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Evaluation of Entomopathogenic nematodes against Termites

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Abstract
The potential efficacy of three species of entomopathogenic nematodes (EPN), *Steinernema siamkayai*, *S. pakistanense* and *Heterorhabditis indica*, against subterranean termites was tested under laboratory conditions. In sand assay method, *S. pakistanense* showed significant results revealed that cent percent mortality of both *Reticulitermes flavipes and Odontotermis hornei* within 24 h than *S. siamkayai* and *H. indica* occurred 48hrs at the rate of 250IJs/ml. A positive correlation was found between dosages as well as exposure time and mortality. Probit analysis indicated that *S. pakistanense* required less number (5IJs/termites) and less time (20h) against *R. flavipes* and whereas *O. hornei*, required less number (5.84IJs/termites) and less time (15.5h).In *H. indica* required less number (5IJs/termites) and less time (20h) against *R. flavipes* and whereas *O. hornei*, required less number (5IJs/termites) and less time (19.8h). *S. pakistanense* more predominant species in our studies both mortality, and offspring production within termites as host.

Keywords: Entomopathogens, *Steinernema*, *Heterorhabditis*, Biological control, Termites

Introduction
The nematodes belonging to genera *Steinernematid* and *Heterorhabditid* are soil organisms. They are ubiquitous, having been isolated from every inhabited continent from a wide range of ecologically diverse soil habitats including cultivated fields, forests, grasslands, deserts, and even ocean beaches. These nematodes have a mutualistic symbiosis with a bacterium (*Xenorhabdus* spp. and *Photorhabdus* spp. for *Steinernematids* and *Heterorhabditids*, respectively). [1] The performance of Entomopathogenic nematodes (EPNs) for successful control of target insect pest is dependent on the motility and persistence of the infective juveniles (IJs). The prevalence of EPNs in natural habitats are influenced intrinsic factors like behavioral, physiological, and genetic characteristics and extrinsic factors such as antibiotics, competition, natural enemies, temperature, soil moisture, pH, soil type, soil texture, relative humidity, UV radiation and desiccation. [2] Temperature is one of the most important factors limiting the success of EPNs [3]. It directly influences host searching, [4] pathogenicity [5] and survival, but pH values characteristics of most field soils do not significantly decrease survival [6]. *Galleria mellonella* have been used as a model insect for the nematode pathogenicity studies. [7]
The termites exhibit a wide diversity in its patterns of social organization in castes, are a major pest of human structures throughout tropic and sub-tropic climate zones, causing billions of dollars in damage worldwide. Subterranean termites are the most economically important known primarily for their destruction of wooden structures and also are agricultural pests. Many of the chlorinated hydrocarbon insecticides recommended previously for the control of termites in sugarcane are banned at present. Hence, there is an urgent need to have an alternate insecticide which is less hazardous to the environment. There has been considerable interest and research on the use of EPNs to control termites [8]. Therefore, it has become necessary to search for alternative way for termite control in the cultivated land as wells as natural diverse habitat so that use of these chemicals can be minimized. Search of potential species/strains of EPNs can act as a promising agent for termite management because of easy to culture and application in open environment.

Materials and Methods
Collection and Maintenance of Termites
Termites were collected from infected sugarcane and its crop residues, dried grasses, wood

pile, dead wood and trees in and around Bharathidasan University campus and nearer farmer lands. They were kept in plastic containers with 1-2 cm deep vermiculite, sand and corrugated wood blocks were added. The termite colonies were kept at room temperature (21-25 °C) in the laboratory. *Reticulitermes flavipes* and *Odontotermis hornei* (workers) were identified [9], at Tamil Nadu Agriculture University, Coimbatore. Before testing, termite workers were taken from containers, counted, and transferred to plastic petri dishes lined with a wet filter paper and vigorous termites selected for bioassay study.

