REPORT ON COMPLETED ITEMS OF WORK

(1978-2000)

Institute code No. : ENT VII (231)

I.C.A.R. Code No. : PI – 78/3 –ICI – H 10/0311

Name and address of research Institute/Centre:

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

Project Title:

BIOLOGICAL CONTROL OF PESTS OF COCONUT

Name and Designation of Project Leaders

G.B.Pillai, Principal Scientist 01-01-1978 to 31-12-1991
B.Sathiamma, Senior Scientist 01-01-1992 to 31-03-1999
Chandrika Mohan, Scientist (Sr.Scale) w.e.f. 01-04 1999

Name(s) and Designation(s) of Project Associates including Project Leader

Sl. No.	Name and Designation	Time spent
1	G.B. Pillai, Principal scientist (Ent.)	1978- 1992
2	J.Antony Scientist S-1 (, (Ent.)	1978- 1979
3	K.S.Mohan, Scientist S-1 (Microbiol.)	1978- 1985
4	Mariamma Daniel (Scientist S-1)	1978-1984
5	S. Devasahayam (Scientist S-1)	1981-1983
6	B.Sathiamma (Sr. Scientist)	1981-1999
7	T.K.Danger (Scientist Sr. scale)	1988-1994
8	C.P. Ramachandran (Scientist S-1)	1989-1994
9	V.A. Abraham (Sr.Scientist)	1990-1994
10	S.K. Bhat (Tech. Officer)	1992-1994
11	K.R. Sreekumar (Scientist)	1992-1993
12	A.S. Sukumaran (Sr.Scientist)	1993-1995
13	Chandrika Mohan (Scientist, Sr. Scale)	1995-*
14	Murali Gopal (Scientist)	1995-*
15	V.K.Sosamma (Sr.Scientist)	1996-*
16	J.Gulsar Banu (Scientist)	1996-1998

*Continuing in the project

Location of the research project with complete address (Division/Section/Sub-center)

Biological control laboratory,

Entomology Section, Division of Crop Protection,

C.P.C.R.I. Regional Station, Kayangulam.

Date of start: 1978

Date of termination:

The present report is on completed items of work under the project. The project is being continued with few items of work

Objective

The project was initiated during 1978. It aims at conducting research on biological suppression of the important pests of the coconut palm by proper utilization of the indigenous and exotic natural enemies such as parasitoids, predators and pathogens; to evaluate the extent of pest suppression exerted by different control agents and to assess the resultant reduction *in* population density of the pests and their damage to the crop.

Practical Utility

Promising bioagents (parasites /predators/ pathogens) for the management of *Oryctes rhinoceros* and *Opisina arenosella* have been successfully established. Research on bioagents for *Rhynchophorus ferrugineus, Leucopholis coneophora*, phytophagous mites, and *Stephanitis typica* are being under taken.

Report of work done

Oryctes rhinoceros L.

Studies on the exotic predator, *Platymeris laevicollis* Dist.

Studies on the biology of *P. laevicollis* when reared on host materials like roaches, *Pyncnoscelus surinamensis*,the coconut leaf eating caterpillar *Nephantis seriopa* (larva) and red palm weevil *Rhynchophorus ferrugineus*(grubs) were made. The nymphs reared on roaches attained adult stage within 100-160 days, where as those reared on *Nephantis* took 150-210 days. Each nymph consumed 25-40 cockroaches and 30-40 *Nephantis* caterpillars respectively during the entire nymphal period. The adult bugs reared on roaches survived for more than 170 days and laid 110-130 eggs. A pre-oviposition period of one month was observed. Each bug consumed a total of 150 cockroaches during its entire life period. It was found that roaches were the most suitable host material for culturing the predator in view of their easy availability and bugs reared on them showed short developmental period, higher longevity and fecundity.

Efforts were made on mass rearing and field colonisation of this exotic reduviid predator. Laboratory reared predators were released on the experimental palms at CPCRI, Regional Station, Vittal. A total of 5190 adult bugs and1851 nymphs were released during 1978-1983. Observations of 100 experimental palms revealed 13.0% leaf attack and 1.0% fresh incidence, nil spathe damage, as against 59.2% leaf attack and 37% fresh incidence and2.5% spathe damage of the initial pre-treatment condition. Detailed observations of 19 experimental palms in the norm fixation plot receiving release of six fully grown nymphs of the predator per palm every quarter, revealed 32.2% leaf attack, nil spathe attack and 5.0% fresh incidence as against 45.7%, 5.0% and 26.2% respectively, of the pre-release condition. The red ant *Oecophylla smaragdina* was observed attacking and killing the nymphs of predator. However there was no buildup of predator population and the predators failed to establish themselves under field conditions

Baculovirus of *Oryctes*

Screening of *O. rhinoceros* for the natural incidence of disease was done using methods such as examination of the morphology of the midgut and its contents, by Giemsa staining of mid gut aspirate, serological detection by immuno-osmophoresis and bioassay tests which showed higher number of hypertrophied nuclei and acute stage exhibited characteristic ring stage formation within the nucleus. EM studies of the infected tissues revealed *Oryctes baculovirus* particles identical in shape and size of the Malayan *Baculovirus of Oryctes*.Field collections of *Oryctes* grubs from 64 locations around Kayangulam showed Baculovirus incidence up to 54.5%during 1983.

Effect of *baculovirus* infection on the longevity and fecundity of *O.rhinoceros* was studied under lab. condition. The 5th replication of trial completed during 1984 revealed that the infected male beetles lived for 31.8 days as against 55.8 days for healthy ones in lab. Diseased female beetles lived for 37.6 days compared to 59.6 days of control females. On an average the longevity of infected beetles were reduced by 40% and in nature this gives ample time for the diseased beetles to transmit the virus through breeding sites to other beetles/grubs. The fecundity of healthy females was on an average 47.3 eggs per beetle and 91.8% hatching rate. Infected females were rendered totally sterile and ovarian eggs could be detected on dissecting.

Evaluation of the longevity and fecundity of Baculovirus infected and healthy beetles collected from the field was done. The infected beetles lived for 10-30 days (average 24.5 days) recording 54.1% reduction in fecundity. The healthy beetles lived for 21-72 days (mean 53.3 days) and laid 0-67 eggs (mean 20.6 eggs). These results are in conformity with the results obtained from laboratory-reared baculovirus infected beetles tested. The L D $_{50}$ of *Oryctes baculovirus* was determined to be3.39x10^{-3} g of virus-killed grub at 30° c. Exposure of virus inoculam to 65,70 and 75° c for 10 minutes completely inactivated the virus suspension.

Method was standardized for mass production of Baculovirus in the lab. A method for the diagnosis of baculovirus disease by examination of the cells excreted by infected beetles was also developed. Infected beetles were confined in plastic basins containing phosphate buffer supplemented with antibiotics, to a depth of 15

mm. Buffer was collected on alternate days and purified by precipitation with 0.4M Nacl and 10% polythene glycol and stored at -10^0c. Based on this, a schedule for diagnosis of virus disease in field-collected beetle was standardized. Baculovirus particles were purified from excreta of infected beetles by precipitation with polyethylene glycol followed by a series of sucrose density gradient centrifugations.

Nucleic acid was extracted using phenol: water: isoproponol method. The yields were:

South pacific virus: 900µg DNA and 11.916 mg protein /2.4lit of faecal matter.

Kerala isolate: 2.484 mg protein and 195µg DNA/. 1.66lit. of faecal matter.

Field release experiments

(a) Minicoy, Lakshadweep

Survey for Baculovirus disease of *Oryctes* in Minicoy Island, Lakshadweep revealed the absence of virus disease in the natural population of beetles and grubs. This observation opened up the feasibility of introducing Baculovirus to the island for the biological suppression of *Oryctes* and work on this line was initiated during May 1983. Detailed pre-release observation on 10% sample palms revealed 56.60% leaf infestation, 31.06% spathe infestation and 39.25% fresh incidence on spindles. A total of 108 beetles (37 females and 71 males) collected from crowns of infested palms in the CPCRI farm Minicoy and adjoining areas were infected with Baculovirus and released in the farm. Post-release observations recorded after eight months revealed indications of establishment of the pathogen in the natural population of beetles and in the breeding sites. Beetles collected from the release site showed more than 40% viral infection. Post-release observations of 80 experimental sample palms showed 44.87% leaf attack, 6.44 % spathe attack and 5.0% fresh incidence.

Baculovirus of *Oryctes* introduced into Minicoy, in April 1983 effected significant reduction in crop damage and increase in the incidence of virus disease in the natural population of beetles as revealed from the data collected during may 1986. After two years of stabilization of the disease, a survey was undertaken to study the situation of palms and intensity of disease incidence in beetle population during December 1988. It was observed that there was further reduction in the leaf

damage and increase in intensity of Baculovirus disease incidence in the natural population of the pest. Fresh infestation and spathe damage was negligible (Table 1.).

Table 1. Performance of Baculovirus at Minicoy, Lakshadweep.

Observation	Leaf Damage (%)	Spathe Damage (%)	Fresh Incidence (%)	Baculovirus disease incidence (%)
Pre-release (April, 1983)	56.6	31.1	39.2	0.0
Post-release (January, 1984)	44.9	6.4	5.0	50.0
Post-release (November, 1984)	20.4	2.3	5.1	25.9
Post-release (September, 1985)	17.5	1.6	9.2	43.3
Post-release (May, 1986)	10.0	0.5	1.2	50.0
Post-release (December, 1988)	7.0	Only 4-5 cases recorded /2,000 palms	Negligible	62.0

A total of 514 breeding sites of beetles such as decaying coconut logs and stumps were scanned for studies on site occupancy of the pest. Out of these 61 sites (11.8%) lodged immature stages of the pest, of which 48 sites (78.3%) had single brood and 13 (21.7%) had multiple broods of the pest. The number of breeding sites having the remains of dead grubs, beetle etc. was only 80.

(b). Androth, Lakshadweep

Oryctes Baculovirus was introduced to Androth Island, Lakshadweep during April 1988. Pre-release observations of palms revealed 55.0% leaf damage, 7.3% spathe damage and 23.5% fresh incidence on spindles. There was no natural

incidence of Baculovirus disease as revealed from the observation made in 350 sample grubs collected from 55 breeding sites of the pest from all over the island. 111 beetles (60 males & 51 females) and 276 grubs were infected with Baculovirus and released in the island. Site occupancy of the pest in breeding sites was also studied. Population of grubs ranged from 5 to 40, pupa 1 to 20 and adult beetles 1 to 2 per breeding site. Adult beetles could be observed only in 10 sites sampled.

First round of post-release observation was recorded during December 1988. Reduction in leaf damage, spathe damage and fresh incidence on a spindle was recorded in two years (Table2.). Site occupancy of the pest also showed slight decline. About 36% of the grub population had contracted viral disease. 31 beetles were infected with Baculovirus and re-released in the low-lying area of Keechery block for augmenting the disease incidence.

Table 2. Performance of *Oryctes* Baculovirus in Andoth island of Lakshadweep.

