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Epizootics and natural enemy complex associated with palm white grubs *Leucopholis* spp. in South India

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Abstract

Leucopholis spp. white grubs are subterranean polyphagous pests of palms in the southern and north-eastern regions of India. Natural enemies play a vital role in effectively suppressing and regulating pest populations, thereby maintaining ecological balance in a sustainable manner. A terrestrial crab was observed predating on arecanut whitegrub, *Leucopholis lepidophora* in Karnataka. Natural parasitism (1.66%) by the ecto-solitary larval parasitoid *Campsomeriella collaris collaris* Fabricius (Hymenoptera: Scoliidae) was recorded on coconut white grubs, *L. coneophora* in organic gardens of Kerala. Additionally, a solitary endo-larval parasitoid, *Prosena* sp. nr. *siberita* (Diptera:Tachinidae), was documented for the first time on *L. coneophora* with 0.83% natural parasitism. Amber disease (2.34%) caused by *Serratia* spp. on *L. coneophora* and *L. burmeisteri*, mortality of second instar grub within 10–12 days of exposure under laboratory assay was also observed. White muscardine fungus (*Beauveria brongniartii*) was isolated and Koch's postulates were established on early instar larvae of *L. coneophora*. Additionally, green muscardine disease (0.18% incidence) due to *Metarhizium anisopliae* and natural infection of (3.93%) of caterpillar fungus (*Cordyceps* spp.) were observed on third instar larvae*of L coneophora*. Entomopathogenic nematodes *Steinernema* sp. showed higher pathogenicity (LT 50 • = 5.104 days at 8000 IJs) compared to *Heterorhabditis indica* (LT 50 • = 14.576 days at 8073 IJs). Despite the identification of diverse natural pathogens associated with white grub populations, their practical exploitation as biological control agent remains limited due to inconsistent performance under natural conditions. Thus, only entomopathogenic nematodes have emerged as promising candidates for effective biocontrol.

Keywords*: Leucopholis, epizootic, muscardine, parasitism, caterpillar fungus, natural enemies, Prosena, Campsomeriella*

Introduction

Palms cultivated in the southern and north-eastern regions of peninsular India are infested by various species of white grubs, regarded as endemic pests of national importance owing to their severe impact on palm health and productivity. The white grub complex includes,three closely related species of genus *Leucopholis* Blanchard, which are *Leucopholis coneophora* Burmeister, *Leucopholis burmeisteri* Brenske *and Leucopholis lepidophora* Blanchard (Veeresh,1981; Veeresh *et al*., 1982; Kumar, 1997; Prathibha, 2015; Prathibha *et al*., 2023). Among these species, *L. coneophora* is predominantly distributed in plains and coastal areas where coconut is extensively cultivated and is commonly referred to as the "coconut white grub (Nirula *et al*., 1952). *Leucopholis burmeisteri* is primarily associated with arecanut gardens in the South Canera district of Karnataka (Nair and Daniel, 1982; Veeresh *et al*., 1982), whereas *L. lepidophora* occurs along the Western Ghats and hilly terrains where arecanut is extensively grown (Veeresh *et al*., 1982). Consequently, the latter two species are collectively denoted as "arecanut white grubs". The grubs cause substantial damage to seedlings and palms, tunneling into the bole and collar region of the seedlings, which often leads to seedling mortality. In coconut palms, the grubs feed on roots, disrupting the vascular conduction which eventually lead to frond yellowing, reduced palm vigour and nut production (Abraham and Kurian, 1970). Second and third instar larvae of *L. burmeisteri* have been reported as voracious feeders, consuming approximately 2.3 g of arecanut root tissue per grub per day. Prolonged feeding results