Nematodes Culture

Three nematode species from the live nematode culture of the Department of Molecular Entomology, University of Bharathidasan University, Tiruchirappalli, Tamil Nadu, were used in this study. They were *Steinernema siamkayai*, *S. pakistanense* and *Heterorhabditis indica*. Prior to the experiments were started, nematodes were propagate in *Galleria mellonella* larvae [10]. Nematode infective juveniles emerging from the *G. mellonella* larvae within 7 d from the first day of emergence were collected using White traps [11] and were kept in a tissue culture flask at 19°C. They were used within 5 d of collection.

Bioassay Studies

The three novel strains were chosen because; they were found to have greater virulence to *G. mellonella* relative to the 25 strain and caused the highest *G. mellonella* mortality among all strains tested. Nematodes virulence was studied in sand assay method. [12]. 10g of dry, autoclaved sand was placed in each Petri dish with 0.2 to 0.5 mm particle size. Approximately 250IJs/ml was applied to each Petri dish. Control dishes received only distilled water. Twenty five worker termites were introduced into the Petri dish after nematode applications. Termite mortality was recorded. All termite-nematode combinations were replicated 3 times and the experiment was repeated twice. Lethal Dose (LD_{50}) and lethal Time (LT_{50}) of nematodes was estimated. Dead termites were dissected after mortality; number of infecting nematodes in each termite and developmental stage of the nematodes were recorded. Number of nematodes emerged from termites were recorded.

Dead *R. flavipes* and *O. hornei* (single worker / dish) were placed in the white trap for IJs production. The dishes were kept in a moist container at 25±2 ºC for up to 21days. Nematodes that emerged from the dead termites were collected and counted using dissecting microscope.

Similar procedure was workout for *R. flavipes* for mortality studies. Nematodes were added to sterilized sand at five rates of 100, 300, 500, 700 and 900 IJs / termites. A total of 25 termites were introduced after nematode introduced into the plate.

Statistical Analysis

The per cent mortality data after corrections were subjected to probit analysis for calculating mean lethal concentrations (LC_{50}, LT_{50}) [13]. Results were corrected for control mortality by using Abbott's formula [14].

Result and Discussion

R. flavipes exposed to the three EPNs exhibited increase mortality within two days. *S. pakistanense* was effective against *R. flavipes* 100% mortality was caused 700 and 900 IJs /ml. *S. siamkayai* was effective at the concentrations of 500IJs, only 100% within two days (Fig.1). *H. indica* recorded maximum mortality 65% was observed. As the level of IJs concentration increases, time of termite mortality also correspondingly increased. According to Poinar & Georgis [15] stated that *S. carpocapsae* caused 80-87% mortality of termite *Reticulitermis* spp. at the concentration of 200IJs. In our study *Steinernema* spp. caused 80-100% at the concentration of 100IJs. Murugan stated that [16] within one day there was 100% mortality after the combined with NSKE and *S. glaseri* at the concentration of 150IJs - 600IJs was observed. Our studies also supported according to above mentioned data within one day *Steinernema* species have the potential to cause cent percent mortality against *Reticulitermis* sp.

