Review Article

Biocontrol of agricultural pests

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Received: 06/08/2024; Revised: 12/10/2024; Accepted: 21/10/2024; Published: 29/01/2025

Abstract
Biological control is an environmentally safe method used in integrated pest management. It involves suppression of pest population using two groups of living organisms referred to as Entomophaga and Entomopathogens. Entomophaga comprise natural predators and parasites of the pest. Entomopathogens are generally microorganisms that include virus, bacteria, fungi, nematodes and protozoa. This review gives an account of various biological control methods including ecological engineering and push-pull strategy with examples and discusses the advantages as well as challenges associated with them.

Keywords: Biological control, Entomophaga, Entomopathogen, Ecological engineering, Push-Pull strategy.

Introduction

The central core to the performance of many ecosystem processes is the insects which in their dominance and long evolutionary background occupy every niche of the earth. Though many insects are beneficial to mankind, some of them interfere with man and his properties aptly termed as “Pests” which could cause direct feeding injury (red palm weevil) or serve as vectors transmitting diseases (Banana aphid transmitting bunchy top disease). Biological control is one of the widely adopted tools in pest management programme that targets environmental and ecological stability by holding biodiversity together. Biological control involves suppression of pests using other living organism which includes two major categories such as (i) Entomophaga encompassing predators and parasitoids and (ii) Enomopathogens involving virus, bacteria, fungi, nematodes, protozoa *etc.*

Types of biological control

Biological control is one of the critical components of Integrated Pest Management programme and owing allegiance to environmental and human safety, this approach formed the centre stage of sustainable pest suppression these days. The word “*Biological control*” was first used by Prof. Harry Scott Smith during 1919; however, its widespread usage was popularized by Prof. Paul H. Debach, a noted citrus entomologist. It could be classical biological control where introduction of natural enemies is usually undertaken from the centre of origin of the invasive pest through standard protocols and get systematically released in the new country of infestation. E.g., Bio-suppression of papaya mealy bug (*Paracoccus marginatus*) in India through introduction of the encyrtid parasitoid, Acerophagous *papaya* from Puerto Rico. Under augmentative biological control, it could be either inoculative with one time release of natural enemies (E.g., Release of green lacewing, *Chrysoperla zastrowii sillemi* for the bio-suppression of sucking pests [Hopper, *Amrasca devastans*; aphid, *Aphis gossypii*] of cotton) or innundative with frequent release of natural enemies based on the pest incursion status (E.g., Frequent release of stage-specific parasitoids, *Goniozus nephantidis* or *Bracon brevicornis*in the bio-suppression of coconut black headed caterpillar, *Opisina arenosella*). Though conservatory biological control is not so prominent and successful in several cropping systems, its silent but significant role in the bio-suppression of two invasive whiteflies, *viz.,* spiralling whitefly (*Aleurodicus dispersus* Russell) and rugose spiraling whitefly (*Aleurodicus rugioperculatus*

Martin) by aphelinid parasitoids *Encarsia dispersa* and *Encarsia guadeloupae* is noteworthy.

Entomophaga

Predators

In general, predators kill the prey for itself and are usually larger than the prey. Predators are mainly free-living species that directly consume a large number of preys during their whole lifetime. The first international shipment of an insect as biological control agent was made by Charles V. Riley in 1873, delivering France the predatory mites *Tyroglyphus phylloxera* to help fight the grapevine phylloxera (*Daktulosphaira vitifoliae*) that was destroying grapevines in France. In 1888–1889 the vedalia beetle, *Rodolia cardinalis*, a lady beetle, was introduced from Australia to California to control the cottony cushion scale, *Icerya purchasi*. This had become a major problem for the newly developed citrus industry in California, but by the end of 1889 the cottony cushion scale population had already declined, and this successful bio-suppression strategy is termed as "*Miracle of Entomology*".

For rodent pests, cats are natural predators and hence, they can be employed for rodent control, in conjunction with reduction of "harborage"/hiding locations. Barn owls are also sometimes used as biological rodent control. Due to the absence of rat snakes and owls in Lakshadweep Islands, rats emerged as a major pest of coconut, reducing yield by about 40%.

