R.P.F. III

FINAL REPORT

1. Institute Code No. **Path XVI (231)**

2. I.C.A.R. Code No. **PI-99/ICI-H20/0311**

3. Name & Address of Research Institute/Centre

CPCRI, Kudlu P.O., Kasaragod – 671 124

4. Project Title

"Integrated control of leaf rot in root (wilt) affected coconut palms" under the mega project "Phytoplasmal diseases of palms"

5. Name & Designation of Principal Investigator

Dr. Alka Gupta, Scientist (Senior Scale)

6. Name(s) and Designation(s) of Associate(s) and Establishment(s) on which borne

(a) Whole time **Nil**

(b) Part time

Dr. Alka Gupta, Scientist (Sr. Scale) – 6 personmonths

Dr. N. Srinivasan, Principal Scientist – 3 manmonths

Mr. M. Gunasekaran, Scientist (SG) – 3 manmonths

7. Location of Research Project with complete address

MLO Section, CPCRI Regional Station, Kayangulam
Krishnapuram – 690 533, Kerala

8. Date of start **Feb. 1999**

9. Date of termination **Feb. 2002**

10. (a) **Objectives**

1. To record the incidence of root (wilt)/leaf rot disease and their interrelationship in Dindigul District of Tamil Nadu
2. *In vitro* antagonism studies against fungal pathogens of coconut leaf rot disease
3. Identification of selected microbial cultures
4. *Ex vitro* biocontrol studies
5. Preliminary field trials with antagonists

10. (b) **Practical utility**

The field survey for root (wilt)-leaf rot incidence in Cumbum and Dindigul Districts of Tamil Nadu will give an idea as to the extent of disease prevalence in various blocks of these districts : The interrelationship of leaf rot with root (wilt) disease also could be established.

Biological control agents against coconut leaf rot pathogens would help in developing an integrated disease management schedule for coconut leaf rot disease.

11. **Technical Programme**

Field survey for root (wilt) –leaf rot incidence in Dindigul district of Tamil Nadu, serological testing of spindle samples collected from root (wilt) affected coconut palms.

Biological control of leaf rot disease using antagonists, *in vitro* antagonism studies, assessment of promising antagonists *ex vitro* in detached coconut leaflets, identifying these selected microbial cultures and preliminary studies under field conditions.

12. **Final Report on the Project**

Final report on the project is furnished in Annexure – I.

13. **Progress of work in relation to the time targeted for completion of work and reasons for non-achievement of targets, if any**

The work progressed as per the schedule.

14. **Publications**

(a) Research papers

(b) Popular articles

(c) Reports

(d) Book chapters

List enclosed in Annexure - II

15. **Details of Field/Laboratory note books and their final location**

Project files/Notebooks Vol. I, II and III maintained at CPCRI, Kasaragod and Regional Station, Kayangulam.

16. **Material developed such as new varieties of crops or breeds of farm animals, implements, products, etc.**

i. Mass multiplication protocols were developed for native antagonists of leaf rot disease pathogens using cheap liquid substrates.

ii. Methods for field application of leaf rot antagonists were standardized.

iii. Talc-based commercial formulation of leaf rot antagonists was developed.

iv. Various isolates of leaf rot pathogens, *Colletotrichum gloeosporioides* and *Exserohilum rostratum*, from different locations in Kerala and Tamil Nadu, were characterized using molecular techniques like RFLP and RAPD (work done at UK).

ANNEXURE – I

FINAL REPORT ON THE PROJECT

Field survey for root (wilt) – leaf rot disease incidence in Dindigul district of Tamil Nadu

The incidence of root (wilt) – leaf rot disease in Cumbum valley of Tamil Nadu came to light in 1998. A sample survey was initiated to gain more information on the distribution of root (wilt)/ leaf rot and their interrelationship in Theni district of Tamil Nadu. Representative gardens in different locations were randomly selected under all blocks of the district *viz.* Cumbum, Uthamapalayam, Chinnamanur, Bodinayakkanur, Theni, Periyakulam, Andipatti and Kadamalaikundu blocks. The palms in these gardens were examined for presence of root (wilt) – leaf rot.

