



Field Bio-Efficacy of Entomopathogenic Nematode-Based Integrated Management of White Grubs in Arecanut

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Abstract

The white grubs (root grubs), a soil-dwelling coleopteron insect pest are major problem for arecanut cultivation in Western Ghats of India. A three year field evaluation (2015– 2017) was conducted to assess the efficacy of integrated white grub management practices, which included the soil drench of a liquid suspension of local isolate of the entomopathogenic nematode (EPN), *Steinernema carpocapsae*, in combinations with other IPM practices. The experiment aimed to develop an integrated pest management system against white grubs in arecanut and to evaluate its impact on arecanut yield and related attributes. The integrated approach, comprising soil drainage, collection and destruction of grubs by digging palm basins (up to 10cm depth and 60cm from the trunk), soil application of neem cake at 2kg/palm, insecticide application (imidacloprid at 0.25ml/L of water), along with soil drenching of liquid suspension of EPN, *S. carpocapsae* at 20lakh IJs/palm, resulted in a significant reduction in grub population (86 to 92%) and an increase in dry arecanut yield (164-332 kgs/acre) by 2017. In contrast, prior to treatment (2015), these gardens had the highest root grub population (11.6grubs/palm) and the lowest yields (48.6–238kgs/acre). These results demonstrate the effectiveness of EPN-based integrated pest management practices for white grub management in arecanut.

Key words: Arecanut, entomopathogenic nematodes, IPM, *Leucopholis* sp. and White grubs.

Introduction

Areca nut is one of the major commercial plantation crops in India, with about 90% of the area and production concentrated in Karnataka, Kerala and Assam, involving over two lakh farm families (Jeer Vinayaka *et al.*, 2018). The crop thrives under diverse agro-climatic conditions, but it is severely affected by pest and diseases. Among these, white grubs, *Leucopholis* spp., (Coleoptera:Scarabaeidae:Melolonthinae) are one of the most destructive soil insect pests, posing a major constraint to production. They are widely distributed in Western Ghats of Karnataka (Shivamogga, Chikmagalur, Uttara Kannada, Udupi and Dakshina Kannada), low altitude coastal Kerala, humid northeastern hills, and the

west coastal peninsula (Veeresh *et al.*, 1982; Padmanaban and Daniel. 2003; Shabbir *et al.*, 2014). The third instar grubs feeds on roots, causing yellowing of leaves, reduced number of bunches, stem tapering, and nut fall ultimately leads to reduced vigour and yield loss and sometimes palm death (Rajamani and Nambiar 1970; Nair and Daniel, 1982; Chandrika Mohan *et al.*, 2022). Three important species of *Leucopholis* infest arecanut (*L. burmeisteri*, *L. coneophora* and *L. lepidophora*) occur in localized tracts in Karnataka. Damage levels of 27.86 to 36.97% with yield losses of 39.79 to 41.60% have been reported (Kalleshwaraswamy *et al.*, 2015). These white grubs are perennial insect pests in arecanut and coconut eco-



systems, having one-to-two year life cycle, and occur mostly in clayey, sandy loam, lateritic and coastal alluvial soils, where water table is high. As all stages of grubs occur in soil, control using insecticides is difficult and needs frequent application of high dosage. Prolonged use of insecticides not only causes soil pollution but also has deleterious effect on soil fauna and flora. Due to the unavailability of successful bio-control agents for white grub management, farmers mainly rely on chemical pesticides.

Hence, field testing of effective biological agents, along with other integrated pest management practices, is one of the major thrust on agricultural research, which can lead to the development of the sustainable pest management strategies at local level. Among the available bio-control agents, entomopathogenic nematodes (EPNs) in the families Steinernematidae and Heterorhabditidae (Rhabditida) are vital in the development of sustainable pest management strategies. These nematodes are lethal insect parasites that infect a wide range of soil insects, occur naturally in agricultural soils (Kaya and Gaugler, 1993). Laboratory experiments and field releases of EPN demonstrated their successful use to control soil insect pests (Klein, 1990; Sharpiro-Illan *et al.*, 2002). Therefore, field trials were conducted during 2015-2017 to evaluate environment friendly, EPN based integrated white grub management strategies in arecanut, and the results are presented here.

