BIOLOGICAL SUPPRESSION OF COCONUT PESTS

Rhinoceros beetle, leaf eating caterpillar, red palm weevil, and white grub are the important pests of coconut palm in India. An Integrated Pest Management (IPM) Schedule has proved to be quite feasible for the control of these pests. The management schedule comprises a combination of proven technologies - mechanical, sanitational, prophylactic, chemical and biological. Insecticides are effective in the control of the pests, but their deleterious effect on the beneficial natural enemies and pollinators and the residues they leave in the edible parts of the nut limit the use of these poisons. Natural enemies were found to be promising in keeping these pests under check and the use of chemicals could be reduced/eliminated from the control schedule. Integrated Pest Management technology now emphasises the use of bioagents which are ecofriendly and self perpetuating.

The pests are listed as per the order of importance.

Rhinoceros Beetle-*Oryctes rhinoceros* (L.)

Damage:

The adult rhinoceros beetle bores through the unopened leaves and inflorescences and causes severe cuts and injuries to the leaves, and drying of the inflorescences. Damage results in 10% loss in yield on an average. However, instances of damage upto 50% have also been observed.

Bioagents:

Baculovirus of *Oryctes* and green muscardine fungus - *Metarhizinm anisopliae* are two promising pathogens of rhinoceros beetle.

***Oryctes baculovirus* (OBV)**

OBV particles consist of rod-shaped nucleocapsid (235 x 110 nm) surrounded by an envelope (Plate 1). The Indian isolate (OBV-KI), and the Philippine isolate (OBV - PV 505) resemble each other morphologically, but differ from each other in protein pattern, SDS-PAGE profiles, glycosylation patterns and immunoblotting tests. (Mohan and Gopinathan, 1989; 1992). The virus is normally non-occluded.

The virus mainly infects the nuclei of the midgut epithelium and fat body. Larvae and adults of the beetle are susceptible to infection. Pupae are not generally affected. Mode of entry of the pathogen is only by oral injestion of virus contaminated feed.

Diagnostic symptoms of the disease are:

(i) Visual symptoms

Infected larvae become lethargic and stop feeding; abdomen becomes turgid and glassy; fat body disintegrates and the quantity of haemolymph increases giving the larvae a translucent appearance. Extroversion of the rectum may also occur. Infected gut becomes devoid of food and gets filled with mucoid white fluid. Diseased grubs die within 15-20 days after infection.

Infected adults become inactive and short-lived and lethal infection leads to total reduction in fecundity and 40% reduction in longevity. No external symptoms are seen in beetles. However, the midgut becomes white and dilated, filled with milky white viscous fluid as compared to the very thin, translucent midgut containing very little clear brown fluid of the healthy adult. Infection causes changes in haemocyte count, particularly in granular cells and plasmatocytes and also in the protein, amino acid and sugar metabolism of the host (Martin Jude Vincent *et al.,* 1988; Biju *et al.,* 1993).

(ii) Giemsa staining of midgut contents:

1. Using a syringe a small volume of infected midgut fluid or a piece of the midgut epithelium is transferred to a clean slide and smeared out. The smear is air dried.

2. The material is fixed for 5-7 min. in acetone free methanol.

3. It is followed by flooding with freshly made 3% Giemsa stain for 45 to 60 min.

4. The slide is then rinsed in water, dried, mounted and examined under oil immersion.

5. In the infected samples, pink coloured hypertrophied nucleus (18-28/µm) (Plate 2) and sparse blue cytoplasm are seen as compared to 7.5 - 12.5/µm of healthy nucleus. In certain cases, the enlarged nucleus is encircled by a dark ring. Vacuolation of the infected nuclei was also observed.

(iii) Immuno-osmophoresis technique:

1. A volume of 4.5 ml of 0.8% warm oxoid agar in 0.045 M phosphate buffer at pH 7.4, containing 0.1% sodium azide, is pipetted on to a Formvar 15/95 E (0.2% w/v in chloroform) coated microscope glass slide.