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in the development of the pencil point symptom characterized by progressive tapering and narrowing of the trunk towards the crown, reduced crown size, absence of inflorescence production, nut fall in bearing palms, and an overall decline in palm vigour and yield (Padmanabhan and Daniel, 2003). In severely affected palms, the entire root system may be destroyed, allowing the palms to be uprooted easily with minimal force. *Leucopholis lepidophora* occurs predominantly along the Western Ghats and is commonly associated with clayey loam soils. (Veeresh *et al.,* 1982). Both *L. burmeisteri* and *L. lepidophora* exhibit a biennial life cycle. These species are highly polyphagous and are considered serious pests of several crops including coconut, arecanut, tapioca, *Dioscorea* spp., rubber, cocoa, banana, fodder grasses, elephant foot yam and sugarcane cultivated along the west coast of India. The eclosion of above mentioned three species coincides with the rainy season. In the case of *L. coneophora* and *L. burmeisteri*, the emergence occurs with the setting of south west of monsoon, whereas *L.lepidophora* emerges during the mid-monsoon period. After emergence, adults mate, feed and oviposit in the soil. The larvae pass through three instars, with the first instar feeding mainly on organic content and roots of grasses. The second instar larvae subsequently migrate to the root zone of palms and begin feeding on fibrous roots. The larval stage is prolonged, lasting approximately 8–15 months. During the larval period, the grubs are susceptible to attack by various natural enemies. According to Kumar (1997) scoliids were the important natural enemies of third instar larvae of *Leucopholis* spp., causing up to 44 per cent mortality along Western Ghats. Indiscrimate application of insecticides is a serious limitation on the incidence of scoliid parasitoid. Application of insecticide in soil after October resulted in absence of parasitism by scoliids. In contrast, farmers predominantly rely on insecticide applications for managing white grubs in coconut gardens. Therefore, the present study was undertaken to explore the natural enemy complex associated with *Leucopholis* white grubs with the objective of identifying potential candidates that could be incorporated into sustainable IPM programmes.

Materials and Methods

Survey and Collection of White Grubs

Field surveys were conducted during 2022 to 2024 in major palm-based cropping systems of Kerala (Alappuzha, Kasaragod, Kollam, Kannur districts) and Karnataka (Uduppi, Dakshina Kannada and Chikkamanagluru districts) to document the species composition of *Leucopholis* whitegrubs and the occurrence of natural enemies. Both healthy larvae and diseased cadavers were collected from the palm root zone. Specimens were placed separately in sterile containers and transported to the laboratory for further examination. Larvae were characterized taxonomically up to species level on the basis of morphological characteristics following the keys of Veeresh *et al.* (1982) and Kumar (1997). Field observations were recorded on the occurrence of predators, parasitoids and pathogens. Parasitized larval cadavers were kept under laboratory conditions for the parasitoid emergence and emerged parasitoids were taxonomically identified.

Isolation of Entomopathogenic Fungi

Entomopathogenic fungi (EPF) were isolated from infected grub cadavers following standard microbiological procedures. Infected specimens were surface sterilized and plated on Potato Dextrose Agar (PDA) medium under aseptic conditions. Developing fungal colonies were sub-cultured and obtained pure cultures. Aqueous spore suspensions prepared from pure cultures were used to establish Koch's postulates by inoculating healthy grubs maintained in sterile soil substrate. Mummified grubs suspected to be infected by caterpillar fungi were incubated in a humid chamber to facilitate complete development of ascocarps. Mature ascocarps were excised from the stipe, surface sterilized, and sectioned longitudinally and transversely before being placed on PDA medium. Fungal growth was monitored, and isolates were identified based on colony characteristics and microscopic morphology.

Isolation of Bacterial Pathogens

Bacterial pathogens were isolated from infected grubs by collecting haemolymph through incision of a larval leg under sterile conditions. The oozing haemolymph was streaked onto Nutrient Agar (NA) plates and incubated under laboratory conditions. Distinct colonies were purified through repeated sub-culturing to obtain pure bacterial isolates.

Apart from native bioagents, pathogenicity of entomopathogenic bacteria, *Bacillus cereus* WGPSB-2 and *Brevibacterium frugoritolerance* that were isolated from *Anomala dimidiata* were tested on first, second and third instar larvae of *L. coneophora* and *L. burmeisteri*. Aqueous suspension of talc based bacterial formulations were mixed into sterile soil substrate and the larvae were introduced. Pathogenicity of green muscardine fungus, *Metarhizium majus* CPCRI MM601 which was isolated from *Oryctes rhinoceros* was also tested on the larvae of *L. coneophora.*

