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Synergism of Entomopathogenic Nematode and Imidacloprid: A Curative Tool to Coconut white Grub, *Leucopholis coniophora* (Coloeptera: Melolonthinae)

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Abstract

Insecticide imidacloprid and entomopathogenic nematode (EPN), *Heterorhabditis indica* alone and in combinations of imidacloprid and EPN were evaluated against early and late 3rd instars of coconut white grub, *Leucopholis coniophora.* Field collected 3rd instars white grubs were placed in pots with sweet potato treated with imidacloprid, the EPN, or both. In all nematode-imidacloprid combinations, both early and late 3rd instars grub mortality was significantly higher than in the *H. indica* alone and imidacloprid alone treatments. Combinations of imidacloprid and nematodes had a strong synergistic effect on mortality at different concentrations of imidacloprid. Nematodes were admixed with various concentrations of imidacloprid ranging from 0.04 to 1.25% could not significantly affect the survival of infective juveniles resulting in only a negligible mortality (3.5%). Results revealed that different imidacloprid concentrations, exposure time and interaction between imidacloprid and exposure time not affected nematodes mortality in bioassay. Combinations of imidacloprid and entomopathogenic nematodes may provide a powerful and economically feasible curative control in white grub management in coconut.

Keywords: Coconut, white grub, entomopathogenic nematodes, *Heterorhabditis indica*, *Leucopholis coniophora*, imidacloprid, early and late instars.

Introduction

Coconut white grub, *Leucopholis coniophora* (Burm.) (Melolonthinae: Coleoptera) has become a menace to coconut and various rhizomatous intercrops grown in palm garden. White grubs or root grubs are pests of coconut in some localised tracts of Kerala and Karnataka. The grubs are brown headed, soft, white bodied ones inhabitating the soil. Adult cockchafers emerge during May-June on receipt of pre-monsoon showers. Beetles lay eggs in soil at different depths. Eggs hatch in about three weeks. The newly emerged grubs feed on the roots of small plants and after a few days they feed on the apical soft region of the coconut roots. Infestation affects the process of absorption of nutrients adversely. Infested palms show yellowing of leaves and immature nut fall. Infestation in a garden continuously for a few years results in stunting of growth of young palms. In nurseries, they cause drying up of leaves and gradual death of seedlings. The life cycle is completed in about an year. Grubs are seen in soil at different depths depending on the soil moisture/water table of the garden and availability of food.

Since the grubs are subterranean, their management has always been problematical. Due to the difficulty in predicting damage by grubs. Several practices to control coconut white grubs have already been utilized and the application of insecticides is the most popular farmer's practice. Unfortunately, the use of them has negative impact both on the environment and on human health. Since the use of pesticide could become a serious problem with concerns of environmental safety. Several insecticides are recommended for the control of white grubs, but because no insecticide is effective unless used at a very high dose, their application is not a sustainable strategy. Several nonchemical control alternatives exists but all of the have limitations. EPNs do not always provide consistent white grub control (Georgis and Gaugler 1991, Klein 1993). Because these limitations, chemical insecticides are still first choice of coconut growers for white grub control. Currently, imidacloprid is one of the most popular insecticides for preventative white grub control because of its high efficacy, relatively low vertebrate toxicity, low application rates, and long systemic persistence (Schroeder & Flattum 1984, Elbert *et al.* 1991). Because its

