the microbial inoculants mixture recorded the highest root characters such as root length (80.1cm), root girth (3.03cm), fresh root weight (86.33g/plant), dry root weight (33.03g/plant) and fresh root yield (7.6 t/ha). This treatment also recorded highest benefit cost ratio (2.73). This experiment shows that the use of bioresources such as Neem Cake and FYM in the ratio 1:4 along with the microbial inoculants as basal dose has a long term effect on the nutrient supplying capacity of the soil thus reducing the cost of production of organic cultivation and enhancing the benefit cost ratio of the farmers.

References

- Gupta, M.L., Mishra, A. and Kanuja, S.P.S. 2003. Root colonization by VAM fungi effects the growth and biomass yield of Periwinkle. Nat. Sem. New Perspectives in Spices, med. Arom. Pl., 27-29 November 2003. Indian Society for Spices, ICAR Research Complex for Goa and Indian Institute of Spices Research, Kozhikode. *Abstract*: 101
- KAU. 2007. *Package of Practices Recommendations : Crops*. Twelfth edition. Kerala Agricultural University, Thrissur, 278 p.
- Kumar, M. and Ali, S.A. 2003. Effect of organic isolates from non-edible oil seed cakes in enhancing urea – NUE in tomato. *Ann. agric. Res. New Series* 24: 681-683
- Nambiar, V.P.K., Jayanthi, A. and Sabu, T.K. 2003. Pharmacognostical studies on *Plumbago indica* Linn. *Aryavaidyan* 17 (1): 7-16.
- Pandey, V. and Kumar, D. 2002. Biofertilizers for sustainable agriculture. *Agric. Today* 5: 44-47.
- Sreekala, G.S. 2004. Effect of organic manures and microbial inoculants on growth, yield and quality in ginger. Ph.D. thesis, Kerala Agricultural University, Thrissur, 312 p.
- Subramanian, S., Rajeswari, E., Chezhiyan, N. and Shiva, K.N. 2003. Effect of *Azospirillum* and graded levels of nitrogenous fertilizers on growth and yield of turmeric (*Curcuma longa* L.). Nat. Sem. New Perspectives in Spices, med. Arom. Pl., 27-29 November 2003. Indian Society for Spices, ICAR Research Complex for Goa and Indian Institute of Spices Research, Kozhikode.

Table 2. Treatment combinations and the quantity of NPK supplied per hectare

Sl. No.	Treatments	NPK supplied (kg ha ⁻¹)
1	T ₁ : Package of practices recommendation(KAU,2002)	50:50:50
2	T ₂ :FYM alone(75 % N)	50:50: 67.2
3	T ₃ :FYM (75 % N)+ microbial inoculants	37.5: 50 :50
4	T ₄ :FYM (50 % N)+ microbial inoculants	25:50:50
5	T ₅ :FYM+ neem cake (100 % N)	50:50:92.5
6	T ₆ :FYM + neem cake (75 % N)+ microbial inoculants	37.5:50:50
7	T ₇ :FYM + neem cake (50 % N)+ microbial inoculants	25:50:50
8	T ₈ :FYM + vermicompost (100 % N)	50:50:75
9	T ₉ :FYM + vermicompost(75 % N) + microbial inoculants	37.5:50:50

10	T ₁₀ : FYM + vermicompost (50 % N) + microbial inoculants	25:50:50
11	T ₁₁ : FYM + neem cake + vermicompost (100 % N)	50:50:50
12	T ₁₂ : FYM + neem cake + vermicompost (75 % N) + microbial inoculants	37.5:50:54.6
13	T ₁₃ : FYM + neem cake + vermicompost (50 % N) + microbial inoculants	25:50:50

Table 3. Growth parameters under treatments

Treat-ments	Fresh weight of roots (g plant ⁻¹)	Dry weight of roots (g plant ⁻¹)	Yield (kg ha ⁻¹) (FW basis)	Number of roots	Length of roots (cm)	Girth of roots (cm)	BC ratio
T ₁	32.47	17.83	2857.07	8.00	45.67	2.60	1.14
T ₂	40.80	19.73	3590.40	7.30	36.43	1.80	1.37
T ₃	68.17	27.80	5998.67	10.30	35.00	3.10	2.20
T ₄	63.83	27.00	5617.30	10.70	64.03	2.73	2.05
T ₅	56.97	22.47	5013.07	9.30	46.40	2.87	1.88
T ₆	60.57	19.50	5229.87	22.00	47.67	2.53	1.83
T ₇	86.33	33.03	7597.30	11.30	80.10	3.03	2.73
T ₈	39.17	26.77	3446.67	8.00	59.37	2.23	1.20
T ₉	47.17	17.73	4150.70	23.30	29.10	2.07	1.47
T ₁₀	60.10	30.63	5288.80	10.70	69.80	3.03	1.93
T ₁₁	58.23	25.33	5124.50	14.30	40.80	2.90	2.00
T ₁₂	57.57	27.70	5065.87	7.30	36.73	3.00	1.80
T ₁₃	49.30	23.73	4338.40	16.00	43.50	2.73	1.53
SE	2.17	1.98	193.17	0.82	1.46	0.047	0.089
C.D. (P=0.05)	6.34	5.79	563.86	2.40	4.25	0.138	0.262

An assessment of species structure and crop diversity in the homestead farms of Kerala, India

V. Krishnakumar, S. Rageena, Jacob John, K. Geetha and D.V.S. Reddy

Central Plantation Crops Research Institute
Regional Station, Kayamkulam-690 533, Kerala, India

Kerala state on the south-western coast of India in the tropical humid zone is one of the smallest states in India. The unique feature of the agricultural scenario of Kerala is the existence of a dispersed pattern of settlement with homestead system of cultivation. These homestead farms are increasingly recognized as loci for *in situ* conservation of agro biodiversity. An attempt was made to assess the status of floral diversity of these homestead farms to formulate appropriate strategies for their future conservation.

The study on the floral diversity of homestead farms of various agro climatic zones of Kerala covering all the 14 districts was carried out through an extensive survey during 2002-04 as part of the National Agricultural Technology Project on "Analysis and Development of Homestead Farms of Kerala and Andaman and Nicobar Islands-A Farmer Participatory Approach". For the survey, the farmers were selected through multistage random sampling technique and from each district, 25 percentage of the panchayats and from each panchayat, 10 farmers were selected. Data were collected from 2,500 farmers through personal interview method with the help of a pre tested questionnaire. The details of homestead farms surveyed are presented in Table 1. Data on floral diversity of each of the homestead farms including crops, trees, ornamental plants, weeds etc. were documented.

Table 1. Details of homestead farms surveyed in Kerala

Name of zone	Name of district	No. of homestead farms surveyed
South zone	Thiruvananthapuram	450
	Kollam	
	Pathanamthitta	
Central zone	Thrissur	660
	Palakkad	
	Idukki	
	Wynadu	
Problem zone	Kottayam	590
	Alappuzha	
	Ernakulam	
North zone	Kozhikkode	800
	Malappuram	
	Kannur	
	Kasaragod	
Total no. of homestead farms surveyed		2,500

Socio economic characteristics of the sample: The size of the homestead farms studied ranged from 0.04 ha to 0.2.40 ha with an average of 0.35 ha, falling well within the range of global inventory of other tropical home gardens reported by Fernandes and Nair (1986). In general, only 43 percentage of the families surveyed were found to depend entirely on agriculture as their source of income and livelihood. Those families which depended on agriculture did most of the farm work using their family labour supplemented with hired labour for works during peak seasons of planting, interculture and harvest.

Floral diversity: All the homestead farms displayed a high ecological importance for herbaceous species, including vegetables, ornamental plants and medicinal crops. The study showed that the total number of cultivated and wild species of plants in the homesteads varied greatly depending upon the crops cultivated and the intensity of management. Increased human intervention has greatly eroded the diversity in some of the home gardens, but those with a lesser degree of management showed as high as 180 different species in an area of 0.4 ha. This includes both planted and non-planted species. The practice of planting several species and varieties of crops ensures nutritional security, diet diversity, risk spreading and maximisation of returns. The details of floral diversity recorded are presented in Table 2.

Table 2. Floral diversity observed in homestead farms (percentage of categories of plants)

Sl.no.	Category of plants	Percentage
1	Food crops(fruits, vegetables, spices and tuber crops)	33.8
2	Ornamentals	19.1
3	Timber and miscellaneous trees	14.6
4	Medicinal plants	12.7
5	Weeds	11.5
6	Plantation crops	3.3
7	Other plants (oil seeds, beverages, cereals, fodder etc.)	5.0

Coconut was the base crop in most of the homestead farms surveyed, in the inter space of which a wide variety of crops ranging from annuals to perennials were cultivated so as to generate cash income after meeting the subsistence needs.

Among the various crop species, the maximum percentage was noticed in the case of food crops (33.8) which consist of fruit crops (13.4); vegetables (10.2); spices (5.7) and tuber crops (4.5). Various types of ornamental plants occupied 19.1 percentage of the species diversity, followed by timber and miscellaneous trees (14.6 percentage) and medicinal plants (12.7 percentage). Plantation crops as a group represented 3.2 percentage of the crop species diversity. Various types of food crops are of seasonal in nature and their inclusion as components of homestead farming is a definite indication of nutritional security of farm families being planned under such a system of cultivation. Timber and miscellaneous trees are perennial in nature and they are being raised either for augmenting income through sale of wood (timber species) or for house hold fuel requirement (miscellaneous trees).

A wide range of crops including trees, shrubs, herbs and creepers could be seen in the interspaces of coconut palms. The common annuals grown are banana, tuber crops like

cassava, elephant foot yam and dioscorea, spices such as ginger and turmeric and a variety of summer vegetables. Amaranth, okra, egg plant, cucumber, chilli and little gourd were some of the vegetables grown. Chikkurmanis, a hedge type green leafy vegetable and curry leaf were noticed in all the homestead farms surveyed.

Though many species of medicinal plants were noticed, none is grown on a commercial basis and some of the species are ingredients of home remedies. The floristic structure of homestead farms is also very variable, but there is a core group of very frequent species, with significant representation of the local flora. This suggests that the homestead farms may contribute to the conservation of native species. Home gardens usually exhibit high diversity as evidenced by reports of 68 tree species in Karnataka home gardens (Shastri *et al.*, 2002), 122 from Assam (Das and Das, 2005) and 127 from Kerala (Kumar *et al.*, 1994).

The intensive poly culture of perennials and annuals in most compatible manner is a highly potential strategy for efficient exploitation of scarce resources of land, water and solar energy. The crop sequence progressing from creepers like cucumber through different canopy layers of vegetables, tubers, banana, lesser fruit trees and tall trees have been reported to give the farm an appearance and structure similar to that of a tropical rain forest (Jose and Shanmugaratnam, 1993). The gradient of light and relative humidity enables various species groups to exploit them (Michon *et al.*, 1983). It also helps to recycle organic waste biomass in the production system and contributes to soil nutrient enrichment through leaf litter and prevents soil erosion. Differential rooting pattern of various crops in the system will enable forage soil at various depths ensuring nutrient balance in soil.

It has been observed that the small and marginal farmers of the State place a high value on their homestead farms as a source of nutritional security, additional income and for risk minimization. They also play a significant role in maintaining the quality of micro environment within the farm. These farms, which can be grouped as low to medium input subsistence system, also promote and conserve diversity. A basket of agricultural and horticultural crops and other miscellaneous plants in the homestead farms indicate a high level of crop diversity. Cultivation of a wide range of traditional and high-yielding varieties of grains, legumes, vegetables, and fruits is a positive feature in terms of sustainability.

There is, therefore, a need to develop policies that will continue to support the rich agro biodiversity of these areas, while providing for the nutritional and economic needs of the population. Coconut-based homesteads of Kerala exemplify an intense land use system, in which plants, animals and man co-exist in a mutually symbiotic manner. The value of home gardens as sources of crops and varieties is assessed by analysis of their species diversity. Each home garden is characterized by a unique set of components. Knowledge on the composition could serve as an effective tool for location specific land use planning and development.

Problems and opportunities

The practice of planting and nurturing different varieties and species of crops on the same piece of land ensures diet diversity, nutrition security and risk spreading. However, if done unscientifically it may mean that no crop receives intensive and scientific management so that its full yield potential is realized. A large number of plants again may lead to overcrowding resulting in under productivity. The large amount of diversity also results in a wide variety of

produces for the market which are most often sold through the village merchant without even any primary processing other than drying. This results in low price to the produce. The survey revealed that most of the homesteads sold products like coconut, arecanut, black pepper and cashew nut to earn cash income. However, all the products are sold as they are harvested without any value addition so that the net income from the system was low. The income could be increased through small scale processing and value addition like production of white pepper, copra and virgin coconut oil etc. This can be brought about through awareness programmes and processing through group approach.

The number of crop species and species composition found in homestead farms may be attributed to the socio-economic conditions of farmers as well as to their needs. The conservation of cultivated plants in homestead farms not only preserves a vital resource for mankind but also provides significant economic and nutritional benefits for the rural farm families. Thus, the homestead farms were found to be complex systems with plant diversity conserved through their use. Data collected from the study could be used in the formulation of agricultural policies and providing technical support. The farmer preferences should be considered when decision-makers provide farmers with agricultural support or the farmers can be helped with better management policies to conserve some of the specific floral diversity in their homestead farms.

Acknowledgement

The authors are grateful to the National Agricultural Technology Project (ICAR) for providing financial assistance for the project work. They are also thankful to all the farmers participated in the study.

References

- Das, T. and Das, A. K. 2005. Inventorying plant diversity in the home gardens: A case study in Barak Valley, Assam, North East India. *Current Sci.* **89**(1): 155-163.
- Jose, D. and Shanmugharatnam, N. 1974. Microfarms and household economy of rural Kerala: An empirical analysis with special reference to the home garden farming system. *Proceedings of the International Congress on Kerala studies*. **4**:86-88.
- Fernandes, E. C. M. and Nair, P.K.R. 1986. An evaluation of the structure and function of tropical home gardens. *Agricultural Systems* **21**:279-310.
- Kumar, B. M., George, S. J. and Chinnamani, S. 1994. Diversity, structure and standing stock of wood in the home gardens of Kerala in Peninsular India. *Agroforestry Systems* **25**: 243-262.
- Michon, M. G., Mary, F. and Bompard, J. 1986. Multistoreyed agroforestry system in West Sumatra, Indonesia. *Agroforestry Systems* **4**:315-338.
- Shastri, C. M., Bhat, D. M., Nagaraja, B. C., Murali, K. S. and Ravindranath, N. H. 2002. Tree species diversity in a village ecosystem in Uttara Kannada district in Western Ghats, Karnataka. *Current Science* **82**: 1080-1084.

A holistic systems approach to identify sustainable perennial crop based systems - a case study

Jacob John, M. Joy, V. Geetha and U. Sheeba

Cropping Systems Research Centre

Kerala Agricultural University, Karamana, Thiruvananthapuram 695 002, Kerala, India

Land, the major production function, is limiting under smallholder farming conditions and it is for these populous small holders of Kerala that integrated land use practices are becoming increasingly important. Homesteads/Home gardens of Kerala exemplify an intense integrated farming system in which plants, animals and man coexist in a mutually symbiotic manner. However, selection and management of enterprises in the homesteads is mostly unscientific and based on farmer's perception and experience. Unless the management is directed in the right path, there is imminent danger to the system's sustainability.

Coconut and arecanut-based systems are dominant in many parts of the world. Farmers cultivate a host of crops including perennials, annuals and seasonal crops along with coconut, without identifying optimum crop combinations. In most cases the evaluation refers primarily to financial criteria and to factors directly underlying those criteria. This emphasis on economics alone decries the importance of several other system attributes properties. A holistic approach is to be adopted to ascertain the sustainability of the systems, identify and recommended most suitable system. Hence, a study was undertaken to identify the most sustainable coconut and arecanut based cropping system using two different approaches.

The study was undertaken in the farmers' fields in Mannarkkad and Sreekrishnapuram Blocks of Palakkad District during 2005-2007. Mannarkkad is a small town in Palakkad district of Kerala state, south India. Mannarkkad has an agri-based economy. Main agriculture produce consists of natural rubber, coconut, arecanut, banana, nutmeg, pepper and rice. Sreekrishnapuram is a small village near Cherpulassery in Palakkad District. Major crops are rice and coconut.

From the study area, certain coconut and arecanut based systems commonly adopted were identified and subjected to detailed systems analysis. The nineteen systems can be broadly classified into arecanut based systems (8 numbers) and coconut based systems (11 numbers). Detailed description of the different systems (plant population of the components, spacing and varieties) are given in Tables 1 to 3.

Table 1. System description: Population of the components

No.	Systems	Plant population per ha
1	Arecanut (A)	(A) = 1111
2	Arecanut (A)+Banana (B)	(A) = 1111 ; (B) = 1111
3	Arecanut (A)+Banana (B)+Xanthosoma(X)	(A) = 1111; (B) = 1111; (X) = 2666
4	Arecanut (A)+Pepper (P)	(A) = 1111; (P) = 1111
5	Arecanut (A)+Pepper (P)+Coffee (Cf)	(A) = 1371; (P) = 1371 (Cf) = 1230

6	Arecanut(A) +Vanilla (V) (Type 1): Vanilla trailed on <i>Gliricidia</i> and Areca	(A) = 1111; (V) = 4524
7	Arecanut(A)+Vanilla (V) (Type2): Vanilla trailed on rubber tyre tied between areca	(A) = 1111; (V) =1111
8	Arecanut (A)+Vanilla (V) (Type 3): Vanilla trailed on <i>Gliricidia</i> only	(A) = 1371; (V) = 1371
9	Coconut (Ct)	(Ct) = 123
10	Coconut (Ct)+Arecanut (A)+Pepper (P)	(Ct) = 123; (A) = 1111; (P) = 1234
11	Coconut(Ct)+Arecanut (A)+Pepper (P)+Banana (B)	(Ct) = 167 ; (A) = 1000, (P) = 167 ; (B) = 1500
12	Coconut (Ct)+Arecanut (A)+Pepper(P)+Nutmeg (N)	(Ct) = 69; (A)= 1111; (P) = 69; (N) = 69
13	Coconut (Ct)+ Arecanut(A)+Pepper(P)+Nutmeg (N)+Vanilla (V) (Systematic)	(Ct) = 129; (A) = 657; (P) =129; (N)=129; (V) = 1371
14	Coconut (Ct)+ Arecanut(A)+Pepper(P)+Nutmeg (N)+Vanilla (V) (Mixed)	(Ct) = 139; (A) = 2500; (P) =816; (N)=139; (V) = 2500
15	Coconut (Ct)+ Arecanut (A) +Pepper (P)+Vanilla (V)	(Ct) = 69; (A)= 555; (P) = 69; (V) = 4444
16	Coconut (Ct)+Pepper (P)+Banana (B)+Nutmeg (N) (Y)	(Ct) = 69; (P) = 69; (B) = 1111; (N) = 69
17	Coconut (Ct)+Pepper (P)+Cocoa (Co)	(Ct) = 92 ; (P) = 92; (Co) = 555
18	Coconut (Ct)+Pepper (P)+Nutmeg (N)	(Ct) = 167; (P) = 167; (N) = 167
19	Coconut (Ct)+Pepper (P)+Vanilla (V)	(Ct) = 159; (P) = 318; (V) = 2380

Table 2. System description: Spacing of the components

Systems	Spacing
A	(A) = 3x3 m
A+B	(A) = 3x3 m ; (B) = 3x3 m
A+B+X	(A) = 3x3 m; (B) = 3x3m; (X) = 2.5x1.5m
A+P	(A) = 3x3m ; (P) = 3x3m
A+P+Cf	(A) = 2.7x2.7m; (P) = 2.7x2.7m; (Cf) = 2.8x2.8m
A+V (Type 1)	(A) = 3x3m ; (V) = 1.3x1.7m
A+V (Type 2)	(A) = 3x3m ; (V) =3x3m
A+V (Type 3)	(A) = 2.7x2.7m; (V) = 2.7x2.7m
Ct	(Ct) = 9x9 m
Ct+A+P	(Ct) = 9x9m ; (A) = 3x3m ; (P) = Trailed on (Ct) and (A)
Ct+A+P+B	(Ct) = 8x7.5m ; (A) = 3.2x3.2m , (P) = 8x7.5m ; (B) = 2.5x2.5m
Ct+A+P+N	(Ct) = 12x12m; (A)= 3x3 m; (P) = 12x12m; (N) = 12x12m

Ct+A+P+N +V (Systematic)	(Ct) = 8.8x8.8m (A) = 3.9x3.9m; (P) =8.8x8.8m; (N) =8.8x8.8m; (V) = 2.7x2.7m
Ct+A+P+N +V (Mixed)	(Ct) = 9x8m; (A) = 2x2m; (P) =3.5x3.5m; (N) =9x8m; (V) = 2x2m
Ct+A+P+V	(Ct) = 12x12m; (A)= 6x3m; (P) = 12x12m; (V) = 1.5x1.5m
Ct+P+B+N young	(Ct) = 12x12m; (P) = 12x12m; (B) = 3x3m; (N) = 12x12m
Ct+P+Co	(Ct) = 9x12m ; (P) = 9x12m; (Co) = 3x6m
Ct+P+N	(Ct) = 7.5x8m; (P) = 7.5x8m; (N) = 7.5x8m
Ct+P+V	(Ct) = 9x7m; (P) = Trailed on (Ct) and Standards; (V) = 2.25x2.25m

Table 3. System description: Varieties of the components

Systems	Variety
A	(A) = Kasaragodan
A+B	(A) = Mangala; (B) = Njalipoovan
A+B+X	(A) = Kasaragodan; (B) = Nendran; (X) = Local
A+P	(A) = Kasaragodan; (P) = Panniyoor-1
A+P+Cf	(A) = Mohitnagar; (P) = Panniyoor-1; (Cf) = Robusta
A+V (Type 1)	(A) = Kasaragodan; (V) = Local
A+V (Type 2)	(A) = Mangala; (V) = Local
A+V (Type 3)	(A) = Kasaragodan; (V) = Local
Ct	(Ct) = West Coast Tall (WCT)
Ct+A+P	(Ct) = WCT; (A) = Mohitnagar; (P) = Panniyoor-1
Ct+A+P+B	(Ct) = WCT; (A) = Mangala; (P) = Panniyoor-1; (B) = Robusta
Ct+A+P+N	(Ct) = WCT; (A)= Mohitnagar; (P) = Panniyoor-1; (N) = Local
Ct+A+P+N +V (Systematic)	(Ct) = WCT; (A) = Mohitnagar; (P) =Panniyoor-1; (N) =Local; (V) = Local
Ct+A+P+N +V (Mixed)	(Ct) = WCT; (A) = Kasaragodan; (P) =Panniyoor-1; (N) =Local; (V) = Local
Ct+A+P+V	(Ct) = WCT; (A)= Mohitnagar; (P) = Panniyoor-1; (V) = Local
Ct+P+B+N young	(Ct) = WCT; (P) = Panniyoor-1; (B) = Nendran; (N) = Local
Ct+P+Co	(Ct) = WCT; (P) = Panniyoor-1; (Co) =Forastero
Ct+P+N	(Ct) = WCT; (P) = Panniyoor-1; (N) = Local
Ct+P+V	(Ct) = Kuttiyadi; (P) = Karimunda; (V) = Local

Two different approaches were adopted for identifying the most sustainable system.

Approach 1: Lightfoot *et al.* (1993) suggested a pragmatic framework for monitoring, analyzing, and comparing the performance of different existing farming systems. It is essential to assess the systems using at least four easily quantified indicators of agricultural sustainability.

They are species diversity, bio-resource recycling, resource system capacity, and economic efficiency.

- **Species diversity** counts the number of cultured and utilized species in the system
- **Bio-resource recycling** measures the number of flows between components of the system.
- **Resource system capacity** describes total farm yield, and is measured as biomass output per hectare.
- **Economic efficiency** represents annual net farming income.

Approach 2: The most sustainable system for the region was identified based on several parameters listed below. Through farmer participatory method, the parameters were ranked as follows.

Rank	Parameter	Weightage factor
1	Profit	16
2	Total cost	15
3	System Pest Score	14
4	System Disease Score	13
5	Labour cost (external/hired)	12
6	Input cost (external)	11
7	Biomass generated	10
8	Biomass recycled	9
9	Bio resource cycles	8
10	System Soil fertility	7
11	Scope for intercropping based on PAR	6
12	System Nutrient Balance	5
13	Biodiversity	4
14	Beneficial microorganisms	3
15	Total Soil Microbial load	2
16	System Energetics	1

The systems were ranked with respect to each parameter and given scores accordingly. The scores were multiplied with the weightage factor of each parameter. For each system, the weighted scores of all the parameters were added to get a final score. Based on the final score, the systems were ranked.

The system that needed maximum male hired labour was coconut+arecanut+pepper+nutmeg+vanilla (Mixed) system (Table 4). This was closely followed by the coconut+arecanut+pepper+banana and coconut+arecanut+pepper systems. Least male labourers were hired in the arecanut and coconut sole crops systems. Maximum female labourers was hired in coconut+pepper+cocoa system followed by

coconut+arecanut+pepper+nutmeg+ vanilla (Mixed) system. Women labour requirement was least in the coconut sole crop system. It was observed that the coconut+pepper+cocoa system generated maximum total employment followed by coconut+arecanut+pepper+nutmeg+vanilla (Mixed) system. Least employment generation was in the sole crop system.

Table 4. Hired labour (gender wise) in the various systems

Systems	Men per ha	Rank	Women per ha	Rank	Total	Rank
A	17	15	17	13	34	17
A+B	28	13	22	11	50	12
A+B+X	38	7	36	5	74	8
A+P	29	12	14	14	43	15
A+P+Cf	60	4	27	10	87	6
A+V (Type 1)	60	4	41	3	101	5
A+V (Type 2)	23	14	29	9	52	11
A+V (Type 3)	32	10	22	11	54	10
Ct	16	16	5	17	21	18
Ct+A+P	71	3	35	6	106	4
Ct+A+P+B	73	2	39	4	112	3
Ct+A+P+N	31	11	33	7	64	9
Ct+A+P+N+V	33	9	31	8	64	9
(Systematic)						
Ct+A+P+N+V	75	1	42	2	117	2
(Mixed)						
Ct+A+P+V	48	6	29	9	77	7
Ct+P+B+N young	35	8	13	15	48	13
Ct+P+Co	55	5	90	1	145	1
Ct+P+N	29	12	18	12	47	14
Ct+P+V	29	12	12	16	41	16

Ct: Coconut; A: Arecanut; P: Pepper; B: Banana; N: Nutmeg; Co: Cocoa; V: Vanilla, X: Xanthosoma; Cf: Coffee

The cost of cultivation comprised of expenditure on input and labour (Table 5). The cost of cultivation was highest in coconut+arecanut+pepper+nutmeg+vanilla (Mixed) system followed by coconut+arecanut+pepper+vanilla and arecanut+vanilla (Type1). The lowest cost of cultivation was for coconut sole crop.

Table 5. Cost of cultivation of the different systems

Systems	Cost of Cultivation (Rs./ha)			
	Input cost	Labour cos	Total cost	Ranking
A	34013	2887	36901	5
A+B	51153	4840	55993	12
A+B+C	62740	5960	68700	16
A+P	36021	4020	40041	6
A+P+Cf	36975	7890	44865	8
A+V (Type 1)	100790	8460	109250	17
A+V (Type 2)	49353	4620	53973	10
A+V (Type 3)	31530	4670	36200	4
Ct	2160	6929	9089	1
Ct+A+P	49278	10610	59888	13
Ct+A+P+B	53021	11840	64861	14
Ct+A+P+N	48208	7261	55469	11
Ct+A+P+N+V (Systematic)	61395	5160	66555	15
Ct+A+P+N+V (Mixed)	163064	10860	173924	19
Ct+A+P+V	106536	6620	113156	18
Ct+P+B+N young	38793	6581	45374	9
Ct+P+Co	11340	11640	22980	2
Ct+P+N	19372	4340	23712	3
Ct+P+V	36970	3860	40830	7

Maximum biomass was generated (Table 6) by coconut+arecanut+pepper+ banana system followed by coconut+arecanut+pepper+nutmeg+vanilla (Mixed) and coconut+arecanut+pepper+nutmeg+vanilla (Systematic). Biomass generated was least in arecanut+vanilla (Type 2) and arecanut sole crop.

Table 6. Total biomass generated in the different systems

Systems	Total biomass generated (Dry weight per hectare)	Rank	Total biomass recycled (Dry weight per hectare)	Rank	% of biomass produced that is recycled
A	8707	18	6222.00	17	71.46
A+B	18108	12	12000.00	12	66.27
A+B+X	20534	7	12000.00	12	58.44
A+P	9635	17	6311.00	16	65.50
A+P+Cf	13385	15	9719.00	15	72.61
A+V (Type 1)	16581	14	14365.00	10	86.64

A+V (Type 2)	8059	19	6222.00	17	77.21
A+V (Type 3)	13273	16	11106.00	14	83.67
Ct	20455	8	17289.00	7	84.52
Ct+A+P	27244	4	22316.00	4	81.91
Ct+A+P+B	43258	1	33844.36	1	78.24
Ct+A+P+N	20361.82	9	15763.00	8	74.05
Ct+A+P+N+V	30152	3	24879.00	3	82.51
(Systematic)					
Ct+A+P+N+V	38759	2	30982.00	2	79.93
(Mixed)					
Ct+A+P+V	17808.82	13	12065.52	11	64.41
Ct+P+B+N young	19581.82	10	14624.52	9	71.32
Ct+P+Co	18965	11	11920.00	13	62.85
Ct+P+N	25439	5	22046.00	5	86.66
Ct+P+V	24829	6	21132.00	6	85.11

Maximum number of bioresource cycles (Table 7) was noticed in coconut+arecanut+pepper+nutmeg+vanilla (Mixed) and coconut+arecanut+pepper+nutmeg (young) followed by coconut+arecanut+pepper+nutmeg+vanilla (Systematic) indicating a high degree of recycling. In sole crop systems, bioresource cycles were less.

Ct: Coconut; A: Arecanut; P: Pepper; B: Banana; N: Nutmeg; Co: Cocoa; V: Vanilla, X: Xanthosoma; Cf: Coffee

Table 7. Number of bioresource cycles in the different systems

Systems	Bioresource cycles	Ranking
A	1	6
A+B	1	6
A+B+X	1	6
A+P	2	5
A+P+Cf	3	4
A+V (Type 1)	3	4
A+V (Type 2)	2	5
A+V (Type 3)	3	4
Ct	1	6
Ct+A+P	4	3
Ct+A+P+B	4	3
Ct+A+P+N	4	3
Ct+A+P+N+V (Systematic)	5	2
Ct+A+P+N+V (Mixed)	7	1
Ct+A+P+V	7	1
Ct+P+B+N young	5	2
Ct+P+Co	4	3
Ct+P+N	4	3
Ct+P+V	3	4

Maximum profit (Table 8) was obtained from coconut+arecanut+pepper+ nutmeg+vanilla (Mixed) system followed by coconut+arecanut+pepper+banana. The net return from coconut sole crop, coconut+arecanut+pepper+vanilla, coconut+pepper+ cocoa, coconut+pepper+nutmeg and arecanut sole crop was comparatively low when compared to the other system. The highest BC ratio was obtained with arecanut+pepper system followed by coconut+arecanut+pepper+banana and arecanut+pepper+coffee.

Table 8. Economics aspects related to the different systems

Systems	Gross Return (Rs./ha)	Net Return (Rs./ha)	Rank (based on Net return)	B:C ratio	Ranking
A	118556.5	81655.25	15	3.21	14
A+B	194554	138561	8	3.47	11
A+B+X	381140	312440	3	5.55	5
A+P	352060	312019	4	8.79	1
A+P+Cf	312200	267335	5	6.96	3
A+V (Type 1)	197170	87920	14	1.80	18
A+V (Type 2)	151610	97637	12	2.81	16
A+V (Type 3)	160490	124290	9	4.43	7
Ct	44280	35191	19	4.87	6
Ct+A+P	211174	151286	7	3.53	10
Ct+A+P+B	454654	389793	2	7.01	2
Ct+A+P+N	164669	109200	10	2.97	15
Ct+A+P+N+V (Systematic)	164072	97517	13	2.47	17
Ct+A+P+N+V (Mixed)	645009	471085	1	3.71	9
Ct+A+P+V	153678	40522	18	1.36	19
Ct+P+B+N young	148011	102637	11	3.26	13
Ct+P+Co	77896	54916	17	3.39	12
Ct+P+N	92470	68758	16	3.90	8
Ct+P+V	269160	228330	6	6.59	4

The difference between the energy input and output of the various systems was worked out (Table 9). The highest energy balance was noticed in the coconut+arecanut+pepper+banana system followed by coconut+arecanut+pepper+ nutmeg+vanilla (Systematic). The energy balance in arecanut based systems was comparatively lower when compared to coconut based systems.

Table 9. Energy balance sheet of the different systems

Systems	Energy Input	Energy Output	Balance	Rank
A	8386.15	22185.66	13799.51	16
A+B	6855.01	79280.82	72425.81	13
A+B+X	16826.61	120629.68	103803.07	12
A+P	13765.82	35089.04	21323.22	14
A+P+Cf	23250.59	38299.28	15048.69	15
A+V (Type 1)	14588.44	23397.4	8808.96	17
A+V (Type 2)	16589.55	19393.56	2804.01	18

A+V (Type 3)	22394.53	22840.62	446.09	19
Ct	1428.25	305100.81	303672.56	3
Ct+A+P	5206.52	269090.36	263883.84	6
Ct+A+P+B	19984.45	496096.74	476112.29	1
Ct+A+P+N	8018.02	170312.118	162294.10	10
Ct+A+P+N+V (Systematic)	13344.45	392935.87	379591.42	2
Ct+A+P+N+V (Mixed)	28796.94	222079.42	193282.48	7
Ct+A+P+V	16105.71	147863.238	131757.53	11
Ct+P+B+N young	4562.53	183697.938	179135.41	9
Ct+P+Co	8395.40	192945.934	184550.53	8
Ct+P+N	6301.21	307568.9	301267.69	4
Ct+P+V	6382.26	273906.16	267523.90	5

Ct: Coconut; A: Arecanut; P: Pepper; B: Banana; N: Nutmeg; Co: Cocoa; V: Vanilla, X: Xanthosoma; Cf: Coffee

Considerable amount of nutrients was recycled (Table 10) in the different systems the greatest recycling of nitrogen and phosphorus was observed in the case of coconut+arecanut+pepper+nutmeg+vanilla (Mixed) followed by coconut+arecanut+pepper+banana system. The quantum of potassium recycled was greatest in arecanut+vanilla (Type 1) system followed by coconut+pepper+vanilla. The quantity of nitrogen, phosphorus and potassium recycled was least in the arecanut+vanilla (Type 2) and arecanut sole crop system. Considering the nutrient balance (Table 11) of nitrogen, phosphorus and potassium together coconut+arecanut+pepper+nutmeg+vanilla (Mixed) ranked first. The systems coconut+pepper+nutmeg and coconut+pepper+banana+nutmeg (young) ranked last.

Table 10. Nutrients recycled in the different systems

Systems	N(kg/ha)	Rank	P (kg/ha)	Rank	K (kg/ha)	Rank
A	104.53	18	9.04	17	11.72	18
A+B	182.69	10	10.01	15	23.54	14
A+B+X	182.69	11	10.01	15	23.54	15
A+P	105.82	17	9.15	16	11.88	17
A+P+Cf	165.89	12	14.50	11	18.44	16
A+V (Type 1)	238.07	5	16.37	9	223.44	1
A+V (Type 2)	104.53	19	9.04	17	11.72	19
A+V (Type 3)	185.21	9	14.24	12	103.59	7
Ct	153.87	15	17.94	8	79.99	10
Ct+A+P	251.71	4	25.68	4	87.36	9
Ct+A+P+B	389.59	2	30.46	2	124.50	4
Ct+A+P+N	190.37	8	18.96	7	54.51	11
Ct+A+P+N+V(Systematic)	258.06	3	27.79	3	129.23	3
Ct+A+P+N+V (Mixed)	396.82	1	37.98	1	105.94	6
Ct+A+P+V	163.07	13	13.19	13	112.93	5
Ct+P+B+N young	159.17	14	10.09	14	53.71	12
Ct+P+Co	125.30	16	16.25	10	49.48	13
Ct+P+N	203.32	7	23.32	5	101.45	8
Ct+P+V	207.08	6	21.54	6	133.80	2

Table 11. Nutrient balance in the different systems

Systems	N (kg/ha)	Rank	P (kg/ha)	Rank	K (kg/ha)	Rank	Compiled Rank
A	62.02	8	101.44	6	98.93	11	9
A+B	-19.92	11	35.20	14	76.25	12	13
A+B+X	25.63	9	324.79	3	233.42	4	3
A+P	139.96	5	27.30	15	223.88	5	10
A+P+Cf	148.79	4	889.20	1	55.85	14	4
A+V (Type 1)	129.05	6	293.31	5	122.38	8	5
A+V (Type 2)	200.00	1	44.76	11	268.81	3	2
A+V (Type 3)	159.94	3	885.87	2	47.47	16	7
Ct	-7.58	10	24.81	16	38.12	18	16
Ct+A+P	-172.67	18	4.39	18	60.34	13	17
Ct+A+P+B	-79.40	14	61.22	8	365.53	2	8
Ct+A+P+N	-50.50	12	37.70	12	101.97	10	12
Ct+A+P+N+V	-186.97	19	314.81	4	40.43	17	15
(Systematic)							
Ct+A+P+N+V	191.99	2	47.89	10	405.30	1	1
(Mixed)							
Ct+A+P+V	68.67	7	70.72	7	209.70	6	6
Ct+P+B+N young	-111.25	15	14.28	17	28.88	19	19
Ct+P+Co	-75.86	13	52.10	9	118.35	9	11
Ct+P+N	-158.16	16	1.89	19	55.67	15	18
Ct+P+V	-158.41	17	36.09	13	163.66	7	14

Ct: Coconut; A: Arecanut; P: Pepper; B: Banana; N: Nutmeg; Co: Cocoa; V: Vanilla, X: Xanthosoma; Cf: Coffee

The soil organic carbon (Table 12) content was highest in coconut+pepper+cocoa system. The available phosphorus content was greatest in arecanut+banana+Xanthosoma system. Available potassium was highest in arecanut+banana system. On considering all these three parameters, maximum soil fertility index was obtained for coconut+pepper+cocoa system followed by arecanut+banana system. Soil fertility index was lowest for coconut+arecanut+pepper+nutmeg+vanilla (Systematic) system.

Table 12. Soil Fertility in the different systems

Systems	Organic Carbon (%)	Rank	Available P (kg/ha)	Rank	Available K (kg/ha)	Rank	Total Rank
A	1.275	9	80.825	2	201.44	9	3
A+B	1.33	7	62.72	4	480.16	1	2
A+B+C	2.2	2	85.12	1	54.88	17	3
A+P	1.41	6	30.24	9	73.6	15	9
A+P+Cf	0.78	14	28	10	63.84	16	13
A+V (Type 1)	0.8	13	34.72	8	208	8	8
A+V (Type 2)	1.25	10	39.2	6	129.92	13	8

A+V (Type 3)	1.6	4	36.96	7	53.76	18	8
Ct	1.45	5	22.4	12	160.16	11	7
Ct+A+P	0.51	17	56	5	372.96	5	6
Ct+A+P+B	1.75	3	30.24	9	160	12	4
Ct+A+P+N	1.29	8	17.92	14	434.56	4	5
Ct+A+P+N+V	0.57	16	25.76	11	47.04	19	14
(Systematic)							
Ct+A+P+N+V	0.92	12	36.96	7	83.48	14	10
(Mixed)							
Ct+A+P+V	0.95	11	36.96	7	452.48	2	3
Ct+P+B+N	1.25	10	14.56	16	219.52	7	10
young							
Ct+P+Co	3.23	1	64.96	3	444.64	3	1
Ct+P+N	0.78	14	17.69	15	232.2	6	11
Ct+P+V	0.76	15	21.44	13	163.52	10	12

This was ascertained by measuring the availability of photosynthetically active radiation. PAR availability (Table 13) was maximum in coconut sole crop system. Despite the large number of components the coconut+arecanut+pepper+nutmeg+vanilla (Systematic) system had ample PAR available and can hence, accommodate more intercrops. Availability of PAR in coconut+arecanut+banana +pepper and coconut+arecanut+pepper+nutmeg systems was very low.

Table 13. Availability of Photosynthetically Active Radiation in the different systems

Systems	Maximum PAR available % of open	Rank	% of open (min)	Rank	Compiled rank
A	65.54	2.00	5.35	9.00	5
A+B	47.23	5.00	2.23	17.00	10
A+B+X	14.40	13.00	5.75	7.00	9
A+P	17.06	10.00	3.00	15.00	13
A+P+Cf	12.19	14.00	3.24	14.00	14
A+V (Type 1)	16.16	12.00	3.45	12.00	12
A+V (Type 2)	10.78	16.00	5.94	6.00	10
A+V (Type 3)	25.40	8.00	6.55	5.00	6
Ct	75.81	1.00	11.10	2.00	1
Ct+A+P	37.33	6.00	3.43	13.00	8
Ct+A+P+B	4.69	18.00	1.35	19.00	18
Ct+A+P+N	10.48	17.00	2.97	16.00	17
Ct+A+P+N+V (Systematic)	62.71	3.00	8.61	3.00	2
Ct+A+P+N+V (Mixed)	4.25	19.00	4.18	11.00	16
Ct+A+P+V	48.11	4.00	6.82	4.00	3
Ct+P+B+N young	19.10	9.00	19.10	1.00	4
Ct+P+Co	17.00	11.00	1.94	18.00	15
Ct+P+N	11.39	15.00	5.61	8.00	11
Ct+P+V	35.67	7.00	4.45	10.00	7

Based on the bacteria, fungi and actinomycetes population, total soil microbial load (Table 14) was calculated. Soil microbial load was highest in coconut+pepper+ banana+nutmeg (young) system followed by coconut+arecanut+pepper+vanilla and arecanut+pepper system. Soil microbial load was lowest in arecanut+banana+Xanthosoma system.

Table 14. Soil microbial load in different systems

Systems	Bacteria (x 10 ⁵)	Fungi (x 10 ³)	Actinomy- cetes(x 10 ³)	Total	Rank
A	44.79	5.14	8.14	4492280	13
A+B	41.04	3.81	7.33	4115135	15
A+B+X	15.90	7.76	21.74	1619099	19
A+P	86.43	3.06	18.79	8664446	3
A+P+Cf	80.76	7.17	14.78	8097442	4
A+V (Type 1)	76.33	8.78	25.17	7667041	6
A+V (Type 2)	23.08	4.56	12.58	2325136	16
A+V (Type 3)	17.93	4.91	8.86	1806770	18
Ct	72.55	4.88	8.89	7269069	7
Ct+A+P	18.16	4.83	17.72	1838656	17
Ct+A+P+B	64.22	4.61	14.06	6440765	8
Ct+A+P+N	58.19	6.08	17.35	5842032	9
Ct+A+P+N+V (Systematic)	55.96	4.12	5.08	5605296	10
Ct+A+P+N+V (Mixed)	54.59	4.19	14.89	5477574	11
Ct+A+P+V	90.93	5.57	10.65	9109216	2
Ct+P+B+N young	105.71	3.97	7.80	10582266	1
Ct+P+Co	43.83	8.47	12.97	4404239	14
Ct+P+N	79.67	8.00	12.00	7987000	5
Ct+P+V	51.48	2.94	10.48	5161523	12

Ct: Coconut; A: Arecanut; P: Pepper; B: Banana; N: Nutmeg; Co: Cocoa; V: Vanilla X: Xanthosoma; Cf: Coffee

The lowest disease intensity was found in coconut+pepper+nutmeg system followed by coconut sole crop and arecanut+pepper system. The highest disease intensity was observed in coconut+arecanut+pepper+ nutmeg+vanilla (Systematic) system. The lowest pest intensity (Table 15) was noticed in coconut+pepper+nutmeg system followed by arecanut+banana, arecanut+banana+ Xanthosoma and arecanut+vanilla (Type 1, 2 & 3) system. In arecanut sole crop, intensity of pest attack was the highest.

Table 15. Disease and Pest intensity in different systems

Systems	Diseases	Rank	Pest	Rank
A	12	5	12	11
A+B	14	7	2	2
A+B+X	15	8	2	2
A+P	10	3	4	3
A+P+Cf	21	11	5	4
A+V (Type 1)	14	7	2	2

A+V (Type 2)	11	4	2	2
A+V (Type 3)	13	6	2	2
Ct	9	2	4	3
Ct+A+P	25	13	7	6
Ct+A+P+B	33	17	11	10
Ct+A+P+N	32.5	16	6.5	5
Ct+A+P+N+V (Systematic)	43	18	10	9
Ct+A+P+N+V (Mixed)	27	14	8	7
Ct+A+P+V	30	15	11	10
Ct+P+B+N young	17	9	8	7
Ct+P+Co	21.5	12	9	8
Ct+P+N	4	1	0	1
Ct+P+V	19	10	4	3

Ct: Coconut; A: Arecanut; P: Pepper; B: Banana; N: Nutmeg; Co: Cocoa; V: Vanilla, X: Xanthosoma; Cf: Coffee

The total population of beneficial microorganisms was highest in arecanut+pepper system followed by arecanut+vanilla (Type 1) and arecanut pure system (Table 16). The lowest population of beneficial microorganisms was recorded in arecanut+banana+Xanthosoma system.

Table 16. Beneficial microorganisms in the different systems

No.	Systems	<i>Pseudo-</i> <i>monas</i> <i>fluore-</i> <i>scens</i> (x 10 ⁵)	<i>Azoto-</i> <i>bacter</i> (x 10 ²)	<i>Azospir-</i> <i>illum</i> (x 10 ²)	<i>Beijer-</i> <i>inkia</i> (x 10 ²)	P Solub- ilising Bact- eria (x 10 ²)	Cellu- lose degra- ding	<i>Tricho-</i> <i>derma</i> (x 10 ³)	Total benef- icial orga-	Rank
1	A	1.69	2.08	65.28	16.08	5.46	0.92	1.52	179502	3
2	A+B	0.55	5.66	51.83	18.88	3.00	5.17	0.78	64233.5	6
3	A+B+X	0.00	2.00	42.66	13.48	0.66	2.44	1.56	7684.2	19
4	A+P	2.05	2.66	95.35	19.21	0.33	0.00	0.72	217474.7	1
5	A+P+Cf	1.22	1.66	78.83	16.82	1.00	0.92	3.06	134982.3	4
6	A+V (Type 1)	1.88	2.00	52.33	10.50	13.00	2.33	0.45	196466	2
7	A+V (Type 2)	0.00	3.50	86.02	29.54	0.33	3.33	1.83	14101.2	7
8	A+V (Type 3)	0.00	2.17	83.83	22.63	0.33	0.00	2.06	12955	8
9	Ct	0.00	2.64	59.30	16.20	23.33	0.33	1.33	11510	9
10	Ct+A+P	0.00	1.36	43.16	12.37	28.00	0.56	0.45	8994.2	16
11	Ct+A+P+B	0.00	2.44	56.00	23.38	1.00	5.33	0.78	9594.8	13
12	Ct+A+P+N	0.00	1.11	57.16	17.46	0.00	3.25	0.28	8177.7	18
13	Ct+A+P+N+V (Systematic)	0.00	2.64	46.33	23.46	8.66	0.93	0.67	8871.7	17
14	Ct+A+P+N+V (Mixed)	0.00	1.43	56.66	21.88	4.66	1.80	0.72	9362.5	15
15	Ct+A+P+V	0.00	2.22	74.50	18.29	0.00	2.41	0.61	10352	11
16	Ct+P+B+N young	1.11	2.77	42.49	18.87	21.33	4.89	0.5	120535.5	5
17	Ct+P+Co	0.00	1.47	49.00	14.33	23.66	4.89	0.39	9725	12
18	Ct+P+N	0.00	0.00	74.66	17.00	0.00	0.00	0.22	9386	14
19	Ct+P+V	0.00	0.89	64.17	27.83	13.00	0.00	0.5	11088.3	10

The ranking cum scoring matrix using the parameters like Species diversity, Bio-resource recycling, Resource system capacity (biomass output per hectare) and Economic efficiency (annual net farming income) is furnished in Table 17.

Table 17. Ranking/Scoring Matrix of Sustainability analysis (Approach 1)

System	Rank based on Net Income	Score	Rank based on Bio-mass generated	Score	Rank based on Bioresource cycles	Score	Rank based on Species diversity	Score	Total Score	Final Rank
Ct+A+P+N+V (Mixed)	1	19	2	18	1	19	2	18	74	1
Ct+A+P+B	2	18	1	19	3	17	3	17	71	2
Ct+A+P	7	13	4	16	3	17	4	16	62	3
Ct+A+P+N+V (Systematic)	13	7	3	17	2	18	1	19	61	4
Ct+P+V	6	14	6	14	4	16	3	17	61	5
A+B+	3	17	7	13	6	14	4	16	60	6
Ct+A+P+N	10	10	9	11	3	17	3	17	55	7
Ct+P+B+N	11	9	10	10	2	18	3	17	54	8
young										
A+P+Cf	5	15	15	5	4	16	4	16	52	9
Ct+P+N	16	4	5	15	3	17	4	16	52	10
A+B	8	12	12	8	6	14	5	15	49	11
A+P	4	16	17	3	5	15	5	15	49	12
A+V (Type 3)	9	11	16	4	4	16	4	16	47	13
Ct+A+P+V	18	2	13	7	1	19	2	18	46	14
Ct+P+Co	17	3	11	9	3	17	4	16	45	15
A+V (Type I)	14	6	14	6	4	16	4	16	44	16
Ct	19	1	8	12	6	14	6	14	41	17
A+V (Type 2)	12	8	19	1	5	15	5	15	39	18
A	15	5	18	2	6	14	6	14	35	19

Ct: Coconut; A: Arecanut; P: Pepper; B: Banana; N: Nutmeg; Co: Cocoa; V: Vanilla, X: Xanthosoma; Cf: Coffee

Based on the ranking, the following inferences can be drawn:

Parameter	Best System	Best Coconut based system	Best Arecanut based System
Species diversity	Ct+A+P+N+V (Systematic)	Ct+A+P+N+V (Systematic)	A+B+X, A+P+Cf, A+V (Type 1) & A+V (Type 3)
Bio-resource recycling	Ct+A+P+N+V (Mixed) & Ct+A+P+V	Ct+A+P+N+V (Mixed) & Ct+A+P+V	A+P+Cf, A+V (Type 1) & A+V (Type 3)
Resource system capacity (biomass output)	Ct+A+P+B	Ct+A+P+B	A+B+X
Economic efficiency (annual net income)	Ct+A+P+N+V (Mixed)	Ct+A+P+N+V (Mixed)	A+B+X
SUSTAINABILITY	Ct+A+P+N+V (Mixed)	Ct+A+P+N+V (Mixed)	A+B+X

Ct: Coconut; A: Arecanut; P: Pepper; B: Banana; N: Nutmeg; Co: Cocoa; V: Vanilla, X: Xanthosoma; Cf: Coffee

The most sustainable system is Coconut+Pepper+Nutmeg. The system Coconut+Pepper+Vanilla ranks second. Among arecanut based systems arecanut+pepper ranks first followed by arecanut+vanilla (Type 3).

The study revealed that profit alone cannot help in ascertaining the sustainability of perennial crop based systems. Long term experiments should aim to generate data on various aspects of the systems viz., profit, total cost of cultivation, system pest score, system disease score, labour cost, input cost (external), biomass generated, biomass recycled, bioresource cycles, system soil fertility, scope for crop diversification based on photosynthetically active radiation (PAR), system nutrient balance, biodiversity, beneficial microorganisms, total soil microbial load and system energetics. All the aspects should be considered together for determining the sustainability of the system. As the components of cropping systems change with location and farmer preferences, adopting a farmer participatory approach is recommended.

Acknowledgement: The research was conducted as part of the *Rashtriya Sama Vikas Yojana* project funded by the Kerala State Planning Board. The authors are thankful to the Board for providing grant for the research work.

Reference

Lightfoot, Clive, M. Bimbao, J. Dalsgaard, and R. Pullin. 1993. Aquaculture and sustainability through integrated resources management. *Outlook on Agriculture* 22 (3): 143-50.

Organic farming as a viable resource management strategy for sustainability in coconut farming

George V. Thomas, V. Krishnakumar, P. Subramanian and Alka Gupta

Central Plantation Crops Research Institute
Kasaragod-671 124, Kerala, India

The farming system is undergoing continuous changes with the development of new technologies, often relying on use of heavy doses of fertilizers and other agrochemicals for higher crop productivity. These practices, even though give increased yield, make the plant system more vulnerable to various biotic and abiotic stresses besides causing deleterious effect on the environment. However, with increasing global awareness on health and environmental issues in the recent years, consumers, the world over, are thus demanding greener, healthy and natural foods as well as other commodities. The demand for organic coconut products and spices (which are usually grown in coconut plantations as mixed crops) is increasing over the years and this trend is likely to continue. It opens up tremendous opportunities for enterprising farmers who produce coconut and various value added products by following organic methods of farming and processing to realize better returns by way of the premium price available for the organic products.

The basic concept of organic farming is development of production system which avoids or excludes the use of synthetically compounded fertilizers, pesticides, GM crops and growth regulators. It relies upon crop rotations, crop residues, animal and green manures, legumes, mechanical cultivation, biofertilizers and biological pest control to maintain soil productivity, to supply nutrients, and to control insects, diseases and weeds. Organic agriculture follows the logic of a living organism in which all elements (soil, plant, farm animals, insects, the farmers etc.) are closely linked with one another. Organic farming, therefore, must be based on a thorough understanding and appropriate management of these interaction and processes.

Suitability of coconut for organic farming: Coconut plays a significant role in the agrarian economy of India, which is one of the largest coconut growing and producing countries in the world. The area under coconut cultivation in India during 2008-09 was 1.895 million hectares with the production of 15.729 million nuts. This important plantation crop contributes around six percent to the national vegetable oil pool and about ten million farm families depend on it for their livelihood. In India most of the production comes from small and marginal farms and more than 90% of the holdings are below one hectare with the average size being 0.22 ha. In the west coast of India, the palm is an essential component in the homestead system of farming, where it is mostly grown as a rainfed crop.

The growth habit and planting methods of coconut make it highly suitable for managing through organic farming. About 74 percent of the roots produced do not go beyond 2 m from the bole and most of the roots also confine to the 30 to 120 cm depth thus utilizing only limited extent of land area for growth of palms and leaving considerable area for inclusion of other crops to maintain crop diversity, an important requirement for organic farming. The orientation of leaves in the coconut crown helps penetration of sunlight into the soil and

provides opportunities for exploitation of land and solar energy for inter/mixed cropping. Such a cropping system approach will also add large quantities of organic wastes to the system and their recycling within the system help to increase organic content of soil and improve microbial activity and make the entire production system more productive even in the absence of external inputs.

Coconut plantations are highly ideally suitable for organic farming as they produce large quantities of waste biomass which, if recycled, can meet the nutrient demand of the crops to a great extent. The total availability of waste biomass from 1.93 million hectare of coconut plantation in the country has been estimated as 14.36 million tonnes annually. The natural decomposition of these wastes and the nutrient release are very slow due to the high lignin content and the nature of lingo-cellulose complex of the coconut waste materials. Substantial saving in terms of fertilizer input is possible through effective recycling of the waste biomass.

Vegetative as well as reproductive growth goes on simultaneously in coconut and hence nutrition is important all the time. Coconut palms export nutrients to the above ground parts continuously from a limited volume of soil throughout its existence. It is, therefore, essential that a nutritionally rich environment is provided in the root zone of coconut all round the year to realize adequate yields.

Organic farming research at CPCRI

Sources of nutrition in organic farming

Nitrogen fixing leguminous crops: Nitrogen fixed symbiotically by legume-rhizobium association can form an important source of nutrients and organic manure for coconut palms. The technique for utilization of leguminous cover crops as green manures to supply biologically fixed nitrogen and easily decomposable biomass to coconut was standardized at CPCRI. It involves cultivation of leguminous creepers such as *Pueraria phaseoloides*, *Mimosa invisa* and *Calopogonium mucunoides* in coconut basins during the monsoon period from June to October and incorporation of legume biomass in respective basins. During a growth period of 140-150 days, the promising legumes generated 15-28 kg of biomass and 102-197 g of nitrogen in the basin of a coconut palm. Besides, there is a significant increase in the population of specific group of beneficial micro organisms and the enzymatic activity modifies the soil environment for the benefit of palm growth.

Glyricidia: Generation of large quantities of nitrogen rich biomass is also possible through the cultivation of the fast growing perennial leguminous green leaf manure tree crop, *Glyricidia* in the coconut plantations. This can be very well grown along the borders of coconut plantation and can generate adequate amount of nitrogen rich green leaves. It can also be raised in littoral sandy soils where no other green manure can establish. Studies conducted at CPCRI has indicated that the best growth and biomass of leaves could be obtained with planting of three rows of *Glyricidia* (at 1x1 m spacing between two rows of coconut) and pruning of leaves during February, June and October. This could produce around 8 t of biomass in one hectare of coconut garden. Application of *Glyricidia* prunings could supply around 90%, 25% and 15% of the requirement of N, P and K, respectively.

Recycling of crop residues: The availability of waste biomass from a well-managed coconut garden with 175 trees/ ha has been estimated as 14 to 16 t per hectare per year in the form of leaves, stipules, spathe, bunch waste and husk. A considerable portion of husk is used for extraction of coir fibre. It has been estimated that 7.5 million tonnes of coir pith, the by-product of coir processing, is available in the country from the various coir defibering units. The natural decomposition of these wastes and the nutrient release are very slow due to the high lignin content and the nature of lingo-cellulose complex of the coconut waste materials. If they are recycled fully, this waste-biomass can meet a major portion of nitrogen and a part of other nutrient requirement of crops. Recycling of coconut waste biomass is possible without affecting the prospects of husk or shell based industries. It also helps to replenish the nutrients exhausted by the palms internally without depending on the external sources.

Vermicomposting of coconut leaves: The low cost vermicomposting technology developed at CPCRI enables production of high quality organic manure from coconut leaves in a period of 60-75 days. Vermicomposting can be done ideally in permanent cement or brick tanks constructed under shaded conditions to maintain appropriate quantity of substrate, optimum moisture and temperature. Partially weathered organic wastes are to be treated with cow dung @ 10 percent by weight in the form of slurry and must be allowed to undergo a preliminary decomposition for about 2 –3 weeks. The earthworms @ 1000 worms per tonne of coconut leaves are to be introduced and the compost bed should be mulched properly using any locally available plant material or gunny bags and has to be protected from direct sun light. Watering is to be done to maintain enough moisture. In about 60- 75 days compost will be ready, leaving behind only a portion of non-decomposed material. On an average, 70 per cent recovery of vermicompost was obtained. Vermicomposting can also be done *in situ* in coconut plantations using coconut leaves and other biomass including wastes from intercrops especially from banana. *In situ* recycling of coconut wastes by vermicomposting in trenches dug in interspaces of four coconut palms yield on an average recovery of 70% in a composting period of 90 days. The average nutrient composition of the vermicompost recovered will be around 1.2-1.8 % (N), 0.1-0.2 % (P) and 0.2-0.4 % (K), organic carbon (17.84%), and C/N (9.95:1.00). The composted palm wastes were also found to contain higher levels of micro nutrients such as Fe, Zn, Cu and Mn when compared to that of the untreated substrate.

The coconut waste used for oyster mushroom production is also found suitable for vermicomposting and it will have higher content of nutrients and low C: N ratio. The composted spent-substrate also contains higher levels of micronutrients such as Fe, Zn, Cu and Mn when compared to that of the untreated substrate. Apart from coconut leaves, other agro-wastes like pine apple waste, banana pseudo-stem and leaves and glyricidia green manure can also be effectively used along with coconut leaves for vermicomposting.

Vermiwash: Production technology for vermiwash, the water soluble components from vermicomposting tanks has also been standardized. This vermiwash is honey- brown in colour with a pH of 8.5 and contains both major and minor nutrients in appreciable quantity. Growth promoting hormones like IAA and GA are also present in vermiwash. Application of appropriately diluted coconut leaf vermiwash (CLV) has found to increase germination and seedling vigour index of cowpea and paddy seeds in laboratory bioassays. Field trials conducted on cowpea, maize and bhendi in the Institute farm during 2004-2005 showed its capacity to

enhance the biomass and yield of the crops accompanied by increase in soil microbial populations, enzyme activities and organic carbon content of the soil.

Bioconversion of coir pith: Coir pith is yet another organic material available in huge quantities from coir processing units. This spongy cork-like material, if left as such, is normally resistant to biodegradation and is a source of environmental pollution. Though coir pith has a number of beneficial properties like improving soil physical properties and moisture holding capacity to a great extent, its direct utilization as manure is not advisable as it contains large amounts of lignin (75 per cent) and phytotoxic polyphenols and less of nitrogen. Hence, it is to be applied to soil only after composting.

Bioconversion of coir pith having a high C: N ratio (100-112:1) to acceptable organic manure with the help of biopolymer degrading micro-organisms is possible. Studies conducted at CPCRI have resulted in the isolation of efficient strains of fungi with high lingo-cellulose degradation capabilities, from naturally decomposing coconut wastes. *Pleurotus sajor-caju* and *Trichoderma* inoculations are effective in production of quality compost from coir pith. Large scale composting has been standardized with the amendment of lime and rock phosphate at 0.5% level each and Glyricidia leaves or alternatively poultry manure (10%) and fungal inoculation at 0.2% level. The raw coir pith with a C: N ratio of 100: 1 can be converted to acceptable manure with a C: N ratio of 17-24:1 within a period of 40-45 days.

For vermicomposting, as coir pith alone is not able to support the growth of earthworms, it has to be first treated with lime and rock phosphate @ 0.5 % each and incubated for three weeks and later mixed with cowdung and fresh vermicompost each @10 %. This mixture has to be layered with uncut coconut leaves @ 20% to facilitate aeration in the bed. The earthworm *Eudrilus* sp. has to be introduced at the rate of 1000 numbers/ tonne of organic materials and the bed should be mulched and protected from direct sunlight. Moisture has to be maintained at 50 % by regular watering. Earthworms form burrows in the bed and vermicastings appear as surface casts. A granular vermicompost with 1.2% nitrogen and 16.7:1 C: N ratio can be obtained in two months.

Microbial resources in organic farming

Use of biofertilizers in coconut gardens can reduce dependency on chemical fertilizers and thus bring both economic and ecological benefits. It has been found that coconut roots harbour several associative nitrogen fixing bacteria and phosphate solubilizers in the root region. The population of phosphate solubilizing bacteria and fungi were higher in coconut based high density multiple species cropping, multi-storied cropping with cacao and mixed farming with Napier grass as compared to that of coconut monocrop. The common genera of P-solubilizing bacteria were *Bacillus*, *Pseudomonas* and *Micrococcus* while those of fungi were *Aspergillus*, *Fusarium* and *Penicillium*. These microscopic beneficial organisms can be mass-multiplied and formulated using locally available materials such as vermicompost and coir pith as carrier media and used as biofertilizers for sustainable organic coconut farming. The inoculation of associative diazotrophs such as *Azospirillum*, *Arthrobacter*, *Azoarcus*, *Herbaspirillum*, *Bacillus*, *Burkholderia* and *Pseudomonas* enhances growth and vigour of polybag raised coconut seedlings. These bioinoculants are highly effective in enhancing root biomass and branching of the secondary roots of the coconut seedlings. Organic amendment along with microbial inoculation brings about a greater level of plant response.

Biofertilizer formulations containing *Azospirillum* spp. and Phosphate solubilising bacteria having 10^8 colony forming units of bacteria prepared in carriers such as lignite are to be applied @100 g per palm. The biofertilizer should be mixed with one kg vermicompost and applied to soil and incorporated with the onset of monsoon especially when the palms are maintained under rainfed condition. However, under irrigated conditions, it can be applied at any time, since maintaining optimum moisture is not a problem.

The beneficial nature of biofertilizers becomes even more important in coconut based mixed cropping/ farming systems as the component crops continually add plant residues to the soil which undergo organic recycling. In mixed cropping, dominant nitrogen-fixing microbial group is the bacterium *Beijerinckia* and phosphate-solubilizers such as *Pseudomonas* sp., *Bacillus* sp., *Aspergillus* sp. and *Penicillium* sp. are present in higher numbers. Higher inhibition potential of resident soil bacteria to phytopathogens is observed in coconut based cropping systems when compared to coconut monocropping systems. When coconut is grown with cocoa, rhizosphere activity is found to increase and a better mobilization of phosphates takes place coupled with nitrogen fixation and production of growth substances such as auxins and gibberellins in rhizosphere.

Coconut based cropping system

Crop diversification rather than mono cropping is to be adopted in an organic production system. Cropping/farming system by raising compatible subsidiary crops and/or integrating with livestock enables to increase the productivity and net returns from unit area of coconut plantations and its sustainability in the long run. Crop diversity involving a number of annual, biennial or perennial crops as inter/mixed crops in perennial stands of coconut also promote the productivity and sustainability of the system.

Coconut offers scope for intercropping in the initial stage of the growth of palms and mixed cropping in the later part of life of palms. In an organic production system, it is essential that all the crops in the field are cultivated and maintained strictly following organic methods of production. In the case of short duration annual crops, a system of crop rotation involving a legume in the cycle would help to restore fertility levels. Crops belonging to the same family (e.g. ginger and turmeric) should be avoided as far as possible in the rotation. Various crops can be used for inter/mixed cropping based on the growth phases of coconut plants.

Based on the experimental results from CPCRI, when intercrops such as cowpea, vanilla and pepper are grown organically, an yield of 2.5 ton/ha for cowpea, 1.0 kg dry pepper /vine and 2 kg vanilla beans for vanilla crop was realized. There is tremendous scope for using vermiwash as manure and pest repellent especially vegetable crops are concerned. Amaranthus responded well to vermiwash application. For Cowpea (trailing type) seeds sown in the pits (3 seeds/pit) at a spacing of 2 m x2 m and bush type sowing is taken up in the channels at a spacing of 30 x15 cm. Black pepper can be planted in the inter space of coconut using live standards or in the coconut basins using palms as the standard.

Coconut based mixed farming system

Integration of crops and livestock can also be widely practiced in coconut gardens to generate not only additional income but also to provide relief from the fluctuating prices of nuts, besides generating more employment. Mixed farming system integrating crop husbandry

and livestock is an integral part of the organic production system. It involves cultivation of shade tolerant fodder crops in the interspaces of coconut and integrating animal enterprises like dairy, poultry, fisheries etc. and recycling the by-products. The animals not only enhance the nutritional status of the household members but also help to augment the farm income by the sale of milk, eggs and other products. While the crop residues and fodder provide animal feed, the manure and litter of the livestock provide renewable sources of organic matter and plant nutrients. They help reduce dependence on inorganic chemical fertilizers and maintain soil health, resulting in a high degree of organic recycling. Such integration will also maximize the beneficial impact of species diversity on soil fertility. Here the system is kept productive by maximizing the complementary and synergistic effects of the components involved.

Experimental results from CPCRI have revealed that intercropping of hybrid bajra Napier Co 3 in coconut and maintained organically resulted in 106 tonnes green fodder/ha/year and this fodder could be sufficient to manage 10 milch animals. By adoption of mixed farming around 35 tonnes of cowdung, 75,000 litres of cow urine could be effectively recycled into the system. Biogas plant of suitable capacity can also be installed in the farm for production of biogas for use in the farm house and slurry for manuring coconut and other component crops.

Soil health benefits in relation to organic farming

The quality of an agricultural soil is a measure of its capacity to sustain crop productivity while preserving environmental quality. Organic farming has positive contribution to soil functions to sustain crop growth and productivity. The augmented microbial build-up and activities, biochemical changes and nutrient transformations mediated by soil microflora and microbial interactions enable sustainability of the system.

Soil health benefits due to organic farming was evident from the results obtained in the coconut based mixed farming system involving cultivation of fodder grass in the interspaces of coconut palms and integration of animal husbandry involving dairy, poultry and pisciculture as components. The system was maintained with three treatments including 100 % organics, 100% inorganic and 50 % organic + 50 % inorganic. The population density of soil microbial groups and function-specific microorganisms of beneficial microorganisms were very high in the organic treatment when compared to the other treatments. Increase in microbial biomass and activities of soil enzymes (phosphatase and dehydrogenase) indicated that the soil biological activity was more in the farming system under organic system of cultivation than under inorganic or integrated treatment.

Soil and water conservation

Conservation of natural resources like soil and water for sustainable crop production under organic farming is very much essential. Coconut leaves, husk and coir pith could be utilized as mulches to reduce the loss of soil moisture and create conditions for proper root growth and proliferation of soil flora and fauna.

Burial of husk in trenches in between the rows of palms is also effective for moisture conservation in coconut gardens. Catch pits can be constructed at slopes, filled with coconut husk and a bund to be made at the downside using the excavated soil and pineapple plants

planted on it. Contour trenches can also be made in between two rows of coconut palms and filled with coconut husk in layers. A bund of 20 cm height and suitable width (>50 cm) is to be made at the downstream using the excavated soil and two layers of pineapple plants are to be planted on the bund with a spacing of 20 cm x 20 cm. Pineapple plants are found to stabilize the bund and provide additional income to the farmer. The runoff water from the upper side along with soil particles will be collected in the trenches. Coconut husk retains the moisture and makes it available for plants during summer months.

Eco-friendly management of pests and diseases

The basis of pest and disease management in organic farming systems is the reliance on the inherent equilibrium in nature. The emphasis on organic plantations should ideally be on the use of varieties resistant to pest and diseases.

Careful management in both time and space of planting of inter crops not only prevents pests, but also increases population of natural predators that have natural capability to control insects, diseases and weeds. Other methods that can be generally employed for the management of pests and diseases are: clean cultivation, improving soil health to resist soil pathogens and promote plant growth; crop rotation; encouraging natural biological agents for control of diseases, insects and weeds; using physical barriers for protection from insects, birds and animals; modifying habitat to encourage pollinators and natural enemies of pests; and using semi-chemicals such as pheromone attractants and trap pests. Biopesticides including microorganisms, parasites, predators and natural plant based pesticides from neem, tobacco and garlic are effective in managing pests of coconut and other intercrops. There are several examples of use of effective bio control agents for suppression of pests and diseases of coconut and other component crops.

Economic feasibility of organic cultivation of coconut: case studies

A field experiment was initiated at the Central Plantation Crops Research Institute, Kasaragod during 2002-03 to evaluate performance of coconut palm under different organic cultivation practices as well as to understand the effect of such treatment on soil chemical and biological properties. The experiment was conducted on West Coast Tall (WCT) and Chandarsankara (COD x WCT). The results revealed that organic cultivation treatments recorded significantly higher nut yield when compared to the control. The mean nut yield of four years (2005-06 to 2008-09) indicated that there was 34 per cent increase in yield over the pre-treatment conversion period under the best treatment of T1 for COD x WCT (Table 1). The mean percentage improvement in yield of organically cultivated coconut over 'no management practice' was 60 and 59 in the case of WCT and COD x WCT. Analysis of soil samples collected from the rhizosphere of coconut palms indicated that total microbial population in general and that of function-specific microbes were observed to be higher under organic treatments.

Coconut based farming system with inter cropping of fodder grass in the inter spaces of coconut with dairy and poultry as components was maintained with organic inputs including the recycling of cowshed wastes and vermicomposting of the biomass generated in the system. The yield of coconut in the organic cultivation was 108 nuts /palm/year and fodder yield was 106 t/ha/year. The system was highly remunerative with net return of Rs. 1,37,164/ha/year achieving economic and environmental advantages under organic farming.

Table1. Effect of organic cultivation treatments on yield of coconut (nuts/ palm/year)

Treatments	2003-04	2004-05	2005-06	2006-07	2007-08	2008-09	Mean (05-06 to 08-09)
T1: Vermicomposting (in basin), application of biofertilizers and cover cropping in interspace	77	72	74	79	11	137	100
T2: Vermicomposting (in trenches) application of biofertilizers and cover cropping in basin	77	81	73	77	93	131	94
T3: Vermicomposting (basin), application of application and vegetables intercrop	68	70	79	74	87	121	90
T4: Vermicomposting (in trench) + application + vanilla & black pepper	94	77	79	78	82	136	94
Control	73	66	55	61	65	72	63
CD(P=0.05)	NS	NS	NS	12.96	24.71	23	

Field experiments conducted under the All India Coordinated Research Project on Palms in different coconut growing tracts of the country had organic treatments such as composted coir pith to supply 100 % N and neem cake + bone meal + ash. The yield under organic cultivation ranged from 80-145 nuts/palm/year in different locations. These results conclusively prove that economically viable coconut cultivation is feasible when palms are managed with organic inputs alone.

There have been significant technological advancements in the development of agro-technologies based on the principles of organic farming in plantation crops. The growth habit and planting methods of crops like coconut make it highly amenable for organic farming based on the low cost technologies utilizing the local resources. Technologies are available for production of sufficient quantities of organic matter, efficient recycling and generation of high quality compost and growth promoting liquid formulations and utilization of microbes to supply the plant nutrients without any ecological damage as is the case with chemical agriculture. The experimental case studies further corroborate the fact that organic cultivation of coconut is an environment-friendly, sustainable and economically viable proposition.

In search of bioinoculants for coconut palm

Sheeja Purushothaman, Sunny Thomas, Mathew George

Central Plantation Crops Research Institute

Regional Station, Kayamkulam-690 533, Kerala, India

Organic agriculture is a production system that avoids the use of synthetic fertilizers, pesticides and growth regulators and relies on crop rotation, crop residues, animal manures, legumes, green manures and on-farm organic wastes. It emphasizes the use of management practices, taking into account the regional conditions, locally adapted to the systems. Organic farming has become popular due to consumer concern with human health and environment (Bingeman, *et al.*, 1953). The beneficial effects of organic fertilizer include enhancement of soil biological activity, enhancement of colonization of mycorrhizae, improved root growth due to better soil structure and slow release of nutrients which contribute to the residual pool of organic nitrogen and phosphorus in the soil, reducing nitrogen leaching and phosphorus fixation. During decomposition of organic matter, non-humic and humic components serve mainly as substrate for soil microbes and contribute to the formation of soil humic contents (Daniel *et al.*, 1990). Biologically fixed N_2 into agricultural system were derived from symbiotic relationship between legume and rhizobium as well as due to non-symbiotic association between free-living diazotrophs and plant roots (Dobb, 1915; Lohins, 1926). Restoring soil health by reverting to non-chemical agriculture has assumed great importance to attain sustainability in production. In the search for eco-friendly and farmer-friendly alternate systems of farming such as natural farming, permaculture, zero budget farming and biodynamic farming are increasingly becoming popular among the farming community. In all these systems, cow plays an important role. All these systems require less financial inputs and place more reliance on natural and human resources available. In promoting organic farming all these systems can be integrated and develop "Jaivik krishi" (JK) and can be promoted in different parts of our country by the common farmers (Reddy and Ram, 2010).

Preparation of consortium of beneficial microbes: Taking cue from the vedic preparations of "Panchagavya" to Karve's super manure and Palekkar's jeevamritham, four different cow dung based preparations (Table 1) were made with the following components. The preparations were kept for natural fermentation for a period of 72 h.

Table 1. Components of raw materials in each batch

Batch I	Batch II	Batch III	Batch IV
Cow dung -100g Cow's urine- 100ml Jaggery -5 g Well water- 1000ml	Cow dung -350g Urea -15g Super phosphate-15g Potash – 5g Ground nut cake-10g Well water-1000ml	Cow dung -350g Urea -15g Super phosphate-15g Potash – 5g Ground nut cake-10g Coconut cake- 10g Well water-1000ml	Cow dung -50g Cow's urine- 25ml Jaggery -10 g Grounded soaked pea- 10g Well water-1000ml

Microbial enumeration: Microbial enumeration and their isolations were done at periodical intervals of 0 h, 24 h, 48 h, and 72 h by dilution and plate count method of Timonin(1940). For serial dilution from the prepared slurry, 10ml of the suspensions were transferred to Erlenmeyer flask(250ml) containing 90 ml sterile water. Before the successive transfers, each flask was shaken thoroughly for 2 min and 1ml aliquote of the appropriate dilution was pipetted into sterile petri plates before the addition of 15-20ml of appropriate sterile cooled medium. Two replications for each group of microorganisms were maintained. Total number of general bacteria was counted on Nutrient agar (Allen, 1959) fungi on Martin's Rose bengal agar (Martin, 1950), actinomycetes on Kenknight's and Munaier's agar (Allen, 1959), nitrogen fixing bacteria on Beijerinckia medium (Becking, 1959) and phosphate solubilizers on Pikovskaya agar (Pikovskaya, 1948). The respective dilutions and periods of incubation of each group of the micro organisms are furnished below and the plates were incubated at room temperature. The individual colonies of the different micro organisms appearing on the dilution plates were counted and the results were given as log cfu/ml.

Preparation of mother culture: As the Batch IV of the preparation harboured higher population of beneficial microbes, the culture was enriched by repeated fermentation in a cascade type of operation. The sediment of the decanted preparation was shade dried and kept for an extended period of 120 days evaluating the population at an interval of 15 days. The enriched preparation of shade dried inoculum was used for further bulk preparation.

Physical and chemical analysis: The biofertilizer was observed for general characteristic such as odour and color at room temperature.

pH analysis: Fifty ml of the fresh solution of biofertilizers, from each batch was taken in 100ml beaker and read with pH meter (Digital analab pH meter) at 27°C.

The number of microorganisms were in between 10^4 - 10^6 cfu/ml, which is in consistent with the microbial analysis result from liquid biofertilizer produced from different plant based raw materials during fermentation as published by DOA(2004), Hasarin Ngampimol *et al.*(2004). The number of microorganisms in Batch 1V was high compared to other batches showing that the quality of raw materials used affected the microbial population since the methods and duration were the same in all the batches (Table 2).

Table 2. Micro organisms in different batches at different intervals (log cfu/ml)

Micro organisms	Batches	0 h	24 h	48 h	72 h
Bacteria	Batch-1	5.49	6.86	7.49	7.61
	Batch-2	5.05	6.18	7.45	7.93
	Batch-3	5.20	6.37	7.15	8.77
	Batch-4	5.14	7.63	8.89	11.07
Fungi	Batch-1	0.00	0.00	5.64	6.62
	Batch-2	0.00	0.77	4.60	6.09
	Batch-3	0.00	0.00	4.38	6.45
	Batch-4	0.00	0.00	5.27	7.92
Actinomycetes	Batch-1	5.08	6.28	7.11	7.27

	Batch-2	5.12	6.19	7.24	7.30
	Batch-3	5.55	6.66	7.13	8.42
	Batch-4	5.34	7.75	8.65	9.63
Asymbiotic nitrogen fixers	Batch-1	6.60	7.45	8.65	8.75
	Batch-2	5.17	6.50	7.50	8.15
	Batch-3	5.06	6.77	8.76	8.76
	Batch-4	5.34	7.72	8.64	9.63
Phosphate solubilizers	Batch-1	5.33	6.18	7.43	8.50
	Batch-2	5.37	6.67	7.45	7.78
	Batch-3	5.21	6.70	7.54	8.66
	Batch-4	5.13	7.65	8.64	9.95

The physical qualities like colour and odour are also important. The colour of all batches of biofertilizers was dark brown at the time of preparation. After 72 h, there was a progressive decrease in the intensity of dark brown colour to less turbid. After 72 h, all the batches gave a fermented smell.

The batches II and III had an alkaline pH range of 7.9 -8.5 and in Batches I and IV the pH was reduced after 72 h of storage compared to the initial pH (Fig.1). This observation corresponds with the result published earlier by DOA (2004) in which the pH of liquid biofertilizer from plant materials was in between 3.3-4.0. The decrease in pH was due to decomposition. During decomposition sugars, hemi cellulose, and cellulose are broken down into organic acids, which further oxidized to CO₂ and H₂O. Proteins undergo hydrolysis and liberate amino acids, which are converted to ammonia. The organic acids liberated combine with ammonia to form ammonium salts and prevent loss of ammonia by volatilization. The acids lower the pH (Daji, 1971). Compared with all the batches, batch IV had low pH(3.5).It is evident that the chemical composition of the raw materials were responsible for the difference in the pH of the biofertilizers produced.

Even though there is no appreciable initial difference in the count of microorganisms in the four preparations

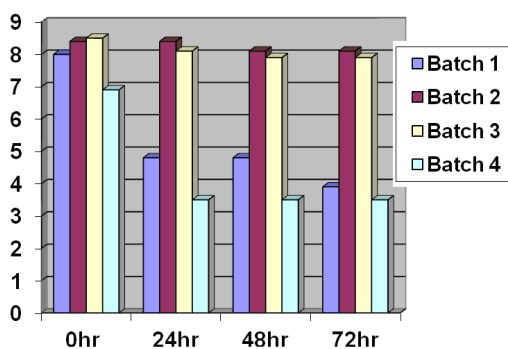


Fig. 1. pH of biofertilisers

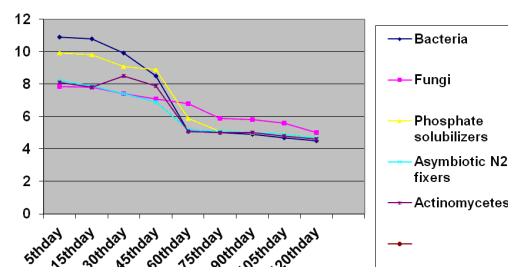


Fig.2. Microbial population of biofertilizer preparation (Batch 4) showed in log no. of viable cells (log cfu/ml) at periodical intervals

employed, Batch IV showed its superiority on fermentation, registering higher population. In the shade dried inoculum prepared from Batch IV lot was tested for an extended period of 120 days at regular interval. There is a gradual reduction in the colony forming units of the organisms till 60th day but then it leveled off. Although much has been heard now about the craze for organic cultivation and super manure, this is perhaps the pioneer attempt to streamline the super manure in a microbial context, facilitating preparation of the biofertilizer in the homestead.

