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Synergism of Imidacloprid and Entomopathogenic Nematodes for the Control of Eastern Subterranean Termite, *Reticulitermes flavipes* (Isoptera: Rhinotermitidae)

Farkhanda Manzoor
Department of Zoology, Lahore College for Women University, Lahore

Abstract.- In laboratory study, the entomopathogenic nematodes, *Steinernema carpocapsae* (Sc) and *Heterorhabditis bacteriophora* (Hb) alone and in combination with the insecticide, imidacloprid were evaluated against workers and nymphs of *Reticulitermes flavipes* (Isoptera: Rhinotermitidae)**.** The degree of interaction varied between the two nematode species. Results revealed that different concentrations, nematode species, exposure time and interaction between entomogenous nematodes and insecticides affected termite mortality in bioassay. It was found that nematodes species Hb and Sc alone had no significant effect on termite mortality but there was synergism between imidacloprid and nematodes species that caused more than 50% mortality in most treatments within all three colonies tested. Further study is warranted on this aspect of termite research so that this synergism between nematodes and insecticides can be applied in the field to control termite colonies and as a substitute for chemical control.

Key words: *Reticulitermes flavipes*, Isoptera, Rhinotermitidae, entomogenous nematodes, biological control, synergism, imidacloprid

INTRODUCTION

Due to recent advances in integrated pest management practices, the use of biological control agents is increasing tremendously throughout the world. Although chemical insecticides are a top priority to control termites and other insect pests, but other alternatives such as entomopathogenic nematodes (Grace, 1997) are also being used. Entomopathogenic nematodes can be referred to as insecticidal nematodes. There are about almost 40 families of nematodes that are associated with insects but two families, Steinernematidae and Heterorhabditidae, contain the most important species of entomopathogenic nematodes used as biological control agents (Gaugler and Kaya, 1990). Compared to insecticides, they are easy to apply, find host actively or passively and are compatible with many pesticides (Smart, 1995). *Reticulitermes flavipes* is a subterranean termite that is widely distributed throughout the U.S.A. It is considered to be a serious pest destroying the wood and causing damage loss to homeowners (Su and Scheffrahn, 1998).

Corresponding author: Doc_farkhanda@yahoo.com
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In this project, the termite species *R. flavipes* was used as the target pest. Experiments were conducted to determine the efficacy of two nematode species, *Steinernema carpocapsae* (*Sc*) and *Heterorhabditis bacteriophora* (Hb), against workers and nymphs of *R. flavipes*. In nature, entomopathogenic nematodes and termites live together in a moist, cool habitat so many experiments were conducted by various laboratories to test the efficacy of different nematode species against different termite species. An attempt to control the Formosan subterranean termite, *Coptotermes formosanus*, with nematodes has already demonstrated the effectiveness of *S. feltiae* nematodes against large field colonies. It was observed that when termites were trapped from field colonies, infected with nematodes, and then returned back to colony, the population of termites was not eliminated. It was therefore concluded there should be direct physical contact between termite and nematode species (Reese, 1971).

Certain similar laboratory experiments have however, contradicted these previous results using the nematode *Neoaplectana carpocapsae* against *Coptotermes, Nasutitermes* and *Termes* which resulted in killing the tested termite species (Poinar, 1979). Recording the infectivity of nematodes against termites in laboratory experiments, 96-98% mortality (n=10) of *Zootermopsis* and

