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# Combined effect of *Azadirachta indica* and the entomopathogenic nematode *Steinernema glaseri* against subterranean termite, *Reticulitermes flavipes*

**Abstract -** Laboratory study has been conducted on the bioactivities of entomopathogenic nematodes and neem seed kernel extract (NSKE) against worker termites of *Reticulitermes flavipes.* Neem at various concentrations did not affect the survivability of nematodes, whereas neem had considerable impact on the survivability of worker termites and this may be due to the presence of active neem compounds (Azadirachtin, salanin etc.). Mortality was 40% on 4th day at lower concentration of 1.0% NSKE treatment; whereas mortality has been increased to 70% at higher concentration (4.0%) on 4$^{th}$ day. There was 100% mortality after the combined treatment with 4.0% NSKE + 600 infective juvenile *Steinernema glaseri*, even at the first day of the experiment. In the present experiment, neem extract does not affected the survival of the nematodes. Hence, nematode and neem extract can be used for soil-insect control particularly for the subterranean termites.

**Key words:** infectivity, neem, *Reticulitermes flavipes*, *Steinernema glaseri.*

## INTRODUCTION

Subterranean termites are economically important pests of dwellings and other human structures where they feed on wood. Because of human health and environmental concerns, organochlorine and organophosphate insecticides are not used in dwellings. Accordingly, there has been great interest in finding alternative biological approaches to control termites (Grace, 1997). In addition, more environmentally friendly insecticides such as imidacloprid are being employed for termite control (Shelton & Grace, 2003). However, imidacloprid has a delayed mode of action and the termites continue to feed. Steinernematids and Heterorhabditids are obligate insect parasites (Poinar, 1979) with associated bacterial symbionts, *Xenorhabdus* spp. and *Photorhabdus* spp., respectively (Akhurst & Boemare, 1990). The infective juvenile (IJ) stage of the nematode remains in the soil until it can invade the body of the susceptible insect after infection, release symbiotic bacteria into the insect hemocoel causing septicemia and death (Kaya & Gaugler, 1993). Entomopathogenic nematodes have been tried against termites, but a high concentration of 400 nematodes / termite is needed to get some degree of control (Wang *et al.*, 2003). Hence, the present paper is to study the control of the subterranean termite, *Reticulitermes flavipes* (Kollar, 1837), a major pest of dwellings in urban

and suburban areas of Tamil Nadu using a combination of biological control agents (entomopathogenic nematodes) and environmentally friendly (neem) insecticides. The principles learned from this research can be adapted to other insect pests and will be useful in India where alternative control approaches are needed to replace the more toxic insecticides that are currently in use.

## MATHERIALS AND METHODS

### Collection and maintenance of termites

Termites (*Reticulitermes flavipes*) were collected directly from their nests by placing wood bait buried near trees in and around the University Campus, Coimbatore, India. They were kept in cylindrical plastic containers with 1-2 cm deep vermiculite and sand (1:1 by volume). Corrugated wood blocks were added as diet. One hundred to 2,000 individuals were collected from each termite colony. The termite colonies were kept for a maximum period of 120 days from field collection date at room temperature (21-25°C) in the laboratory.

### Nematode cultures and extraction

The greater wax moth *Galleria mellonella* (Pyralidae, Lepidoptera) larva was used for the nematode baiting and multiplication. Cultured nematodes were used within one month of collection from the host cadaver. Twenty moth larvae were placed on the artificial diet in cylindrical plastic containers. The containers were kept at 25-28°C with 72-75% relative humidity to avoid fungal contamination (Dutky *et al*., 1964).
A representative sample of 250 ml was placed in a plastic box with ten last instar larvae of *G. mellonella* by baiting technique (White, 1927).

### Preparation of neem seed kernel extract

Neem seed kernels were collected from the Bharathiar University Campus, Coimbatore-641 046, Tamil Nadu, India. Fifty grams of seed kernels of *A. indica* were washed and oven dried to constant weight at 55°C and the dried seeds were pulverized into fine powders and 1.0 g of the neem was stirred in 100 ml of distilled water. After 24 hours, the water extracts were filtered and used for the experiments.

### Mortality bioassay

The wooden blocks were immersed with different concentrations of neem seed kernel extract ranging from 1% to 4% and *Steinernema glaseri* (150 to 600 Ijs). Control wooden blocks were treated with distilled water only and the wooden blocks were allowed to dry at room temperature for ten minutes and then placed in 15 cm in diameter Petri dishes on moist filter paper discs. Newly emerged 24 h starved late instar worker termites were segregated into groups and each group was individually fed with treated and untreated wooden blocks. Each experiment was carried out for 1 d, 2 d, 3 d or 4 d, respectively, with hundred workers per concentration and replicated five times. After 2

d, the termites were transferred to fresh untreated wooden blocks and maintained until they died. The total number of normal workers that survived was noted. The workers were observed for mortality and morphological changes associated with growth disrupting effects.