References

- Allen, O.N. 1959. Experiments in soil Bacteriology, Minneapolis, MN, USA, Burgess, 3rd Edn, pp117.
- Becking, J.H. 1959. Nitrogen fixing bacteria in the genus *Beijerinckia* in S. African soils. *Plant Soil*: 193-206.
- Bingeman, C.V., Varner, E and Martin, W.P. 1953. The effect of the addition of organic materials on the decomposition of organic soil. *Proc. Soil Sci. Am.* **17**:34-38
- Daji, J. A. 1971. Oil cakes. Hand book of manures and fertilizers. ICAR. New Delhi.
- Daniel, H., Mehuys, G.R and Levesque, M. 1990. Influence of humic and fibric materials in the aggregation and aggregate stability of lacustrine silty clay. *Soil Sci.* **151** (2):146-158.
- DOA. 2003. Plant hormones and plant nutrients in liquid biofertilizer. Department of Agriculture, Bangkok, Thai land.
- DOA. 2004. Scientific information of liquid biofertilizer (Part I) Department of Agriculture, Bangkok, Thailand.
- Dobb, A. C. 1915. Green manuring in India . Bull. Indian agriculture. Res. Inst. Pusa:56.
- Hasarin Ngampimol. and Viyada Kunathigan. 2008. The study of shelf life for liquid biofertilizer from vegetable waste. AUJ. T.11: 204-208. Part , J.F; Hornick, S.D.
- Lohins, F. 1926. Nitrogen availability of green manures. *Soil Sci* **22**: 253-290.
- Pikovskaya, R.I. 1948. Mobilization of phosphorus in soil in connection with vital activity of some soil microbial species, *Mikrobiologiya* **17**:362-370.
- Reddy, B.M.C and Ram, R. A. 2010. Emerging technologies on organic production of fruit crops. In: *Organic Horticulture, Principles, Practices, and Technologies*. p.29.
- Timonin, M.I. 1940. The interaction of higher plants and soil microorganisms. I. Microbial population of rhizosphere of seedlings of certain cultivated plants. *Can. J. Res.* **18C**:307-317.

Integrated disease management in coconut

R. ChandraMohan and Merin Babu

Central Plantation Crops Research Institute

Kasaragod 671 124, Kerala, India

Coconut palm (*Cocos nucifera* L.) is susceptible to a number of diseases and pests. Some of them are fatal while others reduce its vigour and finally resulting in economic loss. Among the diseases affecting coconut palm in India, root (wilt), leaf rot, bud rot, stem bleeding and basal stem rot are the major problems owing to the nature of disease and extent of damage. Attempts to protect the crop from diseases date back to ancient times. The Integrated Disease Management (IDM) programme integrates all the suitable techniques and methods in a compatible way to prevent and manage diseases in crops. The profitability and sustainability of coconut production can be enhanced by the adoption of integrated disease management strategies. IDM includes selection of disease-free planting materials, field and plant sanitation, weed management, integrated nutrient management through application of compost or other organic manures along with the recommended dose of chemical fertilizers, bio-control of pathogens etc.

1. Root (wilt)

Root (wilt) disease was first observed in the erstwhile state of Travancore, presently the Kerala state during 1882. The disease was initially reported from three independent locations – Erattupetta area of Meenachil taluk, Kaviyoor and Kallappara of Thiruvalla taluk and Kayangulam of Karthikapally taluk each at a distance of 50 km. Since then the disease has spread to all directions. The disease is non lethal, but debilitating. Palms of all age groups are affected. However, palms contracting the disease in the pre bearing age may not come to flowering and bearing. The disease is prevalent in a contiguous manner in all the 8 southern districts of Kerala starting from Trivandrum to Trichur and in isolated patches in the remaining 6 northern districts of the state. Apart from this the disease is also prevalent in the districts of Tamil Nadu and Karnataka adjoining to Kerala State. Recently the disease has also been recorded in Goa.

Symptoms: The most obvious and diagnostic symptom of the disease is the abnormal inward bending of the leaflets termed ribbing or flaccidity. Yellowing and marginal necrosis of leaflets are the other associated foliar symptoms. Rotting of roots increases with the progress of the disease. Shedding of immature nuts, drying up of spathes and necrosis of spikelets in unopened inflorescence is noticed in certain cases. The husk, kernel and oil of the nuts of the disease affected palms are of poor quality. The disease also causes several internal changes in the palm.



Flaccidity –initial diagnostic symptom of root (wilt) disease

Etiology: Concentrated and intensive research carried out at CPCRI has resulted in the identification of a phloem bound mollicute – phytoplasma as the cause of the disease. Constant association of phytoplasma with the root (wilt) disease has been established beyond doubt.

Spread: The insect vectors transmitting the disease in nature have been identified as lace bug (*Stephanitis typica*) and plant hopper (*Proutista moesta*). The disease also could be experimentally transmitted to phytoplasma indicator host, periwinkle.

Association of leaf rot with root (wilt) disease: Leaf rot, caused by fungi mainly *Exserohilum rostratum* and *Colletotrichum gloeosporioides*, occurs superimposed on root (wilt) affected palms. About 65 % of root (wilt) diseased palms are affected by leaf rot disease caused by fungi. Normally farmers identify a palm as root (wilt) affected only when the leaf rot sets in.



Root (wilt) and leaf rot diseases affected palm

2. Leaf rot

Palms of all ages are susceptible to this disease. The disease causes drastic reduction in photosynthetic area, which in turn causes reduction in yield. Severity of leaf rot symptom is more during monsoons.

Symptoms: Tiny water soaked lesions appear on spindle leaves which gradually enlarge, coalesce freely leading to extensive rotting. The rotting may advance into the interior of the spindle. When the spindle grows, the rotten portions dry up, turn dark brown to black, break and are blown off in the wind. In many cases, the rotten distal portions of leaflet adhere to each other from top to bottom on both sides thereby giving a fish bone appearance. On drying these portions drop off. Hardening of the tissues and development of chlorophyll in the maturing leaflets generally slow down the progress of rotting towards the base of the leaflets so that the basal portions of the affected leaflets remain green and normal giving a fan like appearance. Normally the symptoms would appear on each successive spindle thereby showing symptoms on all leaves. Leaf rot causes reduction in photosynthetic area, disfiguration of the palms and reduction in yield apart from attracting a number of insects that feed, multiply and cause further damage

Etiology: *Exserohilum rostratum* and *Colletotrichum gloeosporioides* are the major pathogens causing leaf rot. Fungi infect the newly emerging spindle leaves and thus leaf rot disease has direct bearing on the decline in yield of palms.

Disease management strategies

Since root (wilt) disease is caused by phytoplasma it is not amenable to conventional plant protection measures. One of the significant features of the disease is that it is not lethal but a debilitating malady which responds to ideal management practices. Two strategies, one for the heavily diseased contiguous area, and another for the mildly affected area have been formulated.

Strategy for heavily diseased tracts

In the heavily diseased area, the yield of palms can be sustained or even improved through adoption of integrated management practices:

- ❑ Removal of disease advanced and juvenile palms
- ❑ Management of leaf rot disease
- ❑ Balanced fertilizer application
- ❑ Addition of organic manures
- ❑ Raising of green manure crops in the basins and incorporation
- ❑ Irrigation during summer months
- ❑ Management of other diseases and pests
- ❑ Adopting inter and mixed cropping.
- ❑ Mixed farming in the diseased gardens involving raising of fodder crops in the inter spaces, maintaining milch cows and recycling of organic waste.

Management of leaf rot is very important in addition to irrigation and recommended dose of fertilizers and manures for the effective management of root (wilt) disease of coconut. The integration of leaf rot management with insect pests and general cultivation practices is very effective in improving the health of the palms and thereby increasing the yield

Leaf rot disease management

- ❑ Cut and remove rotten portions of the spindle and the adjacent two innermost fully opened leaves, if affected by leaf rot. White soft leaflets of the spindle alone are susceptible to fungal attack. Therefore, older leaves that had leaf rot disease earlier need not be removed.
- ❑ Mix Contaf 5 EC (Hexaconazole) 2 ml or Dithane M-45 (Mancozeb) 3 g in 300 ml water and pour into the well around the base of the spindle leaf.
- ❑ Apply 20g Phorate 10G or 30g Furadan 3G mixed with 200g sand around the base of the spindle leaf.



Healthy leaves produced after leaf rot management

- Treat all palms in the garden (healthy and diseased) twice a year, i.e. in April-May and October-November. To make this operation more economical the treatment should be given along with harvest of nuts before and after south - west monsoon.

Residue analysis done for the chemicals, Contaf 5EC, Indofil M45 and Phorate 10 G in mature and tender nuts showed that the nut water, kernel, coconut oil and coconut cake are free from residues after 45 days of application at the rates suggested above

Strategy for mildly affected area

- *Removing all the diseased palms.* The spread of the disease can be arrested by systematic surveillance and rouging of diseased palms as and when identified. Accurate and timely diagnosis of plant diseases is an essential component of integrated disease control. ELISA test has been developed at CPCRI for the early diagnosis of this disease. The disease affected palms can be detected even 24 months before the expression of symptoms and they can be removed to avoid further spread. .
- *Replanting with disease free healthy seedlings.* Sero-diagnostic test is being used extensively in the identification of healthy elite mother palms, screening of the progenies of elite palms, screening nursery seedlings and for confirmation of RWD in new areas of incidence. Replanting with quality seedlings has to be undertaken only in gardens with sufficient space

If the above two programmes are not monitored uninterruptedly the desired goal will not be achieved.

3. Bud rot

Bud rot incidence of coconut was first noticed in Grand Cayman, an island of British West Indies just south of Cuba in 1834. Since then the disease has been reported from every coconut producing region of the world. Now it occurs in all coconut growing countries. In India, bud rot was reported by Butler in 1906. It occurs commonly in west and east coasts of India. Radha and Joseph (1974) reported a disease incidence of 1.2 to 10.9 per cent in Kerala and 35 to 40 per cent was observed in certain gardens having large number of palms. The disease is sporadic in nature in most of the places; however outbreaks of epidemics are also common in certain areas. Heavy incidence of bud rot disease leading to the destruction of thousands of coconut palms has been reported from Kuttiadi and Calicut regions of Kerala State. Bud rot incidence was also noticed in other states like Karnataka, Maharashtra and Goa on a large scale, particularly in the newly planted gardens. In the non-traditional coconut growing areas of Madhya Pradesh like Bastar, Raipur and Durg, bud rot is a problem of serious concern. Since coconut is a perennial crop the loss due to the disease is very high.

Symptoms: Palms of all age groups are susceptible. The incidence was found to be high in palms below the age of 20 years. Mortality rate is also high in palms belonging to the age group of 15-20 years. The first visible symptom is withering of the spindle leaf marked by pale colour. The spindle leaf turns brown and bends down. Farmers often experience difficulties in the detection of the disease at this stage, though it is essential to diagnose the disease at the initial stage to take up curative control measures. The affected spindle leaf can easily be pulled out as the basal portion of the spindle is completely rotten emitting a foul smell. Symptoms

are later observed in younger leaves next to the spindle. Later the inner leaves also fall away one by one leaving only outer whorl of matured leaves in the crown. Ultimately the palm succumbs to the disease with the death of the growing bud (Quillec and Ronard, 1984; Nambiar, 1999; ChandraMohan and Basheer, 2008).

Etiology: Bud rot disease is caused by the fungus *Phytophthora palmivora*.

Disease spread: In endemic areas where bud rot incidence is increasing year after year, the survival of *Phytophthora* propagules as well as the inoculum build up are very high. *P.*

palmivora is reported to overcome the unfavourable weather conditions during the post monsoon period in the disease affected crown tissues and begin to multiply during the subsequent monsoon period. The implication of *Phytophthora* inoculum present in the crown as a potential source of primary inoculum for bud rot is obvious. Further secondary spread of the disease from freshly bud rot affected palms will be very fast under favourable weather conditions. Rain water acts as a carrier for the infectious propagules and plays an important role in the spread of the disease

Reasons for higher incidence of the disease

- Unprecedented high and extended South -West monsoon.
- High rainfall and wind in the disease endemic hilly tracts. In recent years the disease has attained a serious proportion in some of the localities, in South India, especially hilly tracts with high humidity. Rainfall pattern of Calicut district (Kerala State) clearly indicates that the rainfall in heavily bud rot affected areas in the district is much higher than the average rainfall of the district.
- High incidence of attack of rhinoceros beetle.
- Cultural and other management practices including INM were not properly adopted.
- Prophylactic control measures for bud rot management have not been followed in the disease endemic areas.

Integrated disease management

Bud rot disease can be effectively managed by adopting integrated management practices.

a. Cut and removal of severely disease affected palms (beyond recovery) or dead palms

Cut and removal of palms which are in the advanced stage of bud rot or palms dead due to the disease are very important operations to be carried out in all the affected gardens for better control of the disease. This will help to reduce the inoculum load in the garden and thereby checking the fast spread of the disease within the garden and to the neighbouring gardens. Rotten portion of the crown should be destroyed by burning.



Bud rot affected coconut garden

b. Curative treatment

- ❑ To save the palm, curative measures have to be adopted when the disease is in early stages.
- ❑ The appropriate period would be the stage when the spindle has just started showing symptoms of withering.
- ❑ In the early stage of the disease, remove the spindle leaf by pulling it out and cut and remove the infected tissues completely.
- ❑ Two or three healthy leaves adjacent to the spindle may have to be removed if necessary for easy removal of all rotten portions and thorough cleaning.
- ❑ The wound should be treated with Bordeaux paste(10%)/mancozeb
- ❑ The treated wound should be covered with polythene cover to prevent entry of rain water and this protective covering should be retained till normal shoot emerges.
- ❑ In case of advanced stages of infection, the palm should be cut and removed.
- ❑ The diseased tissues should be burnt after their removal.
- ❑ All the healthy palms in the surroundings should be treated with 1% Bordeaux mixture/ mancozeb sachets.

c. Prophylactic treatment

It is important to give prophylactic treatment to all palms before the onset of monsoon, in gardens with a previous history of bud rot incidence. In certain dwarf palms, phytotoxic symptoms like brown sunken spots followed by nut shedding have been observed when Bordeaux mixture is sprayed. Therefore, in copper sensitive palms like Chowghat Orange Dwarf (COD) and Malayan Yellow Dwarf (MYD), keeping perforated sachets containing mancozeb in the innermost leaf axils during rainy season is found to be useful. Large scale field trials conducted recently revealed that pouring mancozeb solution (5g in 300 ml water / palm) well around the base of the spindle followed by placing perforated sachets containing mancozeb (2 sachets/palm; 5 gm mancozeb/sachet) in the inner most leaf axils before the onset of south West monsoon is a very effective prophylactic treatment for bud rot management in disease endemic areas (unpublished).

d. Nutrient management

Besides integrated disease management techniques, nutrient management practices for the affected palms as well as healthy palms are also important for improving the health and vigour of the palms for higher yield. The fertilizer dose generally recommended for an adult palm is 500 g N, 320 g P_2O_5 and 1200 g K_2O per palm per year. Under rainfed condition fertilizer may be applied in two split doses. After the receipt of summer showers, apply one third of the recommended dose of fertilizer around the palm within a radius of 1.8 m and forked in. Dig circular basins of 1.8 m radius and 25 cm depth around the palm in Sept.-Oct. and spread organic manure in the form of compost or green leaves @ 50 kg per palm in the basin. Then, apply two third of the recommended dose of fertilizer over the organic manure and cover the basin with soil.

e. Irrigation

The yield of the palms can be increased by summer irrigation

f. Replanting

In order to maintain optimum population of palms in the gardens from where the palms in the advanced stage of disease or dead palms are cut and removed it is necessary to fill up the gaps by planting good quality coconut seedlings, wherever sufficient space is available.

4. Immature nut fall

Immature nut fall in coconut has been attributed to several factors. The characteristic feature of mother palm is one of the prime reasons for button shedding in coconut. Hence the selection of good yielding mother palm is very important for collection of seed nuts for raising coconut seedlings. Very high soil acidity or alkalinity also leads to high percentage of button shedding and immature nut fall. Drought condition or water shortage for a longer period, water logging and sudden changes in soil moisture also cause heavy button shedding. Imbalance or deficiency of nutrients leads to nut fall. Poor pollination is also one of the major factors responsible for button shedding in coconut. It has been reported that in some coconut palms the production of plant growth regulators was below optimal and in such palms, the nut fall was high when compared to other palms.

Shedding of buttons is also caused by insect attack. Eriophyid mite attack also leads to immature nut fall to some extent. It has been observed that mite injury provides entry points for pathogenic fungi causing rotting and nut fall. In certain cases, even though the mite infestation or injury is negligible, the infection caused by fungal pathogen causes severe rotting and immature nut fall. Such severe incidence of immature nut fall due to rotting of mite infested nuts has been observed in several locations in Kerala state leading to heavy economic loss.

5. Rotting of mite infested nuts

Symptoms: Rotting starts from the point of mite infestation on the nut surface near the perianth as dark brown to black discoloration and gradually extends to the entire surface area. The lesion also spreads deep into the internal tissues. As the lesions spread to about 30% surface area near the perianth region or when the lesion encircles the perianth region, the nut gets detached from the bunch and shed or remains on the bunch in between other nuts.

Mite attack followed by rotting of nuts is noticed in different varieties. Though mite infestation in COD nuts is comparatively low, incidence of rotting and immature nut fall due to fungal infestation is frequently



Rotting and immature nut fall of eriophyid mite infested nuts caused by *L. theobromae* infection

observed in COD. Even slight injury caused by mite paves way for the pathogen to enter and cause severe rotting. Intensity of rotting and immature nut fall varied from locality to locality as well as from garden to garden. In certain cases when the fungal infection starts in nearly mature nuts, it continues even after harvest and storage. The infection goes deep into the husk and spreads to the kernel through the softy eye. The kernel rots assuming a grayish black discoloration and becomes soft.

Etiology: Nut fall or mahali or koleroga caused by *Phytophthora palmivora* is common in high rainfall areas during monsoon season. Though, *P. palmivora* and *Thielaviopsis paradoxa* are also found associated with mite infested nuts, *Lansiodiplodia theobromae* is the main causal organism of rotting and immature nut fall of mite infested nuts.

Management: Rotting or immature nut fall of mite infested nuts caused by *L. theobromae* can be controlled by spraying carbendazim (Bavistin 0.1%) to bunch of the affected palms. If coconut is cultivated under the organic farming system, spraying of garlic bulb extract (10%) can be recommended for the management of the disease.

6. Stem bleeding

Stem bleeding disease is known to occur in all coconut growing regions in the tropics. This disease was first reported from Sri Lanka. Later, the disease was reported from India in 1922. This was followed by reports of the disease occurrence in other countries like the Philippines, Malaysia, Trinidad, Papua New Guinea, Fiji, Ghana and Indonesia. In the early stages of the disease, there is not much yield loss. However, in late stages, there is a steady yield decline causing considerable loss and in advanced stages even death of affected palms occurs. The disease has been found to occur in all soil types, but more in laterite soils and sandy soils on the seashore or backwater areas.

Symptoms: The disease is characterized by development of dark brown patches appearing at the basal portion of the trunk. In course of time, this progresses upwards. A dark reddish brown liquid exudes from the longitudinal growth cracks present on the stem bark and form irregular streaks of exudation. These streaks may coalesce and form larger lesions.



Stem bleeding disease

No oozing is seen from old lesions. The exudates eventually dry up to form black encrustations with brownish orange margin. The tissues beneath the discoloured patch show decay. The internal decay can be observed even in areas beyond the margins of external lesions indicating that the internal decay is not confined to the area of external symptoms. As the decay progresses, the tissues become black and fibrous. This process is fast in young palms. As a result of this, cavities are formed from which liquid comes out, when the bark is pressed or punctured. Serious infection was found to lead to reducing yield and death of young palms.

In the crown also, symptoms appear. The outer whorl of leaves becomes yellow rather prematurely droops and finally dries up. Though nut fall is noticed in later stages of disease, it

is more in palms exposed to drought conditions. The trunk gradually tapers towards the apex and the crown size is reduced. Crown symptoms are more pronounced during the summer season. During the rainy season, as well as in well managed and irrigated gardens, the crown symptoms are not conspicuous till very late.

Etiology: *Thielaviopsis paradoxa* (de Seynes) von Hohnel has been isolated from affected stem tissues. The perithecial stage (*Ceratocystis paradoxa* (Dade) Moreau has also been recorded from the affected palms. The pathogenecity has been established by artificially inoculating healthy palms with *T. paradoxa*. In advanced stages of the disease, infestation with *Diocalandra* weevil can be seen which quickens the deterioration of the palms.

The fungus is a weak pathogen and enters the trunk through wounds/growth cracks. Growth cracks may develop after sudden heavy rains following prolonged dry period or after heavy manuring. Trash burning has also been found to cause damage to the stem. Poor drainage, formation of hard laterite pan leading to crippled root growth, imbalanced mineral nutrition or other physiological disorders are other predisposing factors.

Integrated disease management

The disease can be effectively managed if control measures are adopted in early stages of infection

- ❑ The affected tissues should be completely removed using a chisel. Destroy the chiselled tissues by burning.
- ❑ Treat the wound with Calixin 5% followed by application of hot coal tar after 2 days.
- ❑ Apply 100 ml Calixin 5% through root feeding thrice a year during June, Sept.- Oct. and January
- ❑ Soil application of 5 kg neem cake fortified with *Trichoderma* per palm per year during Sept.- Oct. is also found beneficial
- ❑ Summer irrigation improves the health of the palm as well as yield.
- ❑ Since wounds on the trunks predispose the palms to infection, any type of wounding of palms is to be avoided. Care should be taken not to injure the stem base while ploughing the garden with tractor.

7. Basal stem rot

Basal stem rot is also known as *Ganoderma* wilt or Thanjavur wilt is a very important destructive disease of coconut. In Tamil Nadu, this disease was first observed in Thanjavur district in 1952 and hence called Thanjavur wilt. In Karnataka it is known by the popular Kannada name 'Anabe roga' and in Andhra Pradesh, *Ganoderma* wilt.

In India, Butler first recorded *G. lucidum* in coconut palm in Karnataka State in 1913. Till 1965-66, the disease was confined to the coastal areas of Tamil Nadu and the incidence ranged from 3.3 to 10.8 per cent with the highest incidence (10.8%) in Thanjavur district followed by Kanyakumari (6.3%), Tiruchirappalli (4.0%), Ramanathapuram (4.0%), South Arcot (3.5%) and Chengalpattu (3.3%) districts. In 1978, the disease was noticed in all the districts of Tamil Nadu and the incidence ranged from 0.6 to 4.9 per cent. In Andhra Pradesh, the disease is

mainly prevalent in lighter soils in the coastal districts than in heavy soils. Wide spread occurrence of the disease is noticed in the maidan tracts of Karnataka especially in sandy soils of the southern region.

Symptoms: Five distinct stages can be recognized in the development of Ganoderma wilt. Wilting of leaflets (which sometimes may not be very prominent), yellowing of the leaves of lowest leaf whorl and decay and death of fine roots are the symptoms in the initial stage of disease development. In the second stage bleeding patches appear at the base of the stem near the ground level; the lesions gradually extend upwards; roots decay extensively and there is no new bunch production. Bleeding patches extend in the stem, leaves droop in the outer whorl followed by heavy button shedding and barren nuts. As stem decay traverses upwards; outer leaf whorl dries and drops off; other leaves also droop except the spindle leaf and surrounding two or three young leaves which remain erect and healthy. Ultimately all the leaves droop and fall off leaving the decapitated stem. Stem shrivels and dries up.

The time taken from the initial appearance of bleeding patches in the stem to the death of the palms is from 6 to 54 months, the average being 24 months. In the middle or late stages of disease sometimes the scolytid beetle, *Xyleborus perforans* and the weevil, *Diocalandra stigmaticollis* are found infesting the stem in large numbers at the bleeding patches from which powdery mass is thrown out. The insects accelerate the death of the palm

Etiology: Isolation of the causal organism from different tissues of the affected palms was carried out. *G. applanatum* and *G. lucidum* were isolated from roots irrespective of the extent of bleeding symptom. The pathogenicity has been established by inoculating the fungus in the trunk region.

Integrated disease management

The disease can be effectively contained by following an integrated approach with cultural, chemical and biological methods involving the following operations.

- ❑ Removal of dead palms and palms in advanced stages of the disease and destruction of the bole and root bits of these palms.
- ❑ Isolation of diseased palms from healthy palms by digging isolation trenches of 1 m deep and 30 cm wide.
- ❑ Regular basin irrigation during summer months or moisture conservation by coconut husk burial (250 husks/palm).
- ❑ Avoiding flood irrigation or ploughing in infected gardens to prevent spread of the inoculum.
- ❑ Addition of 50 kg of farmyard manure or green leaves or 200 kg tank silt per palm per year.
- ❑ Application of 5 kg neem cake per palm per year.
- ❑ Raising banana as intercrop wherever irrigation is possible.
- ❑ Soil drenching with 40 L of 1 per cent Bordeaux mixture thrice a year for one year.
- ❑ Root feeding of 2 g of Aureofunginsol + 1 g of Copper sulphate in 100 ml of water thrice a year at quarterly interval. Alternatively, Calixin (Tridemorph) (2 ml/100 ml)

can also be used for root feeding instead of Aureofunginsol. Fungicide treatments will be effective only for palms in early stages of the disease

- If *Xyleborus* attack is found in the stem, smearing with heptachlor may be done.

Conclusion

Integration of various disease management practices depends on several factors like the crop, the disease/pathogen, cropping system, location and climatic factors. Disease management might be viewed as a holistic approach. In all the technology transfer efforts, emphasis should be given to the integrated management practices to achieve the desired goal. However, this is not always easy to accomplish, and “disease management” may be reduced to single ‘disease control’ measures using chemicals. Effective disease management can be achieved only if the integrated plant protection measures are adopted at the right time.

Current status of phytoplasma research

R. Manimekalai

Central Plantation Crops Research Institute
Kasaragod-671 124, Kerala, India

Phytoplasmas were first identified during 1967 as pathogens that cause severe epidemics in major crops. Phytoplasma, an uncultivable mycoplasma like organism (MLO) causes diseases in hundreds of economically important plants and crops such as grapevine, vegetables, corn, sugar beet, oil- seed crops and fruit trees worldwide. '*Phytoplasma*' has become most significant topics for researchers throughout the world. Characterization and diagnosis of the pathogen is possible only through sequencing and analyzing the highly conserved genomic regions of ribosomal DNA and other conserved gene sequences. Molecular diagnostic techniques like PCR (Polymerase Chain Reaction), Real-Time PCR and RFLP (Restriction Fragment Length Polymorphism) analysis are the fundamental techniques currently used for diagnosis and accurate identification of different strains and species of phytoplasma. Recent advances in phytoplasma genomics have generated an impetus for research into control and management of these diseases. This will result in improved diagnostic methods; reduction of disease spread; improvement of insect-vector monitoring and a reduction in the pesticides used for control. Phytoplasma research is widening from development of new diagnostic criteria to genome sequencing projects and its annotation. The greatest drawback in preventing the flow of phytoplasma research is its inability to grow in artificial culture environment.

Phytoplasma- Current research around the world

Phytoplasma are obligate parasitic bacteria resembling mycoplasma in pleomorphic constitution due to the lack of cell wall and are grouped under the class 'Mollicutes'. Phytoplasma are the inhabitant of phloem sieve elements of vascular plants and intestine, salivary gland and other organs of insect vector hosts and are transmitted by the phloem sucking plant insect vectors falling in the order *Hemiptera* (plant and leafhoppers). Phytoplasma is transmitted in a persistent manner by insects belonging to the families Cicadellidae, Cixidae, Psyllidae, Delphacidae, and Derbidae. Phytoplasma can be considered as a potent phytopathogen responsible for huge economic loss all over the world. There are more than 300 phytoplasma strains reported as causatives of various types of plants from shrubs to trees including crops, cereals, vegetables, fruiting and flowering trees, ornamentals and many other plants. Earlier the diseases caused by phytoplasma were considered due to viruses. Uncultivable nature of the phytoplasmas slows down the understanding about them and making any progress in genomics. Full genome of four phytoplasma is presently available and the sequencing projects are nearing completion for the Maize bushy stunt phytoplasma and coconut lethal yellowing phytoplasma.

The application of the genome sequencing and sequence helps in development of diagnostics. Early and sensitive detection and diagnosis of phytoplasmas is of paramount importance for effective prevention strategies, particularly because phytoplasmas may have a very long latency period and most of the phytoplasma disease are seen in perennial high value crops. Micropropagation and vegetative propagation methods such as grafting, cutting help in

the transmission of phytoplasmas. Detection often is hampered by their extremely irregular distribution in host plants. A sensitive, specific and quick diagnostic test would be highly desirable for routine detection, mainly to avoid using infected planting material.

Control of epidemic outbreak can be carried out either by controlling the vector or by eliminating the pathogen from the infected plants by tetracycline, or by other chemicals. However, these protection measures have proved to be quite ineffective under field conditions, and therefore, the only effective way to control phytoplasma infection is to prevent the outbreaks. Identification of alternative control strategies such as the possibility of using biocontrol organisms or phytoplasma mild strains could also provide innovative and promising tools for limiting phytoplasma spread in an environmentally sustainable approach.

Phytoplasma research in India

In India, the phytoplasma diseases are reported in pepper, citrus, coconut, sandal, brassica and sugarcane. Phytoplasma work has been conducted in different laboratories focusing on the molecular detection/phylogeny aspects in different crops. Sandal spike disease is detected through PCR. At Indian Institute of Horticulture Research, a new disease of acid lime was diagnosed in citrus growing tracts of India. The symptoms are premature leaf drop and distorted twigs producing witches' broom. Electron microscopic observation revealed the presence of phytoplasmas in the sieve tubes of leaf midribs. Phytoplasma associated with chilli little leaf disease has been detected by graft transmission and electron microscopy and molecular level. Studies from NBRI detected *Candidatus* Phytoplasma asteris from pigeon pea. Studies done at IARI identified IX group pigeon pea witches broom phytoplasma in *Brassica rapa* showing symptoms of phyllody. Using PCR techniques, the phytoplasma was detected in black pepper (*Piper nigrum*) with phyllody symptoms. 16SrI phytoplasma was identified in Yellowing disease of pepper. Phytoplasmas causing sugarcane grassy shoot disease has been identified and its Phylogenetics was also derived. Phytoplasmas were detected in sugarcane grassy shoot symptomatic samples by the PCR amplification of phytoplasma specific 16S rRNA gene and 16S-23S rRNA spacer region (SR) sequences. A comprehensive database "Phytoplasma database" (PHYTODB) was developed at CPCRI which comprised of genomics and proteomics details of phytoplasma and it has integrated with the analysis tool for phytoplasma classification.

Phytoplasma diseases in coconut

Around the world coconut is affected by phytoplasma and diseases are reported. Root (wilt) in India; Weligama wilt in Sri Lanka; lethal yellowing in Jamaica, Florida, Mexico and East Africa and Nigeria; yellow decline in Malaysia and Kalimantan wilt in Indonesia have been reported by various researchers.

Milestones in phytoplasma research

Root (wilt) disease

The disease was first noticed in Kerala state as early as in 1874. The disease is reported from three independent locations, from Meenachil taluk (1882), Thiruvalla taluk (1907) and Karthikappally taluk (1907). The disease causes enormous yield (960 million nuts) losses in coconut. Detailed studies have been conducted for the detection of Phytoplasma by various

researchers and the methods employed are symptomology, electron microscopy observations, staining of the phytoplasma nucleic acid, grafting on indicator plants to detect symptoms of phytoplasma, insect transmission, ELISA and molecular biology techniques such as PCR.

Phytoplasma Genomics (restrict to one paragraph by giving most salient aspects)

The genome of 4 different phytoplasma strains were sequenced successfully and the genome analysis and comparative studies also performed among them. Phytoplasma genome consists of 14 – 23% repetitive DNA organized in potential mobile units (PMUs), a unique characteristics of phytoplasma genome, contain *tra5* insertion sequences (ISs) and genes for specialized sigma factors and membrane proteins. At first, the whole genome sequencing of a phytoplasma has been successfully accomplished by The University of Tokyo, Japan by sequencing Onion yellows phytoplasma OY-M. The result of their work suggests phytoplasma experienced a reductive evolution as a consequence of its life as an intracellular parasite in a nutrient-rich environment and shows that phytoplasma lacks several genes previously considered to be essential for autonomously replicating cells. Instead, it possesses transporter genes involved in the uptake of substances from the cytoplasm of surrounding host cells. Further, 706,569-bp circular chromosome and four plasmids of aster yellows phytoplasma strain witches' broom (AY-WB) were sequenced by Ohio State University. The AY-WB genome contains long repeating units of DNA. Virulence of AY-WB phytoplasma is suspected by the presence of hemolysin like proteins HlyC and TlyC. The genome sequencing of *Candidatus* phytoplasma mali, AT strain has been accomplished by Federal Biological Research Center for Agriculture and Forestry. '*Ca. P. mali*' genome is of linear chromosome of 601,943 bp in size and GC content of 21.4%. Glycolysis, the major energy-yielding pathway for '*Ca. P. asteris*', is incomplete in '*Ca. P. mali*'.

The chromosome sequence of *Candidatus* Phytoplasma australiense was determined by Max Planck Institute. The circular chromosome has 879,324 nucleotides, a GC content of 27%, and 839 protein-coding genes. Five hundred two of these protein-coding genes were functionally assigned, while 337 genes were hypothetical proteins with unknown function. Potential mobile units containing clusters of DNA repeats comprised 12.1% of the genome. These PMUs encoded genes involved in DNA replication, repair, and recombination; nucleotide transport and metabolism; translation; and ribosomal structure.

Phytoplasma diagnostics

The development of molecular tools has enabled identification and preliminary classification of phytoplasmas. As phytoplasmas have still not been cultured *in vitro*, their diagnosis relies mainly on molecular techniques such as PCR, usually followed by RFLP for assignment to a *Candidatus* Phytoplasma species or to a 16S rDNA group. PCR is mostly used in detection, identification and classification. The 16S ribosomal gene is used as target for designing phytoplasma-universal as well as group-specific primers. In recent past real-time PCR using universal and group specific primers are used in diagnosis of phytoplasmas. All of the researchers exploited a TaqMan probe for detection. *Ca. Phytoplasma mali*, *Ca. Phytoplasma pyri* and *Ca. Phytoplasma prunorum* can be detected through Real time PCR. Q-PCR is applied by many workers to calculate the concentration of several phytoplasmas in their insect vectors. Mixed infections have been reported under field conditions in several important

crops, such as grapevine, palm, potato and ornamentals. Q-PCR would enable detailed study of the interactions of different phytoplasmas in the same host.

Co-operational PCR (co-PCR) has been proposed to detect 16SrX phytoplasmas, avoiding nested PCR. The method uses a tri-primer reaction coupled with dot-blot hybridization with universal and specific probes. Specific detection of phytoplasmas by printing on a microarray was successful. PCR products, obtained with universal primers, were labelled and used as hybridization probes on the array. Low-density PCR arrays are prepared by drying real-time TaqMan PCR reagents (primers and probes) specific for several target genes into different wells of a plate. Primers and probes are reconstituted by adding cDNA together with PCR master mix, and the reaction takes place in a real-time thermal cycler.

Isothermal amplification of nucleic acids has been described as an alternative to PCR such as LAMP which uses a set of four or six primers and a DNA polymerase with strand displacement activity (Bst DNA polymerase) to amplify DNA with high specificity under isothermal conditions in less than 1 h. This method has been used to detect several bacteria and citrus greening bacteria *C. Liberobacter* spp. LAMP products are visualized either by gel electrophoresis or with other methods suitable for use in the field. The synthesis of large amounts of DNA in a LAMP reaction yields a white precipitate of magnesium pyrophosphate, which can be detected either with the naked eye or by real-time turbidimeter. Mait Dickinsen at University of Nottingham, UK, developed a rapid DNA extraction method using lateral flow device (LFD) membranes. And used LAMP assay for the detection of phytoplasma in coconut trunk boring samples.

Phytoplasma and host interaction studies

The phytoplasma and host interaction may be complex or simple. Studies showed that phytoplasma may cause reduced fitness to its insect vectors still some insect vector showed improved over-wintering ability, fertility and longevity. Immunodominant proteins are candidates for host-pathogen interactions such as attachment to host cells.

Antigenic membrane protein (amp) gene was characterized in a few phytoplasmas, belonging to 16SrI, 16SrII, 16SrIII, 16SrX, 16SrXI and 16SrXII groups and can be used for host interaction studies. Immunofluorescence microscopic study focused on the antigenic membrane protein of the OY. Phytoplasma-infected insect showed that the phytoplasma was localized to the microfilaments of the visceral smooth muscle surrounding the insect's intestinal tract and also forms a complex with three insect proteins: actin, myosin heavy chain, and myosin light chain in the affinity column assay. In another study showed that AY-WB phytoplasma produces a protein that targets the nuclei of plant host cells; this protein is a potential phytoplasma effector that may alter plant cell physiology. Recent Italian reports indicate the presence of different phytoplasmas in medicinal herbs showing stunting, witches' broom and yellowing.

Phytoplasma transmission studies also progressing across the world for better understanding of host interaction of phytoplasma. In one study tried to transmit *Candidatus* phytoplasma prunorum through root bridges of diseased and healthy cultivars of apricot plants.

Gaps in current knowledge

The phytoplasma is currently not culturable *in vitro* and it is a mystery how these bacteria use the sugar-rich phloem sap in which they live and how they interact with the host and makes difficult to develop suitable measure for their control. The phytoplasma genomes is highly flexible and evolved to enable to adjust the rapid changes in response to host chemical signals, evasion of host surveillance systems, and adaptation to shifting host environments both in insects vectors and plants. The mechanisms underlying the genome flexibility have yet to be thoroughly explored.

Future thrust

Development of novel diagnostic methods and integrating them into sensitive and simple early detection protocols will be ideal for monitoring propagation material and for screening the plants. For this purpose, marker genes that show sufficient polymorphism will be selected as DNA bar-coding regions, and a database of available collections of phytoplasma strains and/or DNA has to be established. There is need for the development of vector monitoring system for identification of phytoplasma vector species and to monitor their spread. Identification of alternative control strategies against these diseases, such as the possibility to use biocontrol organisms or phytoplasma mild strains could also provide innovative and promising tools for limiting phytoplasma spread in an environmentally sustainable approach. The knowledge of whole genome of different phytoplasma has to be studied for evolving possible control measures.

References

- Phytoplasma Research Begins to Bloom (2009). *Science* 325 388 – 390
- PHYTOPLASMAS Genomes, Plant Hosts and Vectors; Phyllis G. Weintraub & Phil Jones, page: 1-18; CAB International 2010.
- Phytoplasmas: diversity, taxonomy, and epidemiology. *Frontiers in Bioscience* 12, 673–689.
- Development of a one-hour DNA extraction and loop-mediated isothermal amplification assay for rapid detection of phytoplasmas' M. Dickinson, J.A. Tomlinson and N. Boonham, University of Nottingham and Fera, York, UK

A fast and versatile molecular tool for detection of root (wilt) phytoplasma in coconut palms

R. Ramjegathesh, G. Karthikeyan, B. Meena, T. Raguchander and R. Samiyappan

Department of Plant Pathology
Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Coconut palm (*Cocos nucifera* L.) is an economically important perennial crop and provides sustainable income either directly or indirectly to millions of people. India is the third largest producer of coconut in the world (Satya Sundaram 2009). Coconut palm is being affected by more than fifty diseases. In India, especially South India, the root (wilt) disease is most important and it was first noticed during 1882 in Kerala (India). The disease is currently fast spreading in Tamil Nadu state in nearby gardens of Kerala state and cause production constraints in many districts of the state.

Phytoplasma are wall less prokaryotes, live as obligate parasites, small and simplest self replicating organism. It is phloem inhabiting organism at low concentration and has an uneven distribution in woody plants (Weintraub and Beanland, 2006). The molecular diagnosis is most important for phytoplasma, since it has high specificity and sensitivity. Conventional detection of phytoplasma is based on PCR using 16Sr RNA general or group specific primers (Smart *et al.*, 1996), being necessary in many cases a nested PCR in two steps (Gunderson and Lee, 1996) due to low titers of phytoplasma in plants.

Quantitative PCR assay measures the accumulation of amplified products during the reaction through the determination of the fluorescence signal. This method has many advantages over the conventional end point PCR in terms of accuracy, dynamic range, high-throughput capacity and absence of post-PCR manipulations that prevents carry over contamination (Schaad and Frederick, 2002). In this paper we discuss about quantitative PCR conjugated with Taqman® probe for sensitive and simultaneous quantitative detection of different parts of root (wilt) infected coconut palms.

Creamy white colored leaves, trunk and roots were collected from the symptomatic root (wilt) infected coconut palms from southern Tamil Nadu.

Infected sample (0.4 g) was incubated for 10 min in 8 ml of Phytoplasma Grinding Buffer (PGB: 100 mM K_2HPO_4 , 30 mM KH_2PO_4 , 10 % sucrose, 0.15 % bovine serum albumin fraction V, 2% polyvinylpyrrolidone, 25 mM ascorbic acid) in a mortar maintained on ice and then finely crushed with a pestle, adding 5 ml more of PGB (Warrokka *et al.*, 2006). The homogenate was centrifuged for 5 min at 2,500 rpm. The supernatant of each sample was transferred in to clean tubes and centrifuged for 25 min at 15,000 rpm. The pellet was dissolved in 1 ml CTAB buffer (2 % CTAB, 100 mM Tris pH 8, 1.4 M NaCl, 20 mM EDTA). After one-hour incubation at 65°C, the nucleic acids were purified by chloroform-isoamyl alcohol (24:1) extraction and an equal volume of ice cold isopropanol was added to the drawn aqueous phase and then incubated on ice for 1 h. After centrifugation at 10,000 rpm for 10 min, the pellet was dissolved in 400 µl of TE (10 mM Tris-HCl pH 8.0, 1 mM EDTA) to which 40 µl of 3 M sodium acetate and 0.9 ml of 95% ethanol were added. After incubation for 20 min at -20°C,

the mixture was centrifuged for 10 min at 10,000 rpm. Once the supernatant aliquot was eliminated and the tubes washed with 80 % ethanol, the pellet containing the DNA was dissolved in 35 µl of sterile water. The DNA was checked by 0.8 % agarose gel electrophoresis.

For nested PCR, DNA extracted from coconut palm samples were collected from different areas. PCR amplification were performed in 25 µl reactions, each reaction containing 50 ng of template DNA, 1.25 mM Taqbuffer with KCl, 1.25 mM MgCl₂, 2.5 mM dNTP mix, 5U of Taq polymerase (Fermentos, USA), 30 picomoles of forward and reverse primers (Agile life sciences Pvt Ltd, Thane) and sterile water. PCR was performed in a thermal cycler (Eppendorf gradient master cycler) for universal primers R16F2n/R16R2 (Alhudaib *et al.*, 2009) (95°C for 10 min followed 35 cycles of initial denaturation 94°C for 1 min, annealing 60°C for 2 min, extension 72°C for 1 min and final extension of 72°C for 10 min). For the nested PCR, previously amplified PCR products were used as template. The group specific primer pairs FU5/RU3 (Alhudaib *et al.*, 2009 and Nejat *et al.*, 2009) (92°C for 1 min 30 sec followed 35 cycles of initial denaturation 92°C for 30 sec, annealing 57°C for 30 sec, extension 72°C for 50 sec and final extension of 72°C for 10 min) were used for the nested PCR. Aliquots of 10 µl PCR products were analyzed by using 1.5 % agarose gel with horizontal electrophoresis (Bangalore genei) apparatus.

The group specific primers FU5/RU3 were used to amplify 890 bp DNA fragment located at 16S r RNA gene. The primers and probe were designed by following 16sr RNA region (F-5'- GCGTGG GTA GCA AAC AGG ATT AA -3'; R - 5'- CCC GAC ACT TAGTAC TCA TCG TTT -3'; Probe- 5'- FAM - ACG GCG TGG ACT ACC - NFQ-3'). For the establishment of qPCR protocols, a TaqMan MGB method (Aldaghi *et al.*, 2008) QPCR was performed in 48-well optical reaction plates using the Applied step one real time PCR system and data were analysed using the software versions stepone software v2. Ten µl of reaction mixture was pipetted out in each well, containing 2 µl of DNA template, 1 µl of 20X Assay mix (primers and probe), 10 µl of 2X universal Taqman mix (no amprase) (ABI, Foster city, CA, USA)) and 7 µl of sterilized nuclease free water. Controls on DNA free water were run for each experiment. TaqMan probe for fluorescence emission, were carried out in a stepone system programmed to hold at 50°C for 2 min, to hold at 95 °C for 10 min, cycling stage 95 °C for 15 sec and 60°C for 1 min at 40 cycles. Reactions were done in triplicate. PCR results were given as the increase in the fluorescence signal of the reporter dye detected and visualized by stepone software v2. Threshold cycle (Ct) values represent the PCR cycle in which an increase in fluorescence, over a defined threshold, first occurred, for each amplification plot.

For quantification purposes the standard curves were prepared with tenfold serial dilutions of purified 16Sr DNA from coconut root (wilt) phytoplasma in sterilized nuclease free water. DNA concentration was determined by nano drop (Bio Rad laboratories, California, USA). PCR amplifications were carried out in triplicate in three independent experiments. Standard curves generated by plotting genomic DNA coconut root (wilt) phytoplasma against the Ct values exported from the Applied Bio system Step one real time PCR system for each plate. The Ct values for unknown samples were explored from standard curves.

PCR amplification of 16Sr DNA sequence using an universal primer set of R16f2n/R16r2 revealed that DNA fragment approximately 1250 bp were directly amplified from 16Sr DNA gene sequence extracted from coconut root (wilt) infected tissues (Fig. 1). No amplified DNA fragments were observed in DNA samples extracted from nonsymptomatic

palm. Nested PCR for 16Sr DNA gene sequence amplification using a nested primer pairs for the phytoplasma specific 16Sr DNA universal gene sequence. Phytoplasma specific products 890 bp in size were consistently amplified from infected coconut palms, but not from healthy palms (Fig. 2).

Coconut root (wilt) phytoplasma DNA was quantified as a component of the total DNA extracted from different parts (leaves, roots and trunk) coconut palms, using quantitative PCR with phytoplasma genus specific primer/probe set. We noted that at $C_t > 33$, the variation among the triplicated PCR reactions of a single sample was generally less than 0.5 standard deviation, and accurate quantification would require three or more reactions per sample. Therefore, the amount of target DNA that generated a C_t value between 33.14 and 34.21 was considered the quantification limit assays (Fig. 3). There was a statistically significant linear relationship ($R^2 = 0.99$; $Y = -3.08 + 42.77$) between different samples i.e. different parts of coconut palm and DNA estimates using quantitative PCR (Fig. 4). A highest copy numbers of 550069 were observed in leaf samples followed by roots (494224) and trunk samples (293756) (Table 1).

In our study, detection of phytoplasma in different parts of coconut palms was done with the Nested PCR technique, which amplified an amplicon size of 890 bp. It is in accordance with the findings of Alhudaib *et al.* (2009). The organism can be identified by using this initially amplified DNA segment as a template for further PCRs (i.e. nested PCR) using primers that amplify DNA from phytoplasma. Nested PCR assay for more specific detection compared to universal PCR. Identification of infection by phytoplasma was difficult in first round PCR due to low titer of phytoplasma and in which nested PCR yielded positive results (Berges *et al.*, 2000). Confirmation of PCR results by different pairs of primer combinations (generic and group-specific) with subsequent sequencing of PCR amplicons seems to be the way for exact phytoplasma identification in the plant samples.

Recently much interest has been focused on detection and quantification of phytoplasma which is predominantly responsible for severe yield reduction in coconut palms. Various types of detection chemistries can be used for monitoring qPCR products. Probe chemistry was selected for this study. It is highly sensitive and specific based on the monitoring of the fluorescence intensity after each PCR cycle. In the present study a rapid, specific, sensitive qPCR assay for the detection and quantification of phytoplasma on coconut palms was developed. Using such probe, different PCR amplification products can be accurately distinguished by amplification plots. There are reports on the qPCR assay to detect (Baric and Dalla-Via, 2004) and also to quantify (Jarusch *et al.*, 2004) in *Candidatus Phytoplasma Mali*. Both the works allow the specific detection of this phytoplasma, where as probe method described in the present paper allow quantification of coconut root (wilt) phytoplasma.

The sensitivity of the qPCR is similar to that of nested PCR tested with field samples and calibration standards, allowing the detection of 10 copies of the target gene present in the reaction mixture. The qPCR provides an estimation of the phytoplasma concentration in the samples and the standard curves confirm the linearity of the quantification process between exponential increase of DNA copy numbers (from 10^2 to 10^9) and qPCR threshold cycles. The reduced confidence and prediction interval showed the possibility to get adjusted curves with 8 standards and three replicates. In agreement with Rasmussen (2001), repeated run of same

standard curve gave differences in slope and intercept; regression analysis must be done in each run.

The number of phytoplasma per gram of tissue estimates the copy numbers derived from the calibration curve between the ranges of three replications in the different samples. In this work, the concentration of coconut root (wilt) phytoplasma was found to be 5.5×10^4 leaves, 4.9×10^4 in roots and 2.9×10^4 in trunk per gram of tissues. In apple proliferation phytoplasma the concentration ranged from 6.5×10^2 to 1.0×10^8 cells per gram of apple tree tissue (Ester torres *et al.*, 2005). Similarity the concentration of phytoplasma ranged from 9.7×10^3 to 3.0×10^5 cells per gram tissue in apricot, pear and japanese plum (Berges *et al.*, 2000).

Quantitative PCR provides a high security with the positive results due to specificity of the primers and avoid the false positive reaction. Furthermore, quantitative property of the assay can be useful to monitor the phytoplasma kinetics such as the progress of an infection in infected palms and to conduct epidemiology research of this emerging disease of coconut in South India.

References

- Aldaghi, M., Massart, S., Roussel, S., Dutrecq, O. and Jijakli, M.H. 2008. Adaptation of real-time PCR assay for specific detection of apple proliferation phytoplasma. *Acta Hort.* **781**: 387-393.
- Allhudaib, K., Arocha, Y., Wilson, M. and Jones, P. 2009. Molecular identification of potential vectors and alternative hosts of the phytoplasma associated with a lime decline disease in Saudi Arabia. *Crop Protection* **28**: 13-18.
- Baric, S. and Dalla-via, J. 2004. A new approach to apple proliferation detection a highly sensitive real time PCR assay. *J. Microbial Methods* **57**: 135-145.
- Berges, R., Root, M. and See Muller, E. 2000. Range of phytoplasma concentrations in various plant hosts as determined by competitive polymerase chain reaction. *Phytopathology* **90**: 1145-1152.
- Ester Torres., Edson Bertolini., Mariano Carmino Montom. and Maria P. Marti. 2005. Real time PCR for simultaneous and quantitative detection of quarantine phytoplasma from apple proliferation (16Sr X) group. *Molecular and Cellular Probes* **19**: 334-340.
- Gunderson, D.E. and Lee, I.M. 1996. Ultrasensitive detection of phytoplasmas by nested PCR assays using two universal primers. *Phytopathologia mediterranea* **35**: 144-151.
- Jarausch, W., Pececerella, T., Schwind, B., Jarausch, B. and Krezal, G. 2004. Establishment of a quantitative real-time PCR assay for the quantification of apple proliferation phytoplasmas in plants and insects. *Acta Hort.* **657**: 415-20.
- Nejat, N., Sijam, K., Abdullah, S.N.A., Vadamalai, G. and Dickinson, M. 2009. Phytoplasmas associated with disease of coconut in Malaysia: phylogenetic groups and host plant species. *Plant Pathol.* **58**(6):1152-1160.
- Rasmussen, R. 2001. Quantification in the light cycler. In: Meuer, S., Wittwer, C.S., Nakagawara, K.I. (Eds) *Rapid cycle Real-time PCR*. Heidelberg; Springer.
- Satya Sundaram, I. 2009. Coconut Production Escalating. *Market Survey* 1-3.

- Shaad, N.W. and Fredrick, R.D. 2002. Real time PCR and its application for rapid plant disease diagnosis. *Can. J. Plant. Pathol.* **24**: 250-258.
- Smart, C.D., Schneider, B., Blomquist, L., Guerra, J., Harrison, N.A., Ahrens, U., Lorenze K-H, Seemuller, E. and Kirkpatrick, B.C. 1996. Phytoplasma specific PCR primers based on sequences of the 16S-23S rRNA spacer region. *Appl. Environ. Microbiol.* **62**: 2988-2993.
- Warokka, J.S., Jones, P. and Dickinson, M.J. 2006. Detection of phytoplasmas associated with Kalimantan wilt disease of Coconut by the Polymerase chain reaction. *Journal Littri.* **12**(4):154-160.
- Weintraub, P.G. and Beanland, L. 2006. Insect vectors of phytoplasma. *Annual Review of Entomology* **51**: 91-111.

Table 1. Threshold cycles (Ct) values and Copy numbers and numbers of phytoplasma per gram of root (wilt) infected coconut leaves, roots and trunks using Taq® man Probe

Treatments	Ct average ^a	St deviation ^b	Copy number ^c / µl	No. of phytoplasma/g
Leaf sample	33.14	0.59	6286.50	550068.75
Root sample	33.33	0.48	5648.28	494224.50
Trunk sample	34.21	0.42	3357.21	293755.80

^a Threshold cycles (Ct) values obtained by qPCR of the standards used for the quantitative assay and the determination of the phytoplasma concentration in field samples

^b Ct values standard deviation

Copy number of target gene was calculated with the equation of the calibration line obtained with the second run

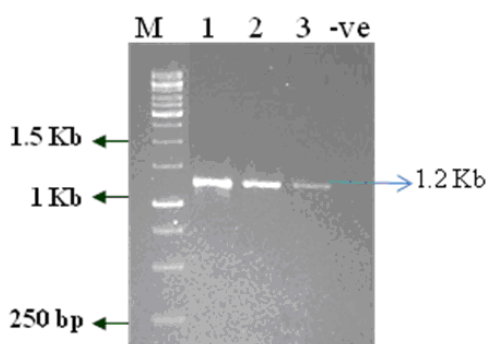


Fig. 1. First round PCR amplification of 16Sr DNA sequences of phytoplasma from naturally infected different parts coconut palm

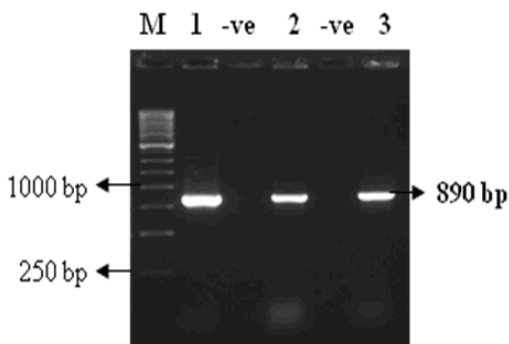


Fig. 2. Nested PCR amplification of 16Sr DNA sequences of phytoplasma from naturally infected different parts coconut palms

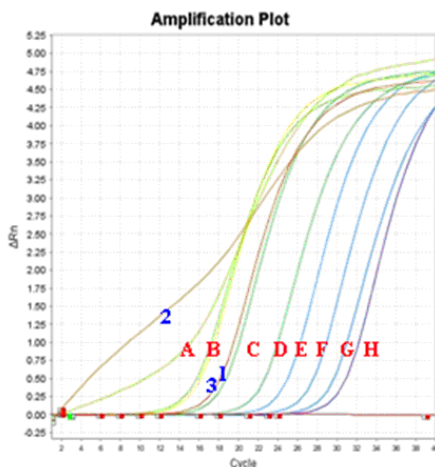
Lane M : 1 kb ladder

Lane 1 : Coconut infected leaf sample

Lane 2 : Coconut infected root sample

Lane 3 : Coconut infected trunk sample

Lane -ve : Negative control (Healthy coconut leaf)



Standards

A – 8.02E9; B – 8.02E8; C – 8.02E7;
D – 8.02E6; E – 8.02E5; F – 8.02E4;
G – 8.02E3; H – 8.02E2

Samples

1 - Trunk 2 - Leaves 3 - Roots

Fig. 3. Amplification plot generated during quantitative PCR of 16Sr DNA gene of phytoplasma (Samples and Standards) using Taqman® probe.

The Relative fluorescence recorded at each cycle of PCR was normalized and plotted

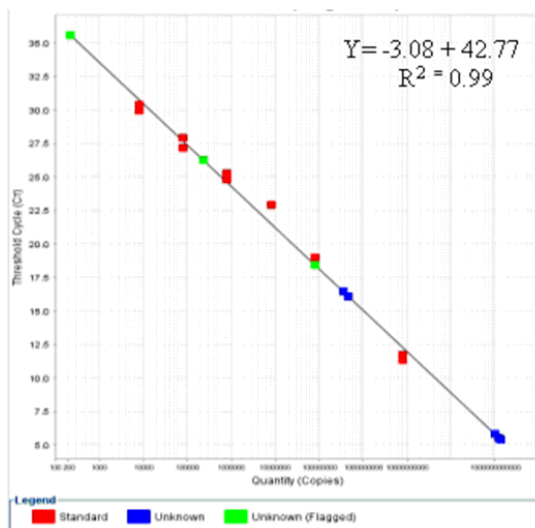


Fig. 4. Standard curve generated during quantitative PCR using Taqman® probe for copy number against threshold cycle (CT)

The standard refers the 10- fold diluted known concentration of 16Sr RNA gene of phytoplasma. Samples refers the amplified product from different parts of coconut samples

Effect of *Pseudomonas fluorescens* and *Trichoderma viride* in the management of basal stem rot disease in coconut

K. Rajappan, M. Surulirajan, C. Natarajan and S. Arulraj

Coconut Research Station

Tamil Nadu Agricultural University, Veppankulam – 614 906, Tamil Nadu

More than 50 diseases have been reported in coconut throughout the world and among them, basal stem rot caused by species of *Ganoderma* has been identified as a serious disease, causing heavy loss in yield. The symptomatology, host range, etiology and epidemiology were reviewed (Bhaskaran *et al.*, 1984), and based on these studies, several control measures were adopted to manage the disease. Various practices like management of soil moisture, application of organic manures, application of fungicides and chemicals etc., were attempted. However, these management practices were found effective only if the disease is detected in early stages (Bhaskaran *et al.*, 1996). The basal stem rot disease causes 15 to 25 percent damage to roots and bole below the ground level by the time external symptoms are visible.

In the present study, efforts were made to collect soil samples from coconut rhizosphere, isolate the biocontrol agents and assess their effectiveness under *in vitro* conditions, to manage the disease using biocontrol agents through root feeding of culture filtrates and soil application and to find out the effect of soil application of biocontrol agents on their population dynamics in the rhizosphere.

Soil samples from the rhizosphere of coconut palms were collected from different places. The samples were processed and *Pseudomonas fluorescens* and *Trichoderma viride* were isolated using King's B Agar (KBA) and *Trichoderma viride* Selective Medium (TSM), respectively. The inhibitory effect of the isolates of *P. fluorescens* and *T. viride* against *Ganoderma lucidum* was assessed under *in vitro* conditions by dual culture method. A field trial was conducted in a farmer's holdings at Mahizhankottai village in Pattukkottai), Thanjavur district, Tamil Nadu to assess the effect of root feeding of culture filtrates of *P. fluorescens* and *T. viride* and also soil application of these biocontrol agents on disease incidence, nut yield and rhizosphere populations of *P. fluorescens* and *T. viride* with the following treatments.

- T₁ - Root feeding of 100% culture filtrate of *Trichoderma viride* 25 ml at quarterly interval.
- T₂ - Root feedings of 100% culture filtrate of *Trichoderma viride* 25 ml at six months interval
- T₃ - Root feeding of 100% culture filtrate of *Trichoderma viride* 25 ml / year
- T₄ - Basal application of *Trichoderma viride* (50 g) + neem cake 5 kg / year
- T₅ - T₁+T₄
- T₆ - T₂+T₄
- T₇ - T₃+T₄
- T₈ - Root feeding of 100% culture filtrate of *Pseudomonas fluorescens* 25 ml at quarterly interval.
- T₉ - Root feeding of 100% culture filtrate of *Pseudomonas fluorescens* 25 ml at six months interval
- T₁₀ - Root feeding of 100% culture filtrate of *Pseudomonas fluorescens* 25 ml / year

- T₁₁ - Basal application of *Pseudomonas fluorescens* + neem cake 5 kg/palm / year
 T₁₂ - T8 + T11
 T₁₃ - T9 + T11
 T₁₄ - T10 + T11
 T₁₅ - Neem cake 5 kg / palm / year
 T₁₆ - Control

The experiment was conducted in the farm of Sri. Ziad Mohammed Hasiar planted with East Coast Tall variety of coconut aged 40 years. Completely Randomized Block design was adopted with two replications with two palms in each plot.

Pre-treatment nut yield, disease index and population levels of *P. fluorescens* and *T. viride* were recorded. The disease intensity was recorded by using the following DI (Disease Index) formula,

$$23.6 + 17.7 \times h + 3.6 \times r - 0.6 \times l$$

where h = height of bleeding
 r = reduction of leaf size
 l = no. of functional leaves

Nut yield, disease index and the population of *P. fluorescens* and *T. viride* after first and second year of treatment were also assessed.

Percent inhibition in the growth of *G. lucidum* by *P. fluorescens* isolates ranged from 61 to 77. The isolates P.f-A, P.f-T, P.f-P and P.f-Ss caused 66 percent inhibition in the growth of basal stem rot pathogen (Table 1).

Table 1. Effect of native isolates of *Pseudomonas fluorescens* on the *in vitro* growth of basal stem rot pathogen *Ganoderma lucidum*

S. No.	<i>Pseudomonas fluorescens</i> (Pf) isolates	Mycelial growth of <i>Ganoderma lucidum</i> (mm)	% reduction over control
1.	P. f-A	19	66
2.	P. f-T	19	66
3.	P. f-V	18	68
4.	P. f-S	13	77
5.	P. f-P	19	66
6.	P. f-B	16	71
7.	P. f-J	15	73
8.	P. f-Bs	15	73
9.	P. f-Ar	22	61
10.	P. f-Ss	19	66
11.	Control	57	-

The inhibitory effect of *T. viride* isolates ranged from 74 percent to 80 percent. Five isolates viz., T.v-S, T.v-Pt, T.v-Bs, T.v-Bsm and T.v-Ja caused 80 percent reduction in the growth of *G. lucidum*, while two isolates viz., T.v-A, and T.v-Ts showed 76 percent inhibition. The isolates T.v-Vs, T.v-Ac and T.v-Ss provided 74 percent inhibition over control (Table 2).

Table 2. Effect of native isolates of *Trichoderma viride* on the *in vitro* growth of *Ganoderma lucidum*

S. No.	Isolate	Growth of <i>Ganoderma lucidum</i> (mm)	Percent reduction over control
1.	T. v. -A	12	76
2.	T.v -Ts	12	76
3.	T.v -Vs	13	74
4.	T.v -S	10	80
5.	T.v. - Pt	10	80
6.	T.v -Bs	10	80
7.	T.v - Bsm	10	80
8.	T.v - Ja	10	80
9.	T. v- Ac	13	74
10.	T.v -Ss	12	74
11.	Control	50	-

In this trial, the initial disease index ranged from 0.92 to 2.33 while the disease index recorded after one year ranged from 1.27 to 2.77. The initial nut yield ranged from 85 to 113. After one year, the nut yield ranged from 81 to 129 (Table 3).

Table 3. Disease index, nut yield and population of biocontrol agents in the BSR management trial using biocontrol agents and neem cake

Treatments	Disease index (mean)		Nut yield(no./ palm/year)		Population of biocontrol agents			
	Initial	After one year	Initial	After one year	<i>T. viride</i> (cfu x 10 ³ / g dry soil)		<i>P. fluorescens</i> (cfu x 10 ⁵ /g dry soil)	
					Initial	After 3 months	Initial	After 3 months
T ₁	1.16	1.45	90	99	2.0	2.5	1.0	1.0
T ₂	1.34	1.52	105	120	2.0	2.0	0.7	1.0
T ₃	1.25	1.70	92	96	2.0	2.5	0.5	1.0
T ₄	1.13	1.32	100	111	1.5	3.5	0.5	1.0
T ₅	1.05	1.27	110	129	2.0	3.5	0.7	1.0
T ₆	0.92	1.27	112	129	1.5	4.0	0.5	1.0
T ₇	1.16	1.40	108	120	1.5	4.5	0.7	1.0
T ₈	1.53	1.80	101	117	1.0	1.5	1.0	1.0
T ₉	1.25	1.55	90	102	1.0	2.0	0.7	1.0
T ₁₀	1.37	1.70	91	105	1.0	2.5	1.0	1.0
T ₁₁	1.32	1.67	102	120	1.0	2.5	0.5	3.0
T ₁₂	1.09	1.42	113	129	1.0	2.0	0.5	2.0
T ₁₃	1.12	1.44	110	120	1.0	1.5	0.7	3.0
T ₁₄	2.02	2.55	103	115	1.0	2.0	1.0	2.0
T ₁₅	2.33	2.77	85	90	1.5	1.5	0.5	1.0
T ₁₆	1.35	2.05	88	81	1.5	2.0	1.0	1.0
CD(p=0.05)	NS	NS	5.69	NS	0.5	1.3	NS	NS

NS= Not significant

The initial population of *T. viride* ranged from 1.0 to 2.5 x 10³ cfu / g dry soil, whereas, after three months of treatment, it ranged from 1.5 to 4.5 x 10³ cfu / g dry soil. The initial population of *P. fluorescens* ranged from 0.5 to 1.05 x 10⁵ cfu / g dry soil, while after 3 months of treatment, it ranged from 1.0 to 3.0 x 10⁵ cfu / g dry soil.

The disease index recorded after two years ranged from 1.27 to 2.77 indicating that there was no further spread of the disease after the imposition of treatments (Table 4).

Table 4. Disease index, nut yield and population of biocontrol agents in the BSR management trial at Mahizhankottai using biocontrol agents and neem cake

Treatments	Disease index (mean)		Nut yield(no./ palm/year)		Population of biocontrol agents			
	Initial	After two years	Initial	After two years	<i>T. viride</i> (cfu x 10 ³ / g dry soil)		<i>P. fluorescens</i> (cfu x 10 ⁵ /g dry soil)	
					Initial	After 3 months	Initial	After 3 months
T ₁	1.16	1.45	90	105	2.0	3.0	1.0	1.5
T ₂	1.34	1.52	105	124	2.0	2.0	0.7	1.0
T ₃	1.25	1.70	92	103	2.0	2.5	0.5	1.0
T ₄	1.13	1.32	100	119	1.5	4.0	0.5	1.5
T ₅	1.05	1.27	110	133	2.0	4.0	0.7	1.0
T ₆	0.92	1.27	112	135	1.5	4.0	0.5	1.0
T ₇	1.16	1.40	108	123	1.5	5.0	0.7	1.5
T ₈	1.53	1.80	101	120	1.0	2.0	1.0	1.0
T ₉	1.25	1.55	90	104	1.0	2.0	0.7	1.0
T ₁₀	1.37	1.70	91	110	1.0	3.0	1.0	1.5
T ₁₁	1.32	1.67	102	128	1.0	3.0	0.5	3.5
T ₁₂	1.09	1.42	113	136	1.0	2.0	0.5	3.0
T ₁₃	1.12	1.45	110	126	1.0	2.0	0.7	3.0
T ₁₄	2.02	2.55	103	122	1.0	2.0	1.0	2.5
T ₁₅	2.33	2.77	85	96	1.5	1.5	0.5	1.0
T ₁₆	1.35	2.70	88	78	1.5	1.5	1.0	1.0
CD (P=0.05)	NS	NS	5.6	19.1	0.5	1.2	NS	0.7

NS=Not significant

The initial nut yield ranged from 85 to 113, whereas, after two years, the nut yield ranged from 96 to 136. The initial population of *T. viride* ranged from 1.0 to 2.5 x 10³ cfu / g dry soil whereas after three months of treatment, in the second year, it ranged from 1.5 to 4.0 x 10³ cfu / g dry soil. The initial population of *P. fluorescens* ranged from 0.5 to 1.0 x 10⁵ cfu / g dry soil whereas, after three months of treatment in the second year it ranged from 1.0 to 3.5 x 10⁵ cfu / g dry soil.

References

- Bhaskaran, R., Ramanathan, T. and Ramiah, M. 1984. Chemical control of Thanjavur wilt of coconut. pp.22-24. In: *Proceedings of All India Symp. On Coconut Diseases*. Coconut Research Station, Veppankulam.
- Bhaskaran, R., Rajamannar, M. and Kumar, S.N.S. 1996. Basal stem rot disease of coconut. Technical Bulletin No.30, Central Plantation Crops Research Institute, Kasaragod. P.15.

Biological management of basal stem rot disease of coconut

L. Rajendran, G. Karthikeyan, T. Raguchander and R. Samiyappan

Centre for Plant Protection Studies, Tamil Nadu Agricultural University
Coimbatore – 641 003, Tamil Nadu, India

Basal Stem Rot (BSR) disease caused by *Ganoderma lucidum* (Leys) Karst. is the most destructive and a major limiting factor in coconut production (Bhaskaran *et al.*, 1989). The BSR is also called as Thanjavur wilt, bole rot, Ganoderma disease and Anabe roga in different states of India. This disease also occurs in numerous perennial, coniferous and palmaceous hosts. This pathogen is soil borne in nature and is serious in sandy or sandy loam soils in coastal areas. The management practices were found effective only when the disease was detected in the early stages (Bhaskaran *et al.*, 1996). Currently, no cost-effective fungicide that gives guaranteed control, although the disease could be delayed by adopting strategic management, which is a labour-intensive procedure. Application of bioagents is the current interest for the management of BSR disease in plantation crops and it has been reported by many workers (Garrett, 1955; Bhaskaran, 1990). Among the bioagents, the endophytes which reside internally in the plant tissues without causing visible harm to their hosts (Hallman *et al.*, 1997) and used in disease management (Wulff, 2000). Many species and strains of the *Bacillus* genus produce a large number of substances with antimicrobial action including antibiotics. Producing more than 70 different antibiotics by *Bacillus subtilis* and some of its metabolites show antifungal or antibacterial activity against a number of phytopathogenic microorganisms (Chung *et al.*, 2008). The utilization of plant own's defense mechanism is a fascinating area of research which can be systemically activated upon exposure of plants to PGPR strains or infection by pathogen (Baker *et al.*, 1997). This phenomenon is called induced systemic resistance (ISR) (Tuzun and Kuc, 1991). The antagonists which differed in their ecology could be combined so that they could effectively utilise the root exudates and survive in association (Baker and Cook, 1974). The use of more than one bacterial biocontrol agent could be more effective than the use of a single one (Raupach and Kloepper, 1998) and each biocontrol agent may use a different mechanism to fight the pathogen, thus better results would be achieved than with a single one (de Boer *et al.*, 1999); might broaden the spectrum of biocontrol activity, enhance the efficacy and reliability of control (Duffy and Weller, 1995) and allow the combination of various mechanisms without the need for genetic engineering (Janisiewicz, 1988). The combination of LS213 [product formed by *Bacillus subtilis* strain GB03 (a growth-promoting agent), *B. amyloliquefaciens* strain IN937a (an inducer of systemic resistance) and chitosan] and *P. fluorescens* CECT 5398 increased all parameters (caliper, fresh weight and height) achieving the highest values in tomato and pepper (Domenech *et al.*, 2006). Incorporation of composted cow manure (5%, vol/vol) into the light peat mix significantly decreased Botrytis Blight of Begonia severity while shoot dry weight and salability were increased (Horst *et al.*, 2005). Though the efficacies of the biocontrol agents Pf1 and *T. viride* has been studied individually (Karunanithi *et al.*, 2004), their combined effect and their molecular mechanisms on the management of BSR of coconut have not been studied.

Hence, the present study was undertaken at the Department of Plant Pathology, Centre for Plant Protection Studies, Tamil Nadu Agricultural University, Coimbatore, India to

investigate the effect of three bioagents viz., *Bacillus*, Pf1 and *T. viride* in combination for the management of basal stem rot disease.

Coconut cultivar “East coast tall” susceptible to *Ganoderma lucidum* was used in all experiments for evaluating the responses of bioagents treated coconut saplings to infection with *Ganoderma lucidum*. The saplings were obtained from Coconut Research Station, Aliyar Nagar, TNAU, Tamil Nadu, India. The virulent *Ganoderma* isolate CRS-1 was used throughout the study.

Coconut root samples were collected and brought to the laboratory. Root sections (2-3 cm long) were made using a sterile scalpel. Root samples were surface sterilized with 1% sodium hypochlorite (NaOCl) in 0.05% triton X-100 for 10 min. and rinsed four times in 0.02 M sterile potassium phosphate buffer pH 7.0 (PB). A 0.1 ml aliquot was taken from the final buffer wash and transferred to 9.9 ml tryptic soy broth (TSB) to serve as sterility check. Samples were discarded if growth was detected in the sterility check samples (agitating samples in TSB, Hi Media Code No. M 011, at 28±2°C) within 48 h. Each sample (0.5g) was triturated with a sterile mortar and pestle in 9.5 ml of the final buffer wash. Serial dilutions up to (1010) of the triturate were made in phosphate buffer. Each dilution of every sample was plated (0.1 ml) on three plates each of three different media; Tryptic soy agar (TSA- Hi Media, Code No. M290). Nutrient agar (NA g/l; peptone 5, beef extract 2 and agar 20, pH 5.0) and King’s B Medium (g/l; proteose peptone 20, K₂HPO₄ 1.5, Mg SO₄. 7H₂O 1.5, glycerol 20 ml and agar 15, pH 7.2) (King *et al.*, 1954). The plates were incubated at 28±2°C for 48-72 h. At each sampling date and for each treatment, one representative of each bacterium, as evident from their colony type and morphology was transferred to fresh King’s B Medium plates to establish pure cultures.

For extraction of antifungal metabolites, the strains grown in potato dextrose broth were centrifuged at 8000g at room temperature. The supernatant was extracted twice with ethyl acetate after adjusting the pH to 2.0 with 6 M HCl. The ethyl acetate fractions were pooled and evaporated on a rotary evaporator at 60°C. The dried fractions were dissolved in minimum quantity of methanol. The metabolites in the methanol fraction were separated by preparative TLC, using chloroform:methanol:ethanol:water (70:30:35:5) solvent system as mobile phase. The spots on the plates were developed with iodine vapour and Rf values were recorded by using following formula.

$$\text{Rf value} = \frac{\text{Distance moved by the solute from the origin}}{\text{Distance moved by the solvent from the origin}}$$

The surfactin sfp 675 bp fragment, corresponding to the *B. subtilis* sfp gene (GenBank accession no. X63158) at positions 167–841, was PCR- amplified using two oligonucleotide primers of sfp-f (5’-ATG AAG ATT TAC GGA ATTTA-3’) and sfp-r (5’-TTA TAA AAG CTC TTC GTA CG-3’). Amplification was accomplished with a DNA Thermal Cycler using the program set for denaturing at 94°C for 1 min, annealing at 46°C for 30 s and extension at 72°C for 1 min, for a total of 25 cycles. Following amplification, the PCR reaction mix was analyzed by 2% agarose gel electrophoresis.

A 0.8-kb fragment of the 5’ region of ituD, which contained up to the ribosome binding site but not the promoter, was obtained by PCR. PCR amplification with primers

ITUP4-F (5'-CCCCTGTTCTAGATGATCGGAGGAATCTC-3') and ITUP5-R (5'-TGCATCGATTCTGTCCATCTAACCGGCATC-3') was performed using the thermal cycler program 96°C for 5 min; 30 cycles of 96°C for 30 s, 60°C for 30 s, and 72°C for 150 s (Tsuge *et al.*, 2001).

The endophytic bacteria were grown on kings broth and nutrient agar with constant shaking at 150 rpm for 48 h at room temperature ($28\pm 2^\circ\text{C}$). The bacterial cells were harvested by centrifugation at 10000 rpm for 15 min, and bacterial cells were resuspended in phosphate buffer (0.01 M, pH 7.0). The concentration was adjusted using a spectrophotometer to approximately 108 cfu/ml ($\text{OD}_{595}=0.3$) and used as bacterial inoculum (Thompson, 1996).

Rice seeds (cv. ADT 46) were surface sterilized with two per cent sodium hypochlorite for 30 sec, rinsed in sterile distilled water and dried overnight under sterile air stream. Endophytic bacterial strains inoculated into respective broth were taken in a conical flask. Required quantity of seeds were soaked in bacterial suspension containing 3×10^8 for 2 h and dried under shade.

Plant growth – promoting activity of bacterial endophytic strains were assessed based on the seedling vigour index by the standard roll towel method (ISTA, 1993). Rice seed bacterization was done as described earlier. Twenty seeds were kept over the presoaked germination paper. The seeds were held in position by placing another presoaked germination paper strip and gently pressed. The polythene sheet along with seeds were then rolled and incubated in growth chamber for 14 days. Three replications were maintained for each treatment. The root and shoot length of individual seedlings were measured and the germination percentage of seeds was also calculated. Plant growth promotion also tested in pot culture method. Bacterized seeds were sown in pots. Twenty seeds were maintained for each treatment. The root and shoot length of individual seedlings were measured and the germination percentage of seeds was also calculated. The vigour index was calculated by using the formula as described by Baki and Anderson (1973). Vigour index = Per cent Germination x seedling length (shoot length + root length).

Bacterial endophytic strains were tested for their inhibition on mycelial growth of *Ganoderma* by following the dual culture technique (Dennis and Webster, 1971). The bacterial culture was streaked at one side of Petridish (1 cm from the edge of the plate) plated with PDA medium and mycelial disc (8 mm diameter) of seven days old culture of *Ganoderma* was placed on the opposite side in the Petridish perpendicular to the bacterial streak. The plates were incubated at room temperature ($28\pm 2^\circ\text{C}$) for four days and the mycelial inhibition of pathogen was measured in millimeter. Biochemical tests were carried out to identify the isolated endophytic bacterial strains. EPC 5 and EPC 8 strains were further confirmed by cloning and sequencing.

A loopful of bacterium was inoculated into the King's broth and incubated in a rotary shaker at 150 rpm for 48 h at room temperature ($28\pm 2^\circ\text{C}$). After 48 h of incubation, the broth containing 9×10^8 cfu/ml was used for the preparation of talc-based formulation. To the 400 ml of bacterial suspension, 1 kg of the purified talc powder (sterilized at 105°C for 12 h), calcium carbonate 15 g (to adjust the pH to neutral) and carboxymethyl cellulose (CMC) 10 g (adhesive) were mixed under sterile conditions, following the method described by Vidhyasekaran and Muthamilan (1995). After shade drying for overnight, it was packed in

polypropylene bag and sealed. At the time of application, the population of bacteria in talc formulation was 2.5 to 3×10^8 cfu/g. The bacterial strains, *Pseudomonas*, *Bacillus*, and fungal strain *T. viride* were grown separately in respective broth. Talc-based formulation prepared for each bioagents and mixed at the time of application.

The organic substrate dried cow dung was tested for the multiplication of *Pseudomonas*, *Bacillus* and *T. viride*. One hundred gram of substrate was weighed in polypropylene bag, moisture content was adjusted to 60% (W/V) by gravimetric method (Dutta and Das, 1999). One set of treatments of the above substrate were sterilized and another set is kept without sterilization. For sterilization the substrates were autoclaved at 15lb psi for 1h on two consecutive days. The substrate was inoculated with 5g of talc based bioformulation of *Pseudomonas*, *Bacillus*, *T. viride*. The substrate was incubated at room temperature. One gram of sample from FYM was derived at regular intervals, and the population (CFU) of *Pseudomonas*, *Bacillus* and *T. viride* in substrate and talc were counted at 10, 20, 30, 40, 50, 60 days intervals after inoculation by serial dilution techniques in selective medium for each bioagents.

The bacterial strains either singly or as mixture were assessed for their efficacy in controlling *Ganoderma* infection under greenhouse conditions. A pot culture experiment was undertaken with the following treatments in completely randomized design (CRD) with two replications.

Sorghum grains (250 g) were put into a polypropylene bag (12 x 8 inch) and sterilized at 121°C for 15 min. The pure culture of *Ganoderma* isolate CRS-1 (Host: *Cocos nucifera*) was inoculated at the rate of three 8 mm mycelial disc separately on sorghum grains and allowed to mass multiply at 30°C under dark room conditions for 12 days. At the time of planting the coconut saplings, *Ganoderma* multiplied in the sorghum grains was added to individual pots.

Bioagents were mixed with fully decomposed farm yard manure and incubated for 15 days and then applied individually and in combination to pots at the interval of one month. Coconut saplings injected with 2ml Tridemorph diluted with 100 ml water served as chemical check, wherever needed foliar spray with distilled water was carried out in the control treatment. Control plants without bioagent treatment were also maintained. All the treatments were replicated two times in CRD.

One gram of root sample was extracted in 0.1M sodium phosphate buffer (pH 7.0) was used for the estimation of peroxidase (PO) (Hammerschmidt *et al.*, 1982), polyphenol oxidase (PPO) (Mayer *et al.*, 1965) and phenylalanine ammonia-lyase (PAL) (Ross and Sederoff, 1992). Enzyme extract was stored in at -70 C until used for biochemical analysis. Protein content in the extracts was determined by the method of Bradford (1976).

The field experiments were carried out in two different BSR endemic locations of Tamil Nadu with 17 treatments. The treatments were replicated twice at three palms per replication to test the efficacy of endophytic consortia bioformulations against basal stem rot disease. A randomized block design was used in the experiments. Production practices were followed as recommended by Tamil Nadu Agricultural University, Coimbatore, India. Disease index for each tree was calculated initially. In the field trials, all the bioagent treatments

except chemical treatment were mixed with FYM, heaped for 15 days with regular watering, proper turning and applied 5kg to individual coconut tree. The bioagents applied at three months interval (four doses per year). The BSR disease intensity was assessed as disease index with the formula $\text{Disease index} = 23.6 + 17.7h + 3.6r - 0.6l$; Where, h – Height of bleeding patches from ground level in metres, l – Number of functional leaves, r – Reduction in leaf size based on 0 – 4 scale. The initial disease index was recorded before imposing the treatments. Then the disease index was recorded periodically after imposing the treatments. Regular observations were made on the bleeding height and number of functional leaves.

The data were statistically analyzed (Rangasamy, 1995) using the IRRISTAT version 92 developed by the International Rice Research Institute Biometrics unit, the Philippines (Gomez and Gomez, 1984). The percentage values of the disease index were arcsine transformed. Data were subjected to analysis of variance (ANOVA) and means were compared by Duncan's Multiple Range Test (DMRT).

Totally fifty five isolates of endophytic bacteria were isolated from healthy coconut roots of Tamil Nadu. Coconut being a perennial crop, growth promotion by endophytic bacteria was tested on rice crop. The growth promotion by bacterial endophytes isolates was assessed by treating the rice seeds with all the fifty five bacterial isolates separately by their suspension. Endophytic bacterial isolates EPC5, EPC8, EPC12, EPC13, EPC15, EPC21, EPC29, EPC32, EPC52 and *Pseudomonas fluorescens* strain Pf1 were found to increase the vigour index of rice seedlings significantly when compared to untreated control. No external symptoms appeared after endophytic bacteria treatment (Table 1).

