

Field Evaluation of the Entomopathogenic Nematodes (EPNs) for Sustainable Management of White Grub, *Leucopholis coneophora* Burm. in Coconut

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ABSTRACT: Coconut, *Cocos nucifera* L. (Palmaeaceae) is an important perennial crop providing livelihood to many small and marginal farmers in the coastal states of India. The major factor hindering crop production is infestation of white grubs (*Leucopholis coneophora* Burm.) in the palm root zone. Currently, management of this pest mostly relies on insecticide application which poses serious environmental hazards. This led to the development of eco-friendly alternative management strategy by utilizing liquid formulation “Kalpa EPN (CPCRI – SC1)” from native entomopathogenic nematode (EPN) *Steinernema carpocapsae*. The present study was devised to evaluate the suppressive effect of native EPN against of white grub, *L. coneophora*. Soil application of ‘Kalpa EPN (CPCRI – SC1) @ 173250, 346500, 693000 and 1039501 IJs/m² palm basin at three monthly intervals per annum over two years (2013 -2014) indicated that treatment with higher dose of 1039501 IJs/m² reduced white grub population by 60.2%. Besides, there was a progressive increase in percent reduction of grubs with increase in EPN concentration and number of years of treatment indicating a dose-induced mortality for the tested formulation. Thus, utilizing EPN as a biological component in white grub IPM programme opens up a new avenue for its sustainable management in coconut.

Key words: Coconut, Kalpa EPN liquid formulation, *Leucopholis coneophora*, *Steinernema carpocapsae*, white grub/root grub

Cocos nucifera L. is known popularly as “Kalpavriksha” by virtue of its wide range of uses. The palm grows in almost 93 countries and cultivated commercially in India, Indonesia, Philippines and Sri Lanka accounting altogether for 78% global coconut production (Chowdappa *et al.*, 2017). The palms have been reported to be infested by more than 900 species of pests including vertebrates and invertebrates throughout the year (Kumara *et al.*, 2015). One among them is *Leucopholis coneophora* L., also known as white grub/ root grub, feeds on the tender palm roots thereby hindering the nutrient uptake resulting in poor growth and low yield. The occurrence of this pest is very prevalent in the sandy, sandy-loamy and lateritic soils of coastal zones of Kerala and Karnataka (Nirula *et al.*, 1952). Three species of white grub namely *Leucopholis coneophora*, *L. burmesterie* and *L. lepidophora* are reported to be occurring in localized tracts of coconut and areca nut

cultivation (Rajkumar *et al.*, 2016). The newly emerged grubs feed and survive on roots of weed plants and later they migrate to feed on the tender apical coconut roots. Grubs are ‘C’- shaped with white body and brown head and the infestation is severe in poorly managed gardens (Josephraj Kumar *et al.*, 2019). The lifecycle is of 1 - 2 years and the beetle emergence coincides with onset of monsoon (Abraham, 1993). The cryptic habitat of these grubs makes it difficult to manage efficiently and the general management tactics include the use of organophosphorus insecticides (Abraham and Mohandas, 1988) which pose serious threat of contaminating water bodies. In addition chemical management is always uneconomical and certain chemicals are banned for use locally (Kasaragod district of Kerala). Considering these factors an alternative biological control strategy using native isolate of entomopathogenic nematode (EPN) could be safe for pest management (Rajkumar and

Subaharan, 2016). EPNs from the families Steinernematidae and Heterorhabditidae are reported as promising bio-control agents for the control of soil-dwelling pests (Rabindra and Hussaini, 2003; Rajkumar *et al.*, 2019) and are considered safe for non target organisms, hence exempted from registration (Kaya and Gaugler, 1993; Patil *et al.* 2015 and Rajkumar *et al.*, 2018). The native EPN species such as *Steinernema scarabaei* Stock and Koppenhofer, *S. longicaudum* Shen and Wang, *S. lanmjungense* Khatri-Chhetri, *Heterorhabditis bacteriophora* Poinar, *H. zealandica* Poinar, *H. indica* Poinar and *S. carpocapsae* (Koppenhofer *et al.*, 2000; Khatri-Chhetri *et al.*, 2011; Rajkumar *et al.*, 2014) were recorded to suppress larvae of many species of white grubs. However, the information on field efficacy of native EPN formulation to manage the white grubs in coconut plantations of affected states is limited. In this context locally available liquid formulation ‘Kalpa EPN’ of *Steinernema carpocapsae* was evaluated against the white grubs in the infested coconut gardens as well as studies on its persistence in soil after application was studied.

MATERIAL AND METHODS

Source of nematodes

The local isolate of *Steinernema carpocapsae* was developed into a liquid formulation and named as “Kapla EPN (CPCRI-SC1)” at the Nematology section, Crop protection division, ICAR-CPCRI, Kasaragod, Kerala, India. Mass production of EPN was carried out on fully grown larvae of Greater wax moth (*Galleria mellonella* L.; Lepidoptera: Pyralidae) at ambient temperature ($28 \pm 1^\circ\text{C}$) under laboratory condition. The emerged IJs from the infected cadavers were harvested using white trap (White, 1927). They were further processed for liquid cultures (Ehlers, 2001) and packaged in polypropylene covers for transportation to the white grub-affected coconut garden. The viability of the nematode liquid formulation was confirmed using a stereomicroscope,

and it was regarded alive if it was actively moving or responding to a needle probe. When more than 90% of IJs were viable only, EPN suspensions were employed in the field trials.