Reticulitermes with 2000 infective stage *N. carpocapsa*e (Breton strain) as well as *Heterorhabditis heliothidis*, respectively, was observed after three days treatment in standard Petri dishes (Georgis and Poinar, 1982). In addition, the live wood tea termite, *Glyptotermes dilatatus* was successfully controlled with *Heterorhabditis* sp. with a dose of 4000 and 8000 ml nematode suspension in doses of 40 and 30 ml per tea bush, respectively. These experiments were performed with the hypodermic needle instead of nozzle for injecting nematodes into galleries inside the exposed tea bush stems (Danthanarayana and Vitarana, 1987). The efficacy of *Neoaplectana carpocapsae* against foraging workers of *Reticulitermes tibialis* was studied in pasture land. The application rate of nematodes was $1x10^7$ per m^2 directly beneath baited traps to the soil. An LD_{50} value for specific nematode-termite combinations was calculated and it was concluded that the entire colony of termites should be treated rather than feeding sites. These results indicated that soil treated under wooden baits reduced termites significantly compared to untreated (controls) within 2-3 weeks (Epsky and Capinera, 1988). However, two strains (Breton and All) of *S. carpocapsae, S. feltiae* and *H. bacteriophora* (=heliothidis) when tested in the laboratory as well as in the field against *Reticulitermes flavipes*, the results showed no significant difference in termite survival rates in the nematode treatments and in the untreated control. In field studies instead of the Breton strain, the Mexican strain of *S. carpocapsae* was used (Mauldin and Beal, 1989). Later on, a new entomopathogenic nematode (EPN) *Neosteinernema longicurvicauda,* was isolated in a laboratory study on naturally infected subterranean termites, *Reticulitermes flavipes.* Adult nematodes were found outside the termite cadaver (Nguyen and Smart, 1994). In a comparative study, four nematode species, *S. carpocapsae* (Breton), *S. riobrave* (TX), *H. bacteriophora* (HP88) and *H. indica* (Coimbatore) were tested against two termite species, *R. flavipes* and *C. formosanus* in the laboratory. Four hundred nematodes/termite were applied and were found that all nematode species were effective against *C. formosanus* while with *R. flavipes*, the results were not successful even at a rate of 2000 nematodes/termite. The highest infectivity was recorded for *H. indica* and *H. bacteriophora* in both termite species (Wang *et al.*, 2002).

Weeks and Baker (2004) evaluated the differences in survivability, detectability and ability of *S. carpocapsae* and *H. bacteriophora,* to kill a subterranean termite *Heterotermes aureus* (Weeks and Baker, 2004). A susceptibility and behavioral response study of the damp wood termite *Zootermopsis angusticollis* to the entomopathogenic nematodes *S. carpocapsae*, indicated that the efficacy of nematodes can be increased if they are applied in combination with some insecticides (Rich *et al.*, 2006). Previously, the synergism test of imidacloprid, a slow acting insecticide, and three entomopathogenic nematodes against mortality of third instar white grubs was carried out in the laboratory. It was accomplished that in such a synergistic interaction, the insecticide usually disrupts nerve function of the grubs, which become sluggish and infective juveniles nematodes can easily infest them. Further, it was also noticed that the degree of interaction in synergism also varies with different species of nematode (Koppenhofer and Kaya, 2000). Various researchers have studied the efficacy of imidacloprid and nematodes separately on different species of termites but not in combination. So in this study for the first time, the two factors, *i.e.,* an insecticide, imidacloprid, and a biological factor, nematodes were used in combination.

MATERIALS AND METHODS

Live nematode cultures of *Steinernema carpocapsae* Breton strain and *Heterorhabditis bacteriophora* HP88 strain were used for this experiment. The method used to rear nematodes was the same as that developed by Dutky and Hough (1955).

Insecticide

Imidacloprid,1-{(6-chloro–3pyridinyl)-methyl}, N-nitro-2-Imidazolidinimine (Miles Inc., Kansas City, MO, USA) was obtained as a wet table powder with 75% active ingredient (AI) (Merit 75 WP) and 25% inert ingredients.