The red spot reduviid predator, *Platymeris laevicolis* imported from Zanzibar was found to be an excellent predator of rhinoceros beetle, *Oryctes rhinoceros* (Figure 1a). A wide array of spiders is recorded as predators of black headed caterpillar, *Opisina arenosella* of which *Rhena*, *Sparassus* and *Cheiracanthium* are the major ones. Many predatory mites including *Amblyseius largoensis*, *Neoseiulus paspalivorus* and *Bdella distincta* were reported as potential predators of coconut eriophyid mite, *Aceria* (Figures 1b and 1c).

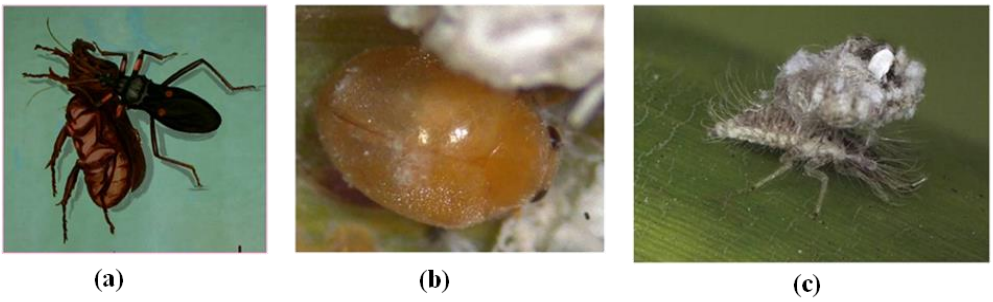


Fig.1. (a) ***Platymerislaevicolis*** **(b)** ***Sasajiscymnusdwipakalpa*** **(c)** ***Dichochrysaastur***

Parasites and parasitoids

A parasite and its host belong to two different classes taxonomically and usually a parasite will never kill the host but could cause extreme level of parasitism. For example, head louse on humans is a parasite. On the other hand, parasitoid and the host belong to the same class and usually a parasitoid kills the host. The bethylid larval parasitoid of *Goniozus nephantidis* parasitizes and kills the host, black headed caterpillar, *O. arenosella* (Figure 2a), while a predator kills the host for itself, and a parasitoid parasitizes the host for its progenies.

The stage specific parasitoids of *O. arenosella viz.*, the larval parasitoids *Goniozus nephantidis* (Bethylidae), *Bracon brevicornis* (Braconidae) (Figure 2b), the prepupal parasitoid, *Elasmus nephantidis* (Elasmidae), and the pupal parasitoid Brachymeria *nosatoi* (Chalcididae) are the most promising ones which are extensively used for augmentative releases for pest suppression (Figure 2c). The major desirable attributes of these parasitoids are their greater host searching ability, production of higher proportion of females, occurrence throughout the year and their distribution in all pest infested areas. A large area field validation of the bio-suppression technology of coconut black headed caterpillar with regular monitoring and release of stage specific parasitoids *viz.*, *G. nephantidis*, *B.brevicornis*, *E. nephantidis* and *B. nosatoi* was taken up during 1999-2002 in different geographic locations in coastal Karnataka and Kerala comprising of a total of 1,400 ha could achieve 93-100 per cent reduction in *O. arenosella* population in a period of two years. Very recently the natural bio-suppression of the rugose spiralling whitefly, *Aleurodicus rugioperculatus* by the aphelinid parasitoid, *Encarsia guadeloupa* (Figure 2d) is a classic example of conservatory biological control which could parasitize the pest to more than 85% in a pest prone area. In this backdrop, a pesticide holiday is advised for the natural buildup of the parasitoid, *E. guadeloupae*.