2. The solidified agar layer is made firm by refrigeration overnight in a moist chamber.

3. Pairs of 2 mm dia. wells are cut 1 cm apart in 2 or 3 vertical rows which serves as antigen and antiserum wells. The aspirate/ homogenate of the infected midgut is directly put into the antigen wells and antiserum from OBV inoculated rabbit in the opposite wells.

4. A current of 12-15 mA and voltage of 10-15 V/cm of agar gel with a running time of 70 min. is used.

5. A specific precipitation line is formed between the antigen and antiserum wells (Mohan and Pillai, 1983).

(iv) Bioassay

Grubs

1. Dissect the test grubs. Excise and homogenate the midgut.

2. Force feed this homogenate orally to healthy grubs and maintain them in moist autoclaved cattle dung-saw dust mixture (2:1 w/w) or sterilized corn waste. (Gopal *et al.*, 1998) and observe for five weeks for the appearance of the disease symptoms.

3. It is bioassay positive if the grubs show any of the symptoms mentioned earlier.

Adults:

1. Allow the freshly emerged beetle to crawl in infected gut homogenate suspension obtained from test beetle for 30 minutes.

2. Keep the beetles in containers with peeled coconut petioles smeared with the homogenate.

3. Dissect the beetle after 15 days and diagnose for infection.

(v) Electron Microscopy:

1. Fix the midgut slices from infected beetles in 2% glutaraldehyde in phosphate buffer (0.2 M, pH 7.2) for 1h at 4°C, wash in buffer thrice and stain in 1% osmium tetraoxide.

2. Electron micrographs show massive infection of the midgut cells with bacilliform virus particles within the nucleus.

(vi) **Analysis of excreta of diseased beetles:**

1. Maintain baculovirus infected beetles individually in plastic container with 5 ml phosphate buffer saline (0.01 M; pH 7.0, Nacl 0.85%). The buffer contains antibiotics (Streptomycin 250; Penicillin 200; Oxytetracycline 100 mg/1) to prevent any possible bacterial action on the excreted gut cells.

2. Collect the faecal matter and centrifuge at 500 rpm for 10 min. and resuspend the sediment in 0.2 ml buffer by gentle pipetting.

3. Then observe the sediment by 3% giemsa stain for hypertrophied nuclei.

Culture medium:
3 to 4 times autoclaved powdered cowdung or coir dust kept in plastic boxes.

Virus culture maintenance

1. Select OBV infected host, kill, dissect and cut the midgut and transfer it to a mortar.

2. Add 3-5 ml of sterile water and homogenize it using a mortar and pestle.

3. Draw the midgut suspension inside a syringe and carefully mouthfeed the healthy grubs with 1 ml of inoculum, (one midgut suspension can be used to inoculate 5-6 healthy grubs).

4. The inoculated grubs are then put in a plastic box (15 cm. dia, 15 cm. ht) containing 200 g of sterilized cowdung or coir dust (plate 3) moistened sufficiently with sterile water. Care should be taken to ventilate the box by making holes in the lid.

5. Check regularly for the OBV infection and the procedure is repeated for maintenance of the viral culture.

Storage and inactivation

The cadavers or virus triturate could be stored at 4°C indefinitely. The infection half-life of the virus in cattle dung is about five days and total inactivation on the eighth day. It could also be stored using skimmed milk (0.25 g/ml) kept at 0 to 7°C (Final Report of adhoc scheme). Formaldehyde or dettol 1% solution inactivates the virus and 54°C is the thermal inactivation point.

Field release of OBV:

1. Allow 10-15 healthy adult rhinoceros beetles to crawl in a baculovirus infected midgut suspension kept in a shallow glass trough (1 g midgut/100 ml 0.001 buffer at pH 8.5) for half an hour.

2. Transfer the beetle into plastic boxes and starve them for 12 to 24 hours.

3. Release the beetle in the field preferably at dusk and observe the field for rhinoceros beetle control once in six months.

Performance of released baculovirus in the field:

On release of baculovirus, the initial inoculum decimated the grubs and adults resulting in the drastic decline in pest population and substantial reduction in crop damage. In India, the baculovirus was introduced into Lakshadweep and Andaman Islands and re-released on the mainland in Chittilappilly (Kerala). The results are given in Tables 1, 2, 3 and 4.