The isolates promoting high growth were further tested for their efficacy by dual plate technique against *G. lucidum* along with Pf1. Among the ten isolates, five strains were found to inhibit the growth of *G. lucidum* *in vitro*. The strain, Pf1 showed high inhibition to *G. lucidum* followed by EPC5 (coconut root isolate) and EPC8 (coconut root isolate). The per cent inhibition was significantly higher in plates streaked with Pf1 (40.67), EPC5 (33.80) and EPC8 (29.16) against control plates (Table 2). The bioagents EPC5, Pf1 and *T. viride* were tested in combination against *G. lucidum* *in vitro*. These three bioagents effectively inhibited the growth of pathogen even upto one month whereas in control, *G. lucidum* covered the plate within 6 days after placing the disc. Biochemical tests showed EPC 5, EPC 8 were belong to *Bacillus* genera. Cloning and sequencing of 16s rDNA of EPC 5 showed that it belongs to *Bacillus subtilis* (Accession No. EF139862).

The extra-cellular diffusible antibiotic was extracted from EPC5, EPC8 and standard check Pf1 and characterized partially by thin layer chromatography. The results showed many spots throughout the chromatogram after iodine vaporization. The extracellular, methanol soluble, ninhydrin-negative spot showed a Rf value of 0.49 (Fig. 1).

1. COMB 1; 2. COMB 3; 3. COMB 7; 4. EPC 5; 5. EPC 8; 6. EPCO16; 7. Pf 1;
8. COPP 17; 9. COGP 35; 10. *Trichoderma viride* 1

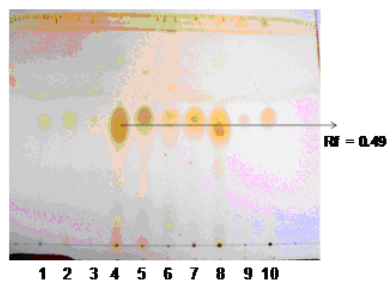


Fig 1. Identification of antibiotic compound using thin layer chromatography

PCR amplification of Surfactin antibiotic showed 675bp amplification in EPC5, EPC8 and COKPB 22 isolates compared to COGB29, COMB1, COMB3, COMB7 and negative in EPCO 16 (Fig 2).

PCR amplification of Iturin D antibiotic showed 800bp amplification in COGB29, COMB1, COMB3 and EPCO 16

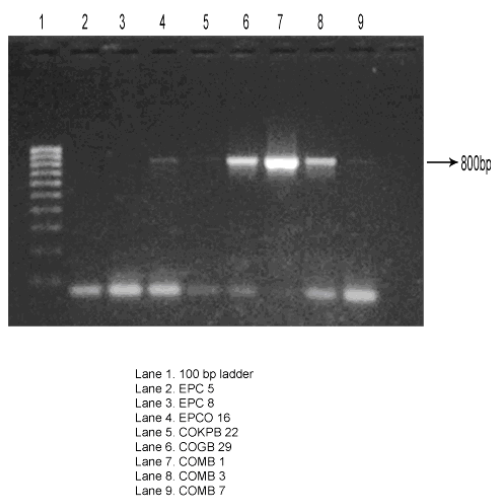


Fig 3. PCR amplification of iturin D antibiotic

separately and in combination and heaped for different days. The population of bioagents was assessed at different days interval *viz.*, 10, 20, 30, 40, 50 and 60 days after mixing with FYM. The results showed that twenty days incubation was optimum for the maximum population buildup of *Bacillus*, *T. viride* and *Pseudomonas* in both sterilized and unsterilized FYM (Fig. 4).

Coconut seedlings treated with endophytic bacteria in different combinations promoted seedling growth under greenhouse conditions. Endophytic *Bacillus* combined with *Pseudomonas* and *T. viride* @ 30g (10g each) per seedling promoted the growth of seedling (height of seedling), girth and number of leaves compared to 15g of individual, combined application and other treatments in ECT coconut seedlings. The bioagents mixture EPC5+Pf1+Tv @ 30g recorded seedling height of 156.5cm, girth of 20cm and seven leaves when compared to the height (123.5cm), girth (11.8cm) and five leaves of inoculated control seedling (Table 3).

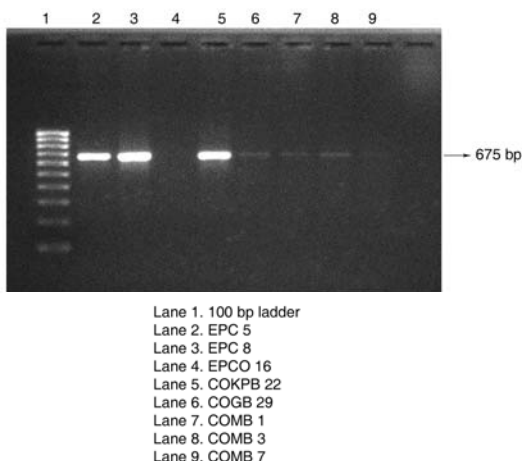


Fig 2. PCR amplification of surfactin antibiotic

followed by COMB7 and negative in EPC5, EPC8 and COKPB 22 isolates (Fig. 3).

To test the population buildup of bioagents *in vitro*, the effective bioagent formulations were mixed with sterilized and unsterilized FYM

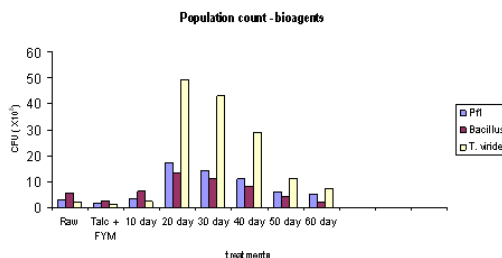


Fig 4. Population load of bioagents in FYM under *invitro* conditions

The assay of defense enzymes revealed that PGPR mixture bioformulations induced a greater amount of enzymes than individual and the control plants. Significant increase in PO activity was observed in EPC5+Pf1+Tv treated coconut seedlings challenged with *G. lucidum* compared to the untreated seedlings. This treatment recorded higher levels of PO activity upto five days of inoculation when inoculated with basal stem rot pathogen and declined thereafter throughout the experimental period of nine days. However, PO activity increased in all the bioagents treated seedlings challenged with *G. lucidum* whereas in healthy control plants, lesser activity was observed (Fig. 5). Combined application of bioagents bioformulation led to the enhanced activity of PPO compared to individual application. EPC5+Pf1+Tv recorded higher PPO activity upto fifth day after challenge inoculation followed by EPC5+Tv compared to other treatments. Inoculated control seedlings recorded less induction of PPO throughout the experimental period of nine days and showed steep decline fifth day after challenge inoculation (Fig. 6). The results showed that samples from coconut seedlings treated with different combinations of bioagents challenged with *G. lucidum* recorded higher induction of PAL activity than the individual treatments. PAL activity was significantly higher in bioagents treated coconut seedlings challenged with *G. lucidum* than the untreated inoculated seedlings. The enzyme activity reached the maximum on third day of challenge inoculation and thereafter it declined. In control seedlings, the activity slowly increased and started declining after third day (Fig. 7).

Soil application of these treatments around the basin of coconut palm and fungicide application by root feeding resulted in arresting the *G. lucidum* pathogen in both the field trials. The initial disease index was calculated for all the trees before imposing the treatments. After the four doses of applications, results showed that EBC5+Pf1+Tv @ 300g (100g each) per palm + 5kg FYM effectively arrested the bleeding symptom in coconut palms compared to the application of bioagents individually and at lower doses in both the field trials. Results of the field experiments from trial I revealed that the palms treated with the mixture of

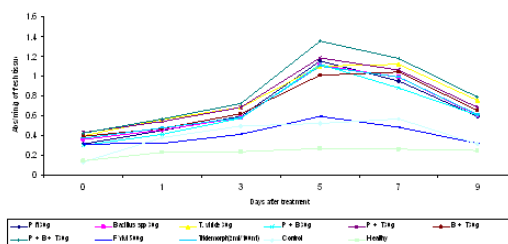


Fig 5. Induction of peroxidase in coconut seedlings treated with bioagents against *G. lucidum*

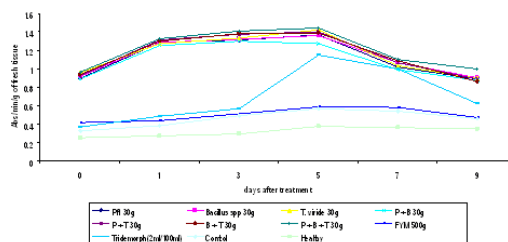


Fig 6. Induction of polyphenol oxidase in coconut seedlings treated with bioagents against *G. lucidum*

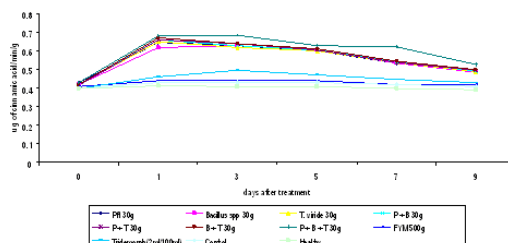


Fig 7. Induction of phenylalanine ammonia lyase oxidase in coconut seedlings treated with bioagents against *G. lucidum*

bioformulation EPC5+Pf1+Tv @ 300g (100g each) per palm + 5kg FYM as soil application recorded the disease index of 12.02 after final dose application as against the disease index of 23.32 in untreated control palms respectively. Similar trend of results were obtained from field trial II, EPC5+Pf1+Tv @ 300g/palm + 5 kg FYM treatment recorded the basal stem rot disease index of 8.87 as against 25.86 after final dose application, respectively. The standard check, root feeding of Tridemorph 2% @ 100 ml/palm recorded the basal stem rot disease index of 11.18 after final application, respectively (Table 4). Thus, the combination of different mixture of bioagents was found to be effective in reducing *G. lucidum* than the individual bioagents.

Coconut palms were affected by many numbers of pests and diseases. Among them, basal stem rot (BSR) disease caused by *Ganoderma lucidum* is the most destructive one which is widespread in nature. The term endophyte refers to microorganism that colonise intercellular spaces and vascular tissues of plants without exhibiting pathogenicity are believed to originate from the rhizosphere or phylloplane microflora (Hallmann *et al.*, 1997). They are found in numerous plant species (Chanway, 1998) with the most being members of common soil bacterial genera such as *Pseudomonas*, *Bacillus* and *Azospirillum* (Chanway, 1996). Colonization of plant roots with certain beneficial microbes causes the induction of a unique physiological and biochemical state in plants called “priming.” Primed plants display either faster, stronger or both activation of the various cellular defense responses that are induced following attack by either pathogens or insects or in response to abiotic stress (Conrath *et al.*, 2006). In the present study, endophytic bacteria from the roots of coconut palms EPC5, EPC8, EPC15, EPC29 and EPC52 were found to increase the vigour index of rice seedlings significantly when compared to untreated control. Hallman *et al.* (1997) reported that most of the endophytic bacterial strains are capable of promoting plant growth. *Bacillus* species are among the most common bacteria found to colonize plants endophytically (Mahaffee and Kloepper, 1997) and play an important role in the biocontrol of vascular plant pathogens. In our study, most of the isolates were characterized as *Bacillus* spp. Whereas, some *Pseudomonas* spp. has also been identified. Several reports indicate the role of bacterial endophytes in the management of plant pathogens. (Barka *et al.*, 2002). In the present study, EPC5, Pf1 effectively inhibited the growth of *Ganoderma lucidum* in vitro. Thus an attempt was carried out in the present study to investigate the efficacy of some of the promising endophytic bacteria against basal stem rot pathogen. Thin layer chromatography of the crude extracts of strains showed many spots throughout the chromatogram after iodine vaporization. The spot at Rf 0.49 that were present in all samples indicating the possible role of these spots in antagonism. The genus *Bacillus* is known to produce lipopeptide antibiotics that are smaller than most proteins. *Bacillus subtilis* strains often produce a variety of antimicrobial cyclic lipopeptides, including iturins, fengycins, and surfactins (Dana and Peypoux, 1994). Amplification of 675 bp with respect to surfactin antibiotic is positive in EPC5 and EPC8 isolates whereas iturin D 800 bp amplification is negative in this isolates. This confirmed that the EPC5 and EPC8 antagonistic property is due to surfactin.

The endophytic bacteria have been reported to improve plant growth (Nejad and Johnson, 2000) and suppress the *Fusarium* spp. (Chen *et al.*, 1995), *Colletotrichum orbiculare* (Kloepper *et al.*, 1992b), *Rhizoctonia solani*, *Sclerotium rolfsii* (Pleban *et al.*, 1995). In the present study, Pf1 and endophytic bacterial strain EPC5 showed maximum mycelial inhibition of *G.*

lucidum. Antagonistic strain of *Pseudomonas putida* (B0) isolated from a sub-alpine exhibited antifungal activity against phytopathogenic fungi in Petri dish assays and produced chitinase, β -1,3-glucanase, salicylic acid, siderophore and hydrogen cyanide (Pandey *et al.*, 2006). The inhibitory action may be due to the production of antifungal or antibacterial agents (Maurhofer *et al.*, 1998), antibiotics (Cazorla *et al.*, 2006; Monteiro *et al.*, 2005; Ramamoorthy and Samiyappan, 2001), siderophore production (Kloepper *et al.*, 1980; Duijff *et al.*, 1999), Competition for nutrition (Sivakumar and Subramaniam, 1999). Seven strains of *Bacillus* isolates showed a high level of in vitro antagonism against *R. solani* and *Macrophomina phaseolina* (Ashour and Afify, 1999). The above facts suggest that the inhibition of basal stem rot pathogen, *G. lucidum* by endophytic bacteria may be due to the production of antibiotics, siderophore mediated competition and lytic enzymes viz., chitinase, β -1,3 glucanase. These antibiotics and lytic enzymes might have degraded the fungal cell wall and restricted the growth of fungus in vitro. Various phenotypic and molecular methods have been developed and used for characterizing bacterial isolates. The result obtained by analyzing primary character and carbon source utilization of different endophytic bacteria revealed that they belong to *Bacillus* spp. Tilak and Srinivasa Reddy (2006) identified isolates from maize as *Bacillus cereus* and *B. circulans* by biochemical characteristics and profile of fatty acids. *Trichoderma* strains are among the most studied fungal biocontrol agents and are successfully used as biopesticides and biofertilizers in greenhouse and field plant production (Harman *et al.*, 2004). The soil borne filamentous fungus *Trichoderma virens* is a biocontrol agent with a well known ability to produce antibiotics, parasitize pathogenic fungi and induced systemic resistance in plants. Pretreatment of cotton cotyledons with *Trichoderma* spp. provided high levels of protection to the foliar pathogen, *Colletotrichum* sp. and involved in the induction of resistance through the activation of plant defense mechanisms (Djonovic *et al.*, 2006). *Trichoderma* spp. are known to produce large quantities of fungistatic metabolites (Bruckner *et al.*, 1990) which are active against many soil borne plant pathogens. In our study, *T. viride* strongly inhibited the growth of *G. lucidum* in vitro. It was observed that soil application of bioagents with different combinations promoted the growth in coconut seedlings significantly compared to untreated control under greenhouse conditions. There was a significant increase in the seedling height, girth, leaf area and number of leaves in the bioagents treated seedling. It was supported by De Silva *et al.* (2000) the leaf area and stem diameter of high bush blueberry were increased by applying *Pseudomonas fluorescens* Pf5. Esitken *et al.* (2003) found that application of *Bacillus* strain OSU 142 as foliar spraying at full bloom stage significantly increased the shoot length, yield and the nutrient element composition in apricot trees compared to control. In the present study, coconut seedlings treated with the bioformulation containing mixture of bioagents and challenged with the basal stem rot pathogen showed higher induction of peroxidase. Induction of eight isozymes viz., PO1, PO2, PO3 PO4, PO5, PO6, PO7 and PO8 was observed in combined application of bioagents *Bacillus*+Pf1+Tv. Recently, several peroxidase genes were cloned and studied in cotton plants during compatible and incompatible interactions with *X. campestris* pv. *malvacearum* (Delannoy *et al.*, 2003). The activity was higher in plants treated with mixture of bioagent bioformulation challenged with the *G. lucidum*. Specific isoforms of PPO were induced in seedlings treated with the combined application of antagonistic formulation after challenge inoculation with pathogen and their expression was prominent when compared to untreated control seedling. The maximum accumulation of PAL upto 5 days of challenge inoculation of pathogen coincides with the time which is normally favourable for the pathogen.

The increased activity of PAL may also be constituted for enhancing the resistance in coconut seedlings against basal stem rot disease. In the present study, increased activity of PAL was recorded in seedlings treated with mixture of bioagents bioformulation EPC5+Pf1+Tv and challenge inoculated with *G. lucidum*. The timing and expression patterns of the defense mechanisms are important for the suppression of pathogen. Higher level expression of defense related proteins and timely accumulation of chemicals at the infection site certainly prevents the entry of pathogen in coconut seedlings treated with mixture of bioagent bioformulations. In our study, endophytic *Bacillus* along with *Pseudomonas fluorescens* strain, Pf1 and *Trichoderma viride* showed enhanced growth, induction of defense enzymes in response to basal stem rot pathogen in coconut seedling than the individual antagonist. Similarly, Raupach and Kloepper (1998) demonstrated that the use of more than one biocontrol agent could be more effective than use of single one. The results revealed that the soil application of talc formulation of *Bacillus* + Pf1 + Tv @ 100g each/palm along with 5kg FYM recorded significantly lower disease index over control after three doses of application. Similarly, a glasshouse and field trial for *Ganoderma* control indicated that treatment with *T. harzianum* and *Gliocladium viride* were superior to *Bacillus* sp. A large scale trial showed that the disease incidence was lower in oilpalm field treated with biological agents than in untreated fields (Susanto *et al.*, 2005). In the present study, soil application of bioagents mixture resulted in enhanced nut yield of coconut in both the field trials. Field studies over a period of two years indicated that stem rot disease in canola was controlled by *Pseudomonas chlororaphis* and *Bacillus amyloliquefaciens* which were comparable with fungicide treatments Fernando *et al.*, 2006).

Exploitation of some biochemical and molecular tools in this study helped to identify and develop the biologically, metabolically and ecologically sustainable competent *Bacillus* strains out of fifty five isolates of endophytes and rhizobacterial strains isolated from different ecosystems of Tamil Nadu. The strains viz., EPC5, Pf1 and Tv individually and in combination performed well against basal stem rot disease in coconut palms. There is a strong need for the comprehensive understanding of the host plant physiology and metabolism mainly in relation to JA, SA and ethylene mediated pathways by bioagents, pathogens and pests and their interactions.

Acknowledgement

Financial support for this work was provided by Department of Science and Technology, New Delhi.

References

- Ashour, A.Z.A. and Afify, A.H. 1999. Use of *Bacillus* strains mixture to improve biological control of cotton seedling disease. *African Journal of Mycology and Biotechnology* 7 (3): 109-120.
- Baker, K. F. and Cook, R. J. 1974. Biological control of plant pathogens, W. H. Freeman and Co., San Francisco. p. 433.
- Baki, A.A.A. and Anderson, J.D. 1973. Vigour determination in soybean seed by multiple criteria. *Crop Sci.* 31: 630-633.
- Barka, E. A., Gognies, S., Nowak, J., Audran, J. and Belarbi, A. 2002. Inhibitory effect of endophyte bacteria on *Botrytis cinerea* and its influence to promote the grapevine growth. *Biol. Control.* 24: 135-142.

- Belid El-Moshaty, F.I.B., Pike, S.M., Novacky, A.J. and Seghal, O.P. 1993. Lipid peroxidation and superoxide production in cowpea (*Vigna unguiculata*) leaves infected with tobacco ring spot virus or southern bean mosaic virus. *Physiol. Mol. Plant Pathol.* 43: 109-119.
- Berger, L.R. and Reynolds, D.M. 1958. The chitinase system of a strain of *Streptomyces griseus*. *Biochem. Biophys. Acta.*, 29: 522-534.
- Bhaskaran, R. 1990. Biological control of Thanjavur wilt disease of coconut. National Symposium on biocontrol of root disease, Annamalai Univ., Annamalainagar (Abstr.) pp. 7-8.
- Bhaskaran, R., Rajamannar, M and Kumar, S.N.S. 1996. Basal stem rot disease of coconut. Technical Bulletin No. 30, Central Plantation Crops Research Institute, Kasaragod, pp. 15.
- Bhaskaran, R., Rethinam, P. and Nambiar, K. K. N. 1989. Thanjavur wilt of coconut. *J. Plant. Crops* 17: 69-79.
- Bruckner, H., Reinecke, C., Kripp, T. And Kieb, M. 1990. Screening isolation and sequence determination of a unique group of polypeptide antibiotics from filamentous fungi. Proc. Int. Mycol. Cong. Regensburg, Federation Republic Germany. 224p.
- Cazorla, M.F., Duckett, S.B., Bergstrom, E.T., Noreen, S., Odijk, R., Lugtenberg, B.J.J., Thomas-Oates, J.E. and Bloemberg, G.V. 2006. Biocontrol of avocado *Dematophora* root rot by antagonistic *Pseudomonas fluorescens* PCL 1606 correlates with the production of 2-Hexyl 5-Propyl Resorcinol. *Mol. Plant-Microbe Interact.* 19(4): 418-428.
- Chanway, C. P. 1996. Endophytes: they're not just fungi? *Canadian J. Bot.* 74: 321-322.
- Chanway, C. P. 1998. Bacterial endophytes: ecological and practical implications. *Sydowia* 50: 149-170.
- Chaparro-Giraldo, A., Barata, R.M., Chabregas, S.M., Azevedo, R.A. and Silva-Filho, M.C. 2000. Soybean leghemoglobin targeted to potato chloroplasts influences growth and development of transgenic plants. *Plant Cell Rep.* 19: 961-965.
- Chen, C., Bauske, E. M., Musson, G., Rodriguez-Kabana, R., and Kloepper, J. W. 1995. Biological control of *Fusarium* wilt on cotton by use of endophytic bacteria. *Biol. Control* 5: 83 -91.
- Cho, S. J., Lim, W. J. Hong, S. Y., Park., S. R and Yun., H. D. 2003. Endophytic colonization of balloon flower by antifungal strain *Bacillus* sp. CY22. *Biosci. Biotechnol. Biochem.* 67(10): 2132-2138.
- Chung, S., Kong, H., Buyer, J.S., Lakshman, D.K., Lydon, J., Kim, S.D and Roberts, D.P. 2008. Isolation and partial characterization of *Bacillus subtilis* ME488 for suppression of soilborne pathogens of cucumber and pepper. *Appl Microbiol Biotechnol* 80(1):115-123.
- Conrath, U., Beckers, G.J.M., Flors, V., García-Agustín, P., Jakab, G., Mauch, F., Newman, M., Pieterse, C.M.J., Poinssot, B., Pozo, M.J., Pugin, A., Schaffrath, U., Ton, J., Wendehenne, D., Zimmerli, L. and Mauch-Mani, B. 2006. Priming: Getting Ready for Battle. *Molecular Plant-Microbe Interaction* 19(10): 1062-1071.
- Dana, R and Peypoux, F. 1994. Iturins, a special class of poreforming lipopeptides: biological and physicochemical properties. *Toxicology* 87:151-174.

- de Boer, M., Van der Sluis, I., van Loon, L.C. and Bakker, P.A.H.M. 1999. Combining fluorescent *Pseudomonas* spp. strains to enhance suppression of fusarium wilt of radish. *Eur. J. Plant Pathol.* 105: 201-219.
- De Silva, A., Petterson, K., Rothrock, C. and Moore, J. 2000. Growth promotion of highbush blueberry by fungal and bacterial inoculants. *Hort. Science* 35: 1228-1230.
- Delannoy, E., Jalloul, A., Assigbetse, K., Marmey, P., Geiger, J.P., Lherminier, J., Daniel, J.F., Martinez, C. and Nicole, M. 2003. Activity of class III peroxidases in the defense of cotton to bacterial blight. *Mol. Plant-Microbe Interact.* 16:1030-1038.
- Dennis, C. and Webster, J. 1971. Antagonistic properties of species groups of *Trichoderma* 1. Production of non-volatile antibiotics. *Trans. Br. Mycol. Soc.* 57: 25-39.
- Dickerson, D. P., Pascholati, S. F., Hagerman, A. E., Butler, L. G. and Nicholson, R. L. 1984. Phenylalanine ammonia-lyase and hydroxy cinnamate CoA ligase in maize mesocotyls inoculated with *Helminthosporium maydis* or *Helminthosporium carbonum*. *Physiol. Plant Pathol.* 25: 111-123.
- Dignum, M. J. W., Kerler, J. and Verpoorte, R. 2001. α - Glucosidase and Peroxidase Stability in Crude Enzyme Extracts from green beans of *Vanilla planifolia* Andrews. *Phytochem. Anal.* 12: 174-179.
- Djonovic, S., Pozo, M.J., Dangott, J.L., Howell, C.R. and Kenerley, C.M. 2006. Sm1, a proteinaceous elicitor secreted by the biocontrol fungus *Trichoderma virens* induces plant defense responses and systemic resistance. *Molecular Plant-Microbe Interactions* 19(8): 838-853.
- Domenech, J., Reddy, M. S., Kloepper, J. W., Ramos, B. and Gutierrez-manero, J. 2006. Combined application of the biological product LS213 with *Bacillus*, *Pseudomonas* or *Chryseobacterium* for growth promotion and biological control of soil-borne diseases in pepper and tomato *Bio Control* 51: 245–258.
- Duffy, B. K., and Weller, D. M. 1995. Use of *Gaeumannomyces graminis* var. *graminis* alone and in combination with fluorescent *Pseudomonas* spp. to suppress take-all of wheat. *Plant Dis.* 79: 907-911.
- Duffy, B. K., Simon, A. and Weller, D. M. 1996. Combination of *Trichoderma koningii* with fluorescent pseudomonads for control of take-all on wheat. *Phytopathology* 86:188-194.
- Duijff, B.J., Recorbet, G., Bakker, P.A.H.M., Loper, J.E. and Lemanceau, P. 1999. Microbial antagonism at the root level is involved in suppression of fusarium wilt by the combination of nonpathogenic *Fusarium oxysporum* Fo47 and *Pseudomonas putida* WCS358. *Phytopathology* 89: 1073-1079.
- Dutta, P. and Das, B.C. 1999. Effect of seed pelleting and soil application of *Trichoderma harzianum* in the management of stem rot of soybean. *J. Mycol. Pl. Pathol.* 29 (3); 317-322
- Elad, Y., Chet, I. and Katan, J. 1980. *Trichoderma harzianum*: A biocontrol agent effective against *Sclerotium rolfsii* and *Rhizoctonia solani*. *Phytopathology* 70: 119-121.
- Esitken, A., Karlidag, H., Ercisli, S., Turan, M. and Shin, F. 2003. The effect of spraying a growth promoting bacterium on the yield, growth and nutrient element composition of leaves of apricot (*Prunus armeniaca* L. cv. Hacıhaliloglu). *Australian J. Agric. Res.* 54: 377-380.

- Fernando, W.G.D., Nakkeeran, S., Zhang, Y and Savchuk, S. 2006. Biological control of *Sclerotinia sclerotiorum* (Lib.) de Bary by *Pseudomonas* and *Bacillus* species on canola petals. *Crop protection* 26 (2007) 100–107
- Garrett, S.D. 1955. A century of root disease investigation. *Ann. Appl. Biol.* 42: 211-219.
- Giannopolitis, C.N. and Ries, S.K. 1977. Superoxide dismutase. *Plant Physiol.* 59: 309-314.
- Gomez, K.A. and Gomez, A.A. 1984. Statistical Procedure for Agricultural Research. John Wiley and Sons, New York.
- Hallmann, J., Quadt-Hallmann, A., Mahaffee, W.F. and Kloepper J.W. 1997. Bacterial endophytes in agricultural crops. *Can. J. Microbiol.* 43: 895-914.
- Harman, G. E., Howell, C. R., Viterbo, A., Chet, I., and Lorito, M. 2004. Trichoderma species—opportunistic, avirulent plant symbionts. *Nat. Rev. Microbiol.* 2: 43-56.
- Horst, L. E., Locke, J., Krause, C. R., McMahon, R. W., Madden, L. V., and Hoitink, H. A. J. 2005. Suppression of Botrytis blight of begonia by *Trichoderma hamatum* 382 in peat and compost - amended potting mixes. *Plant Dis.* 89: 1195-1200.
- ISTA. 1993. Proceedings of the international Seed Testing Association, International Rules for Seed Testing. *Seed Sci. Technol.* 21: 25-30.
- Janisiewicz, W. J. 1988. Biocontrol of postharvest diseases of apples with antagonist mixtures. *Phytopathology* 78:194-198.
- Karunanithi, K., Sarala, L., Rabindran, R., Kalaimani, T. and Manickam, G. 2004. Effect of biocontrol agents on the management of basal stem rot (BSR) of coconut. 26th Annual conference of ISMPP and National symposium on advances in fungal diversity and host pathogen interactions. Goa University, October 7 – 9, p. 53.
- Keel, C. and Defago, G. 1997. Interactions between beneficial soil bacteria and root pathogens: mechanisms and ecological impact. In: Multitrophic interactions in terrestrial system. A.C. Gange and V.K. Brown (eds.) Oxford: Blackwell science, 27-47.
- King, E.O., Ward, M.N. and Raney, D.E. 1954. Two simple media for the demonstration of pyocyanin and fluorescein. *J. Lab Clin. Med.* 44: 301-307.
- Kloepper, J.W., Leong, J., Teintze, M. and Schroth, M.N. 1980. Enhanced plant growth by siderophores produced by plant growth-promoting rhizobacteria. *Nature* 286: 885-886.
- Kloepper, J.W., Wei, G. and Tuzun, S. 1992b. Rhizosphere population dynamics and internal colonization of cucumber by plant growth-promoting rhizobacteria which induce systemic resistance to *Colletotrichum orbiculare*.. In Biological control of plant diseases. E.S. Tjamos.(eds.). Plenum Press, New York. pp. 185-191.
- Mahaffee, W. F. and Kloepper, J.W. 1997. Temporal changes in the bacterial communities of soil, rhizosphere and endorhiza associated with field-grown cucumber (*Cucumis sativus* L.). *Microbial.Ecol.* 34 : 210-223.
- Maurhofer, M., Reimann, C., Sacherer, S. P., Heeb, S., Haas, D. and Defago, G. 1998. Salicylic acid biosynthetic genes expressed in *Pseudomonas fluorescens* strain P3 improve the induction of systemic resistance in tobacco against tobacco necrosis virus. *Phytopathology* 88: 678-684.

- Mayer, A. M., Harel, E. and Shaul, R. B. 1965. Assay of catechol oxidase, a critical comparison of methods. *Phytochem.* 5: 783-789.
- Mguni, C. M. 1996. Bacterial black rot (*Xanthomonas campestris* pv. *campestris*) of vegetable Brassicas in Zimbabwe. Copenhagen, Denmark: The Royal Veterinary and Agricultural University/Danish Government Institute of Seed Pathology for Developing Countries, PhD thesis.
- Monteiro, L., Mariano, R.L and Souto-Maior, A.M. 2005. Antagonism of *Bacillus* spp. against *Xanthomonas campestris* pv. *campestris*. *Brazilian archives of biology and technology* 48: 23-29.
- Nejad, P. and Johnson, P.A. 2000. Endophytic bacteria induce growth promotion and will disease suppression in oilseed rape and tomato. *Biol. Control.* 18: 208-215.
- Pandey, A., Trivedi, P., Kumar, B and Palni, L.S. 2006. Characterization of a Phosphate Solubilizing and Antagonistic Strain of *Pseudomonas putida* (B0) Isolated from a Sub-Alpine Location in the Indian Central Himalaya. *Current Microbiology* 53: 102–107
- Pleban, S., Ingel, F. and Chet, I. 1995. Control of *Rhizoctonia solani* and *Sclerotium rolfsii* in the green house using endophytic *Bacillus* spp. *European J. Plant Pathol.* 101: 665-672.
- Ramamoorthy, V. and Samiyappan, R. 2001. Induction of defense-related genes in *Pseudomonas fluorescens* treated chilli plants in response to infection by *Colletotrichum capsici*. *J. Mycol. Plant Pathol.* 31: 146-155.
- Rangasamy, R. 1995. A Text Book of Agricultural Statistics. New Delhi, India: New Age International publisher Ltd. ISBN 81-224-0758-7.
- Raupach, G. S. and Kloepper, J. W. 1998. Mixtures of plant growth promoting rhizobacteria enhance biological control of multiple cucumber pathogens. *Phytopathology* 88: 1158-1164.
- Reissig, J.L., Strominger, J.L. and Leloir, L.F. 1955. A modified method for the estimation of N-acetyl amino sugars. *J. Biol. Chem.* 217: 959-966.
- Rethinam, P. 2004. Coconut - Making industry competitive. In: The Hindu Survey of Indian agriculture. pp.70-72.
- Sabaratnam, S. and Beattie, G.A. 2003. Differences between *Pseudomonas syringae* B728a and *Pantoea agglomerans* BRT98 in Epiphytic and Endophytic Colonization of Leaves. *Applied and Environmental Microbiology* 69(2): 1220-1228.
- Sivakumar, G. and Subramaniam, P. 1999. *In vitro* screening of *Pseudomonas* isolates against *Rhizoctonia solani* sp. *sasakii*-incitant of Bhendi leaf and sheath blight. Abstract presented in National symposium of Crop Pest and Diseases-Challenges for Next Millennium, November 27-29, 1999 at Society of PPS, IARI, New Delhi.
- Susanto, A., Sudharto, P.S. and Purba, R.Y. 2005. Enhancing biological control of basal stem rot disease (*Ganoderma boninense*) in oil palm plantations. *Mycopathologia* 159: 153–157.
- Thompson, D.C. 1996. Evaluation of bacterial antagonist for reduction of summer patch symptoms in Kentucky blue grass. *Plant Dis.* 80: 856-862.
- Tilak, K.V.B.R and Srinivasa Reddy, B. 2006. *Bacillus cereus* and *B. circulans* – novel inoculants for crops. *Curr. Sci.* 90(5): 642-644.

- Tsuge, K., Akiyama, T., Shoda, M. 2001. Cloning, sequencing, and characterization of the iturin A operon. *J Bacteriol* 183, 6265–6273.
- Vidhyasekaran, P. and Muthamilan, M. 1995. Development of formulations of *Pseudomonas fluorescens* for control of chickpea wilt. *Plant Dis.* 79: 782-786.
- Wulff, E. G. 2000. The use of antagonistic endophytic bacteria for controlling black rot of Brassica in Zimbabwe. Ph. D. Thesis. The Royal Veterinary and Agricultural University, Copenhagen, Denmark. 134p.

Table 1. Effect of bacterial endophytes on Rice seedling growth

Isolate	Vigour Index	
	Pot culture study	Roll towel method
EPC 1	1483.49 ^y	1221.08 ^v
EPC 2	1097.47 ^M	979.98
EPC 3	864.48 ^x	1016.48 ^Y
EPC 4	1043.49 ^Q	1451.48 ^P
EPC 5	1943.37^d	2053.08^s
EPC 6	1615.35 ^o	2021.78 ^w
EPC 7	1056.48 ^P	1146.08 ^x
EPC 8	1934.39^e	2182.88^m
EPC 9	1139.43 ^K	1416.98 ^R
EPC 10	1671.48 ^m	1431.68 ^Q
EPC 11	1487.38 ^x	1625.08 ^L
EPC 12	1862.48^f	3524.48^b
EPC 13	1826.33^g	3549.48^a
EPC 14	1455.30 ^z	2007.48 ^x
EPC 15	1711.47^j	2035.78^{uv}
EPC 16	1294.47 ^F	1602.48 ^M
EPC 17	1575.46 ^q	2093.78 ^P
EPC 18	1431.45 ^B	1803.48 ^F
EPC 19	1663.48 ⁿ	2056.88 ^r
EPC 20	1518.35 ^v	2035.08 ^v
EPC 21	1807.47^h	3028.88^d
EPC 22	1532.46 ^t	2004.98 ^y
EPC 23	1592.28 ^P	1924.28 ^z
EPC 24	1448.48 ^A	2006.38 ^{xy}
EPC 25	1061.48 ^O	1711.68 ^I
EPC 26	904.50 ^V	1002.68 ^Z
EPC 27	1224.34 ^H	1629.08 ^K
EPC 28	1336.45 ^D	2121.88 ⁿ
EPC 29	2006.28^b	3379.48^c
EPC 30	1322.45 ^E	1851.68 ^D
EPC 31	1357.48 ^C	1673.88 ^J

EPC 32	1943.43 ^d	2258.48 ⁱ
EPC 33	1695.46 ^l	2825.48 ^e
EPC 34	1490.48 ^w	2097.88 ^o
EPC 35	1103.46 ^L	1854.48 ^C
EPC 36	1574.46 ^q	2049.08 ^t
EPC 37	1175.40 ^J	2454.48 ^g
EPC 38	1013.47 ^R	1232.98 ^U
EPC 39	1061.32 ^O	1518.08 ^N
EPC 40	1217.48 ^I	1875.48 ^A
EPC 41	1280.40 ^G	1831.48 ^E
EPC 42	764.44 ^Y	979.48
EPC 43	1615.42 ^o	2252.18 ^j
EPC 44	1224.37 ^H	1756.48 ^H
EPC 45	989.46 ^S	1859.38 ^B
EPC 46	1140.41 ^K	1452.08 ^P
EPC 47	983.48 ^T	1797.08 ^G
EPC 48	1084.50 ^N	1473.48 ^O
EPC 49	889.50 ^W	1157.48 ^W
EPC 50	1525.39 ^u	2200.98 ^l
EPC 51	1703.34 ^k	2037.18 ^u
EPC 52	1979.48 ^c	2653.58 ^f
EPC 53	1539.42 ^s	2216.28 ^k
EPC 54	1759.50 ⁱ	2201.08 ^l
EPC 55	1560.49 ^r	2085.48 ^q
Pf1	2024.43^a	2368.28^h
Control 1	939.46 ^U	1403.48 ^S
Control 2	1013.37 ^R	1390.88 ^T

Values are mean of two replications

Data followed by the same letter in a column are not significantly different from each other according to Duncan's multiple range test at $P = 0.05$

Table 2. Antagonistic activity of bacterial endophytic isolates of Coconut against Ganoderma in vitro

Isolates	Per cent inhibition over control
<i>Pseudomonas fluorescens</i> (Pf-1)	40.67 (39.62) ^f
<i>Bacillus</i> (EPC5)	33.80 (35.54) ^e
<i>Bacillus</i> (EPC8)	29.16 (32.68) ^d
<i>Pseudomonas</i> (EPC15)	27.78 (31.80) ^d
<i>Pseudomonas</i> (EPC32)	4.75 (12.58) ^c
<i>Pseudomonas</i> (EPC52)	2.65 (9.37) ^b
Control	0.0 (0.0) ^a

Values are means of three replications, data followed by the same letter in a column are not significantly different from each other according to Duncan's multiple range test at $P = 0.05$

Values in parentheses are arcsine transformed

Table 3. Effect of biocontrol agents on coconut seedling growth parameters under greenhouse conditions

Treatments	First dose			Final dose		
	Height (cm)	Girth (cm)	No.of leaves	Height (cm)	Girth (cm)	No.of leaves
Pf1 15g	119	9.55	2	138 ^c	14.75 ^l	4.5 ^f
<i>Bacillus</i> spp 15g	108.25	10.35	3	118 ^p	15 ^j	5.5 ^d
<i>T. viride</i> 15g	98.25	10.3	2.5	128.5 ^k	14.9 ^k	5.5 ^d
P + B 15g	117	11.6	3.5	132 ⁱ	17.75 ^b	6.5 ^b
P + T 15g	106.5	9.15	3.5	139 ^d	15.25 ⁱ	5.5 ^d
B + T 15g	118.5	9.85	3	131.5 ^j	16.25 ^e	5.5 ^d
P + B + T 15g	129.5	11.05	3.5	154 ^b	17.75 ^b	6 ^c
Pf1 30g	129	10.25	3	135.5 ^f	15.5 ^h	5.5 ^d
<i>Bacillus</i> spp 30g	108	8.9	3	127.5 ^m	16 ^f	6 ^c
<i>T. viride</i> 30g	104	9.25	2.5	127 ⁿ	15.75 ^g	5.5 ^d
P + B 30g	106	10.5	3.5	134.5 ^g	15.25 ⁱ	5 ^e
P + T 30g	118.5	8.65	3.5	133 ^h	16.75 ^d	6 ^c
B + T 30g	103.5	10.25	3	133 ^h	17.5 ^c	6 ^c
P + B + T 30g	130.25	10.65	3.5	156.5^a	20^a	7^a
FYM 500g	126	11.65	3.5	107 ^q	14 ⁿ	5.5 ^d
Tridemorph(2ml/100ml)	97.5	10.10	3	143.5 ^c	14.5 ^m	5.5 ^d
Control	109	9.6	3	123.5 ^o	11.8 ^p	5 ^e
Healthy	116.5	10.6	3	128 ^l	13.75 ^o	5 ^e

P – *Pseudomonas fluorescens* (Pf1), B – *Bacillus subtilis*, T – *Trichoderma viride*.

Values are means of two replications, data followed by the same letter in a column are not significantly different from each other according to Duncan's multiple range test at $P = 0.05$

Table 4. Effect of biocontrol agents on the severity of basal stem rot disease of coconut under field conditions – Trial I and Trial II

Treatments	Disease index *			Disease index *		
	Trial I – Dharapuram			Trial II - Pattukottai		
	Before Application	Final dose after Application	Increase over initial	Before Application	Final dose after Application	Increase over initial
Pf1 150g + 5 kg FYM	9.143 ^l	11.200 ^f	2.057	10.493 ^h	11.985 ⁱ	1.492
<i>Bacillus</i> spp 150g+ 5 kg FYM	3.339 ^c	5.500 ^{ab}	2.161	7.288 ^{cd}	8.875 ^e	1.587

<i>T. viride</i> 150g + 5 kg FYM	4.977 ^f	6.495 ^{bc}	1.518	13.883 ^k	15.565 ^l	1.682
P + B 150g + 5 kg FYM	4.018 ^c	5.295 ^{ab}	1.277	12.047 ⁱ	13.290 ^j	1.243
P + T 150g + 5 kg FYM	6.476 ⁱ	7.730 ^{cd}	1.254	7.483 ^d	8.835 ^e	1.352
B + T 150g + 5 kg FYM	3.385 ^c	4.660 ^a	1.275	9.385 ^g	10.665 ^g	1.280
P + B + T 150g+ 5 kg FYM	3.078 ^a	4.270 ^a	1.192	6.986 ^{cd}	8.185 ^d	1.199
Pf1 300g + 5 kg FYM	3.698 ^d	4.840 ^a	1.142	7.109 ^{cd}	8.145 ^d	1.036
<i>Bacillus</i> spp 300g+ 5 kg FYM	6.796 ^j	7.988 ^{cde}	1.192	5.900 ^b	7.235 ^b	1.335
<i>T. viride</i> 300g + 5 kg FYM	3.189 ^{ab}	4.460 ^a	1.271	4.422 ^a	5.260 ^a	0.838
P + B 300g + 5 kg FYM	3.293 ^{bc}	4.825 ^a	1.532	8.815 ^f	9.665 ^f	0.850
P + T 300g + 5 kg FYM	7.345 ^k	8.675 ^{de}	1.330	6.800 ^c	7.890 ^c	1.090
B + T 300g + 5 kg FYM	6.293 ^h	7.580 ^{cd}	1.287	13.389 ^j	14.220 ^k	0.831
P + B + T 300g+ 5 kg FYM	10.945^m	12.015^f	1.070	8.191^e	8.870^e	0.679
FYM 5kg	6.270 ^h	9.380 ^c	3.110	13.530 ^{jk}	20.070 ^m	6.540
Tridemorph 2/ 100ml	5.955 ^g	7.020 ^c	1.065	10.589 ^h	11.180 ^h	0.591
Control	10.978 ^m	23.320 ^g	12.342	13.075 ^j	25.855 ⁿ	12.780

* Mean of two replications, Data followed by the same letter in a column are not significantly different from each other according to Duncan's multiple range test at P = 0.05, P – *Pseudomonas fluorescens* (Pf1), B – *Bacillus subtilis*, T – *Trichoderma viride*.

Collection of coconut diseases databases and bioinformatics resources in Tami Nadu region

B. Hari Prasath

Vanavarayar Institute of Agriculture
Manakkadavu, Pollachi-642 10, Tamil Nadu

The coconut (*Cocos nucifera* L.) palm is affected by a number of diseases, some of which are lethal while others gradually reduce the vigour of the palm causing severe loss in yield. The major insect pests of the coconut palm are rhinoceros beetle, red palm weevil, black headed caterpillar, cockchafer beetle and coreid bug. The present data include information on ASP.NET, which is a web application framework developed and marketed by Microsoft to allow programmers to build dynamic web sites, web applications and web services. It was first released in January 2002 with version 1.0 of the .NET Framework, and is the successor to Microsoft's Active Server Pages (ASP) technology. ASP.NET is built on the Common Language Runtime (CLR), allowing programmers to write ASP.NET code using any supported .NET language. The ASP.NET SOAP extension framework allows ASP.NET these coconut data are highly useful for rational use of Coimbatore area farmers. Coconut database (diseases, insects) have been implemented and are now accessible for consultation via the internet. Database browsing is available at ASP.NET. Coconut database, web consultation interfaces have been created to allow quick and complex queries, and user-friendly results representation.

Design patterns are recurring solutions to software design problems you find again and again in real-world application development. Patterns are about design and interaction of objects, as well as providing a communication platform concerning elegant, reusable solutions to commonly encountered programming challenges. The Gang of Four (GoF) patterns are generally considered the foundation for all other patterns. They are categorized in three groups: Creational, Structural, and Behavioral. Here you will find information on these important patterns. To give you a head start, the C# source code is provided in 2 forms: 'structural' and 'real-world'. Structural code uses type names as defined in the pattern definition and UML diagrams. Real-world code provides real-world programming situations where you may use these patterns. A third form, '.NET optimized' demonstrates design patterns that exploit built-in .NET 4.0 features, such as, generics, attributes, delegates, object and collection initializes, automatic properties, and reflection. These and much more are available in our Design Pattern Framework 4.0TM. See our Singleton page for a .NET 4.0 Optimized code sample. Coconut database development is based mainly on the ASP.NET database management system version visual studio 2005, running on Web set 24. ASP.NET is an object-oriented system capable of automatically storing and retrieving complex biological information, and is currently used by many databases: Disease database, Pest data base, etc. ASP.NET provides an intuitive object-oriented view of biological data, disease figures, Insect figures, and Symptoms, control measures user interface with many specialized data visualization tools, such as a all types of data's display.

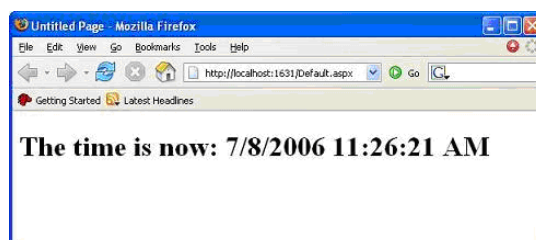
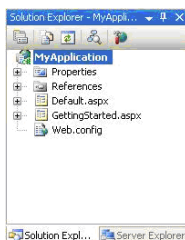
Currently, Coconut database contains around 15 diseases (Bud rot, Leaf rot, Stem bleeding, Root (wilt) disease, Tanjavur wilt, Mahali Crown chocking, Leaf blight or Grey Leaf

[illegible]

The figure consists of two side-by-side screenshots of a Microsoft Internet Explorer browser window. The left screenshot shows a webpage titled "coconut_banana_Diseases" with a large image of a coconut tree and buttons for "Disease" and "Pest". The right screenshot shows a webpage titled "Symptoms" with a list of symptoms for coconut diseases and a small image of a coconut branch with lesions.

Color makes things look good. It pleases the eye and sustains visual interest. The right colors maximize readability and minimize optical fatigue. The wrong colors strain the eyes and lower the level of comprehension. Color communicates. It delivers symbolic meanings that support and enhance the visual experience or individual elements within a design whether it's a web page.

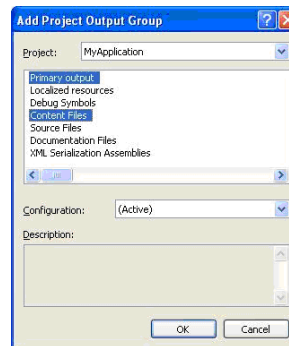
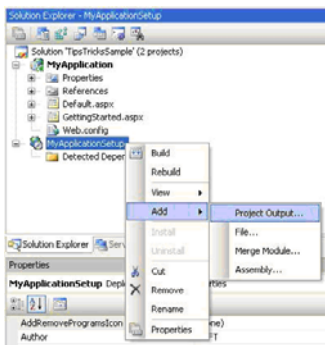
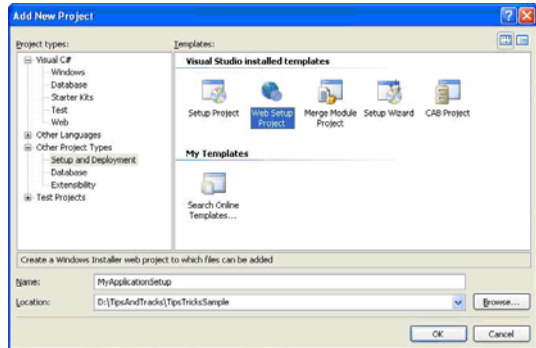
To begin with, we will start with an empty instance of Visual Studio and create a new VS 2005 Web Application project (select File->New Project->ASP.NET Web Application). For the purposes of this simple sample we'll have two pages in the project:



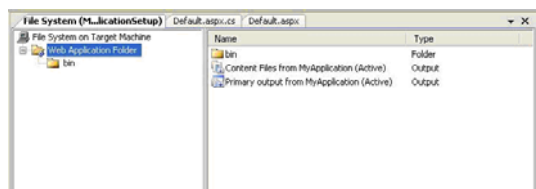
We will add a label to the Default.aspx page and a Page Load event handler in the code-behind to output the current timestamp on each request. When I press F5 to build and run the application, the project will compile and run as I'd expect (and by default use the built-in VS Web Server)

Now that we have a simple ASP.NET application built, we'll want to add a VS 2005 Web Setup Project to the solution. Choose the File->Add->New Project menu item to add one into our solution:

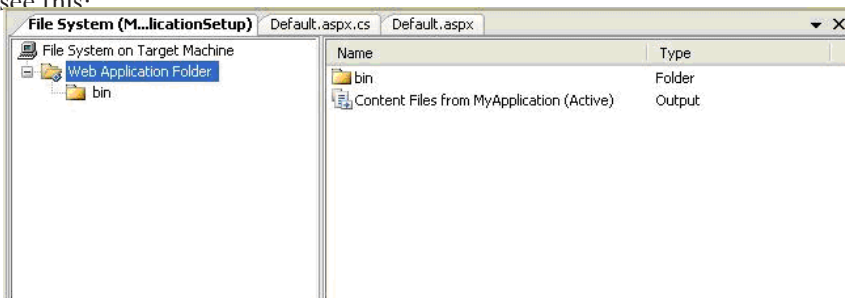
Note that the "Web Setup Project" type shows up under the "Other Project Types->Setup and Deployment" node in the New Project dialog above. Name it whatever you want and hit ok. It will then show up in your solution explorer as a separate project. Our next step will be to configure the web setup project to take the compiled assemblies (\bin directory contents) + content markup (.aspx, .config, etc files) from our Web Application Project and use them as inputs within our setup project. To-do this, right-click on the web setup project node in the solution explorer and choose the "Add->Project Output" context menu item, A dialog will then appear allowing us to select which project in the solution, and which of its project contents, we want to add to the setup package:



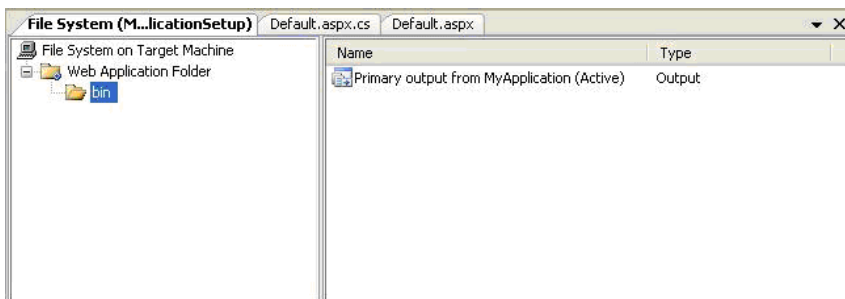
For ASP.NET Web Application Projects it is really important that we select both the "Primary Output" (which are the compiled assemblies for the \bin directory) as well as the "Content Files" (which are the .aspx markup files) within this dialog. By default, the web setup project will copy both of these items into the root of the target Web Application Folder that the setup project will create. You can see that it is configured this way by opening up the "File System" view within the web setup project (right click on the web setup project root and choose View->File System):



This actually isn't what we want to have happen though – since we really want the assemblies (indicated by the Primary Output node) to be copied into the application's \bin directory instead (otherwise ASP.NET won't be able to find them at runtime). To fix this, drag/drop the “Primary Output from My Application” node into the \bin directory. Once this is done you should be able to click on the “Web Application Folder” node on the left-hand side and see this:



And then click on the “bin” folder sub-node and see this:



We now have a basic web setup project created and configured for our ASP.NET Web Application. Next step is to build and run it.

Current Coconut data base have been submitted by different farmers, scientists from other institutions working on tropical crops (a data origin web page is available for Coconut disease and pests). Standard Excel submission files corresponding to the various types of data that can be submitted have been created to allow easy data submission. These files and a web form to deposit the standard data files for their incorporation in Coconut database are currently available on our Database. The internet versions will soon be available. Submitted data quality and integrity are checked by biologist experts of each tropical crop.

It is planned for the Coconut database content to grow quickly. Indeed, new data for a coconut module including sequence, map and primer data, resistance genes, a genetic map QTL data (yield and yield components, bean traits, resistance to various species of coconut) should be soon added to Coconut database. Coconut database model exibility makes it simple to add new types of data as they become available: expressed sequence tag (EST), bacterial artificial chromosome (BAC) data, etc. Future enhancements of Coconut database include the development of other tropical crops modules for rice, cotton, oil palm, rubber tree, pineapple, taro, yam and sorghum. Their interface development will be facilitated by the package.

References

- Anonymous. 1989. All-India co-ordinated research project on palms. Progress Report. Central Plantation Crops Research Institute, Kasaragod: pp.61-78.
- Dakshinamurti, C., Krishnamurthy, B., Summanwar, A. S., Shanta, P., and Pisharoty, P. R. 1971. Remote Sensing for Coconut Wilt. Seventh International Symposium on Remote Sensing, Ann Arbor, Mich.
- MacDonald, Matthew; Szpuszta, Mario Pro *ASP.NET 2.0 in C# 2005* (1st edition ed.). Apress. ISBN 1-59059-496-7, 2005.
- Soon-Hyung Yook, Hawoong Jeong, and Albert-Laslo Barabasi. 2002. Modeling the Internet's large scale topology. *Proceedings of the National Academy of Sciences USA*, **99**(21):13382-13386.
- Summanwar, A. S., Raychandhuri, S. P., Jagdish Chandra, K., Nam Prakash, and Lai, S. B. 1969. Virus associated with coconut wilt (root) disease. *Current Science* **38**: 208-210.
- <http://agritech.tnau.ac.in/>
- <http://coconutboard.nic.in/>
- <http://www.wikipedia.org/>

Comparative genome analysis of different strains of phytoplasma

R. Manimekalai, Lijin, K.G., Roshna, O.M. and Thomas, G.V.

Central Plantation Crops Research Institute

Kasaragod - 671 124, Kerala, India

Phytoplasma are phloem-restricted plant pathogenic mollicutes, causing a variety of diseases in more than a thousand plant species worldwide. They are pleomorphic, cell wall-less bacteria that descended from an acholeplasma-like ancestor and are characterized by small, AT-rich genomes encoding capabilities for transkingdom parasitism and pathogenicity in plants and insects. They are transmitted from plant to plant by phloem feeding homopterous insects mainly leaf hoppers (Cicadellidae), plant hoppers (Fulgoroidea) and psyllids (Psyllidae) and by vegetative multiplication of infected plant material. The difficulties in extraction of DNA from infected plants, and the instability of AT-rich DNA in large-insert genomic libraries slow down genome sequencing projects. For these reasons, genomes of only four phytoplasma strains have been completely sequenced to date. These phytoplasmas include Aster yellows witches'-broom Phytoplasma AYWB, Candidatus Phytoplasma australiense, Candidatus Phytoplasma Mali, and Onion yellows phytoplasma OY-M (Table 1).

Candidatus Phytoplasma mali strain AT was collected in Heidelberg, Germany from a symptomatic apple tree (2). The low amount of potential mobile units and paralogs and the minimal common gene content shared with the other phytoplasma genomes characterize strain AT. Aster yellows witches'-broom phytoplasma cause Aster yellow disease in vegetable crops and is transmitted by the aster leafhopper. Symptoms include a yellowing of the leaves and reduced leaf size, which leads to reduced plant size and lower yields. Candidatus Phytoplasma australiense was identified in diseased Chardonnay grapevines from South Australia. Onion yellows phytoplasma strain OY-M is derived from a wild-type disease-causing strain (onions yellow disease), which was isolated in Saga Prefecture, Japan, in 1982, and shows mild symptoms and does not cause stunting nor phloem hyperplasia.

Table 1. Phytoplasma genome projects

Organism	Host	Disease	Status	Institute
<i>Aster yellows witches'-broom phytoplasma AYWB</i>	Vegetable crops	Aster yellows witches'-broom	Completed	Ohio State University
Candidatus Phytoplasma Australiense	Grapevine	Grapevine yellows	Completed	Max Planck
Candidatus Phytoplasma Mali	Apple proliferation	Apple	Completed	Federal Biological Research Center for Agriculture and Forestry
<i>Onion yellows phytoplasma OY-M</i>	Onion	Onions yellow	Completed	The University of Tokyo

Aster yellows witches'-broom Phytoplasma AYWB (Project ID: 13478, at Ohio State University), Candidatus Phytoplasma australiense (Project ID: 29469, at Max Planck Institute),

Candidatus Phytoplasma Mali (Project ID: 25335, at Federal Biological Research Center for Agriculture and Forestry), and Onion yellows phytoplasma OY-M (Project ID: 13750, at The University of Tokyo) genomes were retrieved from NCBI Genome database (<http://www.ncbi.nlm.nih.gov/>). The protein coding potential of genomes were determined using web online software GeneMark-hmm-p by using species specific parameters of the Markov models of coding and non-coding regions (7). This approach allows delineating local variations of coding potential. A graph also is available which shows details of the protein-coding potential distribution along a sequence. The Open Reading Frames (ORFs) predicted by GeneMark were compared with ORF Finder software in NCBI server.

Molligen 2.0, a complete bacterial genome analysis server with genome data and tools, is used for phytoplasma genome annotation (6). Pairwise genome alignment and protein homologous analysis were done using different tools in the same server. ClustalX, a standalone multiple sequence alignment (MSA) software is used to find closely related proteins from multiple homology and these proteins were identified by phylogenetic analysis tool TreeView. Uneven distribution (skew) of GC content were determined followed by origin of replication site prediction is done using GenSkew software in MIPS server (<http://mips.helmholtz-muenchen.de/services/analysis/genskew>).

A tool, based on BLASTN algorithm was used to check the repetition of transposase and phage associated genes retrieved from NCBI against all completed four genomes of phytoplasma. Different types of ABC transporter genes were analysed in protein level. For that we retrieved five different types of ABC type transporter proteins ie; Aminoacid transporter, Cobalt transporter, Multidrug resistance transporter, spermidine/putrescine transporter and Mn/Zn transporter from four phytoplasmas. We used TopPred software to compare transmembrane topology of all this transporter permease components.

All strains of phytoplasma have circular DNA except Ca (Table 2). *Phytoplasma mali* which has a linear chromosome with 601,943 nt length. *Ca. phytoplasma australiense* has biggest genome (879,959 nt) among three other strains but low GC content than Onion yellows phytoplasma mutant strain (OY-M) (27.74%). Proteins coded by genes (839) are more in *Ca. phytoplasma australiense* even though, Onion yellow phytoplasma (OY-M) consist large number genes (979). Levels of tRNA and rRNA are almost same in all genomes but, some special types of RNAs like transfer-messenger RNA (tmRNA) and non-coding RNA (ncRNA) are present in phytoplasma strains *Ca. australiense* and *Ca. mali*.

Table 2. Genome features of four different strains of phytoplasma

	Aster yellows witches'-broom AYWB	Candidatus phytoplasma australiense	Candidatus Phytoplasma mali	Onion yellows phytoplasma OY-M
<i>Length</i>	706,569 nt	879,959 nt	601,943 nt	860,631 nt
GC Content	26.89%	27.42%	21.39%	27.74%
Topology	Circular	Circular	Linear	Circular
Molecule	DNA	DNA	DNA	DNA
Total ORF	1796	2231	1544	2458
Predicted(3 Frame)				

Genes	692	869	523	979
Protein Coding	671	839	479	754
tRNA	31	35	32	32
rRNA	4	6	6	6
tmRNA	0	1	1	0
ncRNA	0	1	0	0

GenMark predicted ORFs in three frames. The results are as follows, Aster yellows witches'-broom Phytoplasma AYWB (1796), *Candidatus Phytoplasma australiense* (2231), *Candidatus Phytoplasma Mali* (1544), and Onion yellows phytoplasma OY-M (2458). By comparing GenMark result with ORF Finder result we predicted number of genes in each strain.

GenSkew computes the normal and the cumulative skew of two selectable nucleotides for a given sequence. The result is displayed in two different graphs. The normal graph displays every single value related to the particular position in sequence. The cumulative graph adds up the values for all previous windows up to the certain position. The global minimum and maximum are displayed in the cumulative graph. The minimum and maximum of a GC-skew can be used to predict the origin of replication (minimum) and the terminus location (maximum) in prokaryotic genomes (Fig. 1).

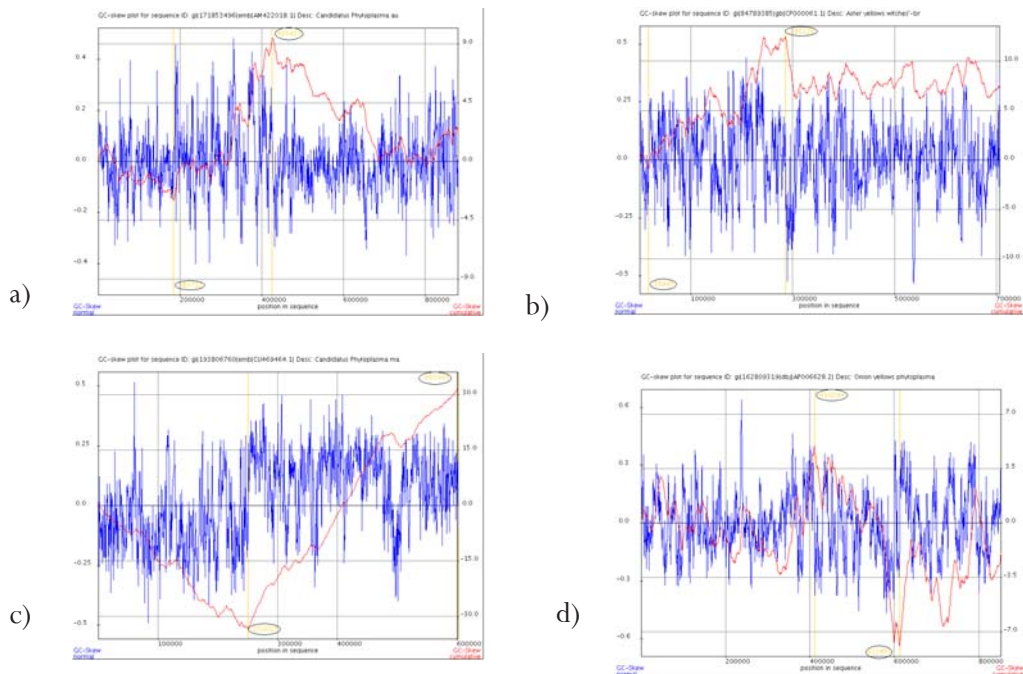


Fig. 1. GC skew analysis of phytoplasma strains a) *Ca. Phytoplasma australiense* b) Aster yellows witches' broom (AY-WB) c) *Ca. Phytoplasma Mali* d) Onion yellows phytoplasma mutant strain (OY-M). The value represented in circle shows predicted regions for origin of replication and terminus location

Cumulative graph for genome predicted regions for origin of replication and terminus location. Aster yellows witches'-broom Phytoplasma AYWB has origin of replication site (16945) in the upstream end of enzyme glycerol-3- phosphate acyl transferase (PLSX). In the same way replication sites for Candidatus Phytoplasma australiense (183712), Candidatus Phytoplasma Mali (250017), and Onion yellows phytoplasma OY-M (620061) are located in the upstream end of 6-phospho fructokinase (pfk), replication initiator protein (dnaA) and transposase (tra5) respectively.

When compared for homologous proteins using multi proteome differential query tool in Moligen database, 448 proteins were found to be common in all the four strains of phytoplasma (Fig. 2). When compared a pair of sequence together some protein showed multiple homologies in other. Number of proteins shared only between two strains was also calculated using same tool. From the results the pair Onion yellows phytoplasma OY-M and Aster yellows witches'-broom Phytoplasma AYWB shared more number of proteins (28) and Aster yellows witches'-broom Phytoplasma AYWB and *Ca*.Phytoplasma mali shared less number. The results filtered for multiple homologies.

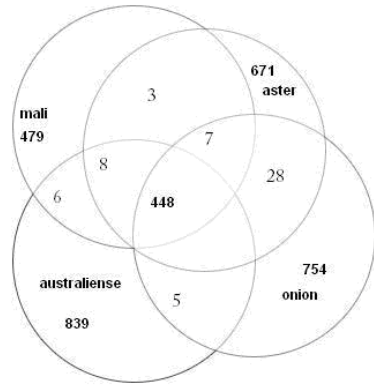


Fig. 2. Protein homologues common between different strains of phytoplasma

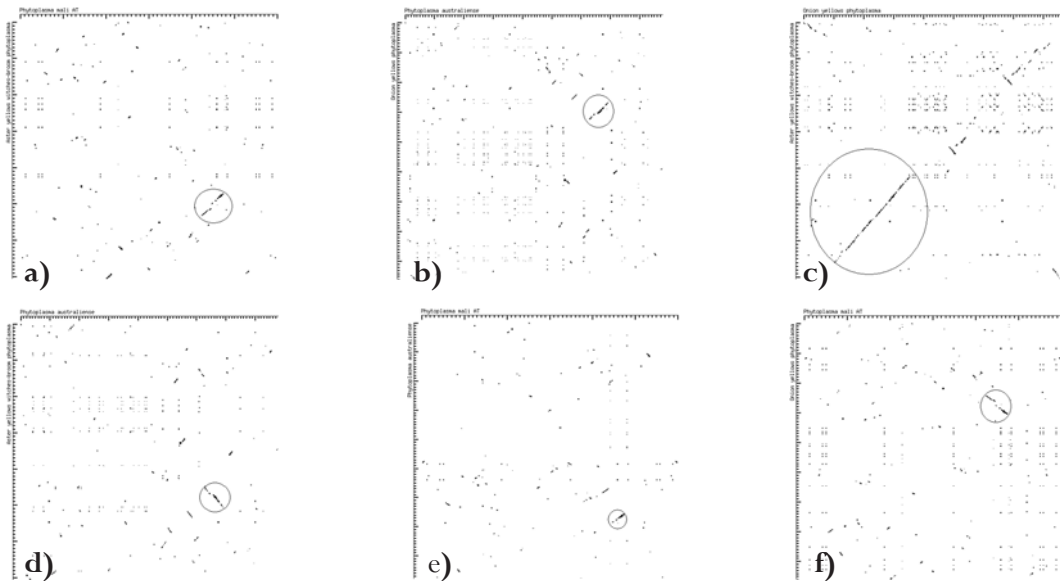


Fig. 3. Pairwise genome alignment between different strains of phytoplasma based upon data from Molligen 2.0 database a) *Ca*.Phytoplasma mali and Aster yellows witches broom (AY-WB) b) *Ca*.Phytoplasma and onion yellows mutant strains (OY-M) c) Onion yellows mutant strains (OY-M) and Aster yellows witches' broom (AY-WB) d) *Ca*. Phytoplasma australiense and Aster yellows witches' broom (AY-WB) e) *Ca*.Phytoplasma mali and *Ca*.Phytoplasma australiense f) *Ca*.Phytoplasma mali and Onion yellows mutant strain (OY-M)

Pair-wise genome alignment and graph were created by selecting gene name as homologous type in the data set of Molligen 2.0 database. The conserved regions between sequences are marked in circle (Fig. 3). The graph showed ~60kb regions were shared between Ca.australiense and AY-WB strain. The pairs Ca.mali - OY-M and Ca.P.mali - AY-WB strain share a region of ~50kb as conserved. Long conserved regions of ~250kb were found between OY-M strain and AY-WB. Ca.mali and Ca.P.australiense have the shortest conserved region of ~25kb size.

Comparative analysis of phage associated gene (Table 3) produces specific results for each genome exception in the case of Candidatus phytoplasma mali where gepA gene of mali showed homologues with 2 region in Candidatus phytoplasma australiense. Aster yellow phytoplasma genome has no phage associated genes and no homologues regions with gepA sequences of other strains.

Table 3. Distribution of phage associated gene in different genomes

Acc.No.	Length	Gene	Taxon	Maximum number of hits			
				Australiense	Aster	Onion	Mali
NC_010544.1	480	gepA	Australiense	4	-	-	-
NC_005303.2	201	gepA	Onion	-	-	1	-
NC_011047.1	654	gepA	Mali	2	-	-	1

Distribution of transposase gene (Table 4) is almost same in Aster yellow phytoplasma and Onion yellow phytoplasma. Transposase gene is not predicted in Candidatus phytoplasma australiense but this analysis predicted 18 and 19 homologues regions for tra5 gene from AY and OY strains respectively. A unique type Tra5 gene is found in Candidatus phytoplasma mali which dont have any homologues region in any other phytoplasmas.

Table 4. Distribution of transposase (tra5) gene in all four different phytoplasmas

Acc. No	Length	Gene	Taxon	Maximum Number of Hits			
				Australiense	Mali	Onion	Aster
NC_007716.1	966	Tra5	Aster	18	-	21	39
NC_005303.2	930	Tra5	Onion	19	-	28	42
NC_011047.1	975	Tra5	Mali	-	1	-	-

Comparative analysis of major seven ABC transporter systems (Table 5) present in the phytoplasma genome concluded in the statement that the presence and number of times the same transporter occupied in the membrane is varied among these strains. Compared to other type systems Amino acid transporter system and Zn/Mn transporter system varies in more number between strains. Onion yellow strain contain 9 amino acid transporter system while mali strain contain only one (ATP_00191). In the same way Zn/Mn transporter system is more (4) in mali strain. Equal presence of transporter systems in various systems indicates its importance in virulence and survival of phytoplasma.

Table 5. Comparative analysis of seven different major ABC transport systems in phytoplasma

Transporter System	Aster Yellow Witches Broom	Candidatus Phytoplasma Mali	Candidatus Phytoplasma Austaliense	Onion Yellow Phytoplasma
Multidrug resistance	AYWB_029 AYWB_028	ATP_00111 ATP_00110	PA0115 PA0114	PAM_059 PAM_058
Spermidine/ Putrescine	AYWB_094 AYWB_093	ATP_00292 ATP_00291	PA0133 PA0134	PAM_661 PAM_660
Dipeptide	AYWB_531 AYWB_530	ATP_00067 ATP_00066	PAa_0718	PAM_189 PAM_190 PAM_022
Aminoacid	AYWB_315 AYWB_265 AYWB_262 AYWB_635	ATP_00191	PA0267 PA0450	PAM490, 494, 077, 588, 487, 639, 464, 456, 078
Sugar	AYWB_668	ATP_00161 ATP_00160	PAa_0023	PAM_751
Cobalt	AYWB_015	ATP_00371	PA0173 PA0139	PAM_180
Zn/Mn	AYWB_622	ATP_00494 ATP_00495 ATP_00004 ATP_00003	PA0483 PA0484	PAM_094

References

- Besemer, J. and Borodovsky, M. 2005. GeneMark: web software for gene finding in prokaryotes, eukaryotes and viruses. *Nucleic Acids Research* 451- 454.
- Barré1, A., Daruvar1 A. D. and A. Blanchard. MolliGen, a database dedicated to the comparative genomics of Mollicutes. *Nucleic Acids Research* 2004: 307- 310.
- Bai, X., Zhang, J. Ewing, A. Miller, S. A. Radek, A. J. Shevchenko, D. V. Tsukerman, K. Walunas, T. Lapidus, A. Campbell, J. W. and Hogenhout. S. A. Living with Genome Instability: the Adaptation of Phytoplasmas to Diverse Environments of Their Insect and Plant Hosts. *J. Bacteriol.* 2006: 3682–3696.
- Kube, M. Schneider, B. Kuhl, H. Dandekar, T. Heitmann, K. Migdoll, A. M. Reinhardt, R. and Seemuller, E. The linear chromosome of the plant-pathogenic mycoplasma ‘*Candidatus Phytoplasma mali*’. *BMC Genomics* 2008: 306-320.
- Lukashin A. V. and Borodovsky, M. GeneMark.hmm: new solutions for gene finding. *Nucleic Acids Research* 1998: 1107-1115.
- Melamed, S. Tanne, E. Ben-Haim, R. Edelbaum, O. Yogeve, D. and Sela, I. Identification and Characterization of Phytoplasmal Genes, Employing a Novel Method of Isolating Phytoplasmal Genomic DNA. *J. Bacteriol.* 2003: 6513–6521.

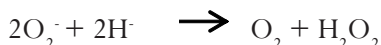
Superoxide dismutase isozymes and their heat stability in coconut (*Cocos nucifera* L.) leaves

Mukesh Kumar, Sugatha P., Muralikrishana K. S., John Sunoj V.S., and Hebbar K. B.

Central Plantation Crops research Institute

Kasaragod -671124, Kerala, India

When plants face abiotic stress, undergo changes in their metabolism in order to cope up with the changing environment. One of the biochemical changes occurring when plant is subjected to biotic and abiotic stress is the production of reactive oxygen species (ROS). ROS are highly reactive and when the scavenging capacity of plant is less than the ROS production, they can seriously disrupt normal metabolism through oxidative damage of lipid, protein and nucleic acids (Halliwell and Gutteridge, 1985; Fridovich, 1986; Davis, 1987). Plants are having a lot of antioxidant systems that protect them against these potential cytotoxic effects. Antioxidant enzymes are the most important components in the scavenging system of ROS. In this antioxidant enzyme system, Superoxide Dismutases (SOD: EC 1.15.1.1) are ubiquitous metalloenzymes (Fridovich, 1975; Jackson *et al.*, 1978) that constitute the first line of defence against reactive oxygen species (ROS). In living cells, SODs catalyze the dismutation of the Superoxide radicals ($O_2^{\cdot -}$) into hydrogen-peroxide (H_2O_2) and oxygen (O_2) and play an important role in protecting the cells against the toxic effect of Superoxide radicals produced in different cell compartments (del Rio *et al.*, 2002).



SODs are divided into four main subtypes and are categorized based upon their cellular locations and metal-ion-binding prosthetic groups. Copper and Zinc SODs (Cu/Zn-SODs) are found primarily in the cytoplasm (Vacuoles) of eukaryotic cells, manganese SODs (Mn-SODs) exist in the mitochondria of eukaryotic cells and the cytoplasm of prokaryotes, iron SODs (Fe-SODs) exist mainly in prokaryotes and the chloroplast of eukaryotic plants, and Nickel SODs (Ni-SODs) exclusively found in prokaryotes only (Jackson *et al.*, 1978; Reddy and Venkaiah, 1982; Werner *et al.* 1992; Youn *et al.* 1996; Pradeepa *et al.* 2009). Each type of SODs having multiple forms, Cu/Zn-SODs are sensitive to CN^- and H_2O_2 , but insensitive to $CHCl_3$ - CH_3CH_2OH , whereas Fe-SODs are sensitive to Cu/Zn -SODs and $CHCl_3$ - CH_3CH_2OH but insensitive to CN^- and Mn-SODs are insensitive to CN^- and H_2O_2 , but sensitive to $CHCl_3$ - CH_3CH_2OH (Fridovich, 1975; Wang *et al.* 2009). This selective inhibition of SODs makes it possible to distinguish the three types of SODs in crude homogenates.

The SOD pattern of various plants and some of its heat stable isoforms (Cu/Zn-SODs) has been described (Bridges and Salin, 1981; Reddy and Venkaiah, 1984; Pan and Yau, 1991; Chopra and Sabrinath, 2004). In plant as well as in mammalian and fungal cells Cu/Zn-SOD is a major SOD (Rotilio, 1986), but plant also contains Mn-SOD and Fe SODs in addition to Cu/Zn-SODs. According to previous thoughts, Fe-SOD was exclusively restricted to prokaryotes and some eukaryotic algae but has also been reported in several higher plant species (Bridges and Salin, 1981; Kwaitowski *et al.* 1985; Pan and Yau, 1991; Rahnama and Ebrahimzadeh, 2006). However, in coconut some work has been done on SODs in relation to drought tolerance and lipid peroxidation (Chempakam, *et al.* 1993). But, still it is not clear

which isozymes of SODs are present and their heat stability in coconut. The present study deals with the identification of SOD isoforms and their heat stability in coconut leaves.

The leaf samples collected from third leaf of three year old coconut (*Cocos nucifera* L.) seedlings of WCT variety grown in poly-bags under Shade net are the material for the observations.

Fresh leaves (2.5g) were cut in to small pieces and ground in pre chilled mortar in 25 ml cold 0.1 M Sodium Phosphate buffer, PVPP (1 g) β -mercaptoethanol (200 μ l) and transferred in to pre chilled centrifuge tubes. The extract was centrifuged at 12000 rpm for 15 minute at 4 °C in refrigerated centrifuge (Hareus, Germany). Took the supernatant and re-centrifuged at the same conditions. The enzyme was partially purified from the supernatant by ammonium sulfate precipitation (final concentration 85%), and subsequent dialysis. The clear pale yellow dialysate after centrifugation was used for further enzyme assay as well as for isozymes identification. An aliquot of the enzyme extract was used to determine its protein content by the method of Lowry *et al.* (1951).

SOD activity was determined by measuring its ability to initiate photochemical reduction of nitro blue tetrazolium (NBT) according to the method of Beauchamp and Fridovich (1971).

Nondenaturing polyacrylamide gels electrophoresis (PAGE) was carried out with 4% stacking gel and 10% separating gel using 0.1M Tris –Glycine electrode buffer (pH-8.3) at 4°C and 160mA according to the method described by Hames and Rickwood's (1990) with some modifications. To visualize the SOD activity, the gels were developed according to the method given by Beauchamp and Fridovich (1971). The gels were incubated in dark for 20 minutes in a solution containing 100 ml 0.05M Tris-HCl (pH-8.2) containing Riboflavin, nitroblue tetrazolium (NBT) and EDTA and illuminated the gels until the white bright bands became apparent.

Detection of SOD isozymes was performed according to the methods described by Luo *et al.* (1996) with some modifications. Gels were incubated separately, in 0.1% H₂O₂ (dissolved in 0.1m Phosphate buffer pH-7.6) and CHCl₃-CH₃CH₂OH (0.3:0.5 v/v) for 30 minutes and then stained according to the method described above.

The effect of high temperature on the SOD activity was studied by incubating the extract in eppendorf tubes at 50°, 60°, 70°, 80°, and 100° C for 10 minutes in water bath with occasional shaking and then cooled by keeping on ice and centrifuge it at 12000 rpm for 15 minutes at 4° C in refrigerated centrifuge (Hareus, Germany). After that, the extract was analyzed for SOD activity and isozymes visualization according to the methods described above (2.3 and 2.4).

Identification of SOD isoforms was done by using native PAGE (Fig. 1 and Fig.2). Using this method, thirteen distinct SOD isoforms were detected in the coconut leaves. Two

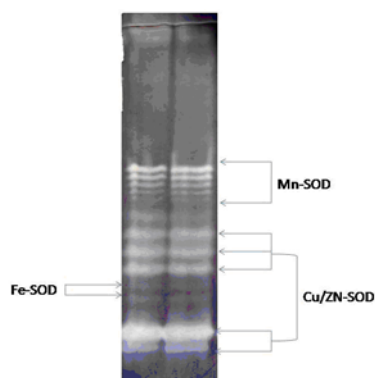


Fig. 1. Superoxide Dismutase isoforms in coconut leaves

of SOD bands were of very low relative mobility than the remaining eleven SODs bands and detected near the junction of separating gel and resolving gels, thus implying that they had very high molecular weight (Fig. 3a, Lane A).

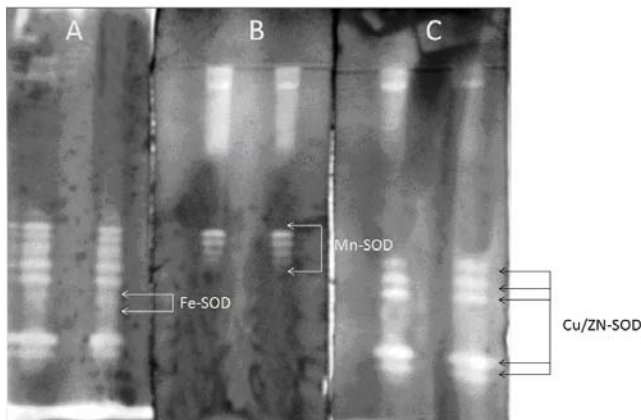


Fig. 2. Identification of Superoxide Dismutase (SOD) isozymes in coconut leaves. After native PAGE, the gels were given the following treatment and then, they were stained with NBT reduction. Lave A: control, untreated. B: H_2O_2 treatment; Lane C: $CHCl_3$ - CH_3CH_2OH treatment. The direction of electrophoresis was top side cathode (-) to bottom side anode (+).

There are four distinct types SODs: Cu/Zn-SOD, Mn-SOD, Fe-SOD and Ni-SOD (detected only in prokaryotes), which are classified according to the metals present at the catalytic site. Each type of SOD (except Ni-SOD) is sensitive to specific inhibitors, allowing discrimination among different forms. For determination of the SOD subtypes among SOD bands from coconut leaves, native gel were treated with different inhibitors and then stained. Two very effective inhibitors, hydrogen peroxide (H_2O_2) and a mixture of chloroform and ethanol ($CHCl_3$ - CH_3CH_2OH) were used to discriminate between SOD isoforms. Cu/Zn-SODs and Fe-SODs are sensitive and lose their activity with H_2O_2 treatment, therefore, utilization of H_2O_2 can identify the Mn-SOD; however the Mn-SOD and Fe-SOD lose their activity with $CHCl_3$ - CH_3CH_2OH treatment. As a result Cu/Zn-SODs can be identified with $CHCl_3$ - CH_3CH_2OH treatment (Wang *et. al.*, 2009). Although, potassium cyanide (KCN) is also a potent SOD inhibitor can cause the inactivation of Cu/Zn-SODs and identify Mn-SODs and Fe-SODs, KCN is highly toxic, therefore, was not used for the safety purpose. In spite of that, the identification of SOD isozymes was achieved by comparison of control gel with other two treatments.