In addition, two entomopathogenic nematodes *viz.*, *Steinernema* spp. and *Heterorhabditis indica* Poinar, were tested against

L. coneophora grubs for its efficacy under laboratory condition. For this, two independent bioassays were performed to find out the median lethal time (LT50 •) of EPNs against late first instar larvae of *L.coneophora*. Nematode suspensions were prepared in sterile distilled water and quantified using a nematode counting dish. For enumeration, ten droplets of 2 µl each were dispensed onto the counting dish, and the number of live infective juveniles (IJs) present in each droplet was counted under a stereomicroscope. The average count was calculated and used to estimate the number of infective juveniles per millilitre of suspension, which was subsequently adjusted to obtain the required experimental concentrations. The LT 50 • of *Steinernema* spp. was evaluated at two concentrations (1000 and 8000 infective juveniles [IJs] per larva) using late first-instar grubs. In each experimental set, 30 larvae were individually maintained in sample containers containing 50 g of sterile soil, into which the required number of infective juveniles was introduced. A control set comprising 30 larvae maintained in sterile soil without nematode inoculation was also included. Observation on mortality were recorded at two-day intervals, and corrected mortality was calculated following Abbott, (1925). Similarly, the LT 50 • of *Heterorhabditis indica* was determined at two doses, *viz*.,1153 and 8073 IJs per larva, following the same procedure.

Results and discussion

The survey indicated that *Leucopholis lepidophora* Blanchard was the predominant species in arecanut gardens of Sringeri, Chikkamagaluru district, Karnataka. A terrestrial crab was observed predating on larvae of *L. lepidophora* in Sringeri (Fig.1). Its feeding studies indicated that, it was capable of consuming an average of three third instar larvae of *L. coneophora* per day. But it could not search out the grubs inside the soil. It also fed on earth worm.

Fig 1. Terrestrial crab

Parasitoids Associated with *Leucopholis* White Grubs

Solitary ecto- larval parasitoid *Campsomeriella collaris collaris* Fabricius was recorded parasitizing third-instar larvae of *L. coneophora* in organic coconut gardens (Fig 2a & 2b), with a parasitism level of 1.66%. The parasitoid larva fed externally on the ventral surface of the grub. Notably, parasitism was absent in insecticide-treated plots, highlighting the negative impact of chemical insecticides on beneficial insects. Insecticide application, particularly after October, eliminated parasitoid activity, emphasizing the need for conservation-based pest management strategies. Earlier studies have reported scoliid wasps as major natural mortality factor, causing up to 44% grub mortality along the Western Ghats (Kumar, 1997). Channabasavanna (1954) and Mohan and Rajan (2010) reported the parasitism by *Campsomeriella collaris* on *L. coneophora* larvae. *Campsomeris* spp. was known to parasitize *L. lepidophora* (Patil and Hapase, 1990) and also *Leucopholis* spp. nr. *Armata*. Kumar (1997) reported the parasitism by two species of scoliids, *Megacampsomeris grossa* and *Colpacampsomeris indica* on *Leucopholis* larvae. It was also reported that, scoliid adult to be highly active during the month of April, surviving on *Bidens pilosa* nectar sources, suggesting that floral resources within plantations play an important role in sustaining parasitoid populations. It emphasizes the importance of habitat management in enhancing parasitoid persistence.

The documentation of solitary endo-larval parasitism (0.83%) by *Prosena* sp. nr. *siberita* (Tachinidae: Diptera) (Fig 3a & 3b) represents the first record of tachinid parasitism on *Leucopholis* spp., expanding the known host range of this parasitoid group. It was further observed that endoparasitic maggots, upon emerging from their host, *L. coneophora*, for pupation, were highly vulnerable to predation by ground-dwelling ants. Its pupal period prolonged for 12.3±1 days. This finding reinforces the ecological principle that conservation biological control may be more effective than augmentative release strategies in perennial cropping systems such as coconut and arecanut.Predation of maggots by ground-dwelling ants, indicates the presence of complex tritrophic interactions which may limit the effectiveness of this parasitoid under field conditions. Although parasitism levels were low, tachinids may contribute to delayed density-dependent mortality, particularly when integrated with other natural enemies.