Period of survey	Leaf damage (%)	Spathe damage (%)	Fresh incidence of spindle (%)	Baculovirus disease incidence
Pre-release (April, 1988)	55.0	7.3	23.5	0.0
Post-release (December, 1988)	43.0	3.0	15.7	35.6
Post-release (January, 1990)	13.5	3.2	5.9	60.6

Entire Island has been surveyed for the incidence of Baculovirus disease in the natural population of beetles/grubs. Visual observations indicated that the grubs collected from all over the island had contracted the disease. Light microscopic observations of the midgut of grubs also showed typical hypertrophied nuclei with characteristic ring formation and confirm with the visual observation. Giemsa stained gut tissues of apparently healthy grubs also showed symptoms of typical Baculovirus infection.

The observations on site occupancy of the pest in breeding sites revealed that the grub population had come down from 80 (pre-release) to about 5 per site

Population of pupae and adults also have been significantly reduced, which are only 0.02 and 0.08 per site respectively. The population of *Baculovirus* infected grubs with typical visual symptoms has also increased from 0.5 (December, 1988 survey) to 11.4 per site (Table3.)

Table 3. Site occupancy of *Oryctes* at Androth

Period of survey	Sites sampled	Number per site			
		Grubs	Pupae	Adults	Diseased grubs
April, 1988	50	80.1	17.8	2.1	0.0
December, 1988	45	7.4	0.1	0.6	0.5
January, 1990	58	5.4	0.02	0.3	11.4

(c) Andamans

Screening of *O.rhinoceros* beetles collected from Andamans for Baculovirus disease was done, 101 beetles collected from areas around PortBlair during 1985 were screened and found to be free of Baculovirus disease. This opened up the possibility of introducing *Oryctes* Baculovirus to Andaman's for the biological suppression of *O.rhinoceros*, a major pest in coconut and oil palm plantations

Eighty-six *Oryctes* beetles collected from Port Blair, Andaman's were infected with Baculovirus inoculum (prepared from infected grubs) and sent to CARI, Port Blair for release in the field. A total of 206 beetles were infected with Baculovirus inoculum and released at two locations in the island during may 1987 by the collaborating scientist from CARI.

(d) Chithilappally, Trichur

For calculation of impact of re-release of Baculovirus in an already infected of contiguous area, virus infected beetles were released in a heavily infested garden at Chittilappally, Trichur during July 1989. Post release survey was carried out during February 1990, which revealed general reduction in pest incidence and crop damage (table 4). Most significant observation is that spathes of the surveyed palms, which emerged after re-release of Baculovirus in July 1989, were not infested.

Two post-release surveys were conduced in July 1990 & march 1991 for the pest Incidence and crop damage. Post-release data revealed that there was drastic reduction in the intensity of pest infestation and crop damage, three years after a single re-release of the viral pathogen. Even though there was slight increase in leaf and spathe damage, there was no fresh incidence on spindles Light microscopic observation of the gut aspirate of beetles revealed presence of baculovirus disease in all the samples.

Table 4. Impact of re-release of baculovirus to an already infected contiguous area at Chittilappilly, Trichur Dist.

Observation	Percentage of infestation			
	Palms	Leaf	Spathe	Spindle
Pre-release July, 1989	100.0	34.44	12.50	68.18
Post-release February, 1990	64.29	23.76	0.00	50.00
July, 1990	64.71	27.07	0.00	17.65
March, 1991	89.47	33.96	6.52	0.00
September, 1991	68.18	27.38	1.89	13.63
August, 1992	22.73	6.66	0.00	0.00

The spathes emerged subsequent to re-release of *baculovirus* in the experimental area were almost free of beetle infestation showing that even in an already infested contiguous area also, re-release of the virus can bring down disease incidence

(e) CPCRI Kasaragod

Survey was conducted to study the intensity of infestation of rhinoceros beetle at CPCRI, Kasaragod farm in December 1990 for the re-release of baculovirus. Data collected from 80 palms in different blocks revealed 91.1% infestation in palms, 20.3% leaf attack, 0.7% spathe attack and 17.7% fresh infestation 50% of the population showed suspected baculovirus infection, 15% confirmed baculovirus infection and 35%were healthy.

(f) Oil palm -Palode

Since *O.rhinoceros* was found to breed in the leaf axils of oil palms having rotten failed bunches, a programme of screening beetle population /breeding sites and to release Baculovirus infected beetles was drawn up for implementation in the oil palm plantation at CPCRI Research Center Palode, Kerala. Among the fifty beetles screened for baculovirus incidence in areas around Palode, 30(60%) showed symptoms of Baculovirus infection. However, 25 *Oryctes* grubs collected from breeding sites such as oil palm bunch, inflorescence heaps, cattle dung pits etc. around the oil palm plantation did not show any symptom of Baculovirus infection. A total of 85 beetles artificially infected with Baculovirus were released in the plantation during July-August 1986. This experiment was the first attempt to evaluate the impact of re-release of Baculovirus in an already infected contiguous area.

Samples of *Oryctes* beetles (40 nos.) received from Agricultural Research station, Ambajipet, Andhra Pradesh were screened for Baculovirus disease incidence during 1988. Studies on the gross morphology of midgut and microscopic examination of smears of gut aspirate of beetles revealed symptoms of Baculovirus disease in 16 beetles, indicating the natural incidence of Baculovirus disease that area.

Other host for OBV

Preliminary studies on the microscopic examination of giemsa-stained smears of gut aspirate and gross morphology of midgut of *Xylotrupes gideon* L, collected from South Kannara dist. of Karnataka had indicated Baculovirus disease incidence in them. Subsequent electron microscopic examination of ultra thin sections of midgut tissues confirmed the occurrence of typical Baculovirus particles. The gut aspirate of infected beetles fed to healthy *Oryctes* grubs by mixing with the feed (cattle dung) also produced typical symptoms of Baculovirus disease. Thus it was confirmed that Baculovirus disease is prevalent in the natural population of *X.gideon*, which also infects coconut palms and causes more or less similar damage as that of rhinoceros beetle. A total of 67 beetles collected from coconut gardens selected at random from eight taluks, of Dakshina Kannada dist of Karnataka were screened for Baculovirus disease. An overall percentage of 52.24 disease incidence was found in the natural population of beetles.

Follow up observations

Follow-up observations on the release of *Oryctes* Baculovirus were recorded at Androth, Lakshadweep during February 1992. The results revealed remarkable reduction in pest incidence and very high occurrence of Baculovirus infection. The percentage of leaf damage has come down to 7.7-spathe damage to 0.3 and spindle damage to zero as compared to 55.0, 7.3 and 29.5% respectively of the pre-treatment condition.

The re-release of Baculovirus of *Oryctes* was continued at chittilappilly, Thrissur district, Kerala and pre and post-release observations on the incidence of the pest and crop damage were recorded. Drastic reduction in the percentage of infested palms, negligible leaf damage and zero spathe damage and fresh incidence was noted during the post release observations recorded during March 1992.

Re-release of Baculovirus of oryctes was continued at CPCRI farm, Kasaragod, an already infected contiguous area. Ten percent sample palms out of 640 palms were observed for the intensity of beetle infection and crop damage. Observations recorded during February 1992 revealed 84.4% infested palms, 21.9% leaf damage, 3.9% spathe damage and 20.3% fresh incidence. Percentage of disease infection recorded was 90.0% as compared to 50% of the pre-release condition.

Further studies.

To revive the work on OBV, from September 1995 onwards *Oryctes* grubs and beetles were collected from Trichur, Kottayam, Alleppey, Kollam, Trivandrum and from Minicoy and Androth Islands of Lakshadweep. The virus was recovered from the Lakshadweep samples during March 1996.Laboratory experiments to find an alternate feed material for maintance of the viral inoculum proved that coir waste, which is available in plenty, is a suitable and better replacement for cow-dung. Sterilized coir waste yielded higher OBV infected grubs at 48% as compared to 32% in sterilized cow dung; however, loss of the grubs and OBV inoculum due to secondary infection by bacterial septicemia was only 52% as compared to 68% in the same treatment. With this study, coir waste is currently being used as feed material for viral inoculated *Oryctes* grubs.

A three-year survey (Sept.1996-Aug. 1999) was initiated at Alleppey, Kollam and Kottayam Districts of Kerala to study the seasonal incidence of OBV,

Metarhizium mycosis and any other pathogen infection on *Oryctes*. Regular collections of this pest from various breeding sites were done from Ayiramthengu, Vavvakavu, Vallikavu, Thodiyoor, Thezhava, Karunagappally Choonad, Krishnapuram Purakkad, Thottapally, Alappuzha and Vazhappally areas. The collected samples were brought to laboratory and screened for diseases. Recording of the weather parameters like Temperature, Rainfall and Relative humidity was also done. From a total of 6627 grubs and 307 adults collected during this 3 years period, it was observed that 5% grubs and 22.1% adults carried natural OBV infection. 3.1% grubs had succumbed to *Metarhizium* mycosis and 19.7% grubs died of an opportunistic bacterial pathogen. The bacterium was identified as *Pseudomanas alcaligenes*. This bacterium is present in the gut of the grubs as a normal gut micro flora. When grubs come under stressed conditions, this bacterium could attain the status of opportunistic pathogen. Baculovirus infection was identified and confirmed as a biotic stress factor. Death of grubs due to *P. alcaligenes* reduced the OBV inoculum turnover during the lab maintenance of this virus pathogen, besides this bacterium also reduces the recycling capacity of the OBV in field limiting its action in the suppression of the pest. Other wise, OBV occurrence in the grubs and adults was negatively correlated to the minimum temperature. Relative humidity was directly related to *Metarhizium* infection while higher temperature suppressed it. *P. alcaligenes* infection was positively correlated to maximum temperature ands negatively to rainfall. The viral infection was observed to peak during September to March period. *Metarhizium* was prevalent during monsoon activity. The coincidental occurrence of the OBV in grubs and adults confirmed the route of transmission of the virus disease from diseased host to healthy host. Also the simultaneous occurrence of OBV and bacterial infection in grub proved that the viral infection acted as one of the stress factors helping *P. alcaligenes* to become pathogenic.

Androtт Assignment: In April 1999 a survey was conducted in the Androtт island of Lakshadweep and it was observed that 12.3% of the rhinoceros beetle grubs carried the baculovirus disease establishing the fact that it exerts control over the pest population over after 10 years of its release. Officials of the Agriculture Department of Androtт were trained on the identification, maintenance, mass – multiplication and field application of baculovirus and *Metarhizium,* during this survey.

Daignostic Method: An SDS- PAGE run of the healthy and OBV diseased midgut content of *Oryctes* grubs gave a clear-cut difference in the protein profile of the samples. This observation could help SDS-PAGE as a diagnostic tool for confirming OBV disease in unknown samples.

Metarhizium anisopliae

Survival of *Metarhizium anisopliae* in the breeding sites of rhinoceros beetle was studied. Culture of *M. anisopliae* containing either carboxyl methyl cellulose (CMC) or microcrystalline cellulose (Avocet) was found to elaborate enzyme associated with cellulose degradation. The most predominant enzyme was exo β-1, 4 glucanase(C 1 enzyme) whose activity on cotton and filter paper was maximum from the 8th day onwards. The levels of B-glycosidase and endo-cellulase were very low. The ability of *M. anisopliae* to produce cellulase-degrading enzyme has lot of significance, since it would help in the growth and proliferation of the fungus in cattle dung and other decaying organic debris.