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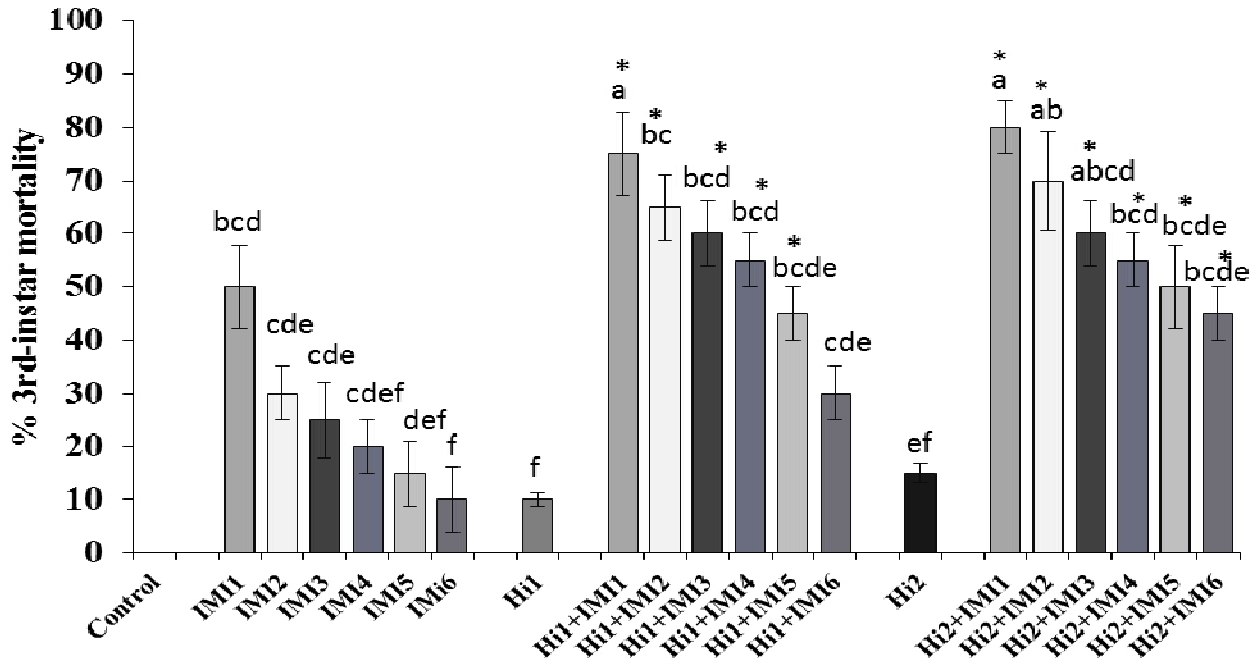
Fig 1. The effect of treatment with imidacloprid concentrations (IMI 1-6), two application rates (Hi1 = 1500 and Hi2 = 3000 infective juveniles/grub) each concentration of the entomopathogenic nematode *Heterorhabditis indica*, or the combination of each concentration of nematode treatment with each concentration of imidacloprid on the mortality (mean of 5 replicates ± SE) of early third instar *Leucopholis coniophora* in pots with potato. Means of columns with the same letter are not significantly different ($P < 0.05$) using Tukey'test. An asterisk indicates significant synergistic interactions between nematode and imidacloprid. An '*' indicates significant synergistic interactions between nematode and the imidacloprid insecticide.



efficacy declines with advancing white grub development (Potter 1998), imidacloprid is applied in a preventative approach, the optimum period for application being during the month preceding egg hatch until the time when grubs are beginning to hatch (Potter 1998). However, white grub outbreaks are difficult to predict because they tend to be localized and sporadic and the eggs and first instars are difficult to sample for. The combination of imidacloprid and nematodes would allow curative treatments against older white grub stages, and because these stages are easier to detect, treatments could be limited to infested areas only, reducing cost and environmental impact.

Nematode combinations with low-environmental impact insecticides such as imidacloprid may offer an efficient alternative that is highly preferable over the use of conventional soil insecticides or widespread applications of imidacloprid. Recently research has shown that imidacloprid enhances the pathogenicity of the *Metarhizium anisopliae* (Paula *et al.* 2011), *Bacillus thuringiensis* (Salem *et al.* 2007), spinosad enhances the pathogenicity of the *Metarhizium anisopliae* (Sharififard *et al.* 2011) and imidacloprid also know to synergists with EPNs against scarab beetle larvae (Koppenhofer and Kaya 1998; Koppenhofer *et al.* 2003). Our hypothesis was that imidacloprid act as stressor on 3rd-instar white grubs and increases their susceptibility to EPNs. A stressor is defined as any stimulus that disrupts the normal homeostasis of an organism (Steinhaus and Martignoni 1970). Specifically, the odjectives of our study were to test the compatibility of imidacloprid and nematodes and to determine the effect of combined applications of imidacloprid and nematodes on early and late 3rd -instar *L. coniophora*.