In this study, pretreatment of the gels with H_2O_2 showed two high molecular weight bands and four medium molecular weight bands (Fig. 3a, lane B) while two high molecular weight bands and five low to medium molecular weight bands were visible on the gels with $CHCl_3$ - CH_3CH_2OH treatment (Fig. 3a lane C). Based on the comparison of gels, we conclude that there are two Fe-SOD isozymes, four Mn-SOD isozymes, five Cu/Zn-SOD isozymes and two unknown (resistant two both the inhibitors) SOD isozymes present in coconut leaves.

In higher plants, Cu/Zn-SODs are the most abundant SOD and are commonly found in the cytosol and chloroplast, Mn-SODs are mainly present in mitochondria and peroxisomes, and Fe-SODs are generally found in chloroplasts (Alscher *et. al.*, 2002). Although Fe-SOD was previously considered to be not very common in higher plants, in roots of red beet (Pradedova *et al.* 2008); in rice flower (Pan and Yau, 1991), carnation petals (Droillard *et. al.*, 1989), and in Brassica leaves has been reported to be present in both mitochondria and peroxisomes (Droillard and Paulin, 1990) thus correlating with an observations in coconut. The significant difference in SOD band width on electrophoregram was also noticeable and it showed that Cu/Zn-SODs in native PAGE have very wide and thick bands while Mn-SODs have medium and Fe- SODs having very thin and faint bands (Fig. 1). The same type of results has also been reported in red beet roots (Pradedova *et. al.*, 2008).

The thermo-stability of SOD in coconut leaves was investigated by incubating the enzyme extract at temperature ranging from 50 °C to 100 °C and detecting the enzymes. The SOD specific activity increased initially from 6.82 to 7.6 by 50 °C and then decreased to 1.88 by 80 °C but was again increased at 100 °C (Fig. 2, II). Chopra and Sabrinath (2004) observed that the SOD specific activity was increased in incubation temperature above 50 °C in *Chinopodium murale*. Similarly, sweet potato SOD showed slight enhancement in the activity at 70 °C incubated for various time intervals (Lin *et. al.*, 1995), while the cabbage SODs lost their activity at 70 °C after 5 minutes (Walker *et. al.*, 1987).

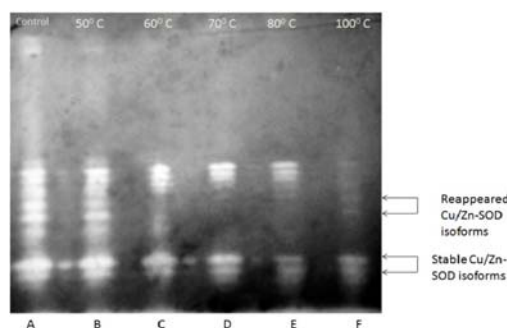


Fig. 3 a. Native PAGE showing SOD activity of coconut leaves extract incubated at different temperatures. Lane A: Control, untreated; Lane B to F: sequential heat treated with 50 °C to 100 °C.

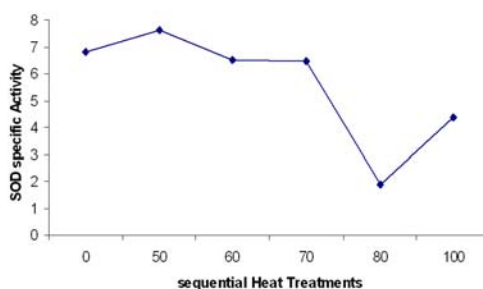


Fig.3 b. Graph showing the effect of sequential heat treatment on SOD specific activity in coconut leaves

When the treated samples were loaded on native PAGE gel and stained the gel for SOD activity, out of 13 isoforms, nine isoforms lost their activity by 80 °C. Only 4 isoforms (two each for Cu/Zn-SOD and Mn-SOD) maintained their original activity up to 80 °C. At 100 °C only two existing isoforms of Cu/Zn-SOD remained stable with increased band strength and two new isoforms of SODs were also detected (Fig. 3a lane F). Chopra and Sabrinath (2004) reported the formation of new SOD isoform in *Chinopodium murale* at 60 °C which was stable even at boiling temperature. The stability of the isozyme, under heat treatment, might be due to the stable confirmation of the protein due to the inter-subunit contacts or increased electrostatic steering of substrate into the active site (Lin *et. al.*, 1995). The two higher molecular weight isoforms (mentioned above) were resistant to both the inhibitors but very unstable under heat treatment and completely lost their activity by 60 °C (Fig. 3a, lane B). We did not get any report about these isoforms in plant kingdom. These isoforms may be containing Nickel ions as reported in some bacteria (*Streptomyces* spp.). This isoform having a molecular mass approximate 60kDa but in general, the bacterial SOD subunits have molecular masses in the range 18-22 kDa. (Youn *et. al.*, 1996). Crystal structure of Ni-SODS in *Streptomyces* spp. revealed that the Ni-SOD was homohexamer consisting of four-helix-bundle subunits (Wuerges *et. al.*, 2004).

In coconut leaf protein extract thirteen SOD isoforms were detected with five Cu/Zn-SODs, four Mn-SODs, two Fe-SODs and two higher molecular weight unknown isoforms. It is also observed in coconut that different isoforms react differently to higher temperature treatments and two new isoforms of Cu/Zn-SODs, appeared in the leaf protein extract heated at 100 °C. Two isoforms of Cu/Zn-SODs were more resistant to higher temperature treatment than the other SOD isoforms. The thermo-stable isoforms of SOD may contribute towards heat tolerance of the plants and identification of higher molecular SOD isoforms may also be helpful in abiotic stress tolerance of the plants. Purification of the thermo-stable Cu/Zn-SODs, identification of unknown isoforms and their gene isolation will be carried out, which could play immense role in the genetic engineering of crops for abiotic stress tolerance.

References

- Alscher, R.U., Erturk, N. and Heath, L.S. 2002. Role of superoxide dismutases (SODs) in controlling oxidative stress in Plants. *J. Exp. Bot.* **53**: 1331-1341.
- Beauchamo, C.O. and Fridovich, I. 1971. Superoxide Dismutase: Improved assay and an assay applicable to acrylamide gels. *Anal. Biochem.* **44**: 276-287.
- Bridges, S.M. and Salin, M. L. 1981. Distribution of iron containing SOD in vascular plants. *Plant Physiol.* **68**: 2758-278.
- Chempakam, B., Kasturi Bai, K.V. and Rajagopal, V. 1993. Lipid Peroxidation in Relation to drought Tolerance in Coconut (*Cocos nucifera* L.), *Plant Physiol. & Biochem.*, **20(1)**: 5-10.
- Chopra, R. K. and Sabrinath, S. 2004. Heat-stable chloroplastic Cu/Zn SOD in *Chinopodium murale*. *Biochem. And Biophys. Res. Commun.* **320**: 1187-1192.
- Davis, K.J.A. 1987. Protein damage and degradation by oxygen radicals. I. General Aspects. *J. Biol. Chem.* **262**: 9895-9901.
- Del Rio L A, Corpas F J, Sandalio L M, Palma J M, Gomez M, Barroso J B 2002. Reactive oxygen species, antioxidants systems and nitric oxide in proxisome. *J. Exp. Bot.* **53**: 1255-1272.

- Droillard, M.J.; Bureau, D. ; Paulin, A. and daussant, J. 1989. Identification of different classes of superoxide dismutase in carnation petals. *Electrophoresis*. **10**: 46-48.
- Droillard, M.J. and Paulin, A. 1990. Isozymes of superoxide dismutase in mitochondria and Carnation (*dianthus caryophyllus*) during senescence. *Plant Physiol.* **94**: 1187-1192.
- Fridovich, I. 1975. Superoxide Dismutase. *Ann. Rev. Biochem.* **44**: 147-159.
- Fridovitch, I. 1986. Biological effect of Superoxide radicals. *Arch. Biochem. Biophys.* **247**: 1-11.
- Halliwell, B. and Gutteridge, J.M.C. 1985. Free Radicals in Biology and Medicine. *Clarendon Press, Oxford*. 29.
- Hames, B.D. and Rickwood, D. 1990. Gel Electrophoresis of Proteins: A practical Approach. *Oxford Univ. Press*, 1-138.
- Jackson, C.A., Moore, B., Halliwell, C.H., Foyer and Hall, D.O. 1978. Subcellular localization and identification of Superoxide Dismutase in the leaves of higher plants. *Eur. J. Biochem.* **91**: 339-344.
- Lin, C.T., Lin, M.T., Chen, Y.-T. and Shaw, J.F. 1995. Subunit interaction enhances enzymatic activity and stability of sweet potato cytosolic Cu/Zn superoxide dismutase purified by a His-tagged recombinant protein method. *Plant Mol. Bio.* **28**: 305-311.
- Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. 1951. Protein measurement with Folin Phenol reagent. *J. Biol. Chem.* **193**: 265-275.
- Luo, G.H., Wang, A.G. and Fu, A.G. 1996. The location staining method for distinguishing different types of SOD. *Progress in Biochem. And Biophysics*, **23**: 356-359.
- Pan, S.-M. and Yau, Y.-Y. 1991. The isozymes of Superoxide Dismutase in rice. *Biochem. J.* 253-257.
- Pradedova, E.V., Isheeva, O.D. and Salyaev, R.K. 2009. Superoxide Dismutase of plant Cell Vacuoles. *Membrane and Cell Biology*. **26** (1): 21-30.
- Rahnama, H. and Ebrahimzadeh, H. 2006. Antioxidant Isozymes Activities in Potato plant under salt stress. *J. Sci. Islam. Rep. of Iran*. **17**(3): 225-230.
- Reddy C. D. and Venkaiah, B. 1984. Purification and characterization of Cu/Zn-SOD from mungbean (*Vigna radiata*) seedlings. *J. Biosci.*, **6**: 115-123.
- Reddy, C.D. and Venkaiah, B. 1982. Isoenzymes of superoxide dismutase from Mung bean (*Phaseolus aureus*) Seedlings. *Curr. Sci.* **20**: 987-988.
- Rotilio, G. 1986. Superoxide and Superoxide Dismutase in chemistry, biology and medicine. *Elsevier Science Publishers, Amsterdam, New York, Oxford*.
- Walker, J.L.; McLellan, K.M. and Robimson, D.S. 1987. Heat stability of superoxide dismutase in Cabbage. *Food Chem.* **23**: 245-256.
- Wang, J., Xuequn, L. and Guanghui, YU. 2009. Identification of Superoxide Dismutase isoenzymes in tobacco pollen. *Front. Biol. China*. **4**(4): 442-445.
- Werner, K., Heinz, R. and Andrea P. 1992. Purification of Two SOD isozymes and Their Sub-cellular Localization in Needles and Roots of Norway spruce (*Picea abies* L.). *Plant Physiol.* **100**: 334-340.

- Wuerges, J., Lee, J.-W., Yim, Y.-W., Yim, H.-S., Kang, S.O. and Carugo, K.D. 2004. Crystal structure of nickel-containing superoxide dismutase reveals another type of active site. *PNAS*. **101**(23): 8569-8574.
- Youn, H.-D.; Kim, E.-J., Roe, J.-H., Hah, Y.-C. and Kang, S.-O. 1996. A novel nickel-containing Superoxide dismutase from *Streptomyces* spp. *Biochem. J.* **318**: 889-896.

Comparison of superoxide dismutase, succinic dehydrogenase, exochitinase and 1, 4- β glucanase activities between healthy and root (wilt) disease affected coconut cultivars – CGD and WCT

V.K.Chaturvedi, N. Srinivasan, M. Sasikala, P.M. Jacob, G. Rajeev, C. Rincy

Susan, M. Monisha and M.S. Jisha

Central Plantations Crop Research Institute

Regional Station, Kayangulam - 690533, Kerala, India

Root (wilt) disease of coconut is widely prevalent, in southern districts of Kerala and also in isolated tracts in northern districts of the State, border districts of Tamil Nadu, and in Goa and Karnataka. The disease is considered to be caused by phytoplasma (Solomon *et al.*, 1983). The main symptoms are flaccidity of leaflets which droop downwards forming the shape of human ribs in the leaves of outer and middle whorls of coconut palms. Later yellowing of leaves and marginal necrosis gradually develops. There is a progressive debilitation of the plant and reduction in nut yield, husk, copra, oil and leaf over a period of 10 years or more. The disease is non-curable but manageable by removing low yielding advanced diseased palms and planting seedlings from disease free mother palms/ disease tolerant varieties etc.

Infection by pathogen has been reported to bring about changes in the metabolic activities of the host plant. Superoxide dismutase (SOD), chitinase (Cht) and glucanase (Gluc) are enzymes which have been reported to be enhanced on infection by various pathogens in different plants (Moghaddam *et al.*, 2006; El-Khallal, 2007; Prasada *et al.*, 1987). But changes in these enzymes in coconut palms in relation to root (wilt) disease have not been studied. Succinate is reported to accumulate in phytoplasma infected *Catharanthus roseus* leaves. (Choi *et al.*, 2004). Succinate is converted to fumarate in the plant by the enzyme succinic dehydrogenase (SucDH). The activity of SucDH in root (wilt) diseased coconut palms has not been studied. Hence, the present study was conducted to assess the changes, if any, in the activities of SOD, Cht, Gluc and SucDH in root (wilt) diseased coconut palms, compared to healthy ones, with a view to find out whether these enzymes had any potential for detection of the disease or in imparting resistance to the host plant.

The enzymes were studied in healthy and RWD palms of disease tolerant - Chowghat Green Dwarf (CGD) - and susceptible - West Coast Tall (WCT) coconut cultivars. The healthy palms and diseased palms of CGD (10-year old) and WCT (40-years old) were selected from the farm of Regional station, CPCRI, Kayangulam., State Nursery farm at Karunagapally, and homestead gardens of farmers at Kottayam. Six to twelve leaflets of spindle leaves were cut from each of the adult palms of WCT and CGD cultivars for serological confirmation of disease and estimation of enzyme activities. Spindle leaves collected from palms in the field were immediately stored between frozen dry ice packs and then kept in refrigerator at -20°C for enzyme analysis and at 8-10°C for serology test. About 7-10 palms in each of the four category *viz.*, healthy, early, middle and advanced stages of the disease, were individually analyzed for all the four enzymes in WCT and only for two enzymes *viz.*, Cht and Gluc, in case of the CGD cultivar. The other two enzymes *viz.*, SOD and SucDH were analyzed in each of 3-9 healthy palms and 3-9 RWD palms (having all the three disease stages) in the CGD cultivar. All the palms were subjected to serological test (Sasikala *et al.*, 2010).

ml of 0.01M sodium phosphate buffer pH 7.6 using 1.5 g acid washed sand and liquid nitrogen. The homogenate was centrifuged at 14,000 (g) for 15 minutes. The supernatant was collected and diluted from 1 to 10 ml with the phosphate buffer (pH 7.6) before enzyme assay. The assay mixture consisted of one ml of 0.03 M sodium phosphate buffer (pH 7.8), 0.2 ml of 750 μ M nitroblue tetrazolium, 0.2 ml of 1.17 mM NADH, an aliquot (0.2 ml and 0.8 ml) of 1 to 10 diluted enzyme and aliquot of water to bring the total volume to 2.8 ml. Then 0.2ml of 150 μ M phenazine methosulfate was added and mixed and spectrophotometer reading taken at 560 nm at exactly 4 minutes after mixing. The blank contained 1.4 ml water and all other reagents and without enzyme. Log of absorbance values obtained for the 0.2 and 0.8 ml of diluted enzyme extract was plotted against the volume of the diluted enzyme. From this plot, the volume of diluted enzyme corresponding to Log of (half the absorbance of Blank) was determined for each sample which represented one enzyme unit.

SucDH. extraction and Assay: The enzyme was extracted and then assayed by the method of Kun and Abood (1949). Leaf lamina (1gm.) was finely shredded with scissors and homogenized in 10 ml of 0.1M sodium phosphate buffer pH 7.2 using 1.5 g acid washed sand and liquid nitrogen. The homogenate was centrifuged at 1500 (g) for 20 minutes. The supernatant was collected and used for assay. The assay mixture contained 1ml of 0.2M sodium succinate, 0.5 ml of 0.1M sodium phosphate buffer pH 7.2, 0.5 ml of 0.1% 2,3,5 triphenyl tetrazolium chloride (TTC) and 1ml of undiluted enzyme. The reaction was allowed for 4 and 6 minutes at 30°C. Thereafter it was stopped by adding 3.5 ml of acetone. In the blank (carried out in duplicates) 3.5 ml of acetone was added and mixed before adding the enzyme. After assay the contents were centrifuged at 2000 (g) at 4°C for 30 minutes. A set of standards was prepared by taking 1ml of 0.2M sodium succinate solution, 0.5 ml of 0.1M sodium phosphate buffer pH 7.2, aliquots of 25 to 100 μ l of 0.1% TTC and then distilled water (400 to 475 μ l) added to make total volume to 2 ml. Thereafter 1ml of freshly prepared sodium dithionite solution (15mg in 10ml distilled water) was added to the standards. Blank was prepared similarly except that 0.1ml of 0.1% TTC and 1.4ml of distilled water was added and sodium dithionite was not added to it. Finally 3.5ml of acetone was added to all the tubes, mixed and absorbance taken at 460 nm in a spectrophotometer. Enzyme activity was calculated for the assay durations of 4 and 6 minutes and their mean value are reported..

Cht. extraction and Assay: The enzyme was extracted and assayed by the method of Abeles *et al.* (1970). Leaf lamina (1 gm) was finely shredded with scissors and homogenized in 5 ml of 0.1M sodium citrate buffer (pH 5.0) along with 40 mg of polyvinylpyrrolidone using 1.5 g acid washed sand and liquid nitrogen. The homogenate was centrifuged at 10,000 (g) for 10 minutes. The supernatant was collected as the enzyme. The enzyme samples as well as enzyme blanks were assayed in duplicates. The reaction mixture in a total volume of 0.5ml contained, 0.06ml of sodium acetate buffer (233.3mM, pH 5.0), 0.04ml sodium azide solution (7.5mM), 0.1ml of chitin suspension (10 mg chitin/ml) and 0.3ml of the crude undiluted enzyme extract. In the enzyme blank, instead of crude enzyme extract, 0.3 ml of heat killed enzyme was added. For preparing the heat killed enzyme 1.0 ml of crude enzyme extract was heated on a burner and as soon as it reached boiling and frothing, it was immediately kept aside to cool to room temperature. The reaction mixture was incubated at 37°C in a shaking water bath for four hours. Then the reaction was stopped by adding 0.1ml sodium borate buffer (0.8M, pH 9.1) to all the microcentrifuge tubes and then centrifuged at 1000 (g) for 5

minutes. An aliquot of 0.3ml of the supernatant was used for the colorimetric determination of N-acetylglucosamine by the method of Reissig *et al.* (1955).

Gluc. extraction and assay : The enzyme was extracted and then assayed by a modified method of Hinton and Pressey (1974). Leaf lamina (1 gm) was finely shredded with scissors and homogenized in 5 ml of 0.1M sodium acetate buffer (pH 5.2) along with 40 mg polyvinylpyrrolidone using 1.5 g acid washed sand and liquid nitrogen. The homogenate was centrifuged at 12,000 (g) for 10 minutes. The supernatant was collected as the enzyme. The enzyme samples as well as enzyme blanks were assayed in duplicates. The reaction mixture consisted of 4 ml of 0.5 % carboxy methyl cellulose , 1 ml of 0.1 M acetate buffer (pH 5.2) and 0.5 ml of crude enzyme extract. In the enzyme blank, 0.5 ml of heat killed enzyme instead of crude enzyme extract was added. For preparing the heat killed enzymes 1.5 ml of crude enzyme extract was heated on a burner and as soon as it reached boiling and frothing, it was immediately kept aside to cool to room temperature. The reaction mixtures in all the test tubes were mixed thoroughly and incubated at 37° C for 60 minutes in a water bath incubator. After incubation an aliquot of 1 ml of the reaction mixture were taken from all the tubes and glucose estimated in it, by the method of Somogyi (1952).

Soluble protein content in enzyme extracts: For protein estimation, 0.5 ml of crude enzyme was mixed with 0.5 ml of 20% TCA in a 1.5 ml microcentrifuge tube and left for 15 minutes and again mixed and left for another 15 minutes. The solution was then centrifuged at 4° C at 3000 rpm for 10 minutes in a biofuge. The supernatant was discarded and the precipitate dissolved in 1 ml of 2N sodium hydroxide solution. From this solution 0.1 and 0.2 ml aliquots were taken and protein estimated by the method of Lowry *et al.* (1951).

SOD activity as well as its specific activity in spindle leaf of healthy and root (wilt) diseased CGD and WCT palms are presented in Fig. 1. No significant difference in these two characters was observed between healthy and root (wilt) diseased coconut palms in both the cultivars. Sunukumar *et al.* (2010) reported significantly higher SOD activity in RWD coconut palms (from diseased tract) compared to healthy ones (from disease free tract). In the present study both healthy and diseased palms were selected from the same tract and no difference in SOD activity were found. Similarly, succinic dehydrogenase and chitinase activities and their specific activities were unaltered due to phytoplasma infection in both the cultivars (Figs. 2 and 3). Succinate is reported to accumulate in phytoplasma infected *Catharanthus roseus* leaves. (Choi *et al.*, 2004). Based on this it was considered that succinic dehydrogenase activity, which converts succinate to fumarate in the plant, may be altered in the plant system due to phytoplasma infection. But the current results indicated that succinic dehydrogenase activity is not altered. El-Khallal (2007) found chitinase activity to increase in tomato leaves on infection with *Fusarium oxysporum* which causes wilt disease of tomato. This response is generally attributed to the presence of chitin in cell walls of fungi which is the substrate for chitinase enzyme. As phytoplasma lack cell wall, similar response of chitinase activity in root (wilt) affected coconut palms could not be expressed and was not observed. Padmaja *et al.* (1981) found significantly higher soluble protein content in leaves of healthy palms (from disease free tract) as compared to RWD palms (from diseased tract). But they found no difference in soluble protein content between healthy and diseased palms from the same diseased tract. Similar results have been found in the present study also, wherein, the soluble protein content of the enzyme extracts was similar in both healthy and diseased palms, irrespective of

the cultivars used (Fig. 1 and 4). But in the case of SucDH and chitinase extract from CGD cultivar (Fig. 2 and 3), healthy palms and/or early diseased stage palms contained significantly higher soluble protein than diseased palms/middle and advanced diseased palms respectively. Glucanase activity as well as its specific activity were significantly higher in advanced stage of RWD in CGD cultivar but no such differences were seen in the WCT cultivar. Generally at the advanced stage of RWD the tips of leaflets start necrotting and at this stage 1,4- α -glucanase is produced to digest the plant cell wall. Probably this may be the case for the higher glucanase activity only in the advanced stage of RWD. These results agree with that reported by Padmaja and Amma (1979) who found that the cellulase (glucanase) specific activity significantly increased in decayed roots of diseased palms as compared to those from healthy palms.

On an average, CGD cultivar had significantly higher SOD and SucDH activities as well as soluble protein content of these enzyme extracts than the WCT cultivar (Figs. 1 and 2), but the reverse was seen in case of SOD specific activity (Fig.1). CGD and WCT cultivars had similar levels of SucDH specific activity, Cht and Gluc activities as well as their specific activities and soluble protein contents (Figs. 3 and 4). The higher SOD and SucDH activities as found in CGD than in WCT indicate a higher potential in clearing superoxide radical and

Fig. 1. Superoxide dismutase activity, specific activity and soluble protein in spindle leaf of healthy and root (wilt) diseased coconut palms (CGD and WCT cultivars)

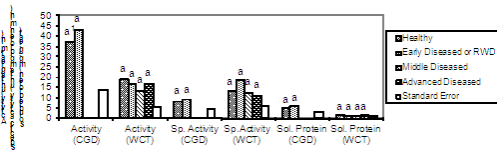
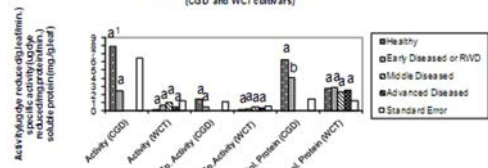


Fig. 2. Succinic dehydrogenase activity, specific activity and soluble protein in spindle leaf of healthy and root (wilt) diseased coconut palms (CGD and WCT cultivars)



succinate and may be contributing towards better tolerance of CGD to RWD than WCT. Moghaddam *et al.* (2006) found no difference in SOD activity in leaves of strawberry cultivars that were resistant and tolerant to *Mycosphaerella fragariae*. They found higher SOD activity,

Fig. 3. Chitinase activity, specific activity and soluble protein in spindle leaf of healthy and root (wilt) diseased coconut palms (CGD and WCT cultivars)

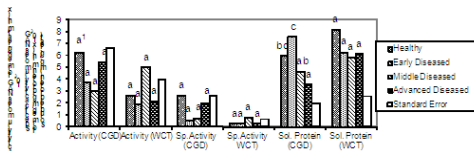
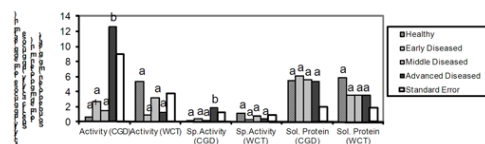


Fig. 4. Glucanase activity, specific activity and soluble protein in spindle leaf of healthy and root (wilt) diseased coconut palms (CGD and WCT cultivars)



only for a short period, in the resistant cultivar as compared to the tolerant one, after infection by pathogen.

The results of the present study, therefore, indicated that changes in the activities of SOD, SucDH, Cht and Gluc due to root (wilt) disease were not consistent in the two cultivars for use in detection of RWD. The changes also do not seem to be imparting resistance against the disease. However, CGD cultivar does have a higher potential of SOD and SucDH activity which may be related to its better tolerance to RWD than WCT. Bars, within each cluster, having any common alphabet above them are not significantly different at 5% probability level NAG=N-acetyl glucosamine

References

- Abeles, F.B., Bosshart, R.P., Forrence, L.E. and Habig, W.H. 1970. Preparation and purification of glucanase and chitinase from bean leaves. *Plant Physiol.* **47**: 129-134.
- Choi, Y.H., Tapias, E.C., Kim, H. K., Lefeber, A.W.M., Erkelens, C., Verhoeven, J.Th.J., Brzin, J., Zel, J., Verpoorte, R. 2004. Metabolic Discrimination of *Catharanthus roseus* Leaves infected by Phytoplasma Using ¹H-NMR Spectroscopy and Multivariate Data Analysis. *Plant Physiol.* **135**: 2398-2410.
- El-Khallal, S. M. 2007. Induction and Modulation of Resistance in Tomato Plants against *Fusarium* wilt disease by Bioagent Fungi (Arbuscular Mycorrhiza) and or hormonal elicitors (Jasmonic acid and Salicylic acid); 2-changes in the antioxidant enzymes, phenolic compounds and pathogen related proteins. *Australian Journal of Basic and Applied Sciences* **1**(4): 717-732.
- Hinton, D.M. and Pressey, R. 1974. Cellulase activity in peaches during ripening. *J. Food Sci.* **39**: 783-785.
- Kun, E. and Abood, L.G. 1949. Colorimetric estimation of succinic dehydrogenase by Triphenyltetrazolium chloride. *Science* **109**: 144-146.
- Lowry, O. H., Rosenbrough, N. J., Farr, A. L. and Randall, R. J. 1951. Protein measurement with the Folin Phenol reagent. *J. Biol. Chem.* **193** : 265.
- Moghaddam, B.E., Charles, M.T., Carisse, O. and Khanizadeh, S. 2006. Superoxide dismutase responses of strawberry cultivars to infection by *Mycosphaerella fragariae*. *J. Plant Physiol.* **163**(2): 147-153.
- Nishikimi, M., Appaji Rao, N. and Yogi, K. 1972. The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. *Biochem. Biophys. Res. Commun.* **46**: 849.
- Padmaja, G. and Amma, B.S.K. 1979. Cellulase activity in the roots of coconut palms affected by root (wilt) disease. *J. Plantn. Crops* **7**(2): 101-104.
- Padmaja, G., Amma, B.S.K., Mathew, C., Nambiar, P.T.N. and Dwivedi, R.S. 1981. Alterations in the leaf protein content of coconut affected by root (wilt) disease. *Ind. J. Pl. Physiol.* **24**: 42-46.
- Prasada, B.K., Sinha, T.S.P. and Shanker, U. 1987. Biochemical changes in tomato fruits caused by *Sclerotium rolfsii*. *Indian Journal of Mycology and Plant Pathology* **17**(3): 316-320.
- Reissig, J.L., Strominger, J.L. and Leloir, L.F. 1955. A modified colorimetric method for the estimation of N-acetyl amino sugars. *J. Biol. Chem.* **217**: 959-966.
- Sasikala, M., Rajeev, G., Prakash, V.R. and Amith, S. 2010. Modified protocol of ELISA for rapid detection of coconut root (wilt) disease. *J. Plantn. Crops* **38**(1): 16-19.
- Solomon, J.J., Govindankutty, M.P. and Nienhaus, F. 1983. Association of mycoplasma-like organisms with coconut root (wilt) disease in India. *Z. Pflkrankh. Pflschutz* **90**(3): 295-297.
- Somogyi, M. 1952. Notes on sugar determination. *J. Biol. Chem.* **195**: 19-23.

Sunukumar, S.S., Thelly, M.T., Murugan, K., Ajitkumar, C.E. and Mohankumar, C. 2010. Phytoplasma induced hypersensitive response of root (wilt) diseased coconut palms of Kerala, India. Golden Jubilee National Symposium 2010: Plant Diversity – Utilization and Management. pp.161-168. Department of Botany, University of Kerala, Thiruvananthapuram-34, Kerala, India.

Root (wilt) disease of coconut was first noticed in the erstwhile princely state of Travancore around 1874 and became very much evident in 1882. The disease was initially reported from

Management strategies for root (wilt) disease of coconut

V. Krishnakumar

Central Plantation Crops Research Institute
Regional Station, Kayamkulam-690 533, Kerala, India

Erattupetta area of Meenachil taluk in Kottayam district (Butler, 1908; Pillai, 1911). Around 1907, the disease was reported from Kaviyoor and Kallooppa areas of Tiruvalla taluk in Pathanamthitta district and later from Kayangulam area of Karthikappally taluk in Alappuzha district. The disease, which appeared almost simultaneously in the above foci, began to spread to adjoining areas (Varghese, 1934).

According to a survey during 1984-85, (Anon., 1985) the disease was prevalent in a contiguous manner in an area of 4.10 lakh ha in eight out of the fourteen districts of Kerala. Now it occurs sporadically in the remaining six northern districts of the State and bordering areas of Tamil Nadu such as Shenkotta, Coimbatore, Cumbam, Pollachi and Kulasekharam; Dakshina Kannada district of Karnataka and in Goa. The disease intensity in the contiguous diseased tract during 1984-85 ranged between 1.5 % in Thiruvananthapuram district to 75.6 % in Kottayam district (Solomon and Geetha, 2004). However, according to a survey of 1996, this disease percentage ranged from 2.09 in Thiruvananthapuram district to 48.03 in Alappuzha district (Anon., 1997). The overall reduction in disease incidence from 32.37 to 24.05 % was attributed to the removal of about 59 lakh diseased palms, replanting with quality seedlings, adoption of management practices and crop conversion from coconut to rubber.

Flaccidity, yellowing and necrosis of margins of leaflets of the leaves of central and outer whorls of coconut palms were considered to be the typical foliar symptoms (Varghese, 1934; Menon and Nair, 1952; Menon and Pandalai, 1958). Radha and Lal (1972) recorded flaccidity, the characteristic bending or ribbing of leaflets as the earliest consistent visual symptom.

Root (wilt) disease of coconut caused by Phytoplasma is non-lethal but debilitating and any definite therapeutic control measures are yet to be developed. However, the bearing palms which are in the initial to middle stage of disease intensity respond well to the management practices. Experiments carried out in the past have clearly brought out results in favour of formulating strategies for containing the disease in its present geographical limits (Anon., 1986) and managing the disease for sustained productivity of the palms (Sahasranaman *et al.*, 1964; Menon and Nayar, 1978; Muralidharan *et al.*, 1986; Rajagopal *et al.*, 1987; Maheswarappa *et al.*, 2005; Krishnakumar and Maheswarappa, 2010). The health and yield of palms could be maintained through the adoption of integrated management practices consisting of balanced fertilizers, addition of organic matter and recycling on farm waste biomass, raising green manure crops in the basin and incorporation, weed control, intercropping and mixed farming, leaf rot control etc.

Some of the predisposing factors considered to be associated with the disease were soil sickness caused by low pH, inadequate drainage, poor aeration, low microbial activity and nutrient imbalances etc. (Pandalai *et al.*, 1958 a, b).

Healthy pockets surrounded by diseased gardens and individual healthy palms in the midst of diseased palms without any spread to neighbouring palms for several years was also noticed (Menon, 1943). Pillai (1981) reported that the rate of spread of the disease to be 1 to 4 km/year from the nearest source of inoculum. Relatively higher disease incidence was noticed in waterlogged low-lying areas adjacent to rivers and canals and in kari soils (Pillai *et al.*, 1973; Cecil and Amma, 1991).

The survey conducted by Pillai *et al.* (1973) indicated that the root (wilt) disease has been found to occur on all major soil types of Kerala under varying ecological conditions ranging from foot hills of Western Ghats to the coastal plains. However, the spread of disease has been faster on light textured sandy, sandy loam and alluvial soils, particularly in low-lying areas and also on heavy textured clays compared to laterite soils. However, subsequent detailed analysis of soil and tissue samples from disease endemic and disease free areas in Kerala have proved that there is no possibility of the direct involvement of any major nutrients in the incidence of the disease (Pillai *et al.*, 1975).

Cecil *et al.* (1991) made a critical evaluation of various earlier studies on the quality of nutrition in relation to disease and concluded that the palms in the disease affected areas, whether apparently health or visibly disease free are in a state of unbalanced nutrition, possibly the result of a relatively higher content of N, P and K on the one hand and a lower content of Ca, Mg and S on the other.

Nutritional studies with different levels of major and secondary nutrients, such as Ca and Mg could neither control the disease nor prevent the fresh incidence of the disease (Cecil and Amma, 1991). Any role of micronutrients in the incidence of the disease has also been ruled out (Mathai and Vasudevan Nair, 1977; Khan *et al.*, 1985; Anon, 1986). Addition of Magnesium sulphate (3.0 kg/palm/year) prevented development of foliar yellowing and increased yield of nuts by about 40 % in the early bearing periods (Amma *et al.* 1983; Anon, 1986). The beneficial effects of Mg were more pronounced on root (wilt) disease affected palms than on healthy palms. The performance of D x T hybrid studied up to 12 years gives indication that this hybrid with adequate management from field planting can perform better than local WCT.

Strategies for root (wilt) disease management

The initial attempt for the management of the problem was eradication of disease affected palms to contain the disease within the contiguously infected geographic limits and it was very successful. Uprooting and burning of root (wilt) disease affected palms which occurred in an isolated garden in Vellum village, Shencottai in Tamil Nadu during 1971 could prevent recurrence of the disease in the area (Anon., 1986). Subsequently a buffer zone in the sparsely affected areas north of Karuvannoor River in Thrissur District was created by eradicating nearly 28,000 diseased palms. Removal of diseased palms was continued as and when noticed in succeeding years. These operations have brought down the recurrence of the disease considerably.

According to Ramadasan *et al.* (1971), juvenile palms that contract the disease before the onset of flowering seldom yield and hence all such palms as well as disease advanced palms are to be removed systematically. Such areas are to be replanted with disease free and quality planting materials. It was found that disease advanced palms never respond to any kind of management practices (Muralidharan *et al.*, 1986).

Root regeneration: Roots of disease affected palms were found to exhibit derangements in root functioning, water relations, mineral nutrition, respiration and photosynthesis (Rajagopal *et al.*, 1998). In order regenerate roots of diseased palms, studies using various growth hormones (IBA and NAA), thiamine (amino acids, vitamins, glutamic acid) and phenols (chlorogenic acid, caffeic acid, gallic acid and coumarin) were conducted. Application of IBA+ phenols (@500 ppm and 400 ppm, respectively) produced 51 fresh roots compared to two roots in the control (Amma and Patil, 1982). A reduction in disease index from 35 to 26 within a period of one year was also observed. Dwivedi *et al.* (1980) reported that ascorbic acid (400 ppm fed through cut root) not only reduced disease symptoms but also improved the yield of palms. However, studies made by Rajagopal *et al.* (1986b) did not find any positive response for application of ascorbic acid in reducing the disease indices or increasing the yield.

Basin management: Raising green manure crops in coconut basins and their incorporation was found to add organic matter and improve soil nutrient supplying capacity. Green manure crops, which are of short duration (around 90-100 days), when grown in the coconut basins could provide about 20-25 kg fresh biomass. These leguminous green manure crops fix atmospheric N in soil and decompose very fast up on incorporation. Glyricidia can also be grown in boundaries of coconut gardens and green leaves used for adding organic matter to soil.

A field experiment was conducted in a farmer's garden with coconut palms in the early and middle stages of root (wilt) disease to study the influence of basin management with green manure legumes along with recommended dose of fertilizers and 500 g MgO on disease (Thomas *et al.*, 1993). It was found that the biomass generated by three green manure crops viz., *Pueraria phaseoloides*, *Mimosa invisa* and *Calopogonium mucunoides* during the a growth period of 130 days in coconut basin was 24.6, 27.9 and 24.9 kg/basin (Table 1).

Table 1. Effect of basin management with green manure legumes on root (wilt) disease and yield of coconut palms

Treatments	Root(wilt) disease index		Nut yield /palm/year	
	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment
<i>Pueraria phaseoloides</i>	12.38	16.58	39.51	63.84
<i>Mimosa invisa</i>	18.00	17.91	32.32	54.46
<i>Calapagonium mucunoides</i>	21.12	20.91	42.96	52.21
Control	19.38	24.18	26.50	38.93
LSD(P=0.05)		NS		17.12

Though no significant change in the disease condition of the palms due to legume treatment was noticed, the yield of palms increased as a result of legume treatment, fertilizer application and adoption of plant protection measures.

Basins of 1.8 m radius away from bole of coconut palm after application of first round of fertilizers may be taken and 100 g seed of green manure crop sown along with receipt of rains in May-June. The plants are to be allowed to grow till they attain maximum vegetative growth (say 90 days). When one or two plants start flowering, the entire plants are to be uprooted and incorporated doe decomposition and addition of organic matter. The green

manure cowpea (*Vigna unguiculata*) plants could supply nutrients such as N,P and @ 130 g, 10 g and 110 g (on dry weight basis), respectively per basin.

Irrigation: Flaccidity is considered to be the main distinguishing symptom of root (wilt) disease. Rajagopal *et al.* (1986 a) opined that the flaccidity on leaflets of root (wilt) affected coconut palms were associated with impaired stomatal mechanism leading to increased rate of transpiration. Summer irrigation was found to increase water up-take and reduced transpiration rate of root (wilt) affected palms, thus bringing about balanced water economy to the disease affected palms (Rajagopal *et al.*, 1986b). Irrigating coconut palms during the summer months @ 250 l water per palm per week resulted in significant decline in disease index (Rajagopal *et al.*, 1987). The irrigation and fertilizer applied palms showed 69.7 % improvement while there was 78.5 % deterioration when both these inputs were not provided (Table 2).

Table 2. Effect of summer irrigation on the condition of root (wilt) disease affected coconut palms

Treatments	No.of palms	No.of palms showing		
		Improv- ement*	Deterior- ation**	No change
Irrigation + fertilizers	33	23(69.7)	5(15.1)	5(15.1)
No irrigation+ fertilizers	46	14(30.4)	20(43.4)	12(26.2)
No fertilizer, no irrigation	14	2(14.3)	11(78.5)	1(17.2)

Values in brackets indicate the percentage on total number of palms observed

*Improvement refers to decline in disease index over pre-treatment

** Deterioration means increase in disease index over pre-treatment

There was significant reduction to the extent of 51, 60, 40, 60 and 45 %, respectively in flaccidity, yellowing, leaf rot, senescence and necrosis due to irrigation over the pre-treatment. There was an overall increase of 53.3 % of the photosynthetic area with an increase in yield of 10 nuts per palm over the pre-treatment.

The coconut palms are to be irrigated, by adopting suitable method, depending on the availability of water. Hose irrigation in coconut basin may be adopted by supplying water @ 200-250 l once in four days for yielding palms and @ 75 to 80 l water for young palms (3-4 years). If drip irrigation could be adopted, water may be supplied @ 30-32 l water/day through four emitters for mature palms and @ 20 l water/day using emitters for young palms.

The coconut basins are also to be protected from summer period, for which mulching with available materials such as coconut leaves, composted coir pith, coconut husk or other dry leaves. Mulching may be done after the application of second dose of chemical fertilizers (post-monsoon) and retained till April-May.

Since water logging hampers root respiration and uptake of nutrients, adequate measures are to be taken to improve drainage in the coconut garden.

Integrated management approaches

Adoption of integrated management practices like irrigation, plant protection measures, mixed cropping and fertilizer application increased the average yield of palms in a

root (wilt) disease affected garden from 17.6 to 46.0 nuts/palm/year (Anon., 1982). A large scale demonstration on the eradication of root (wilt) disease affected palms in a mildly infected area of Thrissur district (Anon., 1986) revealed an improvement in yield from 20.2 to 49.2 nuts/palm due to the adoption of improved management practices over a period of four years (1981 to 1985). Muralidharan *et al.* (1986) reported beneficial effect of integrated management practices on yield and disease condition of palms. An average increase of 24.3 nuts/ palm/ year was observed under rainfed conditions. This response was mainly derived from the apparently healthy and disease early palms. Increase in yield by adopting management practices was also observed under irrigation.

Recycling of biomass generated in the coconut garden through vermicomposting and application to coconut palms helps to reduce the chemical fertilizer requirement. The results of study conducted in this aspect by Krishnakumar and Maheswarappa (2010) indicated that through adoption of integrated nutrient management practices (i.e. application of 50% of recommended chemical fertilizers + 25 kg organic manure/palm as vermicompost + incorporation of basin raised green manure crop), the health of palms could be improved over the years or maintained without further deterioration (Table 3).

Table 3. Percentage of coconut palms under different root (wilt) disease intensity categories

Treatment	DI category	Year			
		2005	2006	2007	2008
50% fertilizers+25 kg organic manure (as vermicompost+incorporation of basin raised green manure crop)	DE (DI<20)	20.5	20.5	29.5	36.4
	DM1 (20<DI<35)	56.7	56.7	61.4	54.5
	DM2 (35<DI<50)	22.8	22.8	9.1	9.1
Recommended dose of fertilizers alone (500:300:1000 g NPK/palm/year)	DE (DI<20)	12.1	15.2	42.4	51.5
	DM1 (20<DI<35)	78.8	75.8	54.5	48.5
	DM2 (35<DI<50)	9.1	9.0	3.1	0

DE= Disease Early DM= Disease Middle DI= Disease Index

The yield of coconut palms under different categories of root (wilt) disease is presented in Table 4.

Table 4. Yield (nuts/palm/year) and number of palms under different disease index categories during 2005 and 2007

Disease index categories and index during 2007	Disease index categories and index during 2005			
	Disease Early (DE)	Disease Middle 1 (DM1) (DI<20)	Disease Middle 2 (DM2) (20<DI<35)	Average (35<DI<50)
Disease Early	65.4 (13)	64.8 (18)	0	65.0 (31)
Disease Middle 1	(0)	53.9 (41)	41.9 (4)	52.8 (45)
Average	65.4 (13)	57.2 (59)	41.9 (4)	57.8 (76)

Figures in parenthesis indicate number of palms

The results also indicated that there was an increase in the percentage of palms from DM2 category to DM 1 or from DM 1 to DE over a period of time. For instance there were 59 palms in the DM1 category during 2005, of which 18 were shifted to disease early in the course of study. Average yield of palms (post-treatment) against the cross classification of disease categories (based on disease index during 2005 and 2007) is also presented in Table 4. There was significant increase in nut yield between Disease Early (65.) and Disease Middle 1 (52.8) palms.

Cropping system approach

Coconut palms, by virtue of its canopy architecture, provide ample scope for inclusion of an array of compatible companion crops in the garden. However, in majority of cases, where homestead type of system is noticed, overcrowding with various tree crops etc. is quite common. In such cases, regulation of shade of trees and other mixed crops is necessary to allow penetration of sufficient light into the coconut garden. Systematically maintained coconut based cropping system was found to increase the overall income from the garden. Adoption of multiple cropping and mixed farming systems in root (wilt) affected coconut gardens have indicated to exert ameliorating effect on the disease affected palms.

Menon and Nayar (1978) reported that intercropping with tuber crops (cassava, elephant foot yam and greater yam) for five years in a 16 year old coconut garden affected by of root (wilt) disease gave an overall increase in nut yield to the extent of 5, 15 and 8, percent respectively. They also reported that though there was an increase in disease intensity (indices 32.1 to 37.3) in plots intercropped with cassava, in other plots where intercropped was done with elephant foot yam and greater yam, there was a decline (37.0 to 33.3).

An increase in yield of disease affected palms (27 to 35 per cent) was reported by Amma *et al.* (1983) as a result of mixed cropping with cocoa. The increase was maximum under double hedge systems of cocoa planting. The superiority of double hedge system in increasing the yield of coconut was also reported earlier by Nair *et al.* (1975).

Adoption of mixed farming, wherein animal husbandry component is also integrated with crop production system, generates considerable quantities of organic waste biomass and they could be effectively recycled for improvement of soil health. Sahasranaman *et al.* (1983) investigated the effect of mixed farming on disease amelioration, yield of root (wilt) affected palms and soil fertility. They observed considerable reduction in foliar yellowing due to mixed farming, but flaccidity and necrosis increased. Beneficial effects like rejuvenation of roots of disease affected palms, increase in soil organic carbon, available P and exchangeable Ca, Mg and K in the sub-soil were also reported. A net increase of 28.1 % in the yield of root (wilt) affected palms was also reported (Table 5). The response was high in disease early palms and low in disease advanced palms.

Table 5. Effect of mixed farming on yield of root (wilt) diseased palms

Disease Index	Nut yield				Increase over pre-treatment (%)		Estimated net response due to mixed farming(%)
	Pre-treatment yield		Post-treatment yield				
	Control	Treatment	Control	Treatment	Control	Treatment	
0-10	58.3	50.3	62.4	68.4	7.0	36.0	31.6
11-25	31.1	33.7	29.2	42.3	-6.1	25.5	
26-50	20.9	22.5	18.1	24.5	-13.4	8.9	
> 51	19.5	19.0	21.4	23.7	9.8	24.7	
Overall	32.5	31.4	29.8	39.6	-2.0	26.1	

Maheswarappa *et al.* (2005) from the High Density Multispecies Cropping System (involving component crops such as black pepper, nutmeg, banana and pineapple) studies at CPCRI, Regional Station from 1998 to 2002 noticed increase in nut yield to the extent of 54.5%, 52.0%, 48.3% and 40.9 % under Apparently Healthy (AH), Disease Early (DE), Disease Middle (DM) and Disease Advanced (DA) indexed palms, respectively compared to pre-experimental yield (Table 6).

Table 6. Coconut yield and copra yield as influenced by integrated management practices in HDMSCS

Disease index	Avg. nut yield (nuts/palm)		Copra content (g/nut)	Copra out turn (kg/palm)
	Pre-experimental (1991-93)	Average (1998-2002)		
AH(12 palms)	44	68.0	179.3	15.6
DE(33 palms)	40	60.8	182.4	13.5
DM(59 palms)	36	53.4	181.7	10.0
DA(8 palms)	21	29.6	180.6	4.7

Improvement in yield has been noticed from third year onwards after imposing integrated nutrient management practices. Apparently healthy palms recorded the highest yield followed by disease early and disease middle palms. Copra content of palms under different disease category did not differ much. Adoption of integrated nutrient management practices resulted in copra development to the optimum potential of palms. However, the copra out turn was the lowest for disease advanced palms, as a result of lower number of nuts produced. They also observed reduction in root (wilt) disease index after four years to the extent of 2.9 in disease early, 5.6 in disease middle and 6.4 in disease advanced palms due the integrated management practices (Table 7).

Table 7. Root (wilt) disease index of coconut palms as influenced by integrated nutrient management practices

Disease index	During June,1998	During July,2002	Increase(+) Decrease(-)
DE(<20) (33 palms)	15.1	12.2	-2.9
DM(20-50) (59m palms)	41.4	35.8	-5.6
DA (>50) (8 palms)	60.8	54.4	-6.4

The performance of root(wilt) disease affected coconut palms applied either with 50% recommended fertilizers + organic manure through vermicompost and incorporation of *in situ* raised green manure cowpea (*Vigna unguiculata*) or 100 % recommended fertilizers alone in a coconut based high density multispecies cropping system was studied by Krishnakumar and Maheswarappa (2010). Though there was a slight improvement in yield in the post-treatment period in both the treatments (Table 8), it was found to be non-significant. However, there was an increase of 17 % in yield in palms receiving both fertilizers and organic manures compared to 7 % increase in palms receiving only fertilizers. Further the correlation analysis indicated negative correlations between yield (2006- 2007) and disease index in the years 2005, 2006 and 2007 and is respectively -0.223, -0.268 and -0.284 ($P < .05$). The general tendency in palms affected by the root (wilt) disease is that yield gradually goes down either due to premature button shedding or due to decrease in production of female flowers.

Table 8. Overall mean yield of coconut palms (nuts/palm/year)

Treatment	Pre-treatment yield(2003-04)	Post-treatment yield(2006-07)
50% Fertilizers +Organic manure	51	60
Fertilizers alone	53	57
CD($P=0.05$)	Not significant	

It is evident that through an integrated nutritional management, the health of palms could be improved over the years or maintained without further deterioration, thereby bringing more palms from the disease middle category to disease early category.

Management of disease and pests

Leaf rot disease often superimposes with root (wilt) and, therefore, control of this disease as a part of root (wilt) management becomes inevitable. Even though the root (wilt) is not amenable to conventional crop protection measures, leaf rot control is possible. A systems approach with integrated measures is suggested in which various methods are integrated (Srinivasan, 2010).

- ❑ Remove all disease advanced and uneconomic palms [both root (wilt) alone and root (wilt) with leaf rot]. If replanting is envisaged, use only healthy seedlings from elite palms and varieties or hybrids tolerant to root (wilt)/leaf rot.
- ❑ Adopt general recommended practices as applicable to root (wilt) affected regions such as application of balanced dose of fertilizers and organic manure and growing green manure crops (for incorporation in the coconut basins); provision of proper drainage and irrigating the palms during summer.
- ❑ Regular monitoring of individual palms, especially the emerging spindle and young leaves. As a prophylactic measure, cut and remove the infected portions of spindle and other young leaves close to it. This helps to reduce fungal inoculum load in the crown, thereby avoiding possibility of infection by about 50%.
- ❑ Use of fungicide – either Hexaconazole (Contaf 5E) @ 2 ml or Mancozeb (Dithane M-45/Indofil M-45) @ 3 g in 300 ml of water and pour into the axil of the spindle leaf of every palm. Alternatively, talc formulation of biological control agents viz., *Pseudomonas*

fluorescens or *Bacillus subtilis* (either singly or in combination) @ 50 g mixed in 500 ml of water may be applied for every palm. Usually no infection occurs in the maturing (green) leaves and hence application may be restricted to the tender susceptible portions only.

The above mentioned management practices are to be taken up at least twice per year (April – May, October – November) for all palms in the garden. Control of leaf rot helps to produce healthy foliage of the root (wilt) infected coconut palms, and hence, adoption of such an approach improves the overall health of the palm and thereby ensures better productivity.

Rhinoceros beetle (*Oryctes rhinoceros* Linn.), and red palm weevil (*Rhynchophorus ferrugineus* Oliv.) are the most important pests infesting coconut and the recommended practices for mechanical, cultural and chemical control measures are to be adopted for their management(6.2b).

Use of seedlings produced from disease tolerant/resistant mother palms

The search for resistance to coconut root (wilt) disease started with Butler (1908) who suggested that there could be resistance to coconut root (wilt) disease in the local cultivars grown in the diseased tracts. Davis (1953) reported occurrence of high yielding palms among the heavily diseased palms. Rawther and Pillai (1972) studied the average yield in both healthy and diseased coconut palms and also disease incidence in various dwarfs, hybrids and WCT. They found that Dwarf x Tall (natural cross) gave higher yield in both healthy and diseased palms compared to other varieties. Dwarf x Tall (natural cross) also had the lowest percentage of disease incidence (4.6). West Coast Tall had the highest disease incidence (48.5 %) and the lowest yield. All the other varieties and hybrids were superior to WCT in disease resistance and yield performance. Ninan (1978) reported from a survey of different varieties such as WCT, Dwarf Green, Dwarf Orange and their hybrids that Chowghat Green Dwarf (CGD) palms were most tolerant to root (wilt) disease with 92.17 % palms free from the disease.

In an intensive survey in large number of farmers' coconut gardens in the 'hot spot' districts of Alappuzha, Pathanamthitta, Kollam and Kottayam, 200 Chowghat Green Dwarf (CGD) palms were observed for their resistance to root (wilt) disease, and among them 75 % were disease-free indicating that CGD is having higher level of resistance to root (wilt) disease when compared to other varieties.

During 1987, a comprehensive breeding programme for evolving resistant / tolerant variety to root (wilt) disease was initiated at CPCRI, Regional Station, Kayamkulam (Nair *et al.*, 1996). The following observations formed the basis of breeding for resistance to coconut root (wilt) disease in coconut.

- ❑ Chowghat Green Dwarf (CGD) is having higher level of resistance compared to other varieties.
- ❑ In the disease endemic areas, in the midst of heavily disease-affected palms, disease-free and high yielding WCT palms were available. These disease-free palms were identified as the base material for the breeding programme.

Seed nuts from such disease-free mother palms were collected and seedlings were produced and distributed to farmers for field planting in root (wilt) disease affected areas.

Technology Transfer of integrated root (wilt) management package – Experiences from the farmer participatory programmes

In order to demonstrate the effectiveness of various integrated root (wilt) management package developed by CPCRI, Participatory Technology Transfer Approach (PTTA) was taken up in Alleppey district (Kerala state) in a contiguous area of 25 ha around the institute with maximum disease intensity and involving 208 farm families with 5000 palms in different growth stages (Anithakumari *et al.*, 2004). The recommended technologies were adopted in the farmers' gardens to improve the productivity and to create awareness and popularize root (wilt) management technologies among the stakeholders. Majority of the households had less than 0.01 ha of land, only 2 per cent had above 0.4 ha area under coconut.

The adoption of various integrated root (wilt) management practices was found to be effective in improving the average yield of root (wilt) affected palms. The average yield increased from 24.17 to 46.30 nuts/ palm/year after three years, recording an improvement of 91.40 %. The improvement of the initiation of flowering of coconuts from 2.0 to 12.2 % indicated the need for scientific management of palms of all stages in the root (wilt) affected gardens. The impact of the root (wilt) disease management technologies is presented in Table 9.

Table 9. Impact in yield of root (wilt) disease affected palms due to management practices in farmers' fields

	Before technology adoption	During project implementation	After technology adoption
Average yield (nuts/palm/year)	24.17	32.5 (34.3 % improvement)	46.3 (91.4% improvement)
B:C ratio	1.03	1.38	1.77
Yield categorization		Before technology adoption (%)	After technology adoption (%)
Started flowering		2.0	12.2
Very low yield (Less than 10 nuts/ palm/year)		6.5	4.3
Low yield(10 – 20 nuts/palm/year)		26.0	13.6
Medium yield (21-50 nuts/palm/year)		19.0	31.3
High yield (Above 50 nuts/palm/year)		7.5	20.7
Non bearing palms		39.0	12.7
Disease advanced palms cut and removed - 5.2%			

There was considerable improvement in the performance of palms under various yielding categories, wherein the high yielding palms increased from 7.5 % to 20.7 %. The increase noticed in the case of medium yielding palms was from 19 % to 31.3%, whereas, both very low and low yielding palms decreased almost 50 % compared to before technology adoption period.

The management of other pests and diseases is also very important in determining the overall performance of the palms of the area. Leaf rot disease was found to occur superimposed on 65 % of the root (wilt) affected palms and hence, most of the farmers could realize the adverse effect of the root (wilt) disease incidence only after the incidence of leaf rot disease was noticed. Adoption the leaf rot management technology recommended by CPCRI brought down the initial severity of the disease from 47.9 % 1.5 % resulting in improvement of overall health and appearance of the palms.

Root (wilt) disease management strategies

Based on the available experimental findings, the following management practices are recommended.

1. Apply balanced doses of fertilizers (500 g N, 300 g P_2O_5 , 1000 g K_2O and 500 g MgO/palm/year) in two splits. One-third dose is to be applied during April-May and two-thirds during September-October for rainfed palms and in four splits during January, April, July and October for irrigated palms. Magnesium sulphate (@1 kg per palm per year) is to be applied along with the second dose of other fertilizers.
2. Adopt control measures for disease (leaf rot) and pest (rhinoceros beetle) as detailed below.
 - a) Cut and remove leaf rot affected portions of only spindle and two top most fully opened leaves. Apply either Hexaconazole (Contaf 5E) @ 2 ml or Mancozeb (Dithane M-45/Indofil M-45) @ 3 g in 300 ml of water and pour into the axil of the spindle leaf of every palm. Alternatively, talc formulation of biological control agents viz., *Pseudomonas fluorescens* or *Bacillus subtilis* (either singly or in combination) @ 50 g mixed in 500 ml of water may be applied for every palm. Usually no infection occurs in the maturing (green) leaves and hence application may be restricted to the tender susceptible portions only.
 - b) As an integrated prophylactic measure against pests, apply a mixture of 200 g sand with equal quantity of Marotti(*Hydnocarpus pentandra*)/neem cake in the spindle axis. Apply this during April-May and October-November. Treat manure pits and other possible breeding sites of rhinoceros beetle with *Metarrhizium anisopliae* culture and plants of *Clerodendron infortunatum*.
3. Recycle the available biomass in the coconut garden. Apply 50 kg organic manure in the form of farm yard manure or vermicompost per palm per year.
4. Grow green manure crops such as *Peuraria phaseoloides*/*Vigna unguiculata* in coconut basins during April-May to September-October and incorporate the biomass.
5. Adopt cropping system approach by raising intercrops in rotation/adopt mixed cropping/mixed farming with recycling of organic matter.
6. Adopt soil moisture conservation measures by mulching during November to May. Irrigate the palms during summer months with water @ 250 l/palm/week. Avoid water logging and provide proper drainage wherever necessary.
7. Remove juvenile palms showing symptoms of root (wilt) disease irrespective of its intensity. In mildly affected areas, all disease affected palms, irrespective of the intensity

of disease and yield of palms, should be removed to eliminate the foci of infection. Also remove all disease advanced and uneconomic palms and replanting with healthy elite seedlings if the planting density is less than 175/ha.

8. Restructure the canopy of other perennial tree crops to provide maximum light for the coconut palms.

References

- Amma, B.S.K. and Patil, K.D. 1982. An attempt to regenerate roots in root (wilt) affected coconut palms by hormones, phenols and amino acids. pp. 55-58. In: *Proc. International Workshop on special problems in physiological investigations of tree crops*. RRIL, Kottayam, India.
- Amma, P.G.K., Cecil, R.S., Pillai, N.G., Mathew, A.S. and Nambiar, P.T.N. 1983. Performance of Dwarf x Tall hybrid coconut in root (wilt) affected areas of Kerala under different fertilizer levels. *Proc. PLACROSYM* 5: 405-410.
- Anithakumari, P., Manoj, S and Arulraj, S. 2004. Participatory technology Transfer (PTT)-an innovative approach in root ((wilt) affected coconut area. *CORD* 20(2): 63-71.
- Anonymous, 1982. Coconut root (wilt) disease-A practical approach “contain the disease and live with it”. Technical Bulletin 8, pp. 5. Central plantation Crops Research Institute, Kasaragod, Kerala, India.
- Anonymous, 1983. Annual Report for 1981. Central Plantation Crops Research Institute, Kasaragod, pp. 279.
- Anonymous. 1985. Ccoconut root (wilt) disease-Intensity, production, loss and future strategy. CPCRI, Kasaragod. p.45.
- Anonymous, 1986. Coconut root (wilt) disease - present status of research and management. Technical Bulletin 14 pp.10. Central Plantation Crops Research Institute, Kasaragod, Kerala, India.
- Anonymous. 1997. Coconut root (wilt) survey-1996. Dept. of Agriculture, Govt. of Kerala. p.55.
- Butler, E.J. 1908. Report on coconut palm disease in Travancore. *Agric. Res. Inst. Pusa Bull.*, No.9: 23 p.
- Cecil, S. R. and Amma, P. G. K 1991. Soil and Nutrition. pp: 44-52. In: *Coconut root (wilt) disease*. Monograph series no.5. Central Plantation Crops Research Institute, Kasaragod.
- Cecil, R. S., Hameed Khan, H and Biddappa, C. C. 1991. Nutrient management for coconut disease and disorders. *J. Plantn. Crops* 19 (2):81-90.
- Davis, T.A. 1953. Breeding in coconut for disease resistance. *Indian Cocon.J.* 6(3):95-100.
- Dwivedi, R.S., Amma, B.S.K., Mathew, C and Ray, P.K. 1980. Control of root (wilt) disease of coconut with micronutrients, phenolic compounds and ascorbic acid. *Plant Disease* 64 (9): 843-844.
- Khan, H.H., Biddappa, C.C., Joshy, O.P. and Cecil, S.R. 1985. Micronutrient distribution in the crowns of healthy and root (wilt) diseased palms. *J. of Plantn. Crops* 13: 65-69.
- Krishnakumar, V. and Maheswarappa, H. P. 2010. Integrated management for root (wilt) diseased coconut (*Cocos nucifera*) palms. *Indian J. Agric. Sci.* 80(5):394-398.

- Maheswarappa, H. P, Anithakumari, P, Kamalakshi Amma, P. G. and Shanavas, M. 2005. Influence of integrated management and high density multi-species cropping systems on soil properties, plant nutrition and yield in root (wilt) affected coconut garden. *Cord* 21(2):18-29.
- Mathai, G. and Vasudevan Nair, K. P. 1977. Effect of soil application of fertilizers and micronutrients on root (wilt) disease and yield of coconut in the back water region of Kerala. *Agric. Res. J. Kerala*. 16: 244-246.
- Menon, K.P.V. 1943. Report on the scheme of investigation on the diseases of coconut in South India. 1942. Dept. of Agri., Travancore, 22 p.
- Menon, K.P.V. and Nair, U.K. 1952. Scheme for investigation of root and leaf diseases of coconut in South India. Consolidated report of work done from 8th March, 1937 to 1st March, 1948. *Indian Cocon. J.*, 5: 81-100.
- Menon, K.P.V. and Pandalai, K.M. 1958. *The Coconut Palm - A Monograph*. Indian Central Coconut Committee, Ernakulam, 384 p.
- Menon, K.S. and Nayar, T.V.R. 1978. Effect of intercropping with tuber crops in root (wilt) affected coconut gardens. *Proc. PLACROSYM* 1 : 416-424.
- Muralidharan, A., Nair, M.G. and Jayasankar, N.P. 1986. Response of coconut root (wilt) disease to management practices. *Indian Cocon. J.* 17 (1): 3-6.
- Nair, P.K.R., Varma, R., Nelliath, E.V. and Bavappa, K.V.A. 1975. Beneficial effect of crop combination of coconut and cocoa. *Indian J. Agric. Sci.* 45: 165-171.
- Nair, M.K., Koshy, P.K., Jacob, P.M., Nair, R.V., Rao, E.V.V.B., Nampoothiri, K.U.K and Iyer, R.D. 1996. A root (wilt) disease resistant coconut hybrid and strategy for resistance breeding. *Indian Cocon. J.* 27(1):2-5.
- Ninan, C.A. 1978. Exploitation of dwarf germplasm in coconut breeding. *Indian Coconut Journal* 8(11):1-4.
- Pandalai, K.M., Sankarasubramoney, H. and Menon, K.P.V. 1958 a. Studies on soil conditions in relation to the root and leaf diseases of the coconut palm in Travancore-Cochin. Part IV. Total and exchangeable calcium and magnesium content of coconut soils. *Indian Cocon. J.* 11: 49-56.
- Pandalai, K.M., Sankarasubramoney, H. and Menon, K.P.V. 1958 b. Studies on soil conditions in relation to the root and leaf diseases of the coconut palm in Travancore-Cochin. Part IV. Exchangeable cation, cation exchanging capacity and pH of coconut soils. *Indian Cocon. J.* 11: 84-101.
- Pillai, K.N. 1911. *Coconut* (in Malayalam). pp. 112. Vidyabhivardhini Press, Quilon.
- Pillai, N.G., Lal, S.B. and Shanta, P. 1973. Distribution and intensity of root (wilt) disease of coconut in Kerala. *J. Plantn. Crops* 1 (suppl.): 107-112.
- Pillai, N.G., Wahid, P.A., Kamaladevi, C.B., Ramanandan, P.I., Cecil, S.R., Kamalaskhi Amma, P.G., Mathew, A. S. and Nambiar, C.K.B. 1975. Mineral nutrition of root (wilt) affected coconut palms. Paper 4th session. FAO Technical working Party, Coconut Prod. & Processing. Kingston.
- Pillai, N.G. 1981. In :Review of Research on Coconut Root (wilt) Disease. Central Plantation Crops Research Institute, Regional Station, Kayangulam, 90 p. (Mimeo).

- Radha, K. and Lal, S.B. 1972. Diagnostic symptoms of root (wilt) disease of coconut. *Indian J. Agric. Sci.*, **42**: 410-413.
- Rajagopal, V., Patil, K.D., Mathew, C., Amma, B.S.K. 1986a. Abnormal stomatal opening in coconut palms affected with root (wilt) disease. *J. Exp. Bot.* **37**:1398-1405.
- Rajagopal, V., Patil, K.D., Mathew, C., Amma, B.S.K. and Varkey, T. 1986b. Water relation studies in the root (wilt) affected coconut palms. In : *Final Report* Central Plantation Crops Research Institute, Regional Station, Kayangulam, Kerala, India pp. 24.
- Rajagopal, V., Patil, K.D. and Amma, B.S.K. 1987. Efficacy of management practices for increasing the productivity of root (wilt) affected palms. *Indian Cocon. J.* **18** (8): 3-6.
- Rajagopal, V., Mathew, C., Varkey, T. And Joseph, K. V. 1998. Physiology and Biochemistry. Pp. 65-73. In: *Coconut Root (Wilt) Disease*. (Eds. Nampoothiri, K.U.K and Koshy, P.K.), CPCRI, Kasaragod.
- Ramadasan, A., Shanta, P. and Lal, S.B. 1971. Resistance or susceptibility, age of bearing and yield in young coconut palms in relation to development of root (wilt) diseases. *Indian J. Agric. Sci.* **41**: 1107-1109.
- Rawther, T.S.S. Pillai, R.V. 1972. Note on the field observations on the reaction of coconut varieties to root (wilt). *Indian J. Agric. Sci.* **42**(8):747-749.
- Sahasranaman, K.N., Radha, K. and Pandalai, K.M. 1964. Effect of manuring and inter cultivation on the yield of coconut in relation to leaf rot and root (wilt) diseases. *Indian Cocon. J.* **8**: 3-11.
- Sahasranaman, K.N., Pillai, N.G. Jayasankar, N.P., Varkey, T., Amma, P.G.K. and Radha, K. 1983. Mixed farming in coconut gardens: Economics and its effect on root (wilt) disease: In: *Coconut Research and Development* (ed. Nayar, N.M.). Wiley Eastern, New Delhi, pp. 160-165.
- Solomon, J.J. and Geetha, L. 2004. Phytoplasma disease of coconut in India – root (wilt) and tatipaka diseases. *Cord* **20**: 21-35.
- Srinivasan, N. 2010. Production of value added coconut seedlings through biopriming with biocontrol agents against leaf rot disease. In: *Abstracts, National Conference on Production of Quality Seeds and Planting Material – Health Management in Horticultural Crops*, 11-14 March 2010, New Delhi, p. 229.
- Thomas, G.V., George, M. and Shantaram, M.V. 1993. Effect of growing green manure legumes in the basins of root (wilt) affected coconut gardens. In: Nair M.K., Khan, H.H., Gopalasundaram, P. and Rao, E.V.V. (Eds.) *Advances in coconut research and development*. Oxford and IBH Publishing Co. Pvt. Ltd. New Delhi. pp. 421-42.
- Varghese, M.K. 1934. *Disease of the coconut palm*. Dept. of Agri. and Fisheries, Travancore. Government Press, Trivandrum 105p.

Integrated pest management in coconut

P. Rajan, Chandrika Mohan and A. Josephraj Kumar

Central Plantation Crops Research Institute

Regional Station, Regional Station, Kayamkulam - 690 533, Kerala, India

Coconut palm (*Cocos nucifera* Linn.) known as *Kalpavriksha* occupies a dominant role among the cultivated palm species in India as it provides livelihood securities to more than 10 million people in 18 States and 3 Union Territories of the country. The crop is cultivated in an area of 1.90319 million ha with a total production of 14,744 million nuts (2007-2008). The four Southern States viz., Kerala, Karnataka, Tamil Nadu and Andhra Pradesh contribute the major share of the area (90%) and production (92%) of the crop in the country.

Although production and productivity of coconut in India has grown up considerably in the past few decades, prevalence of major pests in majority of the coconut areas in the country have adversely affected the coconut industry to a large extent. Coconut palm being a perennial crop grown mostly under homestead gardens provides continuous supply of food and shelter for the buildup of various pests which cause extensive damage to the crop during all stages of its growth. It has been reported that 547 species of insects and mites attack the palm during different phases of growth and incur substantial crop loss. The annual loss due to the pest complex in Kerala State alone has been estimated to be 618.50 million nuts. Constantly changing cropping systems and adoption of agro-technologies for higher productivity without looking into the ecological balance of the nature resulted in instability in biotic and abiotic components of the ecosystem. Such disruptions have created instances of minor pests emerging as major pests and also resulted in large scale spread of major pests to newer areas. In India the pest control strategies in coconut were initially developed with more orientation towards use of insecticides. But increased awareness on the side effects caused by indiscriminate use of chemical pesticides had made integrated pest management (IPM) the need of the present era. IPM is a pest population management system utilizing all suitable technologies in a compatible manner to maintain the pest population below the economic injury level. It is a holistic approach aimed to minimize pest impact and to maintain the integrity of ecosystem. During the past two decades considerable efforts have been made to develop IPM strategies for combating major pests of coconut palm.

Pests

In India, rhinoceros beetle (*Oryctes rhinoceros* L), red palm weevil (*Rhynchophorus ferrugineus* Oliv.), leaf eating caterpillar (*Opisina arenosella* Walk.), white grub (*Leucopholis coneophora* Burm.) and the eriophyid mite (*Aceria guerreronis* Keifer) are the major pests distributed widely in all coconut growing tracts. Rodents also cause heavy crop loss in certain areas like Island ecosystem and lands locked with water bodies. Minor pests include coreid bug, slug caterpillars, scale insects, mealybugs, termites and whiteflies.

a) Rhinoceros beetle (*Oryctes rhinoceros*)

The adult beetle bores into the unopened spindle leaves and spathe causing characteristic “V” shaped geometric cuts on leaves and round to oblong holes on spathes. The

pest breeds in decaying organic debris such as cattle dung, compost, dead and decaying palms etc. Black beetle infestation has to be considered serious as the damage done by this pest provides egg laying sites for another lethal pest viz., red palm weevil and for entry of fungal pathogens.

The photosynthetic efficiency of the palms gets reduced as there is considerable reduction in functional area of young leaves resulting loss of vigour and reduction in yield. It is estimated that this pest on an average causes 10% loss in production of nuts. The damaged spathes will dry up causing complete loss of nuts in affected bunch. Attack on young seedlings and young palms results in stunted growth and delayed flowering and repeated attacks in growing point lead to the death of the seedlings. Petiole damage results in breaking of fronds.

IPM

- (i) The dead and decaying coconut logs and other organic debris in the vicinity of coconut plantations may be properly disposed off, since this act as prolific breeding grounds of the beetle.
- (ii) Hook out beetles from crown during peak periods of pest infestation (June- September) and the bore hole shall be filled with a mixture of Mancozeb and sand @ 3g/ 1kg.
- (iii) Application of powdered oil cakes of neem (*Azadirachta indica* A. Juss.), or marotti (*Hydnocarpus wightiana* Bl.) @ 250g mixed with equal volume of sand, into the top most three leaf axils around the spindle leaf thrice a year during May, September and December is recommended as a prophylactic measure against rhinoceros beetle and red palm weevil. Placement of naphthalene balls in the leaf axils at the base of spindle leaf @ 12g/ palm (3-4 numbers) and covering them with sand to prevent quick evaporation provide good protection against the pest for 45-60 days.
- (iv) Manure pits and other breeding sites are to be treated with the entomopathogenic fungus, *Metarhizium anisopliae* @ 5×10^{11} spores/m³. The pathogen is highly virulent and produces epizootics in the grub population in the breeding material. All stages of the host excepting the eggs are mycosed. Incorporation of the weed plant *Clerodendron infortunatum* is also very effective in the control of the pest build up in the breeding sites. Treatment of breeding sites with carbaryl 0.01% is recommended in cases where adoption of the above two methods are not possible.
- (v) Release of *Oryctes rhinoceros* virus (OrV) infected beetles @ 10-12 beetles / ha is recommended. The virus infects both adults and grubs. The pathogen kills the grubs in 15-20 days and reduces the longevity and fecundity of beetles by 45% and 95%, respectively. Introduction of the pathogen in the natural habitat of the pest causes epizootics in pest population and leads to significant reduction in the larval population in breeding sites and resultant reduction in beetle damage on the coconut palms. Management of the beetle using the OrV has been successfully validated in different regions of the country including Lakshadweep and Andaman Islands.
- (vi) Installing pheromone traps (PVC) using pheromone “Oryctalure” (Ethyl, 4-methyl Octonate) @ 5 traps / 25 ha for collection and destruction of adult beetles. The collected beetles can also be used for virus inoculation and re-release to the pest infested areas.

Rhinoceros beetle



Male and female beetles



Geometrical cuts on leaves



Healthy grubs

*Metarhizium anisopliae* infected grubs

PVC Pheromone trap

b) Eriophyid mite (*Aceria guerreronis*)

The nut infesting eriophyid mite, *A. guerreronis* is one of the most serious pests of coconut causing significant reduction in yield. In India the pest was first reported during 1998 from Kerala State and within a short span of time the mite has spread to all traditional coconut growing states in the West and East coast of the country. Mite infests coconut buttons during early stages of growth soon after pollination. Developing nuts harbour a large population of mite under their perianth. Symptoms appear approximately one month after initial colonization of mites inside the fertilized buttons as white longitudinal patches or triangular yellow patches near the perianth. As nuts grow, these patches turn brown and longitudinal fissures and wartings appear on the nut surface. Drying and shedding of buttons and young nuts are experienced in severe infestation. Dispersal of mites occurs through wind and population of mite reaches high during summer months from March to May.

In India invasion of coconut mite over large areas of coconut plantations in initial years of occurrence has affected up to 70% damage resulting in production of undersized or malformed nuts. It has been observed that as the time advanced there was considerable reduction in the level of incidence and infestation resulting in reduction of economic loss. The yield loss in South India recorded an average crop loss of 30.94% in terms of copra and 41.74% in terms of husk production in the initial years of pest damage. Mite damage also caused significant reduction in quality of fibres in terms of fibre length and tensile strength. It has been reported that fibres from moderately to severely infested nuts suffered 26-53% reduction in length. In recent surveys the loss in terms of copra in South India ranged from 8-12% showing a remarkable reduction in yield loss as compared to initial years of occurrence.

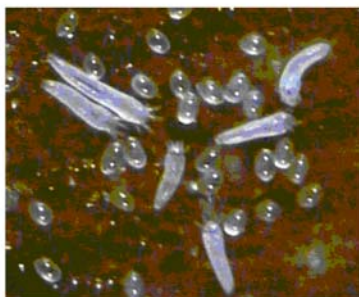
IPM

An integrated management strategy with need based application of botanical pesticides either by spraying or root feeding and adequate nutrient management of affected palms is currently recommended for the management of the pest.

- (i) Phytosanitary measures like crown cleaning and plantation sanitation.
- (ii) Spraying 2% neem oil-garlic-soap emulsion or azadirachtin 10000 ppm @ 0.004% on young bunches / root feeding of neem based pesticides containing Azadirachtin 5% (7.5 ml + 7.5 ml water) or azadirachtin 1% (10 ml + 10 ml water) thrice a year during January, April and September
- (iii) Recycling of organic biomass in coconut ecosystem using *in situ* vermi-composting, raising of suitable green manure crop in coconut basin and its incorporation, application of NPK fertilizers as per recommendation and soil moisture conservation measures.
- (iv) Conservation of predatory mites especially the phytoseiid mite, *Neoseiulus baraki* which is predominantly found along with the mite colonies in all pest infested areas.

Presently, emphasis is given for development of biocontrol strategies as they are safe and ecofriendly and vital in sustainable management of the pest. The fungal pathogen, *Hirsutella thompsonii* has received considerable attention throughout the world as the most effective natural enemy of eriophyid mite of coconut. This acaropathogenic fungus could be collected from all major coconut growing tracts of the country along with mite colonies and a total of 42 indigenous isolates are currently maintained. The potential of the virulent strains of *H. thompsonii* for the management of the eriophyid mite in the field is currently investigated at CPCRI.

Coconut eriophyid mite



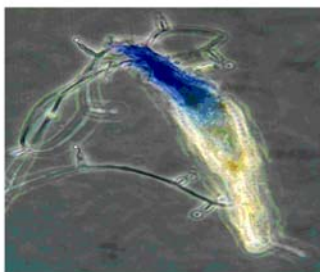
Mite colony



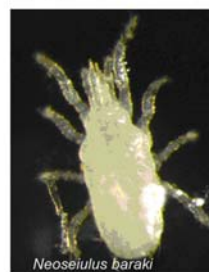
Symptoms on developing nuts



Severely infested bunch



Mite infested by *Hirsutella thompsonii*



Predatory mite
Neoseiulus baraki

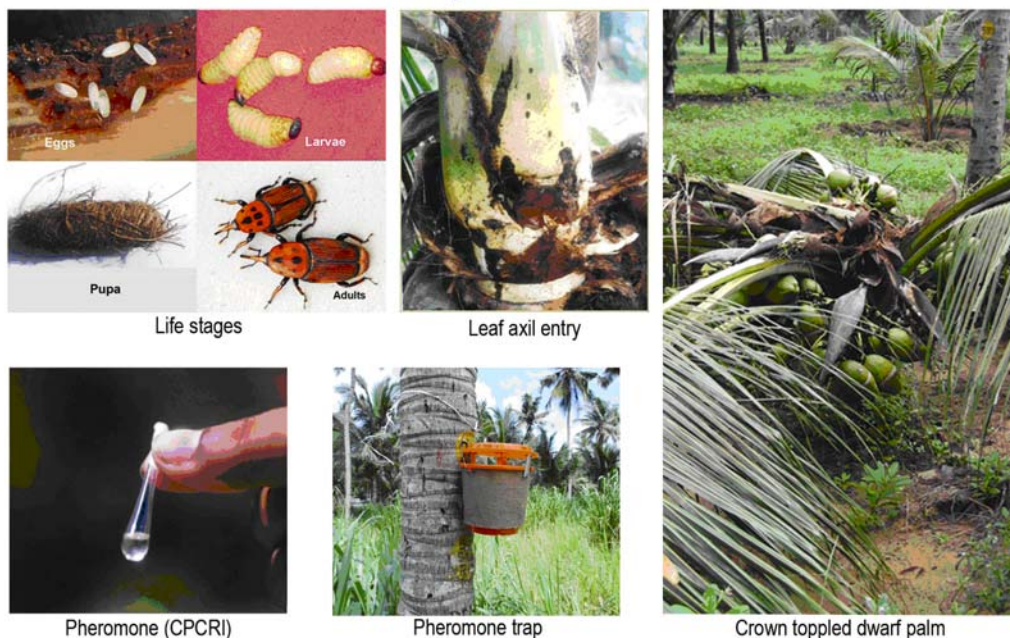
c) Red palm weevil (*Rhynchophorus ferrugineus*)

Red palm weevil is a fatal enemy of coconut palm. The infestation of the pest is predominant on palms of younger age group ranging from 5-20 years. Feeding on the growing point by grubs results in death of the palm. The weevils are attracted to the rotting smell and the pest incidence is quite severe in areas where palms are infected with bud rot/ leaf rot or infested by rhinoceros beetle. The presence of holes on the stem, oozing out of brown viscous fluid through holes, extrusion of frass, wilting of inner leaves, splitting of leaf bases are the visual symptoms of pest attack. The gnawing sound produced by feeding grubs is audible when closely monitored with ears on the infested stem. Entry of the pest through the crown is the most common and vulnerable type of infestation. The grubs in such cases stay very close to the cabbage portion (growing point) of the palm and results in drying of the young heart leaves. It is estimated that about 5-10% of the palms in the age-group of 5-20 years are lost annually due to the pest attack in different parts of the country.

