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Field evaluation of the entomopathogenic nematodes against the white grub, *Leucopholis lepidophora* Blanchard (Coleoptera: Scarabaeidae)

Jagadeesh Patil* and Vijayakumar Rangasamy

Abstract

The ability of entomopathogenic nematodes to suppress larval populations of the white grub, *Leucopholis lepidophora* Blanchard (Coleoptera: Scarabaeidae), infesting the areca nut palm (*Areca catechu*) was investigated under field conditions over a 2-year period (2015–2017). *Heterorhabditis indica* at two application rates (1.7×10^5 and 3.5×10^5 IJs palm $^{-1}$) caused higher percentage reduction of *L. lepidophora* larvae than *Steinernema abbasi* and chlorpyrifos. Chlorpyrifos-treated plots caused higher percentage of reduction of the grub larvae than *S. abbasi* at the lowest rate (1.7×10^5 IJs palm $^{-1}$). However, *S. abbasi* at 3.5×10^5 IJs palm $^{-1}$ performed at least equally well than the chlorpyrifos treatment. The kernel yield from *H. indica*-treated plots at 3.5×10^5 IJ palm $^{-1}$ was 85.4% higher than those from water control and 33.3% higher than that in the chlorpyrifos treatment. The kernel yields varied significantly among different treatments. The cost-benefit analysis showed that *H. indica* is a promising biocontrol agent for the management *L. lepidophora* control in areca nut field.

Keywords: Areca nut, *Leucopholis lepidophora*, Biocontrol, Entomopathogenic nematodes

Background

Root injury caused by the larvae of the white grub, *Leucopholis lepidophora* Blanchard (Coleoptera: Scarabaeidae), threatens the production of areca nut palm or betel nut palm *L. (Areca) catechu* in the Western Ghats, Eastern Ghats and East and North Eastern regions of India. *L. lepidophora* is a basically dominant white grub species in high rainfall and Western Ghats regions of India and is widely regarded as the most serious threat to areca nut production in India (Veeresh 1983), causing yield losses up to 39.8–41.6% (Kalleeshwaraswamy et al. 2015). *L. lepidophora* is also a key pest-causing damage to sugarcane, rice, maize (Adsule and Patil 1994), and peanuts (Ranga Rao and Rameshwar Rao 2013).

Since the white grub larvae are subterranean, the damage prediction is difficult and their management has always been problematic. The control of white grub larvae in India mainly depends on application of chemical

insecticides. However, the control efficacy was not satisfactory because of quick development of insecticide resistance. With more concerns for the environment and human safety, environmentally friendly control strategies for *L. lepidophora* control are urgently needed to replace the highly toxic chemical pesticides.

Entomopathogenic nematodes (EPNs) from the families Steinernematidae and Heterorhabditidae are potential alternatives for the control of soil-dwelling pests (Toepfer et al. 2010), because of their ability to actively search for their hosts (Kaya and Gaugler 1993; Grewal et al. 2005; Georgis et al. 2006 and Yan et al. 2012). Previous studies have shown that EPN species such as *Steinernema scarabaei* Stock and Koppenhofer, *S. longicaudum* Shen and Wang, *S. lanmjungense* Khatri-Chhetri, *Heterorhabditis bacteriophora* Poinar, *H. zealandica* Poinar and *H. indica* Poinar (Koppenhofer et al. 2000; Koppenhofer and Fuzy 2003; Grewal et al. 2004; Du et al. 2009; Khatri-Chhetri et al. 2011; Guo et al. 2015 and Patil et al. 2015) were highly virulent to larvae of many species of white grubs. Therefore, it is expected that EPNs are good candidates for the

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control of *L. lepidophora*. However, research on the potential of EPNs to suppress *L. lepidophora* has been limited.

Therefore, the present study aimed to evaluate the efficacy of two EPN species, *Steinernema abbasi* Elawad and *H. indica* Poinar along with the commonly used chemical insecticide (chlorpyrifos) in an areca nut field infested with *L. lepidophora*.

Methods

Source of EPNs

Nematodes, *Steinernema abbasi* NBAII Sa01, and *Heterorhabditis indica* NBAIIH38, were obtained from the Division of germplasm collection and characterization, National Bureau of Agricultural Insect Resources, Bengaluru, India. Both nematodes were cultured on larvae of the greater wax moth, *Galleria mellonella* L. (Lepidoptera: Pyralidae) at 25 ± 1 °C. Infective juveniles (IJs) emerging from the wax moth larvae were collected thrice a week, using White traps. Nematode viability was assessed under a stereomicroscope and considered alive when actively moving or showing response after probing with a needle. EPN suspensions were used for the experiments when more than 98% of IJs were viable.