Urease was found to be elaborated from clones of *M.anisopliae* on solid medium containing urea as the sole source of nitrogen as described by Hankin & Anagnostrakes (1977). Improvement of the selective medium formulated for isolation of *M anisopliae* was obtained by manipulating the concentration of Cetyl Trimethyl Ammonium Bromide (CTAM). A concentration of 30ppm was found to totally inhibit the growth of many fungal / bacterial contaminants of cow dung and to retard the growth of *M. anisopliae* slightly.

Of the different cereal and tuber substrates tried for mass culturing of *M. anisopliae*, tapioca chips and rice bran with waste fish meal extract / urea as 0.5% nitrogen supplement.(1.08g and 7.34g respectively /100g tapioca –rice bran mixture) and moist autoclaved wheat proved economically viable. This yielded 36.4x 10^9 and 45x 10^9 spores/100g of substrate.

The entomopathogen *Metarhizium anisopoliae* was successfully cultured using coconut water as the medium. The mycelial disc of the fungus (5 mm diameter) was inoculated in 200 ml of sterile 100% coconut water in 500 ml conical flasks under sterile condition and kept at 25^{0}C in an incubator. Spore counts and mycelial weights were recorded on the 10th, 12th & 13th day after inoculation. Average spore counts per ml on the 10th, 20th &30th day post inoculation were

643x10^4, 2457x10^4 and 5528 x10^4 and total dry mycelial weight 0.729 g, 1.63 g and 1.81 g respectively. .

Comparison of the dry mycelial weights and spore counts of the fungus cultured in Potato Dextrose Broth (PDB) showed that higher values of mycelical weight and spore count per ml medium was obtained in coconut water. Details are given in table5.

Table 5. Growth and sporulation of *Metarhizium anisopliae* in potato dextrose broth and Coconut water media (Mean of 10 replications)

Day of observ	Dry mycelial wt. per200ml medium		No. of spores/ml of culture medium		No. of spores/ml of dry mycelical wt.	
	PDB	CW	PDB	CW	PDB	CW
10^{th} day	0.612 g	1.028g	5.42x10^6	6.8x10^6	0.87x 10^4	0.66x 10^4
12^{th} day	1.419 g	1.662g	9.0 x 10^6	21. x 10^6	0.63 x 10^4	1.27x10^4
13^{th} day	2.168 g	2.493g	18.75 x 10^6	52.65 x 10^6	0.86 x10^4	2.11x 10^4

PDB – Potato Dextrose Broth CW – Coconut Water

Further studies indicated higher mycelial weights and spore counts in coconut water medium than those obtained in the potato dextrose broth. Further, the fungus produced more spores in filter sterile coconut water from nuts, than that autoclaved coconut water (table 6).

Trials were also conducted to culture the fungus in unsterile coconut water in the presence of different combinations of antibiotics and/or inhibitors viz. oxytetracycline, penicillin, cycloheximide, grisiofulvin and acetyl-trimethyl ammonium bromide. Though the above combination (except grisiofulvin) was effective for selective growth of the fungus on solid medium, it could not favour the growth of the fungus in a liquid medium like coconut water.

Table 6 Growth and sporulation of *M.anisopliae* in coconut water and potato dextrose broth.

Dry mycelial weight & spore production of *M.anisopliae* at different periods of culturing.						
	10 days		20 days		30 days	
Medium	Dry wt/ 100 ml Medium(mg)	Spores/ml (X 10^6)	Dry wt/ 100 ml Medium(mg)	Spores/ml (X 10^6)	Dry wt/ 100 ml Medium(mg)	Spores/ml (X 10^6)
Autoclaved coconut water	0.73	5.5	1.00	24.2	1.46	34.7
Filter sterile coconut water	0.65	10.7	0.83	27.2	1.45	41.0
Asepti.drawn coconut water from nuts.	0.59	9.6	0.97	29.7	1.56	46.7
Autoclaved coconut water pH-6.5	0.60	4.3	0.91	24.1	1.33	33.6
Potato dextrose broth	0.50	4.5	0.78	7.2	1.15	18.72

It is clear from the above data that coconut water is an ideal medium for mass culturing the fungus. Thus, culturing of a bio control agent is possible in a locally available agricultural material, which is being wasted now.

With a view to isolating more virulent strains of *M.anisopliae*, seven fresh isolates were made from field collected grubs of rhinoceros beetle during 1992 Growth behavior studies of these isolates showed similar characteristics with the isolate already maintained in the laboratory.

Studies were initiated in strain improvement of *M.anisopliae* with emphasis on chitinolytic, proteolytic and lipolytic activities of the fungus. Six strains of *M.anisopliae* were procured from Boyce Thomson institute, USA for study during 1992-93.Five cultures received from USA during1993-94 and local strain were maintained in coconut water medium for their identification by morphological and cultural characteristic. Production of enzymes by these strains on solid medium was

also studied. From the growth, morphological and spore characters, the cultures were tentatively identified as *M.anisopliae var. anisopliae* and the local strain as *M.anisopliae var. major*. No variation in the production of lipase, chitinase and protease was observed when these five strains were grown in solid medium.

Field applications of the coconut water cultured *M. anisopliae*, inoculated @10^{10} spores per m^3 cow dung to 5-cow dung heaps in farmers garden was done. The samples collected from the treated heaps showed the presence of the fungus @103-104 spores/10g soil as compared to the absence of the fungus in the heaps during the pre-treatment studies.

M. anisolpiae was successfully revived during 1996 in different fungal media viz. PDA, Sabourands Agar and Richards Synthetic media. To avoid the virulence loss of this pathogen, it is regularly passed through the host and re – isolated from the dead cadavers.

Evaluation the seasonal occurrence, intensity of infection and interaction if any, of *baculovirus*, green muscardine fungus, bacterium or any other pathogens associated with *O.rhinoceros* in nature

Studies were initiated during1995-96. *Oryctes* grubs from three locations viz. Krishnapuram, Karunagappally and Ayiramthengu were collected at monthly intervals from dead coconut palms/cattle dung pits and screened for the occurrence of the above pathogens and the resultant pathogenicity of grubs. Baculovirus infection varied from 0-60%. Mortality due to bacterial infection varied from 40-100%. None of the samples showed infection by green muscardine fungus. Studies indicated a clear-cut difference in the protein profile of the healthy and Baculovirus infected grubs of rhinoceros beetle.

Entomophilic nematodes

The nematode collected from the haemocoel of beetles during 1984 was tentatively identified as *Rhabditis* sp. Pathogenicity tests with the same carried out on beetles and grubs. There was 53.3% mortality of beetles, one month after treatment. In the case of grubs, 100% mortality was obtained during the course of three weeks.

Work on entomophilic nematodes was re-established during 1999 and is being continued.

Nephantis serinopa Meyr. (= *Opisina arenosella* Wlk.)

Studies on exotic ichneumonid larval parasitoids

***Eriborus trochanteratus* M.**

Laboratory multiplication of the exotic ichneumonid larval parasitoid *Eriborus trochanteratus* (Morley) was done on *Corcyra cephalonica* and *N.serinopa* caterpillars. There was a preponderance of males in the progenies emerged from both the hosts. Feeding adult parasitoids with Proteinx and Vit. E resulted in slight improvement of the female proportion. Those reared on *C.cephalonica* gave 70.87% males and 29.13% females while from *N.serinopa* 57.14% males and 42.86% females emerged. In an attempt to colonise the parasitoid under natural condition a total of 1049 parasitoids (651 females and 398 males) were released in the experimental plot at Thottappally during 1979-80.Fifteen cocoons of parasitoid were recovered from the release site during Aug-Sept1980 indicating their establishment. Four pupae were found to be hyper parasitised by *Brachymeria nephantidis*. Two releases were made during January and June 81.The experiment on colonization of the parasitoid had to be discontinued since June 1981, as there was no fresh build up of pest population in the experimental plot.

***Spoggosia bezziana* B.**

Rearing of the exotic tachinid *Spoggosia bezziana* Bar. was initiated with the nucleus culture obtained from Dr.R.Mahindapala, crop protection officer, coconut research Institute of Srilanka. From consignment of 175 puparia received in October 1978, 106 flies emerged. They were exposed to parasitisation to 1804 *N.serinopa* and *Herculia nigrivitta* caterpillars, from which 1006 puparia could be reared. Out of these 552 flies (368 males and 184 females) emerged. Low fecundity of flies and lower hatching rate of eggs were the problem met with the culturing.

***Bessa remota* A.**

Introduced the exotic tachinid parasite, *Bessa remota* Ald. (Tachinidae) from Malaysia. and reared initially on *Nephantis* was released on *N. serinopa* and *Herculia nigrivitta* caterpillars. Out of 5056 *N. serinopa* caterpillars exposed for parasitisation. 2551 puparia could be reared (50.4% parasitisation) where as 554 *H. nigrivitta* exposed for parasitisation yielded only 134 puparia (24.2% parasitisation). Attempts on colonization of the introduced parasitoid were done at two centers viz.

Ponmana, Quilon dist. and Kandampatti, Salem dist. T.N. At Ponmana center a total of 418 females, 310 males and 159 parasitised larvae were released and in Kandampatti Center. 260 females and 188 males were released. Results revealed no indication of establishment

Laboratory culturing of *B. remota* was done using *O.arenosella* caterpillars as hosts and attempts on field colonization of it were made. No indication of the establishment of released parasite could be obtained from any of the release sites receiving parasite release during 1981-1983. Efforts to parasitise the teak defoliator *Hyblea purea* caterpillars with *B.remota* also did not meet with success.

Studies on indigenous parasitoids

Intensity of natural parasitism

Observations on the intensity of natural parasitisation of *N.serinopa* pupae collected from the coastal and backwater tracts of Quilon and Alleppey districts of Kerala state during 1978-1979 revealed on overall parasitisation ranged from 28.2-59.4%. Among the different species of parasitoids, *Neobrachymeria nostoi* Habu. effected 13.1-38.86%, *Brachymeria nephantidis* Gaban 12.35-16.58%, *Antrocephabus sulcatiscutellum* Girault 0-3.9%, *Trichospilus pupivora* Ferr. 0.9-3.25% and *Xanthopimpla punctata* 0-3.9% (Table 7)

Table 7. Percentage of natural parasitism by different parasitoids on *Opisina*

Period	No. of pupae observ	Overall % parasit.	Parasitisation by species of parasitoids				
			B.nosatoi	*B.nephantidis*	*Androcephalus & chalcids*	*T.pupivora*	*X.punctata*
Jan-Mar`79	193	28.2	13.1	13.1	--	2.0	--
Apr-June`79	612	59.40	38.86	16.58	2.97	0.99	--
July-Sept`79	220	31.17	15.60	13.47	0.70	1.40	--
Oct-Dec`79	210	53.89	30.54	12.35	3.87	3.25	3.90

B. nosatoi was the dominant species of pupal parasitoid of *N. Serinopa* in the coastal tracts of Quilon and Alleppey dists. of Kerala, followed by *B. nephantidis*. A new species of *Xanthopimpla,* (Identified as *X. nana nana* Schulz *by* Prof. V.K. Gupta, Delhi University) was reared from pupae of *N. serinopa* for the first time during1980 and was found to be a dominant species in some localized tracts in coastal Kerala.