Materials and Methods

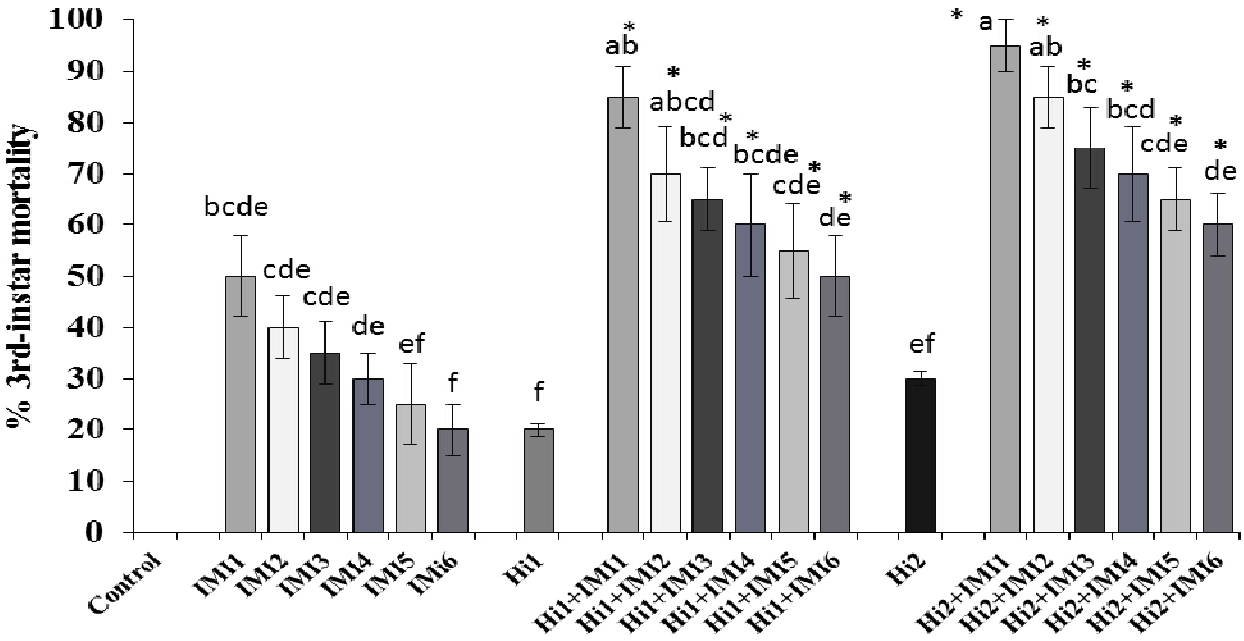
Insects

The field collected early and late 3rd instar coconut white grubs were collected from coconut garden at the Central Plantation Crops Research Institute (CPCRI) farm in Kasaragod and College of Agriculture, farm in Nileshwar, Kerala, India. None of the sites had been treated with either insecticides or nematodes during the previous year. Grubs were kept individually at room temperature (22-25°C) for 1wk 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.

Nematodes

The entomopathogenic nematode, *Heterorhabditis indica* from the live nematode culture of the Department of Crop Protection, CPCRI, Kasaragod, India, was used in this study.

Fig 2. The effect of treatment with imidacloprid concentrations (IMI 1-6), two application rates (Hi1 = 1500 and Hi2 = 3000 infective juveniles/grub) each concentration of the entomopathogenic nematode *Heterorhabditis indica*, or the combination of each concentration of nematode treatment with each concentration of imidacloprid on the mortality (mean of 5 replicates ± SE) of late third instar *Leucopholis coniophora* in pots with potato. Means of columns with the same letter are not significantly different ($P < 0.05$) using Tukey'test. An asterisk indicates significant synergistic interactions between nematode and imidacloprid. An '*' indicates significant synergistic interactions between nematode and the imidacloprid insecticide.



Prior to the assays, *H. indica* was cultured on last instars of the greater wax moth, *Galleria mellonella* (L.) (Lepidoptera: Pyralidae). Prior to the assays, nematodes were reared (Kaya and Stock 1997). After harvesting infective juveniles (IJs) from *G. mellonella*, the nematodes were stored in tap water at 13°C for up to 1 week prior to the assays. Nematode viability was 100%. Unless otherwise stated. A serial dilution of the nematode stock solution was conducted to achieve the necessary concentration of nematodes.

Insecticide

The insecticide, Imidacloprid (Confidor 17.8 SL, Bayer Crop Science Ltd.) was chosen as the insecticide standard because it is alternative use to choloropyriphos insecticide for management of coconut white grub.