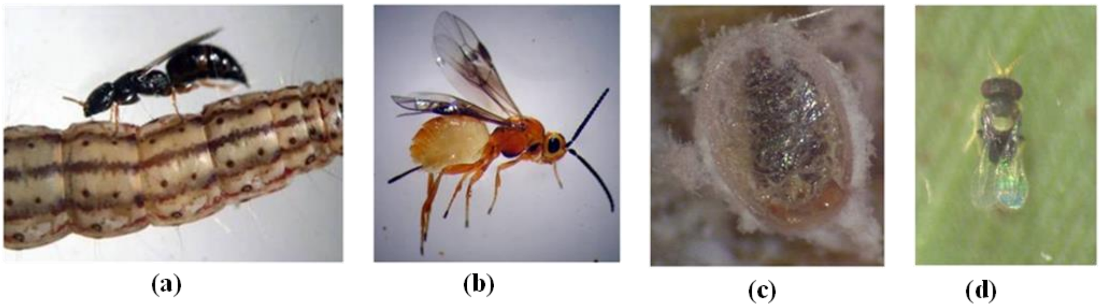


Fig.2. (a) ***Goniozus nephantidis*** **(b)** ***Bracon brevicornis*** **(c) Parasitized pupa (d)** ***Encarsia guadeloupae***

Entomopathogens

This category includes microbial pathogens such as virus, bacteria, fungi, protozoa and nematodes used in biological pest suppression. [1-6]

Virus

Insect viruses (baculovirus) are obligate disease-causing organisms that can only reproduce within a host insect. They can provide safe, effective and sustainable control of a variety of insect pests, although they are most effective as part of a diverse integrated pest management program. They are broadly classified as occluded or non-occluded types based on the occlusion of virion particles. Virus infection begins in the insect's digestive system but spreads throughout the whole body of the host in fatal infections. The body tissues of virus-killed insects are almost completely converted into virus particles. The digestive system is among the last internal organ systems to be destroyed, so the insects usually continue to feed until they die. The occluded viruses such as *Spodoptera litura* nuclear polyhedrosis virus, *Helicoverpa armigera nuclear* polyhedrosis virus, *Cydia pomonella* granulosis virus are very effective and commercially used in biological pest suppression. In coconut, the use of non-occluded virus, *Oryctes rhinoceros* nudivirus (OrNV) is very effective and kills the *O. rhinoceros* grub in 15 -20 days' time significantly suppressing the longevity and fecundity of adult beetles (Figures 3a-c). *Or*NV was first reported from Malaysia by Huger (1966) and named it as rhabdion virus of *O. rhinoceros*. In India, *Or*NV infected grubs after developing the symptoms could be stored in the deep freezer at -40^{o}C indefinitely retaining its virulence. Studies conducted at ICAR - CPCRI indicated that *Or*NV infected grubs become less active and stops active feeding. As a result of virus multiplication in the mid gut epithelium, fat body disintegrates and haemolymph content increases. This causes translucency in the abdominal region which is an important exopathological symptom of the *Or*NV infection and mortality in grubs. The infected beetles disseminate the virus through faecal matter into the surroundings after 3-9 days of inoculation at the rate of 0.3 mg virus adult^{-1} day^{1}. Introduction of *Or*NV in Minicoy and Androth Islands of Lakshadweep, Chittilappilly, Thrissur, Kerala and Sipighat, Andaman Island successfully

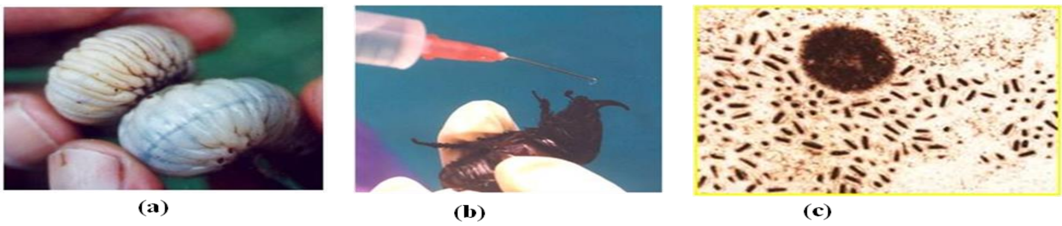


Fig.3. (a) Infected and healthy grub of ***Oryctes rhinoceros*** **(b) nudivirus,** ***Or*****NV inoculation (c)** ***Or*****NV particle under microscope**

reduced the population of *O. rhinoceros* and its damage potential on coconut.