Materials and Methods

Field Trials Location

Field trials to evaluate EPN-based integrated management of white grub were conducted for three consecutive years (2015-2017) in white grub infested arecanut gardens, belonging to four farmers (each one acre), located in the Malnad areas of Sringeri and Sullia Taluks, Karnataka State (Table 1). The age of the palms in the selected gardens ranged between 15 to 20 years, and the soils varied from clayey loam to red sandy types. The annual rainfall in the area

was approximately 3000mm. The plots were selected based on accessibility, and the regions were known to be endemic to white grub infestation, with at least 10-15km distance between each selected plots within the location. Within the four selected plots, a total 40 soil samples (200g each) were randomly collected from the rhizosphere of white grub infested palms to check for the presence of EPN using the *Galleria mellonella* baiting technique (Tarasco *et al.*, 2020). None of the soil samples showed the presence of EPNs before the imposition of treatments.

Table 1. Details of field experiment locations

Plot No	Name and Locations	Geographical Coordinates
1	Mr. Yogisha, S., Sankesha house, Markanje post and village Sullia, Dakshina Kannada, Karnataka, Mob: 9880308903	12°33'29"N 75°23'21"E.
2	Mr. Ramesh H. T., Honnnavalli village, Sringeri, Chikamagalur, Karnataka, Mob: 9449651966	13° 25' 1.128" N, 75° 15' 9.756" E
3	Mr. Jagajeevan Rao, Renjala House, Markanje post and village Sullia, Dakshina Kannada, Karnataka, Mob: 9187706871	12°33'29"N 75°23'21"E.
3	Mr. K. N. Gopala Heggade, Kigga (Bellare) village, Sringeri, Chikamagalur, Karnataka, Mob: 9449620541.	13.4167° N 75.2667° E

Production of EPN, *S. carpocapsae*

The local isolate of EPN, *S. carpocapsae* (Rajkumar *et al.*, 2016) was mass-multiplied on final-instar larvae of Greater wax moth, *G. mellonella* (L.) (Lepidoptera: Pyralidae) at room temperature ($28\pm 2^{\circ}\text{C}$) under laboratory conditions (Kaya and Stock 1997). Galleria larvae were reared on an artificial diet following the standard methodology (Dutky *et al.*, 1964). Dead *Galleria* larvae were collected at three days interval after inoculation with *S. carpocapsae* infective juveniles (IJs) at 5-7 IJs/*Galleria* larvae and transferred to White's traps to collect emerging IJs (White, 1927).

The emerged IJs from the infected cadavers were harvested and further processed into a liquid formulation at the Nematology section, Crop Protection Division, ICAR-CPCRI, Kasaragod, Kerala, India (Fig.1). Processed nematodes were stored at room temperature ($28\pm 2^{\circ}\text{C}$) for no more than one week. The viability of the nematode liquid suspension was confirmed using a stereomicroscope, nematodes were considered alive if actively moving or responding to a needle probe. Only suspension with more than 90% viable IJs were used in the field trials.