Table 1: Impact of the introduction of baculovirus disease into *O. rhinoceros* population in Minicoy, on crop damage in experimental plots

	Mean (%) of experimental plots		
	Leaf damage	Spathe damage	Spindle damage
Pre-release			
April 83	55.83	25.90	29.56
Post-release			
Jan 84	45.43	8.86	8.27
Nov 84	25.57	1.95	1.84
Sept 85 12.89	1.61	2.90	
CD 5%	5.40	6.14	9.72
CV (%)	9.36	16.73	31.32
SEM	1.75	1.88	3.16

(Mohan *et al.* 1989)

Table 2: Performance of *Oryctes* baculovirus in Androth Island of Lakshadweep

Period of Survey	Leaf damage	Spathe damage	Fresh incidence of spindle	OBV incidence
Pre-release (Apr '88)	55.0	7.3	23.5	0.0
Post-release (Dec. '88)	43.0	3.0	15.7	35.6
Post-release (Jan '90)	13.5	3.2	5.9	60.6

(Pillai, 1990)

Table 3: Effect of re-release of baculovirus in an already infected contiguous area at Chittilappilly, Thrissur, Kerala

	% of infestation			
	Palms	Leaf	Spathe	Spindle
Pre-release				
July 1989	100	34.4	12.5	68.18
Post-release				
Feb 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
Aug 1992	22.73	6.66	0.00	0.00

(Biju *et al.* 1995)