IPM

- (i) Maintenance of field sanitation by removal, splitting and burning of dead palms, which harbour various stages of the pest, helps a lot in reducing fresh incidence and hence should be considered as an important step in IPM.
- (ii) Prevention of pest entry into the palm is the major step to be adopted in the IPM package. Fermenting smell emanating from the injured portions of the palm attracts the weevils for egg laying. This can be prevented by avoiding injuries on the palm and by treating the wounds, if any, with coal tar + carbaryl. Prophylactic leaf axil treatment for *O. rhinoceros* keeps away red weevil also. Whenever leaves are cut, a petiole length of 1.2 m should be retained on the palm to prevent the entry of the pest into the trunk through the cut petiole. Timely fungicidal treatment for bud rot and leaf rot also is essential.
- (iii) For infested palms, curative treatment with 1.0% carbaryl / 0.1% endosulfan (Use of endosulfan is banned in Kerala) is recommended as stem injection method or crown pouring. In stem injection method, after plugging all the holes on the lower part of the palm the insecticide solution is administered into the palm with a funnel through the uppermost hole. If the entry of the pest is through the spindle area, cutting the highly rotten part and pouring the insecticide through the crown shall be adopted.
- (iv) Root feeding of monocrotophos (10 ml + 10 ml water) for seedlings and non-bearing palms.
- (v) Trapping of red palm weevil using aggregation pheromone lures “Ferrugineol” (4-methyl, 5-nonanol) in food baited bucket traps forms an important component of the IPM strategy. Pheromone trapping @ 1 trap/ ha for monitoring and mass trapping of adults with adequate servicing of trap baits is recommended. Usually, the traps are serviced once a week and the food bait is replaced. Food bait includes 200 g ripened banana / pine apple plus 5 g yeast and 5 g carbaryl mixed with one litre of water. When the trap is set, the chemical from the sachet gets released (volatilized) into the surroundings, attracting the weevil towards the trap and the food attractant in the bucket orients the attracted weevil into the trap. Once trapped, the insect on contact with the insecticide mixed in the food gets killed. Presently, the aggregation pheromone is synthesized in CPCRI

Red palm weevil



Current investigations on the effect of new molecules for the curative treatment of red palm weevil have given promising results. Three molecules *viz.*, imidacloprid (Confidor 200% SL) @ 0.01%, Spinosad @ 0.013% (Success 2.5% SC) and Indoxacarb @ 0.04% (Avaunt 14.5% SC) when field tested against red palm weevil have given more than 80% recovery of infested palms. The entomopathogenic nematodes belonging to *Heterorhabditis* spp. are also currently evaluated against this pest.

d) Black headed caterpillar (*Opisina arenosella*)

The pest periodically assumes severe proportions in coastal and backwater areas and also in the vicinity of water bodies in the interior parts of peninsular India. Caterpillars construct galleries of silken webs reinforced with excreta and scrapes of leaf bits. Hiding in these galleries they feed on the chlorophyll containing parenchymatous tissues. These affected portions get dried presenting a scorched up appearance. Damage results in drying of outer and middle whorls of leaves. Damage to the leaves affect the photosynthetic efficiency of the palm, which leads to severe decline in yield and in addition renders the leaves unsuitable for other purposes also. Diagnostic symptom of infestation is the presence of galleries on the lower surface with live or dead stages of the pest. Damage results in drying of outer and middle whorl of leaves and such infested palms exhibit a scorched / burnt appearance from distance. A crop loss extending up to 40% loss in yield has been recorded in the endemic areas of the pest infestation.

IPM

- (i) Cutting and burning the heavily infested and fully dried outermost 2-3 leaves removes the pupae and other life stages of the pest.

- (ii) Release of larval / pupal parasitoids such as *Bracon brevicornis*, *Goniozus nephantidis* and *Brachymeria nosatoi*. The larval parasitoid *Goniozus nephantidis* (Bethyridae) is released if the pest is at 3rd larval stage or above @ 20 parasitoid /palm and *Bracon brevicornis* (Braconidae) @ 30 parasitoid/palm. The pre-pupal parasite *Elasmus nephantidis* Rohw. (Elasmidae) and the pupal parasite *Brachymeria nosatoi* Habu. (Chalcididae) are also very effective in managing the pest. They are released @ 49 and 32% respectively for every 100 pre- pupae, pupae estimated to be present on the palm. Before field releasing, the parasitoids should be fed with honey and newly emerged parasitoid can be released after 3 days of emergence. Management of black headed caterpillar through the mass release of stage specific parasitoids has been field validated in various endemic tracts of the pest both in Kerala and Karnataka and it has been a big success story through farmer participatory programmes.
- (iii) In severe sporadic outbreaks, spraying 0.05% diclorvos on under surface of leaves is recommended.
- (iv) Proper irrigation and adequate fertilizer application should be provided to rejuvenate severely pest-affected palms.

Leaf eating caterpillar



Caterpillar feeding on leaf



Severely infested garden



Bracon brevicornis



Goniozus nephantidis



Brachymeria nosatoi

e) White grub (*Leucopholis coneophora*)

White grubs are major pests of coconut palm mostly confined to sandy loam soil tracts of Kerala and Karnataka. Grubs feed on roots of coconut palm as well as other intercrops like cassava, yams, banana, vegetables etc. Adult beetles are chestnut-brown coloured and they emerge out of soil after a few rains during pre-monsoon showers in May-June.

In nursery seedlings the grubs feed on tender roots and also tunnel into the bole and collar regions resulting in the drying of the spindle leaves followed by gradual death of the seedlings. In older coconut plantations continuous infestations by the grubs results in yellowing of leaves, premature nut fall, delayed flowering, retardation of growth, tapering of crown and reduction in yield. Peak grub population is seen in the coconut basin during September-October

IPM

- (i) Deep ploughing and digging of the soil during pre and post monsoon periods for exposing grubs to predators
- (ii) Trapping of adult beetles through light traps during the emergence period (May-June) and their destruction.
- (iii) Soil application of phorate 10G @ 100g / palm or drenching the basin with chlorpyrifos @ 0.05% during June and September. The insecticide has to be applied in the active root zone of the palm evenly leaving a distance of 60 cm from base of the trunk. The phorate granules have to be well raked and mixed with soil. Insecticidal applications have to be continued for at least three consecutive years for effective management of the pest.

Minor Pests

Coreid bug, *Paradasynus rostratus* Dist.

Adults and nymphs feed on the buttons and young nuts causing shedding of buttons and malformation of mature nuts. In many cases, the infested nuts show crinkling, gummosis and become barren. In addition to coconut, the pest also attacks guava, cashew, neem and cocoa. The pest is active in the field from June to February with peak population during September-October. Control of the pest is achieved by insecticidal spraying with 0.1% carbaryl on young bunches or placement of phorate 10G @ 5 g per palm in two polythene sachets at the stalk regions of youngest two bunches close to the leaf axils.

Slug caterpillars

The slug caterpillars are sporadic pests on coconut. Their infestation becomes very severe during summer and post-monsoon months. The caterpillar voraciously feeds on the lamina, leaving only the midrib. These are polyphagous insects and hence the management becomes a problem at times. *Contheyla rotunda* Harm., *Latoia lepida* Cram. and *Macroleptena nararia* M. are reported from coconut in India. *C. rotunda* is more common in the coastal tracts of West Coast of India whereas, the spiny slug, *M. nararia* is predominantly seen in East Coast of India. For the management of the pest during the outbreak season chemical control using carbaryl 0.1% is recommended. Light trapping of adult moths is suggested as an effective monitoring tool and a feasible mechanical control strategy of the pest. For rejuvenating the severely affected palms proper nutrition management and irrigation are highly essential.

Mealybugs

The important species of mealybugs affecting coconut are *Palmiculter palmarum*, *Pseudococcus coccotus* and *Pseudococcus longispinus*. They colonize on tender parts like spear leaves, spadix, inflorescence and inside the perianth of nuts. Mealybug infestation results in poor nut

set, shedding of buttons and drying up of spadix in adult palms. In seedlings failure of heart leaf development and in severe cases the death of seedlings are reported. Management of the pest is accomplished by spraying dimethoate or monocrotophos at 0.05%. In the field the natural enemies especially coccinellids and lygaeids exert a good control of the pest.

Scale insects

Scale insects are usually found in colonies of dense masses on lower surface of leaf, petioles, peduncles and nuts. *Aspidiotus destructor*, *Aonidiella orientalis*, *Lepidosaphes megregori* are the important species of scales infesting coconut palms. The pest incidence is at its peak during summer months. The predators especially coccinellid beetles exert natural control of the pest in the field. In the case of severe infestation spraying with 0.1% fenthion, 0.05% dimethoate or monocrotophos is recommended for the management of the pest.

Termites

Termites (*Odontotermes obesus*) occasionally causes serious damage to nursery seedlings and adult palms. They feed on the collar region of the seedlings and result in wilting of central shoot. In adult palms attack is observed on the bark. Drenching the nursery with 0.05% chlorpyrifos twice at 20-25 days interval manages the pest effectively. In older palms affected trunks are to be swabbed with the chemical.

Whiteflies

Two species of whiteflies viz., the areca whitefly *Aleurocanthus arecae* and the spiralling whitefly, *Aleurodicus dispersus* were found infesting the leaves of middle and lower whorls of young palms. They inhabit on lower surface of leaflets and the presence can be visualized by the development of sooty mould on the upper surface of the leaflets. These insects are naturally controlled by parasitoids and predators present in the ecosystem.

Rodents

Rodents pose a major threat to coconut palms especially in the island ecosystem and water logged lowland tracts. In Lakshadweep 40% of the nuts are lost due to rat damage alone. The arboreal black rat, *Rattus rattus wroughtonii* is the major rat species. This vertebrate pest lives on the crown of the palm and feeds on the inner contents of tender nuts of 3-6 months maturity resulting in shedding of nuts. The burrowing rodents viz., *Bandicota bengalensis*, *Bandicota indica* and the gerbil, *Tatera indica* makes extensive burrows in soils and damages the coconut seedlings by eating away the cabbage portions.

An integrated approach consisting of adequate spacing, mechanical barriers, farm sanitation, trapping and chemical methods are generally followed for rodent management. Poison baiting using single dose anticoagulant rodenticide bromodialone (0.005%) is effective in the management of black rat. For control of bandicoots and gerbils poison baiting with zinc phosphide is very effective. A community approach is highly essential for achieving the desired results in the management of rodents.

Summary

Effective IPM strategies have been developed against major pests of coconut but there is scope for refining these technologies further for making them cost-effective, ecologically

safe and farmer friendly. Emphasis should be given for popularization of biocontrol intensive integrated technologies among farming community through Farmer Field Schools. In depth studies are required for developing feasible biocontrol strategy against coconut eriophyid mite and red palm weevil. Contiguous and homestead nature of coconut gardens and long flight range of various insect pests warrant the necessity for adoption of IPM technologies on a group approach mode.

Biological methods of pest management in coconut

Chandrika Mohan, A. Josephraj Kumar and P. Rajan

Central Plantation Crops Research Institute
Regional Station, Kayamkulam 690 533, Kerala, India

The perennial nature of coconut palm provides a continuous supply of food and shelter for the pest population buildup, which cause considerable damage to the crop during all stages of its growth. The major pests of coconut in India are the rhinoceros beetle (*Oryctes rhinoceros* Linn.), red palm weevil (*Rhynchophorus ferrugineus* Oliv.), leaf eating caterpillar (*Opisina arenosella* Walk.), eriophyid mite (*Aceria guerreronis* Keifer) and the white grub (*Leucopholis coneophora* Burm.). Pests like coreid bug, scale insects, mealy bugs and slug caterpillars cause considerable damage to the crop in certain localities.

An Integrated pest management (IPM) schedule comprising combination of different technologies – mechanical, sanitation, cultural, prophylactic, chemical and biological methods are the existing strategy globally recognized to manage pests of coconut and has proved to be quite feasible. Chemical control mainly with insecticides and repellents are effective in the control of the pests, but their deleterious effect on the beneficial natural enemies and pollinators and the residues they leave in the palm and the environment limit the use of these poisons. In the context of increasing interest in organic farming and production of safer food for consumption, there is a need to develop more sustainable methods of pest management for the benefit of agriculture, people and the environment.

Biological control which utilizes the natural enemies of the pest is an excellent alternative to the chemical pesticides in many ecosystems. Biological control agents broadly involve entomophaga and entomopathogens. Entomophaga mainly consists of parasitoids and predators and entomopathogens include fungi, virus, bacteria and nematodes which cause lethal infections. In India, for coconut pest management, applied biological pest suppression is now widely practiced for two major pests viz., the rhinoceros beetle and leaf eating caterpillar. Biocontrol agents have a range of attractive properties that include host specificity, lack of toxic residue, no phytotoxic effects, ecofriendly, human safety and the potential for pest management to be self sustaining. Biocontrol agents can also be produced locally which is in terms of choosing and matching natural enemies to small scale needs. Successful use requires fundamental knowledge of the ecology of both the pest and natural enemy.

Entomophaga

Parasitoids as effective biocontrol agents of coconut black headed caterpillar:

In the IPM of coconut pests, parasitoids are extensively utilized for the management of the black headed caterpillar *O. arenosella*, which is one of the dominant caterpillar pests of coconut palm. The larvae of *O. arenosella* feed on the parenchymatous tissues on the under surface of leaflets and construct galleries of silken webs reinforced with excreta and scrapes of leaf bits. Diagnostic symptoms of infestation are the presence of galleries on the lower surface of the leaflets with different stages of the pest and the upper epidermis intact. Pest infestation usually starts from the outer and middle whorl of leaves. On an average 40% yield decline is noticed

in severely affected palms and palms of all ages are susceptible to its infestation. Concealed habitat of the pest well covered by the larval galleries makes pesticide spraying practically very difficult. Hence biological suppression with the release of parasitoids provides the best solution for the management of *O. arenosella*.

Among the various parasitoids of *O. arenosella* hymenopterans predominate.

Plate -1. Parasitoids of *O. arenosella*



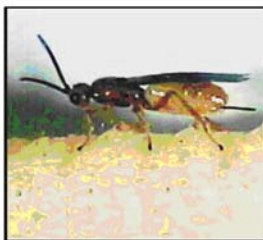
Larvae of *O. arenosella*



Larvae of the parasitoid *G. nephantidis* developing on *O. arenosella*



G. nephantidis



B. brevicornis



E. nephantidis



B. nosatoi

Based on the stage of the pest on which the parasitoids develop they are treated as egg, larval, prepupal, larval-pupal and pupal parasitoids. Among the 40 species of parasitoids reported from black headed caterpillar of coconut in India, the gregarious larval ectoparasitoids *Goniozus nephantidis* Muesebeck and *Bracon brevicornis* Wesmael, the prepupal parasitoid *Elasmus nephantidis* Rohwer and the pupal parasitoid *Brachymeria nosatoi* Habu are the most promising ones. The major desirable attributes of these parasitoids are their greater searching ability, capacity to withstand high temperature, production of higher proportion of female progeny, occurrence throughout the year, and abundance during peak period of the pest and their distribution in all pest infested areas.

Techniques have been developed for mass production of the promising parasitoids. The pest infested area should be monitored regularly and parasitoids releases should be initiated at the post monsoon period during November – December if there is any pest incidence. Parasitoids are to be released at the fixed dosages depending on the target stage of the pest at fortnightly intervals till the pest population is suppressed. As the release should synchronize with the stage of pest in the field, a sample of leaflets should be collected from infested palms at random and examined for live pest stages.

The parasitoid *Goniozus nephantidis* is released if the pest is at 3rd instar larval stage or above @ 20 parasitoid/palm and *Bracon brevicornis* @30 parasitoid/ palm. The prepupal parasitoid *Elasmus nephantidis* and pupal parasitoid *Brachymeria nosatoi* are also very effective in managing the pest. They are released @ 49 and 32 % respectively for every 100 prepupa and pupae estimated to be present on the palm. Before releasing in field, the parasitoids should be fed with honey and newly emerged parasitoid can be released in the field after three days of emergence. *G. nephantidis* and *B. brevicornis* could easily be mass multiplied on larvae of the rice moth *Corcyra cephalonica*. The prepupal parasitoid, *Elasmus nephantidis* is a highly host and stage specific parasitoid and always requires a steady supply of pre-pupa of *O. arenosella* for mass multiplication. This is the major constraint for mass production of *E. nephantidis* in the parasite breeding laboratories.

Pupal parasitism by many species of *Brachymeria* are observed in nature. Among them *B. nosatoi* is most potential for effective biocontrol since it possesses all the desirable attributes with high percentage parasitism, long life span, good searching ability and tolerance to high temperature. *B. nephantidis*, another major *Brachymeria* species in nature sometimes act as hyper parasitoid on primary parasitoids of *O. arenosella*.

Field performance of parasitoids: Using IPM technologies or exclusive release of promising biocontrol agents many demonstrations were laid out in the last two decades by CPCRI in coastal Kerala and Karnataka.

Studies conducted in an endemic area during 1990-1993 at Thodiyoor (Kollam District, Kerala) with the field release of the three stage specific parasitoids at fixed norms and intervals in *O. arenosella* infested coconut garden (2.8 ha) resulted in highly significant reduction (94%) in pest population. Regular monitoring and release of stage specific parasitoids induced 52.6 and 94.7 % reduction in pest population after one and two years, respectively of parasite release in a heavily infested tract at Neendakara, Kollam Dist., Kerala. Large scale (1,400 ha) field validation of the biosuppression technology of coconut black headed caterpillar done during 1999-2002 in different geographic locations in coastal Karnataka (Ullal and Jeppinamogru) and Coastal Kerala (Purakkad and Ayiramthengu) could achieve 93-100 % reduction in *O. arenosella* population in a period of two years with regular monitoring and release of stage specific parasitoids. Reports of biological suppression of *O. arenosella* in coastal districts of Orissa by the release of parasitoids are also available. Successful biocontrol of *O. arenosella* was reported in Andhra Pradesh and timely augmentation of *G. nephantidis* @ 10 adults/palm at fifteen days interval suppressed the pest population in Karnataka. The higher frequency of occurrence of *G. nephantidis* in all the locations during post release period indicated the suitability of this larval parasitoid to adapt in various locations in the coastal belts of Kerala and Karnataka.

Parasitoids reported from other pests: Although parasitoids are reported from other pests of coconut, they were not successfully introduced as effective biocontrol agents. The scoliid wasp *Campsomeriella collaris* Fabr. parasitizes larvae of white grubs in the field. *Scolia cyanipennis* Fabr. and ectoparasitic mites are also observed in association with the different stages of the rhinoceros beetle. The egg parasitoid *Chrysochalcisea indica* was recorded from the coreid bug *Paradasynus rostratus* and the mymarid parasite *Parallelaptera* sp. on the eggs of *Stephanitis typica*. The parasites reported on the scale insect of coconut, *Aonidiella orientalis*,

include *Aphytis chrysomaphali* Marcat and *Cauca parvipennis* Gaham, and *Cocobius reticulatus* (Aphelinidae). More study in this line is required to collect and catalogue more parasitic species and to standardize the techniques for field application and evaluation.

Predators: Insect and spider predators are abundant in the coconut ecosystem. Insect predators viz., *Santalus parallelus* Payk., *Pheropsophus occipitalis* Macleay, *P. issoderus*, *Chelisoches morio* (Fab.) and species of *Scarites*, *Harpalus* and *Agrypnus*. are frequently observed in the breeding grounds of the rhinoceros beetle feeding on the eggs and early instar larvae.

An exotic predator *Platymeris laevicollis* Distant was imported from Zanzibar to India for the control of rhinoceros beetle. As compared to the indigenous predators, *P. laevicollis* feeds on adult beetles. The predator is long lived (170-240 days) and fecundity high (110-170 eggs /female) and could easily be mass multiplied on ground roaches. Field release of predator was done in coconut plantations in Kerala and Karnataka @ 6 bugs/ palm and could achieve significant reduction in beetle population and the damage to the palm. But the predators failed to establish under field conditions and a long term sustainable effect on pest management could not be achieved with this predator.

A variety of predatory fauna is also available in nature in association with *O. arenosella*. The most important insect predators are the carabid beetles, anthocorid bugs, chrysopids coccinellids, ants and spiders. Spiders, though generalist predators and polyphagous, contribute a major group of natural enemies in coconut ecosystem. Spiders are the most dominant group of predators on *O. arenosella*. The efficiency of the predators is neither fully assessed nor widely used in the biological control programmes of *O. arenosella*. Habitat manipulations like conserving ground litter, using safer pesticides etc. contribute to their conservation. The earwig *Chelisoches morio* (Fab.) feeds on the eggs and early instar grubs of red palm weevil in the field.

Predators of scale insects, mealy bugs, lace bug, white flies: Scale insects, mealy bugs, coreid bug, lace bug and whiteflies are observed in most of the coconut plantations as minor pests. Their population is kept under check by the natural predators which are very active in the coconut ecosystem.

Predators play a predominant role in limiting coconut scale population. Reports of a range of predators viz., *Scymnus saverini* Wsc., *Scymnus* sp., *S. apiciflavus* Mots. *Pharellus minutissimus* Sic. *Azyatrinitis* Hshl, *Pentilia insidiosa* Muls. and *Scymnus aenipennis* on scale *Aspidiotus destructor* are available. The coccinellids *Chilocorus nigrinus* Fab. and *Pseudoscymnus dwipakalpa* Ghorpade, *Cybocephalus* sp are predacious on *A. destructor* and *Aonidiella orientalis*.

Fourteen insects and twenty-three spiders are observed as predators on *Stephanitis typica*. The identified insect predators are the mirid *Stethoconus preaeffectus* D., chrysopid *Ankylopteryx octopunctata octopunctata* Fabr., reduvids *Endochus inornatus* Stal., *Rhinocoris fuscipes* Fab., *Euagoras plagiatus* Burm. and earwig *Chelisoches morio*. Fab.

The mealy bugs associated with coconut in India are *Palmiculter palmarum*, *Pseudococcus longispinus*, *Pseudococcus cocotis*, *Dysmicoccus* sp. and *Rhizoecus* sp. *Spalgis epius* (Lycaenidae) and species of *Pullus* and *Scymnus* (Coccinellidae) are the natural enemies from mealy bug colonies recorded. They exert limited check of the pest population. The predators of coreid bug include the ant *Oecophylla smaragdina* and the reduviid *Endochus* sp.

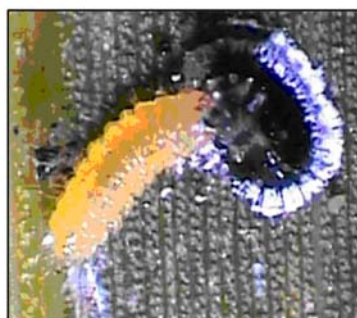
Plate -2. Predators



Serangium parcesetosum



Jauravia pallidula



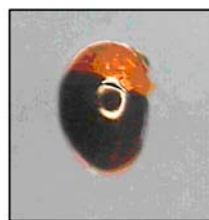
Grub of *Cybocephalus* sp.



Chilocorus subindicus



Scymnorphus sp.



Cybocephalus sp.



Chelisoche morio

Two types of whiteflies viz., areca whitefly, *Aleurocanthus arecae* and spiralling whitefly *Aleurodicus dispersus* have been recorded from coconut in India. Their population is kept under check by the activity of predators viz., *Serangium parcesetosum*, *Jauravia pallidula*, *Cybocephalus* sp. etc. Two species of lady bird beetles namely *Chilocorus subindicus* and *Scymnorphus* sp. were found predatory on spiralling whitefly.

Predators of coconut eriophyid mite: The nut infesting eriophyid mite *A. guerreronis* Keifer has emerged as one of the serious pests of coconut in India in 1998 and has become the major pest of coconut palm in a very short spell. Mites live in colonies on the tender portion of the buttons covered by the perianth and suck sap from the meristematic tissues. The symptom of attack is the appearance of elongated white patches below the perianth, later turning to pale yellow and brown. As the nut grows, the injury forms wartings and longitudinal fissures on the nut surface. Severe infestation results in button shedding, reduced kernel weight and reduction in fiber content. Currently botanical pesticides are recommended for mite management.

A variety of predatory mites and smaller insects are associated with *A. guerreronis* in different parts of the world. Predatory mites belonging to Phytoseiidae, Bdellidae and Tarsonemidae are encountered in various collections. In India the phytoseiid mite *Neoseiulus baraki* is the most dominant predator in the field. Other predatory mites include *Neoseiulus paspalivorus* and *Bdella* species. The insect predators encountered with coconut mite population in the field are thrips, coccinellids and syrphid maggots. But these are found only occasionally and in very few numbers. An increasing trend of incidence and better establishment of predatory mites in the field over the years are observed. The activity of the predators is high during June

to December in the field. Compared to the young developing nuts below three months, more predators are encountered in 4-6 months old nuts. The predatory mites are larger in size compared to the coconut mite and they gain entry only later into the nuts. This is one of the limiting factors for the wider use of the predators. However, conservation of the predatory fauna in the ecosystem is beneficial to regulate the coconut mite in nature.

Entomopathogens: Biological control using entomopathogens is an important component of IPM. It is possible to use specific micro-organisms that kill arthropods. These include entomopathogenic fungi, nematodes, bacteria and viruses. The microbial agents which play a vital role in the bio suppression of various pests of coconut have been examined and in the case of black beetle (*Oryctes rhinoceros* L.) this group of natural enemies has been thoroughly studied.

Fungi: Entomopathogenic fungi are of considerable importance in crop pest control because of their ability to infect a wide range of insect pests through non *per os* infection. In coconut pest management entomopathogenic fungi such as *Beauveria bassiana*, *Metarhizium anisopliae*, *Hirsutella thompsonii* are the best utilized globally. Fungi require relatively high humid microclimate (>70% RH).

Green muscardine fungus as a potential enemy of Rhinoceros beetle: The rhinoceros beetles cause damage to palms of all age groups by boring into the unopened spindles and inflorescence. As the pest bores deeper into the host it pushes out the chewed up tissues, which are seen extruding from the entry points. Once these injured spindles open up, the green leaves present a geometric 'V' shaped cut pattern. The damage to inflorescence is seen as round oblong holes on the spathes, which soon dry up. The pest occurs throughout the year and breeds in cattle dung, compost, dead and decaying organic debris like coconut and other palm trunks, cocoa pod shells, oil palm bunch waste, coir dust, rotting paddy straw and sugar cane waste *etc.* The life cycle is completed in 6 months on an average.

Plate - 4. Entomopathogens of rhinoceros beetle



Rhinoceros beetles
male and female



O.rhinoceros grubs
infected with *M.anisopliae*



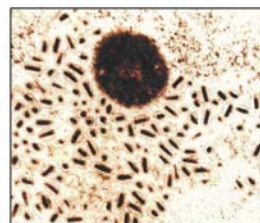
M.anisopliae culture
in rice medium



Oryctes rhinoceros virus
infected and healthy grubs



Inoculation of OrV into
rhinoceros beetle

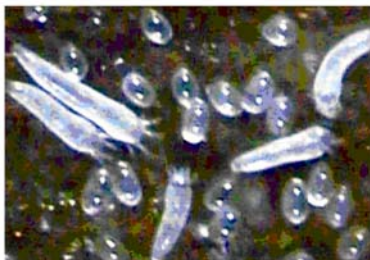


Electron microscopic
view of OrV

Among the entomopathogens the green muscardine fungus *Metarhizium anisopliae* (Metsch.) Sorokin (Deuteromycotina: Hypomycetes) is one of the most effective and successful biocontrol agent in coconut ecosystem as a potential pathogen of *O. rhinoceros*. *M. anisopliae* var. *major* (spore size 10-14 µm) is highly infective variety used widely for the control of this pest. All the stages of the host excepting the eggs are mycosed. The fungus is very active during monsoon when the relative humidity is 70-90% and the temperature is 26-28°C. The infected grub becomes sluggish and mortality occurs within 10-15 days. The body of the infected grub becomes hardened and white powdery fungal colonies appear in the joints of the integument. Within a week green coloured spores are produced and finally the cadavers become black and mummified. The mass production of the fungus has been developed at CPCRI using solid (cassava chips and rice bran mixture supplemented with nitrogen source) and liquid (coconut water) media. Different substrates like broken rice/wheat grains, millets etc. have also are found to be cheaper substrates for multiplication of the fungus. For the field application of the fungus, the fungal spores are mixed with sterile water and used to drench the breeding materials of the rhinoceros beetle @ of 5×10^{11} spores / m³. The fungus survives in the breeding material for long periods. Use of this fungus for biocontrol of rhinoceros beetle has been popularized as a women friendly technology and CPCRI is presently facilitating farm level production of this fungus to cater local needs.

***Hirsutella thompsonii*, a promising fungal pathogen against coconut mite:** The fungus *Hirsutella thompsonii* has been reported as a predominant pathogen among microbial pathogens of eriophyid mite. In India the incidence of *H. thompsonii* was recorded from Kerala, Karnataka, Tamil Nadu, Andhra Pradesh, Pondicherry and Lakshadweep Islands. In Kerala, local strain of *H. thompsonii* var. *synnemetosa* could be isolated from field samples. CPCRI could

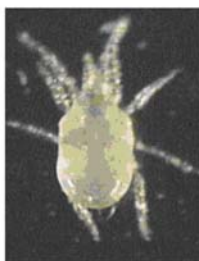
Plate -3. Natural enemies of eriophyid mite



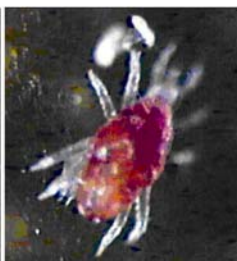
Microscopic view of eriophyid mite colony with adults and eggs



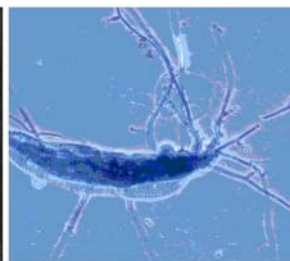
Acaropathogenic fungus *Hirsutella thompsonii*



Predatory mite *Neoseiulus baraki*



Predatory mite *Bdella* sp.



Eriophyid mite infected with *H. thompsonii*

collect virulent native isolates of this fungus from different locations of India. A total of 42 isolates of *H. thompsonii* were collected and maintained. Talc based formulations of the virulent strains of this fungus are being evaluated for the suppression of coconut eriophyid mite in the field and preliminary results indicated 70-80% suppression in pest population. Other fungal species associated with eriophyid mite include species of *Poecilomyces*, *Beauveria*, *Metarhizium*, *Sporothrix*, *Verticillium*, *Acremonium*, *Aspergillus*, *Penicillium* and *Fusarium*. However, the bio-efficacy of these fungi as biocontrol agents of mite in field conditions is not fully studied.

Virus:

***Oryctes rhinoceros* Virus:** The *Oryctes rhinoceros* virus (OrV) was discovered in 1960 in Malaysia and has been effectively used to control the rhinoceros beetle in coconut and oil palm in Southeast Asia and the Pacific. It is a classical example of successful inoculation and long term control of an insect pest. The virus consists of rod shaped virions and replicates in the nuclei of infected cells. On the basis of its (ultra) structure, OrV was previously considered to be a so called non –occluded baculovirus (NOB). Due to the lack of occlusion bodies, it was later removed from the family Baculoviridae. OrV contains a double stranded DNA genome of about 130 kilobase pairs. The virus gains entry in to the host through contaminated food and it multiplies in the mid gut epithelial cells and fat bodies of grubs and adults. Infected grubs become lethargic, stop feeding and move to the surface of the feeding media. With the multiplication of the virus in the mid gut epithelium of the grub the fat body disintegrates and the haemolymph content increases giving a translucent appearance for the grub. The diseased grub dies within 15-20 days. The infection by OrV results in reduction of the longevity of the beetle by 45% and fecundity by 95% relative to the healthy beetles. Diseased adults also become inactive.

Mass production of the virus: The *Oryctes* virus is mass multiplied and maintained in the laboratory on live grubs. The OrV infected grubs are dissected, the swollen midgut is taken out and the viral suspension of the midgut is prepared by grinding with phosphate buffer. This viral inoculum is fed to the healthy grubs to induce infection. The inoculated grubs are reared on sterilized food (cow dung, coir pith or saw dust) moistened sufficiently. The development of OrV infection is to be monitored in the inoculated grubs and the whole procedure of inoculation of healthy grubs is repeated to maintain the viral culture in the host. The grubs showing external symptoms such as translucent gut or extroversion of rectum has to be dissented immediately; otherwise secondary infection with other pathogens like *Pseudomonas* etc. takes over and destroys the OrV infection.

Field release: For field release, either the adult beetles are inoculated orally using a syringe with the OrV inoculum or they are allowed to wade through the virus inoculum (2g of infected larval gut tissues in 1 litre of phosphate buffer) contained in a basin for 30 minutes. After the inoculation, the beetles are confined together in a box containing rotted coconut wood powder mixed with the virus inoculum for 24 hours. The treated beetles were removed the following day and confined for a week in a box containing fresh coconut petioles provided as food, since the infected beetles begin to excrete the virus only a week after infection. The infected beetles were liberated after dusk in the field. 12-15 infected beetles per hectare are recommended to disseminate the virus in nature.

Impact of the release of OrV: In India, the population of *O. rhinoceros* and its damage on coconut palm was checked substantially when the OrV was released in the pest infested areas of Lakshadweep islands. In Minicoy, there was a reduction of 76.9% leaf damage, 93.8% spathe damage in a period of 30 months. In Androth islands leaf, spathe and fresh spindle damage showed reduction of 75.4, 56.1 and 74.8% respectively in a period of two years and the OrV population showed an increase from nil to 60.6 % showing the establishment of the pathogen in the pest infested area. Similar results were observed in the mainland in Chittilappilly, Trichur, Kerala, where pest incidence reduced from 100 to 23% and there was 80.6 %, reduction in leaf and 100% reduction both in spathe and fresh spindle damage when OrV was re-released in a period of 3 years during 1989 to 1992. Establishment of OrV in native population of *O. rhinoceros* at Sipighat, Anadammans by release of the virus is also reported. From nil infection during the pretreatment period in 1989 the infection has increased to 61% in 1991. By augmenting the natural population of virus infected beetles, sustained reduction in beetle incidence and crop damage can be achieved even in already infected contiguous areas. The biggest singular advantage OrV offers over other microbial agents is in being an auto transmissible pathogen capable of passing from generation to generation. The method of its propagation involves the release of diseased beetle and no spraying of the virus to the crop is required.

Significant reduction in rhinoceros beetle damage by the combined use of two potential biocontrol agents (*M. anisopliae* and OrV) was reported from large scale field studies (2,400 ha) done in Alappuzha (75.1 and 79.4% reduction in leaf and spindle damage) and Kasaragod (66.6 and 95.8% reduction in leaf and spindle damage) districts during 1999-2002.

Bacteria: Bacterial pathogens *Acinetobacter calcoaceticus* and *Pseudomonas alcaligenes* have been identified as pathogens of *O. rhinoceros* grubs. *Bacillus thuringiensis* Berliner and *Serratia marcescens* B. are observed to be pathogenic to *O. arensioella* in the field.

Pathogens of red palm weevil and white grubs: So far no effective microbial agent could be identified on red palm weevil which is a fatal enemy to coconut palm. *Pseudomonas aeruginosa* was identified as facultative pathogen of red palm weevil. A yeast isolate has also proved to be a transitional pathogen producing mortality in weevils/grubs. Reports on the infectivity of *Bacillus thuringiensis* subspecies *kurstaki* (Btk.) and a polyhedrosis virus to larvae of red palm weevil are available. These pathogens were isolated from the field in Egypt and stored for 4 years as air dried smears on glass slides, before being tested. Record of eugregarine protozoan pathogen *Pseudomonocystis* sp., which infect 22.7% of the third instar white grubs in the field is available.

Entomopathogenic nematodes:

Entomopathogenic nematodes (EPN) in the families Heterorhabditidae (represented by the genus *Heterorhabditis*) and Steinernematidae (represented by the genera *Steinernema* and *Neosteinernema*) have been used to suppress populations of soil and cryptic insect pests in a variety of agro-ecosystems. EPN are obligate parasites of insects and kill their hosts with the aid of bacteria carried in the nematode's alimentary canal which provide nutrients to the nematodes, produce antibiotics and inhibit competing microbes, and kill the host through septicemia. They are associated with mutualistic bacteria in the genus *Xenorhabdus* for Steinernematidae and *Photorhabdus* for Heterorhabditidae. The positive attributes of these

nematodes as biological control agents are that they have a broad host range, are safe to invertebrates, plants and other non-target organisms, have no known negative effect on the environment, are easy to mass produce *in vivo* and *in vitro*, are easily applied using standard spray equipment, can search for their host, kill rapidly (within 48 h), have the potential to recycle in the environment, are compatible with many chemical and other biological pesticides, are amenable for genetic selection for desirable traits and are exempt from registration in many countries.

Field efficacy: Moisture conditions have been recognized as one of the most important factors in the soil environment affecting survival, virulence and persistence of nematodes. Entomopathogenic nematodes need high relative humidity to survive and a film of free water for movement. They may become dormant at very low soil moistures.

***In vivo* multiplication of entomopathogenic nematodes:** *In vivo* mass production of EPN has been standardized using *Galleria mellonella* larvae in the laboratory by filter paper inoculation technique. *Steinernema* sp. infected cadavers turn creamish-white in colour characteristic of the bacterium (*Xenorhabdus poinarii*) housed in it whereas *Heterorhabditis* sp. infected insects become reddish-brown due to *Photorhabdus luminescens*. After required incubation the cadavers are placed in White's trap for the emergence of nematodes. From the White's trap, the nematodes wriggle out and migrate all over the Petri dishes under minimum moisture content. Nematodes thus emerged out are harvested by adding water and later filtered for concentrating the nematodes. On an average 2-3 lakh nematodes were produced from each insect.

Use of EPN against coconut insect pests: EPN has been found promising in the management of red palm weevil and white grubs infesting coconut. An entomopathogenic heterorhabditid nematode was isolated from Egypt and United Arab Emirates planted with date palm trees and has shown potential for the control of red palm weevil. Among the species of EPN evaluated against the grubs of red palm weevil, *R. ferrugineus*, in filter paper based bioassay, *Heterorhabditis* sp. was found to be more virulent than *Steinernema* sp and the local isolate *H. indicus* was found to be more virulent inducing 92.5% mortality @ 1500 IJ/grub. Talc based EPN formulation of *H. indicus* elicited higher mortality than water suspension based formulation on coconut petiole based bioassay. Among the four species of EPN evaluated against the coconut white grub in soil-column bioassay, *Steinernema abassi* was found to be virulent inducing 37-45% mortality of white grubs @ 5000 IJ/grub in a period of 96-120h. Species specificity against various pests, concentration of IJ and moisture content hold the key in the field success of EPN on a large scale. Field evaluation of the promising EPNs against red palm weevil and white grubs are to be undertaken on priority as these two major pests are not having any effective biocontrol agents.

Limitations in use of biocontrol methods: Under the present market system, many biological control products have not competed well with less expensive and more effective synthetic pesticides. The down sides of Biocontrol agents are that most are niche products, pest control is not immediate, there can be lack of environmental persistence, and efficacy can be low and unpredictable particularly in outdoor environments. Timely availability of quality bioagents, quantity, farmers perception about biological control, short shelf life of bioagents, formulation, storage and registration *etc.* are some of the limiting factors for

popularization of biocontrol technology. Biosystematics of insect groups especially parasitoids and predators is of prime importance in biocontrol. Illustrated easy to use identification guides should be made available for research and extension workers. Identification service is lacking for major groups of crop pests and their natural enemies. Regional approaches to catalogue biodiversity would be ideal.

Future thrust: Research and development opportunities

- ❑ Strain improvement of natural enemies utilizing the natural enemy population from various locations.
- ❑ Tritrophic interaction studies on host-pest and parasitoids/predators and exploring methods for conservation biocontrol, behavioral studies on parasitoids and educating/conditioning parasitoids for better parasitic efficiency and synthesis of kairomones to attract parasitoids and predators and to retain them in the released fields.
- ❑ Cataloguing predators in coconut ecosystem and exploring predatory potential of promising species and evolving mass multiplication techniques for promising predators.
- ❑ Effective formulation and delivery of EPN to be standardized for suppression of white grubs and red palm weevil.
- ❑ Development and upscaling of a mycopesticide using *Metarhizium anisopliae* and *Hirsutella thompsonii* for management of rhinoceros beetle and eriophyid mite of coconut.
- ❑ Farmers Field School (FFS) programmes and farmer participatory demonstrations in larger areas for use of biological control agents under IPM.
- ❑ Studies on the effect of pesticides on the beneficial fauna.
- ❑ Cell lines for production of obligate pathogens

Conclusion

All the proven cases with the promising bioagents have confirmed a meaningful way of pest management utilizing the indigenous fauna for the biological suppression of the pests of coconut. The indigenous natural enemies proved to be quite useful in controlling the pests, particularly rhinoceros beetle and leaf eating caterpillar. The biosuppression of rhinoceros beetle by employing viral pathogen *OrV* is documented as one of the classical examples of biocontrol of an insect pest. Proper monitoring and release of stage specific parasitoids could effectively bring down the population of coconut leaf eating caterpillar in the field. The current scenario warrants an augmentative release of these promising bioagents in areas wherever pest infestation is found. Spider fauna plays an important role in the natural suppression of pests in the field. Conservation of these biocontrol agents of the pests has become quite imperative. Limitation on use of pesticides or to identify safer and ecofriendly pesticides is required to integrate the biocontrol agents in IPM. As predators are mostly polyphagous, release of predators when parasitoids are active has to be avoided. Pollen and nectar bearing plants in the vicinity provides supplementary food for predators such as chrysopids, coccinellids and mites. More detailed studies on the bioecology of different species of parasitoids and predators affecting the key pests of plantation crops are to be undertaken with a view of utilizing them for biocontrol suppression of the pests

References

- Chandrika Mohan and Sujatha, A. 2006. The Coconut leaf caterpillar, *Opisina arenosella* Walker *CORD*: 22 (Special Issue): 25 -78.
- Kaya, H.K. and Gaugler, R. 1993. Entomopathogenic nematodes. *Annu. Rev. Entomol.* **38**: 181-206.
- Kumar, P.S. 2002. Development of a biopesticide for coconut eriophyid mite in India. In: *Proceedings of British Crop Protection Conference- Pests and disease* Vol 1& 2 18-21, Nov, 2002, British Crop Protection Council, Brighton, United Kingdom.
- Mohan, K.S., Jayapal, S.P., and Pillai, G.B. 1989. Biological suppression of coconut rhinoceros beetle, *Oryctes rhinoceros* L. in Minicoy, Lakshadweep by *Oryctes baculovirus* – Impact on pest population and damage. *Journ. Plantn. Crops.* **16**(Suppl.): 163-170.
- Nair, C.P.R., Rajan, P. and Chandrika Mohan. 2005. Coconut eriophyid mite, *Aceria guerreronis* Keifer – An overview. *Indian Journ. Plant Protec.* **33**(1): 1-10.
- Pillai, G.B. 1993. Biological control of insect pests of plantation crops 235-252 In: *Organics in soil health and crop production* (Ed. P.K. Thampan) Peekay Tree Crops Development Foundation, Cochin 254pp.
- Sathiamma, B., Sabu, A.S. and Pillai, G.B. 1996. Field evaluation of the promising species of indigenous parasitoids in the biological suppression of *Opisina arenosella* Wlk. the coconut leaf eating caterpillar. *Journal of Plantation Crops* **24**: 9-15.

Table 1. Important parasitoids of *Opisina arenosella*

Name of parasite	Family	Target pest stage	Nature of parasitoid
<i>Apanteles taragamae</i> Vier.	Braconidae	Early larva	Solitary
<i>Bracon brevicornis</i> Wesmael	Braconidae	Late larva	Gregarious
<i>Goniozus nephantidis</i> Mues.	Bethylidae	Do	Do
<i>Elasmus nephantidis</i> Rohw.	Elasmidae	Pre pupa	Do
<i>Goryphus nursei</i> Lam.	Ichneumonidae	Larva-pupa	Solitary
<i>Meteoridea hutsonii</i> Nixon	Braconidae	Do	Do
<i>Antrocephalus hakonensis</i> Ashm.	Chalcididae	Pupa	Do
<i>Brachymeria nosatoi</i> Habu	Chalcididae	Do	Do
<i>B. nephantidis</i> Gahan	Chalcididae	Do	Do
<i>Xanthopimpla punctuate</i> F.	Ichneumonidae	Do	Do
<i>X. nana nana</i> Schlz.	Ichneumonidae	Do	Do
<i>Trichospilus pupivorus</i> Ferr.	Eulophidae	Do	Gregarious

Table 2. Predators of *O. arenosella* in coconut ecosystem

Species	Order: Family
<i>Calleida splendidula</i> Fabricius	Coleoptera: Carabidae
<i>Parena nigrolineata</i> Chaudoir (= <i>Parena laticincta</i> Bates)	Coleoptera: Carabidae
<i>Jauravia</i> sp	
<i>Cheilomenes sexmaculata</i> Fabricius	
<i>Micraspis discolor</i> Fabricius	

<i>Propylea fallax</i> Khnzorian	Coleoptera: Coccinellida
<i>Jauravia pubescens</i> Fabricius	
<i>Cardiastethus exiguus</i> Poppius	
<i>C.affinis</i> Poppius	Heteroptera: Anthocoridae
<i>C.nazarensus</i> Reuter	
<i>Buchananiella sodalis</i> Buchnan-White	
<i>Ankylopterox octopunctata candida</i> Fabricius	Neuroptera: Chrysopidae
<i>Chrysopa</i> sp.	
<i>Mallada astur</i> Banks	
<i>Monomorium floricola</i> Jerdon	Hymenoptera: Formicidae
<i>Anoplolepis longipes</i> Jerdon	
<i>Monomorium</i> spp.	
<i>Crematogaster</i> sp	Arenae: Araneidae
<i>Tapinoma</i> sp.	
<i>Technomyrmex albipes</i> Smith	
<i>Argiope pulchella</i> Thorell	Arenae: Clubionidae
<i>Gasteracantha</i> sp.	
<i>Neoscona muckerjei</i> Tikader	
<i>Neoscona bengalensis</i> Tikader &Bal	Arenae: Gnaphosidae
<i>N. elliptica</i> Tikader &Bal	
<i>Neoscona</i> sp.	
<i>Argiope catenulate</i> (Doleschall)	Arenae: Salticidae
<i>Larinia jayasankari</i> Biswas	
<i>Cheiracanthium</i> spp.	
<i>C. melanostoma</i> Thorell	Arenae: Sparassidae
<i>Clubiona drassodes</i> Cambridge	
<i>Poecilochroa barmani</i> Tikader	
<i>Marpissa anusuae</i> Tikader & Biswas	Arenae: Tetragnathidae
<i>Marpissa calcuttaensis</i>	
<i>M. dhakuriensis</i> Tikader	
<i>M. tigrina</i> Tikader	Arenae: Therididae
<i>Phidippus indicus</i> Tikader	
<i>Phidippus bengalensis</i> Tikader	
<i>Phidippus pateli</i> Tikader	
<i>Plexippus paykullii</i> (Audnovin)	
<i>Rhene danieli</i> Tikader	
<i>R. indicus</i> Tikader	
<i>R. khandalensis</i> Tikader	
<i>Zygoballus</i> sp.	
<i>Sparassus</i> sp.	
<i>Olios lamarcki</i> Litreille	
<i>Heteropoda leprosa</i> Simon	
<i>Tetragnatha andamanensis</i> Tikader	
<i>Theridion</i> sp.	

Emerging pests of coconut in India

Rajan, P., Josephraj Kumar, A., Chandrika Mohan and Subaharan, K.

Central Plantation Crops Research Institute
Regional Station, Kayangulam 690 533, Kerala, India

Coconut is an important small-holder crop that significantly contributes to food security, improved nutrition, employment and income generation to at least 10 million farm families. A total of 547 species of insects and mites attack the palm during different phases of growth and incur substantial crop loss. The annual loss due to the pest complex in Kerala State alone has been estimated to be 618.50 million nuts (Kurian *et al.*, 1979; Rajan *et al.*, 2010). Emerging pests are those pests reported from an area for a particular crop where population has been increasing over a period of time considerably to cause damage above economic injury level. Mohandas and Remamony (1993) have reviewed the emerging pest problems in coconut and their control measures. Status of these pests has been changing from minor to major or secondary to primary (Nair *et al.*, 1998). Emergence is usually associated with increased population densities resulting from ecological changes consequent to alterations in the environment. Apart from this, changes occurring in cropping patterns, adoption of modern agro-techniques and indiscriminate use of chemical pesticides also add to the biotic imbalance and consequent emergence of new pest problems. Recent reports on the incidence of a few insects on coconut plantations in different parts of India attaining the status of emerging pests are presented.

Periodic surveys and surveillance were undertaken in different South Indian states *viz.*, Kerala, Karnataka, Tamil Nadu, Andhra Pradesh and Union Territory of Lakshadweep Islands during 2008-2010 for recording the incidence of emerging pests in these areas. The symptoms and intensity of damage, nature of damage, loss incurred and biocontrol agents associated with these pests were recorded. Cataloguing and identification of the emerging pests were undertaken at national insect repositories.

Coreid bug: The widespread occurrence of the coreid bug, *Paradasynus rostratus* Dist. (Heteroptera : Coreidae) was reported as a serious emerging pest on coconut from Southern districts of Kerala. Thiruvananthapuram district recorded the highest incidence (40.6%) followed by Kollam (29.8%) and Alappuzha (23.4%). Incidence was found to be lowest in Kozhikode and Thrissur (Table 1). The bug causes heavy crop loss by shedding of developing buttons and immature nuts ranging from 18.2-66.4% in endemic gardens. In addition to coconut, the bugs attack other crops like cashew (tender nuts), guava (tender fruits), custard apple (tender fruits), cocoa (tender pods) and neem (young flushes). *P. rostratus* was first reported from Kerala by Kurian *et al.* (1972). The pest is active in coconut gardens from June to February with peak population during October-December (Rajan and Nair, 2005). An egg parasitoid, *Chrysocalcisea indica* parasitizing egg mass, a reduviid predator, *Endochus inornatus* preying on nymphal stages and weaver ant, *Oecophylla smaragdina* preying all stages of pest were recorded as natural enemies.

Table 1. Incidence of coreid bug, *P. rostratus* in different districts

Districts	Incidence (%)
Thiruvananthapuram	40.6
Kollam	29.8
Alappuzha	23.4
Thrissur	0.05
Kozhikode	0.02

Nut infestation symptoms by coreid bug as well as eriophyid mite were characteristically distinguished by Chandrika Mohan and Nair (2000). Physiological modulation of qualitative chemical parameters of coconut oil from coreid infested nut was well documented (Mayilvaganan and Nair, 2002). Visalakshy *et al.* (1987) reported carbaryl (0.1%) as a effective insecticide against coreid bug. Spraying of neem seed oil-garlic emulsion (2%) and profenophos (0.05%) was found effective for the management of coreid bug (Ambily *et al.*, 2009).

Slug caterpillars: In Eastern and Western Godavari districts of Andhra Pradesh the outbreak of coconut slug caterpillar *Macroleptia nararia* M. (Lepidoptera : Limacodidae) was recorded during March-May, 2009 and the level of infestation ranged from 5-85%. Temperature and moisture are critical factors in the growth of herbivorous insects and their invertebrate predators, parasitoids and pathogens. Outbreaks can be of eruptive in nature driven by intrinsic population processes and trophic-level factors. It can also be gradient in nature as a consequence of changing environmental factors favouring population growth. High temperature (>39°C) coupled with high relative humidity favoured emergence of *M. nararia* in higher population (CPCRI, 2010c). Infested gardens showed a scorched / burnt appearance of the middle and outer whorl of leaves and the crop loss reached as high as 90-95% in severely affected gardens. Feeding damage was exacerbated by grey leaf blight fungus, *Pestalotiopsis palmarum* which infect leaf tissue breached and damaged by early larval instars. This association has been reported from slug caterpillar species such as *Chalcocelis albiguttatus*, *Darna catenatus*, *D. trima*, *Parasa balitkae*, *Setora nitens* and *Thosea lutea* (Howard *et al.*, 2001; Holloway *et al.*, 1987). In the field, some caterpillars were found infected by entomopathogens. Light trapping is suggested as an effective monitoring tool and a feasible mechanical control strategy of the pest.

An outbreak of slug caterpillar, *Conthyla rotunda* Hamp. (Lepidoptera : Limacodidae) was reported from Payyannur, Kerala during February 2010 causing complete defoliation of the leaves resulting in drastic reduction in nut yield. While East coast of India recorded the emergence *M. nararia*, West Coast of India witnessed the outbreak of *C. rotunda*. Outbreak of a particular species corresponded to the geography-induced weather factors as well as the intrinsic biotic factors (Menon and Pandalai, 1960). Occurrence of another slug caterpillar, *Parasa lepida* Cram. (Lepidoptera : Limacodidae) was also noticed in low proportion during the surveillance period from coconut nurseries. Sathiamma *et al.* (1972) described the two slug caterpillars, *C. rotunda* and *M. nararia* as sporadic pests of coconut (Fig. 1).

Slug caterpillar (*Macroleptra nararia*)



Conthyla rotunda



Parasa lepida



Fig. 1. Slug caterpillars of coconut

Whiteflies: Two species of whiteflies, viz., Arecanut whitefly *Aleurocanthus arecae* David (Aleyrodidae : Hemiptera) and spiralling whitefly *Aleurodicus dispersus* Russell (Aleyrodidae : Hemiptera) were recorded in mild to medium intensities in various tracts of Kerala, Tamil Nadu and Lakshadweep Islands (Fig. 2). These soft bodied insects were found in congregation on the under surface of matured coconut leaflets. These whiteflies were suppressed in nature by the natural enemies preventing the pest to reach an outbreak status.

A. arecae is noticed on young coconut palms of both tall and dwarf cultivars generally during April-May. Black envelope of sooty mould fungus is visualized on upper surface of infested leaflets. Adult whiteflies have smoky grey wings and ants are not commonly associated with the pest congregation. Pupae have blackish setae on the body (Chandrika Mohan *et al.*, 2007). As the name suggests, adults of *A. dispersus* has a typical spiralling fashion of egg-laying and found in mild to moderate levels during March-May. It is highly polyphagous pest infesting a wide array of crops in coconut plantations. In Minicoy, the spiraling whitefly nymphs were reported more on papaya, banana, tapioca and castor and were found parasitized by the aphelinid parasitoids. Adults measure about 2 mm length with white wings (Ramani, 2000; Josephraj Kumar *et al.*, 2010a).

Mealybugs (Pseudococcidae : Hemiptera): Five species of mealybugs were recorded as emerging pests in various tracts. They include spindle mealybug, *Palmicultor palmarum* Ehr., spadix mealybug, *Pseudococcus cocotis* Mask., leaf mealybugs, *Dysmicoccus* sp. and *Ferisia virgata* Ckll. and root mealybug, *Nipacoccus nipae* (Mask.) (CPCRI, 2010c) (Fig. 3).

P. palmarum infests mainly on young seedlings and are found in dense aggregations only on leaf axils and base of spear leaves. Red ants are seen associated with this mealybug

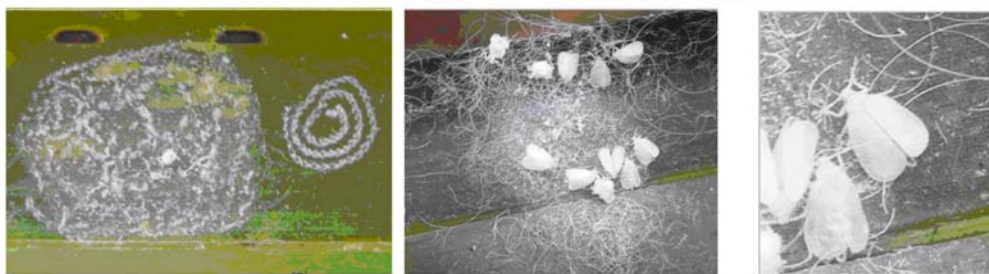
Spiralling whitefly (*Aleurodicus dispersus*)Arecanut whitefly (*Aleurocanthus arecae*)

Fig. 2. Spiralling white fly and arecanut white fly

colony. *P. cocotis* affects spadix and floral parts of palm and severe infestation causing drying of spadix and shedding of immature buttons. Red ant, *Oecophylla smaragdina* is associated with this mealybug colony. *Dysmicoccus* sp. and *F. virgata* infest leaves of coconut seedlings and young palms and infested seedlings show yellowing and loss of vigour. Incidence of buff coconut mealybug, *N. nipae* on tender roots of coconut seedlings was observed for the first time at Kayangulam during August 2010. This mealybug species was not observed on foliage or other arboreal parts of the palm. Adult females are 1.5-2.5 mm long, oval in shape and purplish-brown in colour covered by orange thick wax with 10-12 pairs of marginal pyramid-shaped wax filaments (CPCRI, 2010c).

Mealybugs infesting coconut

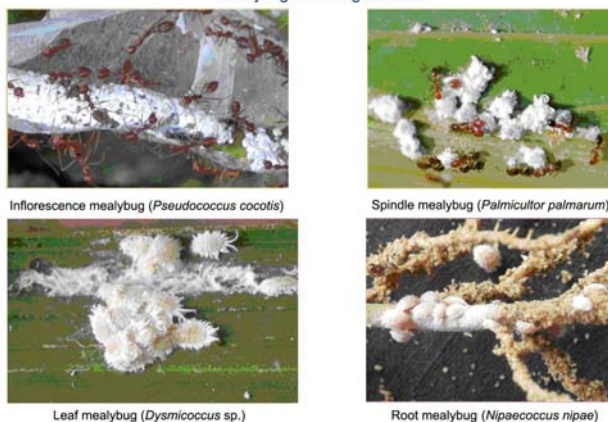


Fig. 3. Mealy bugs infesting coconut

Scales (Hemiptera : Diaspididae & Coccidae): A total of seven species of scale insects were observed infesting coconut palms / seedlings during the survey period (Fig. 4). This includes four species of armoured scales and three species of soft scales. Armoured scales recorded as emerging pests during the period include the coconut scale, *Aspidiotus destructor* Sign., Oriental scale, *Aonidiella orientalis* Newstead, Mussel scale, *Lepidosaphes mcGregori* Banks and Needle scale, *Chionaspis* sp. The three species of soft scales recorded from coconut were wax scale, *Ceroplastes floridensis* Comstock, Brown soft scale, *Coccus hesperidum* Linn. and Stellate scale, *Vinsonia stellifera* Westwood.

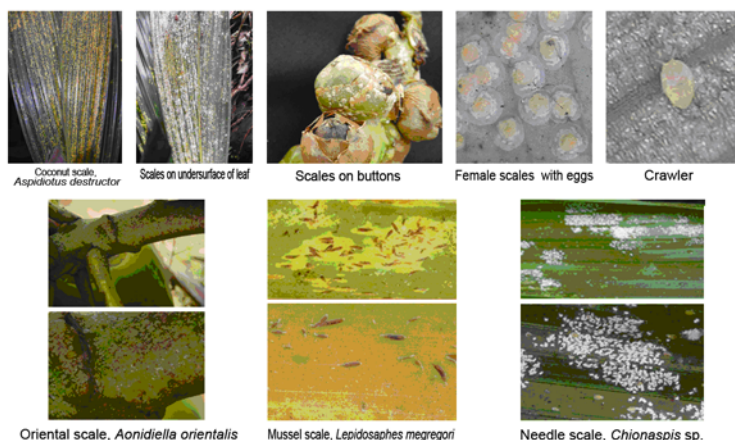


Fig. 4. Armoured scales

The coconut scale *A. destructor* was recorded from all states of South India, Lakshadweep Islands as well as Assam and West Bengal. They inhabit mainly on the undersurface of the leaves and due to their infestation the leaves turn yellow to brown. Vidyasagar (1989) elaborated symptomatology of various scale insects infesting coconut. Kapadia (1984) reported five species of scale insects viz., *Chrysomphalus aonidium* (Linn.), *Pseudoaulacapsis cockerelli* (Cooley), *Aonidiella orientalis* (Newst.), *Memiberlesia lantaniae* (Sign.) and *Lepidosaphes* sp. infesting on nuts, leaf sheaths and leaflets of coconut. Jalaluddin and Mohanasundaram (1989) studied the biology of *A. destructor* and reported three sprays of 2.5% soap at weekly intervals induced cent percent mortality of *A. destructor* in the nursery. *A. orientalis* was also recorded from all major areas of South India and Lakshadweep Islands and are mostly confined to rachillae and nuts. The adult mussel scale feeds from undersurface of leaves and was mainly observed in Kerala and Minicoy Islands. White needle scale, *Chionaspis* sp. was mainly recorded from planted coconut seedlings in both Tamil Nadu and Kerala (Josephraj Kumar *et al.*, 2010b)

Soft scales were seen as emerging pests in Island conditions as well as in few tracts of Kerala and Tamil Nadu (Fig. 5). Wax scale has characteristic mound or projections on its body and usually seen singly on coconut leaflets. The brown soft scales are closely associated with black ants and infested leaves show sooty mould. Stellate scales are star-shaped scales and are seen in few numbers on coconut leaflets. Generally scales are bio-suppressed in nature by their natural enemies and the main predators of this group include coccinellid beetles, cybocephalids. Aphelinid parasitoids also play a key role in suppression of scales along with predators (Josephraj Kumar *et al.*, 2010b).

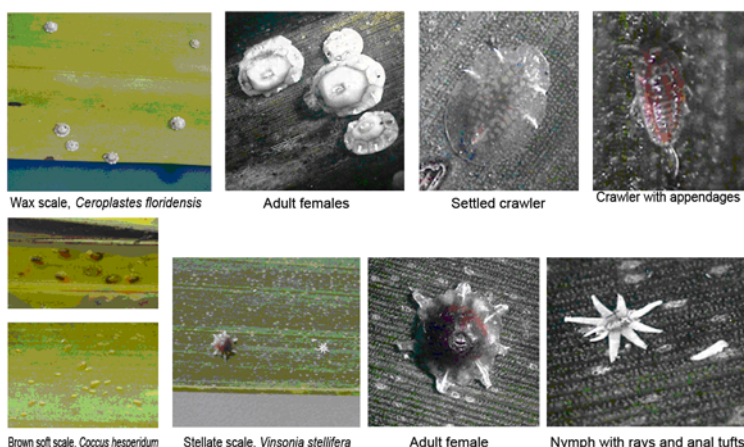


Fig. 5. Soft scales

Aphids: During the year 2010, the palm aphid, *Cerataphis braziliensis* Hempel (Aphididae : Hemiptera) was noticed on unfurled spindle leaf of Malayan Green Dwarf varieties of coconut seedlings at Kayamkulam. These aphids resemble whitefly pupae in many aspects especially the presence of fringe white waxy filaments circumscribing the body. Adult aphids have a mid-dorsal ridge and inconspicuous siphunculi. The aphid colony was found attended by two ant species viz., *Solenopsis* sp. and *Oecophylla smaragdina* (CPCRI, 2010b; CPCRI, 2010c) (Fig. 6).

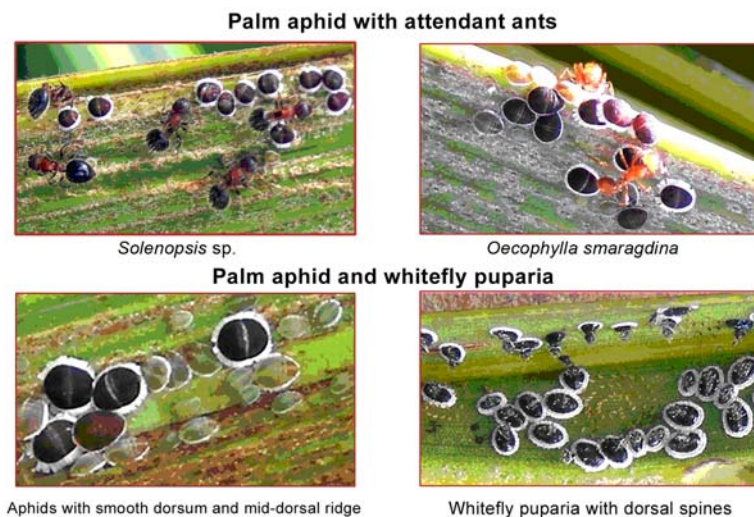


Fig. 6. Aphids infesting coconut

Ash weevils: During 2010 a new species of Asian grey weevil, *Myloccerus undatus* (Curculionidae : Coleoptera) was recorded as an emerging pest of coconut seedlings at Kayamkulam and Karunagapalli, Kerala. Mild to medium level of infestation damaging 5-10% leaf lamina of un-split leaves with typical notching-like symptom along the leaf margins

was noticed on majority of the coconut seedlings in these areas. The characteristic feature of this weevil is the presence of three-spined hind femur and is considered as an invasive pest from Sri Lanka (CPCRI, 2010a; CPCRI, 2010c). Coconut ash weevil, *Mylocherus curvicornis* Fab. was also noticed on coconut leaves of young palms especially on outer whorls in different tracts of Kerala (Ponnamma *et al.*, 1982)(Fig. 7).

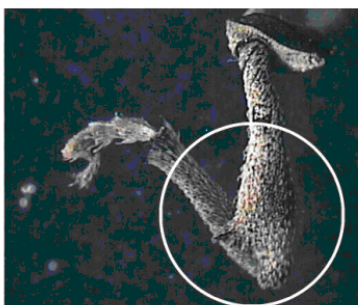
Asian grey weevil, *Mylocherus undatus*



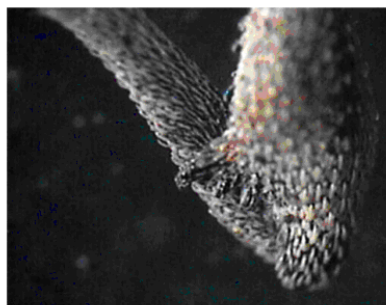
Notching-like damage symptom



Adult weevil



Hind leg with spined femur



Tri-spined hind femur

Fig. 7. Ash weevils infesting coconut

Bagworms: Occurrence of a bagworm, *Pteroma plagiophelps* (Hamps) (Psychidae : Lepidoptera) feeding on mature coconut/ oilpalm leaflets was encountered in West Godavari districts of Andhra Pradesh. The pest was also noticed in very low intensity in some areas of Kerala *viz.*, Palode, Kottayam and Kasaragod. The pest scraps the leaf tissues and feeding areas develop into necrotic lesions and holes. It is emerging as a pest of economic importance in Andhra Pradesh in coconuts and oil palms (CPCRI, 2010c)

Conclusion

Presently most of the emerging pests reported from coconut are suppressed by their natural enemies; however, these pests may develop into a status of key pest if congenial ecological conditions prevail at a particular point of time. Hence, monitoring and surveillance of emerging pests in coconut ecosystem is essentially required in an era of climate change inducing gradient outbreaks. Concerted efforts for conservation of different natural enemies in the ecosystem

are warranted for preventing these pests attaining epidemic levels. Future management of these emerging pests relies on improved monitoring systems, habitat management including ecological engineering and biological pest suppression.

References

- Ambily, P., Nandakumar, C., Hebsy Bai. (2009). Management of coreid bug, *Paradasynus rostratus* Dist. on coconut palm in homesteads having mixed cropping. *Entomon* **34**(2): 63-70.
- CPCRI (2010a). First record of Asian grey weevil, *Myloccerus undatus* Marshall on coconut. *CPCRI News*. **29**(3): 4.
- CPCRI (2010b). Palm aphid in coconut, *CPCRI News*. **29**(4): 4.
- CPCRI (2010c). *Annual Report 2009-10*. Central Plantation Crops Research Institute, Kasaragod, 671 124, Kerala, India, 148p.
- Chandrika Mohan, Rajan, P. and Nair, C.P.R. (2007). Incidence of whitefly (*Aleurocanthus arecae* David) on coconut foliage. *J. Plantn. Crops* **35**: 119-121.
- Chandrika Mohan and Nair, C.P.R. (2000). Distinguish the nut infestation by eriophyid mite and coreid bug in coconut. *Indian Coconut Journal* **30**(3): 60-61.
- Holloway, J.D., Cock, M.J.W. and Desmier de Chenon, R. 1987. Systematic account of South-east Asian pest Limacodidae. In: Cock MJW, Godfray HCJ, Holloway JD (eds) *Slug and Nettle caterpillars*, CAB International, Wallingford, UK, pp 15-117.
- Howard, F.W., Moore, D., Giblin-Davis, R.M. and Abad, R.G. 2001. *Insects on palms*, CAB International, Wallingford, Oxon, UK pp 42-70.
- Jalaluddin, M. and Mohanasundaram, M. (1987a). Biological studies in *Aspidiotus destructor* Sign. a pest of coconut in Tamil Nadu. *Indian Coconut Journal* **19**(11): 14-19.
- Josephrajakumar, A., Rajan, P., Chandrika Mohan and Jacob, P.M. (2010a) Re-invasion and bio-suppression of spiralling whitefly, *Aleurodicus dispersus* Russell on coconut in Minicoy Island. *Indian Coconut Journal* **53**(2): 20-23.
- Josephrajakumar, A., Rajan, P. and Chandrika Mohan. (2010b). Scale insects, mealybugs and whiteflies of coconut palm and their management. In: *In a Nutshell: Essays on Coconut* (Ed.) CV Ananda Bose, Coconut Development Board, Kera Bhavan, Kochi, 199-206 p.
- Kapadia, M.N. (1984) New records of coconut scale insects and their natural enemies in Gujarat. *Indian Coconut Journal* **14**(11): 9-10. .
- Kurian, C., Pillai, G.B., Abraham, V.A., Mathen, K. (1972). Record of a coreid bug (nut crinkler) as a new pest of coconut in India. *Curr. Sci.* **12**: 37.
- Kurian, C., Sathiamma, B. and Pillai, G.B. (1979) . World distribution of pests of coconut. *FAO Technical document* **119**: 53 p.
- Mohandas, N. and K.S. Remamony. (1993). Emerging pest problems in coconut and their control. In: *Advances in Coconut Research and Development*. (Eds.) M.K. Nair, H.H. Khan, P. Gopalasundaram, E.V.V Bhaskara Rao, Oxford and IBH Publishing Co. Pvt. Ltd., 1 493-503.
- Mayilvaganan, M and Nair, C.P.R. (2002). Physico chemical changes due to coreid bug infestation on coconut. *J. Plantn. Crops* **30**(2): 61-63.

- Menon, K.P.V. and Pandalai, K.M. 1960. *The Coconut palm- A monograph*, Indian Central Coconut Committee, Ernakulam 384p.
- Nair, C.P.R., Sathiamma, B., Chandrika Mohan and Murali Gopal (1998) Newer approaches in the integrated management in coconut. *Indian Coconut Journal* **29**(4): 99-103.
- Ponnamma, K.N., Kamalakshyamma, P.G., Kurian, C. and Nambiar, P.T.N. (1982) Incidence of *Myloccerus curvicornis* (Fab.) in relation to nutritional status of coconut. *The Philippines J. of Coconut Studies* 1&2 (VII): 40-41.
- Rajan, P. and Nair, C.P.R. (2005) Coreid bug of coconut. *Indian Coconut Journal* **36**(6): 11-12
- Rajan, P. and Nair, C.P.R. Rajan, P., Chandrika Mohan and Josephraj Kumar, A. (2010) Have coconuts without pest infection. *Indian Horticulture* **55**(5): 33-37.
- Ramani, S. 2000. Fortuitous introduction of an aphelinid parasitoid of the spiraling whitefly *Aleurodicus dispersus* Russell (Aleyrodidae : Homoptera) into the Lakshadweep Islands, with notes on host plants and other natural enemies. *Journal of Biological Control* **14**: 55-60.
- Sathiamma, B., Abraham, V.A. and Kurian, C. (1972) Slug caterpillar-a sporadic pest of coconut. *Indian Farming*. **21**(11): 39-40.
- Vidyasagar, P.S.P.V. (1989) Scale insects and mealybugs of coconut. *CPCRI Extension folder No. 16*, 4p.
- Visalakshy, A., Beevi, S.N., Pillai, K.S., Nair, K.K.R and Das, N.M. (1987) Control of the coreid bug on coconut. *Entomon* **12**(4): 395-396.

Biology of coreid bug, *Paradasynus rostratus* Dist. in different hosts

Ambily Paul and C. Nandakumar

Department of Entomology, College of Agriculture
Kerala Agricultural University, Vellayani, Thiruvananthapuram, Kerala, India

Even though coconut enjoys a prime position as a plantation crop in the country, it is subjected to the vagaries of nature and outbreaks of pests and diseases. The coconut palm is prone to infestation by a large number of insect and non insect pests. Of late, the coreid bug, *Paradasynus rostratus* Dist. has gained notoriety as a major pest of coconut in Kerala. The adults and nymphs of the bug feed from the buttons resulting in immature nut fall. They suck the sap from the immature buttons by thrusting their long needle like mouth part just below the perianth which later develops necrotic lesions. The high temperature, low relative humidity and low rainfall favoured the multiplication of the coreid bug (Paul *et al.*, 2009). Significant reduction in copra and oil was observed in moderately and heavily damaged nuts (Paul *et al.*, 2010). Several plants had been reported as alternative hosts of *P. rostratus* in Kerala. Cashew (Nair and Remamony, 1964), guava (Nair, 1975; Kurian *et al.*, 1976; Beevi *et al.*, 1989), mango, tapioca, rubber (Kurian *et al.*, 1976), tamarind (Kurian *et al.*, 1979), neem (Sundararaju and Babu, 1999), cocoa (CPCRI, 1999), passion fruit (Mohan and Nair, 2000) and black pepper (Lekha and Mohan, 2004) had been identified as alternative hosts of the pest. However, the most potential hosts are guava, cashew and neem. The study on the duration of lifecycle of coreid bug concurrently on different hosts is yet to be done. Study of biology is important to establish the nature of population build up of coreid bug on different hosts and to determine the economic threshold level for effective management of the pest.

Studies on the life cycle of *P. rostratus* on coconut and alternative hosts *viz.*, guava, cashew and neem were conducted in the Department of Entomology, College of Agriculture, Vellayani. The adult bugs collected from the field were confined in cylindrical glass jars (20 cm x 50 cm). The observations on mating, egg laying and egg period were noted. Four cylindrical glass jars (20 cm x 50 cm) were selected. Coconut buttons collected from third bunch along with rachis, guava twigs with tender fruits, twigs of cashew with young nuts and tender twigs of neem were moistened by placing the cut end into conical flasks containing water. These conical flasks were placed inside cylindrical glass jars and the mouths of the jars were covered with muslin cloth. A set of five first instar nymphs were introduced into each glass jar. The number of instars, duration of each instar and adult longevity were observed and recorded.

The adult female laid eggs in clusters containing an average of 43 yellowish oval shaped eggs per cluster. The eggs were 1.65 mm long and 0.85 mm wide at the middle. At maturity (hatching), the eggs turned dark red with a golden tinge. A crescent shaped black marking was present at the cephalic end of the egg. The egg period was 8.20, 8.40, 7.60 and 8.40 days when reared in guava, neem, cashew and coconut respectively. The pest completed five nymphal instars when reared on the alternative hosts (Table 1 and Plate 1). The mean egg period took 8.15 days.

The first instar nymphs were ant like, reddish orange in colour and measured 2.50 mm x 0.70 mm. After hatching, they congregated near the eggshells. Antenna had 4 segments

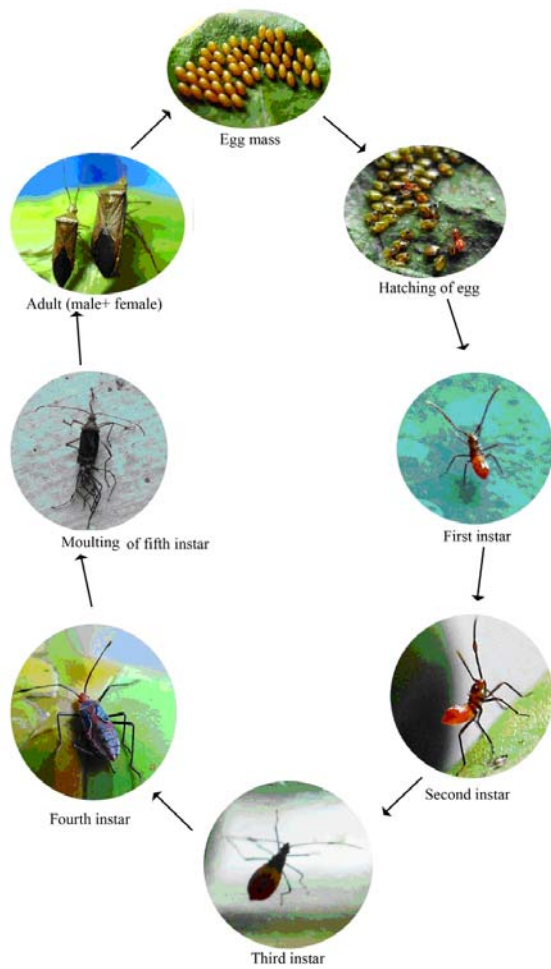


Plate Life cycle of coreid bug, *Prostratus*

Table 1. Developmental duration and biometrics of *Paradasynus rostratus* in different hosts

Life stages	Duration (days)					Size (mm)
	Guava	Neem	Cashew	Coconut	Mean	Length x Breadth
Egg period	8.2	8.4	7.6	8.4	8.15	1.65 x 0.85
I instar	3.6	4.6	3.8	3.6	3.90	2.50 x 0.70
II instar	6.8	5.6	6.4	4.6	5.85	4.50 x 1.00
III instar	4.2	4.0	3.8	4.2	4.05	6.70 x 1.50
IV instar	5.6	5.0	6.4	5.2	5.55	8.50 x 2.00
V instar	4.8	5.6	5.4	5.4	5.30	13.00 x 2.50
Adult longevity						
Male	55	50	62	68	58.75	16.50 x 5.00
Female	60	52	65	68	61.25	17.50 x 6.00

and was 2.50 mm long. The third segment of antenna was laterally compressed. The nymphs took 3.60, 4.60, 3.80 and 3.60 days to moult into second instar when reared in guava, neem, cashew and coconut respectively. The mean number of days taken by the first instar nymphs was 3.90.

The second instar nymphs were 4.50 mm long and 1.00 mm wide. The abdomen was dark reddish and slightly shorter than the thorax. The second instar nymphal period recorded in the different hosts guava, neem, cashew and coconut were 4.20, 4.00, 3.80, 4.20 respectively. The antenna was 6.51 mm in length and reddish brown in colour. The third joint of the antenna was lighter in colour compared to the other parts of the antenna. The second instar nymphs took a mean of 5.85 days to moult into the third instar.

The third instar nymphs measured 6.70 mm x 1.50 mm. The head was as long as the thorax. The antenna was 9.00 mm in length and reddish brown in colour. The instar took 4.20, 4.00, 3.80 and 4.20 days in guava, neem, cashew and coconut respectively to moult into the next instar. The mean number of days taken by the fourth instar nymphs was 4.05 days. From the third instar onwards, the nymphs moved through the foliage and tender branches for feeding.

The fourth instar nymphs were 8.50 mm long, 2.00 mm wide and dark reddish brown in colour. Small wing buds were visible. The duration of the fourth instar nymph was 5.60, 5.00, 6.40 and 5.20 in guava, neem, cashew and coconut respectively. The fourth instar nymphs took a mean of 5.55 days to moult into the next instar.

The fifth instar nymphs were dark reddish brown in colour and measured 13.00 mm x 2.50 mm in size. Wing buds were prominent, 4.00 mm in length and covered the meso and metathorax. Antenna measured 14.50 mm in length. The fifth instar became adult in 4.80, 5.60, 5.40, and 5.40 days in guava, neem, cashew and coconut respectively. The fourth and fifth instar nymphs caused severe damage to the crops they infested. The symptoms of damage caused by the bug on young buttons of coconut are depicted in Plate 2. The fifth instar was completed in 39.00, 40.20, 39.40 and 28.00 days in guava, neem, cashew and coconut respectively. This instar took a mean of 5.30 days to complete the fifth instar.

The female adult coreid bug was slightly bigger than the male with broader abdomen. Female measured 17.50 mm in length and 6.00 mm in width across the prothorax. However, the male measured 16.50 mm x 5.00 mm. The other characters were similar in both male and female coreid bugs. Antenna was reddish brown in colour and nearly as long as the body. The basal one fourth portion of the fourth segment of antenna was whitish. Mating took place six to seven days after emergence. Copulation occurred several times during adult life, oviposition started six to seven days after the first copulation. The longevity of male bugs was 55, 50, 62 and 68 days in guava, neem, cashew and coconut respectively. The female had a life span of 60, 52, 65 and 68 days on guava, neem, cashew and coconut respectively. The average lifespan of the male and female coreid bug was 58.75 and 61.25 days respectively. The longevity of the male bug was shorter compared to females.

Knowledge of the biology of the bug on different host plants is essential to determine the population buildup of the bug in an area with a heterogeneous mix of crops susceptible to bug infestation and also to predict the number of generations completed per year. No significant

difference was observed in the life cycle of the pest when reared on the host crop and alternative hosts. The observations concurred with the findings of Nair and Remamony (1964); Nair (1975); Kurian *et al.* (1976 and 1979); Ponnammam *et al.* (1985); Beevi *et al.* (1989) and Nair *et al.* (2000). Study on the duration of lifecycle of coreid bug in different hosts simultaneously would be helpful for developing a management strategy against this pest.

The coconut based farming system is the most common farming system occupying more than 90 per cent of the homestead farms in the state (Regeena *et al.*, 2004). The availability of food and habitat from the various hosts fuel the growth and development of the pest and consequent infestation. The stratification of the coconut palm and other alternative hosts in the multiple cropping system greatly influence the nature and distribution of the bug. This may be due to the orientation of the bugs to adequate food source in the proximity provided by the principal as well as alternative hosts at different seasons of the year. Starving and the presence of eggs to be laid bring about a remarkable increase in locomotive activity of female bug and these behavioural reactions help the females to find their feeding and egg laying sites. From the above studies, it is clear that the life cycle of coreid bug in different hosts are more or less similar. Hence the chances of coreid bug to breed in different hosts available in the homestead are equal. The uninterrupted supply of food and habitat throughout the year relative to the seasonal cycle of the coreid bug was the most important factor in the coreid bug becoming a major pest. This is perhaps the most important factor to be considered in the process of developing tactics for ecological management of the coreid bug. The influence of different host plants on the rate of development of coreid bug needs further studies.

Acknowledgements

The authors are grateful to the Dean, College of Agriculture, Vellayani and Professor and Head, Instructional farm, Vellayani for the facilities provided to carry out the work. The assistance provided in the form of SRF by CSIR to the first author for conducting the research is thankfully acknowledged.

References

- Beevi, S.N., Visalakshi, A., Nair, K.K.R., Remamony, K.S. and Das, N.M. 1989. Guava as a potential host of *Paradasynus rostratus* D. (Coreidae) the coreid bug of coconut in Kerala. *Entomon* **14**:363-364.
- CPCRI. 1999. *Annual Report*. Central Plantation Crops Research Institute, Kasaragod, Kerala, India, 178.
- Kurian, C., Abraham, V.A. and Koya, K.M.A. 1976. A new enemy of coconut in India. *Indian Farming* **27**:12.
- Kurian, C., Abraham, V.A. and Koya, K.M.A. 1979. Studies on *Paradasynus rostratus* Dist. (Heteroptera: Coreidae) a pest of coconut. pp. 484-503. In: *Proceedings of International Symposium on Plantation Crops, Placrosym 11*, December 12-14, 1979. Ootacamund, Nilgris.
- Lekha, M. and Mohan, P. 2004. Record of coreid bug, *Paradasynus rostratus* Distant (Hemiptera: Coreidae) on black pepper. *Insect Environ.* **10** (3):118.
- Mohan, C. and Nair, C.P.R. 2000. Distinguish the nut infestation by eriophyid mite and coreid bug in coconut. *Indian Cocon. J.* **31**:60-61.