Studies on the position, nature and size of emergence holes made by different species of chalcids and other species of parasitoids gave some definite clue as to the species of parasitoid that emerged from the pupae. Thus evaluation of the extent of natural parasitisation of the pest could be undertaken by examination of pupal cases from the field.

B.hebetor

Biology & behavior of *B.hebetor* was studied in the laboratory on *O. arenosella* caterpillars (.Table.8) There was improvement in sex ratio male: female (5:1) in *B. hebator* when female parasites were allowed to mate once or twice only and each parasitoid was provided with adequate number of host for parasitisation. Mass multiplication can be done on larvae *Opisina* and *Corcyra.* Glass chimney sandwich method is used for this purpose.

Table 8. Biological parameters of *B. hebetor*

Particulars	Period (days)
Egg period	1.25 ± 0.44
Larval period	3.05 ± 0.68
Pupal period	4.38 ± 1.15
Longevity (male)	9.03 ± 1.84
Longevity (female)	8.94 ± 1.88
Fecundity (No. of eggs/ female)	229.1 ± 67.80
Sex ratio (Female: Male)	1:1

For field evaluation of the laboratory reared larval parasite *B. hebetor*, field releases of the parasite were done at fortnightly intervals at Ponmana during 1983. Data collected from the released plot revealed that the reduction in *Opisina* population was not significant as both the released plot and control plot recorded almost equal number of caterpillars. However, the mean number of infested leaves was more on the control palms as compared to that released palms.

Goniozus nephantidis* M. (*Perisierola nephantidis)

The bethylid parasitoid *Goniozus nephantidis* could be reared in large numbers using the fully grows caterpillars of *O.arenosella* and *Corcyra* as hosts. Early 6th instar caterpillars of *Opisina* are the ideal host stage for rearing *Perisierola nephantidis.* As it gave more number of progeny with higher percentage of females The caterpillars were parasitised individually in separate containers and transferred to specimen jar cages for further development of the parasitoid

***Elasmus nephantidis* R.**

Courtship, mating behavior & biology of pre-pupal parasite *Elasmus nephantidis* Roh. was done. Larval period lasts 3.5 days & pupal 6.5 days. Longevity of adults 12-31 days & fecundity 45-79 eggs. Life cycle is completed in 11 days.

***Xanthopimpla* species**

A technique has developed to mass culture *X. nana* and *X. punctata* in the laboratory using *O. arenosella* and *Anadevidia peponis* pupae as hosts. The lab-reared parasitoids were released in the experimental plot at Ponmana for evaluation. Culture material of *X. punctata* was supplied to regional research station, Paiyar, Kaveripattanam, Tamil Nadu for mass multiplication in pest-infested fields.

Another species of *Xanthopimpla* quite different from *X. punctata* and *X. nana nana* was recorded from *O. arenosella* pupae. It has five pairs of abdominal punctuations, while *X. nana nana* has 6 pairs and *X. punctata* 4 pairs. Its ovipositor is the shortest and tarsus and metathoracic legs are dark. Biology of this species was studied under a temperature range of 22-30°c and 45-80% RH. It had an incubation period of 30 hours; there was a pre-oviposition period of 3 days, egg period 29-30 hours, larval period 6-10 days and pupal period 5-9 days. The life cycle of the parasitoid was completed in 12-16 days in host pupae such as *O. arenosella, Sylepta derogata, Margaronia indica* etc where as in *Andevidia peponis* pupae it took 13-20 days. Longevity of adults was recorded as 1-4 months. *Xanthopimpla sp.* is smaller than *X. punctata* and *X. nana nana.* However, the adults reared on *A. peponis* were larger in size than those reared on *O. arenosella* and *S. derogata.* The adults reared on *O. arenosella* lived for longer periods. A lab culture of this parasitoid was built up.

Xanthopimpla punctata, X nana nana and Xanthopimpla sp are the three dominant species of pupal parasitoids affecting a high degree of pest suppression

towards the latter half of the year. These species breed in separate territories, where relative humidity is quite high, *and Xanthopimpla spp.* have greater searching ability and they colonise in areas of high pest population close to seashore or backwaters. Comparative studies on the bioecology of these species were made under identical conditions of temperature and relative humidity; *X. punctate* completed its total developmental period in 10-20 days *X.nana nana* in 12-21 days and *Xanthopimpla.sp* in 10-20 days. *X.punctata* is an outstanding example of a polyphagous species of parasitoid which turned out to be a better killer of the pest than even other host-specific and gregarious species of parasitoids of the pest. Average maximum parasitism by *X.punctata* was 43.59% in the territory occupied by the same

Evaluation of the field performance of lab. reared *Bracon hebator, X. punctata,* and *X. nana nana* was done.

High incidence of natural parasitism of *X. nana nana* was observed at Eravipuram. Out of a total of 52.4% effective natural parasitism, 31.6 % was by *Brachymeria nephantidis*, 4.9%by *Trichospilus pupivora*, 1.3% by *B. nosatoi*, 0.6% by *B. excarinata* and 0.9% by *Eurytoma albotibialis*. It was observed that high intensity of parasitism by *X. nana nana* adversely affected *B. nosatoi*. However the population of *B. nephantidis* was not affected. *Trichospilus pupivora*, which was absent in the tract after the draught during 1983, had regained its position during 1984. It is also significant that the other species of *Xanthopimpla* were not present in the area occupied by *X. nana nana*

***Brachycoryphus nursei* L.**

Bioecology of the inchneumonid parasitoid *Brachycoryphus nursei* Lam. was studied. The solitary ecto- parasitoid showed some interesting peculiarities in host acceptance, oviposition & development. It attacked the larvae, pupae and pre- pupae. Always deposited numerous eggs on a host eventually to produce only one individual parasitoid. Occasionally it developed internally in the host pupa also. Though a high fecund species, it possessed low searching ability, and effected only negligible percentage parasitism in *O. arenosella* in the field. Eggs were laid after keeping the ovipositor touching the paralysed host. Feeding on the hosts haemolymph was observed throughout the long life span of the female parasitoid. The larvae of *B. nursei* were cannibalistic. This parasitoid produced more female progeny than males in the field. However the reverse occurred in the laboratory. Mating took place under laboratory conditions. The females were polyandrous and the males were very

vigorous. Egg to adult stages of the parasitoid was completed in 13-21 days. However majority of the adults emerged in 14-17 days. It had a pre oviposition period of one day, egg period 26-29 hours and larval period 6-9 days. Females mated and feed on then hosts haemolymph on the day of emergence itself. Longevity of adults fed with undiluted honey was 1 to 4 months. In view of the preponderance of males in laboratory culture large-scale multiplication of this parasitoid was not attempted.

***Eurytoma alhotibialis* A.**

The hyper parasitoid, *Eurytoma alhotibialis*. Ashmead was reared from the cocoons of *B nursei* collected from the field .*E. albotibialis* recorded as a hyperparasitoid as well as a primary parasitoid of *O. arenosella* is predominantly a hyper parasitoid on the larval- pupal stages of primary parasitoids of the pest. Its peak activity in nature synchronizes with the peak activity of *Brachymeria spp. and* that of the pest itself during Apr.- May. Egg to adult stages of the hyper parasitoid is completed in 13-19 days. Adult longevity is more than 3 months and sex ratio is highly female biased. (Male: female ratio 1:6). The life cycle of this hyper parasitoid is almost comparable to that of *Brachymeria spp.* .It was observed that during April &May *E. albotibialis* suppressed 13.11%and 43.48% population of *Brachymeria* sp.in the field.

***Brachymeria* species**

A technique has been standardized for the mass culturing of *B. nosatoi.* Field release of laboratory-cultured chalcids was carried out during 1980 in a coconut garden showing severe infestation by the pest at Kuttanadu (Kerala). A total of 305 *B. nosatoi,* 280 *B. nephantidis*, 100 *B. attevae* and 100 *Antrocephalus* sp were released in the plot

Studies on courtship and mating behavior and comparative biology of 4 sps. of *Brachymeria* and *Antrocephalus* sp. associated with *N.serinopa* revealed that *B. nosatoi* possessed the major attributes of an effective bio control agent of the pest. *B. nosatoi* had a greater searching ability, being able to locate host pupae which remain inside cocoons in silken galleries and its ovipositor was sufficiently long to reach the pupae in the cocoons. The parasitoid had a long life span of more than 3 months during which a large number of pupae were parasitised.

Brachymeria sp. reared from *Nephantis* pupae collected from Salem area in Aug. 1981 was subsequently identified as *B. hime attevae* Joseph, Narendran & Joy.

Its intensity of natural parasitism also was high. 7.2% (25/351) as compared to that in West Coast. However, *B. nosatoi* and *Trichopilus pupivora* were totally absent in this tract, Unlike in West Coast, the intensity of parasitism by *Xanthopimpla punctata* was found to be as high as 26.5% out of a total parasitism of 49.3% as revealed from the observation made on 351 sample pupae. Examination of empty pupal cases collected from this area also revealed 26.7% parasitism by *X. punctata.*

***Trichospilus pupivora* F.**

The eulophid gregarious pupal parasitoid, *Trichospilus pupivora* completed life cycle in 16-19 days. The parasite was observed to aestivate in the pupal stage during summer months. Poor searching ability and dispersal ability, low non-optimal temperature tolerance, inability to recognize parasitised pupae renders it ineffective for the natural control of *Opisina*

***Apanteles taragamae* Wilk.**

Attempts to rear the braconid, *Apanteles taragamae* parasitic on the early instar caterpillars of *O.arenosella* in the lab. were initiated during 1983 but were not successful till 1988. The experiment were continued during 1989 by the method developed by Ghosh and Abdurahiman (1988) .Out of a total of 40 cages set with field -colleted or laboratory reared female parasitoids only seven adults (3 males +9 females) emerged .Egg to adult period varied from 12-20 days in the case of males and 14-24 days in the case females .Sex-ratio was 1:1.3 (male: female) .However further egg laying was not observed and as such the maintenance of culture was hampered Techniques were standardized for mass multiplication of this braconid endoparasitoid on coconut seedlings raised in field cage during 1994-95. . Females of *A.taragamae* were allowed to parasitise second instar caterpillars of *O. arenosella* in glass vials. The parasitoid larvae were allowed to grow on the seedlings and the emerged parasitoids collected for field release. Number of days taken for emergence of a brood ranges from 10-24 days. Further studies on this parasitoid is being continued

Egg parasitoids

Opisina infected leaflet samples were collected from the field from two locations viz. Thodiyur and Ayiramthengu at monthly intervals for studying the occurrence of the parasitoids and predators of the eggs of *O.arenosella*.No natural parasitism was noted. Only the cunaxid predators consumed the eggs. Observations indicated that *Cardiastethes* sp. *Amblyseius* sp. and spiders are the dominant natural enemies.

