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Management of White grub (*Leucopholis burmesterie*) infesting Arecanut through Entomopathogenic Nematodes under field conditions

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ABSTRACT

The present investigation was carried out to find out the response of combined application of native strains of EPN and imidacloprid insecticide on suppression of root grub *Leucopholis burmesterie* and other symptomatic parameters in root grub affected arecanut palms and yield. The result indicated that significant reduction in grub population as well as increase yield and emergence of healthy roots in treated palms compare to farmer practice. The combined application of EPN, *Steinernema carpocapsae* @ 1.0×10^7 P^{-1} + imidacloprid @ 0.004% (1 ml / 5 litre water/palm) has resulted in an average 53.1% reduction of grub population within two years of treatment then exclusive application of EPN and imidacloprid ($P<0.05$) in root grub infested arecanut gardens at Karnataka. Resulted, native strains *S. carpocapsae* combination with imidacloprid may be used as an effective curative measure for root grub infestation in arecanut. The infectivity of the tested EPNs against root grub is opens up a new hope of utilizing them in insect pest management in arecanut.

Key words: *Areca catechu*, Imidacloprid, *Leucopholis burmesterie*, *Steinernema carpocapsae.*

Arecanut (*Areca catechu* L.) is one of the highly profitable commercial plantation crop in recent year, growing. Many insect pests such as mites, inflorescence caterpillar, spindle bug, scale, pentatomid bug and white grub severely damage the arecanut palm, among them white grub or root grub, *Leucopholis burmesterie* (Melolonthinae : Coleoptera) is the most destructive pest, specially in coastal and ghats regions of Kerala, Maharashtra and Karnataka. In addition to arecanut these grubs also attacks other crops such as, banana and tuber crops in arecanut based cropping system. There are three different species of white grub (*Leucopholis coneophora*, *L. burmesterie* and *L. lepidophora*) occurring in localized tracts of arecanut growing regions of Karnataka. Chavan *et al.* (2018) applied EPM in the rice root aphid and *Heterorhabditis indica* to control aphid confined in the root zone of rice. Since the grubs are subterranean, management becomes difficult. The application of chlorpyrifos/ phorate insecticides is the most popular farmer's practice in arecanut gardens. Currently, imidacloprid is one of the most popular insecticides for white grub control because of its high efficacy, relatively low vertebrate toxicity, low application rates, and long systemic persistence and imidacloprid also know to synergists with EPNs against scarab beetle larvae. The combination of imidacloprid and EPN would

allow curative treatments against white grub larvae easier to detect in arecanut palms basin, treatments could be limited to infested areas only, reducing cost and environmental impact. The objectives of our study were to test the efficacy of EPN, *S. carpocapsae* application in combination with imidacloprid on white grub population in farmer's arecanut gardens.

Materials and Methods

The 2rd instar white grubs were collected from arecanut garden at Koppa in Chikmagalur district, Karnataka. Selected garden for grub collection were not been treated with either insecticides or EPNs during the previous years. Grubs were kept individually at room temperature (22-25ºC) for a weak in a mixture of organic compost and loamy sand with sweet potato (*Ipomoea batatas* L.) provided as food. Only apparently healthy grubs were used in the bioassays. EPN, *Steinernema carpocapsae* (CPCRI-SC1) collected from nematode culture lab, Department of Crop Protection, ICAR-CPCRI, Kasaragod, was used in this study. Prior to the assays, *S. carpocapsae* was cultured on greater wax moth, *Galleria mellonella* (L.) (Kaya & Stock 1997). The insecticide, Imidacloprid (Confidor 17.8 SL, Bayer Crop Science Ltd.) was chosen as the insecticide standard check and tested whether imidacloprid and *S. carpocapsae* are compatible by assessing nematode viability and infectivity after exposure to various concentrations of imidacloprid. Suspension of *S. carpocapsae* (1000 IJs/ml) containing control (only water), 0.48, 0.24, 0.12, 0.06, 0.03 and 0.02% imidacloprid were filled in microtiter plate (4 ml/well), with 5 replicates. EPN mortality was evaluated at 24, 48 and 72 hrs after exposure to different concentrations of imidacloprid solutions. The IJs were separated from imidacloprid by pouring the suspension through a sieve followed by an additional 500 ml of tap water. Immobile IJs were touched with a probe and considered dead if they did not react. The infectivity of *S. carpocapsae* was determined by adding 1 ml of suspension containing 50 IJs and one wax moth larva to Petri dish lined with filter paper. Wax moth larval mortality was observed after 72 hrs. Dead larvae were kept for emergence of nematodes individually on a white trap and counted the number of nematodes IJs produced/larva.

