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EFFICACY OF ENTOMOPATHOGENIC NEMATODES TO CONTROL UP–COUNTRY LIVE–WOOD TERMITE, *Postelectrotermes militaris*

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Infectivity of some Sri Lankan isolates of entomopathogenic nematodes against up-country live-wood termite *(Postelectrotermes militaris)* was carried out. These isolates were compared with two commercial formulations of entomopathogenic nematodes namely "Biosafe" *(Steinernema carpocapsae)* and "Nemasys" *(S. feltiae)*. This study revealed that Sri Lankan isolate, *Heterorhabditis* spp. (HSL 6) and *S. carpocapsae* were better controlling agents against *P. militaris* than other isolates tested.

INTRODUCTION

Health and environmental hazards associated with chemical pesticides are forcing to find less toxic pest management methods. One would be biological control using entomopathogenic nematodes. Entomopathogenic nematodes are known as effective controlling agents of insect pests and have been widely used against cryptic and soil inhabiting insects (Klein, 1990; Begley, 1990). Third stage infective juveniles of these nematodes are capable of actively searching for their hosts and parasitized consequently. In many occasions, insects in concealed habitats also provide suitable conditions for nematode survival and infectivity (Kaya, 1990). These were assumed to be supported for successful outcome of using such biocontrol agents.

Entomopathogenic nematodes found in different parts of the world have different infectivity levels and also, they are climatically adapted. Therefore, when new nematodes are isolated it is vital to screen them against a target pest. Evaluation of dose response and time response relationships between pathogen and host prior to field application offers vital information for an effective pest control programme. Moreover, the pathogenicity of different strains and species of entomopathogens to the insect can be compared in order to select those which are more virulent while eliminating many unsuitable isolates. There are different techniques for conducting dose response or time response bioassays, all of which need knowledge and understanding of the control and the target host.

This paper presents an analysis of dose and time responses of entomopathogenic nematodes found in Sri Lanka, compared with commercial formulations of *Steinernema carpocapsae* and *S. feltiae*, to evaluate their pathogenicity for the tea termite, *P. militaris*, prior to field applications.

MATERIALS AND METHODS

Sources of nematode materials

Steinernema carpocapsae (Biosafe) and *S. feltiae* (Nemasys) were imported from Biosys, California and the Agriculture Genetic Company, UK, respectively. Sri Lankan isolates, both *Heterorhabditis* (HSL 6 and HSL 105) and *Steinernema* (SSL 8, SSL 82, and SSL 109) were obtained from the soil survey carried out in the year 1992 and mass cultured in the laboratory on *Galleria mellonella* (Lepi; Pyralid) larvae.

Bioassay using tissue papers

A total of 110 plastic dishes (No. 11 dishes) lined with two layers of "Kleenex" tissue papers was prepared. Infective juveniles of *S. carpocapsae, S. feltiae*, or the Sri Lankan isolate of HSL 6, HSL 105, SSL 8, SSL 82, and SSL 109, were introduced into each separately with $5x10^3$, $10x10^3$, and $25x10^3$ dosages in 5 ml of sterilised water (five replicates per nematode per dose) using a micro pipette. Untreated controls (5 replicates) were treated with 5 ml sterilised water. Then, 50 active and healthy termite "workers" were taken from cultures maintained in the laboratory and introduced into each dish immediately. The dishes were closed with the lids. The assay was run at 23 ± 3^0C. It was set up in a completely randomized design. Observations on termite mortality were recorded every day for 10 days. Cadavers were not removed from the dishes during the period of observation.

Bioassay using established termite colonies

An average of 300 active and healthy termite "workers" by weight (previously cultured in the laboratory) were introduced into plastic boxes of 16.5 x 11 x 5.5 cm. Immediately before that, 2–3 pieces of tea wood with galleried portions were placed in each. The pieces were 10–15 cm long and 5–8 cm wide. The covered boxes were kept for 2 weeks at room temperature until the termite colonies established. Then, *S. carpocapsae, S. feltiae*, or the Sri Lankan isolates of HSL 6, and SSL 8 were applied with the following doses in 8 ml of sterilised water: $5x10^3$, $10x10^3$, $25x10^3$ and $100x10^3$ nematodes per treatment. Application was done directly to the galleried wood using a pasture pipette several times. The controls were treated with the same amount of water. All treatments were replicated four times. This was set up in a completely randomized design at room temperature (21–26^{0}C). Termite mortality was recorded after 10 days of exposure by splitting open the galleried wood and counting the number of live and dead termites in each box. Relative humidity was maintained throughout at 100%.