Fig. 2. Parasitism of third-instar larvae of *Leucopholis coneophora* by the solitary ectolarval parasitoid *Campsomeriella collaris collaris* Fig 2a. *Campsomeriella collaris collaris* Fig. 2b *C. collaris* collaris parasitism on *L. coneophora*

Fig. 3. Parasitism of larvae of *Leucopholis* spp by the solitary ectolarval parasitoid *Prosena* **sp. nr.** *Siberite* Fig 3a *Prosena* sp. nr. *siberita* fly and Fig 3b Prosena maggot emerged out

Incidence of Entomopathogenic Bacteria

Amber disease caused by *Serratia* spp.was recorded in third-instar larvae of *L. coneophora* with a natural incidence of 4.32% at Kasaragod and Alappuzha districts of Kerala and from *L. burmeisteri* collected from Sullia in South Canara district of Karnataka (Fig. 4a & 4b). Koch's postulates confirmed the pathogenicity to two species of grubs leading to mortality of the first instar grubs in about 10 to 12 day sunder laboratory condition (room temperature: 25±2º C and RH: 87%). Being a weak pathogen, it lost its infectivity on sub-culturing. *Serratia* spp. are non-spore forming bacteria which cause death over an extended period of 1-3 months in third instar larvae, by this time larvae would gain characteristic amber colouration due to depletion of fat. *Serratia* infection resulted in feeding deterrence, gut clearance, reduced proteolytic enzyme activity in the gut and gradual depletion of fat reserves leading to characteristic amber coloration of third instar larvae. Similar symptoms have been described in scarab grubs infected by *Serratia entomophila* and *S. proteamaculans* (Jackson, *et al.*, 1993; Klein and Kaya, 1995). The relatively slow disease progression suggests that the bacterium acts as a chronic pathogen rather than an acute epizootic agent. Loss of virulence upon subculturing further indicates adaptation to host-associated conditions, a phenomenon (virulence attenuation) commonly reported in entomopathogenic bacteria. Nevertheless, its natural occurrence across multiple locations suggests ecological persistence and possible contribution to long-term population suppression.

Fig. 4a. Amber diseased white grub

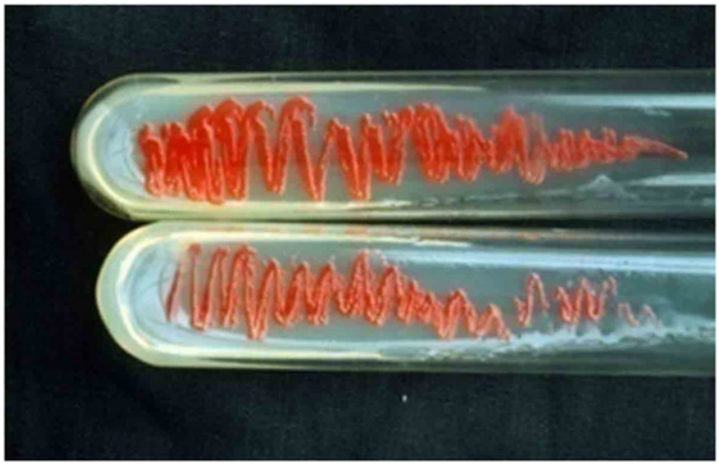

Fig 4b. Serratia spp.

Mycoses Associated with *Leucopholis* spp.

Three species of fungal pathogens were observed to infect coconut white grubs during the course of study. Those are, white muscardine fungus, *Beauveria* spp. which caused white muscardine disease (Fig 5), green muscardine fungus, *Metarhizium anisopliae* Sorokin Metschnikoff, that caused green muscardine disease (Fig 6) and a species of caterpillar fungus, *Cordyceps* spp. isolated from infected cadavers Natural infections of *Metarhizium anisopliae* causing green muscardine disease in early instar larvae of *L. coneophora was observed in coconut ecosystem*. Previous studies (Padmanabhan and Daniel, 2003; Sreekumar *et al*., 2006) also documented *Metarhizium* infections in coconut root grub in endemic regions. Field evaluations elsewhere demonstrated significant reduction of *L. lepidophora* populations following application of *M. anisopliae* (Kesarasingh and Patil 2010; Prabhu *et al.,* 2011), supporting its potential as a microbial biocontrol agent.

