Evaluation of entomopathogenic nematodes for the management of white grub, *Leucopholis lepidophora* Blanchard (Coleoptera: Scarabaeidae)

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**Abstract**

The native strains of entomopathogenic nematode (EPNs) were evaluated against arecanut white grub, *Leucopholis lepidophora* Blanchard during 2015-17 at two locations viz., Koluru (Thirthahalli taluk) and Harakere (Shivamogga taluk) villages of Karnataka district of Karnataka, India. Field trials were conducted under All India Coordinated Research Project (AICRP) on Palms to identify the suitable ecofriendly management measures. The imposition of aqua formulation of EPN, *Steinernema carpocapsae* (CPCRI-SC1) @ 60 lakh IJs/palm during June - July followed by combined application with insecticide imidacloprid 17.8 SL @ 0.0045% during September - October was caused 96.6% and 92.5% reduction in grub population in farmers field at Koluru and Harakere village respectively over pretreatment grub population followed by EPN alone application of *S. carpocapsae* caused 74.3% and 79.1% and talc formulation of *H. indica* caused 54.8% and 51.7% reduction in grub population in the respective villages. The standard insecticide imidacloprid 17.8 SL @ 0.0045% caused a reduction of 77.3% and 80.8% in the respective villages during the period of treatment. The palms treated with EPN and imidacloprid had shown the better emergence of new roots indicates suppression of grub population in the palms root zone and increase in the number of leaves and overall improvement in the palms growth and yield indicates effective uptake of inputs by the palms over untreated palms. It was concluded that EPN can be used as one of the component in IPM of arecanut white grubs with imidacloprid for its effective management in arecanut.

**Keywords:** Arecanut, aqua formulation, *L. lepidophora*, biocontrol, entomopathogenic nematodes and *S. carpocapsae*

1. **Introduction**

The Arecanut, *Areca catechu* L., belongs to family palmae is one of the most important plantation crop grown in western ghats of India. It is largely cultivated in the plains, hilly and coastal regions of South and Eastern parts of India. Arecanut palm is attacked by an array of insect pests, among them, white grub is National Pests and are also called as root grubs. The root infesting Scarabaenid white grub complex of palm ecosystem in South India comprises three closely related species viz., *Leucopholis coneophora* Burmeister, *L. burmeisteri* Brenske, *L. lepidophora* Blanchard (Veeresh et al., 1982) [8]. Among *L. lepidophora* is predominant species which infests arecanut based cropping system and are widely distributed in coastal Western Ghats and hill regions of Karnataka and prefer clay loam soil and has biennial life cycle with grub period of 15 - 16 months (Veeresh et al., 1982) [8]. Second and third instar grubs of this species are voracious feeders, ingesting 2.3 g areca root tissues/grub/day. Considerable yield loss between 39.7 and 41.6 percent due to root grubs in arecanut is reported in Chikkamagaluru, Uttara Kannada, Udupi, Dakshina Kannada and Shivamogga districts of Karnataka (Kalleshwaraswamy et al., 2015) [3]. Root grubs causes extensive damage to the arecanut palm by directly feeding on roots and the affected palms have characteristic symptoms like yellowing and dropping of leaves, stem tapering at the crown region, reduced internodal length, premature nut fall which ultimately leads to reduced palm vigour, yield and death of plant. Under severe infestation, the palms lose their anchorage due to complete loss of roots can be easily lodged with a gentle push, as the entire root system is damaged by the grubs (Padmanabhan and Daniel, 2003) [10]. Since the white grub larvae are subterranean, the damage prediction is difficult and their management has always been troublesome.
So far management of white grub in India mainly depends on repetitive application of conventional insecticides with higher dose are the widely practiced control options. The current recommendation for the management of Leucopholis spp. in palm garden is the application of chlorpyrifos @ 2 kg a.i/ha (Anonymous, 2000) (1) is being adopted by farmers till date. Continuous exposure of an insecticides for long period leads to development of resistance in insects. Prolonged dumping of chemicals not only causes soil pollution but also has deleterious effect on soil fauna and flora hence not a sustainable strategy. Several non chemical control alternatives exist but all of them have limitations. Since the use of pesticides could be concerns for the environment and human safety, environmentally friendly management strategies needs to be evaluated for the management of L. lepidophora. Entomopathogenic nematodes (EPNs) belong to the family Steinernematidae and Heterorhabditidae are potential alternatives for the control of soil-dwelling pests among all the biocontrol agents (Toepfer et al. 2010) (7), because of their ability to actively search for their hosts. Therefore, the present study was aimed to evaluate the native EPN isolate flood formulation of S. carpocapsae (CPCR-SCI) and H. indica Poinar (commercial talc formulation) along with the commonly used chemical insecticide (imidachloprid) in arecanut field infested with L. lepidophora in endemic regions of Shivamogga district of Karnataka.

