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RESEARCH

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Biocontrol potential of the entomopathogenic nematodes (Rhabditida: Steinernematidae and Heterorhabditidae) against the termite species, *Microtermes obesi* (Holmgren) (Blattodea: Termitidae)

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Abstract

Background: Globally, termites cause a great problem for the farmers which eat the stalk of the wheat, maize, and the sugarcane and also affect the growth of vegetables. For ecological farming system, biocontrol agents represent a vital implement in pest management policies. Biological control can play a probable, even more considerable part to reduce the adverse possessions of synthetic chemical pesticides.

Results: The present study aimed to evaluate the biocontrol potential of one heterorhabditid species and 4 steinernematid species against the termite species (*Microtermes obesi* (Holmgren) (Blattodea: Termitidae)) on filter paper bioassay and wooden logs. Among all tested species of the entomopathogenic nematodes, maximum mortality rates 100% were obtained by using *Steinernema pakistanense* N-KA.04 and *S. bifurcatum* N-KA.93 after 48 h of the application. *S. siamkayai* N-KA.12 mortality ranged (85–87%), followed by *S. ceratophorum* N-KA.57 (77–80%) and then *Heterorhabditis indica* N-KA.03 (70–77%) at the highest concentrations (350–650 IJs/ml).

Conclusions: The expansion of appropriate application products for the management of termite effectiveness, observing and timing of application, participation of farmers in checking, and judgment is essential to avert disasters of entomopathogenic nematodes (EPNs) in field conditions and to make farmers' confident with the use of the most promising species of EPNs.

Keywords: Termite, *Microtermes obesi*, Heterorhabditis, Steinernematid, Biocontrol

Background

Biological control program establishes a more ecologically tolerable substitute to traditional chemical control procedures. When efficaciously executed, it can yield long lasting, economical controlling pest populations with negligible ecofriendly disruption. Entomopathogenic nematodes (EPNs) have been found parasitizing the species in the orders of Coleoptera, Diptera, Hemiptera, Hymenoptera, Orthoptera, Lepidoptera, Siphonaptera, Thysanoptera, and Isoptera. These nematodes deal with an ecologically innocuous substitute to chemical insecticides in the controlling of termites (Khan et al. 2016).

Microtermes obesi (Holmgren) (Blattodea: Termitidae) is one of the most communal termite species of Pakistan. It has been found aggressive forest plants as well as timbers which are used as building materials; doors and windows and ventilators are commonly devastated (Manzoor et al. 2014). Synthetic pesticides which are used for the control of agricultural pests are directly board the human

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vigor triggering serious syndromes such as cancer and they are more expensive than natural products (Bounias, 2003). Termites forage and live in environments that are humid, cool, and without direct sunrays such as wood materials or soil. These ecofriendly situations are perfect for the existence and association of steinernematid and heterorhabditid nematodes and afford the origin for the curiosity in their role in control of subterranean termites (Wang et al. 2002). The performance of EPNs for successful control of target insect pest is dependent on the motility and perseverance of the infective juveniles (IJs).

The aim of the present study was to evaluate the biocontrol potential of 5 species of EPNs against the termite species, *M. obesi.*

Methods

Collection of Microtermes obesi

M. obesi termite's colonies were collected from infested trees of Gulshan-e-Maymar, Gadap Town (25° 7′ 55′′ North, 67° 13′ 57′′ East) Sindh, Karachi, Pakistan.

Surveys for entomopathogenic nematodes

Collected EPN species from soil samples (of 500 gm) of Pakistani localities as Karachi, Sindh of Pakistan were identified and confirmed on supervision of NNRC, Uok. All nematodes' isolates were propagated in larvae of *Galleria mellonella* L. greater wax moth. Infective progenies were harvested by White traps (White, 1927), collected, and maintained in 50 ml beaker having distilled water at 12 °C for 15 days to performed experiment.

Filter paper bioassay

Filter paper bioassay was conducted in Petri dishes (90 cm) lined with filter paper Whatman No. 1, with 20 termites in each dish. Nematode species in 3 ml were inoculated with 3 different concentrations of infective juvenile at 150, 250, and 350 IJs/ml. Control treatments received 3ml water without nematodes. Petri dishes were wrapped with Parafilm and kept in chamber maintained at 30 °C and 80% RH.

Spray on infested logs

Ten inch pieces of wooden logs of trees infested with 50 termites were placed in a plastic container (28 × 16 × 8 cm), each EPN species was sprayed by 3 different concentrations; 250, 450, and 650 IJs/ml, separately in 20 ml water suspension. Containers were protected by aerated lids and incubated at 25 °C. Control trial with infested wooden logs was sprayed only with 20 ml water.

Replication and mortality evaluation

Three replicates were used for each species and set of experiment. After 48 h of application, dead cadavers of each treatment were transferred separately to White traps for assessment of mortal source and confirmation by emergence of nematode progeny.

Data analysis

SPSS statistical software, 2002 was used to analyze ANOVA, analysis of variance; significant differences in treatments were elucidated through Duncan's multiple range test ($P \leq 0.05$) (Duncan, 1955).