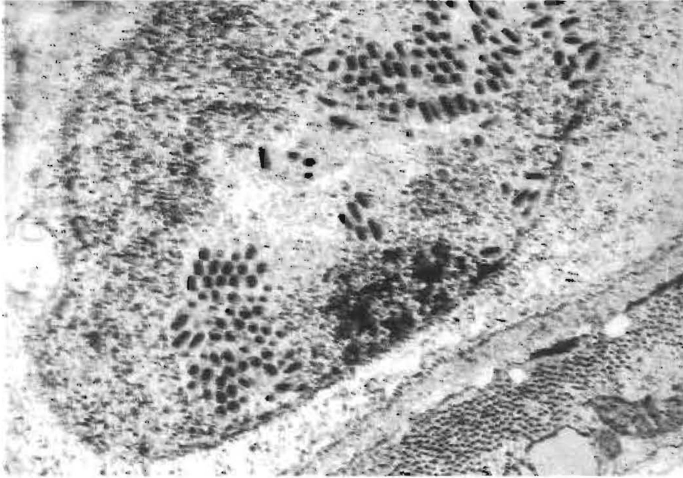

Plate 1.
Baculovirus of *Oryctes*
- EM photograph

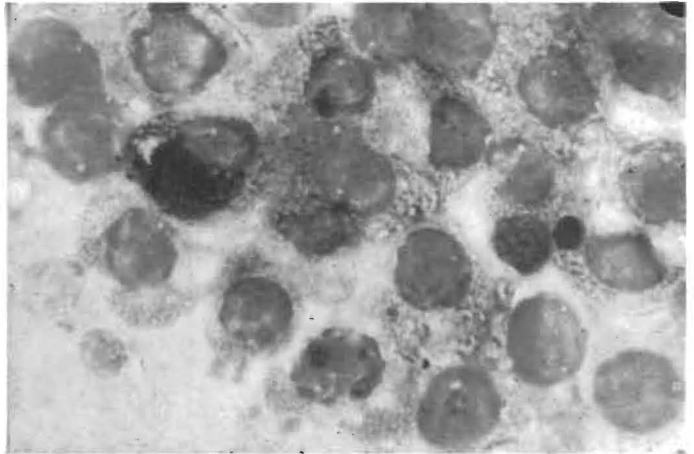

Plate 2.
Hypertrophied nuclei

Plate 3.
Oryctes grubs in coir waste

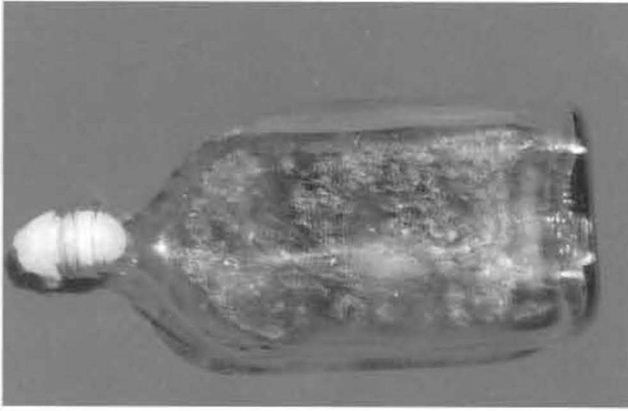

Plate 4.
Metarhizium anisopliae
in coconut water

Plate 5.
Metarhizium anisopliae
in cassava rice bran
mixture

Plate 6.
Reduviid predator
Platymeris laevicollis

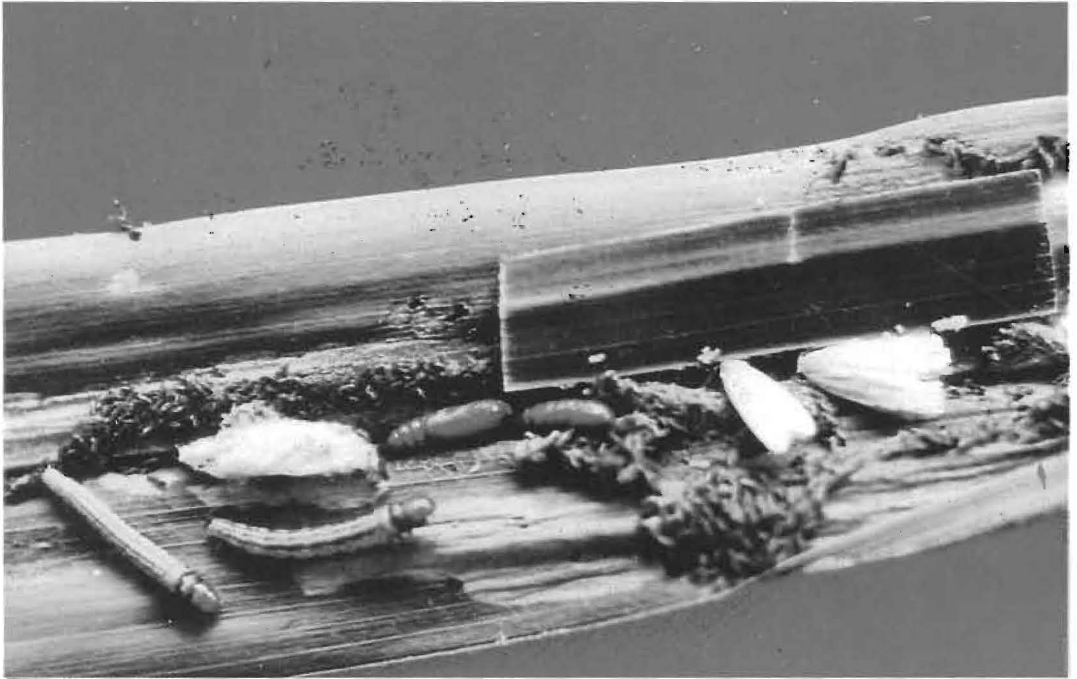

Plate 7. Life stages of *Opisina arenosella*

Plate 8. *Campsomeriella collaris* parasiting white grub

Table 4: Percentage of virus infection and decline in beetle population at Sipighat (Andamans)

Observations	Percent virus infection
Pre-release 1987 (March)	0% (n=81)
Post-release 1987 (December)	53% (n=53)
1988 (Dec)	77% (n=17)
1989 - 1991	61% (n=18)

(Jacob, 1996)

Advantages:

1. This virus is host-specific, self-perpetuating and safe to non-target organisms.
2. It can be easily mass multiplied and can be integrated in IPM.

Metarhizium anisopliae (Metsch) Sorokin

M. anisopliae var. *anisopliae* (spore size 5.8 μm) and *M. anisopliae* var. *major* (spore size 10 - 14 μm) are the two varieties identified, of which the latter is more pathogenic to *Oryctes* grubs.