**Survivability Test**

Effect of NSKE on nematode survivability was studied in the laboratory at 28 ± 1°C in the dark. Standard Petri dish bioassays were conducted to evaluate the effect of NSKE on mortality of nematodes. Different concentrations of neem extract were taken in separate Petri dishes and about 1000 juveniles were introduced into each concentration. Water was taken in control Petri dishes. Every two days of exposure, live nematode juveniles were collected and observed under a binocular microscope.

**Infectivity Test**

The nematode infectivity rate after the neem treatment of *R. flavipes* workers was observed under laboratory conditions. The *R. flavipes* workers were kept in a cylindrical plastic container with 1-2 cm deep vermiculite and sand (1:1 by volume). Corrugated cardboard and wood blocks were added as food treated in different concentrations of neem seed kernel extract. After 24 h, the IJs of *S. glaseri* were then released into the treated container. After 24 h the infectivity and mortality of neem treated worker termites were observed. The number of nematodes in the whole termite cadaver was noted by dissecting the termite and by counting the number of nematodes in the insect body with the help of a dissecting microscope. Similarly, the infectivity rate of nematodes without neem treatment on workers of *R. flavipes* was also studied in separate experiment.

**Statistical Analysis**

For the mortality bioassay, the per cent mortality data after corrections were subjected to probit analysis for calculating mean lethal concentrations ($LC_{50}$, $LC_{90}$) (Finney, 1971). Results were corrected for control mortality by using Abbott's (1925) formula. All other percent mortality, infectivity and percent survivability data were subjected to analysis of variance (ANOVA) and the means were separated using Duncan's multiple range tests (Alder & Rossler, 1977).

## RESULTS

**Effect of NSKE and *S. glaseri* on mortality of *R. flavipes* workers**

Considerable mortality of termites was evident after the treatment with neem seed kernel extract and it was dose dependent. Lethal doses ($LC_{50}$ and $LC_{90}$) were also worked out (Tab. 1). The $LC_{50}$ values after 1.0%, 2.0%, 3.0% and 4.0% NSKE concentration treatment were 4.602, 3.689, 3.164, and 2.606, respectively. Similarly, the $LC_{50}$ values after treatment with 150 Ijs, 300 Ijs, 450 IJs and 600 IJs entomopathogenic nematodes was 694.713, 577.59, 458.15, and 408.58, respectively. The $LC_{50}$ values after combined

| Treatments | No. of workers | Percentage of mortality (days) | | | | $LC_{50}$ | $LC_{90}$ | Chi-square value ($X^2$) |
|---|---|---|---|---|---|---|---|---|
| | | 1st | 2nd | 3rd | 4th | | | |
| Control | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| NSKE (%) | | | | | | | | |
| 1.0 | 100 | $8^{ef}$ | $15^{f}$ | $28^{f}$ | $40^{e}$ | 4.602 | 7.894 | 0.140 |
| 2.0 | 100 | $10^{e}$ | $26^{d}$ | $37^{de}$ | $55^{cd}$ | 3.689 | 6.582 | 0.940 |
| 3.0 | 100 | $21^{b}$ | $38^{ab}$ | $45^{c}$ | $62^{bc}$ | 3.164 | 6.840 | 1.069 |
| 4.0 | 100 | $26^{a}$ | $41^{a}$ | $57^{a}$ | $70^{a}$ | 2.606 | 5.887 | 0.074 |
| Sg (IJs) | | | | | | | | |
| 150 | 100 | $6^{e}$ | $19^{ef}$ | $33^{e}$ | $36^{ef}$ | 694.713 | 1.216 | 4.077 |
| 300 | 100 | $17^{cd}$ | $34^{bc}$ | $42^{cd}$ | $49^{c}$ | 577.59 | 1.239 | 2.086 |
| 450 | 100 | $26^{b}$ | $36^{b}$ | $55^{a}$ | $58^{a}$ | 458.15 | 1.096 | 1.847 |
| 600 | 100 | $32^{a}$ | $41^{a}$ | $57^{a}$ | $61^{a}$ | 408.58 | 1.135 | 0.971 |
| NSKE+Sg ($IJ_S$) | | | | | | | | |
| 1.0+150 | 100 | $49^{de}$ | $77^{f}$ | $99^{a}$ | $100^{a}$ | 165.906 | 355.356 | 3.334 |
| 2.0+300 | 100 | $79^{c}$ | $98^{a}$ | $100^{a}$ | $100^{a}$ | 55.840 | 207.615 | 0.048 |
| 3.0+450 | 100 | $88^{b}$ | $100^{a}$ | $100^{a}$ | $100^{a}$ | 57.343 | 160.738 | 0.129 |
| 4.0+600 | 100 | $97^{a}$ | $100^{a}$ | $100^{a}$ | $100^{a}$ | 19.799 | 97.814 | 0.029 |

Tab. 1 – Mortality of *Reticulitermes flavipes* workers after treatment with neem seed kernel extracts and *Steinernema glaseri.* Within a column, means followed by the same letter(s) are not significantly different at 5% level by DMRT.

treatment of NSKE + *S. glaseri* with 1.0 % + 150 Ijs, 2.0 % + 300 Ijs, 3.0 % + 450 IJs and 4.0 % + 600 IJs was 165.906, 55.840, 57.343, and 19.799, respectively (Tab. 1).