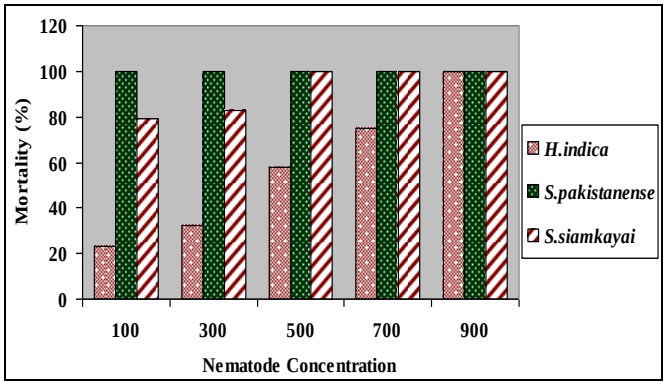

Fig 1: Percentage mortality of *R. flavipes*

Study on the pathogenicity of three native entomopathogenic nematodes, *Heterorhabditis* sp. *Steinernema* spp. by dose response and time exposure assay and determination of lethal dosages (LD_{50}) and lethal time (LT_{50}) was performed. LD_{50} and LT_{50} were calculated using four densities of test EPNs viz., 100,300, 500, 700 and 900 IJs/ml and were calculated. Although the termite was susceptible to test EPNs, there were differences among these EPNs in their ability to kill the insect. Among test EPNs, S. *pakistanense* appears to be the most promising. The per cent mortality of termites increased with the increase the dosages as well as exposure time. A positive correlation was found between dosages as well as exposure time and mortality. Probit analysis indicated that *S. pakistanense* required less number (5 IJs/termites) and less time (20h), whereas *H. indica* took more time (78.3 hrs) for desired mortality with less number (4IJs/termites) of *R. flavipes* (Table 1). Similar species of *H. indica* was studied at 400 infective juveniles per termite resulted in 58 and 73% mortality of *R. flavipes* was reported which support our studies that *H. indica* cause mortality at low rate [17]. Time taken to kill the host larvae varies from species to species, depending on the virulence, may be the probable reason for delayed mortality of *R. flavipes* by *H. indica*. The subterranean termite species have unusual behavioral habits and temperature preferences. EPNs were shaken off the legs of subterranean termites of the genus *Reticulitermes* and *Coptotermes* before the IJs were able to move toward the host's mouth area was reported [18].

Table 1: LD_{50} and LT_{50} EPNs on *R. flavipes*

Nematodes species	Isolates	IJs Dosage/ termite	LD_{50} (No. of IJs/termite)	LT_{50} (Time in hour)
S. siamkayai	**Kar 211**	100	5.01	30.8
		300	5.20	21.2
		500	5.50	18.7
		700	5.84	16.2

		900	6.23	13.1
S. pakistanense	**Kar 05**	100	5.28	20.1
		300	5.61	17.3
		500	5.84	14.8
		700	7.37	12.7
		900	7.37	9.8
H. indica	**Kar 707**	100	4.23	78.3
		300	4.53	61.7
		500	5.13	44.9
		700	5.67	41.8
		900	5.85	20.3

The isolate nematodes studied demonstrated pathogenicity against termites not being mortality dose-dependent, it dependents on strains the mean values varying from 55% to 100% within 24hrs (Figure 2). Three species have the competent to kill the termites within 48h. *S. pakistanense* was found 100% mortality within one day after exposure to dose of 250IJs. *S. pakistanense* found that more pathogenic than *S. siamkayaii* and *H. indica* working on termite. *O. horni* found that 100% mortality occurred within one day after exposure to *S. pakistanense* at the concentration of 100IJs/ml and within two days in the case of *S. siamkayaii* and *H. indica* were observed. Few researchers only worked on species *S. pakistanense* against termites. This result is in agreement with report [19] by Shahina who tested subterranean termite showed 96% mortality in sand assay method at the concentration of 200IJs/ml. When considering foraging strategy, *Steinernema* is an ambushing nematode [20]. Which nictates (stands on its tail and waves most of its body off the surface of the substrate) readily and is most effective at infecting highly mobile insects. *O. hornei* is an active termite, very susceptible to infection by nematodes. Variation of nematodes depends on the species and strains and also species specific symbiotic bacteria. Moreover, behavioural, morphological and physiological defense strategies of insects may also affect the nematode ability to infect and multiply [21].

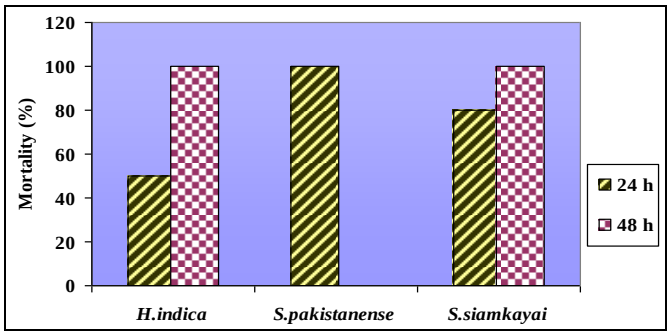

Fig 2: Percentage mortality of *O. hornei*

Table 2: LD_{50} and LT_{50} of *Steinernema* spp. and *Heterorhabditis* sp. on *O. hornei*