Bioassay using uprooted infested tea bushes

Nearly 15 years old and uniform sized tea bushes (vegetatively propagated, clone TRI 2025, and suspected of harbouring live termite colonies), were chosen from field No. 7 of the Brunswick Division of the Brunswick State Plantation. Bushes positive for termites were identified visually by termite gallery openings exposed by very recent prune cuts (pruned not more than 3 months earlier). Fifteen bushes at a time were brought into the laboratory in thick polythene bags soon after uprooting from the field. They were kept vertically positioned in the sampling bags. Infective juveniles were tested in the following doses: $1x10^5$, $5x10^5$, $10x10^5$ and $30x10^5$ per bush.

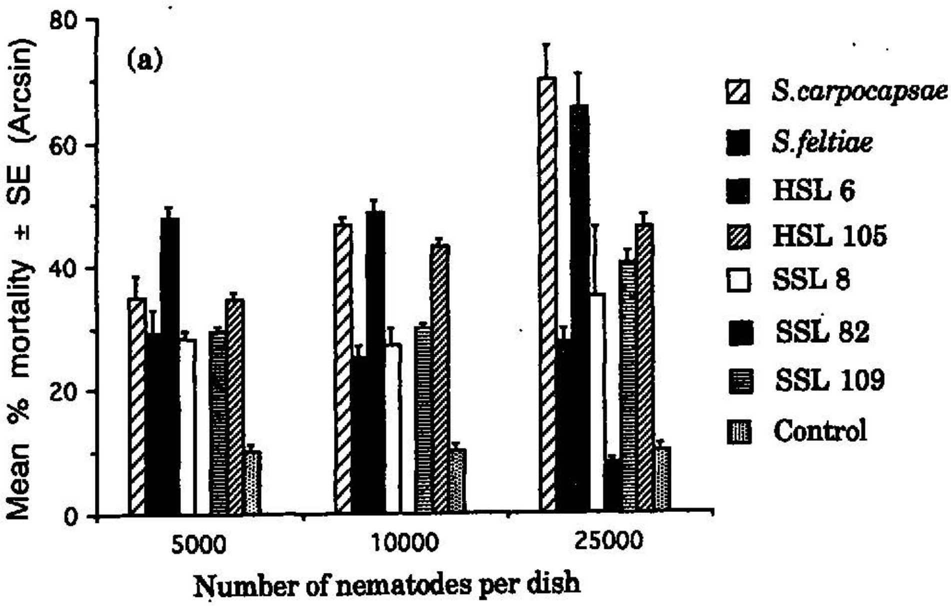
Treatments of each nematode type, *S. carpocapsae*, *S. feltiae*, or the Sri Lankan isolates of HSL 6, and SSL 8 were tested independently under similar laboratory conditions. In each assay, all four nematodes were tested with a set of controls and were replicated three times (15 bushes per treatment). The number of replicates was limited to three due to the heavy labour required to split open bushes and also because the number of tea bushes of similar types is limited in the field. For the SSL 8 nematodes, only the first two treatments were applied due to low production of infectives from the *Galleria* infection. Treatments were applied to randomly selected bushes in the laboratory using a plastic syringe filled with the diluted dose. The syringe was held directly into the exposed gallery openings and pressed so that the suspension was injected along the galleries parts. A volume of 50 to 100 ml suspension was applied per bush was nearly 10–15 min. Treated bushes were kept in the polythene bags with the tops open, at a room temperature of 21–26^{0}C. Termite mortality data was taken after one week in the laboratory by carefully splitting open the bushes using a secateur to avoid injuries to the termites. The number of live and dead termites recovered from each was recorded separately.