Occurrence of root (wilt)- leaf rot in all the blocks of Theni district was detected. Overall comparison of the disease distribution revealed that severely infected gardens occur (even upto cent per cent incidence in certain individual gardens) in Cumbum block (south/ south-west part of the district). Rampant expression of the symptoms of root (wilt)- leaf rot are seen in gardens of the block, especially in the jurisdictions of Lower Camp. Gudalur, Cumbum, Surilipatti and Kamayakaundanpatti. In the other blocks of the district, the disease incidence could be generally considered as sporadic, even as the disease was suspected in several gardens and occasionally severely affected palms observed in various locations.

With Cumbum block as the base, a disease gradient in the district is apparent in Western, Northern and Eastern directions of the district (e.g. Bodinayakkanur, Periyakulam and Varusanadu), where sporadic incidence of root (wilt)/ leaf rot was seen. As such, thousands of palms were found to be infected with root (wilt)-leaf rot in the district. The leaf rot syndrome was invariably sighted in gardens where root (wilt) incidence was clearly seen thus confirming the interrelationship of leaf rot with root (wilt).

Representative spindle leaf samples of root (wilt)/ leaf rot infected palms, from various locations of the district, were collected and processed for serological analysis and laboratory examination. Out of 31 palms sampled from the district, 30 palms reacted positively against the root (wilt) antiserum. Consistent association of leaf rot disease (LRD) fungal complex was observed from the LRD affected spindle samples. The fungi which could be isolated were *Colletotrichum gloeosporioides, Exserohilum rostratum, Fusaroium moniliforme, Thielaviopsis paradoxa*, etc.

A few gardens (at Pattiveeranpatti, Ayyampalayam and Sithayankottai) in Dindigul district of Tamil Nadu were also visited as the incidence of root (wilt) was suspected there also. Sporadic occurrence of root (wilt) disease and superimposition of leaf rot in the affected palms was observed in various gardens of this region. Six samples of spindle leaves were collected from root (wilt) affected palms in the region and subjected to serological testing. All six samples gave strong positive reaction against the root (wilt) antiserum, thus, confirming the spread of root (wilt) disease in coconut gardens of Dindigul district of Tamil Nadu also.

During the course of the sample survey, a special orientation training on root (wilt) – leaf rot was imparted to the officials of Tamil Nadu Agricultural Department (Theni District) for creating a mass awareness about the disease and also to augment more data on the disease distribution in the district.

Table 1: Incidence/general distribution of coconut root (wilt)-leaf rot in different blocks of Theni district, Tamil Nadu

Block	No. of coconut gardens examined	Disease status
Cumbum	20 gardens (Lower camp, Gudalur, K.G.Patti, K.M.Patti, Kamayakaundanpatti, Surulipatti, Cumbum, C.Pudupatti, Hanumanthanpatti)	Severely root (wilt) infected gardens were seen at Lower camp, Gudalur, Cumbum, Surilipatti, Kamayakaundanpatti, etc. Rampant incidence of leaf rot was also seen in these gardens. In other locations, disease occurrence was moderate to low.
Uthamapalayam	12 gardens (Uthamapalayam,Gohilapuram, Rayappanpatti, Kombai, Pannaipuram, Thevaram)	Low to moderate extent of root (wilt) incidence and in certain locations, disease suspects were observed. Leaf rot affected palms with clear symptoms of root(wilt) were seen in some gardens.
Chinnamanur	15 gardens (Chinnamanur, Karunkattankulam, Markayankottai, Kuchanur, Appipatti, Kanniservaipatti, Erasakkanaikanur, Odaipatti, Seepalakottai)	Sporadic incidence of root (wilt) in several locations of the block was evident. Leaf rot was also sighted in gardens where typical symptoms of root (wilt) could be noticed.
Bodinayakkanur	13 gardens (Bodinayakkanur,Kurangani, Kattapalam, Kottakudi, Thoppupatti, Kodangipatti, Meenakshipuram, Boothipuram)	Sporadic incidence of root wilt, but with clear symptoms, was recorded in several places. In one garden at Boothipuram, even severely affected palms were seen. Leaf rot syndrome was visible in most of the root wilt affected gardens.
Theni	16 gardens (Bommaiyakaundanpatti, Allinagaram, Theni, Veerapandi, SPS Coloney, Koduvilanpatti, Sriranga-puram, Venkatachalapuram)	While disease free gardens could be seen in certain localities, sporadic incidence of root wilt (even with severe symptoms) was seen at Bommaiyakaundanpatti, Veerapandi, Koduvilanpatti etc. Clear syndromes of leaf rot was also seen in such gardens.