- Nair, M.R.G.K. 1975. *Insects and Mites of Crops in India*. 3rd edition. Indian Council of Agricultural Research, New Delhi, 404 p.
- Nair, K.R.C., Abraham, V.A., Rajan, P. and Nair, C.P.R. 2000. The nut crinkling coreid bug, *Paradasynus rostratus* Dist. (Heteroptera: Coreidae) – An emerging pest of coconut palm in India. *Indian Cocon. J.* **31**(3): 62-63.
- Nair, M.R.G.K. and Remamony, K.S. 1964. *Paradasynus* sp. (Hemiptera: Coreidae) as a pest of cashew nut in Kerala. *Indian J. Ent.* **26** (4): 461-462.
- Paul, A., Nandakumar, C and Hebsy Bai. 2009. Seasonal abundance of coreid bug, *Paradasynus rostratus* Dist. in coconut. In: *Proceedings of the National workshop on climate and development*, Trivandrum, Kerala. 132p.
- Paul, A., Nandakumar, C and Stephen, R. 2010. Extent of yield loss in coconut due to the infestation of coreid bug, *Paradasynus rostratus* Dist. *J. Plantn. Crops.* **37** (10): 45-50.
- Ponnamma, K.N., Kurian, C., Sukumaran, A.S. and Koya, K.M.A. 1985. Field evaluation of BHC, carbaryl and endosulfan for the control of the coconut coreid bug *Paradasynus rostratus* Distant. *Indian Cocon. J.* **15** :10-11.
- Regeena, S., John, J., Ravi, S., Krishnakumar, V. and Geetha, K. 2004. *Homestead Farms-Agricultural Heritage Systems of Kerala*. Technical Bulletin. Directorate of Extension, Kerala Agricultural University, Thrissur, 24 p
- Sundararaju, D. and Babu, P.C.S. 1999. Neem as a trap for the coconut coreid bug, *Paradasynus rostratus* Dist. *Insect Environ.* **5**: 31.

Natural enemy complex associated with coconut eriophyid mite, *Aceria guerreronis* Keifer

Chandrika Mohan, P. Rajan and C.P.R. Nair

Central Plantation Crops Research Institute
Regional Station, Kayamkulam 690 533, Kerala, India

The coconut eriophyid mite *Aceria guerreronis* Keifer (Acari: Eriophyidae) is one of the major pests of coconut (*Cocos nucifera* Linn.) in South America, Caribbean Islands, India, Africa, and Sri Lanka (Mariau, 1977; Sathiamma *et al.*, 1998; Seguni, 2000 and Fernando *et al.*, 2000). Nymphs and adults feed on the soft meristematic tissues of the buttons by remaining under the perianth. Continuous feeding leads to development of warting and longitudinal fissures on the surface of the nuts. Severe infestation results in poor growth, malformation, reduction in size of the nuts and copra content. The microscopic size of the mite and its hidden habitat underneath the tepals of developing young nuts has hindered effectiveness of pest management programmes. Various chemical and botanical pesticides are currently recommended for management of the pest which results in only short term effect to mite management. The chemical pesticides are hazardous to humans, pollinators, natural enemies and in a long way to the environment. Hence studies were conducted to catalogue natural enemies associated with coconut eriophyid mite from various coconut growing tracts of India with an aim to select the most promising one for an effective, long lasting field level biocontrol. Result of the investigations for the period during 2000-2010 is presented in the paper.

Eriophyid mite infested nut samples were collected by regular surveys from various coconut growing locations of Kerala. Detailed surveys covering 10 panchayat in each district were undertaken in 2004-05. The survey was carried out at 3-4 months interval in all the districts so as to cover various seasons of the year *viz.*, summer (January – April), rainy months (May-August) and post monsoon (September-December). Random surveys were undertaken during 2006-09 in other States of India namely Tamil Nadu, Andhra Pradesh, Orissa, Karnataka, West Bengal, Assam and Lakshadweep Islands.

Nuts of 3-5 month old maturity with different levels of mite infestation were collected from various mite infested palms and were brought to the laboratory at Central Plantation Crops Research Institute, Kayamkulam, Kerala. The mite colonies on nut surface and tepal lobes were observed under microscope for presence of natural enemies. The predators found associated with the eriophyid mite colonies were collected and preserved in 70% alcohol and got identified. The predators were identified by Prof. M. Mohanasundaram, Prof (Retd.), Tamil Nadu Agricultural University, Coimbatore, India and *Neoseiulus baraki* by CRI, Sri Lanka.

The nuts were screened for presence of dead colonies under stereomicroscope. Dead mite colonies were picked to a clean microscopic slide and stained using lacto phenol cotton blue. Microorganisms associated were isolated by plating surface sterilized mite on respective media for fungus/bacteria and incubated at $28 \pm 2^{\circ}\text{C}$ and observed at every 24 hours. The microbes were brought to pure culture and identified. Identification of the promising *Hirsutiella thompsonii* isolates were carried out by CABI. Pathogenicity was assayed on detached nuts with cut end dipped in 2% sucrose solution. Homogenized spores and hyphae / cell suspension were sprayed on the nuts.

Observations recorded on the population of predatory mites present in sample nuts from different locations revealed the presence of six species of predatory mites (Table 1).

Table 1. Predatory mites associated with *Aceria guerreronis* beneath the perianth of coconuts

Sl. No.	Species	Family
1	<i>Neoseiulus baraki</i> Athias-Henriot	Phytoseiidae
2	<i>Neoseiulus paspalivorus</i> De Leon	Phytoseiidae
3	<i>Typhlodromus</i> sp.	Phytoseiidae
4	<i>Chelacaropsis moorei</i> Baker	Cheyletidae
5	<i>Cheletogenes ornatus</i> (Canestrini & Fanzago)	Cheyletidae
6	<i>Bdella</i> sp	Bdellidae

Among the predatory mites, *N. baraki* was the predominant species (75.9%) attacking eriophyid mite colonies in the field in all the years and other predators were recorded only in very few numbers (Fig.1). One species each of syrphid, coccinellid and thrips was also recorded. Other mites collected from the coconut mite infested nuts and tepals include *Polyphagotarsonemus latus* Banks (Tarsonemidae), *Tyrophagus putrescentiae* Schrank (Acaridae), *Blattisocius* sp (Ascidae) and *Opsomyssus* sp (Gastronyssidae).

Observations revealed continued presence of predatory fauna during various years with a steady increase of their population in the field over the years. On an average 37.09 samples showed presence of predators during 2002 which was increased up to 82.7% in 2006 (Fig.2). This indicated that a predatory population was registering an increasing trend of incidence and a better establishment in nature.

Seasonal incidence of the major predator (*N. baraki*) was studied for 2 years during 2001-02 in the initial build up stage of eriophyid mite and predatory fauna and also repeated during 2007-08 when there was steady presence of predators with eriophyid mite colonies. Results revealed that the population of *N. baraki* was present throughout the year with peak during April during 2001-02 and the predator was present in high density for

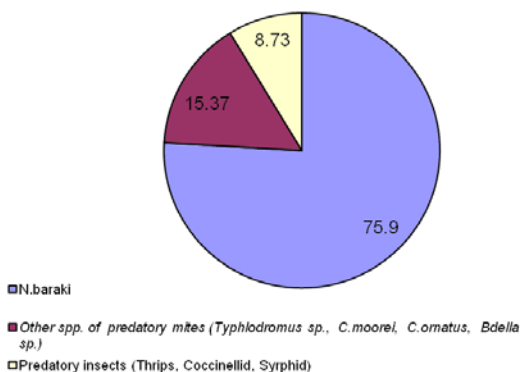


Fig. 1. Extent of natural occurrence of various predators on coconut eriophyid mite

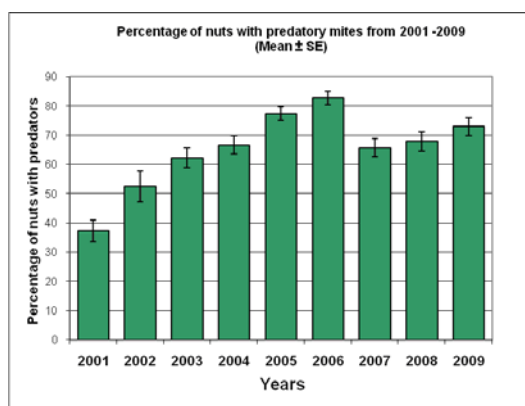


Fig. 2. Percentage of nuts with predatory mites during 2001-2009

more prolonged period during December to June during 2007-08. The average number of *N. baraki* for the 4 years is presented in Fig.3.

Nuts of various months maturity (one month to eight month old) were collected from 10 palms during the month of April for 2 years 2004 and 2005 and observed for presence of eriophyid mite and predators. Population build up of eriophyid mite starts from 2nd month of nut maturity and peak population was observed in 5th month old nut. Population of predatory fauna build up was observed from 3rd month and maximum population was observed on 6th month old nuts. The average population of eriophyid mite and predators for two years is presented in Fig.4. Population of the predatory mites were more in the inner most two tepals compared to mite colonies on nut surface and the outer tepals.

Observations on predatory fauna in nuts collected from different states of India and Lakshadweep also showed their occurrence in all major geographic zones of the country including island ecosystem (Table 2). The percent incidence of predators ranged from 51.0 to 81.3 in different states of the country.

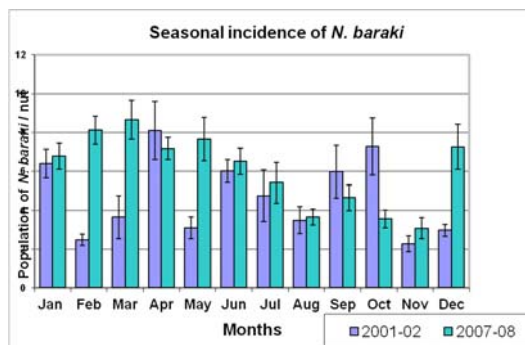


Fig. 3. Seasonal incidence of *N. baraki* on *A. guerreronis* infested nuts (average of four years)

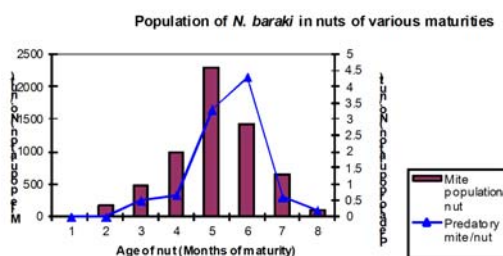


Fig. 4. Average population of eriophyid mite and predatory mites on nuts of different maturity stage

Table 2. Incidence of predatory mites on coconut mite in various locations

States	Percent of nuts with predators	Average number of <i>N. baraki</i> / nut
Tamil Nadu	55.7	8.3
Andhra Pradesh	70.5	6.3
Orissa	78.8	8.2
Lakshadweep	81.3	8.1
Karnataka	73.5	9.6
West Bengal	51.0	3.9
Assam	66.7	4.0
Kerala	74.2	6.0

A variety of predatory mites and smaller insects have been found associated with *A. guerreronis* in different parts of the world. Howard *et al.* (1990) reported *Amblyseius largoensis*

Muma, *Neoseiulus mumai* Denmark, *N. paspalivorus* De Leon attacking mites in Florida. The Bdellidae mites namely *Bdella indicata* and *Bdella distincta* Baker and Balock were reported as predators of mite in Benin by Mariau (1977). Hall *et al.*, (1980) reported tarsonemid *Lupotarsonemus* from Ivory Coast and New Guinea attacking the different stages of coconut eriophyid mite.

Fernando *et al.* (2000) had identified *Neoseiulus paspalivorus* De Leon (phytoseidae) and *Bdella* sp (Bdellidae) as predatory mite fauna on eriophyid mite in Sri Lanka. Marimuthu *et al.* (2003) had reported three species of predatory mites viz. *Amblyseius paspalivorus*, *Bdella* sp and a tarsonemid attacking mite colonies in the field in Tamil Nadu with a population level of only 5-10 per nuts. Predatory mites belonging to phytoseidae, Tarsonemidae and Bdellidae were reported from Kerala by Naseema Beevi *et al.* (2003). Mallik *et al.* (2003) reported that among the phytoseid mites, *A. paspalivorus* and the tarsonemid *Lupotarsonemus* sp are the predatory mites more encountered with mite colonies in Karnataka. The tarsonemid is found attacking on egg and the phytoseid attacking on all stages of mite. The potential of predators in suppression of eriophyid mite colonies has been questioned by many workers on account of the facts that they are observed only occasionally and in very small populations on infested coconut and there is no evidence that they make a significant impact on coconut mite population (Hall *et al.*, 1980; Howard *et al.*, 1990). Fernando *et al.* (2010) had reported suppression of *A. guerreronis* population by inundative release of laboratory bred *Neoseiulus baraki* in Sri Lanka. Present investigation recorded Phytoseiid, Cheyletid and Bdellid mites as predators of *A. guerreronis* with *Neoseiulus baraki* as the prominent predatory mite with maximum predatory population on 6th month old nut.

Attempts to isolate microbial organisms from dead colonies of coconut mite during different periods of the study revealed the presence of various groups of organisms belonging to fungi, actinomycetes, and bacteria (Table 3). Among the various microbes isolated from dead mite colonies *Fusarium* species was the predominant fungus obtained from samples from all the areas.

Table 3. Microorganisms isolated from eriophyid mite

Microorganisms	Frequency of isolation (% of total nut)
<i>Fusarium</i> spp.	55.6
Actinomycetes	18.2
<i>Hirsutella thompsonii</i>	7.9
<i>Aspergillus</i> sp., <i>Mucor</i> , <i>Acremonium</i> ,	18.3
<i>Pencillium</i> , <i>Lecaniu</i> , Bacteria and others	

Percentage of eriophyid mite infested nuts with microbial infections ranged from 20.0 (Assam) to 55.8 (Orissa). From the microbial infected nuts 37 isolates of *H. thompsonii* were recovered from Kerala, 3 from Karnataka and 2 from Orissa accounting for a total of 42 isolates of *H. thompsonii* (Table 4).

Table 4. Occurrence of microbial pathogens with *A. guerreronis* in various states

State	Districts surveyed	Average % of nuts with microbial infection	<i>H. thompsonii</i> isolates obtained (number)
Kerala	Trivandrum, Kollam, Alappuzha, Kottayam, Ernakulam, Calicut	27.4	37
Tamil Nadu	Malappuram, Thrissur, Kasaragod Pollachi, Thanjavoor, Thirunelveli, Kanyakumari	24.05	-
Andhra Pradesh	East Godavari, West Godavari, Krishna	31.55	-
Karnataka	Dakshina Kannada, Hassan, Tumkur	42.0	3
Orissa	Puri	55.8	2
West Bengal	Mohit Nagar	22.3	-
Assam	Kahikuchi	20.0	-
Lakshadweep	Androth, Kalpeni, Kavarathi, Minicoy	23.6	-

Isolation of *H. thompsonii* from eriophyid mite infested nuts was attempted throughout the year during 2005-2006 from South Kerala. Isolation studies revealed that maximum incidence of *H. thompsonii* during post monsoon season namely September to December (Fig 5).

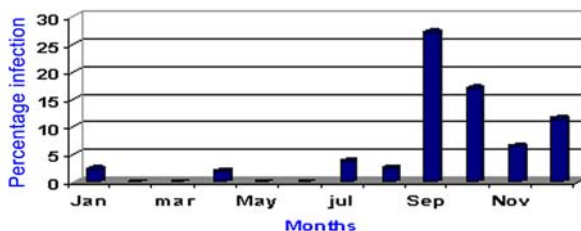


Fig.5. Seasonal incidence of *H. thompsonii* in Kerala (2005-06)

Pathogenicity trials with *Fusarium moniliformae*, Actinomycetes, bacteria and *H. thompsonii* resulted in 34.3 %, 10%, 4.5% and 76% mortality respectively of the mite in treated nuts. *Lecanium* spp and *Penicillium* sp were not pathogenic. Owing to the phyto-infective property of many *Fusarium* specieses no detailed studies were attempted.

Among microbial pathogens, fungal species contribute the predominant pathogens of coconut eriophyid mite. The fungal Pathogen *Hirsutella thompsonii* has received considerable attention throughout the world as the most effective natural enemy of eriophyid mite of coconut.

H. thompsonii has been used both in the laboratory and field for suppressing the coconut mite in different countries of the world (Hall *et al.*, 1980; Moore and Howard, 1996). The potential of this fungus as an effective biocontrol agent against *A. guerreronis* was well documented (Espinosa Becerril and Carrilo Sanchez, 1986; Lampedro and Rosas 1989; Cabrera, 2000). In India, the incidence of the pathogen (*H. thompsonii*) was recorded by many workers (Beevi, *et al.*, 1999; Kumar *et al.* 2001a; Kumar, 2002). Other fungal species associated with eriophyid mite include species of *Paecilomyces*, *Beauveria*, *Metarhizium*, *Sporothrix*, *Verticillium*, *Acremonium*,

Aspergillus, *Penicillium* and *Fusarium* (Kumar *et al.*, 2001 b) and *Scopulariopsis brevicaulis* (Murali Gopal *et al.*, 2002).

Pathogenicity tests were carried out with all the 42 isolates of *H.thompsonii*. Pathogenicity was proved positive by establishing Koch's postulates with 9 isolates when carried out in detached nuts harboring mite colonies. Average percentage mortality of mite with inoculation of the isolates is presented in Table 5.

Table 5. Mortality of coconut mite with various *H. thompsonii* isolates

Isolates	Percentage mortality of mite (7DAT)
CPCRI-19	81.8 (65.2)
CPCRI-33	60.4 (50.9)
CPCRI-11	77.4 (61.6)
CPCRI-51(II)	83.9 (68.0)
AMB-37	60.35 (55.4)
THA-5	70.1 (56.8)
KAN-4	66.4 (54.9)
MARA-27	67.5 (55.3)
KAY-4	82.3 (68.7)
Control	22.8 (28.4)
General mean	56.53
CD (P = 0.05)	9.54

Figures in parenthesis are the angular transformed values

Several morphological variations were observed among the *H.thompsonii* isolates. Colony characters of some of the distinct *H.thompsonii* isolates were studied and the details are given in Table 6.

Table 6. Colony characters of the virulent isolates of *Hirsutella thompsonii* in SDA medium

Sl.No.	Isolate	Radial growth*	Cfu /ml	Growth characters on SDA
1	KAY-4	2.35 cm	18 x10 ⁴	Cement coloured raised cottony growth, no honey dew, highly curly synnemata
2	CPCRI-11	3.71 cm	5 x10 ⁴	Cream colour flat growth, honey dew present, synnematous
3	MARA-27	1.68 cm	12 x10 ⁴	Honey dew present, uniformly cement grey colour, short synnemata of 1-2 cm.
4	CPCRI-19	3.25 cm	22 x10 ⁴	White intermingled with ash surface growth, synnematous, 1-2 honey dew

5	KAN-4	2.81 cm	175 x10 ⁴	White and ash colour in some areas,. slightly raised growth, no honey dew, no synnemata
6	THA-5	3.01 cm	12 x10 ⁴	Cottony white with ash colour, 4-5 cm long synnemata
7	AMB-37	2.60 cm	20 x10 ⁴	Cottony grayish growth, honey dew present, synnematous
8	CPCRI-33	2.78 cm	8 x10 ⁴	Light ash colour, no honey dew, synnematous
9	CPCRI-51(II)	3.50 cm	21 x10 ⁴	Ash raised surface, no synnemata, no honey dew

* Radial growth after 10 days of inoculation at lab. temperature (28°C) in petri plate

Six species of predatory mite, one species each of syrphid, coccinellid and thrips were found in association with coconut mite colonies. *N. baraki* was the predominant species of predatory mite and is showing an increasing trend of establishment in nature over the years. These natural predators has to be conserved by avoiding indiscriminate spray of pesticides in the microhabitat as they can exert good check on the buildup of mite population. Among pathogens, *Hirsutella thompsonii* was the major species pathogenic to coconut mite inflicting 60-84% mortality. There is high morphological diversity in the *H. thompsonii* collections. Studies on formulation of a myco-acaricide with *H. thompsonii*, its shelf life, mode of application and frequency of application in relation with the weather factors are to be explored in detail.

References

- Beevi, S. P., Beena, S., Lyla, K.R., Varma, A.S., Mathew, M.P. and Nadarajan, L. 1999. *Hirsutella thompsonii* var. *Synnematos* Samson, McCoy & O'Donnell on coconut mite *Aceria (Eriophyes) guerreronis* (Keifer) – a new report for India. *J. Tropical Agriculture* **37**(1-2): 91-93
- Cabrera, R.I. 2000. Biological control of the coconut mite, *Aceria guerreronis* (Acari: Eriophyidae) with the fungus *Hirsutella thompsonii* and its integration with other control methods. In: *Proceedings of International Workshop on coconut Mite*, CRI, Sri Lanka, pp. 89-103.
- Espinosa Becerril A. and Carrilo Sanchez, J.L. 1986. The fungus *Hirsutella thompsonii* Fisher of the control of eriophyid *Eriophyes guerreronis* (Keifer). *Agricultura technical en Mexico* **12**: 319-323.
- Fernando, L.C.P., Wickramananda, I.R. and Aratchige, N.S. 2000. Status of coconut mite, *Aceria guerreronis* in Sri Lanka. In: *Proc. of International Workshop on Coconut mite (Aceria guerreronis)*. Eds. L.C.P.Fernando, G.J. de Moraes and Wickranananda, I.R. , CRI, Sri Lanka. pp.1-8.
- Fernando, L.C.P., Waidyaratne, K.P., Perera, K.F.G. and De Silva, P.H.P.R. 2010. Evidence for suppressing coconut mite, *Aceria guerreronis* by inundative release of the predatory mite, *Neoseiulus baraki*. *Biological Control* **53**: 108-111.
- Hall, R.A., Hussey, N.W. and Mariau, D. 1980. Results of a survey of biological control agents of the coconut mite, *Eriophyes guerreronis*. *Oleageneux* **35**: 395 - 400.
- Howard, F.W., Abreu Rodriguez, E. and Denmark, H.A. 1990. Geographical and seasonal distribution of the coconut mite, *Aceria guerreronis* (Acari: Eriophyidae), in Puerto Rico and Florida, USA. *J. Agric. University Puerto Rico*. **74** (3): 237 – 251.

- Kumar, P.S. 2002. Development of a biopesticide for the coconut eriophyid mite in India pp 335-340. In: *Proceedings of the British Crop protection Conference-Pest & diseases Vol 1&2*, 18-21 November 2002. British Crop Protection Council, United Kingdom.
- Kumar P.S., Singh S.P. and Gopi, T. S. 2001a. Natural incidence of *Hirsutella thompsonii* Fisher on the coconut eriophyid mite *Aceria guerreronis* Keifer in certain districts of Karnataka and Tamil Nadu in India. *Journal of Biological Control* **15**: 151-156.
- Kumar, P.S., Singh S.P., Anuroop, C. P. and Ramachandra, C.G. 2001b. Acaropathogenic fungi associated with the coconut eriophyid mite (*Aceria guerreronis*) in south India, pp 99-100. In: *National Seminar on Emerging Trends in Pests and Diseases and Their Management*, 11-13 October, 2001, Tamil Nadu Agricultural University, Coimbatore, India.
- Lampedro, L. and Rosas, J.L. 1989. Selection of *Hirsutella thompsonii* Fisher strains to fight the coconut mite *Eriophis guerreronis* Keifer. 1. Pathogenicity bioexperiments *Revista Mexicana de Micologia* **5** : 225-231.
- Mallik ,B.C., Chinnamade Gowda,, Jayappa, J., Guruprasad, H. and Onkarappa, S. 2003. Coconut Eriophyid mite in India issues and strategies. In: *Coconut Eriophyid Mite - Issues and Strategies Proceedings of the International workshop on coconut Mite held at Bangalore*. Eds. H.P. Singh and P. Rethinam. Coconut Development Board. pp. 27-34.
- Mariau, D. 1977. *Aceria guerreronis*: an important pest of Africa and American coconut plantations. *Oleagineux* **32**(3): 101-111.
- Marimuthu, T., Palanisamy, S. and Ramaraju, K. 2003. Integrated Management of Coconut Eriophyid Mite (*Aceria guerreronis* Keifer) in Tamil Nadu. In: *Coconut Eriophyid Mite - Issues and Strategies Proceedings of the International workshop on coconut Mite held at Bangalore*. (Eds. H.P. Singh and P. Rethinam). Coconut Development Board. Kochi. Pp 81- 86.
- Moore, D and Howard, F.W. 1996. Coconuts In: *Eriophyid Mites. Their Biology, natural enemies and control*. Linquist, E.E.; Sabelis, M.W. and Bruin.J (Eds.) Elsevier, Amsterdam, Netherlands. pp 561-570.
- Murali Gopal., Saleena, A. and McCoy, C.W. 2002. Study of microflora associated with coconut eriophyid mite as a preliminary step towards pathogen isolation. *Current Science*. **82**(1):22-24.
- Naseema Beevi,S., Thomas Biju Mathew, Hebsy Bai and Saradamma, K. .2003. Status of Eriophyid mite in Kerala – Resume of work done. In: *Coconut Eriophyid Mite - Issues and Strategies Proceedings of the International workshop on coconut Mite held at Bangalore*. (Eds. H.P. Singh and P. Rethinam). Coconut Development Board. Kochi pp. 64- 75.
- Sathiamma, B., Nair, C.P.R. and Koshy, P.K. 1998. Out break of a nut infesting eriophyid mite *Eriophyes guerreronis* (K.) in coconut plantations in India. *Ind. Cocon. J.* **29**(2): 1-3.
- Seguni, Z. 2000. Incidence, distribution and economic importance of the coconut eriophyid mite, *Aceria guerreronis* Keifer in Tanzanian coconut based cropping systems. In: *Proceedings of International Workshop on coconut mite* (Eds. Fernando et al., 2000) pp 54-57.

Survey and monitoring of pest problems of coconut in Konkan region of Maharashtra

S.S.Gurav, V.S.Desai, B.N.Bhangare, D.D.Nagwekar and A.L.Narangalkar

Regional Coconut Research Station, Bhatye, Ratnagiri
Dr.Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli, Maharashtra, India

Coconut is one of the important plantation crops of the Maharashtra. The area under this crop has increased from 6,600 ha (Thampan, 1986) to 34,000 ha in 2005 (Anonymous, 2006) after successful implementation of Employment Guarantee Scheme.

Even though, the coconut had a prime position as a plantation crop in the country, it faces many problems like vagaries of nature and sudden out break of pest and diseases. The coconut palm is attacked by 200 insect and non-insect pests (Rabindra, 2000) at different stages of its growth. Among these, the rhinoceros beetle, red palm weevil, black headed caterpillar, eriophyid mite and rats are serious pests and found in many coconut gardens in Konkan region of the Maharashtra.

The roving survey was conducted in different areas of Konkan region of Maharashtra to know the intensity of infestation of coconut rhinoceros beetle, red palm weevil and black headed caterpillar. Out of five districts of Konkan, four districts *viz.*, Thane, Raigadh, Ratnagiri and Sindhudurg were selected for recording observations on pest infestation because; the coconut cultivation is confined to these four districts only. Out of these four districts, the coconut growing tahasils were selected. From each tahasil, two villages were selected and from each village two coconut growers were selected for recording observations. The total number of palms from each tahasil was taken in to consideration for calculating per cent infestation.

The per cent infestation was calculated by using following formula;

$$\text{Per cent infestation} = \frac{\text{Total number of infested palms}}{\text{Total number of palms}} \times 100$$

The same orchards were used for every year survey to know the increase or decrease in pest infestation. The survey was conducted in February-March 2005, November 2005, May-June 2007 and January 2008.

The district wise situation of different pests of coconut is given in Table 1. Data revealed that the rhinoceros beetle and red palm weevil was observed in all districts under study. The black headed caterpillar was observed in Thane district only in all the four periods of survey. The intensity of infestation of rhinoceros beetle was ranged from 14.93 to 19.02 per cent in all the districts. The intensity was more or less similar in all the periods of survey; this indicated that the rhinoceros beetle was present in all the areas of Konkan region of Maharashtra. The infestation of red palm weevil was in the range of 0.61 to 12.36 per cent. The intensity was maximum (12.36 %) in Ratnagiri district only in February-March 2005; this may because of the one infested orchard in the area. But the infestation was decreased in next periods of

survey after adoption of suitable control measures. Otherwise the intensity of the red palm weevil is very less in all the districts.

The intensity of black headed caterpillar was found only in Thane district and it was in the range of 0.70 to 25.36 per cent. In the year 2005 the intensity was more and then it was declined to the extent of 0.70 per cent. The black headed caterpillar was observed in Raigadh district only in the year 2005. Tahasil wise data on infestation of different pests of coconut are given in Table 2.

The infestation of rhinoceros beetle was more or less similar in both coconut growing Vasai and Palghar tahasils and it was in the range of 12.83 to 19.53 per cent. The intensity of infestation of red palm weevil was in the range of 0.43 to 2.56 per cent in both the tahasils. The intensity was 1.92 and 1.97 per cent in February-March 2005 in Vasai and Palghar tahasils respectively, it was somewhat increased in November 2005 and then declined below one per cent in 2007 and 2008. The intensity of black headed caterpillar was in the range of 21.59 to 29.14 per cent in the year 2005. The intensity was drastically reduced to zero in Vasai tahasil in June 2008; this may be because of pre monsoon rains received in first fortnight of May 2008.

The rhinoceros beetle infestation was in the range of 14.43 to 20.90 per cent in all the tahasils of the district. It was more or less similar in all the tahasils over a period of study. The intensity of infestation of red palm weevil was in the range of 0.61 to 2.64 per cent. It increased to some extent (from 2.03 and 1.19 to 2.64 and 2.55 %) in Alibaug and Murud tahasils in February-March 2005 to November 2005 and then reduced to 1.12 and 1.05 per cent, respectively. The infestation of black headed caterpillar was observed in Karjat tahasil only (2.46 %) in February-March 2005. All other tahasils were free from black headed caterpillar infestation over a period of study.

The infestation of rhinoceros beetle was in the range of 13.93 to 19.20 per cent. It was found to be maximum (19.20 and 17.02 %) in Mandangadh tahasil in May-June 2007 and January 2008. The maximum infestation (22.09 and 21.86 per cent) of red palm weevil was observed in Rajapur tahasil, in February-March 2005 and November 2005, respectively. This might be due to one heavily infested orchard in the area. The infestation then reduced due to management of red palm weevil effectively. In other tahasils it was in the range of 1.08 to 2.63 per cent. The black headed caterpillar was not observed in all the areas surveyed in Ratnagiri district.

The rhinoceros beetle infestation was observed in all the areas surveyed in Sindhudurg district during the period of study. The intensity of infestation was from 15.45 to 20.84 per cent. The red palm weevil infestation was observed in all the tahasils surveyed in Sindhudurg district. It was in the range of 1.12 to 2.48 per cent in May-June 2007. The black headed caterpillar infestation was not observed in Sindhudurg district.

Overall results of the survey indicated that the rhinoceros beetle infestation was observed in all coconut growing orchards of Konkan region. The red palm weevil infestation

was less (0.38 to 3.20 %) in all the areas except Rajapur tahasil (22.09 % in Feb-March 2005). The black headed caterpillar is a pest of Thane district and it was not present in all other areas of the other districts except Karjat tahasil of Raigadh district.

References

- Anonymous, 2004. Area and production of coconut in World and India. *Indian Coconut J.* **30**: 32-33.
- Anonymous. 2006. Report of the Directorate of Horticulture, Govt. of Maharashtra, Pune. p 12
- Rabindra, R. 2000. Microbial control of coconut pests. Presented in group meeting on recent advances in management of coconut pests held at CPCRI, Kayangulam from 24-25 May, 2000. p.26
- Thampan, P.K. 1986. Profitable coconut growing. Published by Coconut Development Board, Ministry of Agriculture, Govt. of India. P 2.

Table 1. District wise situation of different pests in Konkan region of Maharashtra

Sl. District	Feb-Mar-05			Nov-05			May-/Jun-07			Jan-08		
	Rhino-ceros beetle	Red palm weevil	Black headed caterpillar	Rhino-ceros beetle	Red palm weevil	Black headed caterpillar	Rhino-ceros beetle	Red palm weevil	Black headed caterpillar	Rhino-ceros beetle	Red palm weevil	Black headed caterpillar
1 Thane	14.93	1.94	25.36	17.22	2.38	25.10	19.02	0.70	0.92	18.41	0.61	0.70
2 Raigad	17.91	1.58	0.61	18.37	2.59	0.0	17.21	0.97	0.00	17.34	1.02	0.0
3 Ratnagiri	18.22	12.36	0.00	17.50	8.72	0.0	15.97	1.29	0.00	15.52	1.32	0.0
4 Sindhudurg	18.0	1.33	0	NS	NS	NS	17.29	1.46	0.0	NS	NS	NS

NS Not surveyed

Table 2. Tahasil wise situation of different pests of coconut in Konkan region of Maharashtra

Sl. District / Rhino-	Feb-Mar-05			Nov-05			May-Jun-07			Jan-08		
	Red ceros beetle	Black palm weevil	Rhino-headed caterpillar	Red ceros beetle	Black palm weevil	Rhino-headed caterpillar	Red ceros beetle	Black palm weevil	Rhino-headed caterpillar	Red ceros beetle	Black palm weevil	Rhino-headed caterpillar
A Thane												
1 Vasai	17.04	1.92	29.14	19.38	2.21	23.64	18.06	0.63	0.0	17.97	0.94	0.0
2 Palghar	12.83	1.97	21.59	15.07	2.56	26.52	19.53	0.74	1.40	18.64	0.43	1.0
B Raigad												
3 Shrivardhan	19.71	2.49	0.0	NS	NS	NS	17.21	0.97	0.00	18.61	0.85	0.02
4 Alibaug	20.90	2.03	0.0	18.89	2.64	0.00	NS	NS	NS	15.90	1.12	0.0
5 Murud	14.43	1.19	0.0	17.85	2.55	0.00	NS	NS	NS	18.17	1.05	0.0
6 Karjat	16.62	0.61	2.46	NS	NS	NS	NS	NS	NS	NS	NS	NS
C Ratnagiri												
7 Mandangad	NS	NS	NS	NS	NS	NS	19.20	3.20	0.0	17.02	2.55	0.0
8 Dapoli	NS	NS	NS	NS	NS	NS	15.96	0.84	0.00	14.94	1.08	0.0
9 Guhagar	NS	NS	NS	17.32	1.86	0.0	13.93	1.11	0.00	15.64	1.21	0.00
10 Ratnagiri	18.83	2.63	0.0	18.43	2.45	0.0	16.74	0.38	0.0	NS	NS	NS
11 Rajapur	17.61	22.09	0.0	16.75	21.86	0.0	16.62	3.04	0.0	NS	NS	NS
D Sindhudurg												
12 Vengurle	NS	NS	NS	NS	NS	NS	16.84	1.33	0.00	NS	NS	NS
13 Sawantwadi	NS	NS	NS	NS	NS	NS	15.45	1.20	0.0	NS	NS	NS
14 Dodamarg	NS	NS	NS	NS	NS	NS	20.84	1.44	0.0	NS	NS	NS
15 Kudal	NS	NS	NS	NS	NS	NS	19.05	2.48	0.0	NS	NS	NS
16 Malvan	18.00	1.33	0	NS	NS	NS	16.24	1.12	0.0	NS	NS	NS
17 Deogad	NS	NS	NS	NS	NS	NS	18.95	2.08	0.0	NS	NS	NS

NS Not surveyed

Reaction of different coconut genotypes against Eriophyid mite, *Aceria guerreronis* Keifer in Konkan region of Maharashtra

S.S.Gurav, V.S.Desai, D.D.Nagwekar and A.L.Narangalkar

Regional Coconut Research Station, Bhatye, Ratnagiri

Dr.Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli, Maharashtra, India

Coconut palm (*Cocos nucifera* L.) is subjected to attack by a number of insect pests viz., Black headed caterpillar, Rhinoceros beetle, Red palm weevil etc. Of late, the widespread incidence of coconut eriophyid mite, *Aceria guerreronis* has become a major threat to coconut palms. In India, it was first observed in the coconut gardens of Ernakulum district of Kerala State in 1998 (Sathiamma *et. al.*, 1998). In the past few years, the pest spread to other areas, covering the whole of Kerala, Tamil Nadu, Karnataka, Andhra Pradesh and Maharashtra. In Maharashtra, the pest was first reported in Thane district. Then it spread throughout the Konkan belt of Maharashtra. Different control measures were recommended for the control of this pest like spraying of Azadirachtin 0.004 percent on young bunches thrice in a year or root feeding of Azadirachtin 5 percent @ 7.5 ml + 7.5 ml water/palm thrice in a year. Along with above measures, use of recommended dose of fertilizers, application of neem cake @ 10 kg/palm/year and micronutrients were recommended for the management of this pest. However, problems in adoption of these measures like requirement of skilled labour, unavailability of the inputs and lack of community based control measures resulted in partial success in the management of this pest.

The use of resistant or tolerant genotype is one of the components of the integrated pest management. This component was not included in the IPM programme because eriophyid mite is a new pest in the region. Hence, to evaluate the reaction of existing genotypes against coconut Eriophyid mite, the present study was carried out at Regional Coconut Research Station, Bhatye, Dist. Ratnagiri (Maharashtra).

The incidence of eriophyid mite on coconut was observed from the year 2003 at Regional Coconut Research Station, Bhatye, Dist. Ratnagiri which is the only coconut research station in Maharashtra. To know the reaction of different coconut genotypes against eriophyid mite, ten coconut genotypes (Sanramon, Bornio, Jamaica, B.S.I, Kenya, Guam, Seychelles, Lono, TxD and Cochin China) available at Regional Coconut Research Station, Bhatye were screened under natural infestation from July 2008 to July 2009 (5 harvests). The nuts from ten trees in each genotype were observed for the mite damage at each harvest. Based on the visible surface damage method as suggested by Julia and Mariau (1979) nuts were classified into following categories (Table 1).

Table 1. Classification of nuts based on visual surface damage method

Grade	Visible surface damage on mature nut	Percent damage
1	Nuts with no mite damage	0
2	Nuts with superficial mite damage	1-10
3	Nuts with significant mite damage but not much smaller	11-25
4	Nuts with significant mite damage, smaller and with some distortion	26-50
5	Nuts very heavily attacked, very much reduced in size and often greatly distorted	51-100

Then mean damage grade index was worked out by using following formula.

$$\frac{(g_1 \times n_1) + (g_2 \times n_2) + (g_3 \times n_3) + (g_4 \times n_4) + (g_5 \times n_5)}{n_1 + n_2 + n_3 + n_4 + n_5}$$

where g_1 to g_5 = damage grade

n_1 to n_5 = Number of nuts exhibiting different damage grade.

Based on the mean damage grade index, the genotypes were classified as follows.

Mean damage grade index Score	Classification
0 – 1.0	Resistant
1.1 – 2.5	Moderately resistant
2.6 – 3.5	Susceptible
3.6 – 5.0	Highly susceptible

The extent of damage caused by eriophyid mite in different genotypes is given in Table 2. The per cent mite infestation on different coconut genotypes varied from 57.76 to 85.65. The maximum percent damaged nuts were in Sanramon (85.65) followed by Bornio (83.69) and the minimum were in Seychelles (57.76).

Table 2. Extent of damage caused by eriophyid mite in different genotypes

Genotype	Percentage of nuts in each damage category					Total no. of nuts	Percent nut damage	Mean damage grade index score
Sanramon	14.35	17.18	21.20	16.23	31.04	844	85.65	3.32
Bornio	16.30	18.15	17.39	18.26	29.89	920	83.69	3.27
Jamaica	27.12	25.40	15.34	10.18	21.96	815	72.88	2.74
B.S.I.	34.17	24.34	15.28	10.15	16.05	916	65.82	2.49
Kenya	21.20	24.58	18.45	17.35	18.43	830	78.79	2.87
Guam	22.45	21.42	16.39	14.19	25.55	775	77.51	2.99
Seychelles	42.23	15.21	17.34	14.15	11.06	940	57.76	2.37
Lono	25.33	14.13	18.15	16.09	26.30	920	74.67	3.04
T x D	32.39	22.09	18.77	13.25	13.50	815	67.61	2.53
Cochin China	23.20	15.31	15.31	13.34	32.84	862	76.80	3.17

Based on the mean damage grade index score, none of the coconut genotypes was found to be resistant to eriophyid mite. However, the genotypes B.S.I., Seychelles and T x D were found to be moderately resistant with a mean damage grade index score of 2.49, 2.37 and 2.53, respectively. These genotypes be used in the hybridization programme for evolving resistant variety for coconut eriophyid mite. All the other seven genotypes *viz.*, Sanramon (3.32), Bornio (3.27), Jamaica (2.74), Kenya (2.87), Guam (2.99), Lono (3.04) and Cochin China (3.17) were found to be susceptible to the coconut eriophyid mite, *Aceria gueronis*.

References

- Julia, J.F and Mariau, D. 1979. Nouvelles recherches en cote. D – Ivoire Sur *Eriophyes guereronis* K., acarien ravage ur des noix du cocotier. *Oleagineux* **34** : 181-189.
- Sathiamma, B., Radhakrishnan Nair, C.P. and Koshy, P.K. 1998. Out break of nut infesting Eriophyid mite *Eriophyes guereronis* K in coconut plantations in India. *Indian coconut Journal* **29** (2): 1-3.

Survey and monitoring of eriophyid mite, *Aceria guerreronis* Keifer of coconut in Konkan region of Maharashtra

S.S. Gurav, V.S. Desai, B.N. Bhangare, D.D. Nagwekar and A.L. Narangalkar

Regional Coconut Research Station, Bhatye, Ratnagiri

Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli, Maharashtra, India

Eriophyid mite (*Aceria guerreronis* Keifer) of coconut, a microscopic pest, observed first from Mexico way back in 1965 as a major pest, has become a serious pest in Asia and Pacific region, which was first observed in Sri Lanka in 1997. In India, the mite was first observed to be causing serious damage from Ernakulam district of Kerala in 1998 (Sathiamma *et al.*, 1998). It was first noticed in Maharashtra from Vasai tahasil of Thane district in 2002 (Anon, 2003). Now it has spread throughout Maharashtra and causes serious damage to the coconut plantations.

The Konkan region is a long narrow strip having seashore of 720 km, particularly known for tourism. The majority of coconut cultivation of Maharashtra is confined to this region. It comprises of five districts, out of which coconut plantations are found in Thane, Raigadh, Ratnagiri and Sindhudurg districts. As this pest was new to this area, a survey has been undertaken after notice of this pest to know the intensity of infestation and also to take the decision for initiation of control measures from different parts of this region.

To know the intensity of infestation of coconut eriophyid mite, a survey was undertaken from coconut growing tahasils of four districts of Konkan region (Thane, Raigadh, Ratnagiri and Sindhudurg) as the coconut cultivation is confined to these four districts only. From each tahasil, two villages were selected and from each village, two to six coconut growers were selected. The total number of palms from each tahasil was taken in to consideration for calculating percent infestation. The percent infestation was calculated by using following formula:

$$\text{Percent infestation} = \frac{\text{Total number of infested palms}}{\text{Total number of palms}} \times 100$$

The same orchards were used for survey from 2003 to 2008 to know the increase or decrease in eriophyid mite infestation.

District wise eriophyid mite situation is given in Table 1. The infestation of eriophyid mite in Thane district was 29.10 percent in April 2003, and it increased to 74.54 percent in May- June 2006 and then declined to 66.35 percent in January 2008. In Raigadh district it was

Table 1. District wise eriophyid mite situation in Konkan region of Maharashtra

Sl. No.	District	Percent infestation								
		April-03	June-03	April-04	Sept-Oct-04	Feb/Mar-05	Nov-05	May/Jun-06	May / Jun-07	Jan-08
1	Thane	29.10	46.32	65.86	NS	63.82	71.28	74.54	70.41	66.35
2	Raigadh	3.66	18.19	1.90	0.56	2.81	13.04	27.79	28.43	42.08
3	Ratnagiri	2.34	15.37	3.33	4.36	7.49	17.24	40.08	20.37	18.09
4	Sindhudurga	4.90	NS	34.09	18.42	46.34	NS	100	89.59	NS

3.66 percent in April 2003, it was slowly increased in next periods of survey and reached to 42.08 percent in January 2008. In Ratnagiri district it was 2.34 percent in April 2003, it was slowly increased to 40.04 percent in May-June 2006 then it was reduced in June 2007 and January 2008.

In April 2003 the eriophyid mite infestation in Sindhudurg district was 4.90 percent, and it increased to the extent of 100 in May- June 2006 and then started declining.

Taluka wise eriophyid mite situation is given in Table 2. The incidence of eriophyid mite was first reported from Vasai tahasils of Thane district. In Vasai tahasil, the infestation was 40.84 percent in April 2003, it was increased steadily and reached to 80.04 percent in May-June 2006 and then started declining. In Palghar tahasils it was 5.90 percent in April 2003 and increased to the extent of 73.13 per cent in May-June 2007 and then reduced to some extent in January 2008.

Table 2. Taluka wise eriophyid mite situation in Konkan region of Maharashtra

Sl. No.	District / Taluka	Percent infestation								
		April-03	June-03	April-04	Sept-Oct-04	Feb/Mar-05	Nov-05	May/Jun-06	May / Jun-07	Jan-08
A	Thane									
1	Vasai	40.84	67.26	78.35	NS	77.66	75.26	80.04	65.24	62.89
2	Palghar	5.90	25.38	53.36	NS	49.97	67.31	64.52	73.13	68.21
B	Raigad									
3	Shrivardhan	2.73	NS	3.85	2.25	1.93	NS	14.67	28.43	40.13
4	Alibaug	2.41	18.19	0.45	1.20	2.26	10.58	29.76	NS	36.21
5	Murud	5.13	NS	1.39	0.59	3.78	15.51	51.16	NS	53.37
6	Panvel	13.33	NS	4.25	NS	NS	NS	NS	NS	NS
7	Karjat	0	NS	NS	2.37	3.25	NS	NS	NS	NS
C	Ratnagiri									
8	Mandangad	NS	NS	NS	NS	NS	NS	NS	4.80	5.10
9	Dapoli	NS	NS	NS	NS	NS	NS	NS	5.14	5.91
10	Guhagar	NS	NS	NS	NS	NS	9.06	15.61	29.20	32.06
11	Ratnagiri	2.51	15.37	9.06	11.12	14.68	39.50	80.50	32.31	NS
12	Rajapur	0	NS	0	0	0.30	3.17	0.51	18.24	NS
D	Sindhudurg									
13	Vengurle	9.59	NS	39.60	NS	55.92	NS	100	89.24	NS
14	Sawantwadi	0	NS	33.61	NS	43.15	NS	100	90.34	NS
15	Dodamarg	0	NS	44.77	NS	57.42	NS	100	92.05	NS
16	Kudal	0.75	NS	18.37	20.35	19.23	NS	100	87.81	NS
17	Malvan	NS	NS	NS	NS	56.00	NS	100	89.76	NS
18	Deogad	NS	NS	NS	NS	NS	NS	100	85.41	NS

NS Not surveyed

In Raigadh district, the infestation of eriophyid mite was in the range of 2.41 to 13.33 percent in April 2003 from all the coconut growing tahasils. It was slowly increased from all the tahasils and reached in between 40.13 to 53.37 percent in January 2008. Overall results indicated that the eriophyid mite infestation was medium in Raigadh district. In Ratnagiri tahasils, the eriophyid mite infestation was 2.51 percent in April 2003, it was increased to 80.50 percent in May-June 2006 and then showed declining trend. In other tahasils of Ratnagiri district, the initial infestation was less and then it was slowly increased. Maximum infestation was 32.06 percent in Guhagar tahasils in January 2008.

In Sindhudurg district, the initial infestation was less in coconut growing tahasils, it was drastically increased to 100 percent in May-June 2007 and then showed declining trend in May-June 2007.

Overall results of the survey showed that, the infestation of coconut eriophyid mite was less initially, increased steadily and then started declining in due course of time. The declining trend of infestation may be due to increase in the population of natural enemies and adoption of control measures.

References

- Anonymous. 2003. AGRESKO report of Dr. B.S. Konkan Krishi Vidyapeeth, Dapoli presented in April 2003.
- Sathiamma, B., Nair, C.P.R. and Koshy, P.K. 1998. Outbreak of nut infesting eriophyid mite *Eriophyes guereronis* (K.) in coconut plantations in India. *Indian Coconut Journal* **29**(8): 1-3.

Product diversification and value-addition of coconut - an overview

K . Madhavan and T. Arumuganathan

Central Plantation Crops Research Institute

Kasaragod - 671 124, Kerala, India

Coconut tree is one of the most important cultivated palm in the world and is grown in more than 80 countries of the tropics. The largest producers of coconut are Indonesia and Philippines followed by India. The Indian consumption pattern indicates that 56 percent of the produce is utilized for domestic and religious purposes, 35 percent for coconut oil production, 7 per cent for making edible copra and 2 per cent for manufacturing coconut powder. The coconut palm also provides a series of by-products such as fiber, charcoal, handicrafts, vinegar, alcohol, sugar, furniture, roofing, fuel, etc. Coconut palm has more than 200 diversified local uses.

Coconut tree fulfils the basic needs of people such as food, drink, shelter, fuel, furniture, medicine, decorative materials and much more. The coconut palm is the most useful palm in the world. Every part of the tree is useful to human life for some purpose or the other. Hence, the coconut palm is endearingly called “kalpavriksha” meaning the tree of heaven. It is the “heavenly tree”, “tree of abundance” and “nature’s supermarket”.

One of the main reasons for the recent fall in price of the coconut and its products is dependency on price of coconut oil which again depends on the cost of other vegetable oils. Thus product diversification of coconut and development of value added products become very important in the coconut industry. The country has been successful in evolving appropriate processing technologies for the profitable utilization of some of the value added products of the coconut palm. As a result, products like packed coconut milk, coconut creams, spray dried coconut milk powder, sweet coconut chips, coconut water based products like snow ball tender coconut, coconut water concentrate, vinegar and nata-de-coco, etc was developed and they also have good commercial potential. Here we have given an overview of the edible products that can be prepared from coconut.

Products derived from fresh kernel of matured coconut

The fresh kernel of ripe coconut constitutes an essential ingredient in the recipes of diverse food preparations in the households as well as in food industries of different countries. In the household food preparation fresh kernel is used extensively in the grated, paste or milk form. The fresh kernel is of low acid nature, meaning extremely prone to microbial degradation, especially in tropical climate. The dairy industry with similar type of products has, by introducing hygienic processing of high standard, managed to control the quality of the end products. Therefore fresh kernel processing plants require the same level of hygiene as the dairies for safe and consistent production. In the fresh kernel processing industries, shelling and removal of testa are the common labour intensive unit operation. As the existing machineries available are not satisfactory, further refinement is required.

Desiccated coconut: Desiccated coconut is the white kernel of fresh mature coconuts, shredded and dried down to about 2.5 % moisture content under strict hygienic conditions.

It is used for human consumption, and it retains the original oil and protein of a fresh nut. Four standard grades based on particle size are produced: extra fine; fine (macaroon); medium and coarse. A limited amount of fancy cuts such as flakes, threads and chips are also produced for special markets. The main uses of desiccated coconut are for the confectionary industry, as a filling for chocolates and candies; the bakery industry for biscuits, cake and nut filling products; direct usage to decorate cakes, biscuits and ice cream and preparation of various snacks.

Major importing countries include the USA, Canada, Europe (UK, the Netherlands, Germany, France, Spain), South Africa, the Middle East and Australia. The major exporting countries are Philippines, Sri Lanka and Malaysia, together accounting for export 90 % of the world demand for Desiccated coconut.

The process of DC manufacture involves a series of steps: selecting, sorting and husking of coconuts, shelling, paring, washing, sterilizing, grinding, drying, sieving, packing, and storage. Selection and sorting of coconuts is very important for the process. Only fully mature nuts are suitable for desiccated coconut, as immature nuts have lower oil content, an off color and poor keeping qualities. Germinated nuts have to be rejected, as they begin to deteriorate due to biochemical changes.

Desiccated coconut is made out of fully matured nuts, which have been stored for about a month before dehusking. This storage facilitates the subsequent deshelling operations because the kernel detaches itself from the shell during the storage period. A special type of small axe carefully chips the dehusked kernels and the shells removed. The whole kernel when removed from shell will be covered with a surface layer of brown testa, which has been removed with a special paring knife. The parings which amount to 12-15 percent of the kernel are usually sun dried, but sometimes oven dried and processed for oil, the yield is being 60 to 62 %. The resulting oil is known as paring oil, is of inferior quality and finds use in soap manufacture.

The pared kernels are placed in tanks and sliced into two to release the coconut water. The sliced kernels are then passed into other tanks through a two-stage washing. The kernels should be immersed in water in order to prevent discoloration. The rate of flow of water into the tanks is regulated to input the rate at which the pared kernels are let in. The coconut water is led into settling tanks and the surface scums when formed are removed and boiled to get an oil of inferior quality. The resulting press cake has a high fertilizer value.

The kernel pieces after thorough washing are sterilized by passing through large tanks containing boiling water. The pieces are carried in wire baskets in a continuous process by mechanical device and the movement of basket is adjusted in such a way that from one end of the tank to other, a set period of one to one and half minutes is available for sterilization. The oil layer that may form on the surface of the boiling water is removed at frequent intervals. In order to prevent discoloration, it is also advised to give a washing spray on the conveyer carrying the coconut pieces away from the tank. Subjecting the disintegrated meat to live steam in stainless steel blanchers at about 80°C for five minutes also effects sterilization.

After sterilization, the kernel pieces are transferred from wire baskets straight to the disintegrator, which shreds the kernel pieces into a fine wet meal. The product that comes out of the disintegrator will have about double the weight of the final product. This is to be

subsequently desiccated or dried to a final moisture content of 2-2.5 per cent. For drying, the wet meal is spread on trays of fine metal mesh supported by a wooden frame. The trays are then mounted in tiers in a hot air drier and the product dried at about 77 to 82°C. Within this temperature range, the drying time is usually 40 to 45 minutes. During this process the trays are moved twice and the content raked over in order to ensure uniform drying and to break up any lumps that might have formed. Continuous drying is also common where the trays are arranged in one tier and the wet meat drops from the top tray to the lower ones by means of a typing mechanism, and, by the time the contents reach the bottom tray, the drying process is over.

The dried product is allowed to cool a galvanized table, sifted into coarse, medium and fine grades. In the fully mechanized plants, the cooling system is integrated into the drying system. When the drying process is over, the product is sucked from the drier and blown along a long pipe to a cyclone cooler mounted on a platform above the grading table and the packing section. At the time of packing, the temperature of the product will be about 49°C. The dried material is then conveyed to a sifting or sieving machine, where different particle sizes are separated and then bagged. Desiccated coconut is packed into 5-ply Kraft paper bags with an inner liner of polyethylene or polypropylene film. The net weight of a bag is 50 kg, the shelf life is about 6 months provided it is stored under cool dry conditions without stacking too high.

The final yield of desiccated coconut will depend on the maturity and weight of the fully matured husked nut. In India, the yield differs from state to state and the range is from 8000 to 9000 nuts to produce 1 ton of desiccated coconut.

Coconut chips: Coconut chips can be prepared by dehydrating the intermediate moisture coconut kernel. Intermediate moisture coconut kernel is the mature coconut kernel after removing the moisture content of the kernel partially by osmotic dehydration by using osmotic mediums like sugar syrup. Coconut Chips is crispy and can be packaged and marketed in laminated aluminium pouches, which will have shelf life of 6 months. Since it is in ready-to-eat form, it could be used as snacks at any time. Coconut chips with different flavour can be prepared by adding the required flavour essence in the osmotic medium. Instead of sweet, salted coconut chips and medicated coconut chips can also be prepared by suitable change in the osmotic medium.



Fig 1. Coconut chips

Coconut milk and related products: Coconut milk is an emulsion of coconut oil in water into which some of the soluble components of the fresh kernel have already been passed. It is milk like liquid obtained from the gratings of the fresh kernel after they are mixed with some water, kneaded and pressed out. The milk is pleasant and sweet with an agreeable flavour. A comparison of the coconut milk with cow's milk has shown that coconut milk is richer in fat, poorer in protein and sugar content. Apart from household culinary uses, coconut milk is utilised as a substitute for dairy milk as evaporated and sweet condensed milk and in the preparation of white soft cheese, yoghurt and many other foodstuffs. Commercial production

of these products has been promoted in the Philippines, Thailand, Indonesia, Western Samoa, Sri Lanka and Malaysia and to some extent in India.

Bottled coconut milk: The processing technology involves extraction of milk from coconut, straining the milk in a cheese cloth into an aluminium kettle with 0.1 percent benzoic acid before placing the kettle in an autoclave at 117°C for three minutes with steam injection. The temperature of the milk in the pot is then brought down to 80-85°C, by running tap water. The milk is then homogenised for about five minutes and bottled at 70°C to 80°C. The final product is as good as cow's milk and is highly nutritious.

Coconut cream: Coconut cream is a white, smooth, liquid cream with excellent coconut flavour and 20-30% fat, aseptically packed. The product is easily pourable and ready for direct serving or to be used in other food preparation. Coconut cream is essentially used as a fat source for the reconstitution of the skimmed dairy milk and as a component of infant milk powders.

Dehydrated coconut milk: This is produced on a commercial scale in the Philippines, Malaysia and India. In the Philippines, the fresh coconut milk is blended with small amounts of additives such as maltodextrin or casein and is spray dried. The final product is marketed in laminated foil bags. The powder easily dissolved in water to form a milky white liquid with the flavour and texture of coconut milk.

Virgin coconut oil: VCO is the oil obtained from fresh, mature endosperm (kernel-meat) of the coconut by mechanical or natural means, with or without use of heat, no chemical refining, bleaching or deodorizing and maintains the natural aroma and nutrients. It is called "virgin" because the oil obtained is pure, raw and pristine. Virgin coconut oil is suitable for human consumption in its natural form. It is the purest form of coconut oil, crystal clear, contains natural vitamin E and with very low, free fatty acid content (0.1 %). It has a fresh coconut aroma ranging from mild to intense depending on extraction process.

Virgin coconut oil can be extracted directly from the fresh coconut meat or from coconut milk or from coconut milk residue. The different methods involved in VCO production are Hot-processing method, Natural fermentation method, Centrifugation process and Extraction from dried grating (EDG) method. The choice of the technology to be adopted depends to a great extent on the scale of operation, the degree of mechanization, the amount of investment available and the market demand. For decades, people in coconut producing countries like India and Philippines boiled coconut milk extracted from freshly grated or comminuted (grated, chopped, granulated) coconut meat with or without the addition of water, to produce coconut oil for hair and body massaging applications.

Edible coconut flour: After expelling the milk, the protein rich residue is dried and powdered to obtain a product called coconut flour. The flour so obtained typically contains 7-8 percent protein, 3-5 percent moisture and 17 percent oil. It can be used as an ingredient in weight control foods because of its high fibre content. The protein contained in the flour is identical to that contained in the original fresh kernel. After blanching the residue has to dry. The dried coconut residue is passed through a special type of screw press under a specified expeller setting to reduce oil content of the residue. The de-fated flakes are dried to reduce its moisture content to 2.5 to 3.0 per cent.

Ready to eat food items

Processes for preparation of ready-to-eat food items such as coconut pickle and coconut chutney powder have been developed and standardized by CPCRI, Kasaragod. These products can be prepared from the byproducts obtained while preparing the coconut chips and virgin coconut oil.



Fig. 2. Coconut chutney powder



Fig. 3. Coconut pickle

Preserved tender coconut water: The water of tender coconut, technically the liquid endosperm, is the most nutritious wholesome beverage that nature has provided for the people of the tropics to fight the sultry heat. It has caloric value of 17.4 per 100 gm. “It is unctuous, sweet, promoting digestion and clearing the urinary path,” says Ayurveda on warm coconut water. Most coconut water is still consumed fresh in tropical coastal areas. Once exposed to air, the liquid rapidly loses most of its organoleptic and nutritional characteristics, and begins to ferment.

Tender coconut water is at its optimum level of acceptability and economic viability for commercial use when nuts are 6-7 months maturity. Later quality and quantity of water decreases. Hence it is ideal to preserve coconut water at this stage of maturity to derive maximum benefits. Investigations carried out at DFRL (Defence Food Research Laboratory), Mysore, to preserve the tender coconut water in plastic pouches of 200 ml / 350 ml capacity. The tender coconut water is highly susceptible to heating. Hence it is subjected to minimum heating by use of additives like Nissin, which helped in maintaining the natural pH of 4.9-5.2 instead of reducing to below 4.5, which reduced the acceptability. The product is found to be generally acceptable up to three months under ambient storage conditions.

Snow ball tender coconut: The tender coconut kernel is good for convalescing patients. It contains good amount of nutrients. An attempt was made to supply the tender coconut water along with tender coconut kernel without any waste material being thrown away after consumption. Snow ball tender coconut is the tender coconut without husk, shell and testa, which is ball in shape and white in color. This white ball will contain tender coconut water, which can be consumed by just inserting a straw through the top white tender coconut kernel. In this process coconut of 8-month maturity is more suitable for making snow ball tender coconut. Making groove in the shell before scooping out the tender kernel with water



Fig. 4. Making of snow ball tender nut

is one of the important unit operations. For this a suitable machine has been developed. It is nutritive and is a drink and a snack at the same time. Since there is no refuse after consumption, there is no scope for littering of the premises. Since snowball tender nut can be individually packaged and refrigerated under hygienic conditions, the shelf life of this product is prolonged and therefore this ready-to-serve product is found to become popular.

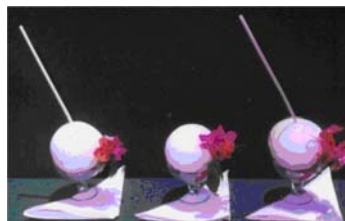


Fig. 5. Snow ball tender nut

Preservation of tender coconut kernel: The kernel of tender coconut is a good source of carbohydrate, fiber and other nutrients. Protein content was high in the eight months old fresh coconut meat. The fresh kernels left after the use of tender nut water is not put to effective use though it has high nutritional qualities. An effort on value addition and product diversification has resulted in the development of new products such as tuty-fruity and candy from fresh kernels. Processed young tender coconut kernel could be in the form of sweetened dehydrated/dried (or) young tender coconut kernel in syrup (or) dried chips. These young coconut kernel products are ideal for dessert (or) snack food. Freshly made preserves are wholesome and attractive. The fruits mixed with cane sugar and subsequently drained and dried are called candied fruits. Candied fruit covered with a thin transparent coating of sugar is called a glazed fruit. When candied fruit is coated with sugar or sugar crystals are allowed to deposit on it, it is called crystallized fruit.

Processing of tender coconut kernel- candy: Young coconut is washed and split open to remove the water. The soft kernel is scooped out and cut into cubes. Pricking should be done with stainless steel forks. In the case of crystallized candy, after pricking, immerse the fruit pieces in dilute limewater (1.5 %) or alum (2 %) for few minutes before further processing. The pieces are washed 3-4 times with fresh water and blanched for 5 minutes in boiling water to make them soft. This assists in absorption of sugar and prevents enzymatic browning. Sugar (50 %) was spread on the blanched fruit pieces in alternative layers. Syrup is drained on next day and enough sugar is added to raise the concentration of the syrup to about 60°Brix. Citric acid is added as preservative. Coconut pieces are added, boiled and kept for 24 hours. The process is repeated every day until the Brix of residual syrup reaches 70-75°. The syrup is drained and pieces are dried in hot air and stored in glass bottles/polyethylene bags. In the case of crystallized candy the concentrates of sugar syrup is continued till Brix value reaches 70-78°. Syrup is drained off and the pieces are rolled in finely ground sugar. Crystallized candy can be stored for 3 months.

Canning of tender kernel: A technology has been developed for the canning/bottling of the kernel of tender nuts of different age groups. It is now possible to preserve kernels of 8 to 10 month old nuts and also jelly- like formations obtained from 6 to 7 months old nuts. For canning, meat from 8-10 months old nuts is first scooped out, the adhering testa removed by using a sharp knife and the pared meat cut into stripes 0.5 cm thick and 6 cm long after washing. The stripes are put in cans to which is added 50° B syrup with 0.01 per cent sodium metabisulphite. The filled cans are then exhausted at 78°C, sealed and processed at 110°C for about 20 minutes. In the processing of jelly like meat obtained from tender nuts 6-7 months old, the meat is scraped out and to every part of the meat a corresponding amount of refined sugar is added. The mixture is cooked in low heat until the sugar is totally dissolved, hot

packed in sterilized bottles and closed tightly. This product is likely to meet with widespread consumer acceptance.

Tender coconut punch and cutter: Tender coconut water is a delicious and nutritious refreshing drink provided by the nature. Though the artificial soft drinks once tended to reduce the importance of tender coconut water, the propaganda in favour of tender coconut water by the Government, the Coconut Development Board and the proponents of naturopathy has now paved the way for its increased consumption. But the main factors deterring the large scale adoption of 'tender coconut parlour' is the absence of simple tools for the easy extraction of tender coconut water. After drinking the tender coconut water some people like to eat the soft meat. For extracting the meat the tender coconut is placed on a wooden block or held in the left



Fig. 7. Tender nut cutter

hand and a heavy machete is used to break it into two halves. The meat is then scooped out with a spoon. Considerable practice is necessary before acquiring the skill for cutting it open with least number of cuts and also for avoiding injury. People having this skill are only a few in number. Because of the risk involved, only a few people learn this craft. In view of this, a simple device has been developed by CPCRI, Kasaragod for making a small hole in the coconut and a cutter to cut open into two halves.



Fig. 6. Tender coconut punch

Dressed tender nuts: It is a partially dehusked cool tender coconut. Since husk constitute major portion of the volume of tender coconut, partial portion of the husk is removed. The 7-8 months old green tender nuts are machine shaved to an attractive and uniform hexagonal shape, the smallest size possible that can retain the fluid safely inside. After removal of husk, tender coconut is wrapped with food grade polyethylene for aesthetic and hygienic purpose. These machine-shaved nuts are kept in cold rooms before serving. This will be the natural drink for several occasions like marriages, rituals, ceremonies, social functions, conferences, seminars, symposia, exhibitions, trade fairs etc.

Products derived from coconut water of matured coconut

In most of the countries coconut water is now a waste product of the coconut industries. As the nut matures, the composition of the water especially the sugar content, also undergoes significant changes. During the early stages of development, invert sugar present in the water increases and reaches a maximum at 220 days. After this stage, sucrose appears in the water and the concentration of total sugars falls. Similarly the concentration of total solids also declines and subsequently disappears during germination.

Bottled coconut water: Bottling of coconut water for use as a soft drink is gaining popularity. Coconut water can be marketed as natural soft drink if preserved and packed. Non-carbonated beverage can be produced from the coconut water of mature nuts. The process involves collection of water, upgradation and pasteurisation, filtration and bottling. Coconut vinegar and nata-de-coco are prepared from coconut water, which has got industry demand.

Nata-de-Coco: Nata-de-Coco is a gelatinous dessert delicacy formed by the action of a microorganism, *Acetobacter xylinum*, in a culture medium of sugared coconut water. The organism can be cultured either in coconut water or skimmed coconut milk.

The culture solution is prepared by mixing coconut water with sugar and acetic acid at a stipulated proportion, which is inoculated with *Acetobacter xylinum* through a culture liquid made from pineapple waste. The cultured solution is distributed either in wide mouthed glass jars or plastic basins and is kept undisturbed for about 2-3 weeks.

Fermentation is completed in 2-3 weeks depending on the environmental conditions. Optimum temperature for nata production is in the range of 23-32°C. During this period a white or cream colored jelly-like substance forms and floats on top of the culture medium. At this stage the jelly-like substance or Nata would be about an inch thick. This surface growth is harvested, sliced into cubes, approximately 1x3 cm or according to requirement. Washed thoroughly to remove the acid taste smell. Drain the nata and add sugar equal to the weight of drained nata, mix thoroughly and kept overnight. The next day stir the mixture to disperse any undissolved sugar. Add small amount of water. Heat the mixture to the boiling point, stirring occasionally. Add flavouring if desired. Set aside overnight and repeat the heating process until the nata is fully penetrated with sugar as evident by the clear and crystalline appearance of the sweetened nata and preserved in either tin containers or bottles. The gelatinous growth is believed to be composed mainly of polysaccharides, probably dextrose and to be cellulose in nature. Nata-de-Coco is very delicious dessert item in the Philippines and other countries, particularly in the United States, which is served either mixed with other fruits or baked into a delicious cream pie or simply served with flavored syrup.

Ready to serve (RTS) coconut water beverage:

Delicious beverages can be made from matured coconut water on blending with lemon juices or other fruit juices. These beverages could be an alternate for synthetic beverages, which are not good for health.

Coconut water squash: Coconut water squash can be prepared from mature coconut water using the ingredients sugar, citric acid, lemon juice, ginger and sodium benzoate. It contains sodium, potassium, Vitamin C and Carbohydrate with calorific value of 300 Kcal.



Fig. 8. Coconut water squash

Process for the production of copra and coconut oil

The dried coconut endosperm is called copra. The copra and the oil it contains are the principal products of coconut palm. With oil content of 65-70 percent, copra is the richest source of fat. The essential requirement of copra drying is to bring down the moisture content of the wet fresh kernel from 45-55 percent to 5-6 percent. There are two types of copra – edible copra and milling copra. Edible copra is available in two forms – ball copra and cup copra. The production of edible copra in India is around 50,000 tonne per annum and the produce is utilised for sweet snacks preparations in households and as an ingredient in the processed foods.

Copra processing

Copra (dried meat or kernel of coconut) may be made both in the forms of 'cups' or 'balls'. In India, 1500 million nuts are used for this purpose. Copra in the form of balls is made from whole unsplit nuts. The process consists in storing coconuts with husk in suitable ball copra store which is usually a two storied brick and mortar building, the floor and the four sides of the top floor being made of wooden bars spaced three to four inches apart. The size of the store varies according to the requirements of the producer. Usually there are five or six compartments 3.66 x 3.66 x 1.83 m, each compartment holding 4000 to 5000 nuts. Small-scale manufacturers also use a wooden platform over the kitchen fireplace. Fully ripe nuts, 12 to 14 months old, are harvested and kept into the wooden upper floor of the store. They are frequently stirred and /or smoked till they are quite dry. The proper stage of drying is determined by shaking the nut when the detached kernel makes the rattling sound. After the rattling stage, the nuts are stored for sometimes and then dehusked and shelled and suitably marketed. The smoking is done by burning paddy husk, coconut husk, dry leaves or cheap firewood in the ground floor of the store. The entire process takes 8 to 12 months.

Cup copra is made either by sun drying or kiln drying. Copra is a highly valued commodity in the world markets for oil seeds, oils and fats, with oil content of about 65- 70 %, copra is the richest source of fat. Though there is variation in the oil content of copra derived from different producing regions, according to Indian standards, one ton of copra corresponds to about 680 kg of oil with an average extraction of over 645 kg of oil. The essential requirement of copra drying is to bring down the moisture content of the wet meat from 50-55% to 5-6%.

The ordinary way of copra processing is by exposing the split halves of the nut to the heat of the sun or artificial heat. The period of drying is related to the temperature under which the copra is dried which in turn depends upon the method employed. 11-12 month old nuts are utilized for copra making to obtain the optimum output of better quality copra. Both freshly harvested and stored nuts are utilized. The final recovery of copra is more if the nuts are stored before dehusking for a period of 3-4 weeks. Husked nuts cannot be stored for long because deterioration of kernel sets in after a week. During rainy season, when conventional practice of sun drying is not possible, drying by artificial method is the only possible solution for processing the produce. The existing direct type kiln dryers are not desirable as the product becomes inferior in quality due to smoking and improper drying. Copra which has been smoke dried using coconut husk or wood is often more highly contaminated with poly cyclic aromatic hydrocarbon than that dried using coconut shell. Hence, most copra produced is relatively of poor quality, principally caused by inadequate drying techniques. To overcome these problems, different types of copra driers were developed by CPCRI and are being used.



Fig.9. CPCRI Solar cum electrical dryer with agri waste as third source of energy

Copra and the coconut oil as well as the cake derived from it are a major source of foreign exchange for many coconut-growing countries in Asia, the Pacific, and Africa. The quality of copra and copra cake is influenced by the method and the manner of drying the coconut kernel. Improperly dried copra gives rise to certain moulds, the most harmful of which is the yellow green mould called *Aspergillus flavus* and other aflatoxin related moulds. Aflatoxin is harmful both for man and animals. It is therefore extremely important that the coconut kernel be properly dried to prevent the attack of aflatoxin related moulds.



Fig.10. CPCRI Copra drier with coconut shell as fuel

Processing of mature nuts to copra has several problems. Improper processing, results in low oil yield and incidence of aflatoxin. Proper post-harvest practices, as well as proper drying and storage can increase the oil yield to about 20 per cent. Proper drying of coconut results in copra with lower moisture content and lower incidence of aflatoxin.

Coconut oil from copra

In most of the coconut producing countries, copra crushing has become a traditional industry. Power driven '*Chekkus*' or rotary mills, expellers and hydraulic presses are used for crushing on a commercial scale. The quality of copra is related to the quality of coconut oil. Copra is cut into small chips in a copra cutter. The chips are fed into steam jacketed kettles and cooked mildly at a temperature of 70°C for 30 minutes. After proper cooking, the cooked material is fed into the expeller continuously to extract oil. This oil is filtered by means of a filter press and stored in MS tanks. Bulk packaging is done in tin containers. HDPE containers and polymeric nylon barrier pouches are used for small consumer packing.



Fig.11. Coconut sap

Other value-added products

Coconut sap: Coconut Sap is a sugar-containing juice, obtained by tapping the unopened spadix / inflorescence of the coconut palm. It contains sugar, minerals and proteins. Sap yield is highly variable and it varies from day to day, season to season, spadix to spadix and tree to tree. Average yield of sap is 1.59 lt. (0.6-3 lt) per palm per day. It is a rich source of ascorbic acid and carbohydrate with sucrose as the major sugar. It cools the body and known as refrigerant drink / health drink. Coconut sap contains high amounts of essential elements such as N, P, K, Mg and micronutrients (B, Zn, Fe, Cu).

Table 1. Amino acid and vitamin contents of fresh coconut sap

Amino acid	Value (g/10g)	Vitamin	Value (mg/dl)
Histidine	1.19	Thiamine (Vit. B1)	77.0
Arginine	0.35	Riboflavin (Vit. B2)	12.20
Aspartic Acid*	11.22	Pyridoxine (Vit. B6)	38.40
Threonine*	15.36	Para-aminobenzoic acid	47.10
Serine*	8.24	Pyridoxal	38.40
Glutamic Acid*	34.20	Pantothenic acid (Vit.B5)	5.20
Proline	3.52	Nicotinic acid (Vit.B3)	40.60
Glycine	0.47	Biotin (Vit. H)	0.17
Alanine	2.56	Folic acid (Vit.B9)	0.24
Valine	2.11	Inositol	127.70
Methionine	-	Choline	9.0
Isoleucine	0.38	Vitamin B ₁₂	Trace
Leucine	0.48		
Tyrosine	0.31		
Phenylalanine	0.78		

Source: Kozaki, 1974

*Dominant amino acids

Coconut sap sugar: Coconut sap sugar is obtained by boiling freshly harvested toddy/sap in moderate heat to evaporate the water. It has a high potential for use as natural sweetener. This product is already exported to Australia, Japan, United Kingdom, France, Canada, Saudi Arabia, Germany, Hongkong, and Malaysia. Thailand is the major producer of coconut sap sugar.



Fig. 12. Coconut sap sugar

Coconut sugar which is the main product of sap is good for both diabetics and non-diabetics. It does not induce hyperglycemia or high blood sugar because it has a low glycemic index (GI). Tests conducted by the Food and Nutrition Research Institute and the Dept. of Science and Technology revealed that the glycemic index (GI) of coconut sugar is only 35, much lower than that of cane sugar that is 50. According to Dr. Trinidad P. Trinidad, a scientist at FNRI, GI is the glucose response of an individual from food relative to a standard glucose solution. Low GI is good for proper control and management of diabetes mellitus and has been shown to lower total and LDL. It is also good for weight maintenance, therefore preventing obesity.

Sap-based food products will be rich in total solids, sugar (sucrose), protein, carbohydrates, phosphorous and amino acids and more over Income obtained from sap based products is 8-10 times that obtained from sale of nuts.

Table 2. Nutritional value of coconut sap sugar

Particulars	Values
Total Energy Content, cal/100g	369.40
Total carbohydrate, g/100g	92.3
Moisture, %	2.2
Ash, %	2.2
Crude Protein, %	1.3
Crude Fat, %	0.12
<i>Vitamins and Minerals</i>	
Vitamin C, mg ascorbic acid/100g	23.4
Thiamine	0.41
<i>Macronutrients, mg/100g</i>	
Potassium	1,300.0
Phosphorus	79.0
Magnesium	29.0
Sulfur	26.0
Calcium	6.0
<i>Micronutrients, mg/100g</i>	
Zinc	2.0
Iron	2.0
Boron	0.63
Copper	0.23
Manganese	0.13

Analyzed by: Philippine Coconut Authority - Plant and Tissue Analysis Laboratory

A comparative analysis made by the PCA Plant Tissue Analysis Laboratory reveals that coconut sugar contains more amounts of nitrogen, phosphorus, potassium, magnesium, sodium, chlorine, sulfur, boron, zinc and copper compared to refined cane or brown sugar. Hence, coconut sap sugar is more nutritious than commonly used sugar products.

Table 3. Comparative analysis of the macro & micro nutrient contents of three types of sugar

Nutrient content	Coconut sap sugar (a)	Brown cane sugar (b)	Refined (white) sugar (c)
Micronutrients mg/L (ppm) in dry matter			
Manganese (Mn)**	1.3	2.0	0
Boron (B)**	0.30	0	0
Zinc (Zn)**	21.20	2.0	1.20

Iron (Fe)**	21.90	12.60	1.20
Copper (Cu)**	2.3	0.6	0.60
Macronutrients mg/L (ppm) in dry matter			
Nitrogen (N)*	2,020	100	0
Phosphorus (P)**	790	30	0.70
Potassium (K)	10,300	650	25
Calcium (Ca)	60	240	60
Magnesium (Mg)	290	70	10
Sodium (Na)	450	20	10
Chlorine (Cl)	4,700	180	100
Sulfur (S)	260	130	20

a Sourced from Zamboanga Research Center; b Brand GK; c Brand Hermano

* Determined through combustion method using Nitrogen Analyzer

** Determined using ICP-AES

Analyzed by: Philippine Coconut Authority - Plant and Tissue Analysis Laboratory

Coconut is a subsistent crop, which has provided the basic needs of a number of countries in the tropics for centuries. With the use of coconut oil in the production of soap and margarine in Europe in the 19th century, it was converted into a commercial crop. In the beginning of 20th century copra was the king among the oil seeds. In East Indies it was known as green gold. However, the period after the Second World War saw the substitution of vegetable oils and oleo chemicals for coconut oil in international trade.

The increase in the output of coconut was marginal. Price of coconut oil fluctuated heavily due to frequent short supply situations. A campaign against coconut oil alleging that it causes cardiovascular diseases aggravated the situation. The newly industrialized countries in the East such as Taiwan, South Korea are fast emerging as key importers of coconut product. One of the main reason for the recent fall in price of the coconut and its products is dependency of price of coconut oil which again depends on the cost of other vegetable oils. Thus product diversification of coconut and development of value added products become very important in the coconut industry. The country has been successful in evolving appropriate processing technologies for the profitable utilization of some of the value added products of the coconut palm. As a result, products like packed coconut milk, coconut creams, spray dried coconut milk powder, sweet coconut chips, coconut water based products like snow ball tender coconut, coconut water concentrate, vinegar and neta-de-coco, etc have been developed and they seem to have good commercial potential. These products have become important as an agro-based raw material for many industries. As a result, it has become possible to encourage product diversification and development of value added product in the industry. Developing new technologies for evolving new and non-traditional coconut products alone will not sustain the coconut processing industry. Effective market promotion activities are to be organized by way of organizing exhibitions, workshops and trade fairs in order to create consumer awareness and boost the demand of coconut products to keep the wheel of the coconut industry to accelerate fast against the present slow pace of development.

Machineries and tools for enhanced efficiency in palms

T. Vidhan Singh and A.C. Mathew

Central Plantation Crops Research Institute

Kasaragod - 671 124, Kerala, India

The coconut palm *Cocos nucifera* L is one of the most useful palms in the world and it provides a variety of useful products like food, fuel and timber. Machinery developed at CPCRI for enhancing efficiency in cultivation and utilization of coconut is discussed.

Basin opener: The basic concept used was to shift the soil to one side using one-sided rotary blades. This was achieved by selecting the blades from two sets of blades. The end blades were modified to avoid touching the frame of the power tiller frame by modifying the curvature of the blade. The basin opener is fabricated using 18 gauge M.S. sheet, 6'80'760 mm spring steel plate and 6'25 mm flat. A rectangular structure with straight surface at the left side (from behind the power tiller) and curved surface at the right side was fabricated using M.S. sheet of 18 gauge thickness, 200 mm width, at left end and 300 mm at the right end is gradually curved from left to right for easy movement of soil. A spring steel plate of 6'80'760 mm, with the bottom end sharpened to cut the soil, is welded to the lower portion of the M.S. sheet. The entire frame is hinged at four points of the rotor frame of the power tiller at an angle of 10° for the displacement of soil towards the right side during anticlockwise movement. The total number of basins that can be opened in one day of 8 hours duration is about 96, whereas in the traditional method of opening by labourer, a maximum of 12-15 basins can only be opened. As this job is difficult, cost/labourer is also high and it works out to be almost double/basin. Also as this is a time bound operation, using a power tiller this operation can be done in the shortest possible time.

Palm sprayer: A power operated sprayer was designed and developed. The developed sprayer comprises of two co-axial pipes of ultra-light weight (0.5 kg / m), which can be used to spray up to a height of 15.25 m from the ground. The pipe height can be locked at any desired level above 6 m. The total cost of the sprayer includes the cost of the pipes (15.25 m), prime mover & pump, tank for carrying the spraying material etc., is around Rs. 75,000/-. For marginal farmers, instead of the prime mover a rocker sprayer can be used, thus bringing down the initial investment to about Rs. 7,500/-.