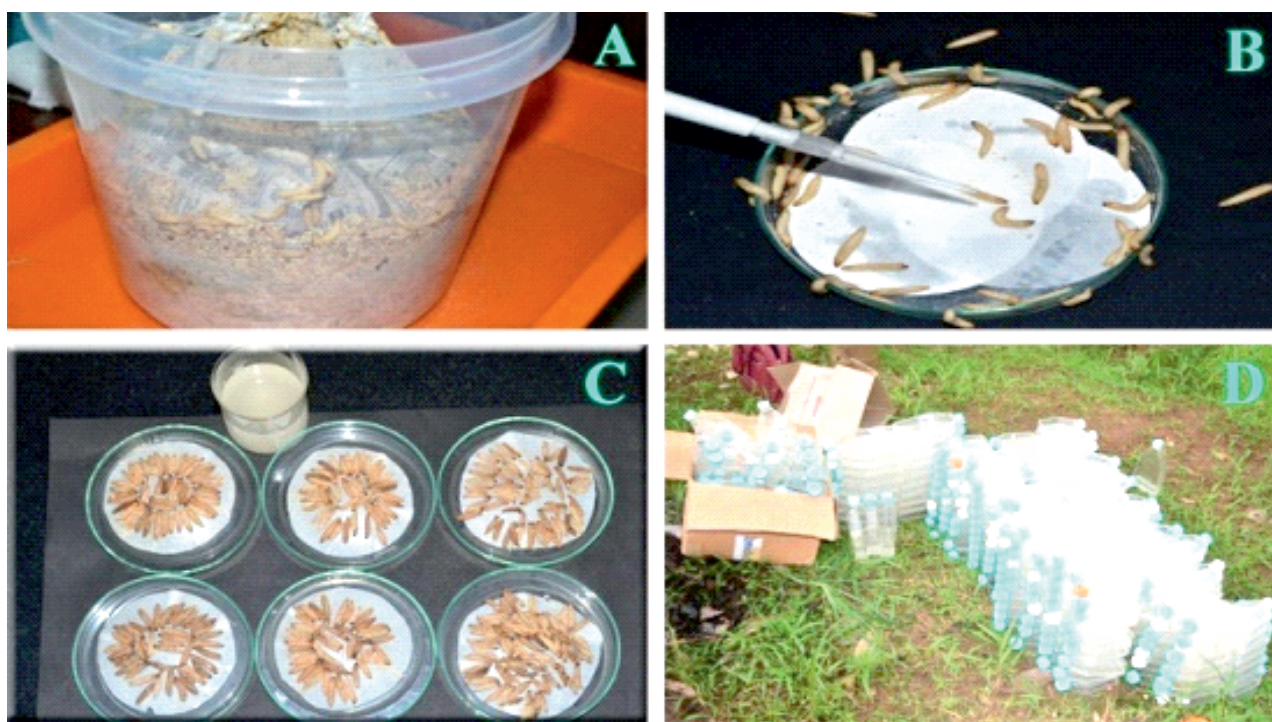


Fig.1. Mass production of *S. carpocapsae* on *G. mellonella*, (A) Rearing larvae on artificial diet, (B) Inoculation with IJs, (C) Extraction of IJs, and (D) Nematode suspension ready for field application.

Application of EPN

EPN treatments were applied at the root zone of arecanut palms, covering a 60 cm radius with liquid suspension of EPN, *S. carpocapsae* at 20 lakh IJs/palm, mixed in three liters of water. Applications were carried out during June – July and again in September – October, in combination with insecticide (imidacloprid at

0.25ml/L of water). For insecticide application, two liters of solution were drenched within a 60cm radius around each palm. Nematode applications were conducted in the morning (9.00–10.0am) at a temperature $24^{\circ}\text{C}\pm 2$ to facilitate nematode movement in to the soil (Fig.2).



Fig.2. Application of *S. carpocapsae* in palm basin and microscopic view of IJs



As part of the IPM strategy, shallow digging of soil up to 10cm depth around the palm and between the interspaces was carried out during September for the manual collection and destruction of grubs concentrated around the root zone of palms (Fig.11). Application of neem cake at 2kg/palm (Fig.3) was undertaken during December–January. Proper drainage facilities

(Fig.4) were provided in the treated plots, and timely phytosanitation measures were implemented throughout the treatment period. All selected plots received soil nutrients, including urea at 60g/palm, rock phosphate at 60g/palm, and muriate of potash at 120g/palm, applied at the root zone of arecanut by opening ring around the tree trunk.



Fig 3. Application of neem cake in arecanut palm basins



Fig 4. Provision of drainage in treated arecanut gardens

Observations

From each plot, 35 palms were randomly selected and tagged for observations, Data recorded included root grub infestation symptoms, number of grubs in the palm basin (0.5 m² per palm) (Fig.5) before and after treatment, and yield (Subaharan *et al.*, 2001). Pre-treatment counts showed an average of

11.6grubs/palm, in early and late larval stages (Fig.6). Larval populations were monitored at 3, 6 and 9 months after application. The applied nematode establishment in treated gardens was confirmed by soil baiting technique with fully grown *G. mellonella* larvae at three monthly intervals by collecting soil samples randomly from the treated palm basins.



Fig 5. Counting number of grubs in an arecanut palm basin



Fig 6. Healthy early and late instar grubs

Statistical analysis

In each field trial, data on the effects of integrated treatments were subjected to analysis of variance (ANOVA) to determine the mean percentage reduction in grub population. Means were compared to detect the significant difference among the treated plots and untreated control (not applied nematodes and not followed IPM practices). The mean data of all the years were pooled and analyzed for further strengthen the results using SAS. Graphs were prepared in MS Excel 2003, and data are presented with standard error.