Nematode species	LD_{50} (No. of IJs/ termite)	LT_{50} (Time in hour)
S. pakistanense	5.84	15.5
S. siamkayai	5.68	16.3
H. indica	5.00	19.8

Lethal doses (LD50) values ranged from 5.84 to 5.00 IJs/ termites (both *Sterinernema* spp. and *Heterorhabditis* sp. respectively). Lethal time values at the dose of 25IJs/termites ranged from 15.5 to 19.8 hrs among the control of termites (Table2). LT_{50} obtained demonstrated that the termites mortality increased very short duration but affecting the reproductive potential of nematodes inside the host.

R. flavipes and *O. hornei* showed brick red color. *H. indica* was seen through the cuticle of dead *R. flavipes* and *O. horni* after 24 and 48h after inoculation under dissecting microscope. Nematodes began to emerge at 5 d after infestation. Average numbers of infective juveniles produced from *R. flavipes* (per worker) was highest *S. pakistanense* 1800IJs and lowest *H. indica* 295±12 IJs (Fig.3). There was evidence in this study that *H. indica* against *R. flavipes* showed same 289±50 IJs was studied by [22] and *O. horni* (workers, respectively) were *S. pakistanense* 1600 IJs, *S. siamkayai* 400 IJs and *H. indica* 185 IJs (Fig.4) respectively.

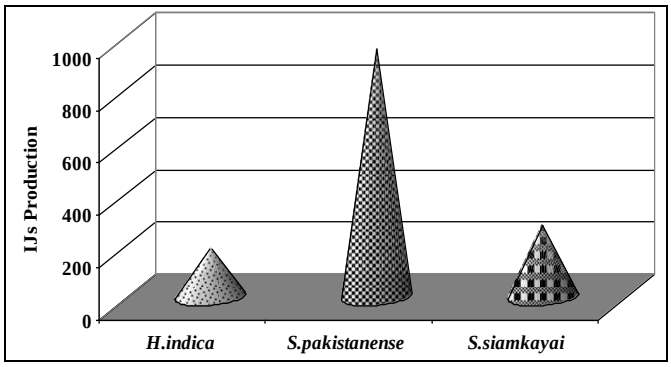

Fig 3: Reproduction of nematodes (*R. flavipus*)

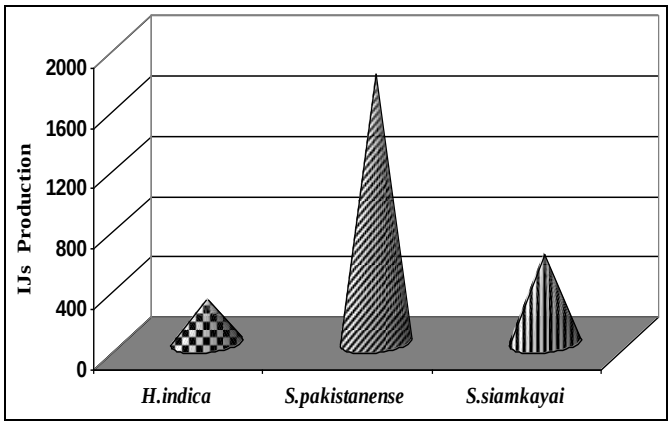

Fig 4: Reproduction of nematodes (*O. hornei*)

The scientific reason in rate of production was reduced due to their size, number of IJs penetration and in addition to that mite *Australhypopus* sp. (Acari: Acaridae) is very common on the *R. flavipes* body [23]. Rate of nematode production depends on number of IJs penetration into the host. The less productivity of IJs by *Steinernema* can be explained as probably being due to the body size of the nematode and proportion of the available food material of host compared to the other insect species [24]. It was hypothesized that among three species of EPNs to find out the potential in terms of dosage dependent and time dependent. Therefore above study revealed S. *pakistanense* as at different dose levels is effective on both two different species of termites. The study has showed that *S. pakistanense* have a potential stains that may be exploited for the sustainable management of termites along with other suitable strategies in integrated pest management.

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