Diagnostic symptoms:

The infected grubs become sluggish, lose appetite and die within 10-15 days. The body gets hardened and white powdery fungal colonies appear at all joints of the integument. After 4-7 days, green coloured spores are produced. Finally, the cadavers become black and mummified (plate 4). Adults also succumb to infection but are less susceptible.

Mode of infection:

The fungal spores attach to the insect epicuticle, they germinate and produce germ tubes. These germ tubes penetrate both the epicuticle and procuticle and enter the body. They spread inside the tissues, produce toxins and arrest the host metabolism resulting in the death of the host. The success of infection depends on an optimum temperature of 27-28°C and relative humidity of 70-90%.

Culture maintenance

1. Transfer *M. anisopliae* spores into the freshly prepared sterilized PDA slants under aseptic conditions.
2. Incubate the slants at 28 ± 2°C until a mat of green spores is produced.
3. Store the slants in refrigerator.

Mass production and field application

a. **Coconut water method** (Plate 5)

1. Fill 25-30 ml sterilized coconut water (from mature nuts) in flat sided glass bottles. Plug the mouth with cotton and transfer *M. anisopliae* spores into the bottle.
2. Incubate the bottle at 28 ± 2°C by keeping it flat on the incubator rack. After 25-30 days of incubation, green coloured spore mass is produced.
3. Mix the spore mass in sterile water and spray it on the rhinoceros beetle breeding sites.

b. **Cassava chips method** (Plate 6)

1. Fill cassava chips and rice bran (8:1 w/w) in an aluminium vessel.
2. Add urea or extract of waste fish meal powder as nitrogen supplement (1.08 g and 7.4 per 100 g of cassava chips: rice bran mixture)
3. Cover the whole unit with a cloth having a small hole in the centre.
4. Sterilize the whole unit.

Table 5: Important parasitoids of *Opisina arenosella*

Name of parasite	Target pest stage	Nature of parasitoid
Apanteles taragamae	Early larva	Solitary
Bracon hebetor	Late larva	Gregarious
Goniozus nephantidis	Do	Do
Elasmus nephantidis	Pre-pupa	Do
Goryphus nursei	Larva-pupa	Solitary
Antrocephalus hakonensis	Pupa	Do
Barachymeria nosatoi	Do	Do
B. nephantidis	Do	Do
B. atteviae	Do	Do
B. lasus	Do	Do
Trichospilus pupivorus	Do	Gregarious
Xanthopimpla punctata	Do	Solitary
X. nana nana	Do	Do

Table 6: Important predators of *Opisina arenosella*

Name of predator	Predator stage	Prey stage
Ankylopteryx octopunctata candida	Nymph	Egg/Early larva
Calleida splendidula	Larva/adult	All stages
Parena nigrolineata	Do	Do
Sphedanolestes aurescens	Do	Do
Cheiracanthium sp.	Do	Do
Cheiracanthium melanostoma	Do	Do
Rhene indicus	Do	Do
Sparassus sp.	Do	Do

5. Add *M. anisopliae* spore suspension in sufficient quantity to the substrate under aseptic condition through the hole in the cloth using a funnel.

6. Incubate for a month until green coloured spores develop.

7. Mix 1:1 w/w the spore substrate with powdered cowdung and sprinkle over the breeding sites of the beetle.

Efficacy of the pathogen

Mortality observed was 100% in grubs and 38% in beetles in the laboratory. In the field the pathogen effected more than 75% death of the pest larvae, when it was applied at the optimum dose during favourable conditions. Once applied, the fungus takes few weeks to establish, but it persists in the site for more than two years. The spores are resistant to hot conditions and germinate during favourable weather.

Advantages

1. *M. anisopliae* suppresses population of rhinoceres beetle during monsoon season, when no pesticide can be either applied or are proved effective.

2. It is safe to non-target organisms, easily mass multiplied and can be included in the IPM.

A number of predators are associated with the beetle in its breeding ground. *Santalus parallelus, Pherpsophus occipitalis, P. lissoderus, Scarites* sp., *Harpalus* sp and *Agrypnus* sp. nr. *bifoveatus* consumed the eggs and early larvae of *Oryctes* under natural conditions. The extent of pest suppression exerted by these predators warrants their conservation in nature. In addition, an exotic reduviid predator *Platymeris laevicollis* (Plate 7) imported from Zanzibar was also found to be effective in killing the adult beetles living on the palm crown. Release of these bugs @ 6 bugs per palm checked the *Oryctes* population and damage to the palm crown. But, this reduviid predator could not establish under the conditions prevailing in Kerala and Karnataka.