To determine effect of imidacloprid and *S. carpocapsae* against 2nd instars on *L. burmesteri* in plastic pots, filled with a potting mixture @ 1:1 ratio (sterilized soil & sand) with sweet potato as food source. Five 2nd instars grubs were placed in each pot and left for 24 h for incubation. Any grub remaining on the soil surface was considered unhealthy and replaced with other healthy grub. After 24 h inoculated with the following treatments: T_1 - imidacloprid at 0.25, 2.5, 100 and 250 ppm; T_2 - *S. carpocapsae* (1225, 2450 and 3850 IJs/ grub); T_3 - combination of each imidacloprid concentration and *S. carpocapsae* (Table 2) along with the control (water only). Treatments were applied 4 ml of water / container. The pot were kept in BoD at 24±1°C. The mortality of grubs were recorded after 72, 96 and 120 h and replicated 5 times in a CRD. At the time of evaluation, all grub were removed from the pot and mortality of grub was recorded. Dead grubs were kept on white traps to observe IJs emergence from dead grubs.

Another experiment was conducted on 10 year old arecanut palms farmer's garden, at Koppa taluka of Karnataka. This garden severely infected with white grubs. EPN and imidacloprid @ 0.004% in five litre of water. Three drenching were done at one year interval during month of September and October from 2012 to 2014, laid out in a RBD, replicated thrice by keeping six palms / replication along with control. Larval population was recorded by digging the soil at the base of the tree and surrounding interspaces and counting the grubs. Observations were recorded at 60 days after imposition of treatments. Data on the % grub reduction was subjected to correction. The treatment means were compared by using Duncan's Multiple Range Test (DMRT).

Results and Discussion

All the treatments significantly reduced root grub population as compared to control and pre-

Table 1. Effect of imidacloprid on survival of IJs of *Steinernema Carpocapsae*

Imidacloprid (ppm)	% mortality of IJs at different intervals N=1000		
	24 hrs	48 hrs	72 hrs
4800	8.2 (16.2)[a]	13.3 (21.0)[a]	21.5 (27.4)[a]
2400	5.8 (13.6)[a]	12.6 (20.6)[a]	18.6 (25.4)[a]
1200	4.5 (11.8)[ab]	7.9 (16.2)[ab]	15.4 (23.0)[ab]
600	2.4 (6.9) [cb]	5.5 (12.2)[cb]	11.8 (19.9)[cb]
300	0.9 (3.4)[cd]	2.6 (7.1)[c]	9.7 (18.0)[c]
200	0.0 (0.0)[d]	1.5 (5.4)[c]	4.5 (12.2)[d]
Control (ster. water)	0.0	0.0	0.0

[#]Values in the parenthesis are arc sine transformation; Values in each column are the mean number of five replications, Mean followed by different letter indicate significant difference (P=0.05).

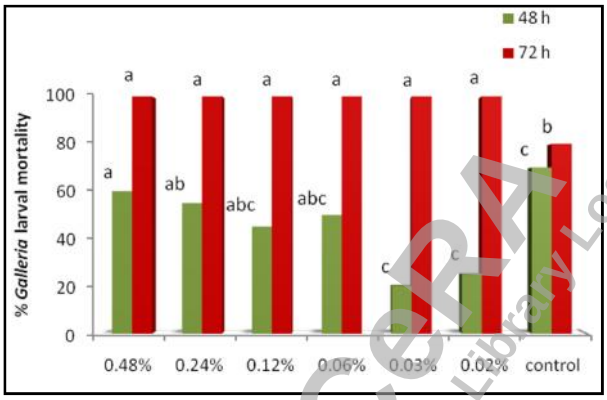

Fig. 1. Effect of imidacloprid on the infectivity of IJs of *S. carpocapsae* against 3rd instar *Galleria mellonella*

treatment population. The present study indicate that root grub can be effectively managed by removing the severely infested palms followed by one time drenching the combination of EPN + imidacloprid during month of September - October. There was significant difference in the mortality of *S. carpocapsae* following direct exposure to the different concentrations of imidacloprid. EPN mortality after 24 hrs (0.9 to 8.2%), 48 hrs (1.5 & 13.3%) and 72 hrs (4.5 to 21.5%) (Table 1). Similarly, there was significant difference in infectivity of imidacloprid treated IJs against *G. mellonella* larvae (Fig. 1). Gupta *et al.* (2011)

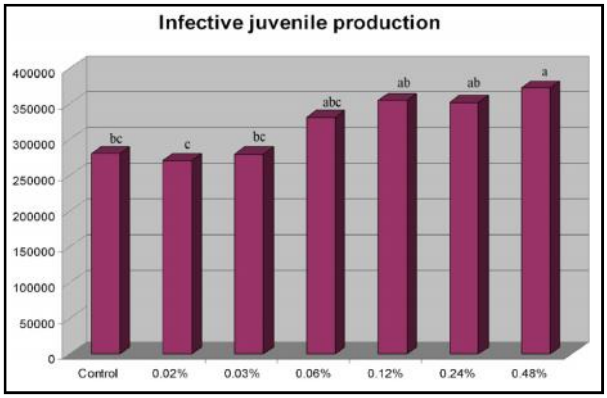

Fig. 2. Effect of imidacloprid on multiplication of IJs

Table 2. Effect of imidacloprid on *Steinernema carpocpasae* against *Leucopholis burmesteri*