For each colony,180 plastic containers, (90 for 2-day breakdown and 90 for 4-day breakdown with one piece of What man No.1 filter paper were prepared, 20 g sand and 5ml water were added to each container and mixed well using a metal spatula. Using a vacuum aspirator, 40 termites (workers and occasional nymphs) were counted and added into each container. So for colony 1 a total of 7200 termites were used in the experiment. The same process was repeated for colony 2 and colony 3. Termites were counted 1-2 days before treatment, any sluggish and unhealthy termites were removed and replaced with healthy termites one day before treatment. The termites were allowed to forage into the sand for one day before the treatment was applied. The experimental unit code was assigned to each container randomly. These 180 containers were randomly assigned to 36 treatment conditions (18 treatments per 90 containers) composed of all possible combinations of the following:

Five concentrations of imidacloprid Sc: *Heterorhabditid* Hb, Control (No nematodes), two species (0.001%, 0.005%, 0.01%, 0.05%, 0.1%) of nematodes, five concentrations of imidacloprid + Sc (0.001%, 0.005%, 0.01%, 0.05%, 0.1%), five concentrations of imidacloprid + Hb (0.001%, 0.005%, 0.01%, 0.05%, 0.1% (one control.

Each of these treatment conditions were applied to five containers as there were five replicates of each treatment. The experiments were conducted at room temperature (20-24˚C). As the termite could feed on the cellulose pad at the bottom of the container, their feeding behavior was also noted. The insecticidal concentrations were prepared by following procedure:

An amount of 0.544 mg of the powdered imidacloprid was dissolved in 200 ml distilled water to prepare a stock solution of 0.2% concentration. Further concentrations were prepared from the stock solution. As for experiment, 0.001%, 0.005%, 0.01%, 0.05% and 0.1% concentrations were required and for each concentration treatment, there were 30 containers, so 5 treatments x 30 container =150 treatments

Hb treatment

As mentioned earlier, the nematodes were counted and there were also 10 containers used for treatment with Hb (5 for 2 day and 5 for 4 day breakdown). According to project design, 500 nematodes were required for each container and there were 40 termites in each container, so this is equivalent to12.5 nematodes per termite. For this treatment, 10 containers were used. 1ml nematode suspension was added into each container the suspension was prepared by the following procedure: As for Hb, 10 µl suspensions contain 34 nematodes and 143 µl would have 500 nematodes.

Imidacloprid Hb treatment

For this treatment, a new suspension was prepared as per required concentration. 500 nematodes were counted in 143 µl suspension and diluted to a final volume to 500 µl using distilled water for the control treatment; only 1 ml water was added to each container. All of these treatments were kept at room temperature and observations were made after 2 and 4 days.

Sc treatment

As mentioned for Hb treatment, 500 nematodes per container were counted and used for 10 containers (5 for 2 day and 5 for 4 day breakdown) with 40 termites, so as 12.5 nematodes per termite were used. 1ml nematode suspension was added into each container. For the control treatment, only 1 ml water was added to each container. All of these treatments were kept at room temperature and observations were made after 2 and 4 days.

Data collection

There were two periods of data collection for each colony, 2 days after treatment and 4 days after treatment. The termites were assessed to determine the impact of the treatments after the designated time. Each of the termites that could be located was classified into one of the following categories: OK (healthy, no visible signs of treatment impact): Sluggish (slow in movements): Ataxic (movement without coordination, falling over and righting itself slowly): Moribund (lying on back or side with slight movement of appendages), Dead but not infected by nematodes, and Dead infected by nematodes based on color of the termite (red= Hb; yellow=Sc).

To observe the emergence of infected

juveniles (IJs), IJs were extracted using the White trap method (White, 1927). Dead termites from each container were taken out and put on a piece of filter paper into Petri dish. Using pipette, distilled water was added and observations were taken for 1-14 days. From white traps, 1 ml suspension was taken into separate Petri dish, this suspension was observed under microscope to note after how many days IJs emerged from dead termites. It was observed that IJs normally emerged 2-4 days after infection and were very active. After 7 days their number increased and they were actively moving and settled at bottom of Petri dish, but after 14 days they became sluggish and eventually died. Hb was smaller and more active than Sc IJs.