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Results

Surveyed entomopathogenic nematodes

EPNs; *H. indica* N-KA.03, *S. pakistanense* N-KA.04, *S. siamkayai* N-KA.12, *S. ceratophorum* N-KA.57, and *S. bifutcatum* N-KA.93 were recovered from soil samples (500 gm) collected from different localities of Karachi, Sindh, Pakistan as mentioned in Table 1.

Filter paper bioassay

In filter paper bioassay with 20 termites per Petri dish, the corrected mortalities were significantly affected by using EPN species (F = 51.032, df = 3, 24, P < 0.001) and the tested concentrations (F = 7.247, df = 2, 24, P = 0.003), with a significant interaction between the 2 factors (F = 2.674, df = 6, 24, P = 0.039). Control treatment showed non-response against termite. *S. pakistanense* N-KA.04 and *S. bifurcatum* N-KA.93 showed the highest mortality rates up 100% at 250 and 350 IJs/ml, with non-significant difference. The second highest mortal rate of termite achieved by *S. siamkayai* N-KA.12, followed by *S. ceratophorum* N-KA.57and *H. indica* N-KA.03 at the 3 tested concentrations after 48 h (Fig. 1).

Spray on infested logs

In the spray method on infested wooden logs, 50 termites were treated by different EPN species at different concentrations. The corrected mortalities of the termite were significantly influenced by both the EPN species (F = 48.15, df = 3, 24, P < 0.001) and their concentrations (F = 10.20, df = 2, 24, P = 0.004), with a significant interaction between the 2 factors (F = 1.05, df = 6, 24, P = 0.029). All the 5 species of EPNs were found significantly effective against termites by spray on infested logs (Fig. 2). No mortality response was found in the control treatment. Maximum mortality 100% was observed by *S. pakistanense* (ANOVA: F = 64.7; df = 2, 9; P < 0.01) and *S. bifutcatum* (ANOVA: F = 14.5; df = 2, 9; P < 0.01) at 250 and 350 IJs/ml after 48 h of application (Fig. 2). *H. indica* (ANOVA: F = 76. 02; df = 2, 9; P < 0.01) showed the least mortality rate as than the other EPN species, when a maximum mortality rate (77%) was achieved at 350 IJs/ml. On both methods, increase of EPNs' concentration significantly increased the mortality rates of the termites

Table 1 Surveyed localities and host for collection of entomopathogenic nematodes

Localities	Coordinates	Host	Sample code	EPNs species
Nursery park No. 5 Liaquatabad	24° 54′ 8″ N 67° 3′ 3″ E	*Cynodon dactylon*	N-KA.03	*Heterorhabditis indica*
Nursery Liaquatabad Dak khana	24°54′ 8″ N 67°3′ 3″ E	*Carica papaya*	N-KA.04	*Steinernema pakistanense*
Nursery park No. 2 Liaquatabad No. 8	24° 54′ 8″ N 67°3′ 3″ E	*Rosa indica*	N-KA.12	*S. siamkayai*
Askari Park Purani Sabzimandi	24° 53′ 41″ N 67° 3′ 47″ E	*Cynodon dactylon*	N-KA.57	*S. ceratophorum*
Korangi 5/1.2 Model park	24° 46′ 59.99″ N 67°07′ 60″ E	*Zoysia japonica*	N-KA.93	*S. bifurcatum*

Discussion

Use of different species of EPNs for pest control that occur in cryptic habitats including termites is a rapid, defensible, ecologically nontoxic, and cost-effective method (Vashish et al. 2013). They are capable to reprocess in the atmosphere, are willing to genetic selection for desirable traits, and are exempt from registration in many countries (Shahina et al. 2017). The nematodes can be practical with customary spray equipment under field conditions (Hazir et al. 2004). Numerous trials revealed the efficiency of EPNs to control termites. Laboratory trials with termites and EPNs were generally tested out in Eppendorf tubes, packed cell volume (PCV) tubes, containers, Petri dishes bottom with sterile sand, or wet filter paper. For termites, under all circumstances, corrugated wood blocks and straw piece of filter paper are commonly used in to serve as nourishment (Baïmey et al. 2017). In the present investigation, 1 heterorhabditid and 4 steinernematid species showed the best biocontrol potential as compared to other species. Similar results were found by same species against *Coptotermes heimi* (Tabassum and Salma, 2020). In sand assay method, *S. pakistanense* showed also significant results, causing 100% mortality of *Reticulitermes flavipes* and *Odontotermis hornei* within 24 h as reported by Razia and Sivaramakrishnan (2016). Wang et al. (2002) reported that *H. indica* was more efficient against *R. flavipes*. Zadji et al. (2014) tested the pathogenicity against the workers of *Macrotermes bellicosus* with 1 *H. indica* and 29 Benin isolates of *H. sonorensis* in Eppendorf tubes. The outcomes of the trial presented that 73% of the nematode isolates killed more than 80% of the termite. In the present study experiment, mortality of *H. indica* N-KA.03 reached up to 77% at the highest concentration. Jawad et al. (2020) tested *S. carpocapsae*, *H. bacteriophora*, and *H. bacteriophora* (IRQ.1 strain) against *Microcerotermes diversus* using 6 concentrations conducted in filter paper and wood bioassays. The percent mortality caused by native *H. bacteriophora* against termites was higher showed 43.6 ± 2.7% than both commercial strains of *S. carpocapsae* and *H. bacteriophora* 36.9 ± 1.6% and 29.9 ± 1.4% respectively.