Bacteria

A small number of entomopathogenic bacteria have been commercially developed for control of insect pests. These include several *Bacillus thuringiensis* sub-species, *Lysinibacillus* (*Bacillus*) *sphaericus*, *Paenibacillus* spp. and *Serratia entomophila*. *B. thuringiensis* sub-species *kurstak*i is the most widely used for control of pest insects of crops and forests, and *B. thuringiensis* sub-species *israelensis* and *L. sphaericus* are the primary pathogens used for control of medically important pests including dipteran vectors. These pathogens combine the advantages of chemical pesticides and are fast acting, easy to produce at a relatively low cost, easy to formulate, have a long shelf life and allow delivery using conventional application equipment and systemics (i.e. in transgenic plants).

Mode of action

B. thuringiensis (Bt) is an aerobic or anaerobic facultative and sporulating bacterium (Figure 4). It can be found in soil, insects and their habitats, stored products, plants, forest, and aquatic environments. It produces a parasporal inclusion body (crystal) of protein origin, formed during sporulation and this crystal is composed of Cry proteins which are encoded by Cry genes. After ingestion, the crystals are solubilized in the alkaline (*p*H 9 to 12) midgut environment. Some toxins are activated under alkaline conditions (CryII1A) and others are activated under conditions of neutral to acid *p*H (Cry1b). Cleavage of Cry toxins is a crucial step in the activation of the toxin and also in its specificity in different insects.

membranes of the columnar cells of the lepidopteran gut. The binding of Cry toxins to the apical microvillus of the membrane vesicles of the insect determines the specificity of the Cry toxins. The Cry toxins cross the peritrophic membrane by binding to specific receptors on the apical membranes of intestinal cells causing opening or pore formation followed by vacuolation of the cytoplasm by osmotic imbalance between the intracellular and extracellular environments and cell disruption. This destroys the microvilli, causing the insect to stop feeding, leading to its death.

Success

Unlike broad spectrum chemical pesticides, *Bt* toxins are selective and negative environmental impact is very limited. Of the several commercially produced microbial control agents, *Bt* has more than 50% of market share. Extensive research, particularly on the molecular mode of action of Bt toxins, has been conducted over the past two decades. The Bt genes used in insect-resistant transgenic crops belong to the Cry and vegetative insecticidal protein families of toxins. Bt has been highly efficacious in pest management of corn and cotton, drastically reducing the amount of broad-spectrum chemical insecticides used while being safe for consumers and non-target organisms. Despite successes, the adoption of Bt crops has not been without controversy. In addition to discovery of more efficacious isolates and toxins, an increase in the use of Bt products and transgenes will rely on innovations in formulation, better delivery systems and ultimately, wider public acceptance of transgenic plants expressing insect-specific Bt toxins.

(a) (b) (c)

Fig. 4. (a) *B. thuringiensis* (b) Bt infected larva (c) Recombinant DNA technique

When toxins are solubilized, protoxins are released through the action of proteases resulting in active proteins of 60–70kDa. The protoxins are activated by digestive enzymes in the midgut and bind to specific receptors in the microvilli of the apical

Fungi

Entomopathogenic fungi do not require *per os* infection and could enter insect system through the cuticle. Examples include *Metarhizium anisopliae*, *Verticillium lecanii*, *Beauveria bassiana*, *Nomuraea rileyi*etcwell exploited in pest management. *Metarhizium anisopliae* (Metchinkoff) Sorokin is an entomopathogenic fungus which kills *O. rhinoceros* in conditions of low temperature and high humidity. The fungus could be mass multiplied using cheaper substrates in both solid and liquid media and the spores could be harvested and treated in the breeding site at 5 x 10^{11} spores m^{-3}. In vermicomposting sites, treatments with *M. anisopliae* spores killed all third - instar larvae, with the highest dose giving the fastest kill, taking eight days when favoured by high humidity (Figure 5.a and b). This technology through farmer - participatory and women group approach has created a great impact on the long-term bio-suppression of the pest in farmer-participatory and community-mode. Mass production technology of *M. anisopliae* was standardized in semi - cooked rice grains yielding a spore count of $3x10^{7}$cfu / g of the culture.