The egg parasitoid *Trichogramma embryophagum* was mass cultured on the eggs of *Opisina* and *Corcyra* in the laboratory. Egg to adult period was observed to be 10 days and 56.9% parasitism on *Opisina* eggs under laboratory condition. Field release revealed only 5-8% parasitism on *Opisina*.

Predators

Spiders

Twenty-six species of spiders belonging to twelve genera and six families were collected from the coconut palms infested with *Opisina arenosella*. (Table 9) The hunting spiders including species of *Cheiracanthium, Clubiona, Marpissa, Plexippus, Rhene* and *Sparassus* and the weaving spider *Tatragnatha* were widely distributed. Cheiracanthium constituted nearly 21% of the total spider fauna of the coconut palms. The spider associated with *O. arenosella* galleries on coconut leaves was identified as *Larinia jayasangari* Biswas in 1984. *Cheriancanthim sps.* consumed 131 *Nephantis* caterpillars in 92 days, followed by *Sparassus* (124 caterpillar), *Rhene indicus* (68) and *Plexippus payakulli* Aud.(50 caterpillars). Life history studies of four types that were found to be good predators were done. Studies on the biology of *Rhene indicus* showed that the female spider completed its life cycle in 70-98 days. Adult female spider had a life span of 71-266 days. Male spider completed its life cycle in 71-98 days and lived for 25-77 days.

Table 9. Spider fauna associated with *Opisina arenosella* infested coconut palms

Araneidae (=Argiopidae)	*Argiope catenulata* (Doleschall) *Larinia jayasankari* Biswas *Neoscona bengalensis* Tikader and Bal *N. elliptica* Tikader and Bal
Clubionidae	*Cheiracanthium melonostoma* Thorell *Cheiracanthium* sp. *Clubiona drassodes* Cambridge
Gnaphosidae	*Poecilochroa barmani* Tikader
Salticidae	*Marpissa anusuae* Tikader and Biswas *M. dhakuriensis* Tikader *M. tigrina* Tikader *Marpissa sp.* (Coll.No. 67) *Marpissa sp.* (Coll.No. 68) *Phidippus bengalensis* Tikader *Phidippus* sp. (Coll.No.7) *Phidippus sp.* (Coll.No.26) *Phidippus sp.* (Coll.No. 61) *Plexippus paykulli* (Aud) *Rhene danieli* Tikader *R. indicus* Tikader *R. khandalensis* Tikader
Sparassidae	*Sparassus* sp
Tetragnathidae	*Tetragnatha andamanensis* Tikader.

Studies on the seasonal abundance of spiders associated with *Opisina* infested coconut palms revealed that spider population was abundant from July to October, particularly the species of *Cheiracanthium, Sparassus, Rhene indicus* and *Marpissa tigrina.*

Predatory beetles

Studies on rate of predation, longevity and fecundity of carabid beetle *Parena laticincta* indicated that the beetles lived for more than 440 days, during which each beetle consumed 130 *N.serinopa* caterpillars and laid 230 eggs. Techniques for rearing the carabid predator *Parena nigrolineata* in the lab were developed and lab reared predators were released at Kannetty for evaluation of their performance. A total of 165 beetles were released in the plot since June 1983. Periodic releases of the predator and monitoring of the field was done till 1987. Analysis of data revealed that the mean population of *Opisina* in the release site was 7.37 as against 35.48 in the control. Biology of the predator *Calleida splendidula* Fabr. was studied and its feeding potential evaluated. The adult predator consumed 1 *Opisina* IVth instar caterpillars in every three days and 11-13 II ndinstar *Opisina* caterpillars during the entire grub phase. The adult beetles lived for a period of 6-14 months in the laboratory cages. The lab culture of the predator was built up. *C. splendidula* had an incubation period of 4-7 days.

Four species of coccinellid beetles such as *Microaspis discolor* (Fabr.), *Menochilus sexmaculatus* F., *Profilia fallax* Khnzorian and *Jauravia pubescens* (F.) were identified as egg predators. Other predators identified were *Idgia dimelaena* (Walk.) (Melyridae) grubs as pupal predators, *Creagris labrosa* Nietner (Carabeidae) as larval predators and *Ankylopteryx octopunctata candida* (Fabr.) (Neuroptera : Chrysopidae) as egg and larval predators of *O. arenosella.*

Mass rearing on artificial diet

Experiments on mass rearing on artificial diet were initiated in 1981. More than 25% of moths emerged showed wing deformity. The caterpillars completed life in 41-45 days (26-34° C and 41-87% RH) in the laboratory. The larval and pupal phases were completed in the diet in 42-48 days. Wing deformity in moths observed in earlier trials could be reduced by the addition of coconut oil into the diet. Fungal contamination of the culture during the rainy season was a major problem and this hampered the growth of caterpillars in the diet.

Sampling technique

A sampling technique was evolved for assessing population of *N. serinopa* and its parasite complex. Observations of 10 sample palms at Eravipuram centre at fortnightly intervals for developing a simple sampling technique to monitor Opisina population and its natural enemy complex in the field were concluded in Dec. 1983.

The data on estimated population and observed population during different periods are given in Table 10.

Table 10.Estimated and observed populations of *O. arenosella* *

Period	Estimated population	Observed population
Feb – Mar	1394	1495
Apr – June	2020	1399
July – Oct	1628	1256
Nov – Jan	1672	1659
Total	6724	5809

*** (sum of 46 observations)**

The 't' test indicated that the difference between the estimated and the observed populations was just significant (t = 2.49).

For assessment of the pest population in an infested garden, population is to be ascertained on 20% of the sample palms In each of the sample palm larvae, prepupae and pupae of the pest and associated parasitoids and predators present on 41-60% of leaf lets of 20% leaves from the lower or middle whorl are to be counted. Population appears in different intensities during different months and hence population was estimated for the periods of the year using the following formula.

Periods	Sampling
February to March	Y= 22.59+5.75x
April to June	Y=38.40+9.70x
July to October	Y=20.87+6.20x
November to January	Y=6.36+8.99x
Y= Estimated population X= Population count on sample leaflets	

Dosages for release of parasites

Experiment on working out dosages for release of parasites viz. *Perisierola nephantidis*, *Elasmus nephantidis* and *Brachymeria nosatoi* individually each at two levels viz. 25% and 50% of target stages of pest was concluded during 1983 and data presented in table 11.

Table 11. Dosages for release of parasites of *O.arenosella*

Level of parasite release (%)	Mean number of infested leaves			Mean pest population		
	Perisierola nephantidis	*Elasmus nephantidis*	*Brachymeria nosatoi*	*P. nephantidis*	*E. nephantidis*	*B. nosatoi*
0	26.67	26.67	26.67	20.08	20.08	20.08
25	5.25	2.69	6.75	3.96	3.21	7.48
50	4.88	7.08	9.58	34.14	4.19	11.58
CD 5%	5.86**	4.50**	5.21**	19.82**	6.83**	9.94**

The experiment on fixation of doses for release of parasites in combination was concluded during Dec. 1984. Data collected from the experimental plots at Eravipuram, Kannetty, where the 3 species of parasitoids were released individually and in combination were analyzed. Quadratic response curve were fitted for the host-parasitoid relationship and the optimum dose for release of the parasitoid for biosuppression of *Opisina* were worked out. Optimum dose for release of *Goniozus (Perisierola) nephantidis* Mues. was 20.5%, *E. nephantidis* Rohw. 49.4% and *B. nosatoi* Gahan 31.9% of the target stage of the pest, when these parasitoids were released individually and 40% when all the 3 species were released in combination.

Field release

. Seven consignments of laboratory reared parasitoids such as *Goniozus nephantidis*, *Elasmus nephantidis, Brachyrmeria nosatoi* and *Xanthopimpla punctata* were released in highly *Opisina* infested plantation at Kayamkulam kayal farm during 1986-1988 and at Trikkakara during Aug & Sept 1987. Three consignments were released at Thottappally where heavy incidence of *O.arenosella* was observed during 1988. Periodic monitoring of pest population and release of appropriate species of parasitoids in adequate numbers resulted in pest suppression. Fresh infection of *O.arenosella* was observed in the plantation at Thottapally during 1989, where the laboratory reared parasitoid were released previously for evaluation of their field performance .*G nephantidis, Elasmus nephantidis* and *Brachymeria*

nostoi were mass multiplied and two consignments of parasitoids were released during January and Feb, 1990.

The braconid parasitoid *Apanteles taragamae* Wilkinson and the bethylid parasitoid *Goniozus nephantidis* (Mues.) were mass reared and used for field evaluation. The nucleus culture was supplied to the zonal parasite breeding laboratories in the coconut growing states.

A field experiment on the performance of laboratory-reared parasitoids viz *Goniozus nephantidis* (Mues.) *Elasmus nephantidis* Rohn *and Brachyameria nostoi* Habu was stated in Thodiyoor Kollam dist Kerala. The natural enemies recorded from the area were *Apanteles taragamae* Wilkinson *B. nostoi* Habu *Xanthopimple punctata*, and *Parena* nigrolineata (Chaudoir). There was 61.7 emergence of moths 29.5% parasitoids and 6.1% hyper parasitoids when 747 field collected sample pupae of O. *arenosella* were kept under observation in laboratory. Pre and post release observations on the population of the pest and natural enemies were recorded. Release of the parasitoids was done @20.5% *Brachymeria nephantidis*, 49.4% *Elasmus nephantidis* and 31.9% *Brachymeria nostoi* in relation to the target stage of the pest. Sample palms (20%)were observed and population of *O arenosella* and the associated natural enemies recorded. Observations revealed significant reduction in the population of pest and a high percentage parasitism by the released parasitoids (Table 12)

Table 12. Performance of laboratory reared parasitoids on *Opisina arenosella*

Months	Infested leaves (%)	Mean population / sample leaf					
		Pest			Parasitoid		
		Larva	Prepupa	Pupa	Bethylid	Elasmid	Chalcid
Oct.1990	29.2	8.8	0.1	0.2	-	-	0.04
Mar.1991	26.7	4.3	0.3	0.5	0.01	-	0.20
Sep.1991	9.9	3.2	0.02	0.1	-	-	-
Mar.1992	6.2	3.2	0.1	-	-	-	-

The results revealed very significant reduction in the total population (larva+ pupa) and larval population.

Observations were recorded from a second garden comprising 300 palms where the bethylid parasitoid *G.nephanidis* alone at the fixed norm was released.

Significant reduction was noted in the percentage of infested leaves, and mean population of *O. arenosella.* In addition to the released parasitoids, natural incidence of *A. taragamae.* and *X. punctata* were observed at this garden also. *P.nigrolineata.*and many species of spiders were also recorded in the experimental palms. Among the hyper parasitoids, only *Eurytoma albotibialis* and *Pediobius sp.* were noted.