Location of the field experiment

Field experiment was conducted during 2013 - 2014 in the white grub infested coconut garden at Kasaragod ($\text{N}12^\circ31.550' \text{E}074^\circ58.081'$). The soil type was sandy and annual rainfall was 3000 mm. The experiment was designed using randomized complete block design, with 5 treatments and 4 replicates, each replicate consisted of 3 palms (a total of 12 palms per treatment). Larval population of *L. coneophora* was recorded before imposing EPN treatments. To estimate the larval population of *L. coneophora*, eight soil samples (0.3×0.3 m surface area to a depth of 0.3 m) were obtained randomly from the interspaces and palm basin within each block and the population density of larvae was assessed by counting. Similarly, the natural EPN population in the field was also checked by baiting soil samples with greater wax moth larvae before initiating the experiment. No EPN populations were recovered from the experimental field.

According to pre - treatment sampling, the larval population of *L. coneophora* was estimated to be of 6.2 ± 0.60 grubs m^2 , and they were in the early larval developmental stage (1st instar) at the time of treatment imposition.

Soil application of EPN

The soil application of ‘Kalpa EPN (CPCRI – SC1) liquid formulation of entomopathogenic nematode *Steinernema carpocapsae* was evaluated at different concentrations *viz.*, 173250 (T1), 346500 (T2), 693000 (T3) and 1039501 (T4) IJs/ m^2 in palm basin twice a year during mid June – July and September – October over two year period (2013 - 2014). Water without IJs was used as control (T5). The soil application of IJs was

carried out by mixing each IJs concentration in 5 liters of water and poured around the each palm base covering 2.5 meter radius. The application was carried out during the morning hours (9.00 – 10.0 am) with temperature of $24^{\circ}\text{C} \pm 2$ to favor the nematodes for easily reaching the root zone. To estimate the reduction in the larval population, observation was taken at 3, 6 and 9 months after EPNs' application.

Treatment details

T1: Kalpa EPN liquid formulation of *S. carpocapsae* @ 173250 IJs/ m² per palm basin

T2: Kalpa EPN liquid formulation of *S. carpocapsae* @ 346500 IJs/ m² per palm basin

T3: Kalpa EPN liquid formulation of *S. carpocapsae* @ 693000 IJs / m² per palm basin

T4: Kalpa EPN liquid formulation of *S. carpocapsae* @ 1039501 IJs/ m² per palm basin

T5: Control (without EPN soil application)

Establishment and persistence of EPN

In order to assess the EPN, *S. carpocapsae* establishment and persistence in the soil, randomly 10 soil samples were collected from each treatment block during 2013 and 2014 (A total of 20 samples per year). From each soil sample, 150 g of soil was filled into two plastic containers (height -7 cm x width - 4 cm) (Total 8 samples from each treatment) and released four final instar larvae of *G. mellonella* and checked the larval mortality at 24 h intervals for six days. The infected larval cadaver were incubated in clean petri dishes for two days using BOD at $28 \pm 2^{\circ}\text{C}$ and these larvae were placed over white trap on 3rd day for nematodes emergence. The emerged nematodes were confirmed morphologically with pure cultures of *S. carpocapsae* maintained at Crop protection division of ICAR – Central

Plantation Crops Research Institute Kasaragod, Kerala (Rajkumar *et al.*, 2016).

Statistical analysis

The data were subjected to analysis of variance (ANOVA) and means were compared using critical difference (CD $P = 0.05$) to detect the significant difference among the treatments and with untreated control. The mean data of both the years were pooled and analyzed for further strengthen the results using SAS.

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

$$\text{Percent Reduction (\%)} = \frac{\text{Mean of Pre-treatment value} - \text{Mean of Post treatment value}}{\text{Mean of Pre-treatment value}} \times 100$$

RESULT AND DISCUSSION

Suppression of white grub population

Field studies for determination of efficacy of Kalpa EPN application/ formulation revealed that all the treatments were found significantly superior than control in reducing *L. coneophora* grub population in the infested coconut palms over a period of two years. Maximum reduction in grub population (60.27%) was observed at a higher nematode density of 1039501 IJs/m² followed by (39.85%) 693000 IJs/m² whereas, minimum reduction in grub population (1.29%) was observed in control (without nematode application) followed by (28.02%) lower nematode density of 173250 IJs/ m² (Table. 1). The present findings thus could lead to a viable and eco-friendly option for management of white grubs, especially in Kasaragod, where some classes of synthetic insecticides are banned.