Beauveria spp. was isolated from an unidentified melolonthinae larva collected from sugar cane field in Kannur and the pure culture was obtained. The pathogenicity of fungus could be proved through Koch's postulates that to be infective to *L. coneophora*. However, the pathogen caused mycosis in 29 days after inoculation on the first instar larvae suggesting poor infectivity potential.

Natural mycosis caused by *Cordyceps* spp. was recorded on third-instar larvae of *L. coneophora* and *L. lepidophora* collected from coconut fields in Kasaragod and Sringeri. Infection was identified by stromatal emergence through the epicranial suture of infected grubs. Three types of stromatal growths were observed in these infected larvae that sported single double or triple stromata (Fig 7a & 7b). Spores derived from sexual reproduction are produced in specialized cells, called asci, which are housed in flask shaped fruiting body called perithecium. The perithecia are embedded in club like structure called stroma. Their life cycle may comprise more than one (teleomorphic) meiotic stage and zero to many mitotic (anamorphic) stages. In the present study, the teleomorphic *Cordyceps* from infected *L. coneophora* on PDA medium was observed to be morphologically and microscopically closely allied to the anamorphic *Paecilomyces* spp. It produced white coloured colony that turned grey after one week. Microscopic morphology of the fungus exhibited series of single celled phialoconidia (ameroconidia) formed in basipetal sequence from a phialide (Fig 8). Phialides were engorged at the bottom, progressively tapering towards the apices and form a penicillate structure (penicillus) indicating the anamorphic stage to be potentially *Paecilomyces* spp. However, the holomorphic identity of the fungus and its correct nomenclature needs elucidation. The occurrence of both sexual and asexual stages suggests ecological adaptability and persistence of the pathogen in soil ecosystems. Veeresh *et al.*(1982) documented *Cordyceps* infection on *Leucopholis* grubs. Santhoshkumar and Aparna (2014) documented natural incidence of *Cordyceps* spp. (Accession No: DMRO-526) on coconut root grub, *L. coneophora* in Kasaragod.

Apart from native bioagents, fungal and bacterial pathogens which were isolated from other scarabaeids and entomopathogenic nematodes (EPNs) were evaluated for the pathogenicity.