Database design

Each of the above mentioned treatments was given once to each colony. The database was designed on a factorial analysis with three factors i.e. composed of 3 colonies, 2 and 4 days treatments and 5 different concentrations. Each combination of these treatments was replicated five times. The results of the experiments were recorded in a series of excel database sheets with the observations for each of the workers recorded as OK, Sluggish, Ataxic, Moribund, dead not infected by nematodes and dead infected by nematodes. However, further attention was given only to mortality data, and mortality data (proportional) was normalized by transforming into arcsine of the square root prior to statistical analysis and data from each colony. Each treatment was analyzed for significance by using two way analysis of variance ANOVA factorial analysis with three factors (SPS,13.0) program, in which dependent variable was number of dead termites and fixed factors were different treatments, concentrations colonies, days and replicates. The graphic presentation of the data was done using Graph pad prism 4. Differences among means in all experiments were considered significant at $P<0.001$ and non-significant at $P>0.001$.

RESULTS

The results documented the efficacy of imidacloprid, entomopathogenic nematodes, Sc, Hb alone and in combination with imidacloprid entomogenous nematodes + Sc and imidacloprid + Hb, against the termite, *R. flavipes*. The percentage mortalities (arcsine transformed) of the termite workers and nymphs are shown in table 1, which clearly indicates differences after 2 and 4 days exposure to insecticide, Hb, Sc alone and synergism for colony 1, 2 and 3. The mortality percentage of control was also noted and compared with nematode species. We found that different concentrations, nematode species, exposure time and synergism between insecticides and nematode species all had influence on termite worker mortality.

Infectivity of R. flavipes *with nematodes*

When the effect of treatment for the two nematode species was compared with mortality of control treatment, it was seen that control for colony 3 after 4 days treatment had higher percentage of mortality (22.78 ±4.88) than Hb and Sc, and when the effects of Hb and Sc in all the three colonies were compared, colony 3 for 4 day treatment had highest mean percentage mortality for Sc (16.39 ± 6.61) and Hb for colony 1 for 4 days had highest mean percentage mortality (13.90± 5.08).

The effects of the two nematode species were similar for the different colonies of *R. flavipes* and no one had significant effect on termite mortality at 2 or 4 day treatments for colony 1, 2 and 3. Both nematode species had significantly lower mortality than control. However, better results were obtained when nematodes were used in combination with different concentrations of imidacloprid, and even more than 50% mortality was recorded at the different doses (Table I).

Imidacloprid + Sc treatment

When the results from three different colonies were compared for the effects of imidacloprid in combination of Sc for 2 and 4 day treatments, the three colonies differed significantly ($F=30.39$; df $=2,120$; S) from one another. When 2 and 4 days were compared separately for significance for different colonies, the results revealed that for 2 days exposure there were significant ($F=39.55$; df=2, 60; S) differences among colonies 1, 2 and 3 but for this treatment after 4 days exposure, there were non-significant ($F=5.03$; df=2, 60; NS) differences in mortality for three different colonies of *R. flavipes*.

Table I.- Percent mortality (Mean±SE) of *R. flavipes* induced by different treatments (1) Imidacloprid + Sc (2), *S. carpocapsae,* (3) Imidacloprid+ Hb (4) *H. bacteriophora* (Hb), (5) Imidacloprid and Control, at different concentrations after 2 and 4 days exposure to three different colonies.