Follow up observation in parasite-released plot was done at Thodiyoor during 1997. Observations were recorded at every quarter .10-12 larvae/ palm (medium population) was observed and release of one dose (20.5%) of *Goniozus nephantidis* controlled the pest. The population remained at low level and no further release were made. Survey was conducted in the *Opisina* infested tracts of the coastal areas from Mangalore to Kundapura of Dakshina Kannada Dist, Karanataka in collaboration with the Dept of Horticulture Karnataka, Work was taken up in the two experimental centers at Kotepura (for biological suppression of the pest) and at Mangalore and Kundapura (for IPM).

Pathogens

Work carried out during 1979 indicated that 'Dipel', a commercial formulation of *Bacillus thurengiensis*, showed a mortality of the order of 60.94% for *Nephantis serinopa* caterpillars on spraying the infected coconut leaves at 0.5% concentrations. *Serratia* sp. was isolated from field-collected caterpillars.

(*Rhynchophorus ferrugineus* Oliv.)

Regular monitoring of field collected dead grubs were done for isolation of pathogens. Grubs, which showed some deformity, were studied in the lab. for expression of disease sympyoms,.General external symptoms were accumulation of fluid in the haemocoel, yellowing of fat, sluggishness, refusal to feed,diarrhoea and dilation of gut.Giemsa stained midgut smear was found to contain large cells with hypertrophied nuclei, per nuclear halo formation, vacuolation of cytoplasm and disorganization of chromatin. Trials carried out to reproduce the disease in grubs, the triturate of cadavers were force fed to healthy weevils.30-40 grubs showed typical enlarged nuclei of the gut tissues and other symptoms. Chemical inducers like formaidehyde, sodium nitrate, sodium azide and phosphate were tested to enhance the disease, however these could not produce any positive effect.

Several bacteria were isolated (227 isolates) from the haemolymph of field collected red palm weevil. Studies on morphology, staining and sporulation of bacteria isolated from the field collected samples were carried out .One of the bacteria was found to be a potent pathogen which was identified as *Pseudomonas aeroginosa*. The organism has an L D 50 value between 10^3- 10^4 when injected into the haemocoel and inflicted mortality in healthy grubs within 72 hours. This entomopathogen on forced oral feeding affected 80% mortality of healthy grubs. Another pathogen isolated is Entomobacter.

A rod shaped nuclear viral infection (NPV) was noticed and polyhydra like bodies were seen under EM. Purification and characterization of the polyhydra and the virions were tried by different physico- chemical techniques, both qualitative and quantitave Variation of protein could be noticed in diseased and healthy grubs

The cross inoculation of *Bacillus thuringiensis* and *Bacillus popillae* to red palm grubs did not give convincing results. A yeast like organism was isolated from the haemolymph and fat body of different stages of red palm weevil.

Ectoparasitic mite *Centrouropoda* sp.were isolated from the live pupae and adults. More than80% of the mite population was found concentrated on the dorsal side of the body particularly thorax and wings. Average number of mite /weevil was380, females weevils lodged more number of mites (average 458/female) than males (285/male) The mites caused scabes on the area of infection. The exact role of these mites was not ascertained.

The nematodes collected from cocoon fibers and haemocoel of red palm weevil during 1982 were identified as *Teratorhabditis* sp., *Mikoletzkke* sp., *Monoechoides* sp. and *Acrostichus* sp.

Since 1996, a total of 29 bacterial isolates, 4-yeast isolate and 1 fungal isolate have been obtained from field collected dead red palm weevil. All these isolates have been purified and sub cultured regularly for conducting pathogenicity trials against the pest. From all these isolates tested two gram negative bacterial isolates (RPW *PSI, RPW* -PS2) could give 70-100% mortality on grubs within 48 hours when injected into the haemocoel. They also elicited 30-40% mortality in 7-9 days period when applied on the grubs maintained inside coconut petiole. The other bacterial, yeast and the fungal isolates failed to produce any appreciable mortality. Further studies on isolation of pathogens and pathogenicity trials are being continued.

Leucopholis coneophora Burm.

The parasitic wasp, *Campsomeriella collaris* (Hymenoptera: Scoliidea) were reared in the laboratory on full grown white grubs and the biology was studied. The parasite when reared on other species of white grubs appeared smaller in size as compared to those reared on *L. coneophora*. *C. collaris* does not parasitise the grubs of red palm weevil and rhinoceros beetle.

The fully-grown white grub showed symptoms of infection when the milky disease bacterium, *Bacillus popillae* Dutky was inoculated by force-feeding, injection and soil contamination methods. The presence of rod shaped bacteria with typical spores was observed when haemolymph was examined under microscope. The grubs died in 28-40 days of inoculation.

A gregarine protozoan parasite, *Pseudomonocystis sp.* was detected in the grubs of *L. coneophora* collected from the field. They were detected in the cyst form. The cyst was thin walled and contained a large number of navicular spores with caps at both ends. The infected grubs succumbed to the infection within 27.62±6.28 days.

Predacious mites

Phytoseiid mite, Amblysieus sp (*A. paraaerialis*, *A. eucalypticus*). and cheyletid mites were commonly observed in association with *Oligonychus spp.* and *Raoiella indica*, which are the important phytophagous mites infesting coconut foliage. These mites were identified upto generic level by Dr,. D. Macfarlane, CIE, London. *Lasiosieus sp.* (Mesostigmata: Ascidae), *Amblysieus sp.* (Mesostigmata: Phytoseiidae) and *Agistemus sp.* (Prostigmata: Stigmaedae) were the major predators observed on different stages of *Oligonychus iseilemae*. The predatory behavior of *Amblysieus sp.* on *O. iseleimae* was studied in detail in the lab. *Amblyseius sp.* completed its life cycle in 4-5 days, as compared to that of 7-10 days of the prey, *O. iseilemae*. The predator prey ratio was observed to be 1:6 with a maximum population of the pest and its predator during May. *Amblysieus sp.* consumed 19-42 eggs of *O. iseilemae* during the entire life period. The Cunaxid predator consumed the immature and adult stages of *O. iseilemae*. Rate of prey consumption ranged

from 3.3 – 31.0 mites per female predator. Fecundity ranged from 1.2 – 12 eggs per female and longevity of female is 13-17 days under lab conditions. *Amblyseius* sp. occurred throughout the year except in Jan. and July. The rate of prey consumption ranged from 1.2-3.2 prey per predator per day. Cunaxid also occurred during all months of the year and the prey consumption rate ranged from 2.7-5.3 prey per predator per day. The prey consumption, predator prey ratio and peak period of abundance of important mite predators of *O. iseilemae* are presented in the table 13

Table 13. The prey consumption, predator prey ratio and peak period of abundance of important mite predators of *O. iseilemae*

Predator	Prey consumed		Predator-Prey ratio	Peak period of abundance
	Mean	Range		
Amblyseius paraaerialis	14.7± 1.8	6-38	1:7.4	May
A.eucalypticus	21.3± 2.1	13-34	11:10.6	May
Cunaxa setirostris	35.5± 4.2	9-76	1:10.7	March

Studies on fluctuations in population density of the phytophagous mite, *Oligonichus iseilemae* in relation to natural enemies and meteorological factors revealed that there was significant correlation between predacious mites, Phytoseiidae and Cunaxidae, and weather parameters like maximum rainfall. Maximum temperature (32-34° C) favoured the build up of population. Lower relative humidity (88.52-91.5%) also favoured population build up, but the results were not significant. Almost 71% of variations in populations could be attributed to predators and weather factors (R^2 = 0.706). Consignment of the exotic predator, *Phytoseiulus persimilis* obtained from the biocontrol laboratory, IIHR, Bangalore during 1989-90 could not be cultured because of mortality.

Associated with the pests of the coconut palms also a few species of mites could be collected. The mite associated with *O. rhinoceros* included *Centrouropoda* sp. (Mesostigmata: Uropodidae), *Macrochelus* sp. (Mesostigmata: Macrocheldae) and a mite belonging to family Diplogynidae (Mesostigmata). Species of

Caloglyphus (Mesostigmata: Acaridae) were associated with the cockchafer beetle, *Leucopholis coneophora*. *Suidasia pontifica* (Oudemans) (Prostigmata: Saproglyphidae) were collected from the laboratory cultures of larval parasites of *O. arenosella*.

Stephanitis typica Dist.

Studies on the biological suppression of the tinged bug, *S. typica*, the foliage pest and vector of MLO associated with the coconut root (wilt) disease were initiated during 1985. The mirid predator *Stethoconus praefectes* (Dist.) and spiders were observed in the lace bug colony. The chrysophyid predator was identified as *Ankylopteryx octopunctata* Fabr.

Observations on the seasonal abundance of lace bug *S. typica* and the predators *S. praefectes* and *A. octopunctata* and spiders were recorded from 250 seedlings of coconut each at Cheppad and Nangiarkulangara. at weekly intervals. The data collected from 20% sample palms revealed that the highest pest population was observed during March, June- July followed by October –November. High predator population was observed during May, June – July and minimum in September. There was positive correlation between the prey and predator in the field. Attempts on standerdising techniques for culturing the mired predator *S. prefectes* was initiated during1987. Artificial diet and on the *Corcyra* eggs were not successful. Longevity of *S. praefectes* was poor when it was reared on *Corcyra*. The predator could be reared on *S. typica* colonies established on potted coconut seedlings as well as on detached leaflets in bell jars, provided with adequate moisture. The predator laid average 5.6 eggs / female, however hatching was very poor. Arrowroot was found to be the best host plant for rearing lace bug when compared to *Curcuma* and oil palm seedlings. Predators released in lots of 5, 10, & 20 per caged coconut leaflets infested with *S. typica* effected 70-100% mortality of the prey against 36% mortality in the unchallenged control.

In order to explore the possibility of rearing the mired predator on artificial diet, three basic diet materials such as Haemolymph of red palm weevil grubs, *S. typical* extract and diet containing, casein hydrolysate, cholesterol and agar with

vitamins and antibiotics were tested. Feeding from soaked sponge was found to be good

The chrysopid predator *A. octopunctata octopunctata* occurs in the field during all months with peak population during April, medium population during August and low population from Oct.- December. The chrysopid larva consumed on an average 12± 1 (range 6-38) lace bugs / day. It also feeds on *Corcyra* eggs @98± 29(range11-185) / day. The larva to adult period is on an average 26(22-27) days.

A unidentified predatory caterpillar was collected from the lace bug colony during 1995 from the field. A full grown caterpillar consumed 59-82 lace bugs under lab. conditions. A haerobid predator feeding on the adults and nymphs of *S. typica* was collected from Minicoy during 1985. Rate of prey consumption was observed to be 6 adults or 9 nymphs of *S. typica* / predator/ day.

A fungal pathogen was isolated from field-collected bugs during 1985. Trials conducted to infect the adults and nymphs with the sporulated culture did not yield any positive result.

Survey conducted in Mysore and Nanjaangud areas of Karnataka revealed that in these areas lace bugs were abundant on banana than on coconut, whereas in Kerala, they were more on coconut and rare on banana.