Fig. 5 White muscardine disease on white grub

Fig. 6 Green muscardine disease on white grub

Fig 7a. *Cordyceps* spp. infection with single stromata on *L.coneophora*

Fig 7b. *Cordyceps* infection with double and triple stroma

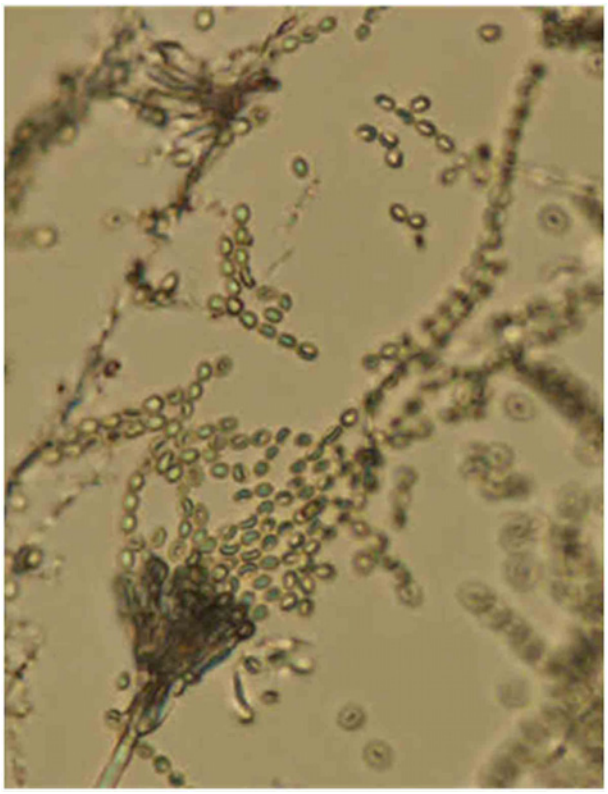

Fig 8. Sporangiophore of anamorphic *Paecilomyces* spp

Efficacy of Entomopathogenic Nematodes

Bioassays were performed in order to find out the LT_{50} of EPN on late first instar larvae of *L. coneophora*. *Steinernema* spp. was found to be more pathogenic to grub as it recorded low median lethal time period (LT_{50} = 10.998 days at 1000 infective juveniles (IJs) concentration). At 8000 IJs / larva its LT_{50} was 5.104 days. At the same time, *H. indica* recorded an LT_{50} of 18.314 days at a dose of 1153 IJs / larva and 14.576 days at 8073 IJs / larva (Table 1). Among the EPNs screened against *L. coneophora*, *Steinernema* spp. exhibited elevated pathogenicity over *H. indica*. Higher penetration rate of *Steinernema* spp. than that of *H. bacteriophora*. to white grubs may have attributed to the elevated pathogenicity (Rosa *et al.*, 2002). As EPNs and white grubs inhabit the same soil environment, it opens up the scope to exploit EPN as potential biocontrol tool against it. Increase in dose resulted in reduction in lethal time taken by both the nematode species. Similar trend was observed by Sankaranarayanan *et al*. (2006), with lowest LT_{50} for *S.glaseri* (24.9h) followed by *H.indica* (27.3 h) at a dose of 1000 infective juveniles/pupa of *Holotrichiaserrata*, indicating elevated pathogenic potential of *S. glaseri.* Glazer (1992) demonstrated substantial variation in invasion capacity among nematode isolates, proposing invasion capacity as a useful criterion for selecting nematodes for specific hosts.

Table 1. Bioassay of entomopathogenic nematodes against *L. coneophora* larvae

Nematode	Dose (IJs / 50g soil)	LT_{50} (Days)	Fiducial limit		Chi^2
			Lower	Upper	
Steinernema spp.	1000	10.998	09.560	12.114	8.844
	8000	05.104	04.260	07.403	0.616
Heterorhabditis indica	1153	18.314	17.361	19.474	1.415
	8073	14.576	12.985	16.428	7.453

Conclusion

The prolonged larval period of *Leucopholis* larvae (8–15 months) represents the most vulnerable phase in the life cycle and provides an extended window for action by natural enemies. In the present study, one predatory crab, two solitary hymenopteran larval parasitoids, one bacterial pathogen, three fungal pathogens were isolated from *Leucopholis* larvae associated with coconut and arecanut ecosystem. The diversity of parasitoids, pathogens, and predators recorded in this study indicates that white grub populations are naturally subjected to multiple mortality factors operating simultaneously within the soil ecosystem. However, the relatively low levels of natural parasitism suggest that these biological agents function primarily as regulatory rather than suppressive forces under field conditions. Collectively, the findings indicate that *Leucopholis* populations are regulated by a consortium of natural enemies rather than a single dominant control agent. The low but consistent incidence of multiple pathogens and parasitoids suggests additive or synergistic mortality effects operating within the soil ecosystem. Conservation of these natural enemies through judicious use of chemical pesticides, giving thrust to organic amendments, and habitat diversification could substantially enhance biological regulation. Entomopathogenic nematodes demonstrated considerable promise as biological control agent, with *Steinernema* spp. exhibiting significantly lower LT50 values compared to *Heterorhabditis indica*. In the current IPM strategy for white grubs in coconut, two rounds of insecticide application are recommended: the first as a patch application in interspaces during the second week of August, targeting first instar grubs, and the second at 45 days after, as a root zone application when the grubs reach second / third instars and actively congregate for feeding. However, the second round of insecticide application can be effectively substituted with root zone application of *Steinernema* spp., which can encourage the establishment of scoliid parasitism on third instar grub. Besides, EPNs and white grubs share the same soil habitat making EPNs as apt biological control agents against white grubs. From an IPM perspective, integrating entomopathogenic nematodes with conservation of scoliid parasitoids offers a promising ecological approach for sustainable management of palm white grubs. Future research should focus on understanding multitrophic interactions, environmental thresholds for epizootic development, and landscape-level factors influencing natural enemy persistence. Long-term field validation will be essential to translate these findings into practical biological control strategies.

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