RESULTS AND DISCUSSION

The mortality of recently dead termites was decided throughout this study by noting motionless legs and antennae to a tactile stimulus. Both active and moribund or unconscious termites were counted as live individuals. Proportional data was normalised using the arcsin transformation prior to statistical analysis. Significant differences were tested using ANOVA. Linear regression models were generated with "Stat Work" package and significance of the model was tested at $P<0.05$.

Bioassay using tissue papers

This is the first attempt to document efficacy of Sri Lankan entomopathogenic nematode isolates against the tea termite, *P. militaris,* with a comparison of two commercial formulations of nematodes namely, *S. carpocapsae* ("Biosafe") and *S. feltiae* ("Nemasys"). The arcsin transformed percentage mortalities of termites are shown in Fig. 1 which show the differences in termite mortality after 3 and 10 days exposure to nematodes. It is clear from these that the dose of nematodes, the time of exposure and isolate/species of nematodes used, all had an effect on the mortality of the termites.



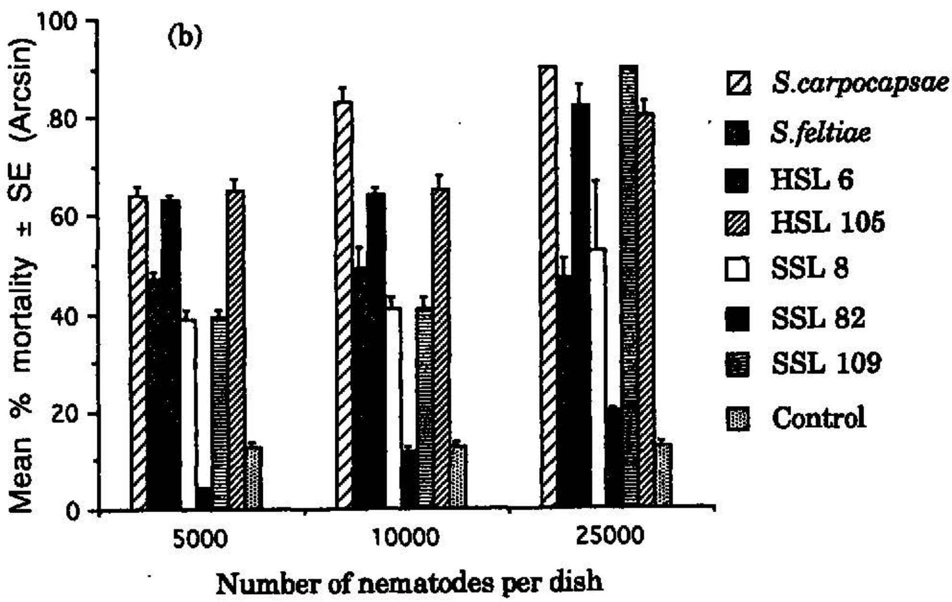


Fig. 1–*Influence of dose of nematodes on percentage (Arcsin transformed) mortality of 'worker' termites (50 per replicate) after (a) 3 days and (b) 10 days (n=5).*

Pathogenicity of the two heterorhabditids was greater than that of SSL 8 and SSL 82 of the Sri Lankan steinernematids at all dosages, but lower than SSL 109 at the highest dose after 10 days. The effects of *S. feltiae* and SSL 8 were similar to each other and these nematodes had a significantly lower effect on termite mortality compared to the heterorhabditids at both time and for all doses. *S. carpocapsae* performed better than *S. feltiae.*