Entomopathogenic nematodes also are found fatal to the grubs of rhinoceros beetle. DD-136 *Steinernema carpocapsae* tested @ 10,000 nemas/third instar *Oryctes* grubs produced 88% mortality against nil in control in the laboratory. The same dose also effected significant reduction in *Oryctes* population in breeding sites for over a month of the treatment.

Species of *Rhabditis* collected from Kerala and Lakshadweep produced 100% mortality of the adult beetle after few weeks of treatment against nil in control.

Leaf Eating Caterpillar – *Opisina arenosella* Wlk.

Opisina arenosella is an important caterpillar pest and all stages are present on the palm foliage (Plate 8). Larvae are the destructive stages. They live in galleries on the lower surface of the leaves and feed on the chlorophyll containing parenchymatous tissues. The upper epidermis is kept intact, so that after the feeding injury the affected leaves appear dry. Usually the lower one or two whorls of leaves are affected, but under epidemic outbreaks all the green leaves, petioles, spathes or even nuts are eaten up. Damage to the photosynthetic tissues result in decline in yield. Egg to adult period is completed in 2 to 2.5 months; nearly 42 days is spent as larva.

Natural enemies:

The list comprises 40 insects and one mite as parasites, and 20 insects and 20 spiders as predators. The important parasites and predators are listed in Tables 5 and 6. A bacterium and a fungus are observed as pathogens.

Sampling technique :

O. arenosella occurs in different intensities throughout the year. On the west coast of Kerala, low population occurs during November - January; medium during February - March and September - October and maximum during April - June. With a view to studying the population in a particular area 20% of the palms are to be selected and 205 leaves from the lower or middle whorl are to be observed as sample from each of these palms. Population of *Opisina* and the associated natural enemies are to be recorded at fortnightly intervals and population estimated based on the following formula:

Month	Sampling formula
February - March	Y= 22.59 + 5.75 X
April - June	Y= 38.40 + 9.70 X
July - October	Y= 20.57 + 6.20 X
November - January	Y= 6.36 + 8.99 X

Where 'X' is the pest count in sample leaflets and 'Y' is the estimated population.

Mass multiplication:

Techniques were standardized for the mass multiplication of important parasitoids (Tables 7 and 8).

Table 7: Mass multiplication of larval parasitoids *Apanteles taragamae* and *Goniozus nephantidis*

Particulars	Parasitoid	
	A. taragamae (Braconidae)	*G. nephantidis* (Bethylidae)
Rearing cages	Cylindrical specimen tube glass jar (10x5 cm) mouth covered cloth.	(7.5 x 2.5 cm) with cotton plug.
Host/stage	*Opisina* or *Corcyra* larva (second instar) 15-20 Nos. on leafbit with gallery *in situ*	*Opisina* or *Corcyra* larva (4th to 7th instar) 1-2 Nos.
Number of parasitoids to be used	Male and female one each (newly emerged)	One or two mated females (2-3 days old)
Days of adult emergence	10-25	10-14
Progeny	Female biased	Female biased
Remarks	Parasitised larvae to be removed after 12 h to fresh leaf-let (for *Opisina* and to 'semolina' (for *Corcyra*) feeding and development	Parasitoid stings and paralyses third instar host larvae, but egg laying occurs from fourth to further instar.