Field experiments

A field experiment was conducted in areca nut plantation, naturally infested with *L. lepidophora* in the North Canara district, Karnataka ($14^{\circ} 78' N$, $74^{\circ} 13' E$; 2.88 m above sea level), India, during the period of August 2015 to October 2017. The experiment was conducted on 22-year-old palms planted in sandy clay loam (52–56% sand, 12–14% silt, 31–35% clay, 2% organic matter, EC 0.21–0.39, and approximately 17% moisture). Before the experimentation, natural EPN populations were detected by baiting soil samples with wax moth larvae. No EPN populations were recovered from the experimental field.

The experiment was conducted in a 6912 m^2 areca nut field, with a palm spacing of 2.4×2.4 m. The experiment was conducted with a total of 1500 palms per hectare, some palms were not established properly due to the white grubs' infestation. The experiment was organized as a randomized complete block design, with 6 treatments and 15 replicates (blocks), with 10 palms per replicate (a total of 150 palms per treatment). 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 estimated. Pre-application sampling indicated that the site had a resident larval population of *L. lepidophora* with a density of 2.4 ± 0.40 grubs m^{-2} , and the grubs were mainly in the third larval instar at the time of the experiment.

Soil applications were carried out at 3:00 p.m., light rain, an air temperature of 25 °C, and a soil temperature of 23 °C at 5 cm depth. Both *S. abbasi* and *H. indica* were applied at the concentrations of 1.7×10^5 and 3.5×10^5 IJs palm^{-1} and 3.0×10^8 and 6.0×10^8 IJs ha^{-1} , respectively. Chlorpyrifos (20% EC-emulsifiable concentrate) was used as a positive control at a rate of 6 ml palm^{-1} (equal to 10,000 ml ha^{-1}). Water without nematodes or insecticide was used as a negative control. Infective juveniles, in 5 l of water, were poured around the base of each tree and covered 1.2 m area. A similar volume was used for chlorpyrifos treatment and water control. Watering can and sprinkler can be used to apply the EPNs and insecticides, respectively. Larval populations of the white grub were monitored 2 and 4 weeks after EPNs' application as described before. The effect of treatments on kernel yield was recorded, and estimated the cost of EPNs and chemical insecticide applications were also estimated.

Nematode persistence

In order to assess the EPNs' persistence in the soil, the mortality of *G. mellonella* larvae buried in the field was evaluated after 60, 90, and 120 days post-application. To study the nematode persistence, perforated plastic boxes containing four larvae in a mixture of sterilized soil and sand (1:1, moisture 13%) were buried around the root zone of each palm at a depth of 15 cm. Six days later, they were retrieved and taken back to the laboratory. Live and dead *G. mellonella* larvae were counted. Dead larvae were incubated in clean Petri dishes with moist filter paper and dissected 3 days later to estimate IJs invasion. There were 15 replicates for each treatment, and the experiment was randomized complete block design. The whole experiment was repeated once with the same number of replicates.

Statistical analysis

Percentage reduction of the grubs was calculated for each treatment using the equation: $R_A (\%) = (A_c - A_t)/A_c \times 100$, where R_A is the percentage reduction of the white grub larvae in the treatment and A_c and A_t indicate the number of larvae in the control and the treatment, respectively (Liu et al. 2007 and Guo et al. 2013). The cost and benefit of different treatments were estimated based on an IJ application rate of 3.5×10^5 IJ palm^{-1} (6.0×10^8 IJ ha^{-1}). Average kernel yield was calculated kilograms per hectare. The cost for the treatments was estimated based on the cost of IJs production on *G. mellonella* and retail price of the chlorpyrifos on 7 August 2015. Net profit was estimated based on the income of kernels (USD\$ 3.1 per kilogram on 4 October 2017) and the cost per hectare from different treatments. The arcsine

transformation (% data) was used for normalization of data before an ANOVA was conducted. Analysis was undertaken on the transformed data, and untransformed means \pm SE are presented. All data were analyzed using PROC GLM (SAS version 9.3; SAS Institute 2011). When ANOVA was significant, comparisons of relevant means were made using Tukey's post-hoc significance test at a significance level 5%.

