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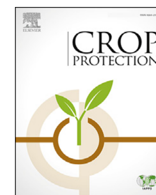

# Efficacy of entomopathogenic *Heterorhabditis* and *Steinernema* nematodes against the white grub, *Leucopholis lepidophora* Blanchard (Coleoptera: Scarabaeidae)

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The white grub *Leucopholis lepidophora* Blanchard (Coleoptera: Scarabaeidae) is a serious pest of arecanut and other commercial crops in India. The development of an environmentally-friendly control method for this pest is urgently needed. The efficacy of two species of entomopathogenic nematodes (EPN), namely *Steinernema abbasi* Elawad and *Heterorhabditis indica* Poinar (Rhabditida: Steinernematidae and Heterorhabditidae) was evaluated against this pest in the laboratory and in arecanut fields. In the laboratory assays, *H. indica* caused significantly higher mortality rates (37.5–93.8%) than *S. abbasi* (22.5–75.0%) in second instar *L. lepidophora* larvae. *Heterorhabditis indica* caused 20.0–77.5% mortality and *S. abbasi* 12.5–65.0% mortality in third instar larvae. Significant differences were observed in $LC_{50}$ and $LC_{90}$ values of the nematode species against different life stages of *L. lepidophora*. Both nematode species and a standard insecticide (chlorpyrifos) were tested against this insect in two field experiments. Grubs were mainly in the second instar stage and third instar stage. Both laboratory and field experimental results indicated that second instar grubs were more susceptible to the nematode species tested than third instar grubs and that the efficacy of EPNs against *L. lepidophora* larvae varies with nematode species. In both the field experiments, *H. indica* ($3.5 \times 10^5$ IJ palm$^{-1}$) showed a significantly higher percentage mortality rate of the white grub larvae compared to that of *S. abbasi* and chlorpyrifos treatments. Chlorpyrifos application, however, was more effective in reducing the grub population compared to *S. abbasi* ($1.7 \times 10^5$ IJ palm$^{-1}$). Our results show that *H. indica* has good potential as an alternative management tool for the management of *L. lepidophora* in areacnut production.

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## 1. Introduction

Arecanut palm or betel nut palm (*Areca catechu* L.) is grown extensively in the tropical Pacific, Asia and East Africa (Manimekalai et al., 2013). In India, arecanut is grown in around 4.0 hundred thousand hectares with a production of 4.7 hundred thousand tonnes (Ramappa and Manjunatha, 2013) and involved in a trade of about Rs.15.0 thousand million annually (Kannan and Jose, 2009). Arecanut is chewed as a stimulant by at least 5% of the world's population (Hegde and Deal, 2014) and chewing is as familiar as chewing gum to Americans (Raghavan and Baruch, 1958). This is believed to aid in better digestion (Prathibha et al., 2017). However, dangers to human health when arecanut is blended with tobacco and consumed in the form gutkha (Rehan et al., 2017). Arecanut extract also possess potential anti-oxidative activity (Kim et al., 1997) and anti-aging component for cosmetics (Lee and Choi, 1999). Apart from the nut usage, the leaf sheath of arecanut is used in making disposable cups and plates etc. The husks of arecanut are used in the manufacture of wrapping paper and cushions. In India there is no legal prohibition on arecanut cultivation for commercial purposes.

Numerous pest and diseases are known to affect arecanut production in India including the root-feeding, immature stages of scarabaeid beetles (Coleoptera: Scarabaeidae), also referred to as white grubs. The three white grub species that mainly occur on arecanut and coconut palms include *Leucopholis lepidophora* Blanchard, *L. burmeisteri* Brenske and *L. coneophora* Burmeister (Veeresh et al., 1982). *Leucopholis lepidophora* is predominant in high rainfall and Western Ghat regions of India, causing 27.9–37.0% damage with a yield losses up to 39.8–41.6% (Kalleshwaraswamy et al.,

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2015) and is widely regarded as the most serious threat to arecanut (Veeresh, 1983), sugarcane and summer peanut production (Adsule and Patil, 1994).

*Leucopholis lepidophora* has one generation every two years. The majority of its life cycle is spent as a grub. Adults mate during the night where the females return to the ground to deposit eggs at a depth of 2.5–5 cm in the soil at dawn. Eggs hatch 12–15 days later and the young larvae feed upon grass roots and decaying vegetation. The duration of the larval stages of the first, second and third instar grubs ranges from 30–40 days, 35–60 days and 400–550 days, respectively, while the duration of the pupal stage is 15 days (Kumar, 1997). Pupation occurs in the soil at a depth of 60 cm. Adults emerge at night from June to November (Kumar, 1997) and live for 20–45 days (Adsule and Patil, 1994; Naik et al., 2009). Arecanut palms show white grub damage symptoms when sufficient damage is done to the roots. The feeding of white grub larvae causes a reduced number of roots, loss of anchorage, stem tapering, reduced number of fronds, chlorosis, yield reduction and in rare cases, death of the plants (Adarsha et al., 2015).