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Block	No. of coconut gardens examined	Disease status
Periyakulam	30 gardens (Chothuparai, Vadakarai, Thenkarai, Periyakulam, Lakshmipuram, Kailasapatti, T.Kallupatti, Kanguvarpatti, Vadugapatti, Themaraikulam, Silarpatti, Jayamangalam, Koilpuram, Gullapuram, Vaigai Dam)	In this block among the gardens examined a mixed situations like disease-free gardens, disease suspects and sporadic root wilt incidence etc. were recorded. In few gardens (eg. at Periyakulam, Jayamangalam) even root wilt severely affected palms were seen. Leaf rot was also found to occur in a similar pattern.
Andipatti	6 gardens (Andipatti, Prathukaranpatti, Kunnur)	While certain gardens were found disese free, stray incidence of root wilt was seen in others. In one garden (at Prathukaranpatti) root wilt-leaf rot symptoms were observed in several palms.
Kadamalaikundu	8 gardens (La Thottam, Ayyanapuram, Kadamalaikundu, Mayiladumparai, Dharmarajapuram, Varusanadu)	In this block also while certain gardens were found disease-free, disease suspects and stray incidence of root wilt were seen in few others. In two gardens, clear symptoms of root wilt-leaf rot in few palms were observed. Co-occurrence of root wilt-leaf rot here also was evident.

Table 2 : Sampling of root wilt affected coconut palms from various locations of Theni Dt. and from Dindigul Dt. of Tamil Nadu, and their serological reaction to root wilt antiserum*

District/ Block	Specific place from where spindle leaf sampled	No. of spindles collected/subjected to serology	No. of spindles reacted positively**
Theni dt.			
Cumbum	Cumbum	9	9
	Kamayakaundampatti	3	3
Uthamapalayam	Rayappanpatti	3	2
Bodinayakkanur	Bodinayakkanur	5	5
	Kattapalam	1	1
	Boothipuram	1	1
Theni	Veerapandi	3	3
	Bommaiyakaundanpatti	3	3
Periyakulam	Periyakulam	3	3
	Total	31	30
Dindigul dt.	Sithayankottai	7	7
	Grand Total	38	37

*In most of the root wilt sampled palms, the leaf rot syndrome was also observed

**The positive reaction of these samples to root wilt antiserum was graded as strong to very strong

Biological control of leaf rot disease using antagonistic microorganisms

Microorganisms (bacteria, fungi and actinomycetes) were isolated from leaf rot affected leaves of coconut and from the rhizotic zones of leaf rot affected coconut palms. A total of 96 bacterial isolates from phylloplane and 21 bacterial isolates from rhizosphere were obtained and purified. In addition to this, 58 fungi (9 from leaf rot affected leaves and 49 from rhizosphere soil) and four actinomycetes cultures (from leaf rot affected leaves) were also purified. Both selective and non-selective media were used for isolation.

All the isolates were subjected to antagonism studies (by dual culture method) against the two main fungal pathogens of leaf rot disease – *Colletotrichum gloeosporioides* and *Exserohilum rostratum*. After repeated screenings, four bacterial isolates (two from rhizosphere and two from phylloplane) and two actinomycetes (from phylloplane of LRD affected coconut palms) were selected for further studies.

The bacterial isolates were biochemically characterized and identified as fluorescent *Pseudomonas* spp. (two in number, from rhizosphere) and *Bacillus* spp. (two isolates from phylloplane). The actinomycetes isolates were identified as *Streptomyces* sp. and *Thermomonospora mesophila* based on morphology and marker chemical constituents of cell wall membrane and whole cell hydrolysates. The *T. mesophila* is gram negative, free-living, produced vegetative branching filaments (0.5 – 1.0 mm in dia.) that were stable (i.e. did not fragment) and which bore aerial hyphae bearing single spores. On agar surface, it formed leathery colonies. The isolate is aerobic, lacked any diagnostic sugar/amino acid and optimum temperature requirement for growth ranged from 38 to 41 °C.