Regular field collection of *Opisina* and *Stephanitis* yielded dead larvae, adults and nymphs with fungal outgrowth. This fungus was isolated and identified as *Aspergillus flavus*. Laboratory studies revealed that AF1 at 10 ^{6}spores/ ml. (from *Stephanitis*) and AF2 (from *Opisina*) at 10^{5}- 10^{6}spores/ml could give 100% mortality by 5-6 days period. Cross infection studies showed that AF1 isolated from *Stephanitis* could kill *Opisina* and vice-versa.

Other works associated with the Project

1. **Maintenance and supply of biocontrol agents:** Maintaining the nucleus culture of the pathogens of *Oryctes rhinoceros* and parasitoids of *Opisina arenosella.* These cultures are supplied to the different agencies that are interested in the biological control of these pests.
2. **Training programmes:** Efforts were made to popularize the biocontrol programme by offering frequent Institutional training programmes to researchers, extension workers, progressive farmers and students.

Technical progammes continuing

1. Studies on *Apanteles taragamae*
2. *Rhynchophorus ferrugineus- isolation of pathogens and pathogenicity trials*
3. *Studies on Entomophilic Nematodes of pests of coconut*

LIST OF PUBLICATIONS

1. Antony .J., Daniel, Mariamma, Kurien, Chandy and Pillai, G.B. 1979. Attempts on introduction and colonization of the exotic reduviid predator, *Platymeris laevicollis* Dist. of the biological suppression of the coconut rhinoceros beetle *Oryctes rhinoceros* L. In: *Proc. PLACROSYM II* (C. S. Venketaraman and others Eds. P. 445.454.
2. Banerjee, A. and Danger, T.K. 1995. *Pseudomanas aeruginosa*, a facultative pathogen of red palm weevil *Rhynchophorus ferrugineus World J. Microbiol. Biotech.* **11**:618-620
3. Chandrika Mohan 1999. Biological control against coconut pests (Mal) *Extension folder*. CPCRI Kayamkulam.
4. Chandrika Mohan and Sathiamma, B. 1999 Leaf eating caterpillar and biological control (Mal.) *Ind Naleekera J.* **28**(4): 1-3.
5. Chandrika Mohan; Sathiamma, B.and Sabu, A.S. 2001. Observations on laboratory mass multiplication of braconid endoparasitoid *Apanteles taragamae* Wilk. On early instar caterpillars of *Opisina arenosella* Walk. on coconut. Accepted *Entomon* (In Press)
6. Danger, T.K.1997.Infection of red palm weevil, *Rhynchophorus ferruginous*, by a yeast. *J.Plantn. Crops* **25**: 193-196.
7. Danger, T.K. and Abraham, V.A. 1997. *Psuedomonocystis* sp., an eugregarine protozoan pathogen of the coconut root grub *Leucopholis coneophora. Biocontrol Science and Technology*. 7: 385-391.

8. Danger, T.K., Geetha, L., Jayapal, S.P. and Pillai, G.B. 1991. Mass production of the entomopathogen *Metarhizium anisopliae* in coconut water wasted from copra making industry. *J.Plantn.Crops* **19**: 54-69.s

9. Danger, T.K., Solomon, J.J. and Pillai, G.B. 1994. Infection of the coconut palm beetle *Xylotrupes gideon* (Coleoptera: Scarabaeidae) by a non-accluded Baculovirus *Z.Pflankranh. Pflschutz.* **101**:561-566.

10. George, M.V.; Sathiamma, B. and Vijayakumar, K. 1984. A sampling technique to estimate the field population of *Opisina arenosella* Walk. (*Nephantis serinopa* Mey.) ,a pest of coconut palm. In: Bhavappa and others (eds.) *Proc. PLACROSYM V* : 638-643.

11. Gopal, Murali, 2000. Glimpses pf coconut cultivation in Andrott Island of Lakshadeep. *Indian Coconut J* .**XXX** (10): 1-3.

12. Gopal, Murali; Gupta, Alka. 2000. *Pseudomonas alcaligenes* infection in grubs hampers natural transmission of Baculovirus on *Oryctes rhinoceros* L. Paper presented in " *International conference on Managing Natural Resources for sustainable Agricultural production in the 21st century*. New Delhi, Feb 14- 18, 2000. P. 722.

13. Gopal, Murali and Sathiamma, B. 2000. Coir waste: an alternate feed material for maintenance of Baculovirus inoculated rhinoceros beetle, Oryctes rhinoceros (L). In: Proceedings PLACROSYM XIII ' *Recent Advances in Plantation Crops Research'* N. Muraleedharan and R RajKumar (Eds.) 339-341.Allied Publishers Ltd. Chennai.

14. Gopal, Murali; Gupta, Alka and Sathiamma, B. 1999. Coconut palm beetle: Microbial control. *The Hindu*, Thursday, April 22, 1999.

15. Gopal, Murali; Gupta, Alka and Sathiamma, B. 2000. *Baculovirus oryctes* for controlling r*hinoceros* beetle of coconut palm. Indian Fmg. **50** (4): 17-19.

16. Gopal, Murali; Gupta, Alka; Rajan, P. and Radhakrishnan Nair, C.P 1999. Effect of systemic soil insecticides and a plant product on microbial load of soil in root (wilt) affected coconut monocropping ecosystem. *CORD* (accepted)

17. Gopal, Murali; Gupta, Alka; Chandramohanan Nair, K.R., Sathiamma, B. and RadhakrishanNair, C.P. 1999. Studies on cross infectivity with entomofungal isolates of *Aspergillus flavus* Link. Against *Stephantis typica*

D. and *Opisina arenosella* W., two pests of coconut. *Indian Cocon. J.* **XXXI** (7): 12-15

18. Gopal, Murali; Gupta, Alka; Sathiamma, B.; Chandrika Mohan,; Chandra Mohanan Nair, K.R and Soniya, V.P. 1999. A fungal pathogen of lace bug and leaf eating caterpillar, two insect pests of coconut palm. *CORD.* **XVI** (1) 49-60.
19. Gopal, Murali; Nair, KRC; Sathiamma, B.and Nair CPR. 1999. Use of a fungus against coconut palm rhinoceros beetle. *Weekly Udayaniki Jeevan.* Vol. **46**, June 6 pp.5
20. Gopal, Murali; Sathiamma, B and Nair, CPR. 1998 Baculovirus *Oryctes*, a classical microbial control agent of the coconut pest *Oryctes rhinoceros. National symposium on Biopesticides and Insect Pest Management*, Entomology Research Institute, Loyola College, Chennai, 27- 28 Feb 1998.
21. Gopal, Murali; Sathiamma, B. and Nair, CPR. 1999. Microbial control of *Oryctes rhinoceros (L)* with Baculovirus *Oryctes.* In " *Biopesticides Insect Pest Management* (eds.) S. Ignacimuthu and Alok Sen. Phoenix Publish House Pvt. Ltd. New Delhi, P: 196-198
22. Gopal, Murali; Sathiamma, B. and Nair, CPR. 2000. Ecology of microbial pathogen of the rhinoceros beetle, an insect pest of coconut In. Symposium on *'Biotechnology of Plant protection* " BHU, Varanasi, 25-27, Feb, 2000. Abstracts of paper. p. 74.
23. Gopal, Murali; Sathiamma, B; Nair, KRC. and Soniya, V.P. 1998. A fungal pathogen of lace bug and leaf eating caterpillar, two insect pests of coconut palm. *39th Annual Conference of Association of Microbiologists of India.* Fisheries College, Mangalore 5-6th Dec. 1998, Abstract of paper: p. 275
24. Gupta, Alka and Gopal, Murali .1999. The prospect of using " EPN" as biopesticide. *Weekly Udaniki Jeevan.* Vol. **50**. Sunday, July 4,pp .3
25. Gupta, Alka and Gopal, Murali. 2000. Biochemical characterization and identification of a bacterial isolates from septicemic grubs of *Oryctes rhinoceros* .In " *National seminar on Recent Advances on Plant Biology*" – CPCRI, Kasaragod, Feb 3-5, 2000. P. 127.
26. Gupta, Alka, Gopal, Murali and Sathiamma, B 1998. Biological control of rhinoceros beetle by entomopathogenic fungus *Metarhizium anisopliae* (Hindi) *Bharatiya Nariyal Patrika* **IX** (3)

27. Kurien, C., Pillai, G.B., Antony, J., Abraham, V.A and Natarajan, P. 1983. Biological control of insect pest of coconut. In. *Coconut Research and Development* Proc. Of the International Symposium I. CPCRI, Kasaragod Dec27-31, 1976. Wiley Earstern. New Delhi p. 361-375.

28. Mohan, K.S and Gopinathan, K.P. 1989a. On quantitation of serological cross reactivity between two geographical isolates of *Oryctes baculovirus* by a modified ELISA. *J. Virol. Meth.***24**: 203-214.

29. Mohan K.S and Gopinathan, K.P. 1989b. Characterisation of viral proteins of *Oryctes baculovirus* and comparison between two geographical isolates. *Arch. Virol.* **109**: 207-222.

30. Mohan, K.S and Gopinathan, K.P .1991.Physical mapping of the genomic DNA of the *Oryctes rhinoceros* baculovirus, KI. *Gene* **107**: 343-344.

31. Mohan, K.S and Gopinathan, K.P. 1992. Characterization of the genome of *Oryctes baculovirus*, a viral biocide of the pest *Oryctes rhinoceros. J. Biosci.* **17**(4): 421-430.

32. Mohan, K.S and Pillai G, B. 1982a. A method for laboratory scale mass cultivation of *Metarhizium anisopliae. Folia Microbiologica* **27**(4): 281-283.

33. Mohan, K, S and Pillai G, B.1982b. A selective medium for isolation of *Metarhizium anisopliae* from cattle dung. *Trans. Br. Mycol. Soc.* (**1**): 181-182

34. Mohan K.S and Pillai, G.B 1983. Immuno- osmophoresis technique for quick diagnosis of *Oryctes* virus of Rhinocreos beetle *Oryctes rhinoceros* L..*Indian J. Exptl. Biol.* **221:** 470- 471.

35. Mohan, K, S and Pillai G, B 1993. Biological control of *Oryctes rhinoceros* (L) using an Indian isolate of *Oryctes* baculovirus. *Insect Sci. Applic.* 14(5-6): 551-558

36. Mohan K.S, Jayapal, S.P and Pillai, G.B .1983. Baculovirus diseases in *Oryctes rhinoceros* population in Kerala. *J. Plant. Crops.* 11: 24-32.

37. Mohan, K.S., Jayapal, S.P and Pillai, G.B. 1985a. Response of Oryctes rhinoceros larvae to infection by *Oryctes* baculovirus. *J. Plantn Crops* **13**(2): 116-124.

38. Mohan, K.S., Jayapal, S.P Pillai, G.B. 1985b. Diagnosis of baculovirus infection in coconut rhinoceros beetle by examination of excreta. *Z. Pflkanh. Pflschutz.* **93**(4): 379-383.