Damage prediction is difficult because the grubs occur below the ground and management of these grubs has always been difficult. Current management relies on summer ploughing and application of chemical insecticides to manage the grubs of *L. lepidophora* with varying degrees of success. However, indiscriminate use of chemical insecticides for the management of white grubs causes harmful effects on soil biodiversity. The Western Ghat region is one of the biodiversity hot spots (Myers, 1990). Thus, there is a need to identify effective alternative control strategies for this pest. Entomopathogenic nematodes (EPN) from the families Steinernematidae and Heterorhabditidae show potential as biocontrol agents for the management of soil-dwelling pests (Toepfer et al., 2010). In previous studies, EPN species such as *Steinernema scarabaei* Stock and Koppenhofer (Koppenhofer and Fuzy, 2003); *Heterorhabditis bacteriophora* Poinar (Koppenhofer et al., 2000); *Hetrorhabditis zealandica* Poinar (Grewal et al., 2004) and *Heterorhabditis indica* Poinar (Patil et al., 2015) show potential to control different species of white grub. However, research on the potential of EPNs to suppress *L. lepidophora* has been limited and the susceptibility of different *L. lepidophora* life stages to these nematodes has not yet been determined. The aim of the study reported here was to evaluate the efficacy and application timing of *Steinernema abbasi* Elawad NBAIISa01 and *Heterorhabditis indica* Poinar NBAIIH38 for the control of *L. lepidophora* in the laboratory and in arecanut field conditions.

## 2. Materials and methods

### 2.1. Nematode cultures

*Steinernema abbasi* NBAIISa01 and *Heterorhabditis indica* NBAIIH38 were obtained from the collection of the Division of Insect Systematics National Bureau of Agricultural Insect Resources, Bengaluru, India. Both species were identified using molecular methods and populations initiated on late instar larvae of the greater wax moth, *Galleria mellonella* (L.) kept at 26 ± 1 °C in a growth cabinet (Woodring and Kaya, 1988). Infective juveniles (IJ) that emerged from the wax moth larvae within the first three days of emergence were collected using White traps (White, 1927). Nematode viability was 100%, unless otherwise stated.

### 2.2. Insect cultures

The second and third instars of *L. lepidophora* were collected from arecanut fields at Uttara Kannada district (14° 36′N, 74° 51′E), Karnataka, India during September and November 2014. None of the sites were treated with either insecticides or EPNs during the previous year. Each grub was individually placed in a plastic container filled with 150 $cm^3$ autoclaved mixture of organic compost and sandy loam for one week. Four potato pieces were placed in each container for the grubs to feed on. Only actively feeding grubs were selected for laboratory experiments. Greater wax moth larvae were reared at 26 ± 1 °C in a growth cabinet on standard artificial diet. Similarly sized late instar wax moth larvae were chosen to test nematode persistence in the soil by burying the larvae in the field during field experiments (Patil et al., 2016).

### 2.3. Laboratory experiment

To assess the efficacy of EPNs on second and third instar grubs of *L. lepidophora,* plastic containers (5.8 cm diam., 200 $cm^3$ soil capacity) were filled with 150 $cm^3$ autoclaved sandy loam soil (75% sand, 15% silt, 7% clay). A single grub (second; 379 ± 37 mg or third instar; 1035 ± 42 mg) was released into each container. Grubs that did not enter the soil within 2 h were considered unhealthy and replaced with other grubs. A soil moisture of 15% was maintained throughout the trial. Potato pieces were added to each container to feed the white grubs. After 48 h, each grub was inoculated with the relevant EPN species at an inoculum rate of 500, 1000, 1500, 3000, and 6000 IJ $grub^{-1}$ in five millilitre of distilled water. The control treatments received five millilitre of distilled water only. The plastic containers were kept in the dark an incubator at 24 ± 2 °C. There were twenty replicates per treatment laid out in a completely randomized design. Larval mortality was assessed at fourteen days after treatment (DAT). Cadavers were kept on White traps (White, 1927) to confirm nematode infection. The experiment was repeated.

### 2.4. Field experiments

Two field experiments were conducted in arecanut plantations located at two different sites in Karnataka, India. The sites were naturally infested with *L. lepidophora.* Before field application, native EPN populations were checked in the field by baiting soil samples with greater wax moth larvae as described by Bedding and Akhurst (1975). No EPN populations were recovered from the experimental fields before onset of the trails. The field experiment was conducted in a 5184 $m^2$ arecanut field with palm spacing of 2.4 × 2.4 m with 1736 palms $ha^{-1}$. Experimental layout was a randomized complete block design with six treatments. There were fifteen replicates (blocks) per treatment and ten palms per replicate (total of 150 palms per treatment). Only palms with noticeable signs of active *L. lepidophora* infestation were selected for application of treatments. Ten palms receiving the same treatment within each block were grouped but not contiguous (Cottrell and Shapiro-Ilan, 2006). To estimate the larval populations of *L. lepidophora*, five soil samples of 0.3 × 0.3 m surface area to a depth of 0.3 m, taken at least 0.3 m distance from palms, were sampled randomly within each block. The number of white grub larvae was determined and the population density of the grub larvae calculated.

The first experiment was conducted in Sirsi (14° 34′N, 74° 56′E), on fifteen year old palms planted in sandy loam soil (56–60% sand, 10–18% silt, 21–29% clay, 2% organic matter, EC 0.20–0.40 and approximately 14% moisture). Applications were carried out on 24 July 2015 at 1.00 p.m. in a light rain with an air temperature of 25 °C and a soil temperature of 28 °C at 5 cm depth. Pre-application sampling indicated that site had a resident *L. lepidophora* larval population with a density of 4.4 ± 1.03 grubs $m^2$. The grubs were mainly in the second instar stage at onset of the experiment.

The second experiment was conducted in Banavasi (14° 31′N, 75° 0′E), on twenty year old palms planted in sandy loam soil

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