**Side-effect of NSKE on the survivability of *S. glaseri***

Survivability of *S. glaseri* after treatment with NSKE, even at the higher concentrations, was not affected. At 4.0% NSKE, the percentage survivability was 98% in the 4th week. At the higher concentration of NSKE (8%), 98% survival was noted in the 2nd week and the average survival of *S. glaseri* was 95.5% (Tab. 2).

| Concentration NSKE (%) | No. of Ijs | Survivability / Week | | | | % Average survival |
|---|---|---|---|---|---|---|
| | | 1st week | 2nd week | 3rd week | 4th week | |
| Control | 1000 | 100[a] | 100[a] | 100[a] | 100[a] | 100[a] |
| 2.00 | 1000 | 100[a] | 100[a] | 100[a] | 99[ab] | 99.7[ab] |
| 4.00 | 1000 | 100[a] | 100[a] | 100[a] | 98[ab] | 99.5[ab] |
| 8.00 | 1000 | 100[a] | 98[a] | 95[a] | 89[b] | 95.5[ab] |

Tab. 2 - Efficacy of neem seed kernel extract on the percentage survivability of *S. glaseri.* Within a column means followed by the same letter (s) are not significantly different at 5% level by DMRT.

| No. of nematode (IJs) | Mortality (%) | |
|---|---|---|
| | Treated | Untreated |
| 500 | 20[e] | 15[e] |
| 1500 | 39[d] | 28[d] |
| 2000 | 47[c] | 34[c] |
| 2500 | 69[b] | 52[b] |
| 3000 | 100[a] | 81[a] |

Tab. 3 - Infectivity of *S. glaseri* on *R. flavipes* worker communities after the treatment with 1% NSKE, or untreated. Within a column means followed by the same letter (s) are not significantly different at 5% level by DMRT.

**Infectivity of treated and untreated *S. glaseri* on worker communities of *R. flavipes*.**

The infectivity of nematodes on NSKE-treated and untreated workers of *R. flavipes* is given in Tab. 3. The percentage of *S. glaseri*-infected workers was considerably higher in NSKE- treated *R. flavipes* compared to untreated termites. There was 100% infectivity after the combined treatment of NSKE (1%) and nematodes (3000 IJs), and the percentage of infectivity was lower (81%) in the NSKE-untreated control (Tab. 3).

## DISCUSSION AND CONCLUSION

Plants are the store-house of bio-active chemicals that have antifeedant, antiovipositional, growth disrupting and fecundity reducing properties towards different insects (Mordue (Luntz) & Blackwell, 1993). In the present study the neem seed kernel extract (NSKE) in combination with entomopathogenic nematodes have shown toxicity against subterranean termites. Earlier studies demonstrated that, to enhance nematode infection against these soil insects, combinations of nematodes with other control agents can be synergistic and provide better control than each agent alone (Koppenhöfer & Kaya, 1998; Koppenhöfer *et al.*, 2000; 2003). Thus, Koppenhöfer & Kaya (1998) demonstrated that the combination of a low concentration of a neonicotinoid (i.e., imidacloprid) insecticide and a low concentration of entomopathogenic nematodes provided excellent control of scarab larvae. The neonicotinoid insecticides are considered to be more environmentally friendly, have low vertebrate toxicity, low application rates, and longer persistence (Elbert *et al*., 1991) than the more toxic, persistent organochlorine or organophosphate insecticides. In the present study, also after application of NSKE, a higher infectivity of nematodes on termites was shown. Previous studies combining Bt and a nematode species against a pest showed that their combination was better than the use of nematodes alone (Bari & Kaya, 1984). Hence, neem can be used to enhance the activity of nematodes for infectivity on termites.

Extracts of *A. indica* and many other plants are known to exert multiple, acute and chronic effects such as growth regulatory and antifeedants, etc., on the same or different insects (Jacobson, 1988; Saxena, 1989; Schmutterer, 1990). Murugan & Vanithakumari (2009) studied the bioactivities of neem products against insects.

Moreover, in the present study infectivity by nematodes was higher in the NSKE-treatment compared to the untreated control, and at the same time the NSKE treatment did not affect the survival and infectivity of the entomopathogenic nematode, *S. glaseri.* Soil insect pests like termites have their own behavioral mechanism to prevent entry of nematodes into the body by grooming activity. In the present study, the application of neem seed kernel extract affected the physiological activity and thereby arrested the grooming activity and it facilitated the easy entry of nematodes and their infectivity.

The principles learned from this research can be adapted to other insect pests and will be useful in India where alternative control approaches are needed to replace the more toxic insecticides that are currently in use.

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