Treatment	Combination of EPN and Pesticide (ppm & IJs)	% mortality of *L. burmesterie*		
		72 hrs	96 hrs	120 hrs
S. carpocpasae alone	1225	0.0	0.0	6.0
	2450	0.0	6.0	22.0
	3850	0.0	16.0	38.0
Imidacloprid + *S. carpocapsae*	0.25 +1225	0.0	0.0	14.0
	0.25 + 2450	0.0	3.0	26.0
	0.25 + 3850	0.0	14.0	48.0
	2.5 + 1225	0.0	2.0	22.0
	2.5 + 2450	0.0	18.0	42.0
	2.5 + 3850	24.0	40.0	58.0
	100 + 1225	0.0	16.0	38.0
	100 + 2450	14.0	34.0	50.0
	100 + 3850	20.0	46.0	86.0
	250 + 1225	12.0	22.0	46.0
	250 + 2450	22.0	38.0	68.0
	250 + 3850	48.0	72.0	100
Control	0.0	0.0	0.0	0.0
CD (P=0.05)	2.6	7.7	6.1	

reported endosulfan with EPN was found most effective against *Plutella xylostella* under field condition.

Significantly higher multiplication of IJs on *G. mellonella* compared to control were observed (Fig. 2). This imidacloprid had no negative effects on EPN survival and reproduction in *G. mellonella*. Similarly, Koppenhofer and Fuzy (2008) reported

Table 3. Field efficacy of *Steinernema carpocapsae* in combination of imidacloprid in management of Arecanut root grub, *Leuchopholis burmesterie* at Kopp, Sringeri, Karnataka

Treatments	Per cent reduction of grubs			Corrected % reduction of grubs		
	2012	2013	Mean	2012	2013	Mean
SC alone	24.7	49.8	37.2[b]	20.4	39.6	30.0[b]
SC + 1ml imida.	47.0	59.2	53.1[a]	42.6	50.9	46.7[a]
SC + 2ml imida.	40.1	54.3	47.2[ab]	35.1	44.7	39.9[ab]
1ml imida.	28.9	45.8	37.4[b]	23.0	34.7	28.9[b]
2ml imida.	31.3	55.0	43.1[ab]	25.5	43.6	34.6[ab]
Control	6.2	8.3	11.9[c]	0.0	0.0	0.0[c]
Mean	30.0[b]	45.1[a]	38.3	24.4[b]	35.6[a]	30.0

Mean showing different letter indicates significant difference between the treatments and year by DMRT.

that imidacloprid had no negative effects on nematode reproduction in white grubs and fitness of the emerging progeny. The combination of *S. carpocapsae* with imidacloprid would allow for the use of lower EPN application rates. Similar such results was reported by Seal *et al.* (2010). The results documented the efficacy of *S. carpocapsae* alone, imidacloprid and in combination with *S. carpocapsae* + imidacloprid against 2nd instar of white grub. In the present investigation 2nd instar of *L. burmesteri* mortality was significantly affected (Hussaini *et al.*, 2005). As far as interaction between EPN and imidacloprid is concerned, it was verified that the main reason for successful application of imidacloprid and EPN combination, was due to slow movement of grubs under the influence of imidacloprid, allowing nematodes to easily penetrate the host insect. In the study, the synergism between *S. carpocapsae* and imidacloprid was consistent and obtained good results under field conditions. Similarly, Koppenhofer *et al.* (2000) reported that EPN and imdacloprid combination resulted more successful in the field rather than in laboratory.

When 2nd instar grubs were inoculated with 1225, 2450 and 3850 IJs/grub, the mortality was only 6, 22 and 38%, respectively. But both the grub stages were treated with 2.5 ppm of imidacloprid 58% mortality was noted (Table 2). In all EPN + imidacloprid combinations, mortality was significantly higher than in the *S. carpocapsae* alone. When different concentrations of imidacloprid were applied simultaneously with *S. carpocapsae* against *L. burmesteri*, mortality differed significantly between treatments (Banu *et al.*, 2003). Imidacloprid disrupts the normal defensive and evasive behaviour that white grubs displayed in response to EPN attack and thereby increased the white grubs' nematode-susceptibility (Koppenhofer *et al.*, 2000). However, Koppenhofer & Fuzy (2008) showed that synergistic interactions occurred in *H. bacteriophora* and imidacloprid were applied against younger larvae (i.e., second instar & early third instar), and that similar control levels could be achieved with reduced EPN and imidacloprid doses.

The results over two years were almost consistent. The mean pooled data reveals that, infective juveniles (150 ml) of *S. carpocapsae* in combination of imidacloprid (1 ml/palm) was highly effective treatment with 53.1% reduction of grubs and was significantly superior, followed by 2 ml imidacloprid in combination of EPN with 47.2% reduction of grubs (Table 3). Hussaini *et al.* (2005) also reported somewhat similar results while controlling *Plutella xylostella*.

The combinations treatment suggest that EPN and imidacloprid had strong combined effect on reduction of white grub population and may provide a powerful and economically feasible approach for white grub control.

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