Coconut bunch support: Partial severing of the stalk from the trunk is known as buckling. A large number of nuts in the bunch and/or big sized nuts, long and less sturdy peduncle, wider angle between the leaf and the inflorescence and weak leaf petiole are some of the causes for buckling of bunches. To prevent the coconut bunches from buckling, a mechanical support has been developed which consists of a trunk-clamp, support-clamps and telescopic support-rods. The trunk-clamp is made in



six different sizes to suit varying trunk girths. Support-clamps can be placed at any point on the trunk-clamp. Telescopic support-rods are made in two different sizes to suit bunches located at various heights and positions on the crown. The support can easily be fixed on the trunk of the palm either by standing on the ground. The fork end of the support-rod is inserted towards the tail end of the bunch (about one-fourth distance from the tip) to support the peduncle. The rod is slightly lifted up and the other end of the rod is inserted in the support-clamp hole. One man can easily do the operation, and it is possible to support as many as 6 to 8 bunches at a time. Harvesting of nuts can be done without removing the support-rod by severing the nuts first and the stalk last. After the nuts from the bunch are harvested, the support-rod can be taken out and used for another bunch that might require support. As the palm grows taller, the trunk-clamp has to be refixed at a higher level. Before removing the trunk-clamp, which is already fixed, another is fixed above the old clamp. The bunch already under support is then provided with another support-rod and the clamp, support fixed to the new trunk-clamp. The old ones can be removed and used for some other palm. This operation may be necessary only once in two years.

Manually operated coconut husking machine: Husking is an important operation in coconut processing. Traditionally it is done by impaling the coconuts on a sharp iron spike fixed in the ground at a slight angle from the vertical. Coconut husking is a hard job requiring considerable skill and involves the risk of physical injury. A manually operated coconut-husking machine has been developed. The device consists of a platform at waist level, with a frame. A shaft, which moves up and down on the platform, is operated by foot through linkages at the bottom end. The top end of the shaft is fixed with a circular plate on which a set of three fixed blades is provided. A rotating plate with guide slots is fitted in alignment and just below the bottom plate. The scooping blades move out radially when the rotating plate is rotated by a handle fixed on it. Another plate with three fixed blades is fixed at the top in such a way that the blades are faced in vertical alignment with guide slots by means of guide rods welded to them. The guide rods move freely in the radial guide slots in the bottom rotating plate. The plate can be rotated by pulling the handle. Coconut is kept inverted in between the top and bottom plates. The bottom plate assembly is lifted up by pressing the foot pedal; pressing it further, the bottom and top blades pierce into the husk. After piercing to the maximum, the handle is pulled out to rotate the free plate which in turn will actuate the scooping blades. Upon scooping, the husk gets detached from the nut into three portions. The device can be easily operated by any unskilled person. It requires less effort to dehusk the coconut using this device when compared with the conventional method. It can be fabricated locally. It is simple in design and safe to operate. The cost of fabrication of this husking machine is Rs.2,000/- approx. And the out turn of this machine is 100 nuts/hr whereas the outturn by traditional way is about 160nuts/hour. But the main advantage of this machine is that an unskilled man can do this job with out the risk of injury.

Coconut splitting device: To solve the problem of splitting of coconuts a manually operated splitting device was designed and developed, the working part of it being a knife made of spring steel and bevel angle was 25 degree. As the cutting blade is made of spring steel, it does not require frequent re-sharpening. The impact force splits the

CPCRI Coconut Dehusker



Capacity 120 – 130 nuts per hour.
For small scale industries.
Cost : Rs. 2000.

nut. The nut water is collected automatically. The average time taken to split 100 nuts is 8 minutes. The total cost of the unit comes around Rs. 2000/-.

De- shelling machine

A cylindrical de-shelling machine was designed and fabricated for separating the kernel from the partially dried coconut cups. The RPM of the de-shelling machine was optimized and found to be 10. The impact force caused by this fall separates the partially dried coconut kernel from the shell. The capacity of the prototype model is 200 coconuts (400 half cups). It is found that, for partially dried coconut with 35 % average moisture content, with an average 40 rotations @10 rpm, the de-shelling efficiency is 80 %.

Coconut and arecanut palm climbing device:

Climbing coconut and Arecanut palm remains the biggest problem among coconut and arecanut farmers. Traditional coconut and arecanut palm climbers are becoming very rare. Timely harvesting and other plant protection measures become very difficult because of this. Even when labour is available, the charge for climbing operation would be very high. The difficulties in coconut and arecanut palm climbing and the requirement of certain amount of skill and prior experience in that field stops other labourers from this job. Coconut and arecanut climbing devices do exist in the market to help such people. However, because of various reasons like complicated design, high cost, requirement of prior experience etc., none of the devices are popular among the farmers.

It was under these circumstances the Central Plantation Crops Research Institute, Kasaragod developed a coconut and arecanut palm climbing device that is simpler and easy to use than all the existing climbing devices available in the market.

A simple coconut and arecanut palm climbing device has been developed by Central Plantation Crops Research Institute, Kasaragod. It consists of a pair of U shaped frames with a foot rest each at one side of the frames near to the open end. The U shaped frames are laminated with vulcanized rubber to get good grip with the coconut and arecanut tree trunk. Provision is given on the foot rests for the user to fix a pair of shoes of his size and choice. Heel of each shoe is at the open end of the longer arm of the device. Length of the middle arm of the U shaped frame can be altered to suit the size, diameter, of the coconut and arecanut tree trunk. The user can fix the climbing device to his feet by wearing the shoes attached to the device and can move up and down the coconut and arecanut tree as easily as he climbs a vertical ladder.

Operation: The user fixes a pair of shoes of his size and choice to each one of the foot rest on the U shaped frames for left leg and right leg. This remains fixed to the device till the shoes get



damaged due to wear and tear or when another user of different shoe size use the same climbing device. The climbing device can be carried by hand or may be taken by hanging the frames on the user's shoulder, in the same way the coir rope rings or bamboo ladders carried by traditional coconut and arecanut palm climbers. Keeping the climbing device on the ground near the coconut and arecanut palm, the user wears the shoes attached to the frames. The climbing device is now attached to his feet. He then moves his one leg along with the device to the opposite side of the coconut or arecanut tree trunk keeping the device horizontal and inserts the device to it, till the middle arm touches the tree trunk. Then he pulls the device towards the side of his that leg till the opposite arm touches the coconut and arecanut tree trunk and applies his body weight on the frame. When he applies his body weight the device tilts towards the side where the weight is being applied. The arm of the device where the foot rest is provided lowers and the clearance between the coconut and arecanut tree trunk and the device gets reduced at these two opposite sides. At this position the device gets locked to the coconut or arecanut tree trunk. Since the heel of the shoe is at the open end of the frame, the frame bends, lowers, to the side of the coconut or arecanut tree trunk where the climber stands. This tilt towards the climber, towards the open end of the device, prevents the climbing device from detaching the coconut or arecanut tree trunk. At this time all the three arms of the frame of that leg come in to contact with the coconut or arecanut tree trunk and the device gets fixed to it. The climber now stands on this device and fixes the device connected to the other leg at a higher elevation on the tree by repeating the same steps. By repeating these movements he can move up and down the coconut or arecanut palm as easily as he climbs a vertical ladder.



Tender coconut punch and cutter: Tender coconut water ('Elaneer' in Malayalam) is a delicious and nutritious refreshing drink provided by the nature. Though the artificial soft drinks once tended to reduce the importance of tender coconut water, the propaganda in favour of tender coconut water by the Government, the Coconut Development Board by advertising in leading national weekly and through popular TV channels and the proponents of naturopathy has now paved the way for its increased consumption. The lead taken by the Coconut Development Board in establishing 'tender coconut parlours' or 'Elaneer Pandals' at different locations is accelerating and enhancing the consumption of tender coconut water in a big way.

But, one of the main factors deterring the large scale adoption of 'Elaneer Pandals' is the absence of simple tools for the easy extraction of tender coconut water. It is an art to use a machete to remove the husk from one end of a tender coconut to expose a part of its shell and to cut open a small hole in the exposed portion of the shell to extract its water or liquid endosperm. While doing so, the coconut is held in one hand and the machete in the other. The husk is sliced off in bits by repeated heavy strokes of the machete. This is difficult and requires considerable practice before the skill is acquired. Some times it leads to injury. The traditional method of cutting is difficult, time consuming, and risky, wherein the tender coconut is held in one hand and struck a few times with a heavy knife or machete cutting it open into two halves. The meat is then scooped out with a spoon. Considerable practice is necessary before

acquiring the skill for cutting it open with least number of cuts and also for avoiding injury. Because of the risk involved, very a few people are available. This is also a major factor that deters people from establishing Elaneer Parlours ('Elaneer pandals) in most of the places. Simpler methods for cutting the tender coconut in an easier way also do not exist at present. With this in mind a simple manually operated tender coconut punch and cutter has been developed.



Copra dryers: A shell fired copra dryer was designed and developed to dry coconut in 24 h which works on indirect heating and natural convection principles using coconut shell as fuel. The capacity of the dryer developed was 1000 nuts per batch. The drying air temperature in the drying chamber was 80 °C. The unique burner designed generated heat for 5 hours without tending and the heat is retained for one more hour. No electrical energy is used in this dryer making it farmer friendly. Once the fuel is charged it produces heat for 6 hours thereby allowing the farmer to do other useful work as compared to other dryers where in fuel is loaded once in 15-20 minutes. Smoke does not come into contact with the copra; hence the copra produced is of good quality. About 100 grams of shell charcoal is also produced during the final phase of heating.

Future thrust

In view of shortage of labour for climbing the palms, simple harvesting device needs urgent attention especially for harvesting tender coconut and tapping Neera which has great potential in enhancing the income generation coconut cultivation.

Development and performance evaluation of Virgin Coconut Oil Cooker

A.C Mathew, K. Madhavan and T. Arumuganathan

Central Plantation Crops Research Institute
Kasaragod - 671 124, Kerala, India

Virgin coconut oil is the unrefined purest premium grade coconut oil obtained from the fresh and mature kernel of coconut (*Cocos nucifera* L.) by mechanical or natural means with or without the application of heat, which does not lead to alteration of the oil. To protect the oil's essential properties, the production of virgin coconut oil does not undergo chemical refining, bleaching, or deodorizing. Said to be high in vitamins and minerals, it is fit for human consumption without the need for further processing (Villarino *et al.*, 2007). Virgin coconut oil (VCO) is growing in popularity as functional food oil and the public awareness of it is increasing.

Various methods have been developed to extract coconut oil, either through dry or wet processing. Dry processing is the most widely used form of extraction. Clean, ground and steamed copra is pressed by wedge press, screw press or hydraulic press to obtain coconut oil, which then goes through the refining, bleaching, and deodorizing (RBD) processes. During the RBD process, heating process is applied especially during deodorization process, which is carried out at high temperature between 204 and 245°C (O'Brien, 2004). The copra industry also faced some problems such as contamination by aflatoxin in copra and cake and presence of high free fatty acids due to high moisture content (Guarte *et al.*, 1996). Recently, there is a trend towards producing coconut oil which does not have to go through the RBD process. Rather than going to the normal dry process, this oil is obtained by wet processing which entails the extraction of the cream from the fresh coconut milk and consequently breaking the cream emulsion. This process is more desirable as no chemical or high heat treatment is imposed on the oil. The coconut oil produced through the wet method is known as virgin coconut oil (VCO).

VCO is generally produced from the coconut milk extracted from fresh coconut. Coconut milk is an emulsion of oil and water that is stabilized by protein. To recover the oil from coconut milk, the protein bond has to be broken either by heat or by enzymes or some other mechanical means. The different processes involved in VCO production are Hot-processing method, Natural fermentation method, Centrifugation process and Direct micro expelling method. One of the different processes involved in VCO production is the hot process wherein the coconut milk is heated to produce coconut oil within the shorter period of time when compared to cold process. Virgin coconut oil is prepared conventionally by heating coconut milk in a container at low flame with continuous stirring. It is said that only half portion of a coconut spathe needs to be burned at a time, indicating the requirement of heating coconut milk at a very low flame. It is done manually and the constant stirring is a laborious process. Many a times the milk gets charred and the charred milk stick to the bottom and the sides of the vessel. This happens when the stirring is not proper or when excess fuel is burnt.

In order to avoid these limitations of the traditional Virgin Coconut Oil (VCO) production, a VCO cooker has been designed and fabricated to extract the VCO by hot

processing. This paper discusses the development and performance evaluation of this equipment for extracting virgin coconut oil from coconut milk.

A virgin coconut oil cooker was designed and developed at the Workshop of Central Plantation Crops Research Institute, Kasaragod, Kerala. It is a batch type cooker and is operated mechanically using an electric motor and reduction gear box. VCO cooker is a double jacketed container made of stainless steel. The schematic diagram showing the parts of the VCO cooker is shown in Fig. 1.

Thermic fluid is filled in between the outer and inner walls of the cooker at the bottom. Thermic fluid is a mixture of synthetic hydrocarbons and is intended for use in the liquid phase for indirect process heating. It offers increased thermal and oxidation stability that translates in to more efficient heat transfer, longer fluid life and optimum operating economics. It has an optimum economic bulk operating range of -10°C to 305°C . It exhibits thermal stability markedly superior to that of mineral oils used for the same purpose. Thermic fluid resists the effect of oxidation up to ten times better than mineral oils. Less oxidation means less solids formation and much less fouling.

Proper insulation is provided between the outer and inner walls of the cooker to prevent heat dissipation. A mechanical stirrer is provided in the chamber of the cooker. The stirrer is having four arms. These four mixing arms, together cover the entire bottom and the sides of the stirrer where coconut milk is kept for heating. Contact portions of the stirrer are laminated with Teflon. Teflon is very non-reactive and reduces friction & wear when it comes in contact with moving part. The Teflon coated stirrer is connected to an electric motor (1 hp, 1440 rpm) through a reduction gear (W63/ U P60/B5). An inlet is provided at one side of the cooker to fill the double jacketed vessel with thermic fluid. An expansion tank (17.5 cm diameter and 45 cm height) is also provided at the inlet. The expansion tank makes it easy to pour the thermic fluid and also helps to monitor the oil level in the cooker. A pressure release valve that acts as a safety valve is provided to the thermic fluid chamber to release the pressure build up, if any, occurs in it. An industrial, dial type, thermometer is provided in the thermic fluid chamber to monitor the temperature of the thermic fluid. An outlet is provided at the bottom of the milk chamber to take out oil. A three inch diameter outlet valve with a rubber washer to make it leak proof is provided in the bottom of the milk container. The outlet valve is opened and closed manually using a lever. Two LPG burners are provided at the bottom of the cooker to heat thermic fluid chamber directly and in turn the thermic fluid chamber heats the bottom of the milk container. The entire cooker is supported by four legs. The schematic diagram showing the front and side view of the newly developed VCO cooker is shown in Fig. 2.

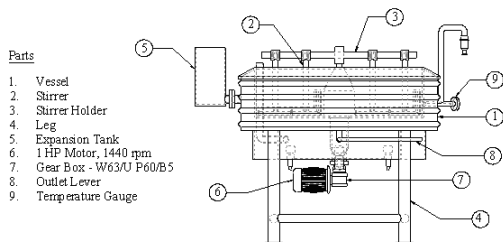


Fig. 1. Schematic diagram of Virgin Coconut Oil Cooker

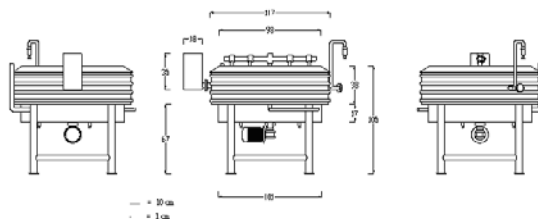


Fig. 2. Schematic diagram showing front view and side views of Virgin Coconut Oil Cooker

Power requirement of mixing arm

The horse power of motor required to rotate the mixing arm was calculated by using the following formula suggested by Khurmi and Gupta (2006).

$$\text{Power required, hp} = \frac{2 \pi NT}{4500}$$

where,

hp = horse power of motor

N = RPM of the mixing arm

T = torque, kg-m

Self weight of the mixing arm = 6 kg

Coconut cream/milk to be mixed = 125 kg per batch

Speed of the mixing arm (assumed) = 25 rpm

∴ Coconut cream to be mixed per revolution = 40 kg

Total weight = self weight + material weight

$$= 6 + 40$$

$$= 46 \text{ kg}$$

Torque required = Load x Distance

$$= 46 \times 0.60$$

$$= 27.6 \text{ kg-m}$$

(Distance = contact distance in the container where the mixing is done = 0.60 m)

$$\text{hp} = \frac{2 \times 3.14 \times 25 \times 27.6}{4500}$$

hp of motor required = 0.963 @ 1 hp.

The design details of the developed VCO cooker are illustrated in the Table 1.

Table 1. Design details of the VCO cooker

Parameter	Dimension
Milk container diameter	Inner dia - 95 cm Outer dia – 109 cm
Milk container height	24.5 cm
Material used for milk vessel/container	Stainless steel-food grade
Milk container thickness	20 gauge
Stirrer arm	4 no.
Stirrer speed	25 rpm
Motor	1 hp, 1440 rpm
Legs	4 No.
Expansion tank	17.5 cm dia and 45 cm height

Coconut milk was extracted from fully matured coconuts. The extracted coconut milk was poured in to the VCO cooker. Though, the cooker could be filled full, for operational convenience it was filled only up to 3/4th capacity. The reason was to avoid any possible splashing when the stirrer is stopped and restarted in between operation. The cooker could be ignited even before loading. The cooker was heated by igniting the gas burners. The burners were burnt with full flame initially. After attaining the required temperature it was reduced as per requirement and quantity of coconut milk. Coconut milk was heated in the cooker till all the water gets evaporated and oil gets separated. The flame was reduced to minimum and finally it was switched off towards the end of the process. The separated oil and the residue, *Kalkam*, were taken out by opening the outlet.

The oil recovery was calculated as per the following formula.

$$\text{OilYield (\%)} = \frac{\text{Quantity of VCO obtained}}{\text{Total quantity of coconut kernel}} \times 100$$

A Virgin Coconut Oil cooker was developed and evaluated for extracting virgin coconut oil from coconut milk. Fig 3 shows the working of the newly developed VCO cooker for extracting VCO from coconut milk.

Coconut milk was extracted from fully matured coconuts. Virgin coconut oil can be extracted from the milk by two different ways. Fresh coconut milk can be directly poured in to the cooker and it can be heated for extracting the oil. In the other method, coconut milk is allowed to settle for 3 hours to separate the skim milk from the cream. If the settling is done under refrigerated condition the time taken is only 1 hour and coco skim milk which is a nutritious beverage containing protein and micronutrients can be recovered for human consumption (Bawalan, 2003). The coconut cream is further separated from the skim milk and can be heated in the



Fig. 3. Working of newly developed VCO cooker

specially designed VCO cooker under controlled temperature to extract virgin coconut oil. The double walled VCO cooker under slow heat coagulates the protein and release the oil. Heat was provided through LPG by heating the thermic fluid chamber. Temperature was monitored and accordingly the heat was regulated. A mechanical stirrer is provided to stir the milk to avoid the cream sticking to the bottom. For the first hour of heating, temperature was allowed to reach 120°C. Further the temperature was brought down to 90°C until the protein begins to coagulate and the temperature was reduced to 70°C when the oil starts to separate. Oil was separated from the oil residue (*kalkam*) by straining the mixture through a stainless steel screen with fine mesh. This oil was colourless, transparent and with pleasant aroma and is known as class A oil. The remaining residue '*kalkam*' was brown in colour and was further heated at a temperature of 80 to 90°C so that maximum oil could be recovered. This oil was slightly dark in colour and is known as class B oil and is suitable for skin care and massage

purpose. Total time taken for cooking was about 2½ h for extracting class A VCO and the time required to extract class B VCO was 3 h. The cream temperature at which the class A Oil started to separate was 90°C where as the thermic fluid temperature was 170°C during the class A oil separation. After collecting the class A oil, Subsequently the thermic fluid temperature was reduced to maintain the cream temperature at 100°C to extract class B oil. Class A and class B oil were separately dried /heated to remove the moisture completely so that the shelf life of the VCO could be enhanced up to 1 year. The VCO yield obtained was 18.6 %.

A Virgin coconut oil cooker with a capacity of 125 l of coconut milk per batch was developed. Premium quality virgin coconut oil could be extracted from coconut oil by hot process method utilizing the newly developed VCO cooker and the oil recovery was around 18.6 % in three hours processing time.

Acknowledgement

The authors are thankful to Dr. George V. Thomas, Director, Central Plantation Crops Research Institute, Kasaragod for providing necessary research facilities for this study. The financial support provided by the Indian Council of Agricultural Research, New Delhi under the NAIP project entitled “Value chain in Coconut” is gratefully acknowledged.

References

- Bawalan D.D.** 2003. Coconut processing modules for micro and village scale enterprises, proceedings of the XLCOCOTECH meeting, Colombo (Ed. P. Rethinam), APCC, Jakarta, Indonesia
- Khurmi, R.S. and Gupta, J.K.** 2006. A textbook of machine design. Eurasia Publishing House (Pvt.) Limited, New Delhi.
- O'Brien, R.D.** 2004. Fats and oils: Formulating and processing for applications, CRC Press, New York.
- Villarino, B.J., Dy, L.M. and Lizada, C.C.** 2007. Descriptive sensory evaluation of virgin coconut oil and refined, bleached and deodorized coconut oil. *LWT-Food Science and Technology* **40**: 193–199.
- Guarte, R.C., Muhlbauer, W. and Kellert, M.** 1996. Drying characteristics of copra and quality of copra and coconut oil. *Postharvest Biology and Technology* **9**: 361–372.

Livelihood security through coconut farming

K.V. Ahamed Bavappa and V. Krishnakumar

Former Chief Technical Advisor UNDP/FAO Projects and Former Director
Central Plantation Crops Research Institute, Kasaragod - 671 124, Kerala, India

Coconut, Kalpavriksha, the tree of life, cultivated in about 95 countries of the tropical world has a very wide range of adaptability and performance features. Unlike in annuals, the potential for increasing the productivity per unit area of land, time and inputs through high density cropping is considerably higher in perennial crops. Studies have revealed that the roots of coconut planted at a spacing of 7.5 m apart effectively use only 22.3% of land area occupied by the crop, while the average air space utilisation by the canopy is about 30%. Thus even on simple physical considerations of soil and air space utilization, a monocrop of coconut can effectively support other plant species in the interspaces. It can accommodate a wide range of crops, annuals, semi-perennials and perennials in the interspaces. Grass and legumes grown in the coconut gardens enable integration of dairy industry providing higher employment and enhanced income. By appropriately choosing the inter crops, a continued supply of greens, tubers, pulses, vegetables and fruits can be ensured giving food security to the coconut farming family. By recycling the crop residues, soil health and fertility status is substantially improved and unit area productivity increased on a sustainable basis. Coconut is also amenable to growing under minimum tillage conditions. The crop thus provides a very wide range of options for the farmer to choose and improve his living conditions.

Coconut by itself is a food crop, amenable for the preparation of a variety of food products. Coconut oil, the healthiest oil on earth and coconut meat fibre as a dietary fibre and the diverse food products that could be prepared from coconut indicate the importance of this crop and the support that coconut and coconut based farming system offer to the livelihood security of millions farmers who cultivate it. It is imperative that the yield potential of all the crops of the system is holistically exploited so as to maximise the per unit area production.

The estimate of worldwide number of hungry people stands at 930 million (2009 estimate) which is on the increase. The present severe hunger that is prevalent in S. Africa is as severe as the 1984 extreme poverty of Ethiopia. In Nigeria, which is one of the poorest countries of the world, 200,000 children are facing death due to malnutrition while over ten million face extreme hunger. The report by the International Food Policy Research Institute, (IFPRI), Washington, has placed India in the 66th position out of 88 countries for Global Hunger Index (GHI), 25 to 28% of the population coming under this category.

Yield potential of coconut: It has to be admitted that the yield potential of coconut has not been properly understood by the coconut farmers and even the development agencies. The occurrence in nature of super yielding elite palms which produce 200 to 471 nuts in a year under rain fed average management conditions speaks for itself the yielding ability of Tall coconut genotype. The nine palms located during the survey undertaken by CPCRI had 35 to 50 leaves and 19 to 24 spathes. As such these palms are capable of producing about double the number of bunches which contributed for their super yielding ability. However, the exploitation

of this character through prepotency testing and multiplication through tissue culture technique has not been realized so far.

While the elite Tall palms yield over 200 nuts, the unselected palms planted in the back yard of houses and receiving household wastes and water yield over 120 nuts continuously. West Coast Tall palms yielding on an average 62 nuts/palm/year planted over an area of 1.2 ha and put under a High Density Multi Species (HDMS) planting system with regular irrigation improved the yield of the palms to 150 nuts maintaining this yield level for over 22 years. The agronomic disirability of coconut based farming systems designed on scientific principles of land space and solar energy utilization, the wide range of crops and animals that could be integrated into the system and the management techniques needed to make the cropping system a success have be reviewed by Bavappa (1990 a, b and c). In Lakshadweep, where fertilizer application is banned, the yield is 19,630 nuts/ha/year (98 nuts/palm at a planting density of 200 palms/ha). Incidentally this is the highest yield in coconut anywhere in the world. The above facts go to show that the yield potential of coconut under good management is at least 3 to 4 times more than what is now being realized.

Increasing the yield of coconut: Coconut palm has a wide range of attributes which make it fit for cultivation under diverse conditions and ecological situations. Taking advantage of the ability of coconut to produce organic matter in large quantities when planted with different crops under HDMS, a number of high density models and farming systems were laid out from 1980 onwards. Two such models planted at CPCRI, Kasaragod gave 28 and 31 tons of organic matter per ha while another model planted at RARS Ambajipeta yielded 40 tons/ha. The direct benefits of organic matter are the nutrients supply from these materials to crops and micro flora, promotion of soil biological activity which is the driving force in the decomposition of organic matter, formation of humus, nutrient transformation and evolution and maintenance of stable soil structure.

Nutrient availability from Organic matter: A study of the nutrient availability from the above sources of organic materials showed that barring for K; the available N and P were adequate to meet the nutrient needs of coconut (Table 1).

Table 1. Nutrient availability in the organic matter

System	Biomass (t)	Nutrient (kg/ha/year)		
		N	P	K
HDMS –CPCR I	28	125	78	115
HDMS – Ambajipeta	40	269	68.6	77.8
Nutrient requirement/ha for coconut	85	54	204	

System effect

Manurial inputs and yield of crops: The crops in the HDMS cropping system had received doses of fertilizers as per the requirement of each crop in the system, all applied at three levels: full, 2/3 and 1/3 of the full dose. The total quantity of nutrients applied to each plot and the yields of crops obtained are given in Table 2.

Table 2. Manurial inputs and yield of crops

Manurial dose	HDMS			Monocrop
	Full	2/3	1/3	
Total nutrients applied to the syste(N, P, K kg/ha)	792.7	534.2	276.6	317.1
Crop yield				
Coconut (mean of 14 years) nuts/tree	151	149	151	129
Clove (g/tree)*	419	614	327	
Banana kg/bunch*	5.54	5.10	4.54	
Pineapple kg/tree*	0.82	0.79	0.70	

*Mean of 5 years

Coconut in the HDMS plot which received 1/3 quantity of the full dose (276.6 kg NPK), yielded 22 nuts/palm more compared to the monocrop which received 317 kg NPK. The extra yield in spite of a lower level of applied nutrients present interesting discussion.

After a period of 10 years, the above manurial trial in the HDMS plot was modified to include five manurial doses (full, 2/3, 1/3, 1/4, and 1/5) and a no manure control. The yield of Coconut and intercrops obtained from different treatment plots is given in Table 3.

Table 3. Output from different crops under coconut based cropping system model at CPCRI, Kasaragod (1999-2005)

Treatment	Coconut (nos.)	Pineapple (kg/fruit)	Clove (dry kg/tree)	Banana (kg/bunch)	Black pepper	BC ratio
Full dose of fertilizers	145	0.89	1.44	5.76	0.87	2.05
2/3 recommended dose	147	0.70	2.55	5.43	1.66	2.18
1/3 recommended dose	147	0.57	1.25	4.70	0.90	2.16
¼ recommended dose	137	0.43	1.12	4.36	1.06	2.13
1/5 recommended dose	129	0.48	1.00	3.91	0.42	2.01
No fertilizer(control)	127	0.45	1.32	3.86	0/46	1.89

Yield reduction of 10 and 18 nuts in ¼ and 1/5 fertilizer dozes respectively go to show that in systems involving diverse intercrops, nutrients supply through fertilizers can be reduced only up to 1/3 of the full dose. A more or less similar trend is seen in the yield of inter crops as well as in BC ratio.

Animal integration: Mixed farming which integrates other enterprises such as dairying, poultry and pisci-culture with coconut by raising fodder crops, banana, pepper, etc.in the interspaces is a highly successful system. Such a system would enable recycling of the cattle

manure, urine and animal feed wastes to the fodder crops and coconut. Mixed farming provides additional employment to the tune of 600 man days per ha and ensures good returns. The mixed farming trial undertaken in 1.40 ha Coconut garden at CPCRI Kasaragod gave a net return of Rs.73, 142/-per annum (year 2000).

Zero or Minimum tillage: The amenability of coconut for adoption of minimum tillage technology is yet another factor that helps to improve soil health and fertility and reduce the cost of production. Cultivation practices such as ploughing, digging, opening the basins, forming mounds etc. expose the earthworms and microbial fauna to solar radiation and weathering, adversely affecting their growth and population. These operations also aid soil erosion. Slashing the weed growth as and when needed and using the organic matter as mulch, application of coconut leaf to the base of the palms, burying the coconut husk and raising the legumes in the coconut palm basins and interspaces promote the earthworm and microbial activity making soil porous, healthy and rich in organic matter and nutrients. Under such a management system, there is continued availability of nutrients and better moisture due to the mulching effect, coconut giving higher yield at lower cost of production. Adoption of this technology also reduces CO₂ emission by immobilizing carbon in incremental soil organic matter and surface residues. A farming system approach especially in the HDMS system also eliminates the need for tillage.

The response of coconut for organic cultivation coupled with zero tillage was significant. Vermicomposting in the coconut basin, application of biofertilizers and cover cropping the interspace increased the yield of West Coast Tall coconut from 58 nuts/ palm to 106 nuts over a period of five years. During the same period, yield of Dwarf x Tall hybrid, increased from 77 nuts / palm to 137 nuts.

Livelihood Security at farm level: While the integrated coconut based farming system models laid out under experimental conditions had given encouraging results, their performance under conditions existing in farmers' field was tested by Central Plantation Crops Research Institute as a global strategy for sustaining the livelihoods of marginal coconut growers under a networked project entitled 'Overcoming Poverty in Coconut-Growing Communities: Coconut Genetic Resources for Sustainable Livelihoods in India', supported by IFAD / COGENT and Biodiversity International over a period of five years. The project was to increase income from coconut gardens and to improve food and nutritional security of farm family members through a Coconut-Based Farming System (CBFS) model integrated with compatible intercrops, animal husbandry or mixed farming activities, and diversification of products and conservation of genetic resources.

The activities were carried out through registered Community Based Organizations (CBOs) on the basis of annual action plans prepared by the members. Major thrust was given for training and capacity building for the CBO members in developing their individual as well as group capacities in terms of technological interventions, arrangement of inputs and management of CBOs. The major interventions included intercropping with cash, food and nutritional security crops, livestock integration, diversification of coconut products and

conservation of genetic resources. This not only confirmed the income generating potential of the models, but also their ability to improve the quality of life. Pre and post data related to demographic, socio-economic as well as food security and nutrition aspects of the clients and income from coconut, intercrops, livestock and household level processing were recorded. Data were collected through personal interview with 150 CBO members and by referring the profitability records kept by the CBO members. The collected data was coded and analysed using SPSS.

Tangible impacts

Several indicators like income from coconut, intercrops, livestock and household level processing and food and nutritional security status were measured before and after the project period to see the tangible impacts of the project.

Improvements in yield of coconut and area under intercrops: The average yield of coconut per palm per year increased from 30 to 37 over the project period, recording a 23% improvement. Even though the project had no component for managing the palms, the manuring and intercultural operations done for the intercrops coupled with the recycled organic matter from intercrops might have contributed for the increase in the yield of coconut. The area under intercrops also doubled during the period, which showed a shift from 3.25 cents during the pre-project period to 7 cents after the project.

Improvements in income from various sources: Significant changes were recorded in the levels of income generated through various sources (Table 4).

Table 4. Comparison of means of income from coconut, intercrops, livestock, off-farm and total income (Rs.)

Source of income	Mean income	
	Pre-project	Post-project
Coconut	2780	4197
Intercrops	806	4287
Livestock	1843	14090
Off-farm	71	2750
Total income	25617	59018

The income from coconut recorded an increase of 50% over the project period. The project efforts could bring in considerable increase in income from intercrops, livestock and household level processing. The total annual income per homestead enhanced from Rs.25, 617 to Rs.59, 017 over the project period.

Change in poverty status of the communities: Change in the poverty status of the communities is the most important factor which decides the overall impact of the project. This was measured in terms of the number of holdings below poverty line in each of the communities before and after the project and presented in (Table 5).

Table 5 . Comparison of poverty threshold level

Name of CBO	Holdings below poverty threshold level (%) (<IUS\$ per head per day)		Percentage change
	Pre-project	Post-project	
Pathiyoor	94	46	48
Thodiyoor	96	62	34
Devikulangara	96	60	36
Mean	95	56	39

Significant change was observed in the poverty status, in terms of the percentage of holdings below the poverty threshold level, which got reduced from 95 to 56%. The highest reduction in poverty incidence was recorded in Pathiyoor CBO (48%), followed by Devikulangara (36%) and Thodiyoor (34%).

Change in food and nutritional security status: Apart from the enhanced income levels, significant improvement was seen in the food and nutritional security of the family members, especially children. Regarding food security, there was an increase in the completely secure category from 67 to 96%, whereas, in the nutritional security, it was from 8 to 72% at the end of the project. The totally insecure category was completely wiped off with project interventions.

Sustainable coconut based farming systems approach through community based organizations is thus, an effective strategy for improving the livelihood security and quality of life of farmers.

Gaps in current knowledge: The ability of coconut to give much higher yields than what is now being obtained has been proved beyond doubt. However, the mechanisms behind such performances are not clearly understood, thus limiting the usability of such information. Increase in yield of tropical perennial plantation crops is possible through increase in planting density to achieve early ground cover, improvements in light interception through shade regulation, use of varieties with greater tolerance to exposure to sun light, reduced maintenance respiration, higher conversion efficiency and better harvest index. The HDMS model laid out at CPCRI had by and large these aspects in view and the yield increase of the coconut from 62 nuts to a steady 150 nuts for nearly 25 years in succession, confirms this contention. However, the yield of 150 nuts obtained with 1/3 the manurial dose which is on a par with full and 2/3 doses indicates that there are factors other than the directly applied nutrients that contribute to the yield increase. This assumption is also supported by the yield difference observed between palms with inter crops and receiving 1/3 the fertilizer dose of nutrients (276.6 kg), yielding 150 nuts and monocrop of coconut which received 317.1 kg of nutrients giving only 129 nuts/palm. The increase of 22 nuts over that obtained by the monocrop has been attributed to be due to a response factor outside the nutrients which is the 'System effect'. It appears that the organic matter from different crops being recycled promotes a dynamic microbial interaction accelerated by rhizosphere biomass and exudates of different crops, promoting the production of a wide range of plant growth promoting substances. Information available

in this sector is meagre. It is also evident from this data that it may not be possible to get yield increase beyond a limit in monocrop of coconut with higher input of nutrients through fertilizer alone.

Future thrust: Research and Development Opportunities

The available information strongly suggest that in coconut, a higher yield of 3 to 4 times of what is now being obtained is possible if proper varieties are chosen and appropriate cultivation techniques, particularly a system approach adopted. The immediate task of the Development agencies and farmers is, therefore, to increase the present level of productivity to an achievable targeted yield of at least 80 nuts/palm. It will be worthwhile to work out Technology Packages with compatible crops that would meet the food needs of the family and generate employment and income on a sustainable basis by the Coconut Development Board, CPCRI and Agricultural Universities and undertake an aggressive productivity improvement and livelihood security programme joining hands with Dept. of Agriculture, Horticulture Mission and other related development agencies.

The growing health consciousness and the preference for natural products need to be fully exploited in promoting tender nut water as a health drink, coconut oil, as the healthiest vegetable fat, coconut meat fibre as a healthy dietary fibre, coconut chips and the wide range of food products that could be prepared from coconut as support to livelihood security.

The carbon sequestration potential of Coconut based cropping system is estimated as 126.75 tons CO₂ / ha/year, which is substantially higher compared to other plantation crops and annuals. Coconut, under high density cropping system will, thus qualify for payment under carbon trading since the difference between CO₂ absorption and CO₂ emission is given benefit of payment by the CDM authorities. They have recognized that CO₂ absorbers like sugarcane need to be awarded CER (Certified Emission Reduction). Each CER representing one ton of CO₂ presently is valued at US\$ 17. Getting this benefit for coconut even under a modified form of CDM is worth serious consideration, when sugarcane is being awarded this benefit.

There are knowledge gaps and inadequacies needing follow-ups in areas related to reducing the juvenile phase of coconut, prepotency testing, vegetative propagation, yield performance of hybrids under farming system conditions, microbial interphase of different fauna among themselves and with rhizosphere of crops in the system and with root exudates and amenability of the crop to zero tillage. Research is to be strengthened in these areas to obtain a quantum jump in the performance of the versatile coconut palm.

Community based interventions in coconut sector for enhanced profitability

P. K. Thampan and Remany Gopalakrishnan

Peekay Tree Crops Development Foundation

Gandhi Nagar, Kochi-682020, Kerala, India

Coconut farming in India is in the hands of small and marginal farmers. These farmers in most cases do not enjoy community support or guidance in adopting economically viable activities for augmenting on-farm income and profitability. Small and marginal farmers do not have access to appropriate production technologies nor the capacity to benefit themselves from the available opportunities in the spheres of production, value addition and marketing.

Now-a-days community-based organizations (CBOs) are being promoted in the coconut sector to give leadership to the farmers for solving common problems through self-help and community effort. The CBOs are registered organizations with membership open to all categories of farmers in the community. The members elect office bearers periodically who are entrusted with the responsibility of identifying local needs and aspirations of the farming community and evolving measures for addressing them through community initiative. Both big and small CBOs with varying membership pattern are presently functioning in some of the Village Panchayats falling within the Pattanakkad Block Panchayat, Alappuzha District. This Block comprises eight Village Panchayats with viable CBOs in some of them. Of these, the Vayalar Community Development Centre (VCDC), the CBO functioning in Vayalar Panchayat since 2002, has over 1,000 farm-households as members. Among the other notable ones the Vayalar Farm Club and Haritha Samrudhi Farmer's Self-Help Group of Pattanakkad, which came into existence in 2006, have coverage of about 200 households each.

Under the aegis of CBOs efforts and resources in a community are harnessed for stabilizing the coconut-based economy of the members. In villages CBOs provide leadership in functional areas such as production of quality planting material, integrated farming under coconut, product utilization for value addition, household and community level employment generation for women and efficient marketing of the farm produce and value-added products. In this regard, the CBOs in the Pattanakkad Block Panchayat have been successful in inculcating in the minds of the members the importance of self-help and community effort in solving the existing problems that hinder the growth in coconut production and product utilization. The changes in the attitude of the members towards this direction have become evident from their increasing participation in community gatherings for identifying local needs, formulating action plans and for efficient implementation both at the individual and group levels. The interventions made by the CBOs in the Village Panchayats and the impact caused on the socioeconomic progress of rural farm-households, traditionally engaged in coconut farming and product utilization for their livelihoods, are summarized in this paper.

Interventions of CBOs

Farmer participatory study: The needs and aspirations of the farming community are first identified in farmer participatory meets where the representatives of the concerned CBO interact with the farmer participants based on a semi-structured questionnaire. The Vayalar

Community Development Centre (VCDC) conducted a farmer participatory study in three different locations of Vayalar Panchayat in 2003 to study and record farmer's perception of coconut varieties, conservation and improvement as well as their assessment of opportunities for increasing on-farm income and profitability from coconut farming.

The important recommendations emanated from the study were (1) to implement a decentralized nursery programme for satisfying the requirement of quality planting material of coconut and other crops in the Panchayat for which coconut root wilt disease free palms in each garden are to be identified to serve as source of seed nuts followed by establishing community nurseries for producing seedlings for general distribution, (2) to promote intensive integrated farming in coconut gardens involving diverse species of trees and shrubs of economic value and agricultural crops with or without livestock components for enhancing on-farm income and profitability, (3) to establish one coconut husk processing plant under community ownership to facilitate local availability of coir fibre for sustaining the household coir spinning sector, (4) to replace manually operated coir spinning wheels in households with motorized ones to reduce the drudgery of working women and to enhance their daily earnings, (5) to introduce facilities for skill development training for local youth possessing entrepreneurial aptitude and (6) to extend techno-economic support to women's groups or their cooperatives to produce value added products from coconut at the farm-household and community levels and organize efficient marketing.

VCDC has formulated programmes in accordance with the recommendations of the participatory study and provided leadership to the community members for field level execution. The CBO has established close working relationship with the different R&D organizations functioning locally as well as at State, national and international levels. Techno-economic support from these organizations could also be harnessed for the efficient implementation of the projects taken up. The supporting organizations included International Coconut Genetic Resources Network (COGENT), Malaysia; UNDP-GEF-SGP; Council for Advancement of People's Action and Rural Technology (CAPART), Government of India; Coconut Development Board; Coir Board; Alappuzha Coir Cluster Development Society; and Coir Directorate, Government of Kerala.

Establishing community nurseries: VCDC has assigned importance to the direct participation of community members in raising and distributing quality planting material of improved coconut cultivars/ecotypes.

The CBO organized a survey of coconut holdings in the Vayalar Panchayat where the area under coconut is around 900 ha. In the surveyed holdings disease free and high yielding palms were marked out to serve as seed source. With the support and assistance of community members over 15,000 seed nuts were procured from the identified mother palms, which included the elite tall and green and orange dwarf types. The seed nuts were distributed among community nurseries formed under the aegis of self-help groups of men and women. In the Vayalar Panchayat 8-10 community nurseries were organized for the production and distribution of quality seedlings.

Apart from the nurseries set up for producing coconut seedlings more community nurseries were organized in all the Panchayats in the Pattanakad Block for raising seedlings of medicinally important tree species for planting as intercropped in coconut holdings. Both VCDC and the CBOs in other Panchayats took lead in this direction to create opportunity for

additional income generation from the intercropped holdings at a later stage. Over 55,000 seedlings of different species of medicinally important trees were made available to the farmers from about 15 community nurseries. The tree species selected for multiplication were *Acacia catechu*, *Aegle marmelos*, *Azadirachta indica*, *Cesalpina sappan*, *Garcinia camboga*, *Gmelina arborea*, *Melia dubia*, *Myristica fragrans*, *Phyllanthus emblica*, *Pongamia pinnata*, *Pterocarpus marsupium*, *Strychnas nux-vomica*, *Terminalia bellarica*, *Terminalia chebula*, *Terminalia arjun*, *Punica granatum* and *Tamarindus indica*.

Promoting coconut based farming system: The CBOs functioning in different Panchayats have evolved and put into effect appropriate measures for promoting coconut-based farming system in homesteads. The community members have been provided with techno-economic support for planting compatible combinations of woody species of economic importance along with vegetables, root and tuber crops, banana, annual spices etc with or without livestock components under coconut in the same operational holding. This system of integrated farming in the inter-and intra-row spaces of coconut is expected to generate multiple sources of food, income and employment for the dependent farm-households besides rendering ecological benefits for the local community.

Over a period of five years since 2004, around 55,000 seedlings of 17 medicinally important tree species were planted in coconut holdings under the leadership of the CBOs functioning in different Panchayats. In the Vayalar Panchayat alone VCDC and the Farmer's Club have been successful in covering all the 7,000 farm-households under the tree planting programme. The plants on reaching harvestable stage within the next 10 years would serve as the major source of genuine raw material for Ayurvedic medicines, which is presently in short supply in the State. This will cause substantial increase in the income from holdings where the tree species have been integrated.

In all the Panchayats in the Pattanakkad Block, the CBOs have promoted the integration of diverse cropping models and livestock components in coconut holdings. This has strengthened on-farm biodiversity and contributed to enhanced income and employment per unit area, time and inputs as well as availability of nutritious foods. In the Pattanakkad Village Panchayat the CBO, Haritha Samruthy Farmer's Self-Help Group, has been instrumental in mobilizing farmers and encouraging them to adopt intensive cropping system under coconut. In 2008 more than 200 farmers were engaged in the cultivation of vegetables and root and tuber crops in their holdings which resulted in an output of 15 tonnes of farm produce valued at Rs.3.0 lakh. The cropping system generated 2,000 man-days of additional employment, which benefited mostly household women. The management practices adopted for intercropping also benefited the coconut palms. To sustain the interests of the farmers in coconut-based cropping system the CBO has formulated a programme for awarding incentive prizes to the best farmers in the Panchayat on yearly basis.

In the nearby Thuravoor Panchayat with support from a local CBO intensive integrated farming system has been practised by a four-member family in a marginal holding of 0.075 ha. The average annual income generated from this holding is Rs.1,20,000, which is equivalent to Rs.16,00,000 per ha per year. The components included in the system are coconut palm, tree spices, root and tuber crops, vegetables, ornamental fish, table fish, love birds and rabbit. One biogas unit, run by rabbit droppings, is also part of the system. The farmer, Mr.K.P.Viswanathan, has been honoured by the Kerala State Agriculture Department for the model farming system developed by him by conferring on him the Best Farmer Award in 2009.

In the Block Panchayat as a whole about 500 holdings have adopted intensive integrated cropping models and in another 165 holdings cattle, goat and poultry formed additional components. In a follow-up study in the Vayalar Panchayat covering 82 intercropped models with average holding size of 0.27 ha the total net income generated from intercrops over a period of 12 months was found to be Rs.659,835 or an average net income per holding of Rs.8,047, which was in addition to that derived from coconut, the main crop. This level of income corresponds to Rs.29,635 per ha per year. The recorded income from intercrops was for the marketable surplus after satisfying the household dietary needs. This shows that cropping system in coconut holdings makes significant contribution to the household food and nutrition security as well. In case studies on eight holdings the net income was found to vary between Rs.9,868 per ha and as high as Rs.74,750 per ha from the intercrops. The cropping system was also found to create additional employment opportunities, which ranged between 150 and 180 man-days per ha per year. In the holdings devoid of crop mixing the range was 60-80 man days.

In the study covering 36 holdings where livestock components were also integrated in the system the total net income from the holdings of the same size group as that of intercrops for the same period was Rs.1,84,680 or Rs.5,130 per holding. The case studies on six livestock rearing units revealed a range in net income from Rs.15,333 and Rs.88,333 per ha. The average net income from cattle rearing was Rs.8,775 per household, which was in addition to that derived from coconut and intercrops. These results revealed that systematic integration of woody species and arable crops along with livestock components will convert even small sized coconut holdings into economically viable operational units. The study also disclosed that among the livestock components cattle rearing was more profitable than goat and poultry rearing in the Vayalar Panchayat. With the integration of livestock in the system the additional employment created was 200-250 man-days per ha per year. The results of 10 case studies conducted in Vayalar Panchayat are shown in Table 1.

Table 1. Results of case studies conducted in Vayalar Panchayat*

Name of farmer	Area of holding (ha)	Activity	Net income per holding (Rs.)	Net income per ha (Rs.)
Mr.V.K.Sukumaran	0.4	Cropping System	11,450	28,625
Mr.D.Krishnan Nair	0.4	-do-	8,650	21,625
Mr.G.Soman	0.2	-do-	13,600	68,000
Mr.V.Asokan	0.4	Mixed Farming	29,900	74,750
Ms.Preethi Panicker	0.24	Cropping System	9,800	40,833
Mr.N.Soman	0.2	-do-	10,700	53,500
Mr.N.J.Xavier	0.2	-do-	11,100	55,500
Mr.Paul Richard	0.38	-do-	3,750	9,868
Mr.P.D.Prabhakaran	0.18	Mixed Farming	15,900	88,333
Ms.Pankajakshi Amma	0.6	-do-	40,524	67,540

**The income shown against each farmer is exclusive of that from coconut*

Apart from offering socioeconomic benefits to the farmers who convert monocropped coconut holdings into integrated farming units the system will support important ecosystem services including climate change mitigation. The population of diverse tree species and the under storey plant components when spread over a large area minimizes the hazards of soil and water erosion, stimulates the underlying productive capacity of the soil resource, improves the quality of local environment and strengthens biological diversity. The woody species in the system and other growing plants can function as effective sink for carbon dioxide and many air pollutants, both gases and particulates. They can remove substantial amounts of carbon dioxide from the atmosphere and store it in vegetation and soils, thus stabilizing the local climate. Thus, the integrated farming system involving 55,000 tree species and miscellaneous under storey crops as promoted by the CBOs will confer on the coconut farming community social, economic and ecological benefits.

Facilitating community owned husk processing initiatives: Coir spinning is the major source of income and employment for over 45 percent of the households in the Vayalar Panchayat with women forming the exclusive workforce. The raw material for spinning is coir fibre, which is extracted from coconut husk. Though coconut husk is available in plenty in the Panchayat facilities were lacking for processing the same into fibre. Consequently, the entire raw material for spinning has to become available from the neighbouring coconut growing States. This situation used to cause frequent shortage of coir fibre and disruption of spinning work leading to erosion in income of the working women. To overcome this situation VDC took lead in getting one husk processing unit established to benefit the local community. As a result, one self-help group comprising 10 members came forward to establish the processing unit. The facility so created is known by the name 'Vayalar Fibre Mills', the project cost of which is Rs. 50 lakhs. The required finance has been mobilized from the community and from institutional sources. The project also received generous support from the Coir Board, Alappuzha Coir Cluster Development Society and Coir Directorate, Government of Kerala. The unit has now 370 coir spinning households involving 740 women members as share holders.

Vayalar Fibre Mills has the capacity to process around 25,000 husks per day which are being supplied daily by 25,000 households of Vayalar and nearby Panchayats. This regular supply not only sustains uninterrupted work of the Mills but also serves as a regular income source @ Rs.0.35-0.40 per husk per day for each household. In the absence of such opportunity, the local availability of coconut husk would otherwise have remained unutilized. The daily output of coir fibre from the processing unit is around 1,750 kg. This will feed 58 household coir spinning units providing full time employment to 116 women and part-time employment for 56 men. The unit also ensures direct employment to 20 men and 10 women.

One coir mats and matting unit including geotextiles and one community coir yarn spinning unit are also functioning under the aegis of Vayalar Fibre Mills. The coir fibre produced in the milling unit is spun into coir yarn in community spinning sheds to serve as the raw material for the manufacture of mats and matting. The coir geotextiles are gaining wider acceptance for controlling soil erosion, establishing restorative vegetation and for engineering applications. The units provide direct employment to over 25 women and five men.

Producing organic inputs in support of green farming: Vayalar Fibre Mills has taken up the composting of coir pith, the waste product generated in its husk processing site, with

the technical support of the Central Coir Research Institute under the Coir Board to facilitate waste disposal from the husk processing site and to make available a rich source of plant nutrients for the use of coconut farmers. From one ha of coconut holding with 175 palms under average management the expected annual availability of nuts is 10,500. On an average, husk constitutes 45 percent of the weight of nut and on this basis a nut weighing 1,100 g will have 495 g of husk with about 20 percent moisture. From 10,500 husks or 5,197 kg, expected to be obtained from one ha, 70 percent or 3,638.25 kg will be pith, which after composting will yield 2,182.95 kg of compost.

From the husk processing facility of Vayalar Fibre Mills the average daily output of coir pith from 25,000 husks processed is around 8,663 kg, which on composting yields 5,198 kg of compost. Coir pith compost has an average nutrient composition of 1.06 N, 0.06% P₂O₅, 1.20% K₂O, 0.5% CaO, and 0.48% MgO besides having appreciable contents of minor elements. The production which commenced during the latter half of 2008 has now reached commercial scale benefiting the coconut farmers who have opted for green farming. Although coconut husk by itself is an excellent material for recycling in coconut holdings, its value as a source of plant nutrients does not decrease even after extracting the commercially valuable coir fibre.

VCDC and similar CBOs in other Panchayats have facilitated on-farm organic recycling by promoting vermiculture technology. Towards this direction 100 standard vermiculture tanks (3m×1.5m×0.75m) have been constructed on the holdings of community members in different villages. This approach could inculcate in the minds of the community members the importance of maintaining clean surroundings and utilizing the organic waste generated in homesteads for producing valuable manure.

Conducting skill development trainings and seminars: The CBOs have taken initiative in organizing trainings and seminars in different Panchayats for the benefit of the community members. In this endeavour the CBOs could access technical and financial support from the concerned development departments in the State. The trainings already conducted covered subjects like appropriate processing technologies for value addition in the coconut sector, coconut-based farming system, palm climbing using mechanical devices, coir spinning, entrepreneurial development, and in miscellaneous rural technologies offering opportunity for self-employment and income generation. Apart from these trainings the CBOs have also organized seminars and workshops on regular basis in the different Panchayats. These covered subjects like sustainable farming, community participation in local level planning and implementation of development projects, cultivation of medicinal plants, marketing strategies etc.

VCDC was also successful in getting deputed a processing expert from Vietnam, Ms. Nguyen Thile Thuy, to train the local women in coconut food processing. She was available for a week and during the period imparted training to around 20 women. Her visit was facilitated by the International Coconut Genetic Resources Network, Malaysia. In the Vayalar and Aroor Panchayats the local CBOs are also extending post-training support to those who exhibit entrepreneurial skill to start viable micro-enterprises or to strengthen the existing ones in which they are involved. Some members of the CBOs who have received training have formed self-help groups and setup profitable enterprises in the fields of catering, coconut-based foods and beverages, mushroom culture etc.

In recognition of the importance of skill development training, particularly for enterprising women, a group of local women in the Aroor Panchayat has established one permanent training centre by name 'Rural Technology Training Centre' (RTCC) for conducting regular training courses for farmers, processors, artisans, extension workers of development departments etc. This centre which received funding support from UNDP-GEF-SGP started functioning from the 1st of December 2008. The objectives of this training centre are to (1) organize periodical skill development training for the benefit of potential entrepreneurs, (2) facilitate the establishment of technically and economically viable micro-enterprises by the trained entrepreneurs, (3) train self-help groups of educated women and men for establishing micro-enterprises in the processing sector, (4) organize periodical seminars and workshops and to bring out publications to diffuse knowledge in appropriate technologies, (5) conduct training visits to recognized R&D Institutions and successful processing industries for the benefit of the trainees as well as the entrepreneurs, (6) arrange special training for the needy in selected trades in major production units, (7) maintain close rapport with national and international organizations engaged in research and development in crop sciences and end use applications, (8) institute Endowments to honour and encourage outstanding entrepreneurs, and (9) conduct studies and surveys and undertake rural development activities.

Creating income and employment opportunities for women: The CBOs in the Vayalar, Pattanakkad and Aroor Panchayats have developed action plans with the primary objective of creating income and employment opportunities for the women members of the community. The essential components of the action plan are (1) modernizing coir spinning, (2) coconut-based food processing, (3) livestock management comprising cattle and goat rearing, poultry keeping etc. (4) catering services, (5) dispensing display-cum sales outlets and (6) miscellaneous other income generating enterprises. The related activities are being organized by the respective CBOs through women's self-help groups formed specifically for each activity. The self-help groups are presently engaged in coir spinning, food processing, mushroom culture, poultry, duck and goat rearing, tailoring, day care units for children, catering services, coir pith composting and managing community nurseries. Already over 350 women organized into 30 groups are engaged in diverse income earning activities.

Household level coir spinning serves the major source of income and employment for women in most of the Panchayats within the Pattanakkad Block. This sector is the exclusive domain of women workers who used to handle manually operated spinning wheels. By spinning in these wheels the workers are exposed to drudgery without commensurate benefits as they could earn not more than Rs.50 per head in a day of arduous toil. In order to improve the working conditions and the earning capacity of the workers, the CBOs such as VCDC, Vayalar Farm Club and Haritha Samruthy Farmer's Self-Help Group accessed financial support from different sources and introduced motorized spinning wheels and replaced the traditional ones from 370 households. As a result, 740 women could get regular and full time employment with higher earnings of Rs.80-100 per worker per day as against the earlier earning of Rs.50 a day. All the 740 beneficiaries have also become shareholders of Vayalar Fibre Mills after contributing Rs.120,000, being 10 percent of the cost of the spinning wheels supplied to them. This is an example of the method adopted by the CBOs for the economic emancipation of the poor women in the coir spinning sector.

The same group of women which organized the Rural Technology Training Centre has promoted a registered society for the welfare of women within and outside the Pattanakkad Block Panchayat. This society named as 'Karunya Women's Charitable Society' has started functioning from the last week of August 2009 with the objectives of (1) rendering financial support to needy women belonging to socially and economically deprived sections of the society for medical, educational and matrimonial purposes, (2) providing vocational training for unemployed women, (3) encouraging and facilitating self-employment by offering techno-economic support to resource poor women possessing entrepreneurial skill, (4) promoting women-managed rural enterprises in processing and marketing sectors, (5) establishing and dispensing shelter for destitute women and old age home for women, (6) providing home away from home facilities for the kids of employed women, and (7) conducting socio-economic studies to identify the needs and aspirations of the women and to enlighten them on the opportunities available for progress by holding seminars, publishing books, periodicals etc. and organizing visits to successful women-managed enterprises.

Shares important social responsibilities: CBOs like Vayalar Community Development Centre (VCDC) and Vayalar Farm Club have established partnership with some State level NGOs in managing social welfare initiatives introduced in the Vayalar Panchayat. These CBOs are presently managing two long-term endowments known as 'Peekay Prizes' and Dr.P.Rethinam Prizes' instituted in 2005 and 2006 respectively by the NGO 'Peekay Tree Crops Development Foundation'. Both the endowments offer incentives and other forms of support to coconut farmers and coir workers on an annual basis. These include cash prizes of (1) Rs.1,500 to one model coconut farmer who has successfully integrated diverse crops and livestock components in his garden, (2) Rs.1,500 to a farmer's self-help-group that has demonstrated good models of coconut-based cropping/farming system, (3) Rs.1,500 to one woman entrepreneur/group involved in the production and marketing of coconut-based foods and beverages, (4) Rs.1,000 each to four outstanding students, (5) Rs.500 each to three students belonging to the families of coir workers falling below poverty line and (6) Rs. 1,000 each to three families of coir workers for marriage/treatment of members. The selection of eligible members/families is made by a seven member committee nominated by the CBOs and headed by the President of Vayalar Panchayat.

CBOs are important functional groups in the coconut sector constituted by the farmers themselves to serve as their representative body in all the development initiatives introduced by the local Panchayat as well as other agencies, both public and private. In most instances the CBOs function in partnership with other organizations to promote the interests of the farming community and others in the coconut sector. Their primary functions are (1) interfacing with the public organizations and the local community to develop a strong public-private partnership, (2) enlisting the members of the local community as effective participants in and beneficiaries of the development programmes taken up in the area, (3) facilitating capacity development of community members, (4) stimulating community interest and assuming leadership role in promoting joint endeavors in production, processing and efficient marketing for common benefit and (5) monitoring the progress of various activities promoted in the community at different stages.

Although CBOs are expected to strive for local development in partnership with other organizations the success will, however, depend on the general acceptance by the

community members of the quality of the office bearers elected by them periodically. This is particularly so in communities plagued by political and communal rivalries. While promoting CBOs, people are to be apprised of the importance of electing only those members as functionaries who enjoy general acceptance of the community members despite their diverse socio-political affiliations. Otherwise the CBOs are likely to fail and those which were once deemed to be model organizations will no more be regarded by the community members as their representative body.

Success stories of technology adoption in coconut

C.Thmaban

Central Plantation Crops Research Institute
Kasaragod - 671 124, Kerala, India

Cultivation of coconut varieties for tender nut

Chowghat Orange Dwarf (COD) is an indigenous coconut cultivar found sparsely cultivated throughout the west coast region of India, particularly in the Chavakkad area of Thrissur district of Kerala. The palm has a thin stem with closely arranged leaf scars, a small compact crown with characteristic orange colour on leaf petioles, inflorescences and nuts. This is an early flowering cultivar and takes about 3-4 years for initial flowering. The average annual yield is 65 nuts/palm with a range of 50-120 nuts. The cultivar is also known as 'Gowrigathram' or 'Chenthengu' and 'Kentthali' in Kerala and Karnataka respectively. This is largely a self-pollinated cultivar. Chowghat Orange Dwarf is best suited for tender nut water. The tender nut water of 7-month old nut is sweet with a total sugar content of 7.0 g/100 ml. Sodium and potassium contents in tender nut water are 20 and 2000 ppm respectively. This cultivar was released by CPCRI in 1991 for large scale cultivation in the states of Kerala and Karnataka as tender nut variety.

Sri. D. Chandrasekhar Chowta, a renowned farmer of Kasaragod is a frequent visitor to Central Plantation Crops Research Institute and the agricultural technology information centre. He is an energetic 65 year old man who is keen on adopting new technologies. He along with his brothers own the 'chowtharu thotta' (chowta estate) in Miyapadav village, Meenja Grama Panchayat of Kasaragod district. He has a land holding of 40 acres under the joint family system in which a variety of crops are being cultivated. In his land he cultivates plantation crops like coconut, cocoa, cashew, vanilla, arecanut, and rubber. Banana is also extensively cultivated as an intercrop. He has also taken up subsidiary enterprises such as dairy, vermicomposting and bee keeping.

During the year 2000, he procured 150 coconut seedlings of Chowghat Orange Dwarf variety from CPCRI through ATIC to establish a coconut garden exclusively for producing and marketing tender coconuts. Chowghat Orange Dwarf is the most ideal tender coconut variety. He has exclusively planted it in 2 acres of land. By 2004 the dwarf coconut palms started yielding, though now a days coconuts do not fetch a remunerative price, there is a good market



Coconut cultivation with chowghat orange dwarf variety for tendernut purpose in Chouta's farm

for tender coconut. retail/wholesale dealers of tender coconut regularly visit chouta's farm and purchase tender coconut and chouta family gets a very remunerative price for their produce. On an average 50-60 per tree is harvested for sale in Chouta's farm and an average price of Rs 8-10 per tendernut is obtained while the market price for coconut is only Rs.3-4 per nut. Mr. Chouta and family is quite appreciative of the services provided to the farmers through ATIC at CPCRI, Kasaragod.

Vermicompost production using coconut leaves

Vermicompost produced using coconut leaves

There is a growing awareness among farmers about organic farming. However, lack of availability of quality organic manure is a major difficulty experienced by farmers. Adoption of vermicomposting of organic wastes available in the farm would be a potential way to overcome this difficulty. Technology has been standardised at Central Plantation Crops Research Institute (CPCRI) for the production of vermicompost as a quality organic manure using coconut leaves. ATIC at CPCRI has been supplying the earthworm, *Eudrilus* spp to farmers and large number of farmers in various parts of the country have started vermicompost production using on farm wastes including coconut leaves. On an average one lakh number of earth worms are being supplied from ATIC to farmers every year at a nominal cost. Success story of Mr. Chandrasekharan, Sivam House, Adukkathuparamba, Madikkai post in Kasaragod District is a testimony to the effectiveness of technology service provided by ATIC. Mr. Chandrasekharan is a farmer having one acre of land and he cultivates coconut, banana and pepper in his farm. Lack of availability of quality organic manure was a major difficulty experienced by Mr Chandrasekharan in the efforts for sustainable crop production. During 2008 he became a member of the coconut farmers' cluster formed under the NAIP project on 'Value chain in coconut' implemented by CPCRI. He attended various training programmes on sustainable coconut production technologies including vermicompost production using on farm wastes including coconut leaves at CPCRI. ATIC supplied nucleus cultures of the local strain of *Eudrilus* spp. capable of composting coconut plantation wastes to Mr. Chandrasekharan. In one production cycle he is able to produce 250 kg vermicompost from his vermicompost unit in about 90 days and apply the same to coconut, banana and pepper. Besides, he also collects vermiwash which he applies to vegetables. According to him there is perceptible improvement in the growth and yield of crops due to the application of vermicompost.



Mr. Chandrasekharan near his vermicompost production unit



Vermicompost produced using coconut leaves

Coconut based mixed farming

Coconut is predominantly cultivated in small and marginal holdings. Most of these holdings neither provide gainful employment opportunities for the family labour throughout the year nor generate sufficient income to meet the family requirement. Presently coconut growers are more exposed to economic risks and uncertainties owing to the rapid price fluctuations. In this context it is needless to emphasize the importance of adopting coconut based cropping/farming system to achieve resource use intensification in order to attain a socio economic stability. Research at Central Plantation Crops Research Institute (CPCRI) has proved that there are possibilities of increasing the productivity and net return from coconut gardens through adoption of coconut based farming/cropping systems. The agronomic feasibility and economic viability of adoption of coconut based farming systems have been amply demonstrated in research stations and farmers' fields. Mr. P.V. Koran, Pallippuzha House, Pallikkara is a 66 year old farmer who has demonstrated the viability of adopting an intensive integrated farming in his 0.32 ha coconut farm. He is a respected farmer in the community for his vast experiences and achievements in coconut based integrated farming system. He has attended all the training programmes related to coconut based farming systems organised by CPCRI. Mr. P.V. Koran had his formal education up to 7th standard. Farming is his primary source of income. After the IPGRI/COGENT funded project on "Developing sustainable coconut based income generating technologies" implemented by CPCRI in the Pallikkara coconut community was initiated, his level of extension and research contact increased considerably. There are 56 bearing coconut palms of 30 years age in his farm. By adopting mixed farming methods in which he has successfully integrated livestock production with coconut farming he is able to realize a gross income of Rs.93775/- from farming in his 0.32 ha coconut farm. An average yield of 100 nuts per palm is obtained from his small coconut garden. He cultivates different fodder grass species in the interspace of coconut palms. Pepper, banana, turmeric are the other crops integrated in the coconut garden. Coconut leaves and other farm wastes are thus effectively recycled in his coconut garden to maintain the soil fertility. The unique feature of mixed farming in Mr. Koran's coconut garden is the cultivation of different species of fodder grass. He cultivates fodder grass like Congo signal, Para grass and Hybrid Napier in about 50 cents of his coconut garden. He adopts scientific management practices for the fodder grass cultivated and from each cent of land he gets an average fodder grass yield of 140 kg for Congo signal, 60 kg each for Para grass and Co-3 Hybrid Napier. He also supplies the slips to other needy farmers free of cost. He maintains five cows (Jersy breed) in his mixed farming unit, out of which two are now milch cows. Apart from the green fodder, concentrates are also fed to the cattle as per the requirement for better milk yield. On an average, he gets 10 lit milk per cow per day. Cow dung obtained is partly recycled as manure to coconut palms and apportion is used for making vermicompost which again is applied to coconut palms and intercrops. Sri Koran got the prestigious award 'Best coconut farmer award for livestock integration and fodder production with coconut farming' from COGENT. Taking into account his achievements in coconut farming, CPCRI felicitated him as one of best cultivators in the District. Similarly, Krishibhavan, the local office of the State department of Agriculture also felicitated him on his success in farming. The adoption of integrated farming methods has enhanced his income from farming thereby enhancing the economic status of his family. His achievements in integrating livestock production/fodder cultivation with coconut farming has become a model for small and marginal farmers.



P.V. Koran in his coconut garden

Mass production and use of biocontrol agents in the integrated management of coconut leaf rot disease – technology popularization through participatory programmes in disease affected regions

N. Srinivasan, R. Chandramohan, R. Bharathi, N. S. Radhika and Shanty Issak

Central Plantation Crops Research Institute
Regional Station, Kayangulam - 690 533, Kerala, India

Coconut (*Cocos nucifera* L.) is a principal plantation as well as an important social crop of livelihood concern for millions of small-medium farmers. Several constraints are reported to affect the crop productivity in the country and among them the incidence of pests and diseases are considered very important. Leaf rot disease (LRD) in association with root (wilt) disease (RWD) is extensively prevalent in southern districts of Kerala state and also heavily in the adjoining state of Tamil Nadu – particularly Theni – Dindigul districts (Srinivasan *et al.*, 2000; Srinivasan and Sasikala, 2001). It is brought out that the RWD affected palm is commonly infected by LRD - a strong interrelationship of leaf rot with RWD firmly established (Srinivasan, 1991). The destruction of the photosynthetic area of leaves in the palm due to LRD is quite clear and conspicuous, and hence RWD affected palms come to the farmers' attention instantly with the onset of LRD. Therefore, the RWD in association with LRD is an economically very important disease complex in coconut. Integrated management of root (wilt) - leaf rot complex has been recommended for optimizing the productivity of the crop in the disease affected regions. However, the knowledge and adoption of scientific parameters practiced are thought to be lower. It is viewed that innovation and technology evolving play a contributory role in stabilizing yield potential of the crop in the disease affected regions and thereby sustainable activities and reducing risk perceptions.

Central Plantation Crops Research Institute (CPCRI) established etiology of root (wilt) and leaf rot diseases. While root (wilt) was considered to be due to phytoplasma (Solomon *et al.*, 1999), the leaf rot has been proved to be caused by a group of fungi wherein *Colletotrichum gloeosporioides*, *Exserohilum rostratum* and *Fusarium solani* are the major pathogens (Srinivasan 1991, 2004, 2008; Srinivasan and Gunasekaran, 1996a, 2000). The Institute also developed the package of integrated management practices for improving the health and productivity of the palm crop. Even though RWD was not amenable to therapeutic control measures, leaf rot control could be evolved and leaf rot control formed an integral part in the management of RWD (Srinivasan *et al.*, 2001). Srinivasan and Gunasekaran (1996b) determined methods of application of fungicides on to coconut palms. This simple measure of pouring the chemical into the spindle axil was standardized and the measure satisfactorily suppressed the disease recurrences in newly emerged leaves. Accordingly, pouring of Contaf into the axil of spindles along with phytosanitation measures on the spindle and other young leaves significantly reduced the incidence of LRD in newly emerging spindle leaves effecting cure and also application of the fungicide on RWD palms prior to the onset of LRD offered prophylactic protection to emerging leaves against leaf rot (Srinivasan and Gunasekharan, 2003).

Biological control has emerged as an important component in the plant disease management system. Biological control agents – certain antagonistic bacteria (*Pseudomonas fluorescens*, *Bacillus subtilis*) and fungi (*Trichoderma viride*) have been found to kill or prevent the growth of the major pathogens of leaf rot (Srinivasan and Bharathi, 2006). Biological control technology could be extended to field level also with a talc-based *P. fluorescens* and thus management of LRD has been found possible (Srinivasan, 2003; Srinivasan *et al.*, 2006c). The technology is new and needed to be widely popularized. Therefore, integrated approach is considered the best option for adoption especially by small-marginal farmers in the disease affected regions. Application of biocontrol agents involving women and other rural population in the areas was felt necessary. A strategy (with structured programmes/methodology for creation of widespread awareness on ecofriendly management of the disease complex and in realizing human resource potential) was required to be adopted in the disease affected regions especially in southern districts of Kerala besides adjoining districts in Tamil Nadu (Theni, Dindigul). A participatory approach programme was implemented to create awareness on biocontrol measures and its beneficial effect with integrated management practices and environmental safety; conducting mass production of antagonists for supplying to rural community; conducting field demonstration in collaboration with extension agencies; producing-distribution of promotional literature; training rural and unemployed people especially women in mass production; reducing cost of cultivation and improving economics of rural people. The programme was implemented in the form of a project as sanctioned by the Department of Biotechnology, Government of India, New Delhi.

Soil/rhizosphere samples of coconut were collected and utilized for isolations of bacterial and fungal biocontrol agents *viz.*, *Bacillus subtilis*, *Pseudomonas fluorescens*, *Trichoderma viride* and *T. harzianum* utilizing specific media (Nutrient agar/King's B agar for bacteria; Potato dextrose agar/*Trichoderma* selective media for fungal isolates). A vast number of isolates were made out and bioassay of pure isolates of bioagents against major pathogens of leaf rot (*Colletotrichum gloeosporioides*, *Exserohilum rostratum* and *Fusarium solani*) performed *in vitro*. One effective isolate was selected from the short-listed antagonists and used. Subsequently *B. subtilis*, *P. fluorescens* and *T. viride* individually and in combinations (*B. subtilis* + *P. fluorescens*, *B. subtilis* + *T. viride*, *P. fluorescens* + *T. viride* and *B. subtilis* + *P. fluorescens* + *T. viride*) were assessed for confirmatory reactions for exploitation. This formed the basis for mass multiplication/development of bioformulations/mass production of each antagonist, thereby utilization of the technology. Evaluation of compatibility among biocontrol agents was also carried out. The techniques for multiplication/mass production of biocontrol agents were also standardized.

Surveys were conducted in the disease endemic districts of Kerala besides certain districts (Theni, Dindigul) in Tamil Nadu, where focus warranted for identifying representative rural groups and gardens. Accordingly, rural people in eight districts of southern Kerala (Thiruvananthapuram, Kollam, Alappuzha, Pathanamthitta, Kottayam, Idukki, Ernakulam and Thrissur), in various blocks of Theni District (Cumbum, Uthamapalayam, Chinnamanur, Bodinayakkanur, Theni, Periyakulam, Andipatti, Kadamalaikundu) and in specified blocks (Athoor, Palani) of Dindigul district of Tamil Nadu were contacted and relevant details especially know-how status with rural people on biocontrol agents/biocontrol technology collected.

Contacts were made with extension and developmental agencies like state departments of Agriculture to reach out to rural groups in these regions and their collaboration availed for conduct of awareness *cum* training programmes on mass production and utilization of biocontrol agents. Services voluntary agencies, self-help groups, NGOs, Krishi Vigyan Kendras of Alappuzha (CPCRI,RS,Kayangulam) and Theni Districts (CENDECT Krishi Vigyan Kendra, Kamatchipuram, Theni) etc. were also availed for knowledge delivery thereby technology percolation to rural people.

Various models/ modes of need based programmes *viz.*, On- and off-campus trainings, Household/Field contacts of rural people and other Awareness programmes encompassing lectures, interface, field school concepts etc. were implemented to enlighten rural people representing various locations/regions concerned. Development of promotional literature on disease *vis-à-vis* biocontrol technology formed an integral part. Hence training folders on biocontrol agents in integrated management of coconut leaf rot disease was developed.

The coconut farmers, particularly in Kerala, are small-marginal and farming as such commonly is as homestead gardens. The crop invariably requires climbing for operations such as plant protection measures that requires especially skilled climbers. Therefore, strategic approaches were adopted in demonstrations and in such a manner to enable rural people to imbibe the know-how for combating the disease problem in environmentally safe manner.

Selection of biocontrol agents and their evaluation: Isolates of *B. subtilis*, *P. fluorescens* and *T. viride* were tested and one representing each selected. *B. subtilis* and *P. fluorescens* were found significantly inhibitory to all pathogens. While the fungal bioagent *T. viride* grew faster limiting the pathogens the inhibition zones developed by bacterial antagonists were invariably higher and more effective (inhibitory principle) and therefore the bacterial antagonists received higher priority. *B. subtilis*, *P. fluorescens* and *T. viride* individually and in their combinations were also evaluated *in vitro* for compatibility. It was found that *B. subtilis* and *P. fluorescens* as well as *B. subtilis* and *T. viride* to be compatible; *P. fluorescens* and *T. viride* not compatible; strains of each species compatible among them; bioagents compatible with minimal concentration of a popular fungicide, Contaf (Hexaconazole). The features were used for bioformulations in implementation of leaf rot disease management system. Utilization of biocontrol agents in consortium mode bearing synergism is important.

Multiplication techniques and mass production of biocontrol agents: The bacterial and fungal bioagents could be multiplied in conventional media as well as in coconut water based liquid culture media. The coconut water derived particularly from developed nuts performed as a potential multiplication medium for mass multiplication of biocontrol agents. Method was standardized for processing the bioagents into bioformulations principally using talc as a carrier material. Mass production of the bacterial bioagents was undertaken in Fermentor System also, that aided in enriched yield of the organisms and such enriched cultures were also processed in to bioformulations. The mass production of different biopesticides was undertaken during various programmes of implementation through out the period and a total of 2.0 t of biopesticides thus produced was utilized in various programmes-field demonstrations, as training kits to

beneficiary participants etc. Novel methods of production of admixture of talc-based biopesticides with neem cake, organics/farm derivatives and also along with various biofertilizers (neem cake: about 1.5 t; biofertilizers: about 200 kg) were also adopted and utilized in programmes.

Gardens and rural groups in Kerala and Tamil Nadu: Analysis of responses from women and men of the region showed prevalence of relatively a low level of knowledge with the rural people on biocontrol technology and therefore the scope, and necessity of enlightening rural masses on the production-utilization of biocontrol agents. Extension material/Technical bulletin on the disease complex *vis-à-vis* utilization of bioagents (biopesticides) was provided to rural women and men through various modes of programme implementation. Disease affected coconut gardens on selected locations were also identified for demonstration of management technology with biocontrol agents. These measures enlightened rural people on mass production and utilization of biocontrol agents.

Trainings and allied programmes: A total of 15 batches of each one-day on campus trainings on mass production and use of biocontrol agents covering aspects of root (wilt)-leaf rot complex, demonstration *cum* practical session on the mass preparation of biopesticides, packaging techniques, fortification of bioagents with organics, demonstration of application of biocontrol agents into coconut system, were conducted for rural men and women in Kerala. Group discussions, supply of training materials, sample biopesticides, and biotreated coconut seedlings were also organized. A total of 396 rural people, out of which 175 are women beneficiaries, benefited through these on campus trainings.

A total of 22 batches of each one-day off-campus programmes were conducted for rural women and men in Kerala and Tamil Nadu-1854 rural people out of which 450 women beneficiaries benefited through these off campus training programmes.

Representative rural households/homesteads/fields in various districts of southern Kerala were contacted and details on root (wilt)-leaf rot, production/use of biocontrol agents in management of the disease complex presented and 689 rural people out of which 237 were women beneficiaries benefited through these measures.

Lectures interface meetings, field school systems etc. were also implemented and in such programme rural people/women/farmers, youths (Agriculture and other graduate students), Agriculture Department Officials (representing various districts of Kerala and specified districts of Tamil Nadu) and certain other Officials were involved-661 persons got benefited out of which women constituted 50%.

Training folders on biocontrol agents in integrated management of coconut leaf rot disease was developed and copies of the same were widely distributed during various stages of programmes implementation to stack holders in the disease-affected region. Various other promotional literatures on coconut root (wilt) -leaf rot disease complex were also utilized for dissemination of knowledge to various beneficiaries of the programmes (Srinivasan *et al.*, 2005, 2006, 2007).

Multilocal field demonstrations of technology involving biocontrol agents were conducted during 2005-06 to 2007-08 in various locations of Kerala and Tamil Nadu states. The details are presented in Table 1.

Table1. Multolocational field trials conducted during 2005-06 to 2007-08

Location	No.of units	
2005-06		
Kerala state		
Thiruvananthapuram		3
Kollam		6
Alappuzha		6
Tamil Nadu state		
Cumbum		1
Uthamapalayam		1
Chinnamanur		1
Bodinayakkanur		1
2006-07		
Kerala state		
Elanthoor, Thiruvalla (Pathanamthitta district)		2
Alappuzha, Cherthala (Alappuzha district)		2
Kaduthuruthy, Pallom (Kottayam district)		2
Tamil Nadu state		
Periyakulam, Theni, Andipatti, 2		
Kadamalaikundu (Theni district)		
Athoor and Palani (Dindigul district)		2
2007-08		
Kerala state		
Muthukulam, Mavelikara, Haripad,		1 each
Bharanikavu, Ambalapuzha (Alappuzha district)		
Oachira (Kollam district) 1		
Peermade (Idukki district) 2		
Paravoor and Narakkal (Ernakulam district)		2 each

Demonstrations in clusters of coconut based homesteads in Alappuzha district and in compact gardens in Theni district (total of 1850 palms) were also done by treatment of biocontrol agents. Integration of organics – neem cake, coir pith compost, vermicompost, *in situ* farm wastes etc. and their effective utilization was also elaborated to stakeholders.

Evaluation from rural community showed enhancement of knowledge on biopesticide production-utilization with the programme implementation. The rural people could be encouraged through programme activities on biopesticides *cum* organics production. The byproduct of coir pith could also be integrated with biopesticide. The need for effective

percolation of scientific know-how for adoption is critical and research and development efforts could be channeled in bridging the gap (Anithakumari and Manoj, 2004). This programme on the biocontrol technology forms first of its kind in disease affected region (Table 2). Amelioration in biotreated palms from the disease was evidenced to the stakeholders for keeping palms free from the disease. The programme also created widespread awareness among rural people about the use of biocontrol agents in integrated disease management, and its environmental benefits.

Acknowledgements

The authors are thankful to the Department of Biotechnology, Ministry of Science and Technology, Government of India for award of a Project on mass production and use of biocontrol agents through which the current work/programme could be implemented.

References

- Anithakumari, P. and Manoj, S. 2004. Technology transfer of integrated root (wilt) management package-Experiences from the participatory programme on coconut. *J. Plantn. Crops* 32: 474-479.
- Solomon, J.J., Nair, C.P.R., Srinivasan, N., Gunasekaran, M. and Sasikala, M. 1999. Coconut root wilt - the malady and remedy. *J. Plantn. Crops* 27 (2): 71 - 92.
- Srinivasan, N. 1991. Occurrence of coconut leaf rot in relation to root (wilt) disease. *Indian Coconut Journal* 21 (10): 14 - 18.
- Srinivasan, N. 2003. Efficacy of *Pseudomonas fluorescens* against leaf rot in root (wilt) affected coconut palms. *Indian Phytopathology* 56 (2): 210-211.
- Srinivasan, N. 2004. Incidence of leaf rot disease in relation to root (wilt) in young coconut palms and fungi in petiole and lamina lesions. *CORD* 20 (2): 28-36.
- Srinivasan, N. 2008. Management of coconut leaf rot disease, *Technical Bulletin No. 58*, Central Plantation Crops Research Institute, Kasaragod, India, 10 pp.
- Srinivasan, N. 2010a. Production of value added coconut seedlings through biopriming with biocontrol agents against leaf rot disease. In: *Abstracts, National Conference on Production of Quality Seeds and Planting Material – Health Management in Horticultural Crops*. 11-14 March 2010, New Delhi, p. 229.
- Srinivasan, N. 2010b. Advances in coconut leaf rot disease and its management measures. In: *Plant Diseases and its management* (Ed) Trivedi, P. C. Pointer Publishers, Jaipur, India, pp. 53-92.
- Srinivasan, N. and Bharathi, R. 2006. Biocontrol agents against pathogens of coconut leaf rot disease. *J. Plantn. Crops* 34 (3): 494-499.
- Srinivasan, N., Bharathi, R. and Prakash, V. R 2005. Biocontrol agents in integrated management of coconut leaf rot-root (wilt) disease – A guide for preparation and use of biopesticides, Training Folder, Central Plantation Crops Research Institute, Regional Station, Kayangulam, Kerala.
- Srinivasan, N., Chandramohan, R. and Bharathi, R. 2006. Biocontrol agents in integrated management of coconut leaf rot disease – A guide for preparation and use of biopesticides, Training Folder, Central Plantation Crops Research Institute, Regional Station, Kayangulam, Kerala.