These microbial isolates were taken up for further studies on their biocontrol effects on two main fungal pathogens of leaf rot disease. Two fluorescent *Pseudomonas* spp. were tested *ex vitro* in detached coconut leaflets under laboratory conditions. Both the isolates checked the infection of the coconut leaflets by the two fungi to a good extent. However, the combination of the two isolates gave best results.

The effect of epiphytic *Bacillus* spp. was studied on disease development on detached coconut leaves. Both the antagonists significantly inhibited the lesion development by the two main pathogens of LRD – *Colletotrichum gloeosporioides* and *Exserohilum rostratum*, which were applied separately as well as in combination. The two antagonists were found to act synergistically (when applied together) against the combined inoculum of both the fungal pathogens, inhibiting lesion development by them to an extent of 82 %.

The bacterial antagonists of leaf rot pathogens, namely, *Pseudomonas* spp. and *Bacillus* spp. were screened for antagonism against fungal pathogens of leaf rot disease, other than the two main pathogens, by employing dual culture method. The fungal pathogens tested were *Fusarium solani*, *Rhizoctonia solani* and *Cylindrocladium scoparium* and were isolated from leaf rot affected leaf samples collected from various locations in Kerala and Tamil Nadu. Different isolates of *Fusarium solani*, *Rhizoctonia solani* and *Cylindrocladium scoparium* varied in their antagonistic responses towards *Pseudomonas* spp. and *Bacillus* spp.

For ecological monitoring of introduced antagonistic bacterial strains and to study their survival on coconut leaves, intrinsic antibiotic resistance markers were developed. The epiphytic bacterial antagonist, identified as *Bacilllus* sp., was susceptible to Gen 20, Str 250, Cm 50, Pen 100, Neo 100, Tet 100, Nal 100, Kan 100, Rif 2 and resistant to Tmp 1000 and Amp 1000, whereas *Pseudomonas* sp. was Gen –250, Tmp –250, Amp +1000, Str +1000 and Cm +1000.

A field observation that wherever prophylactic leaf axil filling with neem and marotti cake is being undertaken for *Rhynchophorus ferrugineus* (red weevil), the severity of leaf rot is reduced, prompted us to initiate a laboratory experiment, whereby effect of different concentrations of neem and marotti cake was studied on growth of *Colletotrichum gloeosporioides* and *Exserohilum rostratum*. Both neem and marotti cakes were found to stimulate the growth of both the fungal pathogens, although to varying degrees (ranging from 32 % to 100 % increase in fungal biomass). The oil cakes

might have been providing nutrients for the growth of fungi and at the same time might have boosted the level of antagonistic saprophytes to a much higher degree which in turn would have caused reduction in the disease severity.

Table 3: Isolation and collection of *Fusarium solani*, *Rhizoctonia solani* and *Cylindrocladium scoparium* isolates from various locations in Kerala and Tamil Nadu

Fungal Pathogen	Location	District	State
Fusarium solani			
KTM-1	Meenachil	Kottayam	Kerala
KTM-2	Meenachil	Kottayam	Kerala
KLM-1	Elampal	Kollam	Kerala
ALP-1	CPCRI farm	Alleppey	Kerala
TCR-1	Ayyanthol	Trichur	Kerala
TCR-2	Urakam	Trichur	Kerala
TCR-3	Vallachira	Trichur	Kerala
TCR-4	----	Trichur	Kerala
TNI-1	Bodi	Theni	Tamil Nadu
Rhizoctonia solani			
		Trichur	Kerala
TCR-1	Ayyanthol	Alleppey	Kerala
ALP-1	CPCRI Farm		
Cylindrocladium scoparium			
TCR-1	Ayyanthol	Trichur	Kerala
KLM-1	Vellakudi	Kollam	Kerala