Table 8: Mass multiplication of pre-pupal parasitoids of *Opisina arenosella*

Particulars	Parasitoid		
	E. nephantidis (Elasmidae)	*B. nosatoi* (Chalicididae)	*X. punctata* X. *nana nana* (Ichneumonidae)
Rearing cages	Specimen tube (7.5 x 2.5 cm) with cotton plug	Cylindrical glass jar (117.5 x 6.8 cm) with mouth covered with muslin cloth	Glass chimney (22 x 4.5 cm) with muslin cloth covering of the openings
Host/stage	*Opisina*-prepupa e - Nos.	*Opisina* pupae with cocoons and silken galleries- 20-30 Nos. placed on cardboard pieces.	*Opisina/ Anadevidia* pupae with cocoons by sandwich method 5 or 6 Nos.
Number of parasitoids	2 or 3 mated females (1-2 days old)	30 to 50 Nos. of both sexes	1 or 2 mated females (4-5 days old
Days of adult emergence	11	12-20	10-12
Progeny	Female biased	Female biased	Female biased
Remarks	Highly host specific and stage specific	Host specific Exposure period 4-5 h	Exposure period 1-3 h.

Impact of release of parasitoids:

Promising parasitoids (bethylid, elasmid and chalcidid) are to be released at fixed norms and intervals depending on the target stages of the pest. As such, *G. nephantidis* is released @ 20.5%, *E. nephantidis* @ 49.4% and *B. nosatoi* @ 31.9%, respectively, depending on the larval, pre-pupal and pupal population of *Opisina*. Release of the larval and pre-pupal parasitoids can lead to 83% and 81% reduction in the population of the pest, respectively (Sathiamma *et al.* 1986). Large scale releases of these parasitoids, in an area of nearly 2.7 ha comprising 500 palms, at the fixed doses and fortnightly intervals for a period of five years revealed significant reduction (94%) in *Opisina* population and the damage to palms. Follow-up observations revealed only a low population, without any further release of parasitoids.

Red Palm Weevil– ***Rhynchophorus ferrugineus* F.**

Red Palm Weevil is a deadly tissue borer pest of coconut palm. Grubs are the destructive stages of the pest and they tunnel into the palm trunk or crown and feed on the soft tissues inside. Adult is a prolific breeder. They are attracted to cuts or injuries or rotten tissues on palm trunk

or crown and lay eggs. Life cycle is completed in about three months.

Damage symptoms:

Early detection of infestation is by observing:

- Holes on the stem/petioles
- Extrusion of frass or oozing out of a brown viscous fluid.
- Longitudinal splitting of leaf base
- Wilting/yellowing of inner leaves
- Rotting crown with characteristic odour
- Easily coming off of the green leaves.
- Presence of cocoon/fibres at the palm base, and,
- Gnawing sound of feeding grubs.

Bioagents:

The recorded bioagents are:

a. Parasite - Pyemotid mite kills eggs and early grubs.

b. Predator - Earwig *Chelisoches moris* feeds on eggs.

c. Pathogen -

  i) The bacterium *Pseudomonas aeruginosa* causes 69% mortality of the grubs in 6 days.

  ii) Yeast causes mortality of early stage grubs.

  iii) Nuclear polyhedrosis virus kills the grubs.

  iv) Cyloplasmic polyhedrosis virus infects grubs which emerges as malformed adults.

  v) Nematodes such as species of *Teratorhabditis, Mikoletzkya, Monochoides, Acrostichus* and an aphelenchoidid are associated with grubs and pupal fibres.

White grub – *Leucopholis coneophora*

This is a major pest of coconut in sandy loam areas of Kerala and Karnataka. Grubs feed on the growing roots of the palm. They are also pests of tuber crops which are grown as intercrops in coconut gardens. The pest has an annual life cycle and the peak emergence of adult beetle occurs at dusk time from June to August.

Damage symptoms:

Yellowing of leaves, premature nut fall, delayed flowering, reduction in yield and feeding damage on live roots.

Bio-agents:

Campsomeriella collaris is a scoliid parasite on grubs (plate 9). Pupation in soil and adults are free living, which feed on honey from flowers.

Pseudomonocystis sp., a eugregarine protozoan pathogen infecting nearly 23% of the third instar grub in the field.

Beauveria bassiona, B. brogniartii and *M. anisopliae* are pathogenic to grubs of *Leucopholis* spp.

The nematode *Coenorhabditis* associated with the grubs is found to be non-pathogenic.

Source of nucleus culture of bioagents:

Biological Control Laboratory,
Central Plantation Crops Research Institute,
Regional Station, Kayangulam,
Krishnapuram - 690 533, Kerala, India.

* * * * *