Results and discussion

Reduction of white grub larvae

Results indicated that application of *H. indica* at 1.7 and 3.5×10^5 IJs palm^{-1} caused significantly higher percentage of reduction of the grub larvae than other treatments at 3 and 6 weeks after application ($F = 70.57$, $df = 4$, 126, $P < 0.0001$) (Fig. 1). Six weeks post-application, *H. indica* caused $> 80\%$ reduction of the grub larvae, which was significantly higher than the plots treated with *S. abbasi* and chlorpyrifos ($F = 70.57$, $df = 1$, 126, $P < 0.0001$). However, treatment with *S. abbasi* was less efficient, and fewer larvae were detected in nematode- and chlorpyrifos-treated plots than that in the water control. These results showed that *H. indica* was more virulent to white grub larvae than *S. abbasi*. Different host-finding strategies exhibited by the "cruiser" *H. indica* (Campbell and Lewis 2002) and "ambusher" *S. abbasi* could explain our findings. *H. indica* had excellent potential for the control of *L. lepidophora* as it provided 89% reduction of *L. lepidophora* larvae, at 6 weeks post application, even at 1.7×10^5 IJ palm^{-1} . These

results indicated that such high efficacy, even in the lowest rate of *H. indica* application, may suggest additional grub mortality caused by the *H. indica* progeny emerged from grub larvae infected by the originally applied nematodes.

Six weeks post-applications, percentage reduction of grub larvae was the highest (89.23%) by *H. indica* and lowest (43.21%) by *S. abbasi* at the rate of 3.5×10^5 IJs palm^{-1} , whereas in the plots treated with chlorpyrifos, 60.25% reduction of the grub larvae was recorded (Fig. 1). Similar results showed that using nematodes could control different white grub species but with various control efficacies, i.e., *S. scarabaei* (100%), *H. bacteriophora* (40–50%), and *H. zealandica* (73–98%), against *Popillia japonica* (Koppenhofer and Fuzy 2003; Grewal et al. 2004; Koppenhofer et al. 2006). Guo et al. (2013 and 2015) reported that *S. longicaudum* and *H. bacteriophora* application caused $> 94\%$ reduction of *Holotrichia oblita* and *S. carpopcapsae* caused $< 20\%$ reduction in peanut fields. Laznik and Trdan 2015 recorded the lowest efficacy of *H. bacteriophora*, when used for the control of mixed populations of Scarabaeidae species. Therefore, for the effective management of target pests, choosing the appropriate nematode species to be matched with the particular target pest is a paramount importance (Shapiro-Ilan et al. 2012). As the extension of sample time, percentage reductions of *L. lepidophora* significantly increased in the plot treated with *H. indica*, *S. abbasi*, and chlorpyrifos ($F = 23.14$, $df = 1$, 266, $P < 0$.

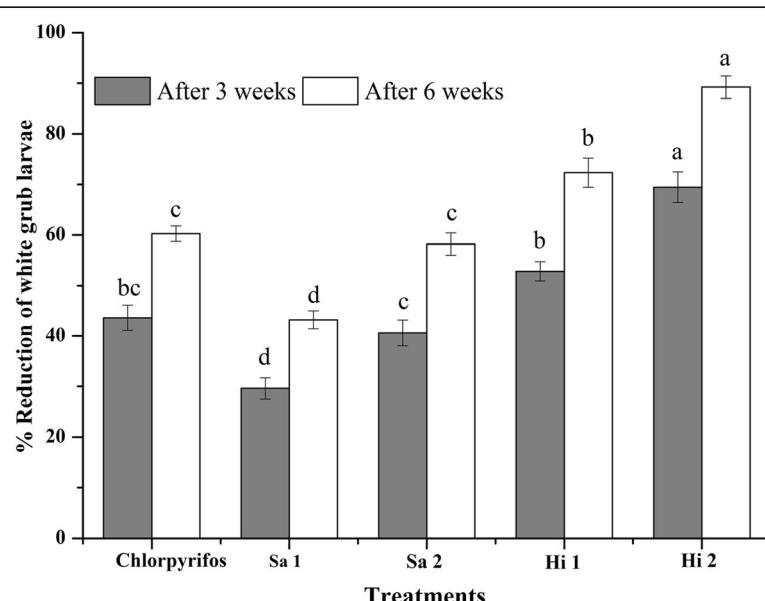


Fig. 1 Cumulative percentage reduction of third-instar larvae of *Leucopholis lepidophora* in areca nut field with different treatments 3 and 6 weeks after application in North Canara district, Karnataka, India during 2015–2017. Different letters on the top of error bars indicate statistically different values for different nematode concentrations using Tukey's test ($P < 0.05$). Bars = standard error. Sa, *Steinernema abbasi*; Hi, *Heterorhabditis indica*; 1 = 1.7×10^5 IJs palm^{-1} , 2 = 3.5×10^5 IJs palm^{-1} . Chlorpyrifos was used at the rate of 6 ml palm^{-1} as a drench application