Result and Discussion

The results indicated that in all treated plots, integrated management practices involving liquid suspension of *S. carpocapsae* significantly reduced root grub populations compared to both the pre-treatment levels and untreated control plot.

Reduction of grub population

Application of *S. carpocapsae* liquid suspension at 20lakh IJs/palm during June–July and in September–October, again in combination with insecticide (imidacloprid at 0.25ml/L) and other IPM strategies (Soil drainage (Fig.4), manual collection and destruction of grubs (Fig.11), and

application of neem cake at 2kg/palm (Fig.3) proved to be the most effective IPM package. This treatment achieved an 86 to 92% reduction in white grub population (Fig.7 and 10). These findings corroborate earlier reports that liquid suspension of EPN (*Steinernema* and *Heterorhabditis*) applied to soil can control a wide range of economically important insect pests (Karunakar *et al.*, 2000; Grewal, 2002; Sankarnarayana *et al.*, 2006; Shahina and Salma 2011; Bhatnagar 2011). Also recorded early curative control when combinations of nematodes and the neonicotinoid (imidacloprid) were applied, demonstrating enhanced efficacy against both early and late instar grubs (Koppenhofer and Fuzy 2008). The effectiveness of EPNs against 3rd instar scarabs can be further enhanced when integrated with complementary strategies, which increase larval susceptibility to nematode infection (Thurston *et al.* 1993, 1994).

Earlier studies demonstrated that, among tested EPN species tested, *S. carpocapsae* was the more virulent against *Holotrichia longpennis* (Scarabaeidae and Melolonthidae) (Khatri-Chhetri *et al.*, 2011). The efficacy of EPNs depends on both application concentrations and species-specific traits (Hominick and Reid 1990). Several researchers have evaluated EPNs

for managing maize fall army worm under greenhouse and field conditions (Andalo *et al.*, 2010). Patil *et al.*, (2014, 2022) reported high pupal mortality (87%) in fall army worm and highlighted the efficacy and reproductive potential of indigenous EPN isolates for managing *Oryctes rhinoceros* grubs in coconut.

However, laboratory experiments often demonstrate high efficacy of EPNs, but these results are not always replicated under field

conditions, as several environmental factors influence their performance (Cantelo and Nickle 1992). For instance, *Steinernema carpocapsae* exhibited 100% control of Colorado potato beetle adults, pupae, and larvae in laboratory trials. However, when the same experiment was conducted outdoors, efficacy declined substantially, with only a 31% reduction in pest populations recorded (Stewart *et al.*, 1998).

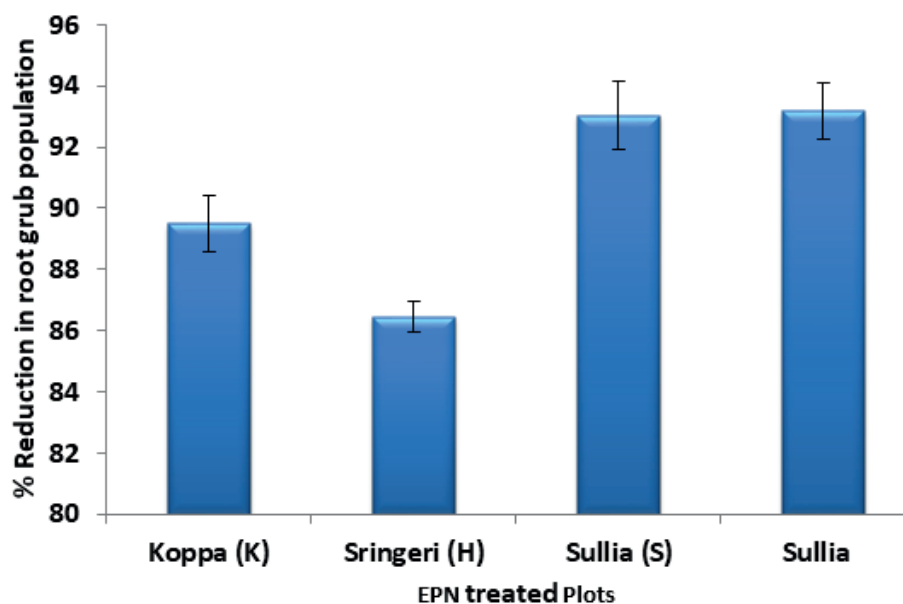


Fig. 7. Mean percentage reduction of *Leucopholis* population after application of *S. carpocapsae* based integrated practices in arecanut.