Compatibility of imidacloprid and nematodes

Compatibility of imidacloprid and *H. indica* was tested by assessing nematode viability and infectivity after exposure to various concentrations of imidacloprid. Suspension of *H. indica* (500 IJs/ml) containing 0, 0.04, 0.08, 0.16, 0.31, 0.63 or 1.25% imidacloprid were filled in 50-ml Earlinemeyer flasks (20 ml per flask) and agitated on a shaker at 65 rpm for 24 or 48 h. There were 5 replicates per treatment. Nematode mortality was evaluated 24 and 48 h after their exposure to various 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. The IJs were washed from the sieve into clean flask and resuspended in 10 ml of sterilized water. To assess the nematode mortality after 24 h or 48 h, 200 µl samples were taken from each treatment and placed in a 100-mm Petri dish lid to count the number of living and dead nematodes by using dissecting microscope. Immobile IJs were touched with a probe and considered dead if they did not react. The IJs were washed 3 times in sterilized, distilled water. The infectivity of *H. indica* was determined by adding 1 ml of suspension containing 30 IJs and one wax moth larva to a 100-mm Petri dish lined with Whatman tissue paper. There were 5 Petri dishes per treatment. Wax moth larval mortality was observed after 4 days. Dead larvae were dissected individually in a Petri dish and digested in a pepsin solution (Mauleon *et al.* 1993) to count the number of nematodes that had penetrated the wax moth larva.

Efficacy of imdiacloprid and EPN mixture to control coconut white grub

To determine effect of imidacloprid and on *H. indica* against early and late 3rd instars of *L. coniophora*, a laboratory experiment was con-

Table 1. Survival and infectivity of *Heterorhabditis indica* infective juveniles for agitated 24 and 48h in imidacloprid solution.

Imidacloprid (%)	**Survival (%)[a]**		**Infectivity[b]**	
	24h	**48h**	**24h**	**48h**
0.0	98.0 ± 0.4	97.8 ± 0.5	9.8 ± 0.5	9.5 ± 0.6
0.04	97.8 ± 0.3	97.5 ± 0.3	9.5 ± 0.6	9.8 ± 0.6
0.08	97.5 ± 0.3	97.3 ± 0.5	9.8 ± 0.5	9.8 ± 0.5
0.16	78.3 ± 0.5	97.0 ± 0.4	9.8 ± 0.9	9.5 ± 0.6
0.31	97.0 ± 0.4	97.0 ± 0.4	10.0 ± 0.4	9.8 ± 0.9
0.63	96.8 ± 0.8	96.8 ± 0.9	9.5 ± 0.6	9.5 ± 1.0
1.25	96.8 ± 0.5	96.5 ± 0.6	9.8 ± 0.5	10.0 ± 0.9
***P* value**				
Imidacloprid (I)	0.3041		0.9967	
Time (T)	0.7368		0.8851	
IxT	0.9968		0.9993	

[a] Survival was assessed in subsamples of approximately 100 infective juveniles.
[b] Mean number of nematodes recovered from a wax moth larva after 3 d of exposure to 30 infective juveniles

ducted. Half liter plastic pots (14 cm diameter by 11 cm height) filled with a soil and sand mixture (1:1) with sweet potato as food source. Four early and late 3rd instars grubs were placed in each pot separately 1 d before the start of an experiment. Any grub remaining on the soil surface was considered unhealthy and replaced with other grub. After 1 d pots were inoculated with the following treatments: 1. imidacloprid at different concentrations (0.04, 0.08, 0.16, 0.31, 0.63 or 1.25%); 2. *H. indica* (1500 or 3000 IJs/grub); 3. a combination of each imidacloprid concentration and *H. indica*; 4. control (water only). Treatment were applied 50 ml of water per pot followed by 30 ml of water to rinse the treatments into soil. The pots were kept in an incubator at 24 ± 1° C in the dark. The pots were destructively sampled 7 d after treatments. Each treatment was replicated five times in a completely random design. At the time of evaluation, all larvae were removed from the containers and mortality was recorded. Cadavers were examined for signs of nematode infection (i.e., coloration) (Woodring and Kaya 1988). Dead grubs were kept on White traps to observe nematode emergence from nematode-killed insects.

Statistical analysis

To normalize data before analysis, percentage of IJs survival and number of IJs penetrating into wax moth larvae were arcsin-square root-transformed. Analysis was undertaken on the transformed data and back transformed data only is presented. Nematode inoculation rates, imidacloprid concentration and time their interactive effects on nematode survival and early and late 3rd instar grubs mortality were subjected to

Table 2. Interaction between insecticide and entomopathogenic nematode, *Heterorhabditis indica* over mortality of *Leucopholis coniophora* seven days after treatment in laboratory.