Treatment		Termite colonies					
		Colony 1		Colony 2		Colony 3	
	Conc.	2 days	4days	2 days	4 days	2days	4days
Treatment 1	0.001	54.00±13.25	90.00±0.00	12.71±6.14	58.64±12.83	72.87±10.43	75.12±12.72
Imidacloprid + Sc	0.005	85.44±4.55	90.00±0.00	29.27±16.47	78.32±11.67	71.24±13.16	80.71±9.28
	0.01	90.00±0.00	90.00±0.00	37.30±9.36	81.00±0.00	58.07±10.81	57.92±13.37
	0.05	80.71±9.28	90.00±0.00	27.03±5.34	90.00±0.00	9.09± 5.13	75.00±9.48
	0.1	74.31±9.64	90.00±0.00	40.65±4.58	79.75±8.16	74.22±5.01	83.67±6.32
Treatment 2 S.*carpocapsae* (*Sc*)		11.60±8.22	6.72±4.29	6.22±2.63	4.40± 2.76	10.04± 4.12	16.39±6.61
Treatment 3	0.001	55.55±14.51	90.00±0.00	10.93±2.79	64.07±11.54	56.64±8.95	69.12±10.61
Imidacloprid+Hb	0.005	62.19±8.13	90.00±0.00	33.79±1.16	61.08±7.52	37.29±6.74	79.25±10.74
	0.01	79.18±8.72	90.00±0.00	25.79±2.64	84.68±5.31	66.28±11.13	80.86±4.16
	0.05	65.94±7.84	90.00±0.00	68.50±6.75	90.00±0.00	66.84±6.69	90.00±0.00
	0.1	5.46±6.77	90.00±0.00	57.45±1.06	85.85±4.14	55.54±6.41	6.36±2.22
Treatment 4 *H.bacteriophora*(Hb)		4.95± 4.94	13.90± 5.08	4.14± 4.14	10.36±3.34	10.11±4.65	13.08±3.50
Treatment 5	0.001	21.63±17.21	90.00±0.00	0.00±0.00	57.08±13.54	46.77±6.67	88.18±1.81
Imidacloprid	0.005	55.11±14.44	90.00±0.00	5.96±4.08	90.00±0.00	73.81±16.18	83.27±4.29
	0.01	82.86±5.16	90.00±0.00	45.15±14.65	90.00±0.00	70.39±9.45	82.74±7.25
	0.05	82.86±5.16	90.00±0.00	75.23±6.12	90.00±0.00	61.55±15.03	74.58±15.41
	0.1	76.50±5.74	90.00±0.00	55.25±12.20	90.00±0.00	45.37±9.89	72.59±14.39
Control		5.45±2.22	15.04±4.58	13.85±1.57	13.13±3.43	12.22±4.92	22.78±4.88

As far as concentrations are concerned, different concentrations did not significantly affect the mortality for 2 days (F=1.76; DF= 2, 60; NS) and 4 days (F=1.05; DF=2, 60; NS) treatment.

Imidacloprid + Hb treatment

The effect of the treatment with Hb in combination with imidacloprid, for three different colonies after 2 and 4 days were also significantly (F=72.94; DF= 2,120; S) different from each other and concentrations for three colonies were also significantly different (F=5.54; DF=2,120; S).

When 2 and 4 day treatments for this combinations were compared separately, for 2 days the three colonies differed significantly (F=17.84; DF=2, 60; S) but for 4 days exposure the colonies were not significantly (F=5.76; DF=2, 60; NS) different.

Similarly, when different concentrations for this treatment were compared for 2 and 4 days, for 2 days different concentrations differed significantly (F=6.77;df=2,60;S) but for 4 days exposure different concentration did not differ significantly (F=1.13; df=2,60;NS).

When the effect of two nematode species in combination with the imidacloprid (imidacloprid+ Sc and imidacloprid + Hb) were compared for different colonies we found significant difference for 2 days (F=54.21; df=2,120;S) and for 4 days (F=10.26; df=2,120;S) treatments for colonies 1, 2 and 3 but the effective concentrations differed significantly (F=5.86;df=2,120;S) for 2 days exposure for colonies 1, 2 and 3. However, for 4 days exposure, different concentrations did not significantly affected the mortality of the termite workers (F=2.87; DF=2,120; NS).

Comparison of imidacloprid + Sc and imidacloprid+Hb treatment

For comparison of these two treatments, *i.e.* imidacloprid+*Sc* and imidacloprid+Hb, these two treatments did not differ significantly for 2 days (F=2.51; DF =2,120;NS) and 4 days (F=783; DF=2,120; NS for colony I 2, and 3).

Comparison of two nematode species Hb and Sc

When nematode species Hb and Sc were compared for mortality Hb treatment had no significant effects on different colonies of termites for 2 (F=498; df= 2, 120; NS) and 4 days (F=2 92;df= 2,60;NS). Similarly treatment of three different colonies with *Sc* only had no significant effects on the survival of the termites for 2 days (F=251; DF= 2,120; NS) and 4 days (F=1 73; DF=2,120; NS) after the treatment.