Searching for natural enemies of coconut eriophyid mite *A. guerreronis*, ICAR-CPCRI could collect more than 40 isolates of the acaropathogenic fungus, *Hirsutella thompsonii* (Figure 5c). Based on the bio-efficacy studies, one virulent isolate collected from Kayamkulam was characterized through molecular tools confirming species identity. Coconut water was found as an ideal medium for mass production of *H. thompsonii*. Talc-preparation of this *H. thompsonii* at 20 g L^{-1} of water $palm^{-1}$ containing 1.6 x 10^{8}cfu with a frequency of three spraying per year resulted in 63-81 per cent reduction in mite incidence. It was found effective in many locations, though seasonal variation in efficacy existed.

metazoans on earth and second only to insects, in terms of diversity of forms (species) is concerned. Nematodes dwell in all types of habitats on the earth. Most of the nematodes are free living which feeds on microorganisms. Nematodes also parasitize plants, animals and human beings. There is hardly any animal on earth which is free from one or the other kind of nematode infection.

Entomopathogenic nematodes (EPN) of the families Steinernematidae and Heterorhabditidae are soil inhabiting insect pathogens that possess potential as biological control agents. These nematodes, working with their symbiotic bacteria (Xenorhabdus for steinernematids and Photorhabdus for heterorhabditids), kill insects within 24 to 48 hour (Figure 6). They are safe for the plant health, human health, soil and the environment. There is a huge potential for the utilization of these nematodes for the management of many coconut pests like white grub, rhinoceros beetle, red palm weevil etc.

(a) (b)

Figure 6. (a) Larvae killed by Steinernematid nematodes (b) Infective juveniles of EPN

(a) (b) (c)

Fig. 5. (a) *M. anisopliae* infected grub (b) *M. anisopliae* in semi-cooked rice (c) *H. thompsonii* infected mite

Entomopathogenic nematodes (EPN)

The word ‘nematode’ means "thread-like” and they are also known as thread worms or round worms. Nematodes are numerically the most abundant

Protozoa and Microsporidia

Protozoans are one-celled forms. One group the Microsporadia, contains many species that have promise for biological control. Microsporidian infections in insects are thought to be common and responsible for naturally occurring low to moderate insect mortality. But these are relatively slow acting organisms, taking days or weeks to debilitate their host. Infected insects may be sluggish and smaller than normal, sometimes with reduced feeding and reproduction, and molting problems. Death may follow if the level of infection is high. *Nosema locustae* is the only commercially available species of microsporidium, marketed under several labels for the control of grasshoppers and crickets. It is applied with insect-attractant bait. Because of its slow mode of action, this product is better suited to long-term management of locusts than to the more intensive demands of commercial crop or even home garden production.

Bio-scavenging

When plants and animals die, the organic matter that makes their bodies possesses energy in the form of chemical bond. That energy is released by sequential breakdown of body tissue constituents. Insects, in feeding on dead plant and animal tissues, often carry out the first stage in the decomposition process by pre-disposing matter for enhanced decay and ultimate break down by microorganisms. Some prominent examples of insect decomposers include termites that break down wood, spring tails that assist in the decomposition of dead leaves, carrion beetles and fly maggots that feed on dead animals aiding scavenging and enhanced disintegration.

Rugose spiraling whitefly

Emergence of the exotic rugose spiraling whitefly (RSW), *Aleurodicus rugioperculatus* (Figure 7a) on coconut palm during 2016 from Pollachi, Tamil Nadu and Palakkad, Kerala was the latest new addition of invasive pest introduced into our country ably aided by inadequate quarantine approach. The pest could have been introduced from Florida, USA either through ornamental palms or RSW-infested coconut seedlings. Being non-native, RSW sounded alarm initially due to extensive feeding potential and high population build-up of the bug on coconut palms. RSW feeds from undersurface of palm leaflet and the enormous honey dew excreted get deposited on the upper surface of palm attracting the sooty mould, *Leptoxyphium* sp. Growth of sooty mould affects the photosynthetic efficiency of palms and served as a characteristic feature of pest identification (Figure 7c).