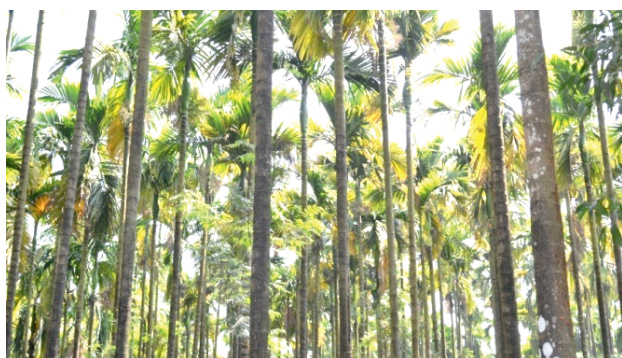


Fig 8. Palms before treatment (2015) showing yellowing foliage and no nut set



Fig. 9. Palms after treatment (2017) showing improved flowering and fruit set



Fig.10. *S.carpocapsae*- infected white grub larvae

Reduction in root grub infestation symptoms

The integrated *S. carpocapsae*-based management strategies significantly improved palms growth, evident from the emergence of healthy green fronds, and reduced root grub symptoms (Fig.8, 9). The decline in root grub population preserved newly emerging roots, enhancing nutrient and water uptake, which increased leaf production and bunch opening, ultimately leading to a notable yield improvement. Stem tapering decreased to 3.5 – 5.6%, palm mortality was reduced to 0.3–1.2%. In addition treated palms recorded the highest number of leaves (7–9 leaves/palm) and bunches (4-5 bunches/palm) compared to un-treated control plot, even though the values are statistically insignificant (Table 2) and maximum yield increases up to 332kgs/acre was recorded in Koppa village followed by 309kgs/acre in Sullia (Table 3 and Fig.12). These results of EPN based integrated strategies in line with the Krishnaya and Grewal (2002); Padmanabhan *et al.*, (1997), who reported EPNs are known to be compatible with

chemical pesticides, fertilizers, and soil amendments such as neem cake. Hanumanthaswamy *et al.*, (2017) observed the combined application of imidacloprid and neem cake with IPM practices over five years consistently improved palm growth and reduced pest incidence compared to untreated control plots. In the treated gardens, palms exhibited untapered stems, larger internodes, healthy green leaves, and were free from white grub infestation, resulting in a 27.17% yield enhancement over untreated control plot. Neem cake contributed through its repellent and anti-feeding effects against root.

Roopa Patil (2017) emphasized that, in addition to plant protection measures, proper drainage management is crucial for effective integrated control of white grub (*Leucopholis lepidophora* Blanchard) in arecanut plantations. While yield levels may not directly reflect reductions in grub populations, the emergence of new healthy green fronds and improved palm growth after three year treatments serve as reliable visual indicators of success.

Table 2. Effect of three years of *S.carpocapsae*-based integrated treatments on arecanut growth and white grub infestation symptoms

Location	Av. No. of palms showing stem tapering (%)	Av. No. of palms showing death (%)	Av. No. of leaves/palm	Av. No. of bunches/palm
Kigga (Sringeri)	5.6	0.3	9.0	5.0
Honnnavalli (Sringeri)	4.8	1.2	7.0	4.0
Renjala (Sullia)	8.3	0.5	7.0	4.0
Sankesha (Sullia)	3.5	0.0	8.0	4.0
Control (Not applied nematodes)	22.7	5.6	5.0	2.0
CD@5%	4.9	1.3	4.1	3.0



Fig. 11. Collection and destruction of grubs from the palm basin and interspaces

Table 3. Effect of three years of *S.carpocapsae*-based integrated treatments on arecanut yield

Location	Before treatment yield (kgs/acre) (2015)	After treatment yield (kgs/acre) (2017)	% Change
Koppa (K)	238.0	332.0	39.4
Sringeri (H)	48.6	164.0	237.5
Sullia (S)	162.8	309.0	89.8
Sullia (R.)	125.5	204.9	62.2
P value		0.0000065583	