0001) (Fig. 1). *S. abbasi* at the concentration of 3.5×10^5 IJs palm $^{-1}$ performed at least equally well than the chlorpyrifos treatment, but chlorpyrifos treatment reduced significantly higher percentage of grub larvae than *S. abbasi* at 1.7×10^5 IJs palm $^{-1}$. *H. indica* was the most efficient in reducing the grub population, followed by the chlorpyrifos and *S. abbasi* treatment.

Nematode persistence

After the successful establishment in the host, EPNs undergo several cycles of multiplication. The success of this process enhanced their ability to be established in the field. In the present study, because of the successful multiplication of both nematode species, *H. indica* and *S. abbasi* in *L. lepidophora* larvae, their persistence was recorded in the soil, even 90 days after application. Moreover, the soil moisture of approximately 17% and temperature of 23 °C during the control period were favorable for the IJs survival and persistence. Mortality of *G. mellonella* larvae in nematode-treated soil samples ranged from 28.0 ± 5.65 to $37.33 \pm 5.12\%$, and the differences were insignificant ($P > 0.05$) between treatments at different sample days ($F = 0.93$, $df = 3, 162$, $P \geq 0.4278$).

Kernel yield of areca nut

To estimate the areca nut kernel yield, seventy-five palms were selected randomly from each treatment. Ripe nuts were harvested and dried to 8% moisture. Dried nuts were de-husked, and kernel weight (kg/palm) of areca nut, i.e., chali (marketable produce), was recorded

for computing the yield. Cumulative kernel weights of areca nuts in the plots treated with different treatments during the year are shown in Fig. 2. The kernel yields (kg/palm) varied significantly among different treatments ($F = 200.73$; $df = 5, 70$; $P < 0.0001$). The kernel yield harvested from the areca nut plots treated with *H. indica* at 3.5×10^5 IJs palm $^{-1}$ was 85.4% higher than water control and 33.3% higher than that from chlorpyrifos treatment. That was similar to Guo et al. (2013) who obtained 82.9% of higher peanut yield in the *Holotrichia parallela*-infested plots treated with *H. bacteriophora* H06 at a rate of 1.0×10^4 IJs plant $^{-1}$ and 30% higher than that in the chlorpyrifos treatment. Chlorpyrifos-treated palms showed significantly higher yield than *S. abbasi* at 1.7×10^5 IJs palm $^{-1}$, but insignificant difference of the kernel yield was found between the chlorpyrifos and *S. abbasi* at 3.5×10^5 IJs palm $^{-1}$. Plots treated with chlorpyrifos showed 39% of increase in kernel yield over control treatment, whereas in plots treated with *S. abbasi* and *H. indica* at 3.5×10^5 IJs palm $^{-1}$, the increase rate of kernel yield was 37.27 and 85.4%, respectively.

Cost estimation of the EPN applications

The wide use of nematodes for the control of the white grubs is still limited because of less availability of nematode products and the high cost. Therefore, in the present study, the cost benefit of the white grub control in areca nut field was estimated (Table 1), where 1500 areca nut palms per hectare were tested. Both nematode