Table 4: Antagonistic response of epiphytic bacterial isolates towards different isolates of LRD fungal pathogens (mean of 4 replications)

LRD pathogen	Inhibition zone (mm*) in PDA	
	B.brevis	*B. coagulans*
Fusarium solani		
KTM -1	3	0
KTM-2	4	2
KLM-1	2	3
ALP-1	2	0
TCR-1	0	4
TCR-2	3	2
TCR-3	4	5
TCR-4	2	4
TNI-1	0	2
Rhizoctonia solani		
TCR-1	<2	0
ALP-1	<2	0
Cylindrocladium scoparium		
TCR-1	<2	0
KLM-1	0	0

Table 5: Antagonistic response of rhizospheric bacterial isolates towards different isolates of LRD fungal pathogens (mean of 4 replications)

LRD pathogen	Inhibition zone (mm*) in PDA	
	P. aeruginosa 1	*P. aeruginosa* 2
Fusarium solani		
KTM -1	2	5
KTM-2	0	3
KLM-1	0	<2
ALP-1	4	<2
TCR-1	4	5
TCR-2	<2	3
TCR-3	0	<2
TCR-4	4	<2
TNI-1	5	7
Rhizoctonia solani	0	0
TCR-1	<2	<2
ALP-1		
Cylindrocladium scoparium	0	0
TCR-1	0	0
KLM-1		

Field trial of leaf rot disease using *Pseudomonas fluorescens* culture

Field trial for biological control of leaf rot disease of coconut with talc-based commercial preparation of the bacterial antagonist – *Pseudomonas fluorescens* (Pf_1), obtained from Tamil Nadu Agricultural University, Coimbatore, was conducted in adult coconut palms. The pre-treatment status of leaf rot in 25-year old leaf rot disease affected palms was recorded under the categories of treatment and control (20 palms/category). The antagonist (commercial formulation) was applied onto the axil of the spindle region of the treatment palms @ 50 g of material suspended in 1 litre of water per palm. The antagonist material was also applied to the basins of these palms @ 100 g/palm. After pre-treatment evaluation, the first round of application was given in April- May 2001 and the second round was completed in September 2001. The control palms were maintained and observed without the application of the antagonist material. The disease incidence level in the emerging leaves of experimental palms was monitored. The preliminary observations indicated that the antagonist material does not have any deleterious effect on the spindle leaf tissues of the treated palms. Further observations are being recorded under the new institute project no. Path XX (231) : Management of leaf rot diseased in root (wilt) affected coconut palms.

Another field trial was initiated in coconut seedlings, using the commercial preparation of *Pseudomonas fluorescens* (Pf_1). The formulation was applied to the spindle region of five coconut seedlings @ 10g/seedling. The observations are being recorded under the new institute project no. Path XX (231) : Management of leaf rot diseased in root (wilt) affected coconut palms.

Field trial of leaf rot disease using native antagonists

Another field trial was initiated to study the effect of native bacterial antagonists on leaf rot affected coconut palms. For this purpose, experimental palms were identified in Blocks V, VI and VII of CPCRI Kayangulam Farm. These palms were marked and

indexed for leaf rot and root (wilt) disease intensity. The cultures of *Bacillus* spp. were mass- multiplied in liquid medium and applied in field palms. The treatments consisted of basin and spindle application separately as well as in combination. Control palms did not receive any application. Fifteen palms were maintained for each treatment category.

Pre-treatment observations were recorded for indexing of leaf rot disease, root (wilt) disease index and nut yield. Indexing for root (wilt) disease was based on visual observation of intensity and frequency of occurrence of three major symptoms of root (wilt) disease i.e. flaccidity, yellowing and marginal necrosis. Leaf rot disease intensity was indexed as per Srinivasan and Gunasekaran (1996). The treatments were first imposed in Aug.-Sept., 2001 and second application was done in Dec.2001- Jan 2002. Every three months after treatment, the palms were observed for nut yield, emergence of new inflorescences and leaf rot disease index. The results showed general improvement in the condition of the treated palms. Spindle application of *Bacillus* spp. was found to give the best results, with 57 % decrease in leaf rot disease index. The newly emerged spindle leaves were completely free of any rotting symptom in this treatment. Basin + spindle application of *Bacillus* spp. was also on par with this treatment.