When 2 day and 4 days treatment with Hb or Sc was compared for three colonies no significant differences were observed (F=475; DF=2, 24;NS for 2 day treatment; F=1.38; DF= 2, 24; NS for 4 day treatment).

Comparison of infectivity of three different colonies with imidacloprid

When termites were treated with the insecticide, imidacloprid alone, the three colonies of *R, flavipes* were significantly affected after 2 and 4 day treatments (F=9.30; DF=2,120; S).

Different concentrations of imidacloprid significantly affected the survival of the termites (F=9.87;df=2,120;S) There was also significant variance component for the three different colonies (F=10.13;df=2,60;S) and different concentrations (F=10.99;df=2,60;S) for two day treatment, but when the three colonies were compared for 4 days treatment, there was non significant variance component (F=2.24;df=2,60;NS), similarly the for concentrations there were non significant variance component (F=930;df=2,60;NS) for termites from the three different colonies.

Table I clearly depicts that nematodes alone had very low impact on termite mortality. The effects of Hb and Sc were similar to one another with significantly lower effect on termite mortality compared to nematodes in combination with insecticide. Entomopathogenic nematodes can have significant effect on mortality of termites when there is interaction between imidacloprid and nematodes.

DISCUSSION

In the life cycle of entomopathogenic nematodes, third stage infective juveniles (IJs) usually invade a proper host insect and infect them. It was also found that of the applied dose of nematodes, only 30-40% of IJs infect their most susceptible host *Galleria mellonella,* as not all IJs are capable of finding a proper host. (Fan, 1989: Fan and Hominick, 1991). Termites are not considered a favorite host for nematodes and their infectivity against termites was not proved so successful as when Sc, and Hb were evaluated against *H. aureus* and showed poor results (Weeks and Baker, 2004) This may be due to several reasons *e.g.,* strain specificity of nematodes, temperature and exposure time to host.

Our data from this study confirms that both nematodes species are not effective in controlling termites in laboratory conditions, and efficacy rate of both species was similar, as shown in results of previous research had indicated five different species of nematodes were infective against termites, *e.g., S. feltiae* and *H. bacteriophora* (=heliothidis) (Mauldin and Beal, 1989); *Heterorhabditis* sp, *Steinernema glaseri* (Danthanarayana and Vitarana, 1987); *Steinernema carpocapsae* (Reese, 1971; Epsky and Capinera, 1988), and can cause mortality under laboratory conditions but in the field the efficacy of nematodes is not so successful. In view of that we can presume that nematodes alone are not successful biological control agents.

Healthy termites normally eat infected, weak or dead termites yet as we observed for several days after death, nematodes were not seen emerging from dead termites. Perhaps, in this study, the dose of nematodes given was not adequate (*i.e.* 12.5 per termite). As was studied in previous research that when there was 10 nematodes per termite, there was little mortality, but when the number of nematodes was increased *i.e.,* 100 nematodes per termites, 58.2% mortality was achieved after 3 days (Epsky and Capinera, 1988). Further study is needed to

apply higher doses of Hb and Sc against *R. flavipes* to see higher mortality percentages.

As far as interaction between imidacloprid and entomopathogenic nematode is concerned, application on white grubs larvae and the degree of interactions differs for different nematode species has already been successfully proved (Koppenhofer and Kaya, 1998). In another similar research carried out, 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, and results were more successful in the field rather than in laboratory (Koppenhofer *et al*., 2000). So this combination of imidacloprid and nematodes can significantly reduce the hazardous effects of insecticide and has been recommended to apply on other insect pests and our study substantiate that in the laboratory we can successful apply this on termites, but in the field there is no evidence that has revealed its efficacy against termite colonies again depending upon soil type, moisture pH and nematode species. So in conclusion we can say that for successful application of imidacloprid and nematode against termites, we should give emphasis on nematode biology, application techniques and selection for more infective nematode species.

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