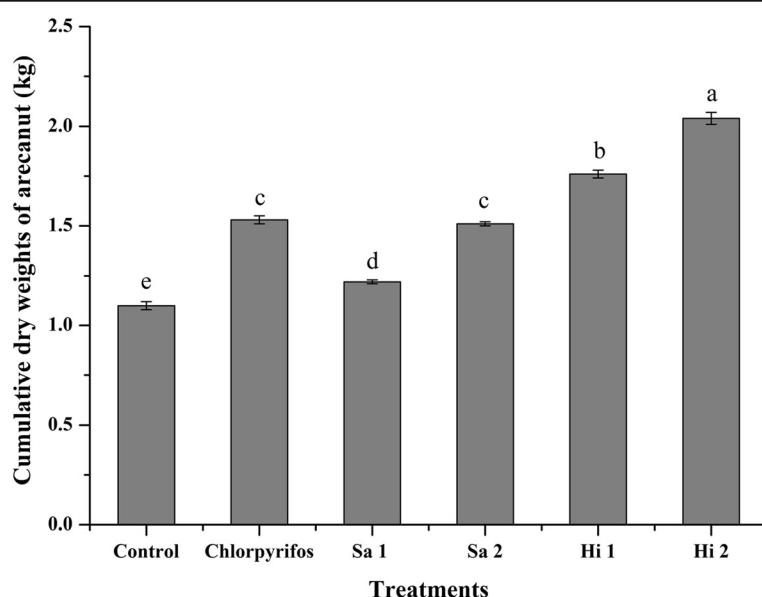


Fig. 2 Cumulative kernel weights of areca nut palms treated with different treatments in North Canara district, Karnataka, India, during 2015–2017. Different letters on the top of error bars indicate statistically different values for different nematode concentrations using Tukey's test ($P < 0.05$). Bars = standard error. Sa, *Steinernema abbasi*; Hi, *Heterorhabditis indica*; 1 = 1.7×10^5 IJs palm $^{-1}$, 2 = 3.5×10^5 IJs palm $^{-1}$. Chlorpyrifos was used at the rate of 6 ml palm $^{-1}$ as a drench application

Table 1 Cost and benefit for the control of third-instar larvae of *Leucopholis lepidophora* with *Heterorhabditis indica*, *Steinernema abbasi*, and chlorpyrifos in the areca nut field

Treatments	Rates ha ⁻¹	Cost of products during 2015–2017 (US\$ ha ⁻¹) ^a	Kernels yield ^b (kg ha ⁻¹)	Income of kernels ha ⁻¹ ^c (US\$)	Net profit ^d (US\$ ha ⁻¹)
<i>H. indica</i>	6.0×10^8 IJs	370.4	1860.0	5792.0	5421.6
<i>S. abbasi</i>	6.0×10^8 IJs	563.8	1140.0	3549.0	2985.2
Chlorpyrifos	9000 ml	68.6	1245.0	3876.9	3808.3
Control	0	0	885.0	2755.9	2755.9

Exchange rate used for conversion to U.S. Dollar: US\$ 1 = 65.19 INR (4 October 2017)

^aCost for the treatments was estimated based on the cost of IJs production on wax moth larvae and retail price of the chlorpyrifos in 7 August 2015

^bCumulative kernel yield during 2015–2017

^cLocal purchase price of the processed kernels (Chali) was (US\$ 3.1/kg) on 4 October 2017

^dNet profit was estimated only based on the income of kernels and the cost per hectare from different treatments

species were used at the rate of 6.0×10^8 IJs ha⁻¹. The average profits estimated, based on the price of the kernel (chali) and the cost per hectare for different treatments, were in the order of water control < *S. abbasi* < chlorpyrifos < *H. indica*.

Georgis et al. (2006) estimated the cost involved in control of *P. japonica* in the USA using in vitro cultured heterorhabditis products as higher than US\$ 500 ha⁻¹, which was four times as much as similarly effective organophosphate and carbamate insecticides and twice as much as the preventatives imidacloprid, clothianidin, and halofenozide control. Similarly, Laznik et al. (2010) reported that even after efficient control of *Leptinotarsa decemlineata* Say, using *S. feltiae* isolate B30 Laznik and the commercial products of *S. feltiae* (Entonem) in potato cultivation in Slovenia, broader implementation of these nematode strains in potato cultivation was difficult because of their high price. In the present study, although the cost for *H. indica* (US\$ 370.4 ha⁻¹) at a rate of 6.0×10^8 IJs ha⁻¹ is almost five times as much as chlorpyrifos (US\$ 68.6 ha⁻¹), but control efficacy, profit, and safety concerns encourage the use of *H. indica* for the management of areca nut white grubs. Therefore, management of the white grubs by using *H. indica* in areca nut fields in India has good prospects.

Conclusions

The present study showed the potential of *H. indica* for the control of *L. lepidophora* grubs in the areca nut field. More studies are needed on the control efficacy of the combined applications of nematode species and chemical insecticides that will increase the efficacy of the EPNs to bring down their cost.

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Availability of data and materials

All data are available at the end of the article and the materials used in this work are of high quality and grade.

Authors' contributions

JP designed the research. JP and VR conducted the experiments. JP analyzed the data and wrote the manuscript. JP obtained the funding. Both authors read and approved the final manuscript.

Ethics approval and consent to participate

All experimental works were approved by Science and Engineering Research Board (SERB) of the Department of Science and Technology (DST), Government of India and ICAR-National Bureau of Agricultural Insect Resources, Bengaluru, India.

Competing interests

The authors declare that they have no competing interests.

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