Various mechanisms employed by native antagonists to effect biological control of leaf rot pathogens were also investigated. The results are presented in tables 6, 7 and 8.

Table 6 : Plant disease suppression mechanisms operated by biocontrol agents of leaf rot disease of coconut

Biocontrol agent	Antibiosis	Mycoparasitism	Production of			
			HCN	Siderophores	Volatile metabolites	Non- volatile metabolites
Pseudomonas aeruginosa	+	+	+	+	+	+
Pseudomonas sp.	+	+	+	+	+	+
Bacillus sp. LP-8	+	--	--	--	--	+
Bacillus sp. CR-17	+	--	--	--	--	+
Thermomonospora mesophila	+	+	--	--	--	+
Streptomyces sp.	+	+	--	--	--	+
Unidentified bacterium	+	--	+	+	+	+

Table 7: A summary of the biocontrol traits possesed by bacterial antagonists of leaf rot disease

Pseudomonas aeruginosa	*Pseudomonas* sp.	*Bacillus* sp. LP-8	*Bacillus* sp. CR-17	Unidentified bacterium
Produces fluorescent siderophores with very high affinity for ferric iron under low- iron conditions.	--	--	--	--
Also produces blue-green, water soluble siderophores. Initial studies suggest that these possess both hydroxamate and phenolate groups and hence, fall under the category of pyoverdins.	Produces water soluble and diffusible siderophores.	--	--	Siderophores are produced under certain growth conditions.
Produces more than one type of antibiotics including pyocyanin.	Produces more than one type of antibiotic compounds.	Antibiotics (water soluble) are secreted into the growth medium.	Antibiotics appear to be the main mode of inhibitory action.	A mixture of antibiotic compounds are produced.

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Produces HCN which may work through plant- induced resistance to certain pathogens.	Also produces hydrogen cyanide.	--	--	Hydrogen cyanide is produced.
Produces both volatile and non- volatile inhibitory secondary metabolites.	Produces volatile and non-volatile metabolites during the stationary phase of the growth.	Non- volatile, antifungal in character, secondary metabolites are produced.	Non- volatile secondary metabolites are produced.	Both volatile and non-volatile metabolites are synthesized.
Some kind of antifungal secondary metabolite – mediated hyphal interference is evident.	Hyphal interference in the zone of antibiotics elaboration and diffusion is seen.	--	--	--
Diversified nutrient utilization capability may confer competitive advantage for colonization on foliar surfaces.	Ability to use diverse substrates for its growth.	--	Able to utilize an array of growth substrates.	--

Table 8 : A summary of the biocontrol traits possessed by antagonistic actinomycetes

Thermomonospora mesophila	*Streptomyces* sp.
Produces red- coloured, water soluble, diffusible antifungal compound	Produces diffusible and water soluble antifungal compound, also produces pinkish- red coloured non- diffusible compound
Shows mycoparasitism/ hyperparasitism	Mycoparasitizes the plant pathogen (hyphal interactions)
Produces some non- volatile secondary metabolites which are inhibitory to fungal pathogens	Non- volatile secondary metabolites are produced

Molecular characterization and detection of the coconut leaf rot pathogens

Genomic DNA was extracted from 10 isolates of *Colletotrichum gloeosporioides* (Cg) and 7 isolates of *Exserohilum rostratum* (Er), the two important fungal pathogens of coconut leaf rot disease (LRD). Initial polymerase chain reaction (PCR) amplifications of the internal transcribed spacer (ITS) region of the rDNA was done using ITS4 and ITS5 primers. The length of the PCR product from both Cg and Er was estimated at 590 basepairs (bp).

PCR amplified internal transcribed spacer regions (ITS4 and ITS5) of Cg and Er were digested with 9 endonucleases (RE). The RE digests yielded banding patterns corresponding to two groups in Er and three groups in Cg. These were further confirmed by sequence analysis.

RAPD profiles of Cg and Er isolates with 10 random decamer oligonucleotides produced varying degrees of polymorphism in Cg whereas only the TNI-1 isolate had low similarity with other isolates of Er. The banding patterns were highly specific at the isolate level; so they could be used as 'fingerprints'.