39. Mohan, K.S; Jayapal, S.P and Pillai, G. B. 1986. Diagnosis pf baculovirus infection ion coconut rhinoceros beetle by examination of excreta. *J. Plant Disease and Protection.* **93**(4): 379-338

40. 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. *J. Plantn Crops* **16**(Suppl.) 163-170

41. Nair, C.P.R.; Sathiamma, B.; Chandrika Mohan, and Gopal, Murali. 1998. Newer approaches in the IPM in coconut. *Indian Coconut J.* **29**(4): 99-103

42. Nair, CPR, Sathiamma, B; Chandrika Mohan, and Gopal, Murali. 1998. IPM in coconut new outlook (Malayalam) *Indian Nalikera J.* **27** (10) 34-39: 66-67

43. Pillai G.B. 1985a. Biological control of pests of plantation crops. *National seminar on Entomophagous Insects and other Arthropods and their potential in Biological Control.* Oct. 9-11, 1985. University of Calicut.

44. Pillai, G, B. 1985b. Coconut pest of National Importance. Paper presented at the National seminar on' *Integrated pest and Disease Management* TNAU, Coimbatore. 5-6Sept- 1984) in: Integrated pest and disease Management edited S. Jayaraj pp. 166-173.

45. Pillai, G.B. 1987. Biological pest suppression in plantation and tuber crops . An overview. Proc. *Seminar cum sixth workshop of Boicontrol of crop pest and weeds.* 29 June 1987, GAU, Anand. Pp. 91-107.

46. Pillai, G.B. 1989. Biological pest suppression in plantation crops. Seminar cum Seventh workshop on Biological control of Crop pests and weeds. Indian institute of sugarcane Reasearch. Lucknow. (22-25 Oct. 1989).

47. Pillai, G.B. 1991. Role of entomophagous in pest management. *National seminar on Biological control on plantation crops.* 27-28 July, RRII, Kottayam.

48. Pillai, G.B and Bhat, S.K. 1986. Parasitism, development of progeny and sex ratio of *Parasierola nephantidis Mues.* (Bethylidae) on different instars of *Opisina arenosella Indian Cocon. J.* **14** (11): 3-5.

49. Pillai, G.B and Kesava Bhatt, S. 1987. Biology and feeding potential of *Parena nigrolineata* a predator of the coconut caterpillar, *Opisina arenosella* Wlk. *Indian cocon .J.* **17**(2) 6-10.

50. Pillai, G.B and Nair, K.R. 1981. Role of pupal parasitoids in the natural suppression of coconut caterpillar, *Nephantis serinopa. J. Plant. Crops* **9**:*84*-87

51. Pillai, G.B and Nair, K R. 1982a. A technique for laboratory multiplication of *Brachimeria nosatoi* and other species of chalacid parasitoids of *Nephantis serinopa. Entomon* . **7:** 213-217.

52. Pillai, G, B and Nair, K.R. 1982b. . Mating behavior and biology of *Elasmus nephantidis* Rohw. (Hymenoptera: Elasmidae) a parasitoid of *Nephantis serinopa.* Meyrick. *J. Plant. Crops.* **10** (1): 33-38.

53. Pillai, G.B and Nair, K.R. 1982c .A note on *Trichopsilus pupivora*, a parasite of *Nephantis serinopa. Entomon* . **7**: 25-26.

54. Pillai, G.B. and Nair, K.R. 1982d. A method to estimate the intensity of natural pupal parasitism of *Opisina arenosella. J. Plant. Crops.***10**: 86-91.

55. Pillai, G.B and Nair, K.R. 1983. *Xanthopimpla nana nana* (Hymenoptera: Ichneumonidae). A new pupal parasitoid of *Opisina arenosella* on coconut *J. Plant. Crops.* **11:** 65-69.

56. Pillai, G.B and Nair, K, R. 1985a. Mating and host paralyzing behaviors of *Goniozus (Parasierola) nephantidis (Mues)* . (Hymenoptera: Bethylidae) on *Opisina arenosella* on coconut. *National seminar on Entomophagous Insects and other Arhtropods and their Potential in Biological control* . Oct. 9-11,1985 PP123-125. University of Calicut

57. Pillai, G.B and Nair, K.R 1985b. Observation on *Eurytoma albotibialia Ashm.* (Hymenoptera: Eurytomidae) a hyperparasitiod of the coconut leaf eating caterpillar, *Opisina arenosella* Wlk. *National Seminar on Entomophagous insects and other Arthropods and their Potential in Biological control.* Oct. 9-11, 1985, University of Calicut

58. Pillai, G.B. and Nair, K.R. 1986a. Addition to the natural enemy complex of the coconut caterpillar *Opisina arenosella* Wlk. *J. Plant. Crops* **14**(2): 138-140.

59. Pillai, G.B and Ramachandran Nair, K. 1986b. Observations on *Xantho pimpla puntata* (Hymenoptera: *Ichnuemonidae*) a pupal parasitoid of

Opisina arenosella. Wlk. PLACRROSYM-V11. Oct. 16-19, 19986. Coonoor.

60. Pillai, G.B and Nair, K.R 1987a. Observations on *Xanthopimpla sp.* (Hymenoptera: *Ichnuemonidae*) a pupal parasitoid of *Opisina arenosella.* Wlk. on coconut in Kerala *J. Biol. Control* **1**(1) 7-10
61. Pillai, G.B and Nair, K.R. 1987b *Brachycoryphus nursei* (Cameron) (Hymenoptera:Ichneumonidae) parasitoid of *Opisina arenosella* Wlk. on coconut in Kerala, India. J. Plantn Crops .15(1): 9-14.
62. Pillai, G.B and Nair, K,R . 1989. Observation on *Xanthopimpla punctata* F. (Hymenoptera: Ichnuemonidae), a papal parasitoid of *Opisina arenosella* Wlk. *J. Plant. Crops.* **16** (Suppl.) 173-177
63. Pillai, G.B and Nair, K.R. 1990a. On the biology of *Calleida spendidula* (F) (Coleoptera; Carabidae), predator of the coconut leaf eating caterpillar, *Opisina arenosella* Wlk. *Indian Coconut J.* **20** (12): 14-17.
64. Pillai, G.B and Nair, K.R. 1990b Role of Ichneumonid parasitoids in the biological suppression of the coconut leaf-eating caterpillar, *Opisina arenosella* Wlk. *Indian Cocon. J.* **21**(4): 3-10
65. Pillai, G.B and Nair, K.R. 1991. Superiority if solitary parasitoids over gregarious species in the biological suppression on the coconut caterpillar, *Opisina arenosella.* Wlk. *National Seminar on Biological control in plantation crops.* 27-28 July, RRII, Kottayam.
66. Pillai G.B and Nair, K.R. 1992. A note on the biology of *Xanthopimpla nana nana* Schluz. (Hymenoptera: Ichneumonodae), a pupal parasitoid of *Opisina arenosella* Wlk. on coconut. *Indian coconut. J* **.23**(2): 2-4`
67. Pillai, G.B and Nair, KR. 1993a .A check list parasitoids and predators of *Opisina arernosella* Wlk. on coconut. *Indian cocon J.* **23**(9): 2-4.
68. Pillai, G.B and Nair, K.R. 1993b. Studies on the Chalcidid pupal parasitoids of the coconut caterpillar *Opisina arenosella* Wlk. in Kerala, India. *Entomon* **17**: 183- 192.
69. Sathiamma.B. 1992. Role of predacious and insects in the biological suppression of spider mites on coconut foliage. PLACROSYM-X, 2-4 Dec. 1992. CPCRI, Kasargod
70. Sathiamma.B. 1993. *Opisina arenosella* Walk.,the leaf eating caterpillar of coconut palm Tech. Bull. 10 CPCRI. Kasaragod. 12pp

71. Sathiamma, B. 1995. Biological suppression of the white spider mite *Olygonychis iseilemae* on coconut foliage. *Entomon.* **20:** 237-243.

72. Sathiamma, B. 1998. Biological suppression of rhinoceros and leaf eating caterpillar. *Indian coconut J.* **28**(12): 4-8.

73. Sathiamma, B and Nair C.P.R. 1994. Pest control through Biological methods. *Kerala Karshakan* **40**(4): 11- 13 and 30.

74. Sathiamma, B.; Abraham, V.A. and Kurian,C. 1982. Integrated pest management of the major pests of coconut. *Ind. Cocon. J.* **12**(6-9): 27-29

75. Sathiamma, B.; George, M.V.and Kurian, C. 1974. Seasonal variations in the larval populations of *Nephantis serinopa* Meyrick in the field as correlated with meteorological factors. *J. Plantn. Crops.* **1**(suppl.): 161-163.

76. Sathiamma, B, Gopal, Murali and Chandrika Mohan 1998. Biological control preferred against *rhinoceros beetle* coconut (Malayalam) *Indian Nakera.J.* **27** (9): 1-5

77. Sathiamma, B.; Chandrika Mohan; Gopal, Murali; Abraham , V.A and Nair , CPR . 1999. Biological suppression of Coconut Pests. Technical Bulletin No. **37**, CPCRI, Kasargod.

78. Sathiamma, B Jayapal, S.P. and Pillai, G,B . 1985a. Spider fauna on coconut palms and their role in biological suppression of coconut caterpillar *Opisina arenosella* Wlk. Nat. Seminar on *Entomophagous insects and other Arthropods and their potential in Biological control.* October 9-11, 1985. University of Calicut Abs. of papers p.11.

79. Sathiamma, B. Jayapal, S.P. and Pillai, G.B. 1985b. Record of *Anylopteryx octopuntata candida*, as egg and larval predator on *Opisina arenosella*, the leaf-eating caterpillar of the coconut palm. *Curr. Sci.* **54:** 1128

80. Sathiamma, B.; Pillai, G.B. and Jayapal, S, P., 1986. Bioecology of *Bracon hebator* (Hymenoptera: Braconidae) an indigenous larval parasitoid of the leaf eating caterpillar *Opisina arenosella* Wlk. (Lepidotera: Crytophasidae). *J. Plant. Crops.* **14** (2) : 140- 145

81. Sathiamma, B.; Pillai, G.B. and Jayapal, S, P., 1987a. Observations on spiders (Order; Araneae) predacious on the leaf eating caterpillar Opisina arenosella Wlk. (Nephantis serinopa M.) in Kerala. *Entomon* **12**(1): 45-47.

82. Sathiamma, B. Pillai, G.B. Abraham Jose, Bhat, S.K. Jayapal, S.P. and Nair, K. R. 1987b. Norms for release of larval, prepupal and pupal

parasitoids of *Opisina arenosella* Wlk., the leaf-eating caterpillar of coconut palms. *J. Plantn. Crops*.15 (20: 118-122

83. 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. *J. Plant. Crops* **24**(1): 9-15.
84. Sosamma, V.K. 2000. Entomophilic nematodes in coconut pest management. Paper presented in the group meeting on recent advances in the management of coconut pests..24-25 May 2000,CPCRI,Kayamkulam. Abstr. Of papers p.28

Signature with Name of Project Leader and associates

15/02/2001

CHANDRIKA MOHAN
Project Leader

MURALI GOPAL **V.K. SOSAMMA**
Project associates

19.02.2001

Signature (with comments, if any) of Head of Division/ Section/ Station.

CENTRAL PLANTATION CROPS RESEARCH INSTITUTE
P. O. KUDLU, KASARAGOD 671124

Signature (with comments, if any) of Director.