Fig.12. Green nut bunches of arecanut harvested from *S.carpocapsae* treated plots

Field establishment and nematode persistence

Field studies indicated successful establishment and persistence of native strains of *S. carpocapsae* in treated plots over a period of three years treatment. Reduction in root grub population and nematode persistence was increased with increase in treatment duration (Fig.13). At the end of the three year experiment, maximum persistence of EPN was observed in 33% of the collected soil samples, compared to 12.6% in the first year of treatment. These results of nematodes recovery from the treated plots indicates the successful establishment of nematodes in soil and it's self-perpetuating in effective management of white grubs.

Differences in *S. carpocapsae* establishment and persistence can be attributed

to soil structure and texture. This aligns with the reported persistence rates of 44.9, 38.8, 32.9, and 26.7% in sandy loam, sand, clay loam, and clay soils respectively (Shang Ping Kung *et al.*, 1990). Observed maximum persistence of 77% in pasture and 78% in potato fields (Khan and Javed 2018). In addition, nematode species and strains differ in persistence potential, with steinernematids generally surviving longer than heterorhabditids. Persistence is also influenced by the presence of host plants and associated pest insects, which facilitate nematode recycling (Strong, 2002). Several studies have shown that direct spot application of nematodes to the soil host niche results higher grub mortality (Shapiro-Ilan *et al.*, 2006) that eventually resulted in higher nematode dispersal, infectivity and survival (Vicente-Diez *et al.*, 2021).

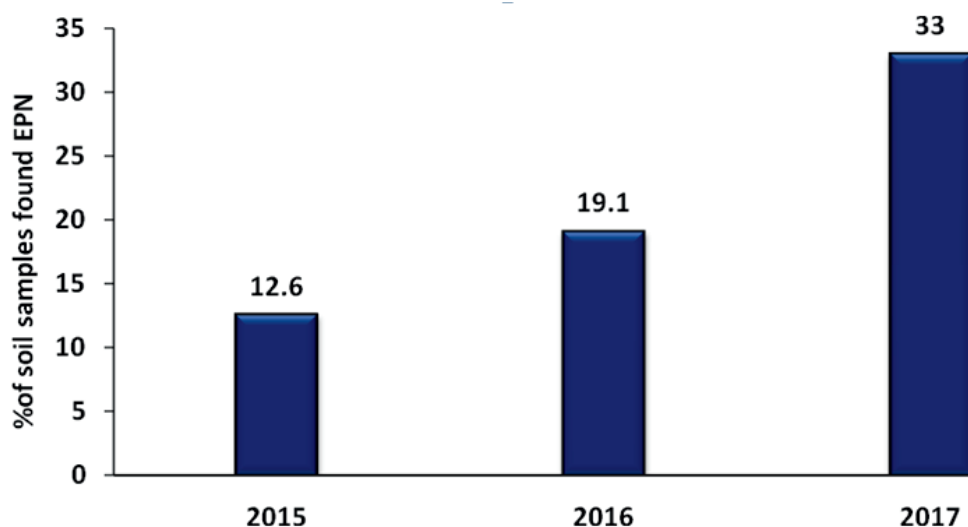


Fig. 13. Percentage of soil samples exhibiting nematode persistence in treated plots

Conclusion

The current recommendations for management of white grub in arecanut involve applying insecticides such as phorate, carbofuran and chlorpyrifos. Adults of white grub beetle emergence and oviposition occur mainly in the beginning of monsoon, while peak grub population in the root zone of palms is

observed during post-monsoon. Considering the pest life cycle, application of *S. carpocapsae* liquid suspension during June – July, followed by a repeat application with a low dose of imidacloprid in September – October, combined with improved soil drainage, manual removal of grubs, and neem cake application, proved more effective



Insecticide use has been increasingly associated with negative effects on beneficial soil microbes. In this context, EPN based integrated management offers a safe, sustainable alternative for the management of white grubs in arecanut plantations. However, despite its effectiveness, there remains a strong need to promote and demonstrate this EPN-based integrated management package through a community-based approach to ensure wider adoption and long-term suppression of this devastating pest.

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