Sequencing of the ITS4 to ITS5 region of 6 isolates of Cg and 4 isolates of Er was done. The nucleotide sequences of Cg isolates confirmed the separation into 3 groups and between groups the isolates show 99 % homology or more to one another with the exception of TNI-1 which was again shown to be quite different.

The sequence data was utilized to design the specific primers. A species-specific primer pair for Er, ERFWD1/ERREV1 was designed. The sequences of Cg isolates had only a few bp differences from those of other Cg pathogens from different host plants, found in the database. Hence, the specific primer pair CgInt/ITS4, already available for this

fungus, was adopted. The PCR conditions (concentrations of Mg++, primers, template, different Taq polymerases, cycling conditions and annealing temperatures) were

optimized for each pair of target primers. These primer combinations, CgInt/ITS4 and ERFWD1/ERREV1, amplified only the target fungus, Cg and Er respectively, and no amplification of the product was observed with DNA from any other fungi.

Methods have been standardized for extraction of genomic DNA from LRD infected dry samples and PCR amplification of the extracted DNA using target (specific) primers. PCR products identical in size to those amplified from pure fungal DNA were produced when primers CgInt/ITS4 and ERFWD1/ERREV1 were used with DNA extracted from LRD infected coconut leaf. No amplification product was observed with DNA extracted from healthy tissue.

The sensitivity of PCR detection was assessed using the PCR product of fungal DNA and DNA from LRD infected leaf. The amplified products from Er and Cg (if both were in the same infected leaf) could be visualized as separate bands at different distances in agarose gels.

ANNEXURE – II

LIST OF PUBLICATIONS

Research papers

1) N. Srinivasan and M. Gunasekaran. 1999. Coconut leaf rot disease complex – a review. *CORD*. **15**(1) : 33-65.

2) N. Srinivasan and M. Gunasekaran. 1999. *Fusarium solani* and *Fusarium moniliforme* in coconut leaf rot disease. *Indian Phytopathology*. **52**(2) : 160-162.

3) Gupta, Alka and Gopal, Murali. 1999. Plant growth – inhibiting rhizosphere microorganisms : a review. *Agricultural Reviews* **20**(2) : 117-122.

4) Gupta, Alka, Gunasekaran, M. Srinivasan, N. 2000. Isolation of bacterial antagonists from rhizosphere and their *in vitro* evaluation against pathogens of coconut leaf rot disease. pp. 132-133. In *Plant Diseases and their Control*. Eds. Shimai, Z., Guanghe, Z. and Huaifang, L. China Agricultural Scientech Press, Beijing. 171 p., ISBN 7-80167-243-7/S.165.

5) Gupta, Alka, Gopal, Murali and Tilak, K.V.B.R. 2000. Mechanism of plant growth promotion by rhizobacteria. *Indian Journal of Experimental Biology* **38**: 856-862.

6) Gunasekaran, M., Gupta, Alka and Srinivasan, N. 2001. Biological control of coconut leaf rot pathogens with *Pseudomonas fluorescens*. Paper presented in National Symposium on Eco-friendly Approaches for Plant Disease Management, CAS in Botany, University of Madras, Chennai, Jan. 22-24, 2001. p. 66.

7) Gupta, Alka. 2002. Important biocontrol traits of microbial antagonists of leaf rot disease of coconut. *Coconut Research and Development (CORD)* (in press).

Popular articles

1) N. Srinivasan and M. Sasikala. 1999. Coconut leaf rot disease. *Kerala Karshakan*. **45**(1) : 23-24 (Malayalam).

2) N. Srinivasan, J.J. Solomon, M. Sasikala, M. Gunasekaran, L. Geetha and G. Rajeev. 2001. Managing coconut leaf rot disease. *Indian Horticulture*. **46**(3) : 28-29.

3) Gupta, Alka, Gopal, Murali and Iyer, Rohini. 2001. Need for introducing the concept of PGPR in coconut crop production system for improved plant growth and establishment. *Indian Coconut Journal* **31**(11) : 12-14.