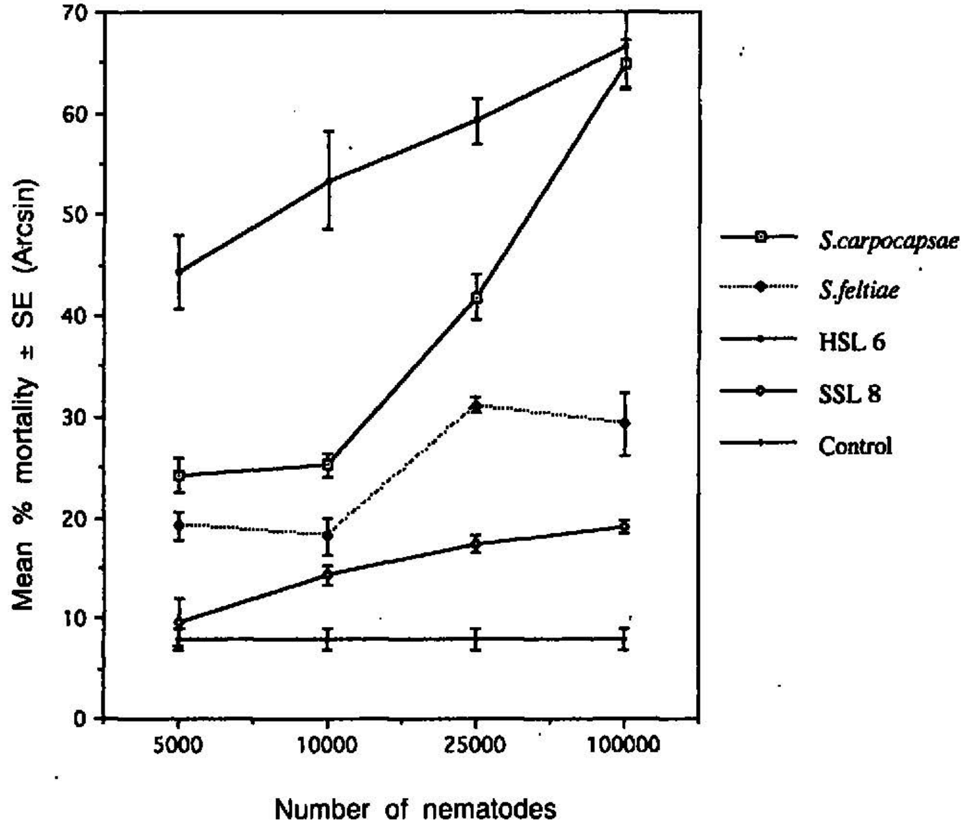
SSL 82 efficacy was the least and it never attained 50% mortality of the insects at any dose for up to 10 days exposure. Its efficacy was found well below that of the control. These results reveal that isolate SSL 82 is not promising as control agent for this termite in this study. The SSL 109 isolate produced encouraging results at its highest dose of $25x10^3$, but at lower doses of $5x10^3$ and $10x10^3$, termite mortality, was below 50% with values similar to that of the SSL 8 isolate. HSL 6 and HSL 105 produced promising results, comparable to *S. carpocapsae.*

The *Steinernema* infected termite cadavers were often seen fetid and mortality of the termites was probably induced by bacterial infection or some other reason in the latter stages of the assay. Then, nematodes failed to develop or reproduce successfully within the cadavers or they may have been ejected from the cadaver through broken parts of the body before they developed to adults. Except for *S. carpocapsae,* HSL 6 and HSL 105 infective juveniles of all the other steinernematid nematodes tested (*S. feltiae,* SSL 8, SSL 82, SSL 109) were seen gathering around dead cadavers at the latter part of the assay. However, there were no clear differences in behaviour of attraction towards the same host. A report of infectives clustering around adult lepidopteran spiracles suggested that they might have located the host by carbon dioxide attraction (Triggiani and Poinar, 1976). It was also noticed in this study that the infectives which gathered often developed to adults and produced progeny while out side the cadaver. This could be explained if the body fluid of dead termites leaked onto tissue papers through separated body parts and acted as a food source for them to develop to adults.

Bioassay using established termite colonies

This bioassay was conducted as a follow up to the earlier highly artificial bioassay conducted on tissue papers. In this, more natural conditions were provided using galleried tea wood as a substrate instead of tissue papers. The assay on tissues revealed that SSL 82 had the least efficacy against the termites (Fig. 1), so further experimentation against the same host was not needed. Therefore, this study intended to examine the efficacy of HSL 6 and SSL 8 to represent Sri Lankan heterorhabditids and steinernematids respectively, compared with the two commercial products of *S. carpocapsae* and *S. feltiae.*

The results for termite mortality in respect to the nematode type and their dosages are shown in Fig. 2. These results further show that different nematodes have different efficacy levels in killing termites. For example, the SSL 8 nematode remained comparatively ineffective compared to the others and there was no significant difference