2. Materials and Methods

Field study was carried out in two arecanut gardens of each one acre area with an age between 15 and 18 year old palms with a spacing of 9ft x 9ft for three consecutive years during 2015-2017. The gardens had an incidence of root grub about 20 and 40 percent at Koluru (Thirtahalli taluk) (N12°, 639572 E 77°, 110043) and Harakere (Shivamogga taluk) (N 13°, 907652 E 75°, 561148) villages, respectively. The experiment was initiated with six treatments by adopting randomized block design (RCBD) with four replications. Each treatment consists of 60 palms which were selected for sampling root grubs. Entomopathogenic nematodes (S. carpocapsae) aqua formulation application was made at two times during June-July and September-October @ 60 lakhs infective juveniles (IJs)/palm followed by insecticide, imidacloprid 17.8 SL application once @ 0.0045% (0.5 ml/2 lt water/palm) during September - October by drenching around the root zone. EPN talc formulation was applied in two times viz., Heterorhabditis indica @ 25g/palm during June - July and September – October and Metarrhizium - talc formulation (20 g/palm) followed by S. carpocapsae @ 60 lakh IJs/palm during June - July and September - October. Imidacloprid 17.8 SL @ 0.0045% (0.5 ml/2 lt water/palm) used as check during June - July and September - October. The EPN, fungal bio-agent and insecticides were compared with each other and untreated control. Three random samples mean grub population per palm basin were recorded before and after imposition of treatment at 30, 60 and 90 days in each year during treatment by digging the soil around at the base of the palm (30 cm away from the palm to a depth of 60 cm and width of 30 cm). Growth characters viz., number of leaves and yield per palm were also recorded. The data were subjected to square root transformation and means were compared using critical difference (CD P=0.05). The results of the three year field studies pooled together to get the most relevant trend are presented in Table 1.

Per cent reduction of root grubs was calculated by using following formula given below:

\[
\text{Percent Reduction} (\%) = \frac{\text{Mean larval population before treatment} - \text{Mean larval population after treatment}}{\text{Mean larval population before treatment}} \times 100
\]

2.1 Treatment details

T1: Aqua formulation of EPN, S. carpocapsae (ICAR-CPCR isolate) @ 60 lakh IJs/palm
T2: Talc formulation of EPN, H. indica (25g/palm)
T3: Imidacloprid 17.8 SL @ 0.0045% (0.5 ml/2 lt water) + S. carpocapsae @ 60 lakh IJs/palm (@ 1.5 billion IJs/ha)
T4: Talc formulation of EPN, Metarrhizium (20 g/palm) + S. carpocapsae @ 60 lakh IJs/palm
T5: Imidacloprid 17.8 SL @ 0.0045% (0.5 ml/2 lt water/palm) (Standard check)
T6: Control

3. Results and Discussion

This is first field studies to evaluate the different strains and formulations of EPN against white grub management in arecanut at Shivamogga district of Karnataka. The results revealed that significant reduction in root grub population in the palm basin and steady improvement of palm health and yield during three year treatments explained in following heads.

3.1 Reduction of white grub population

The mean white grub population in all the treated plots in both the locations (Koluru, Harakere) was found significantly lower than the untreated palms (control). Among all the treatments, application of imidacloprid 17.8 SL @ 0.0045% (0.5 ml/2 lt water) + S. carpocapsae @ 60 lakh IJs/palm (0.75 billion IJs/ha) (T3) was superior in reduction white grub population 96.6% (0.44 grubs/palm from 13.04 grubs/palm) and 92.5% (1.28 grubs/palm from 17.15 grubs/palm) at Koluru and Harakere locations respectively followed by application of imidacloprid alone @ 0.0045% (T5) caused reduction of 77.3% and 79.1% and S. carpocapsae alone @ 60 lakh IJs/palm (1.5 billion IJs/ha) (T1) caused 74.3% and 79.1% reduction in grub population in respective localities after three years of treatments (Fig. 1 & 2). The findings of the present study confirm the superiority of aqua formulation of EPN with imidacloprid application over other biocontrol agents evaluated. This is in conformity with the findings of Mahmoud et al. (2016) (8) showed synergistic effect between Imidacloprid, Thiamethoxam, NeemAzal, Neemix and S. carpocapsae when applied against the Black cutworms, Agrotis ipsilon. Also, Koppenhofer & Fuzzy, (2008) demonstrated application of EPN with neonicotinoid insecticide imidacloprid was more effective in controlling the grubs of Agrotis orientalis, Cyclocephala borealis and P. japonica Newman. Patil et al. (2015) (6) mentioned that the combinations of imidacloprid and nematodes, Heterorhabditis indica, had a strong synergistic effect on mortality of early and late 3rd instars of coconut white grub, Leucopholis
coniophora at different concentrations of imidacloprid. Combinations of imidacloprid and entomopathogenic nematodes may provide a powerful and economically feasible curative control in white grub management in coconut. Similar field studies trend was observed on the inundative application of S. scarabaei @ 0.25 to 2.5 × 10⁹ IJs/ha against white grub populations (A. orientalis) caused 77-100% control within one month of application at rates of and 86-100% control in the following spring at rates of 0.1 to 2.5 × 10⁹ IJs/ha. Increase in efficacy of Steinernema spp. to white grub may be due to its higher penetration rate than that of H. bacteriophora (Rosa et al., 2002) [7].