These findings are in close conformity with the findings of Supekar and Mohite (2015) wherein they

Table 1. Effect of soil application of Kalpa EPN, *S. carpocapsae* liquid formulation on white grub population in coconut

Treatments (IJs/m ²)	Pre treatment (No. of grubs/treatment)		Post treatment (No. of grubs/treatment)		% reduction of grubs		Average % reduction of grubs
	2013	2014	2013	2014	2013	2014	
173250	113	110	88.3	72.3	21.85	34.20	28.02
346500	110	112	87.6	71.0	20.36	36.60	28.48
693000	108	116	75.6	58.3	30.0	49.70	39.85
1039501	114	110	60.0	29.5	47.36	73.18	60.27
Control	113	112	112.0	110.0	0.88	1.7	1.29
SEM	2.0	2.3	4.10	4.5	5.3	6.3	5.9
CD (P = 0.05)	NS	NS	12.3	13.4	15.8	18.7	17.8

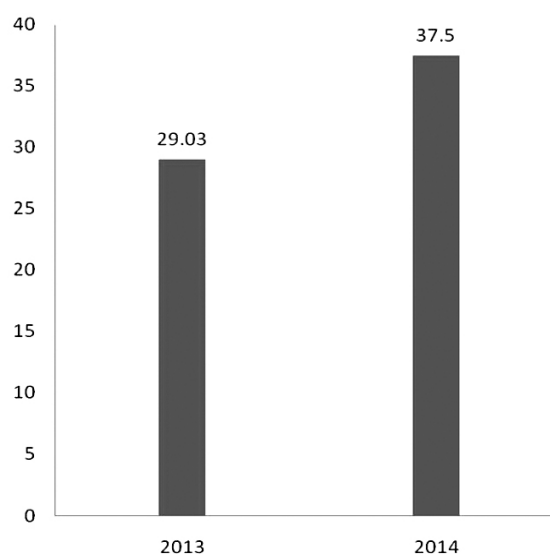
reported that the treatment with *Steinernema carpocapsae* @ 200 IJs/ml against *Holotrichia serrata* recorded 50.70 per cent grub mortality. Rajkumar *et al.*, (2016 & 2019) and Gangadhar *et al.*, (2019) also recorded similar results wherein native EPN was applied to the soil. Application of *Steinernema carpocapsae* @ 1.0×10^7 /palm basin resulted in 53.1% reduction of white grub (*L. burmesterie*) population in white grub infested arecanut gardens and 73.3% mortality of late instar grubs @ 6000IJs/larva in post inoculation were recorded under laboratory condition.

The *S. longicaudum* and *S. glaseri* when applied at $\sim 5.0 \times 10^3$ infective juveniles (IJs) plant⁻¹, *i.e.*, 7.5×10^8 IJ ha⁻¹ exhibited not only control efficacy against *Anomala corpulenta* a major white grub causing damage to peanuts but also reduced root grub population to the tune of 92.7% with 5.6% damaged pods in China (Wenxiu Guo *et al.*, 2020). Application of EPN twice a year based on the biology of the insect targeting both early instars and later instars was effective in suppressing the white grub population.

Field establishment and persistence of EPN

The reduction of grub population and persistence of nematodes is increased with increase in nematode density

per palm and duration of treatment (Fig 1.). At the end of the experiment, maximum persistence of EPN was found in 37.5% of the collected soil samples over first year application 29.03%. Differences in establishment and persistence of *S. carpocapsae* in field can be attributed to nematode density and soil structure and texture. This is in conformity with results of Kung *et al.*, (1990) which reported the persistence *S. carpocapsae*

Percent of soil samples intercepted with EPN**Fig. 1. Percentage of collected soil samples intercepted with EPN from nematode treated plot**

to be 44.9, 38.8, 32.9, and 26.7% in sandy loam, sand, clay loam, and clay type soils respectively. Persistence of *S. carpocapsae* decreased as the proportion of clay increased. Khan and Javed (2018) noticed maximum EPNs (36%) were recovered from sandy loam soil, 77% in pasture and 78% in potatoes of the applied nematodes of *Heterorhabditis bacteriophora* established and persistence month after application and percentage of nematode positive soil samples dropped considerably after tillage (Susuluk and Ehlers, 2007). The longest persistence of *H. bacteriophora* was detected 23 months after release in beans followed by wheat with red clover as cover crop grown in Turkey. Nematodes species and strains differ in their potential to persist and steinernematids usually survive longer than heterorhabditids. Persistence is also influenced by the plant and related pest insect to recycle (Strong, 2002). Our field studies indicated the establishment and persistence of native strains of *S. carpocapsae* (CPCRI – SC1) in soil over a period of two years by applying EPN twice a year under coastal sandy soils of Kasaragod, Kerala.

CONCLUSION

The results of our study indicated that the application of native Kalpa EPN (CPCRI – SC1) liquid formulation was found promising as bio-control agent in bio-suppression of white grubs in coconut ecosystem. Based on the obtained results, it can be concluded that Kalpa EPN liquid formulation of *S. carpocapsae* @ 1039501 IJs/m² in palm basin twice a year is effective in reducing the root grub population and also improve palm health over use. Considering the controversial health issues with insecticide application in Kasaragod, Kerala, EPN might be a promising viable alternative strategy for the control of white grubs.

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