Regression equations for

S. carpocapsae	y	=	4.35	+	13.87 X	(R^2 = 0.884)
S. feltiae	y	=	13.6	+	4.33 X	(R^2 = 0.691)
HSL 6	y	=	37.7	+	7.24 X	(R^2 = 0.993)
SSL 8	y	=	7.2	+	3.16 X	(R^2 = 0.957)

Fig. 2–*Mean percentage mortality (Arcsin transformed) of termites in galleried tea wood, after 10 days exposure to nematodes (n=4).*

between the dosages applied for this isolate ($F=0.49$, $P>0.05$). These results agreed well with those in the preliminary study. It was found that HSL 6 had a significantly higher efficacy than *S. carpocapsae* at lower dosages, but there was no significant difference at the higher dose of 100 x 10^3. Therefore, while the highest efficacy was observed for both *S. carpocapsae* and HSL 6 in this study, the heterorhabditid was by far the most effective nematode tested at lower doses. Significantly lower mortality was observed for *S. feltiae*, compared to *S. carpocapsae* and HSL 6 and this steinernematid showed little response to increasing concentrations of infectives (Fig. 2). This lack of response to concentration was observed in the bioassay using tissues.

It was noticed that some dead infected termites were dragged off by healthy termites from the treated galleries to the outside. Since these galleries were opened to the outside from both ends, having dead termites dragged out by live individuals from the galleries was unavoidable. However, there were no occasions when parasitized termites were removed from galleries before they died. Nevertheless, many dead termites were still found inside the galleries and no attempts were made to drag them out by live individuals.

Bioassay using infested uprooted tea bushes

Both commercial formulations of *S. carpocapsae* and *S. feltiae* and the Sri Lankan isolates of HSL 6 and SSL 8 were tested against entire termite colonies in tea bushes while maintaining laboratory conditions. Encouraging results were provided by the first three nematode types in this assay. By contrast the effect of SSL 8 was the same as untreated control bushes (Fig. 3). These results reveal that SSL 8 is not a good controlling agent against this termite with the given dosages and conditions (Regression ANOVA, $F=1.04$, $P>0.05$). These results confirm the earlier bioassays.

An increasing trend for termite mortality in relation to dosage was observed in the applications of *S. feltiae*, *S. carpocapsae*, and HSL 6 (Fig.3) (Regression ANOVA, $F=11.2$, $P=0.02$, $F=4.86$, $P=0.02$ and $F=10.2$, $P=0.001$ respectively). The latter two showed a tendency towards higher pathogenicity at 100x10^3 (the lowest dose tested) when compared to *S. feltiae*. However, due to the high variability within the replicates there was no significant differences between *S. carpocapsae*, *S. feltiae* and HSL 6 at the highest dose of 30x10^5.

Dead individuals were not observed to be dragged out from the termite galleries in this assay. The termite galleries were not exposed to the exterior since the complete root system with termite galleries was left intact. This would be the situation that would be expected in the field.

In this study, an attempt was made to simulate natural conditions by means of using tea wood pieces and natural bushes containing termites after the preliminary bioassay using tissue papers. The study has indicated some susceptibility of *Postelectrotermes militaris* to entomopathogenic nematodes under more natural conditions. Under these conditions, HSL 6, which represents Sri Lankan heterorhabditids