	Early 3rd instar					**Late 3rd instar**				
Treatments	**M_N[a]**	**M_I[b]**	**M_{IN}[c]**	**% M_E[d]**	**I[e]**	**M_N**	**M_I**	**M_{In}**	**% M_E**	**I**
0.04 + Hi1	10.0	10.0	30.0	19.0	+	20.0	20.0	50.0	36.0	+
0.08 + Hi1	10.0	15.0	45.0	23.5	+	20.0	25.0	55.0	40.0	+
0.16 + Hi1	10.0	20.0	55.0	28.0	+	20.0	30.0	60.0	44.0	+
0.31 + Hi1	10.0	25.0	60.0	32.5	+	20.0	35.0	65.0	48.0	+
0.63 + Hi1	10.0	30.0	65.0	37.0	+	20.0	40.0	70.0	52.0	+
1.25 + Hi1	10.0	50.0	75.0	55.0	+	20.0	50.0	85.0	60.0	+

a Observed proportional percentage mortality caused by nematodes alone.
b Observed proportional percentage mortality caused by imidacloprid alone.
c expected additive proportional mortality for the nematode–imidacloprid combinations.
d expected additive proportional mortality for the nematode–imidacloprid combinations.
e Interaction between treatments : antagonist ($X^2 > 3.84$ & M_{IN}-M_E = a negative value), additive ($X^2 < 3.84$), synergistic ($X^2 > 3.84$ & M_{IN}-M_E = a positive value), being 3,84 correspondent to1liberty degree at $P < 0.05$.
'+' indicates synergistic interactions between nematode and the imidacloprid insecticide.
Hi1 = 1500 infective juveniles/grub.

Table 3. Interaction between insecticide and entomopathogenic nematode, *Heterorhabditis indica* over mortality of *Leucopholis coniophora* seven days after treatment in laboratory.

	Early 3rd instar					Late 3rd instar				
Treatments	**M_N[a]**	**M_I[b]**	**M_{IN}[c]**	**% M_E[d]**	**I[e]**	**M_N**	**M_I**	**M_{IN}**	**% M_E**	**I**
0.04 + Hi2	15.0	10.0	45.0	19.0	+	30.0	20.0	60.0	36.0	+
0.08 + Hi2	15.0	15.0	50.0	23.5	+	30.0	25.0	65.0	40.0	+
0.16 + Hi2	15.0	20.0	55.0	28.0	+	30.0	30.0	70.0	44.0	+
0.31 + Hi2	15.0	25.0	60.0	32.5	+	30.0	35.0	75.0	48.0	+
0.63 + Hi2	15.0	30.0	70.0	37.0	+	30.0	40.0	85.0	52.0	+
1.25 + Hi2	15.0	50.0	80.0	55.0	+	30.0	50.0	95.0	60.0	+

a Observed proportional percentage mortality caused by nematodes alone.
b Observed proportional percentage mortality caused by imidacloprid alone.
c expected additive proportional mortality for the nematode–imidacloprid combinations.
d expected additive proportional mortality for the nematode–imidacloprid combinations.
e Interaction between treatments : antagonist (X^2 > 3.84 & M_{IN}-M_E = a negative value), additive (X^2 < 3.84), synergistic (X^2 > 3.84 & M_{IN}-M_E = a positive value), being 3,84 correspondent to1liberty degree at P < 0.05.
'+' indicates synergistic interactions between nematode and the imidacloprid insecticide.
Hi2 = 3000 infective juveniles/grub.

analysis of variance (ANOVA). When ANOVA was significant, comparisons of relevant means were made using the Turkey's significance test values at the 5% level of significance. Synergistic, additive, or antagonistic interactions between agents in the combination treatments were determined using a X^2 test (Finney 1964, McVay *et al.* 1977, Koppenhofer *et al.* 1998). Grub mortality was calculated by subtracting the number of surviving grubs from the number of grubs released for each replicate. The expected additive proportional mortality M_E for the nematode-imidacloprid combinations was calculated by $M_E = M_N + M_I (1 - M_N)$, where M_N and M_I are the observed proportional mortalities caused by nematodes and imidacloprid alone, respectively. Results from a X^2 test, $X^2 = (M_{IN} - M_E)^2/M_E$, where M_{IN} is the observed mortality for the nematode-imidacloprid combinations, were compared to the X^2 table value for 1 df. If the calculated X^2 value exceeded the table value, a non-additive effect between the two agents was suspected (Finney 1964). If the difference $M_{NC} - M_E$ had a positive value, a significant interaction was considered synergistic. If the difference had a negative value, a significant interaction was considered antagonistic. Differences among means in all experiments were considered significant at $P < 0.05$. Means ± SE are presented. This methodology was followed many researchers (Koppenhofer *et al.* 1998, 2000) All statistical evaluations were performed using PROC. GLM (SAS software, version 9.3, SAS institute).