Farmers are worried due to black encrustation of sooty mould on palms and other under story intercrops in the system. RSW bred fast reaching as high as 15 egg spirals on a leaflet and with the white flocculent masses and sooty mould development could panic any farmer in the initial phase. Gradient outbreak of RSW could be realized interlinked with higher maximum temperature with lower humidity and rainfall as well. However, in a period of four to five months, there has been tremendous build-up of the aphelinid parasitoid, *Encarsia guadeloupae* (Figure 7b) which could bio-suppress RSW quite efficiently and significantly. From a modest 10% parasitism initially registered, natural parasitism rose as high as 82% in a period of four-five months. This is one of the classical success stories of conservatory biological control well documented and by this observation, ICAR-CPCRI has recommended pesticide holiday to effectively conserve the natural enemy involved in RSW suppression. Though not well received, this strategy was well appreciated by the ecological benefit realized to nature as well as to human health. The Organic Policy of the Government of Kerala is another boost in this direction that may also encourage biodiversity preservation.

Sooty mould scavenger beetle

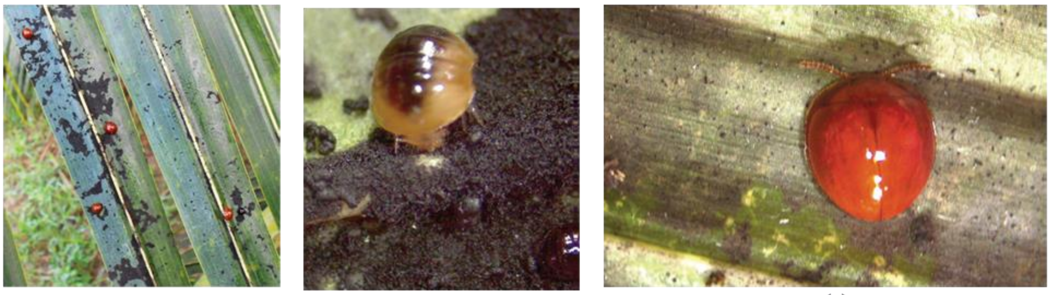
For the first time at the international level, scientists from ICAR-Central Plantation Crops Research Institute, and ICAR-National Research Centre on Banana, Trichy have reported the occurrence of a sooty mould scavenging beetle, *Leiochrinus nilgirianus* Kaszab (Coleoptera: Tenebrionidae) on coconut palms infested by the rugose spiraling whitefly (RSW) (Josephrajkumar

(a) (b) (c)

Fig. 7. (a) *Aleurodicus rugioperculatus* (b) *Encarsia guadeloupae* (c) Sooty mould laden palm leaflet

et al., 2018). This beetle, which resembles the predatory lady beetle in appearance, are found to feed on the sooty mould deposits developed on the honey dew excreted by the rugose spiraling whitefly (Figure 8). *L. nilgirianus* could be first located from sooty mould laden coconut palms at ICAR-CPCRI, Regional Station, Kayamkulam during July-August period which coincided with the south-west monsoon phase providing high humidity and morning wetness, that favoured the accurate niche for enhanced survival and feeding.

All stages of the beetles *viz.*, eggs, grubs, pupae and adult beetles were found on the palm leaflets and other intercrops with sooty mould. Generally, the grubs and adult *L. nilgirianus* beetles are restricted on the under surface of the leaves. But they move on to the upper surface of the leaves with sooty mould deposits during early morning hours with dew drops and feed on the sooty mould. Eggs of this beetleare purplish, laid in groups and emerging neonates are transparent and turn black, assume spherical and cylindrical shapes in movement due to the presence of elastic inter-segmental membrane. Creamish patches are developed during pre-pupating stages and pupae with distinct cream patches are also confined on the under surface of palm leaflet indicating its photo-sensitiveness. During our observations, more than five beetles (Average 2.07±0.9) and immature grubs could be noticed on sooty mould laden leaves. Bristle-like tarsomeres and tarsal claws of adult beetles encouraged effective adhesion, scooping of mould and swift movement on fungal growth. *L. nilgirianus* population was found very high and subsequently got reduced with the decline in RSW population and the sooty mould deposits. Mouth parts and gut lining of the beetle with fungal deposits could be located confirming the scavenging action of the beetle. Though ecosystem services provided by insects include food for wildlife, pest destruction, crop pollination, scavenging, etc.which is estimated at around $57 billion in the United States of America alone, a thorough cleaning action on an economically significant crop like coconut is reported for the first time at global level. Habitat conservation of the sooty mould feeding scavenger beetle, *L. nilgirianus*in the palm ecosystem is very crucial and the conducive weather factors of high humidity and high rainfall prevailing in Kerala during monsoon phase are key factors for the survival and feeding efficiency of the beetle. Though report on scavenging action by insects such as termites disintegrating wood, springtail decomposing dead larvae and fly maggots feeding on dead animals exist in nature, the present instance appears to be unique in which sooty mould deposition is totally cleared by an insect scavenger on palms. Conservation of *E. guadeloupae* by limited or zero pesticide usage coupled with *in situ* habitat preservation of scavenger beetles (*L. nilgirianus*) appears to be a very effective strategy that would help in controlling *A. rugioperculatus* and in clearing sooty mould from coconut palms at no cost in the most natural and eco-friendly manner avoiding chemical management options and other expensive methods. Furthermore, the Organic Policy adopted by the State Government of Kerala could have encouraged the sustenance of the beetle and emerged in need for scavenging action.