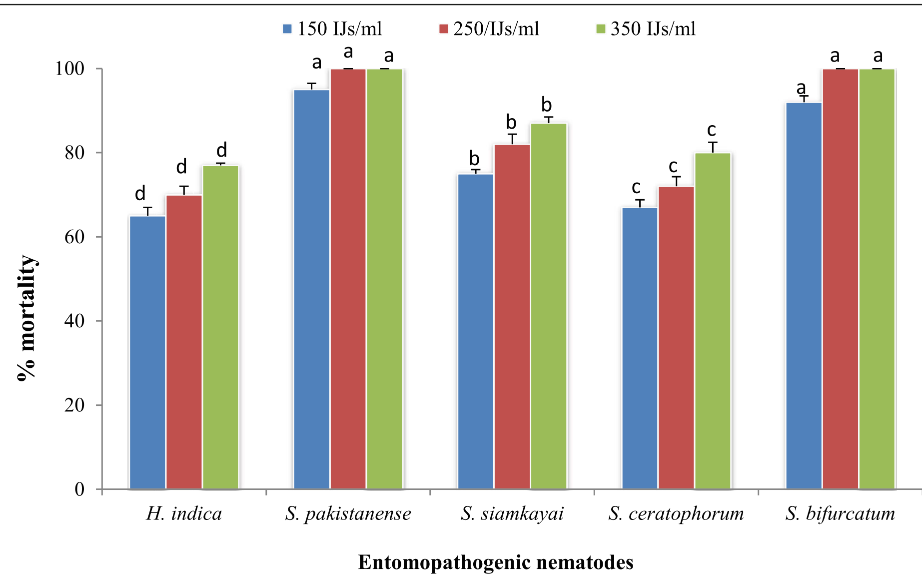

Fig. 1 Biocontrol potential of entomopathogenic nematodes against *Microtermes obesi* on filter paper

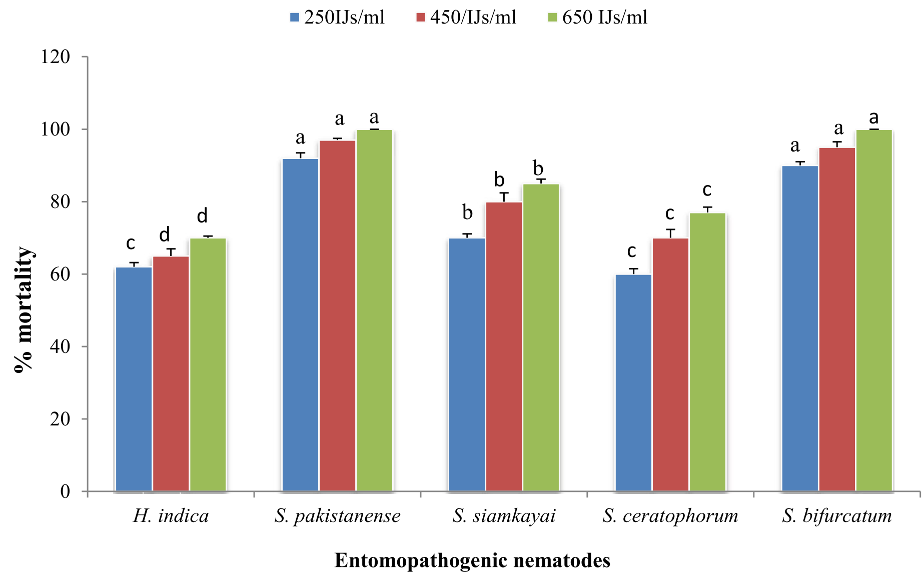

Fig. 2 Biocontrol potential of entomopathogenic nematodes against *Microtermes obesi* on wooden logs

After EPNs' application, termite shell can be remodeled being ruined subsequently, so nematode persistence in the nest zone can obligatory evading regular disruptions. For fruitful management, repeated applications of the EPNs must acquire (Baïmey et al. 2017).

Conclusion

Practical application of effective species of EPNs is obligatory for environmentally sound and sustainable termite control alternative to synthetic chemicals.

Abbreviations

EPNs: Entomopathogenic nematodes; IJs: Infective juveniles; ANOVA: Analysis of variance; *M. obesi*: *Microtermes obesi*

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Authors' contributions

AA carried out the experiment, designed and wrote the manuscript, SJ & TAK analyzed the data. All authors read and approved the final manuscript.

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