4) Koshy, P.K. and Gupta, Alka. 2001. Important pests and diseases of coconut in non-traditional coconut growing areas. *Bharatiya Nariyal Patrika* **12**(1) : 25-32.

5) Gupta, Alka, Gopal, Murali and Iyer, Rohini. 2002. Biological control – a promising biotherapy for plant disease management. *Indian Coconut Journal* (in press).

6) Srinivasan, N., Gunasekaran, M. and Gupta, Alka. 2002. Substantive evidences of complex fungal etiology of coconut leaf rot disease. *Indian Coconut Journal* **32**(12) : 12-15.

7) N. Srinivasan. 2002. Coconut leaf rot complex and perspective for the disease control – status report. *Indian Coconut Journal*. **32**(9) : 2-9.

8) N. Srinivasan. 2002. Coconut leaf rot complex and perspectives for the disease control – status report (Reproduced). *The Planter*. **78** : 203-216.

Papers presented in Conferences/Symposia/Seminars/Workshops/etc.

1) Gupta, Alka, Gunasekaran, M. and Srinivasan, N. 2000. Isolation of bacterial antagonists from rhizosphere and their *in vitro* evaluation against pathogens of coconut leaf rot disease. Paper presented in the First "Asian

Conference on Plant Pathology" held at Beijing, China from Aug. 25-28, 2000. Published in the Proceedings of the Conference ACPP 2000, China Agricultural Scientech Press, Beijing, People's Republic of China, pp. 268. ISBN 7-80167-010-8 (357 p.).

2) Gupta, Alka and Gopal, Murali. 2001. Microorganisms for biological control of pests and diseases in coconut plantation crop – achievements and problems (Oct. 29, 2001). Paper presented during the course on "Challenges and Opportunities of Biological Control in Plant Disease Management", Centre for Advanced Studies in Plant Pathology, IARI, New Delhi, Oct. 16-Nov. 5, 2001.

3) Gupta, Alka. 2002. Mechanisms involved in biocontrol activity of antagonists of fungal pathogens of leaf rot disease of coconut. In "National Symposium on Crop Protection and WTO- an Indian perspective", CPCRI, Kasaragod, Jan. 22-25, 2002. p.32.

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1) Reported to CPCRI Annual Report for 1999.

2) Alka Gupta. 1999. Microbiological Applications in Agriculture. *In* : Training Programme on "Coconut – Production, Improvement and Protection, CPCRI (RS) Kayangulam, June 7-18, 1999.

3) Deputation Report of visit to Beijing, China for attending and presenting a research paper in 1st Asian Conference on Plant Pathology 2000 (Aug. 25-28, 2000).

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6) Alka Gupta. 2000. Microbial control of plant diseases. *In* : Training manual "Pests, Diseases and Nematode Management in Coconut", CPCRI (RS) Kayangulam, Nov. 21-25, 2000.

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1) Gupta, Alka and Gopal, Murali. 2001. Microbial control strategies for disease management in context of coconut plantation crop, pp.101-122. In *Biological Control of Pests* (Ed. A.M. Deshmukh), Milind Publishers, Maharashtra, 193 p.

2) N. Srinivasan. 2001. Diseases and disorders of coconut and their management. In *Plant Pathology* (Ed. P.C. Trivedi), Pointer Publishers, Jaipur, pp. 194-254.

Deputations Abroad during the period of the project

1) Dr. Alka Gupta, Project Leader, was deputed to Beijing, China from 25.8.2000 to 29.8.2000 to participate in the First Asian Conference on Plant Pathology (ACPP 2000). She presented the output of the project in the form of a research paper in the conference.

2) Mr. M. Gunasekaran, Project Associate, was deputed to Rothamsted International, United Kingdom, for a period of one year from 1.10.1999 to 30.9.2000, for molecular characterization and detection of coconut leaf rot pathogens, *Colletotrichum gloeosporioides* and *Exserohilum rostratum* isolated from various locations in Kerala and Tamil Nadu.

3) Dr. N. Srinivasan, Project Associate, was deputed to Maldives from 6.6.2000 to 23.6.2000 for collection of coconut germplasm. He collected eight distinct accessions in the form of embryos.