3.2 Growth characters
Results shown in (table 1) showed significant improvement in the plant growth in terms of number of leaves, green nut production and reduction in the number of palms infested by root grub due to the inundative soil application of EPN. The increase in number of leaves and green nut production was more pronounced by the application of imidacloprid 17.8 SL @ 0.0045% (0.5 ml/2 lt water) + S. carpocapsae @ 60 lakh IJs/palm (0.75 billion IJs/ha) 7.88 leaves/palm and 7.28 leaves/palm; green nut yield production 28.33 kg/palm and 21.50 kg/palm at Koluru and Harakere, respectively followed by T5 - imidacloprid 17.8 SL @ 0.0045% showing leaves 7.55/palm and 7.49/palm at respective localities over untreated palms. This may be once nematode released in root grub infested soil, start actively seek out their insect hosts. When a host has been located, the nematodes penetrate the insect through body openings and release symbiotic bacteria that multiply and rapidly kill the insect (Forschler & Gardner, 1991) [2]. Nematode recovery was observed in treated plots indicates the successful establishment of nematodes in soil and its self-perpetuating in effective management of root grubs. Newly emerging leaves was noticed in all the treated plots except the control. Application of EPN and insecticide imidacloprid resulted in better root growth enhance the uptake of inputs by palms resulted increase in number of leaves and bunches opening resulted significant increase in yield (green nuts) of the treated palms.

Table 1: Effect of EPN application on arecanut growth and yield at Shivamogga

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Mean No. of leaves/palm (before treatment)</th>
<th>Cumulative mean No. of leaves/palm (after treatment)</th>
<th>Mean green nuts weight (kg/palm) (after treatment)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Koluru</td>
<td>Harakere</td>
<td>Koluru</td>
</tr>
<tr>
<td>T1</td>
<td>6.56</td>
<td>6.33</td>
<td>7.39</td>
</tr>
<tr>
<td>T2</td>
<td>6.92</td>
<td>6.08</td>
<td>7.42</td>
</tr>
<tr>
<td>T3</td>
<td>6.20</td>
<td>6.58</td>
<td>7.88</td>
</tr>
<tr>
<td>T4</td>
<td>6.83</td>
<td>7.00</td>
<td>6.88</td>
</tr>
<tr>
<td>T5</td>
<td>6.25</td>
<td>7.00</td>
<td>7.54</td>
</tr>
<tr>
<td>T6</td>
<td>5.08</td>
<td>5.17</td>
<td>5.67</td>
</tr>
<tr>
<td>CD @ 0.5%</td>
<td>0.38</td>
<td>0.92</td>
<td>0.50</td>
</tr>
<tr>
<td>CV%</td>
<td>3.31</td>
<td>8.23</td>
<td>2.82</td>
</tr>
</tbody>
</table>

Fig 1: Effect of entomopathogenic nematodes on root grub population (Leucopholis lepidophora) during three years of experiment (2015-17) at Koluru of Shivamogga, Karnataka.
4. Conclusion

Based on the results, it can be concluded that T3 [Imidacloprid 17.8 SL @ 0.0045% (0.5 ml/2 lt water) + S. carpocapsae @ 60 lakh IJs/palm (0.75 billion IJs/ha)] was found effective in reducing the root grub population at both the locations and also resulted in better root growth. The uptake of inputs enhanced by the treated palms resulted increase in number of leaves and bunches opening which significant showed increase in yield (green nut production). There was a variation in reduction of larval population across two different locations. These variations may be due to soil properties, percolation power of the chemicals in soil, residual effect of chemicals and also variation in microclimate of the garden. Since the EPNs found insecticidal against white grub and they can be easily applied with insecticides, and are compatible with insecticides. Overall, the future use of EPNs is promising, given all the advantages they possess, as well as the increasing demand for any virulent microbial pathogen to
help mitigate the environment and resistance pressure of synthetic chemical insecticides.

5. Acknowledgement
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6. References
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