(a) (b) (c)

Fig.8. (a) *L. nilgirianus* (b) Pre-pupating grub (c) Adult Leiochrini beetle

Ecological Engineering

Ecological engineering defined first by Odum (1962) is a human activity that modifies the environment according to ecological principles. Accordingly, it is a useful conceptual framework for considering the practice of habitat manipulation for arthropod pest management. Habitat manipulation aims to provide the natural enemies of pests with resources such as nectar, pollen, physical refugia, alternative prey, alternative hosts etc. The development of ecological engineering ranged from a simple first approximation that diversity is beneficial, to contemporary understanding that diversity can have adverse effects on pest management. In the recent era of agricultural intensification, the potential for using crop diversity to manage insect and microbial pests has not been extensively exploited.

Push-pull strategy

Push-pull strategies use a combination of behavior-modifying stimuli to manipulate the distribution and abundance of pest and/or beneficial insects for pest management. Strategies targeted against pests try to reduce their abundance on the protected resource, for example, a crop or farm animal. The pests are repelled or deterred away from this resource (push) by using stimuli that mask host apparency or are repellent or deterrent. The pests are simultaneously attracted (pull), using highly apparent and attractive stimuli, to other areas such as traps or trap crops where they are concentrated, facilitating their elimination. The strategy is a useful tool for integrated pest management programs reducing pesticide input.

An agro-ecosystem based pest regression strategy by including compatible intercrops in coconut-based cropping system has registered lower pest incidence (Figure 9). The influence of mixed-volatile cues of crop plurality (rambutan, nut meg, curry leaf, banana, turmeric, red gram, papaya) inflicted lesser rhinoceros beetle damage (15.8%) compared to palms in outer whorls (30%). *In situ* stimulo-deterrent diversionary strategy infused less pest damage and encouraged more defender population through eco-feast crops (*Antigonon leptopus*) and diversity in fruit crops. In addition to pest regression, continuous employment and income is generated fostering closer care to palms complementing the concept of an “inch of land and a bunch of crops”.

Figure 9. An agro-ecosystem based pest regression strategy for coconut cultivation by including compatible intercrops with the objective of

Plant health management

Plant health management (PHM) is the science and practice of understanding and overcoming the succession of biotic and abiotic factors that limit plants from achieving their full genetic potential as crops, ornamentals, timber trees, or other uses. Although practiced as long as agriculture itself, as a science-based concept, PHM is even younger than integrated pest management (IPM) and includes and builds upon IPM; but is not a replacement for IPM. PHM is a moving target, like a football game, where one team is science and technology and the other is nature, where the S & T team is only beginning to know nature's rules while playing itself with the three sets of rules written to, respectively, satisfy the laws of economics, protect the environment, and gain social acceptance. In a nutshell, PHM includes integrated pest, disease and weed management, integrated nutrient management and soil health management in a holistic manner as system approach.

From the ecological engineering concept, health management is revered as holistic and at the same time sustainable providing continuous income and employment as well. Systematic care would thus make you cheer with coconut and empower your health infusing bio-happiness.

Financial support and sponsorship:
Nil

Conflict of interest :

There are no conflict of interest.

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