- Srinivasan, N., Chandramohan, R. and Bharathi, R. 2007. Biocontrol agents in integrated management of coconut leaf rot disease – A guide for preparation and use of biopesticides, Training Folder, Central Plantation Crops Research Institute, Regional Station, Kayangulam, Kerala.
- Srinivasan, N. and Gunasekaran, M. 2003. Curative and prophylactic control of fungal leaf rot complex in root (wilt) affected coconut palms in India. *CORD* 19 (1): 17-26.
- Srinivasan, N. and Gunasekaran, M. 1996a. Incidence of fungal species associated with leaf rot disease of coconut palms in relation to weather and the stage of lesion development. *Ann. Appl. Biology* 129 (3): 433 - 449.
- Srinivasan, N. and Gunasekaran, M. 1996b. Field control of leaf rot disease of coconut with fungicides. *CORD* 12 (2): 34-42.
- Srinivasan, N. and Gunasekaran, M. 2000. Leaf rot disease of coconut (Ed) Rohini Iyer, *Technical Bulletin No. 38*, Central Plantation Crops Research Institute, Kasaragod, India, 14 pp.
- Srinivasan, N., Jyothi Rahna, S. and Anishkumar, V. K. 2006. Evaluation of fungicides and antagonistic organisms against major pathogens of leaf rot disease of coconut and their eco-friendly management. *CORD* 22 (1): 27-50.
- Srinivasan, N., Koshy, P.K., Kamalakshy Amma, P.G., Sasikala, M., Gunasekaran, M. and Solomon, J.J. 2000. Appraisal of the distribution of coconut root wilt and heavy incidence of the disease in Cumbum Valley of Tamil Nadu. *Indian Coconut Journal* 31 (1): 1 - 5.
- Srinivasan, N. and Sasikala, M. 2001. Spread and distribution of coconut root (wilt) disease in the region of Theni - Dindigul districts of Tamil Nadu. *Indian Coconut Journal* 31 (11): 7 - 11.
- Srinivasan, N., Solomon, J.J., Sasikala, M., Gunasekaran, M., Geetha, L. and Rajeev, G. 2001. Managing coconut root wilt and leaf rot diseases. *Indian Horticulture* 46 (3): 28 – 29.

Table 2. Consolidated data on number of participants/beneficiaries availed through various programmes of the project implementation

<i>First year (2005-06):</i>				
S. No.	Type of training	No. of participants/ beneficiaries		
		Men	Women	Total
1	On campus trainings	037	045	082
2	Off campus trainings	423	108	531
3	House hold/Field visits	072	077	149
4	Awareness programmes	070	129	199
	Total	602	359	961
<i>Second year (2006-07):</i>				
1	On campus trainings	0	43	43
2	Off campus trainings	838	258	1096

3	House hold/Field visits	202	61	263
4	Awareness programmes	151	69	220
	Total	1191	431	1622
<i>Third year (2007-08):</i>				
1	On campus trainings	184	087	271
2	Off campus trainings	135	092	227
3	House hold/Field visits	178	099	277
4	Awareness programmes	117	125	242
	Total	614	403	1017
	Grand total for all years	2407	1193	3600

Successful user friendly rodent management techniques in coconut plantations as a frontline research in Mathilakom Block Panchayath in Thrissur District

Jim Thomas, Mani Chellappan and Haseena Bhaskar

College of Horticulture

Kerala Agricultural University, Thrissur, Kerala, India

There was a lot of hue and cry in the mass media about the tender nut damage in coconut palms during 2007-08 in the Mathilakom Block areas near Kodungalloor in Thrissur District, Kerala. It was alleged to be due to bat feeding as reported by the farmers in the locality through the news media which prompted both the Govt. of Kerala and Kerala Agricultural University to expedite upon the issue by constituting a fact finding mission and an action research. After ascertaining the causal agent, a frontline action research and demonstration was organized by the KAU personnel employing a user friendly technique of specially designed self sticking polythene bands tied around the trunks at the ground level which yielded good results in reducing the nut damage.

Detailed field observations, investigations and night expedition studies were conducted in the area to affirm the primary cause of wide spread nut fall in the Mathilakom Block areas near Kodungalloor in Thrissur District, Kerala. On ascertaining the causal agent, in order to convince the farmers about the cause of the malady and the efficacy of some *ad-hoc* management strategies, based on the bio-ecology and ethology of the problematic vertebrate species, four frontline demonstrations were organized in four panchayats, viz., Mathilakom, Kodungalloor, Perinjanam and Kaipamangalom areas in Mathilakom Block. A block level meeting was organized for the farmers from all the Krishi Bhavan areas in the Block Panchayat and the situation was reviewed about the causal agent and their role in the present crisis. Four homestead plots were selected by the farmers themselves based on the severity of the nut damage. Ten severely affected palms with serious nut fall along with nut boring damage were selected from each homestead farms and were subjected to integrated management techniques. The experiment was executed during the first week of December 2008 and thereafter the observations on further nut fall were made at weekly intervals consecutively for two months.

The measures adopted in all the ten palms in each homestead farms under study were as follows:-

1. Manual disinfestation by clearing and sanitizing the coconut crowns
2. Plastic sheet wrapping of 45 cm width at a height of 1.5 m above the ground level to prevent the climbing access of rodents from below with specially designed sticky polythene sheet rolls
3. Application of bromodiolone based rodent bait cakes in the crown as well as tied up on the trunks

Observations were taken on the cumulative total number of freshly fallen coconuts with boring marks of uniform nature irrespective of the nut size at weekly intervals since the treatment application. The pre treatment counts were considered based on the mean total number of the freshly fallen nuts during the prior week before the application.

The night observations on the activity in the crown region of coconut palms revealed that the currently experienced economic loss of coconut with widespread nut fall was primarily due to the rodent depredations followed by bats as a secondary causal agent. The rats belonging to the genus *Rattus* and two species viz., *rufescence* (roof rat or tree rat) and *norevigicus* (port rat) were of primary concern for the present malady with subsequent depredations by the flying foxes and other common bat species.

The behavioral observations on the rodent species have proved that the problematic rat species are largely nocturnal in character and climb up the tree only after sunset to escape from the predatory animals and birds, they mostly hide within the tunnels and burrows during day time on the ground and cannot remain on the crown. Rarely a few female rats may colonize in the crown during the breeding season for nesting the young litter or to wean them occasionally. Normally the adult and the juvenile rats climb up the tree trunk during the early hours of night fall from 7 to 10 pm before the nocturnal birds of prey such as owl and other species become active. The rodents climb up vertical and upright tree trunks with their nails through the rough and cracky bark of the coconut palms. They normally prefer such palms which are medium to tall in nature, bearing tender nuts with sweet flesh and water when there are no alternate fruits such as *Hydnocarpus*, arecanut, cocoa or guava etc., during the season especially during June-July as well as September-October periods.

The data on the nut fall at weekly intervals along with the pre-treatment counts are presented in Tables 1 to 5.

An analysis of the data from the respective sites in all the four panchayats as well from the pooled mean revealed the same trend with respect to the drastic reduction in the nut fall and damage due to rodent infestation after resorting to tree banding and the bait cake application. There was an average damage of 4.85 nuts per palm per week before the treatment application with the highest level of 5.9 at SN Puram and 4.7 each at Perinjanam and Kaipamangalam. Within one week after the treatment itself, the mean number of damage dropped down to 1.09 nuts /palm/week bringing out a percentage reduction of 77.6 over that of the pretreatment nut fall counts. From the second week onwards, the reduction of damage rose above 93 per cent reaching to about 96 per cent reduction after the sixth week.

This data evidentially highlighted the importance of the mechanical exclusion principles to prevent the access of the climbing rodents onto the palms to reach the crown for tender nut damage by boring and consequent nut fall. The potential of the bromodiolone bait cakes applied in the crown as well as fastened around the tree trunk below might have also played their role in reducing the population of the rodents and consequent low nut damages.

However, a perusal on the data over six weeks showed that there was no 100 per cent reduction in nut damage in spite of the treatments. Hence, it could be inferred that there were alternate means for the rodents to climb upon the palms through the overlapping fronds of the adjacent palms or by other contacts through tree branches or connecting wires and ropes across which should also be taken care of.

The above field level action research on tender nut damage and loss in coconut plantations in the coastal Panchayaths under Mathilakom Block areas of Thrissur District in Kerala could be confirmed to be due to tree climbing rodent species and not by any other depredatory animals such as bats or any other species. The damage level caused was found to be around 4-5 nuts per week per palm which could be reduced by all most 90 per cent by resorting to simple techniques of management strategies such as tree banding and bait cake application.

Table 1. Data from site 1 at Mathilakom

Weeks after treatment	Weekly total of freshly fallen nuts from 10 palms										Mean nut fall/palm/week
	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	
Pre-count	7	3	5	6	4	7	6	4	4	4	5
I week	1	1	2	2	1	3	3	2	2	2	2.1
II week	1	0	0	1	0	1	0	1	1	0	0.5
III week	0	0	1	0	0	1	0	0	0	1	0.3
IV week	0	1	0	1	0	0	0	1	0	0	0.3
V week	0	0	1	0	1	0	1	0	1	0	0.5
VI week	0	1	0	1	0	0	0	1	0	1	0.4

Table 2. Data from site 2 at S.N.Puram

Weeks after treatment	Weekly total of freshly fallen nuts from 10 palms										Mean nut fall/palm/week
	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	
Pre-count	12	5	7	6	3	8	3	5	4	6	5.9
I week	1	2	0	1	0	0	0	1	0	0	0.5
II week	0	0	0	1	0	0	1	0	0	0	0.2
III week	0	0	0	0	1	0	0	1	0	0	0.2
IV week	0	0	1	0	0	0	0	0	0	0	0.1
V week	0	0	0	0	0	0	3	0	0	0	0
VI week	1	0	0	0	0	0	0	0	0	0	0.1

Table 3. Data from site 3 at Perijanam

Weeks after treatment	Weekly total of freshly fallen nuts from 10 palms										Mean nut fall/palm/week
	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	
Pre-count	10	5	6	3	5	3	4	3	4	4	4.7
I week	0	1	2	1	0	1	2	0	0	1	0.8
II week	0	0	1	1	0	1	0	0	1	0	0.4
III week	1	0	1	0	0	0	0	0	2	0	0.4
IV week	0	0	0	0	0	0	0	1	0	0	0.1
V week	0	0	0	1	0	0	0	0	0	0	0.1
VI week	0	0	0	1	0	0	0	0	0	0	0.1

Table 4. Data from site 4 at Kaipamangalam

Weeks after treatment	Weekly total of freshly fallen nuts from 10 palms										Mean nut fall/palm/week
	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	
Pre-count	4	4	6	7	5	4	4	6	4	3	4.7
I week	1	1	1	0	0	0	2	1	2	1	0.9
II week	0	0	0	0	1	0	0	10	0	0	0.2
III week	0	0	0	0	1	0	0	0	0	0	0.1
IV week	0	0	0	0	0	1	0	0	0	0	0.1
V week	0	0	0	0	0	0	0	0	0	0	0
VI week	0	0	0	0	0	0	0	0	1	0	0.1

Table 5.Reduction of nut damage by rodent infestation due to tree banding and Rodenticide Bait application in Mathilakom Block Panchayath (Pooled data)

Weeks after treatment	Mean number of fallen nuts/palm/week before and after the treatment application					
	Mathilakom	SN Puram	Perinj-anam	Kaipam-angalom	Mean nut fall count	% Reduction
Pre-count	5	5.9	4.7	4.7	4.85	0
I week	2.1	0.5	0.8	0.9	1.085	77.6
II week	0.5	0.2	0.4	0.2	0.325	93.3
III week	0.3	0.2	0.4	0.1	0.25	94.8
IV week	0.3	0.1	0.1	0.1	0.15	96.9
V week	0.5	0	0.1	0	0.15	96.9
VI week	0.4	0.1	0.1	0.1	0.175	96.4
Weekly Mean	0.68	0.18	0.32	0.23	0.36	
damage						
Mean per cent damage reduction	86.4	96.9	93.2	95.1	92.6	92.6

Bio-control of rhinoceros beetle – Participatory adaptation of low input multiplication of *Metarhizium anisopliae* for bridging adoption gap

P.Anithakumari and Chandrika Mohan

Central Plantation Crops Research Institute
Regional Station, Kayankulam - 690 533, Kerala, India

Coconut (*Cocos nucifera*) is major crop of India providing livelihood to more than 10 million farm families, which are mostly resource poor. Coconut is being cultivated in 93 countries in an area of 12.61 million hectares producing 61.08 billion nuts. Seventy nine percent of the coconut production in the world is from Philippines, Indonesia, India and Sri Lanka. In India, the crop is grown in 18 states and 3 Union Territories in an area of 18.95 lakh hectares producing 15729.70 million nuts with productivity of 8303 nuts per hectare per annum. The state of Kerala occupies 58 percent of production and 58 percent of area under coconut in India. Out of the 5 million coconut holdings in the country, 98 percent are below 2 ha in size and out of these, 3.5 million holdings are in Kerala State (Mathew, 2011). The low productivity of coconut in India could be attributed to the fragmented holdings restricting scale of economy, incidence of pests and diseases, low level of awareness and adoption of recommended technologies as well as fluctuating price of the primary produce besides social factors. The cost of regular management of coconut palms are also increasing in terms of external inputs like chemical fertilizers, pesticides, labour cost etc. Farmers prefer low cost options which are ecologically friendly and sustainable.

Rhinoceros beetle, commonly known as black beetle, is the major pest of coconut palm, present in all coconut growing countries of the world. It attacks all stages of the palm from nursery seedlings, juvenile and adult palms. Rajagopal and Arulraj (2003) reported that the pest causes yield reduction of 6.5 percent in bearing palms. They also indicated that in South Kerala, 36.5 percent of the palms were affected by rhinoceros beetle with an annual loss of Rs.672 per hectare per annum. The pest indirectly affects the growth of seedlings and pre bearing palms and sometimes results in the destruction of seedlings.

The pest could be effectively managed by adopting biocontrol measures, viz., use of *Metarhizium* fungus and baculo virus. The major constraint in continued adoption of bio control practice is the non availability of bio agents against rhinoceros beetle. Hence, this study was taken up to identify the status of rhinoceros beetle infestation, knowledge and adoption among the coconut farmers, to bridge the gap in awareness and adoption of bio control of rhinoceros beetle among coconut farmers in participatory and gender sensitive approach and to share the learning from the action research.

The study was conducted during the period 2009-2010 in three panchayaths i.e. Thekkekara and Devikulangara of Alappuzha district and Edava of Thiruvananthapuram district in an area of 2,000 ha involving 4,000 farmers under coconut clusters. The knowledge and adoption of farmers were documented using pre tested questionnaire prepared in local language for personal interview. The 120 respondents were selected using random sampling technique.

The knowledge items were finalized after judges rating. A score of one was given for correct knowledge/ adoption and zero for incorrect knowledge/no adoption. The incidence of the rhinoceros beetle symptoms were recorded from 10 percent of the total seedlings, pre bearing and adult palms. Nuclear culture of the green muscardine fungus (GMF) *Metarhizium anisopliae* (Metsch.) Sorokin for the rural scale production by women group was provided from Entomology laboratory of the Regional Station, Central Plantation Crops Research Institute, . The basic methodology utilized for this study for farm level multiplication of GMF was developed by Coconut Research Institute, Sri Lanka (2007). This was further modified by Mohan *et al.* (2010). The farmers were selected through surveys, group meetings and discussions with the local extension officials. Skill acquisition and up gradation was provided in farm level GMF production through continuous session and after monitoring the production process. Even though both the genders were given the trainings, only women groups were willing to take up the activity. During the process, due to the participatory nature of the programme, several refinements were proposed and incorporated in the process. The short term outcome of the programme was assessed through survey among the participants.

The socio economic factors of the farmers indicated that all of them were having marginal land holding size only. The average area under coconut per farm family was found to be 0.14 ha (34.21 cents). All the sample farmers were literate including 8.3 percent of them graduates. Average age of the farmers was 52 years with a range of 34-73 years. Majority of the farmers (64 percent) indicated that they depend coconut climbers for information regarding plant protection in coconut. Fourteen percent depend on local extension officials and the rest depend on multiple sources like extension officials, climbers, farm magazines and mass media. None of them were reported to be utilizing ICT as information source.

One third (30 percent) of the households had breeding sites of rhinoceros beetle including cow dung pits, vermicompost units and decaying coconut wood / debris. Less than one fifth (18.7 percent) of the farmers attended any training programme indicating low training intensity in the community.

Rhinoceros beetle incidences were recorded based on the symptoms of infestation in seedlings, pre bearing and bearing palms in farmers' field. Data indicated that coconut seedlings were the most susceptible growth stage with symptoms of pest attack in 48.31 percent, whereas, there were 22.7 percent each of pre bearing and bearing palms with symptoms. Around one third of the total fronds were found to be affected by the pest attack. Aravindakshan and Venkitachalam (1997) reported 66 percent of total palms in Kumbalam panchayath and 50 percent in Kadamakudy panchayath of Ernakulam district were infested by rhinoceros beetle. Palm to palm survey in the NATP project area of Alleppey district showed that 45 per cent of the palms were having rhinoceros beetle infestation. (NATP Final report, 2003). These data indicated that the pest infests all growth

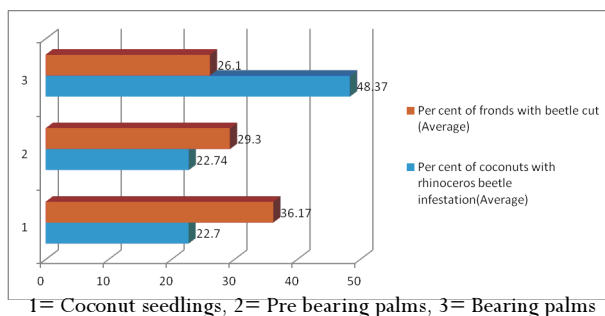


Fig.1. Incidence of rhinoceros beetle in various growth stages of coconut palm

stages causing direct and indirect loss to coconut farmers and the incidence vary with different field factors.

The knowledge and adoption of recommended technologies indicated low level. Anithakumari and Kalavathi (2001) found that even though coconut farmers were well aware of the identification of rhinoceros beetle and symptoms of attack on fronds, only 10 percent of them were having knowledge and a mere one per cent of them adopt any of the biological control methods for black beetle management. The bio control aspects were categorized as low knowledge low adoption technologies in their study. Anithakumari *et al.* (2007) reported that the knowledge on the bio agents, symptoms, breeding sites, non pesticide management of rhinoceros beetle improved by 51 to 100 percent after Farmer Field Schools (FFS). The results of the knowledge of farmers regarding the identification of the pests were almost equal among both the genders. Since this is the most prevalent pest in coconut based homesteads, women were also involved in the management of seedling stage. Around half of the respondents were fully aware of the various symptoms in opened fronds, spindle, seedlings and inflorescence. Due to the intense technology awareness created in case of use of a common weed plant *Clerodendron infortunatum* in vermicompost pits and FYM pits, the use was found to be popular among one third of the farmers. This is a no cost, simple, women friendly technique and the plant is very familiar to the users. The women farmers prefer and have knowledge of this no cost simple practice compared to the chemical use. But the knowledge and use of bio agents were absent among the coconut farming community (Table 1), which was tried to overcome through the present study.

Table 1. Knowledge on rhinoceros beetle and integrated management

Items	Men (%)	Women (%)
Identification of various stages of rhinoceros beetle	70.42	71.08
Identification of symptoms of rhinoceros beetle infestation	54.05	42.31
Use of <i>Clerodendron infortunatum</i> as a botanical method	28.91	29.45
Bio control agents of rhinoceros beetle	0.00	0.00
Chemical control measures for rhinoceros beetle management	12.40	3.67
Knowledge on leaf axil filling/ mechanical removing of beetles	40.84	28.92

The possibility of GMF as potential bio control agent was first reported by Nirula *et al.* (1955). Subsequently, technologies were developed in CPCRI for mass multiplication in coconut water in glass bottles (Pillai, 1993). However, technology diffusion among the farming community was very meager which could be attributed to the non availability of the GMF for adoption and lack of adequate promotional efforts from relevant stakeholders. Moreover, farmers also prefer handy packing of bio agents. Bio control of pests will be more effective and observable if participatory group / community approach is adopted. The superiority of area-wide IPM programmes over the conventional farm-by-farm management of insect pests has been previously recommended by many (Knippling 1992; Lindquist 1998; Mumford 1998; Yu and Leung 2006). Such IPM programmes consider the spatial and temporal distribution of the pest, and are long term in nature and attempt to persistently reduce the pest population in

a relatively large area to non-economic status (Lindquist, 1998). CPCRI also established that participatory technology transfer approaches, clustering farmers for collective decision making and actions enhanced technology utilization and income generation (Anithakumari, 2008). Hence, in this action research programme, the basic unit for technology dissemination was taken as one panchayath i.e. 700-1,000 ha. Farmers themselves were involved in the refinement and production of GMF, campaign for treatment of breeding sites in the whole area and monitoring outcome of adoption. Peoples representatives, women self help groups, extension officials, coconut cluster leaders, progressive farmers, media etc were active stakeholders. Subashini *et al.* (2003) also reported bio agent production at village level as a women friendly technique with very less labour enabling the involved person to engage in their regular activities also. They further found that the activity generated 750 labour days per year and saves 35 per cent on chemical pesticides.

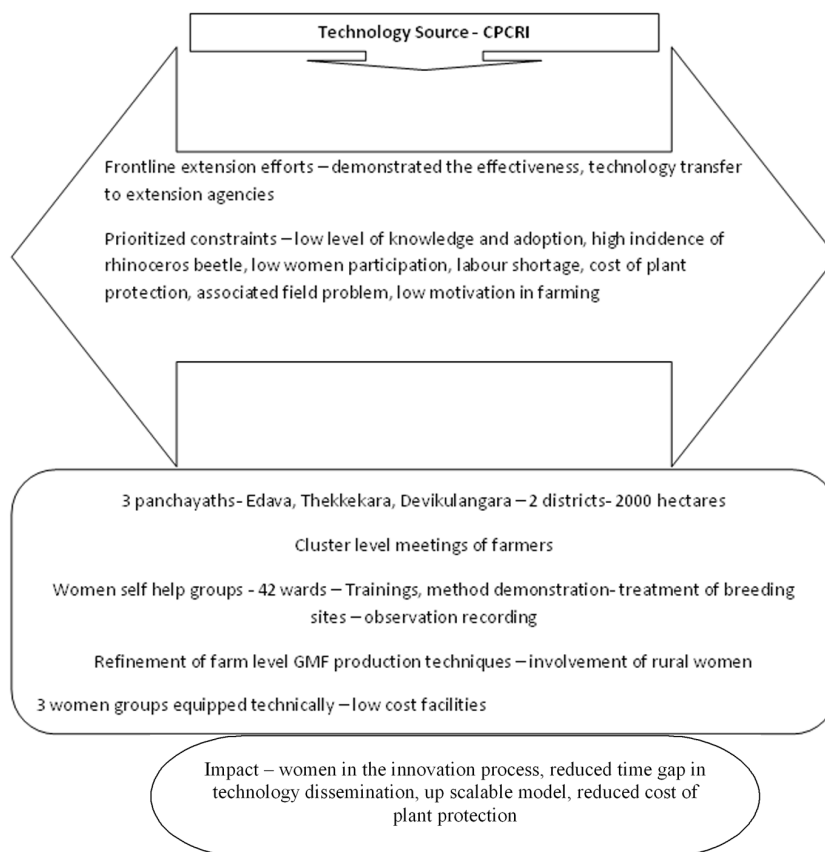


Fig 2. Gender participatory extension strategy in improving community adoption of GMF

The model is up scaled in one more panchayath and the same strategy adopted for multiplication and utilization for other bio agents like *Trichoderma* also among coconut farmers. Through this

approach opportunities for women in the innovation process could be more evident. Escobar *et al.* (2006) also reported that women were receptive than men in their programme on tissue culture in cassava.

Farm level GM production was attempted to bridge the gap in the awareness and adoption of the bio control technologies among coconut communities. Tripp and Ali (2001) in their study opined that widespread use of village level production of bio agents will be encouraged by allowing farmers more opportunities to experiment with them and supporting the commercial manufacture of these products. In this study also the farmers were equipped with the skill and knowledge in farm level GMF production using basic culture from CPCRI. Our study showed that a well organized group can adopt low cost, small scale multiplication technique and establish an informal farmer decentralized system to provide quality product in sufficient quantities. This was similar to the results reported by Subashini *et al.* (2003). For ensuring sustainability, adequate linkages were established with stakeholders like local government bodies, extension agencies, farmer groups, mass media and print media, promotional literature, women self help groups etc. The farm level multiplication was initiated and the programme was successful in ensuring critical input at farmers' level. The participatory efforts could lead to certain refinement which is given below (Table 2).

Table 2. Farmer level refinements made in farm level GMF production

Items of activities adopted initially	Refinements made by groups involved
Lab room, air condition, inoculation chamber, laminar flow, spirit lamp, autoclave, PP cover	Clean room, candle lit space, maintaining high level hygiene, 20 liter pressure cooker (As per CRI method)- no refinements made as such
Inoculation needle for culture transmission	Direct transfer from culture packet within close range of flame
PVC pipe pieces of one inch to keep at the mouth of the PP cover and cotton	Women tried with careful folding of PP cover and stapling to avoid this expenditure without compromising results
Medium of growth – maize and sorghum not easily available locally	Replaced with half cooked rice grain as suggested by entomology experts, experimented with various textures of tapioca and cow dung – finally opted for rice grain
Sterilizing in pressure cooker	Keeping a lining of newspaper in the sides of the cooker before arranging inoculated packets
Skill transfer and experience sharing strategy- linear	Women group already established serving as master trainers in skill up gradation
Quality ascertaining	Women group maintains grub and adult beetles, apply produced GMF and verify the infection, contamination identified by colours. CPCRI, Entomology experts facilitates quality control of farm level GMF produced

Tripp and Ali (2001) found that when farmers adopt the new products, they use criteria of cost and efficiency rather than health and environmental consideration emphasized in IPM. It was found to be applicable in this study also. Hence, the refinement was attempted by the group members to reduce the cost by 20-35 percent and made it simpler.

The visibility of the application of GMF in breeding sites was quick as perceived by 88.4 percent farmers. They recorded that within a week they could recover affected grubs. Altogether 634 potential breeding sites of rhinoceros beetles were treated with GMF. Based on one year of technology adoption, farmers opined that the treatment may be made once in a year for better results. They could recover infected beetles also from pheromone traps installed in the field indicating the presence of inoculums in breeding sites. The cost of production of one packet of 100 g of fungus in the media was up to Rs. 10-12 and one group could produce 30-40 packets per day utilizing their free time. The impact of the technology in reduction of rhinoceros beetle incidence on palms was not evident within this short period, which will be documented in due course.

There were constraints and practical problems that deserve consideration during the process. In a local level effort for production of bio agents, there will be little concern about deliberate fraud. But the process requires careful oversight and stringent supervision, which was being done by the technically qualified Agricultural officer and experts of CPCRI in this case. Marketing was another problem related to the pricing and distribution. Presently the product may be used within 10 days if kept in room temperature. This may be overcome through research support for evolving better formulations for longer keeping quality. When the model will be up scaled access to basic culture, quality control facilitation, provision of continuous and need based technical support, erroneous selection of activity groups leading to low level of motivation/ confidence etc will be barriers that need to be looked into and bridged up.

Dissemination and utilization is a social and dynamic process involving several actors with multiple roles. To ensure the maximum transformation potential of research and impact on productivity improvement, food security and ecological sustainability technologies needs to be put into use among the farm lands. Participatory field based models could enable to bridge the gap in technology utilization through social system based interventions as in the case of green muscardine fungus as bio control agent against rhinoceros beetle, the major pest of coconut. This should be an evolving process with relevant space for various actors to participate and contribute, rather than a linear technology transfer intervention.

References

- Aravindakshan, M. and Venkatachalam, V. 1997. Report on the socio economic survey of coconut holdings in Kumbalam and Kadamakudy panchayaths in Ernakulam district. Coconut Development Board, Ministry of Agriculture, GOI, Kerabhavan, Kochi. (Publn. No.57) pp. 29-41.
- Anithakumari, P. and Kalavathy, S. 2001. Knowledge and adoption of recommended practices by coconut cultivators of root (wilt) affected area. *Indian Coconut Journal*. **31** (9): 14-16.
- Anithakumari, P., Nair, C.P.R., Rajan, P. and Chandrika Mohan, 2007. In. Final report, CFC/DFID/APCC/FAO project on coconut IPM, Jakarta, Indonesia.

- Anithakumari, P. 2008. Clustering coconut farmers- A successful Extension approach for enhancing adoption and income from small and marginal holdings. *CORD* 24 (2): 29-39.
- Chandrika Mohan. , Rajan, P. and Anithakumari, P. 2010. Farm Level production of the green muscardine fungus for management of rhinoceros beetle. Technical Booklet. CPCRI, Regional Station, Kayamkulam.pp.
- CRI, Sri Lanka. 2007. On farm production of green muscardine fungus for management of black beetle. Crop Protection Division, Coconut Research Institute, Sri Lanka, March 2007. 10 pp
- Final Report. 2003. NATP on Etiology and Management of Coconut root (wilt) disease. Central Plantation Crops Research Institute, Regional station, Kayamkulam, pp. 25
- Knipling, E.F. 1992. Principles of insect parasitism analyzed from new perspectives: Practical implications for regulating insect populations by biological means. *Agriculture Handbook*. 693. Washington DCUS Department of Agriculture, Agricultural Research Service.
- Lindquist, D.A. 1998. Pest management strategies: area-wide and conventional. Area-wide control of fruit flies and other insect pests. 13–19. Oxford University. pp.
- Mumford, J.D.Tan, H.K. 1998. Economics of area-wide pest control. Area-wide control of fruit flies and other insect pests. 39–48. Oxford University. pp
- Thomas,M. 2011. Thirty years of triumphs and tribulations in Integrated Development of Coconut Industry in India. *Indian Coconut Journal*. 43(9): 14-24.
- Nirula, K. K., Radha, K. and Menon, K.P.V.1955.The green muscardine disease of *Oryctes rhinoceros* L.I. symptamatology, epizootology and economic importance. *Indian Coconut Journal* 9: 3-10.
- Pillai, G. B. 1993. Biological Control of insect pests of plantation crops. pp.235-252. In: *Organics in soil health and crop production*. (Ed. P.K.Thampan) Peekay Tree Crops Development Foundation, Cochin. 254pp.
- Subashini, H. D., Anand alias Venkateshan and Malarvannan, S. 2003. Trichogramma bio pesticides - A viable micro enterprise – progress and prospective. pp.239-293. In: *Proceedings of the international seminar on downsizing technology for rural development*. (Eds. Khuntia, S., Parida, A. and Mishra. V.N) Bhubaneswar, 2003.
- Tripp Robert and Arif Ali. 2001. Farmers access to natural pest control practices: Experience from an IPM project in India. AgREN- Odi Agricultural research and Extension Network. Network paper No. 113.
- Yu, R. and Leung, P. 2006. Optimal pest management: A reproductive pollutant perspective. *International Journal of Pest Management* .52: 3155-3166.

Extension approaches to enhance technology utilisation and income generation in coconut farming

C. Thamban

Central Plantation Crops Research Institute
Kasaragod 671124, Kerala, India

Coconut palm provides livelihood to millions of farm families in the world, especially in developing countries. Eulogised as “Kalpavriksha” (the tree of heaven), coconut palm is considered a valuable gift to the mankind, as it is a unique crop as a source of food, drink, shelter and a variety of raw materials for industrial exploitation. Coconut plays an important and vital role in the socio-economic life of large number of small and marginal farmers in various parts of the world. It is estimated that more than 10 million people in India are dependent on coconut, as they are engaged in coconut cultivation, processing, marketing and other related activities. Through the systematic research conducted during the last few decades, a substantial number of viable technologies related to crop improvement, production and protection have been evolved for enhancing coconut production. However, farmers are not able to exploit the production potential from these technologies to the extent desirable. Extent of adoption of the recommended practices plays a crucial role in improving productivity and income from coconut farming. The present scenario of technology adoption in coconut calls for the technology generation and dissemination programmes based on viable extension strategies with the active participation of beneficiaries.

Efforts taken in the areas of technology generation and dissemination have immensely contributed for the development of coconut crop especially with reference to production and productivity. Despite all the sincere efforts, the extent of adoption of the available technologies at the cultivators' level is not that satisfactory. A comparison between the best managed gardens and national average of productivity of coconut crop will reveal the fact that there still exists a wide gap between the technologies generated and their utilization by the growers in coconut crop sector, especially in small holdings. The national average of coconut productivity in India is 8,303 nuts per ha per year and in Kerala state the productivity is 7,365 nuts per ha per year while the local West Coast Variety maintained in the HDMSCS experimental plot at CPCRI Kasaragod has an average yield of 30,625 nuts per ha per year. One of the important reasons for this glaring yield gap in coconut is the low level of technology utilisation at farmers' fields.

The process of research could be considered as successful only when the results reach the ultimate users. However, the acceptance of a new idea by the members of a social system is rather slow and difficult. Each individual has to pass through different stages of adoption process. Various communication channels used by research and extension systems help him to pass through these stages in a rapid and positive manner. Studies have been conducted in different regions to assess the extent of adoption of improved coconut cultivation technologies. One of the surveys conducted by CPCRI among the coconut growers in Northern Kerala in India indicated the low to medium level of adoption of various recommended technologies. (Thamban and Venugopalan, 2002). Studies on the extent of technology utilisation by coconut growers in Kerala indicated the following pattern.

Table 1. Technology utilisation pattern by coconut growers in Kerala

Extent of adoption	Recommended technologies
25% and below	Hybrids, fertiliser application in split doses, basin management with green manure plants, irrigation, systematic intercropping/ mixed cropping, plant protection measures, biological control of pests, post harvest technologies
26-50%	Regular planting with adequate spacing, application of organic manures, soil and moisture conservation techniques
51-75%	Planting of seedlings, selection of seedlings, green manuring
76-100%	Local WCT variety, planting method, mother palm selection, basin opening

Coconut farmers experience many constraints in adopting the recommended practices for higher productivity and income from farming. The problems may be of socio-economic, technological, management or infrastructure in nature. Studies conducted at CPCRI show that even in a state like Kerala which boasts of 100 per cent literacy, there still exists a situation where many farmers do not possess sufficient knowledge about the available technologies for enhancing the production and productivity of coconut. Lack of availability of location specific technologies poses an important hurdle in the improvement of coconut productivity to enhance income. Low and fluctuating price of coconut is the most important problems faced by coconut growers. Lack of sufficient labour for climbing palms and high wage rate also create much problem to farmers. Incidence of pests and diseases is another important problem experienced by coconut farmers. High cost of inputs, lack of availability of quality planting materials etc are often reported as constraints in adopting the recommended practices of coconut cultivation (Thampan, 1999).

Socio-economic problems faced by the farmers may slow down transfer of technology efforts as well as extent of adoption. Low efficacy of TOT and feedback system is another important constraint in improving technology utilisation by farmers to earn more income from coconut farming. Majority of the coconut growers belong to small and marginal holding categories and the uneconomic holding size limits the adoption of recommended technologies for higher productivity and income from coconut farming. Planting coconut in unsuitable locations, poor spacing due to fragmented holdings, low availability and utilization of organic manure due to reduced adoption of livestock integrated farming, lack of irrigation/ drainage facilities etc also adversely affects effective utilization of technologies in coconut farming (Anithakumari *et al*, 2003). The adoption process is also hindered by lack of effective extension support in time, in adequate quantity and quality. Poor management and low adoption of recommended cultivation practices reduces income from coconut farming.

Lack of adoption of scientific cultivation practices has been stated to be one of the important reasons for low productivity of coconut. A comparison between the best managed gardens and national average of productivity of coconut will reveal the fact that there still exists a wide gap between the technologies generated and their utilization by the growers, especially in small holdings (Rajagopal *et al*, 2004). The low level of technology utilisation at farmers' fields calls for formulating effective extension strategies suitable to the heterogeneous

farming situations in coconut cultivation. Various programmes are implemented by research and development agencies on a regular basis as part of the efforts to disseminate the research results among the cultivators.

The important transfer of technology activities for coconut development implemented by CPCRI include training programmes, front line demonstrations, information communication through mass media like radio, television, newspapers and farm magazines, extension pamphlets, CD ROMs, video cassettes etc., arranging exhibitions, seminars, Kisan Melas and group meetings, providing consultancy through field visits and replying postal queries etc.. Besides, few innovative extension approaches have also been pilot tested through action research with farmers' participation by CPCRI for improving technology utilisation by coconut farmers.

A substantial number of appropriate technologies relating to production, protection and processing of coconut have been evolved at various coconut research Institutes. To be meaningful, the technologies generated at the research stations should reach the ultimate users, the farmers. On campus/off-campus training programmes for farmers and extension personnel on specific topics related to technologies to improve coconut scenario form an important component of TOT programmes to enhance their knowledge and skill for better technology utilisation.

Organising need based training programmes enhances the technology utilization and thereby income of coconut farmers. Assessment of training needs of the coconut growers is to be done to streamline the training programme. Participatory methods can be effectively employed to unearth the areas of improved technologies in which farmers require exposure. Depending on the techno-socio-economic situations of coconut farming prevailing in the locality, the training needs of farmers would be varying. Studies have shown that coconut farmers' topmost preference was for off-campus training followed by institutional and other types of training. Off-campus training programmes are organised in villages in the most realistic situations for a short duration. Farmers' preferences with respect to the methodology, duration, venue, season and frequency of training are also to be given adequate attention while formulating training programmes for coconut farmers. For example, CPCRI offers various types of training programmes for farmers on topics such as Production Technology on coconut, Management of coconut diseases, Integrated pest management in coconut, Nursery management in coconut, Organic farming technologies for coconut based cropping systems and Post Harvest Technology (Training Schedule 2012-13, CPCRI). These training programmes are offered in regional languages like Malayalam, Kannada, Tamil, Telugu and Hindi as desired by the farmers. Both Institutional training programmes and off-campus training programmes related to coconut farming are organised regularly at CPCRI and through the Krishi Vigyan Kendras (Farm Science Centres) at Kasaragod and Kayangulam.

Lack of availability of labour, especially skilled labour for coconut climbing, and high wage rate are important problems faced by growers in adopting timely crop management practices. To tackle this problem, the Coconut Development Board has initiated an innovative training programme viz., 'Friends of Coconut Trees (FoCT)' to develop a professional group of youth for harvesting and plant protection operations in coconut. The training targeted the group of unemployed youth in developing technical skills, entrepreneurship capacity, leadership qualities and communication skills to address the needs of the coconut growers. More than 5000 rural youth have been trained in the state under this programme which started during August 2011.

Extension personnel of different organisations are directly involved in organising various technology transfer programmes and implementing development schemes to improve the income generation from coconut farming. Training of extension personnel enhance their knowledge and skill, inculcate appropriate attitude, provide on-the-job experience and develop professional behaviour thus enabling them to extend better service to clients, the farmers, which is the ultimate aim of all training interventions. Taking into cognizance the importance of technology transfer, systematic training programmes catering to the needs of extension personnel engaged in the development of coconut are organised at research organisations such as CPCRI. Short duration training courses for research, teaching and extension staff provides opportunities for sharing of information and experiences at different levels, apart from imparting knowledge and skill. The study conducted among extension personnel who had participated in the training programmes conducted at CPCRI during 1995-98, indicated that the efforts for organising the training programmes were highly successful in achieving the objectives as reflected by the high Training Effectiveness Index (TEI) values (Table 2).

Table 2. Effectiveness of training programmes as perceived by participants

Course title	No. of respondents	Training Effectiveness Index (TEI)
Production technology of coconut	45	80.93
Coconut based farming systems	41	79.81
Hybridization technique in coconut	39	75.12
Pest, disease and nematode management in coconut	37	73.21
Biological suppression of coconut pests	40	80.83

(CPCRI Annual Report, 1999).

In all the training programmes there was significant improvement in the knowledge, skill and attitude of extension personnel involved. The study also revealed that lack of essential teaching and communication equipments, lack of adequate transport facilities to reach farmers and lack of training in extension methods and communication skills were perceived as the most important constraints faced by the extension personnel in implementing educational programmes on the topic of training they attended to benefit the ultimate users viz., the farmers. Unless effective measures are taken to overcome these difficulties as experienced by the extension personnel, the training programmes organised may not serve the basic objective of disseminating the latest technologies evolved at the research institutes to the farmers.

Demonstration is a very effective method of transfer of technology in a community. It is a group method of extension usually conducted to educate and motivate groups of farmers. Demonstration may stimulate farmers to try out innovations themselves, or may even replace a test of the innovation by the farmer. A great advantage of demonstration is seeing how an innovation works in practice. Demonstrations to be effective should be integrated with the total extension programme. Result demonstration attempts to motivate the people for adoption of a new practice by showing its distinctly superior result. A method demonstration is given before a groups of people to show how to carry out an entirely new practice or an old practice in a better way. It is essentially a skill training, where the emphasis is on effectively carrying

out a job, which shall improve upon the result. It involves seeing, hearing, participating and practicing in a group which shall stimulate interest and action. Method demonstration is sometimes used as complementary to result demonstration. Frontline demonstration of various improved coconut production technologies with the active participation of farmers has been proved to be an effective method transfer of technology for improving the yield and income from coconut farming.

Demonstrations on thematic areas related to hybrids and high yielding varieties, agro techniques, cropping/farming systems, integrated pest and disease management in coconut can be effectively laid out as an important TOT activity. CPCRI has been organizing front line demonstrations in farmers' field on different coconut cultivation technologies such as coconut based farming systems, management of root (wilt) affected coconut gardens etc. Such demonstration programmes have proved to be effective in convincing the farmers about the technical feasibility and economic viability of the technologies. Further, these demonstration programmes have multiplier effects in the dissemination of recommended technologies among cultivators at large. The adoption of the integrated root (wilt) management practice was effective in improving the average yield of root (wilt) affected palms in farmers' fields. The average yield of the palms increased from 24.17 to 46.3 nuts/palm/year after three years, recording an improvement of 91.4 per cent. Observations on yield of coconut revealed an increase in productivity of palms from a pre-demonstration yield of 95 nuts per palm per year under mono crop situation to 122 nuts per palm per year in coconut based High Density Multi Species Cropping System (CPCRI annual report, 1997). Thus, there was an additional income of Rs 108/- per palm per year due to the adoption of technology on HDMSCS demonstrated.

Low efficacy of transfer of technology and feedback system is an important constraint in improving technology utilisation by farmers to earn more income. The low level of utilisation of technologies in farmers' fields calls for formulating effective extension strategies suitable to the heterogeneous coconut farming situations. Some innovative extension approaches have been pilot tested through action research with farmers' participation by Central Plantation Crops Research Institute (CPCRI) for improving technology utilisation by farmers as described below.

1. Research-Extension-Farmer Interface facilitated through Cyber Extension Programmes

Cyber Extension include effective use of information and communication technology (ICT), national and international information networks, internet expert systems, multimedia learning systems and computer based training systems to improve information access to the farmers, extension workers, research scientists and extension managers. CPCRI has been implementing various cyber extension activities as part of strengthening the technology transfer programmes of the Institute in mandate crops viz., coconut, arecanut and cocoa.

As part of the cyber extension activities, a group video conferencing system through ISDN was installed at the ATIC, CPCRI, Kasaragod to facilitate interaction between various stakeholders for enhancing technology utilization in coconut, arecanut and cocoa. The Research-Extension-Farmer Interface facilitated through video conferencing under Cyber Extension Project was formally inaugurated at CPCRI, Kasaragod on 7.4.2007. Facilities at the District Collectorates for videoconferencing were utilized for the interaction through the collaboration with State IT Mission. Since then, the Institute has been organizing interface

programmes involving different categories of stakeholders at regular intervals to strengthen the technology transfer efforts.

In the year 2010, the videoconferencing facility for organizing interface programmes was strengthened by procuring a mobile CODEC. The additional facility of mobile CODEC enhanced the scope of interface programmes by linking the scientists at the Institute head quarters with farmers and other stakeholders in distant locations, which do not have videoconferencing facility, either through ISDN or IP-network. This facility provides more opportunities for the researchers at the Institute to have interactions on field problems with farmers and other stakeholders located in distant places.

The details of interface programmes facilitated through videoconferencing organized by CPCRI are furnished below.

Table 3. Research-Extension-Farmer interface programmes facilitated by videoconferencing organized by CPCRI

Type of interface programme	Participant at the Institute HQ	Participants at the off-campus location	No. of interface programmes conducted during 2007-2012 (up to October)
Type -1	Scientists	Extension personnel and Farmers	16
Type -2	Scientists	Farmers	12
Type -3	Scientists	Extension personnel	17
Type -4	Extension personnel	Scientists	06
Type -5	Scientists and members of SHGs	Scientists and members of SHGs	04
		Total	55

A total of 55 interface programmes facilitated through videoconferencing were organized by CPCRI during the period from April 2007 to October 2012. The programmes thus organized can be broadly categorized as follows.

Type 1. In this type of interface programme, scientists of the institute interact on themes related to production technologies of coconut, arecanut, cocoa and palm based cropping systems with extension personnel and farmers located in distant places. Mostly this type of interface programmes have been initiated in collaboration with commodity boards such as Coconut development Board (CDB), State Department of Agriculture/ Horticulture and Krishi Vigyan Kendras (KVKs).

Type 2. In this type of interface programme, scientists of the institute interact with farmers located in distant places. Mostly this type of interface programmes have been initiated at the request of commodity based farmers' groups such as coconut farmers groups.

Type 3. In this type of interface programme, scientists of the institute interact with extension personnel attending training programmes at other horticulture research institutes on topics related to palm based cropping systems.

Type 4. In this type, interaction is facilitated between extension personnel attending training programmes at CPCRI and scientists at other horticulture research institutes on themes related to improved varieties and agro techniques of horticulture crops suitable for inter/mixed cropping in coconut/arecanut gardens.

Type 5. In this type, interaction between SHGs are facilitated mainly to share their experiences in production and marketing of value added agro based products and also to facilitate demonstration of techniques involved in the production to benefit the members of SHGs at the other end of interaction.

Effective linkages are established with various agencies such as ICAR institutes, Commodity Boards like CDB, State Department of Agriculture/Horticulture, State IT Mission, NGOs like MSSRF, people's representatives and farmer organizations for the effective conduct of the interactive videoconferencing programmes by CPCRI to strengthen technology transfer programmes in coconut, arecanut and cocoa under the cyber extension project.

Since launching the facilities, interactive videoconferencing involving various stakeholders have been conducted between CPCRI headquarters at Kasaragod and various locations in the country. All the four southern States viz., Kerala, Tamil Nadu, Karnataka and Andhra Pradesh where the mandate crops of the Institute are mostly cultivated have been covered through the interface programmes besides other states such as Assam, Bihar and Orissa. In Kerala, more number of locations have been covered through the interactive videoconferencing due to the comparative advantage for arranging the logistics for the use of mobile CODEC equipments.

2. Community Based Organisations (CBOs) in coconut communities for sustainable income enhancement

Livelihood of a substantial number of families in rural poor communities in India depends on coconut farming. Many a times, the income generated from coconut farming in small and marginal holdings does not provide enough for meeting the requirements of such families. Technology options for enhancing income from coconut farming in such poor rural communities do exist, but not fully realised in field situation. The fragmented holdings don't render themselves viable for the optimum utilization of resources and the adoption of improved technologies by the cultivators. To augment the production and productivity of such small and marginal holdings it was suggested to have group management of resources which helps to overcome the inherent weaknesses of the fragmented holdings. The concept of organizing coconut farmers into Community Based Organisations (CBO) for sustainable income enhancement with the objective of efficient management of farmers' resources to reduce cost of cultivation and to increase productivity through integration of technologies even in very small farm holdings have been demonstrated by CPCRI in selected localities under some projects.

A project sponsored by IPGRI for developing sustainable coconut based income generating technologies in poor rural communities was implemented by the CPCRI in two selected

coconut communities, at Pallikkara in West Coast region and Ariyankuppam in East Coast region. The three-pronged strategy for the project included i) growing suitable inter/mixed crops in coconut gardens and integrating animal husbandry and other subsidiary enterprises with coconut farming ii) cultivating high yielding cultivars of coconut to enhance the yield and income and iii) promote the diversification of coconut products. The implementation of the strategies envisaged in the project was routed through Community Based Organisation of coconut growers in the selected communities. Micro-credit for introducing the interventions envisaged under the project was routed through the CBO. Joint, participatory analysis of the coconut farming scenario in the community was undertaken by the team of scientists and farmers under the CBO to design the technological interventions to be implemented. A close linkage was developed between the CBO and scientists from CPCRI for the effective implementation of interventions. Arrangements for procuring planting materials, inputs and organising training programmes on CBO management and relevant technologies were done through the CBO with close collaboration with CPCRI and other agencies. Monitoring and evaluation of the interventions implemented in farmers' field were done through CBO. Table 4 below depicts the additional income generated through coconut based intercropping under the project in the Ariyankuppam coconut community in the East Coast of India.

Table 4. Net returns realised through intercropping in coconut gardens

Sl.No.	Cropping system	Net returns (Rs. /acre)	Net returns above monocrop(Rs. /acre)
1.	Coconut monocrop	21500	
2.	Coconut + tapioca	27500	6000
3.	Coconut + groundnut	25550	4000
5.	Coconut + bhindi	28500	7000
6.	Coconut + rice	26500	5000
7.	Coconut + banana	31500	10000
8.	Coconut + bittergourd	28000	6500

This innovative extension methodology is being adopted for the implementation of other similar projects also.

Under the project on 'Cluster approach among coconut farming community for improving productivity and income from small and marginal coconut based homesteads' in the root (wilt) affected coconut area the farm family members of 25 hectare area clustered together for deciding the farm strategies; both individual and group ventures for improving productivity and income. The average yield of coconut was doubled after technology package implementation for three years.

The cluster approach has been scaled up by other agencies like Coconut Development Board (CDB) among coconut farming communities through their development schemes. The Board has initiated the formation of Coconut Producer Societies (CPS) by associating

40-100 coconut growers in a contiguous area with a consolidated minimum of 4000-5000 palms. The objective is socio economic upliftment of the farmers through productivity improvement, cost reduction, efficient collective marketing and processing and product diversification. A farmer equity contribution is also proposed to be mobilized. A matching equity contribution will be sought from the state Government as a one time assistance for making the CPS effective (<http://coconutboard.nic.in/cps.htm>)

Under the NAIP project on 'Value chain in coconut', implemented by CPCRI a methodology was evolved for participatory group approach through Community Based Organisations (CBOs) for sustainable income enhancement in small and marginal coconut holdings. The methodology involves facilitating CBOs of farmers and women SHGs for effective integration of production and processing technologies in coconut holdings for higher income. Under the project, 10 CBOs comprising of 534 farmers in clusters of 25 ha each were formed. Technological interventions on soil and water conservation, soil health management, INM, inter/mixed cropping, IPM and IDM were implemented in farmers' gardens. Four women SHGs were facilitated to take up microenterprises on production and marketing of coconut value added products like coconut chips. The group approach in coconut farming for income enhancement in small holdings is being scaled up by other agencies like Coconut Development Board, State Department of Agriculture and Local Self Governments.

3. Other extension approaches in coconut

Appropriate extension strategies are very critical in technology transfer and capacity building of the farming communities of resource poor coconut growing areas like root (wilt) disease affected areas. The following were the major approaches thus implemented by CPCRI.

- a. Participatory technology transfer approach (PTT) for technology assessment with participation of farm families in a contiguous area affected by coconut root (wilt) disease : Under this approach the farm families of manageable contiguous area (50-100 ha) will be the participating farmers and this approach suits well for first line technology assessment and transfer. Multidisciplinary team of scientists, extension officials, farmer representatives, local village representatives, women, youths, groups etc form the stakeholders. Participatory rural appraisal tools were employed for pre implementation, appraisal at 6 monthly interval and also post implementation of the project. The awareness, knowledge, adoption and attitude of farmers towards the technology package for the integrated root (wilt) disease management technologies) were improved by 40-85 per cent over the pre implementation level. The monitoring, appraisal and evaluation of the technology implementation and impact were done with the stakeholder participation and documented in 'farmer – scientist-extension dialogue session' for further scaling up by other agencies.
- b. Farmer field schools for IPM of coconut: The innovative concept of farmer field schools (FFS) was implemented by CPCRI Regional Station, Kayamkulam for the IPM of rhinoceros beetle in coconut organized in 15 locations. It was observed that the knowledge and awareness of farmers were improved by 100 per cent over pre FFS level.
- c. Promoting women's self help groups: It is generally assessed that there is immense scope for introducing interventions related to the promotion of women's self help

groups for processing of diversified coconut products at the farm household and community level. Hence, Entrepreneurship Development Programme can be organised for women to train them on the various opportunities for value addition in coconut. Topics such as concept and practices of Entrepreneurship Development, group approach for micro level interventions on product diversification in coconut, production of quality copra using copra dryers, coconut kernel based food products, preparation of coconut candies, production of Snow Ball Tender Nut, production of coconut chips, Oyster mushroom cultivation on coconut wastes and production of vermicompost using coconut leaves etc can be included in the Entrepreneurship Development Programme.

Under the COGENT-IPGRI project on “Developing sustainable coconut based income generating technologies in poor rural communities in India”, implemented by CPCRI, Entrepreneurship Development Programmes on ‘Value addition in Coconut’ were organised under the project for women. The experiences and impact of organising the Entrepreneurship Development Programme on value addition in coconut for women under the COGENT-IPGRI project clearly indicate the scope and importance for enhancing the income of resource-poor coconut farmers and socio-economically disadvantaged rural women through product diversification in coconut. The documentation of outputs of the COGENT sponsored project on ‘Developing sustainable coconut based income generating technologies in poor rural communities’ in different coconut growing countries also revealed that women members of the Community Based Organisations under the project increased their income by 3-5 times through the production and marketing of coconut high value products compared to their previous income from copra, securing for them a steady source of additional income and helping them rise above poverty line. Equally important, this project intervention provided employment opportunities to formerly unemployed and under employed rural women resulting in enhanced self esteem, and economic and social empowerment (Batugal and Oliver 2003).

Under the NAIP project on ‘Value chain in coconut’, implemented by CPCRI four women SHGs were facilitated to take up microenterprises on production and marketing of coconut value added products. The SHG members were trained on technologies for the production of coconut chips, VCO and a range of other coconut based value added products. Capacity development programmes were also organized for the SHG members on themes like good processing practices, quality control, procurement of raw material, maintenance of machinery, marketing and maintenance of accounts and audit etc. Besides the economic empowerment, the members of women SHGS were also benefited through more recognition in the society, improvement in communication skills, knowledge in financial management etc by involving in the management of coconut based microenterprises.

d. Farmers’ participation in coconut research: Ensuring farmer participation in research and extension invariably enhances the extent of technology utilisation at farm level. There is scope for participatory technology assessment and refinement in coconut for achieving higher productivity. Indian Council of Agricultural Research (ICAR) implemented a project in 1995 for Technology Assessment and Refinement through

Institution-Village Linkage Programme (IVLP) under National Agricultural Technology Project (NATP). CPCRI was one of the selected centres to implement the project. IVLP is a novel front line extension programme, the implementation of which begins with the selection of a suitable village, that is followed by a detailed agro-ecosystem analysis of the selected village, diagnosing the problems of each production system and prioritising these problems; identification of technological interventions based on problem-cause relationship; the development of action plans and their implementation; and detailed socio-economic evaluation including farmers' reactions and perceptions about the interventions. This was carried out using various PRA tools and techniques. In all these stages, the active participation of farmers was ensured. The TAR-IVLP project was implemented by CPCRI in three villages, viz. Pady, Edneer and Nekraje of Kasaragod, Kerala with coconut as one of the important crops in the production system of the villages.

Keeping the problem-cause relationship as the basis, action plans were prepared for each technological interventions with the active participation of farmers facilitated by IVLP Core Team. Each intervention may be either in the form of On Farm Trial (OFT) or Verification Trial (VT). For those problems wherein solutions are experimentally proved elsewhere were taken as VTs and for those technology for which the results are yet to be confirmed under farmers' field conditions are taken as OFTs. Farmers' practice was included as control in all the trials undertaken. The action plan gives an account of the micro-farming situation, problem and its causes, intervention points, potential solutions, nature of intervention, treatments, critical inputs etc. The technological interventions contemplated under the action plans are implemented in the selected farmers field either as OFT or VT. At each and every stage of the trial, the participating farmers are involved in assessing the interventions using various performance indicators such as technical observations, economic observations, farmers reaction etc. The details of technological interventions implemented is furnished in Table 5.

Table 5. Details of technological interventions implemented in coconut - based farming systems under IVLP

Sl. No.	Technological intervention	Nature of intervention	No. of farmers involved in the trial
1.	Assessing the performance of improved coconut varieties under rainfed conditions	OFT*	20
2.	Assessing the performance of improved coconut hybrids under irrigated conditions	OFT	20
3.	Assessing the performance of tapioca as an intercrop in adult coconut gardens under rainfed conditions	VT**	43
4.	Assessing the performance of ginger as an intercrop in adult coconut gardens under rainfed condition	VT	35

5.	Assessing the performance of turmeric as an intercrop in adult coconut gardens under rainfed condition	VT	37
6.	Assessing the performance of elephant foot yam as an intercrop in adult coconut gardens under rainfed conditions	VT	20
7.	Assessing the performance of amaranthus as intercrop in adult coconut gardens under rainfed conditions	VT	20
8.	Assessing the performance of Bhendi varieties as intercrop in adult coconut gardens under rainfed conditions	OFT	20
9.	Assessing the performance of green manure crops in the basins of adult coconut palms under rainfed conditions	OFT	20
10.	Assessing the performance of integrated control measures for stem bleeding disease in coconut	VT	12
11.	Rat control in coconut gardens	VT	19
12.	Assessing the performance of integrated management of rhinoceros beetle in coconut	VT	19
13.	Pitcher irrigation in coconut	VT	19
14.	Assessing the efficiency of composting of farm waste in coconut - based homesteads through earth worms	VT	20
15.	Assessing the efficiency of copra dryer for household use	VT	3 groups of farmers

*On Farm Trial

**Verification Trial

Implementation of the project revealed the effectiveness of participatory approach in the performance assessment of various technologies related to high yielding varieties, intercropping, nutrient management and crop protection in coconut (Thamban *et al*, 2004).

e. Strengthening research-extension-farmer linkage in coconut sec

As has been already described, there are various agencies both from research and extension systems striving for the development of coconut. Invariably some overlaps do occur in their functioning. But taking into account the enormous number of farm families with small holdings, the duplication will only have a multiplier effect in dissemination of technologies for enhancing coconut production. For better impact, the extension activities of the research and extension/ development agencies are to be co-ordinated at different levels. Technology dissemination process will be accelerated if concerted efforts are made by the different agencies having proper co-ordination among

them. The experiences of CPCRI in implementing the interface programme to strengthen the research-extension-farmer linkage in coconut sector indicate the relevance of strengthening research-extension-farmer linkage in coconut sector.

Interface programme for coconut is an approach for strengthening the transfer of technology efforts for the development of coconut sector in Kerala state. In this approach, the research and extension personnel and farmers are brought in a common platform to streamline activities for the sustainable development of coconut. The programme was implemented during the year 2001-02 and 2002-03. Thematic sessions relating to crop improvement, crop production, crop protection and post harvest processing technologies were included in these interface programmes, in which scientists from CPCRI and KAU, extension personnel from Department of Agriculture and Commodity Boards, people's representatives and selected farmers participated.

The results of impact analysis indicated the effectiveness of the interface programmes in enhancing the awareness and knowledge about the technologies for improving coconut productivity and income of farmers.

Table 6. Perception of extension personnel about the impact of the programme on awareness and knowledge level on improved coconut production technologies (n=150)

Sl. No.	Topic covered	Improvement in awareness		Improvement in knowledge	
		No. of respondents		No. of respondents	
		Very much Improved	Improved	Very much Improved	Improved
1.	Crop improvement technologies-HYV/hybrids	87	63	88	62
2.	Crop production technologies				
a.	Integrated nutrient management	88	62	98	52
b.	Organic recycling in coconut garden	125	25	136	14
c.	Water management in coconut	96	54	99	51
d.	Coconut based cropping/ farming systems	102	48	106	44
3.	Crop protection technologies				
a.	Integrated Pest Management in coconut	87	63	90	60
b.	Integrated Disease Management in coconut	89	61	88	62
4.	Value addition in coconut through product diversification	114	36	118	32

The impact study also revealed that there is vast scope for planning decentralised schemes based on improved coconut production technologies dealt in the interface programme for implementation under decentralised planning programme. The experiences gained during the district level interface programme and the results of analysis of the impact of interface programmes, revealed that the concept of research-extension-farmer interface is sure to enhance the adoption of technologies ultimately leading to coconut growers' own benefit. Hence, it was suggested that the interface programme on coconut may be continued for strengthening transfer of technology efforts for improving coconut sector in Kerala state. Similar ventures may have to be initiated in other states as well to serve as a catalyst in the promotion of coconut technologies.

Coconut plays a vital role in the agrarian economy of many regions in the world. Though large number of technologies has been generated for the improvement of coconut at various research institutes, and various agencies are involved in the transfer of technologies in coconut by implementing various extension activities and development programmes, the extent of utilization of the available technologies at farmers' level is not at a satisfactory level. The present scenario of technology adoption in coconut calls for the technology generation and dissemination programmes based on a viable extension strategy with the active participation of stakeholders. Further, effective linkage is to be established among different research, and extension agencies and coconut farming community through well co-ordinated participatory research/extension programmes for ensuring a meaningful technology generation and transfer in coconut.

References

- Anithakumari, P., C. Thamban and S. Arulraj. 2002. Constraint analysis in coconut cultivation. *Indian Coconut Journal* 33 (9): 6-8.
- Batugal, P. and Oliver, J.T. 2003. Poverty Reduction in Coconut Growing Communities Vol I: The Framework and Project Plan. International Plant Genetic Research Institute Regional Office for Asia, the Pacific and Oceania (IPGRI-APO), Serdang, Selangor, Malaysia.
- Rajagopal, V., Arulraj S. and Thamban, C. 2004. Research-Extension interface for transfer of technology on coconut. CPCRI, Kasaragod. p 33
- Thamban, C. and Venugopalan, M. (2002). Extent of adoption of recommended package of practices for coconut cultivation. In: Rethinam, P., Khan, H.H., Reddy, V.M., Mandal, P.K. and Suresh, K. (Eds.) Research and Development in Plantation Crops in the new Millennium. Proceedings of the International Conference on Plantation Crops (PLACROSYM XIV) held during December 12-15 at Hyderabad. pp: 218-223.
- Thamban, C., S. Arulraj and C.V. Sairam. 2004. Farmer participatory technology assessment and refinement in coconut through Institution Village Linkage programme- experiences of CPCRI in India. *Journal of Plantation Crops* 32 (supl.) : 459-464.
- Thampan, P.K. 1999. Enhancing the income and employment in the coconut sector through conservation and use of special coconut ecotypes in India – Report on farmer participatory survey. Peekay Tree Crops Development Foundation, Kochi.

Impact of training programmes on adoption of vermicomposting technology utilizing palm wastes, among farmers of Kasaragod district, Kerala

**B.T.Rayudu, S.Leena , K.Manikandan, R.Sanal Kumar,
M.P.Jayashre, and Manojkumar,T.S**

Krishi Vigyan Kendra

Central Plantation Crops Research Institute, Kasaragod 671 124, Kerala, India

Now there is worldwide awareness for adopting organic agriculture due to the deterioration of soil health and increased environmental pollution caused by indiscriminate use of inorganic fertilizers and pesticides. Therefore, it is highly essential to maintain our soil fertile as well as organic rich and to stop its any further degradation for the well-being of our future generations. To restore the soil composition, farmers are now showing inclination to revert to organic farming. Efficient utilization of basic natural resources viz. soil, water and biodiversity as well as their appropriate management for ensuring sustainability of the entire system and enhancing productivity is the basic principle of organic farming. It is a system of farming where the soil is made physically fit, chemically balanced and biologically active. If the soil is fed properly with organic manures, nature will be encouraged to build up soil biodiversity and thus develop its own capabilities to support plant growth for sustainable production. Earth worms are true bio agents for soil health. In this context, the Central Plantation Crops Research Institute has made tremendous achievement towards building soil health by identifying a local earthworm closely related to the African night crawler, *Eudrilus* sp. and standardized the methodology of vermicomposting of coconut and arecanut palm wastes using this earth worm species. This vermicomposting technology is being promoted and popularized among the farmers of Kasaragod district through the Krishi Vigyan Kendra of the Institute by way of training programmes and other transfer of technology activities of the Institute.

A total of 66 training programmes were conducted for the past five years wherein 2241 farmers trained. Out of which 23 training programmes are on-campus with the participation of 208 men and 247 women participants and 43 training programmes are off-campus with the participation of 1141 men and 645 women. To know the effectiveness of these training programmes on the knowledge level of trained and untrained farmers as well as adoption of the technology, an evaluation study was conducted with the following objectives:

- to find out the profile of trained and untrained farmers,
- to find out the difference of knowledge level on vermicomposting technology between trained and untrained farmers, and
- to find out the adoption level of the vermicompost technology among trained and untrained farmers

Research design: Ex-post-facto research design was used for the study.

Sample size: Keeping in view the fact that the trained participants are distributed in all the 39 panchayats of Kasaragod district, one panchayat each from four blocks of Kasaragod district was selected purposively. In each panchayat, one village was selected randomly. Data base on trained and untrained farmers were prepared in each panchayat and representative sample has been drawn randomly for the study. The details of the sample population are presented in Table.1. Accordingly, the total sample size of participants for the present study becomes 220.

Table1. Details of sample size of the study

Sl.No.	Name of the block	Name of the panchayat	Name of the village	Sample size	
				Trained	Untrained
1	Manjeswar	Mangalpady	Mangalpady	25	25
2	Kasaragod	Muliyar	Muliyar	25	25
3	Kanhangad	Kallar	Rajapuram	30	30
4	Nileswar	West-Eleri	Beemanady	30	30
	Total			110	110
	Grand total			220	

Variables: Age, education, occupation and communication behaviour were selected as personal profile of the respondents and the knowledge as well as adoption of vermicomposting technology were selected as dependent variables. The selected variables and indicators are quantified based on the standard procedures.

Data collection: A well structured interview schedule prepared based on the variables and indicators selected. The desired information was collected through individual personal interviews. The collected data were processed by suitable statistical tools like frequency, percentage, mean, standard deviation and t-test.

The information pertaining to the profile of the respondent farmers both trained and untrained like age, education, occupation and communication behaviour were collected, processed and presented in Table 2. The data indicates that majority of the farmers were (48.18%) followed by old and young aged. In case of education level, majority of the farmers had high school to primary education in both the categories of trained (70.00%) and untrained (76.36%). With regard to occupation, majority of the trained farmers had occupation of agriculture alone (56.36%) followed by Agriculture + Allied sectors (40.00%) whereas majority of untrained farmers had occupation of Agriculture + Allied sectors (55.45%) followed by Agriculture alone (39.09%). In the case of communication behavior, majority of farmers in both categories had medium level 73.65% and 77.27% in trained and untrained, respectively. Pooled data revealed that majority of the farmers belonging to 35-50 year of age with high school education (48.64%) and medium level of communication behavior (74.45%). They were doing occupation of agriculture (47.72%) and Agriculture + allied sectors (47.72%).

Table 2. Distribution of respondent farmers based on their age, education, occupation and communication behaviour

N = 220 (110 + 110)

Sl.No. Category	Respondent farmers					
	Trained		Untrained		Pooled	
	F	%	F	%	F	%
1. Age						
Young (< 35 years)	20	18.18	22	20	42	19.09
Middle (35-50 years)	48	43.64	53	48.18	101	45.91
Old (> 50 years)	42	38.18	35	31.82	77	35
Total	110	100	110	100	220	100
2. Education						
Illiterate	11	10	5	4.55	16	7.27
Primary	30	27.27	24	21.82	54	24.55
High School	47	42.73	60	54.54	107	48.64
Secondary	12	10.91	14	12.73	26	11.82
College	10	9.09	7	6.36	17	7.72
Total	110	100	110	100	220	100
3. Occupation						
Agriculture alone	62	56.36	43	39.09	105	47.72
Agriculture + Allied sectors	44	40.00	61	55.45	105	47.72
Agriculture + Service	2	1.82	2	1.82	4	1.82
Agriculture + Business	2	1.82	4	3.64	6	2.73
Total	110	100	110	100	220	100
4. Communication behaviour						
Low (< M - SD)	6	5.45	11	10	17	7.72
Medium (Betw. M $\pm$ SD)	81	73.65	85	77.27	166	74.45
High (> M + SD)	23	20.90	14	12.73	37	16.83
Total	110	100	110	100	220	100

Knowledge level on vermicomposting technology among the respondent farmers

A bird eye view of the data presented in the Table 3 reveals that majority of the trained farmers have medium level of knowledge on vermicomposting technology (79.09%) followed by high (10.91%) and low (10%). In case of untrained farmers, majority of them have low knowledge level (53.64%) followed by medium (42.73%) and high (3.63%) which distinctly low.

Table 3. Distribution of respondent farmers based on their knowledge level

N = 220 (110 + 110)

Sl.No.	Knowledge category	Respondent farmers					
		Trained		Untrained		Pooled	
		F	%	F	%	F	%
1	Low (< M - SD)	11	10	59	53.64	70	31.82
2	Medium (Betw. M $\pm$ SD)	87	79.09	47	42.73	134	60.91
3	High (> M + SD)	12	10.91	4	3.63	16	7.27
	Total	110	100	110	100	220	100

From the Table 4, the computed 't' values on the knowledge level about vermicomposting technology between trained and untrained farmers of Mangalpady, Muliya and Kallar panchayats were found significant at 1% level of probability where as it was at 5% level of probability for the farmers of West-Eleri panchayat. Further, there was significant difference between the mean scores of trained and untrained farmers of all four panchayats.

Table 4. Differences among the respondent farmers in respect of their knowledge level on vermicomposting technology

Sl.No.	Knowledge level	Mean	SD	Computed ‘t’ value
Mangalpady				
1	Trained farmers	5.44	0.44	8.34**
2	Untrained farmers	1.78	1.33	
Muliyar				
1	Trained farmers	6.68	1.74	7.51**
2	Untrained farmers	2.72	1.96	
Kallar				
1	Trained farmers	5.80	3.52	4.52**
2	Untrained farmers	2.66	1.39	
West-Eleri				
1	Trained farmers	7.48	1.78	3.68*
2	Untrained farmers	5.63	2.05	

** Significant at 1% level of probability; * Significant at 5 % level of probability

Adoption of vermicompost technology by the respondent farmers

The data presented in Table 5 illustrate the percentage adoption of vermicomposting technology among trained and untrained farmers. Among trained farmers, 32.73 % adopted the technology where as from untrained category the percentage of adoption is 4.55. The adoption among untrained category reflects technology diffusion from farmer to farmer i.e from trained farmers to fellow farmers..

Table 5. Distribution of respondent farmers based on their adoption of vermicompost technology

N = 220 (110 + 110)

Sl.No.	Category	Respondent farmers					
		Trained		Untrained		Pooled	
		F	%	F	%	F	%
1	Adopted	36	32.73	5	4.55	41	18.64
2	Non - adopted	74	67.27	105	95.45	179	81.36
Total		110	100	110	100	220	100



A view of vermicompost units established by the trained farmers under the technical guidance of KVK

Majority of the respondent farmers in both trained and untrained categories are middle aged, possess high school education, earning their lively hood from agriculture alone and shows fairly well communication behaviour towards learning the information on agriculture and allied sectors. There was a significant difference on knowledge about vermicomposting technology between trained and untrained farmers. Thus clearly reveal that the differential scores on knowledge level obtained by the trained farmers may be due to their participation in the training courses on vermicomposting technology organized by Krishi Vigyan Kendra of CPCRI. Among trained farmers, 32.73 % adopted the technology where as from untrained category the percentage of adoption is 4.55. Easily adoptable technology is the main factor for its adoption. This shows that the training programmes organized by KVK has created significant

impact both qualitatively and quantitatively towards the betterment of socio-economic conditions and farming situation of the farmers of Kasaragod district

References

- George V. Thomas 2001. Vermicomposting of coconut wastes. Manual for the training programme CPCRI, Kasaragod. 4-6 Dec. 2001.
- George V. Thomas 2004. Organic farming practices in coconut with emphasis on vermicomposting of wastes. In *Recent Advances in Organic farming technologies in Plantation Crops*, (eds. G.V. Thomas and C. Palaniswami) CPCRI, Kasaragod pp 16-24.
- Prabhu, S.R, Thomas, G. V. and Gopal, Murali. 2003. Vermicomposting of coconut leaves. CPCRI Extension Publication No. 107. CPCRI, Kasaragod.
- Nageswara Rao, G (1983). Statistics for agricultural sciences. Oxford and IBH publishing Co. 66 Janpath, New Delhi-110001.
- Venkatramaiah P. (1983). Socioeconomic status scale, Ph.D Thesis , UAS, Bangalore.

Application of spatial technique in field experiments with coconut

C.T. Jose, K.S. Ananda and K.Muralidharan

Central Plantation Crops Research Institute
Regional Station, Vittal – 574 243, Karnataka, India

The most important principle of field experimentation technique is to control the experimental error or the unexplained variation. We generally use block designs in field experiments to control the experimental error due to positional variations. The underlying assumption of homogeneity of experimental area within the block may not satisfy always, particularly when the block size is large. Field experiments with perennial tree crops like coconut require large experimental area, and it is grown in both plane and hilly terrains and getting large homogeneous area is difficult. Also we may not know in advance the soil fertility gradient and other factors influencing the response variable to divide the experimental area into homogeneous blocks. Gilmour et al, (1997) suggested the covariance modeling technique to tackle this problem. In this paper, we have used spatial modeling technique suggested by Jose et al, (2009) to estimate/eliminate the positional effect in field experiment with coconut.

The semiparametric regression model considered for the study is given by

$$Y = m + Xb + f(U, V) + e, \quad (1)$$

$\hat{\beta} - \hat{\mu}$

where $Y = [y_1 y_2 \dots y_n]^T$ is the observation vector, m is the general mean, $X = [x_1 x_2 \dots x_n]^T$ is the design matrix, $b = [b_1 b_2 \dots b_p]^T$ is the treatment effect vector, $f(U, V) = [f(u_1, v_1) \dots f(u_n, v_n)]^T$ is the nonparametric spatial function representing the positional effect and e is the independently and identically distributed (iid) random error vector with mean zero. It is assumed that $f(U, V)$ is a smooth function. The backfitting algorithm of Hastie and Tibshirani (1990) is used to compute the estimates for the semiparametric regression model. The backfitting estimators for b and f are equivalent to

$$\hat{\mu} = \bar{Y}, \quad \hat{\beta} = (X^T(I-S)X)^{-1}X^T(I-S)(Y - \hat{f}) \text{ and } \hat{f} = S(Y - X\hat{\beta}),$$

where, S is the smoothing matrix derived using local linear regression (Ruppert and Wand, 1994). An estimate of s^2 is given by

$$\hat{\sigma}_1^2 = \frac{1}{(n - p - 1 - \text{trace}(S))} [Y - \hat{\mu} - X\hat{\beta} - \hat{f}]^T [Y - \hat{\mu} - X\hat{\beta} - \hat{f}]$$

The variance of $\hat{\beta}$ is estimated by

$$\text{where, } P = (X^T(I-S)X)^{-1}X^T(I-S).$$

Additive model for positional effect: When the number of experimental units is comparatively small, estimating the spatial function using the bivariate smoother is inadequate. In such situations, bivariate additive model is better than the two dimensional spatial function used in model (1). By using bivariate additive function, the model (1) becomes

$$Y = m + Xb + f_1(U) + f_2(V) + e, \quad (2)$$

where, f_1 and f_2 are the univariate nonparametric functions representing the positional effect in the U and V directions. The backfitting algorithm will provide an explicit solution to the above semiparametric regression model and the estimates are given by

$$= (X^T (I-Q) X)^{-1} X^T (I-Q) (Y) \quad \text{and}$$

The matrix Q and the estimates $\hat{\mu}$ and $\hat{\beta}$ are obtained by solving the set of equations

$$\text{and} \quad Q = Q_1 + Q_2$$

An estimate of s^2 under model (2) is given by

The variance of $\hat{\mu}$ is estimated by

where, $P = (X^T (I-Q) X)^{-1} X^T (I-Q)$.

The semi-parametric spatial regression model (1) and model (2) are applied to the data of spacing trial of coconut at CPCRI Research Center, Kidu and compared with the traditional method of eliminating the positional effect by blocking the experimental area. The experiment was laid out in randomized block design with 9 treatments (spacing). The main objective of the experiment is to compare the effect of different spacing on the yield of coconut. Two years average yield data has been taken as the response variable. Observations on a total of 560 coconut palms were considered for the study. The spatial location is taken based on the U-V coordinate axis.

The estimated treatment means with standard errors obtained from the analysis of coconut yield data based on the method of blocking and spatial techniques are given in Table 1. The fitted spatial function for the positional effect (Fig 1) indicates that the blocks are not homogeneous. The spatial function for the positional effect is fitted after eliminating the treatment and block effects from each observation. If the blocking is effective to eliminate the positional effect, the fitted spatial function will be a plane without showing any spatial trend.

Table 1. Estimated parameters with standard errors

Spacing	Method of blocking		Spatial model (1)		Spatial model (2)	
	$(\mu+\beta)$	SE	$(\mu+\beta)$	SE	$(\mu+\beta)$	SE
6mx8m	48.0	3.6	42.3	3.6	42.3	3.5
6mx9m	53.1	2.8	56.0	2.8	55.9	2.7
9mx9m	58.3	4.5	56.4	4.0	56.5	3.9
5mx8m	46.7	4.0	46.7	3.9	46.6	3.8
5mx9m	43.0	3.1	52.8	3.0	52.8	3.0
7mx7m	41.6	4.9	41.5	3.9	41.5	3.7
4mx9m	27.9	2.6	25.0	2.6	25.0	2.4
8mx8m	44.5	2.8	47.6	2.7	47.7	2.7
4mx8m	18.8	2.5	18.1	1.9	18.1	1.8

But in the present case, the fitted function shows clear spatial trend and it indicate that the traditional method of blocking to eliminate positional effect is inadequate. The standard errors of the estimates obtained using spatial methods are comparatively smaller than that of method of blocking (Table 1). In the case of blocking, it is assumed that the area within the block is homogeneous and this might not be true always particularly when the experiment with coconut require large area. Taking the same positional effect value for all the palms within a large block will increase the experimental error particularly when

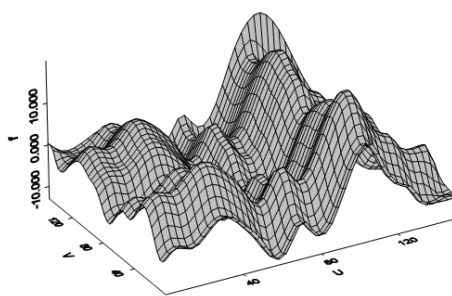


Fig.1. Spatial model (2) fitted for the positional effect

there is trend in soil fertility or any other factor which affects the parameter under study. In the case of spatial technique the positional effect is considered as a smooth spatial function and the positional effect of each experimental unit (palm) is estimated separately based on the performance of the nearby experimental units. If the number of observations is very large, spatial model (1) is appropriate to eliminate positional effect in field experiments and if the number of observations is less, model (2) is more adequate. The performance of spatial model (1) and spatial model (2) are almost same even though model (2) performs slightly better than model (1) (Table 1). This shows that the in the present study, the model (2) with two smooth functions in U and V directions is more adequate to explain the spatial effect than model (1). The graphical representation of the fitted spatial model (2) is shown in Fig. 1. The shape of the fitted model indicates that, dividing the experimental area into homogeneous blocks is very difficult.

Positional effect is a major factor for the experimental error in field experiments. We generally use block designs in field experiments to control the experimental error due to positional variations. The underlying assumption in classical block designs that the homogeneity of experimental area within the block may not satisfy always, particularly when the block size is large. Also we may not know in advance the soil fertility gradient and other factors influencing the response variable to divide the experimental area into homogeneous blocks. Field experiments with coconut require large experimental area, and it is grown generally in hilly areas where getting large homogeneous area is difficult. Under the above situations, the spatial smoothing technique which does not require much assumption about the distribution of soil fertility or other characters influencing the positional effect, is more useful. The analysis of coconut field experimental data shows that there is considerable reduction in standard errors for comparing treatment means by using spatial technique than the traditional method of blocking.

References

- Gilmour, A.R., Cullis, B.R. and Verbyla, A.P. (1997). Accounting for natural and extraneous variation in analysis of field experiments. *J. Agric. Biol. Envir. Stats* 2:269-273
- Hastie, T.J. and Tibshirani, R.J. (1990). *Generalized Additive Models*. Chapman & Hall, London

- Jose, C.T. and Ismail, B. (2001). Nonparametric inference on jump regression surface. *Journal of Nonparametric Statistics* **13**:791-813.
- Ruppert, D. and Wand, M.P. (1994). Multivariate locally weighted least squares regression. *Annals of Statistics* **22**:1346-70.

International Conference on Coconut Biodiversity for Prosperity

International Committee

International Advisory Committee

Dr. Emile Frison, DG, Bioversity International
Dr. C. V. Ananda Bose, IAS, Chairman, CDB, Kochi
Dr. H. P. Singh, DDG(Hort.), ICAR, India
Dr. Carlos B. Carpio, Dy. Administrator, ARI, Philippines
Dr. Hugh Harries, UK, Dr. Carlos Oropeza, CICLY, Mexico
Dr. Mike Foale, Australia

Organizing Committee

Chairman:

Dr. H. P. Singh, DDG(Hort.), ICAR, India

Secretary:

Dr. George V. Thomas, Director, CPCRI, Kasaragod, India

Members:

Dr. Stephan Weise, Bioversity International
Dr. Arancon Jr. Romulo, Executive Director, APCC, Indonesia
Dr. C. Jayasekara, Director, CRI, Sri Lanka
Dr. Hengky Novariantio, Director, ICOPRI, Indonesia
Dr. Jean Louis Konan, Coconut Programme, Cote de' Ivoire
Dr. K. V. Ahamed Bavappa, Former Director, CPCRI
Dr. P. Rethinam, Former Executive Director, APCC
Mr. Thomas Mathew, CCDO, CDB, India
Mr. P. K. Thampan, President, PTCDF, India
Dr. Prem Mathur, Bioversity International
Dr. D. Balasimha, President, ISPC, India