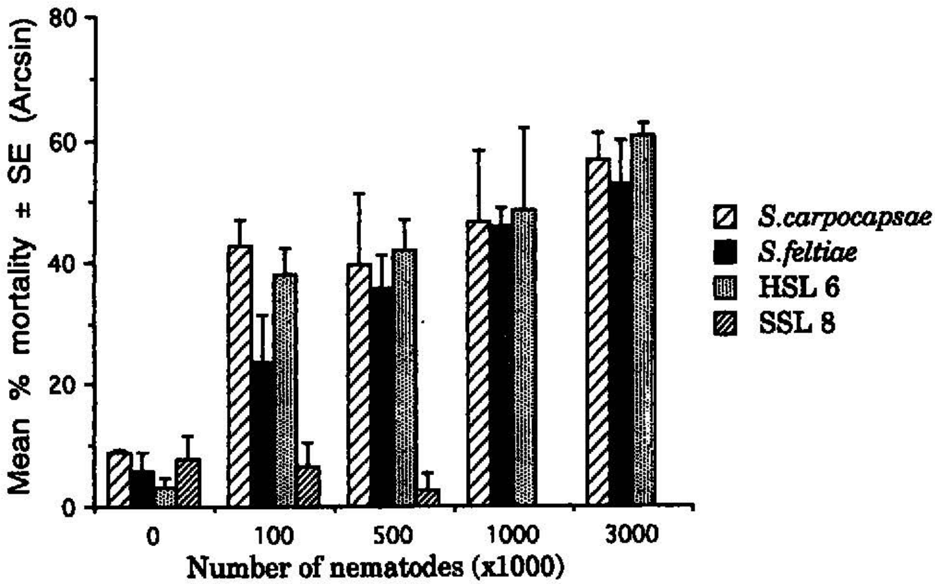


Fig. 3– Mean percentage mortality (Arcsin transformed) of termites in uprooted tea-bushes, after 6 days exposure to nematodes (n=3).

and *S. carpocapsae* were more effective than native steinernematid isolates tested in the laboratory. The SSL 8 isolate did not produce consistent results, and was not effective against this termite when the application was done to a whole bush. Both SSL 109 and HSL 105 used in the first assay using tissue papers produced encouraging results. The SSL 82 isolate was not effective under any circustances. It was shown that bioassays using either established termite colonies or uprooted infested bushes, did not provide 100% mortality at the doses tested. However, increased doses were required to obtain similar mortality rates when the bioassay changed from established colonies to uprooted bushes.

Entomopathogenic nematode third stage "dauer larvae" are usually termed "infective juveniles". However, all such juveniles are not capable of infecting an insect host. Fan (1989) and Fan and Hominick (1991) found that regardless of nematode strain, dose or exposure to single or several hosts, only about 30–40% of the applied infective stages were able to successfully parasitise *Galleria mellonella* larvae, one of the most susceptible insect hosts, in sand tubes. It is unknown why the majority of nematodes failed to parasitise when conditions are optimal. Therefore, the same principle was applied in deciding the preliminary doses for killing termites in these assays. That is, it was assumed that no more than 40% of the juveniles would prove infective.

Infectivity of nematode strains is highly variable and is related to the temperature at which the assay was carried out. The temperature thresholds for infectivity vary for strains and species and this may be more important in laboratory tests on infectivity (Molyneux *et al.*, 1983). However, the temperature conditions during these assays (23±3° C) were chosen to be within the presumed temperature thresholds for infectivity and also representative of field conditions.

The exposure time of hosts to the nematodes must be considered as an important influence on nematode infectivity because more nematodes will contact and infect the insect host when the exposure time is increased (Fig. 1). Since the termites are not highly susceptible to the nematodes tested, mortality of hosts was assessed rather than the number of nematodes able to parasitise each host. Also, the dose and exposure time were highly correlated with the effectiveness of the nematode isolates tested. The bioassay using tissue papers shows that 6–10 days were required to obtain maximum parasitism. By contrast, lepidopteran larval pests, which can be very susceptible to nematode infection, can show maximum mortality within 48 h of initial exposure. For example, Kondo and Ishibashi (1986) found that infectivity of steinernematid nematodes to the *Spodoptera litura,* the common cut worm, by means of counting the number of nematodes in each host increased over a period of 48 h. However, there were dissimilarities between nematode species tested.

Finally, this study indicates that a Sri Lankan isolate of a heterorhabditid, namely HSL 6, has similar efficacy levels as *S. carpocapsae* for controlling tea termites in the laboratory. Similarly, the first bioassay using tissue papers showed that the HSL 6 and HSL 105, both have similar infectivity levels against *P. militaris.* However, the steinernematid isolates were not consistent, and very poor efficacy was given by SSL 82. The *S. feltiae* product also gave inconsistent results. However, it did produce much more encouraging results when used in tea bushes with high doses of nematodes. Therefore, *S. carpocapsae* and *S. feltiae* remain good candidates for evaluation under field conditions where comparatively high dosages will be necessary. However, field evaluations of the Sri Lankan heterorhabditids remains for future studies because more work is needed to test formulations for reducing their activity during storage and for stabilising their infectivity.

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