Results and Discussion

Compatibility of imidacloprid and nematodes

There was no significant (F= 1.24; df = 42, 6; P = 0.3041) difference in the moratlity of *H. indica* following direct exposure to the different concentrations of imidacloprid. Nematode mortality after 24h of agitation in imidacloprid solutions ranged between 2.2 and 3.2% and for 48h ranged between 2.5 and 3.5%, and was not significantly affected by imidacloprid (Table 1). Similarly, there was no significant (F= 0.11; df = 42, 6; P = 0.7368) difference between the exposure times. At the time of dissection, there was also no significant (F= 0.09; df = 42, 6; P = 0.9967) differences in the development of the nematodes, most nematodes were in the adult stage. Similarly, there was no significant (F= 0.02; df = 42, 6; P = 0.8851) difference between the exposure times. This data show that imidacloprid has no negative effects on nematode survival and reproduction in white grubs. Similarly, Koppenhofer and Fuzy (2008) also reported that imidacloprid has no negative effects on nematode reproduction in white grubs and fitness of the emerging progeny.

Infectivity, that is, capacity of *H. indica* to cause *Galleria mellonella* larval death was not statistically different after being exposed to the insecticides data not shown. The analysis of variance revealed there was no significant two-way interaction between imidacloprid concentrations and exposure time. (Table 1). Presently, *H. indica* appears to be the nematode of choice for combinations with imidacloprid for curative treatments because of the limited availability of *H. indica.* However, the stronger interaction of *H. indica* with imidacloprid would allow for the use of lower nematode application rates and may make this nematode more attractive for commercial production.

Efficacy of imdiacloprid and EPN mixture to control coconut white grub

The results documented the efficacy of imidacloprid, EPN, *H. indica* alone and in combination with imidacloprid + *H. indica* against early and late 3rd instar of white grub, *L. coniophora*. In present investigation early and late 3rd instar *L. conoiphora* mortality was significantly affected by imidacloprid rate (F= 6.75; df = 46, 8; P = 0.0001) and *H. indica* (F= 8.42; df = 46, 2; P = 0.0008), but there was no interaction between imidacloprid rate and *H. indica.* As far as interaction between imidacloprid and EPN is concerned, it was verified that the main reason for successful application of imidacloprid and nematode synergism is the slow movement of grubs under the influence of imidacloprid, allowing nematodes to easily penetrate the host insect (Koppenhofer *et al.* 2000). In our study the synergism between imidacloprid and *H. indica* was consistent, we may accept good results even in field conditions. Similarly, (Koppenhofer *et al.* 2000) reported that imdacloprid and EPN combination results were more successful in the field rather than in laboratory.

When both early and late 3rd instar grubs were inoculated with 2000 and 3000 IJs/grub, we could only 10, 20, 15 and 30% mortality respectively. But both the grub stages were treated with 1.25% of imidacloprid we found 50% mortality (Fig 1 & 2). In all nematode-imidacloprid combinations, mortality was significantly higher than in the *H. indica* alone and imidacloprid alone treatments. When different concentrations of imidacloprid were applied simultaneously with *H. indica* against *L. coniophora*, mortality differed significantly between treatments (F=12.06; df =20, 84; P =0.0001). We did not study the mechanism responsible for the interaction between imidacloprid and *H. indica*. Imidacloprid disrupts the normal defensive and evasive behaviors that white grubs display in response to EPN attack and thereby increases the white grubs' nematode -susceptibility (Koppenhofer *et al.,* 2000). Combinations of *H. indica* and imidacloprid had a strong synergistic effect on early and late 3rd instar mortality (Table 2 & 3). However, Koppenhofer and Fuzy (2008) showed that synergistic interactions also occur when *H. bacteriophora* and imidacloprid are applied against younger larvae (i.e., second instar and early third instar), and that similar control levels could be achieved with reduced nematode and imidacloprid rates.

To achieve sustainable coconut cropping systems with minimal reliance on chemical pesticides, imidacloprid–nematode combinations could be tested in noncurative control approaches. For example, imidacloprid could improve nematode establishment after augmentative releases. Imidacloprid could also be used to trigger epizootics of endemic nematode populations that are common in white grub populations (Campbell *et al.* 1998). Finally, imidacloprid–nematode combinations may also be applicable to other cropping systems and insect pests. In this study we have not worked out the economics of the examined combinations, and field experiments are necessary to give a better idea about the economics.

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