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Virulence of four *Steinernema* species as a biological control agent in controlling the termite, *Coptotermes heimi* (Wasmann) (Isoptera: Rhinotermitidae)

Tabassum Ara Khanum and Salma Javed*

Abstract

Subterranean termites are an ancient group of social insects, broadly spread, known primarily as economically important pests for the destruction of wooden structures and also as agricultural pests. Many of the banned chlorinated hydrocarbon insecticides used to be recommended for the control of termites. Hence, it has become necessary to find alternative measures for termite control in the natural diverse habitats as well as in the cultivated soil to diminish use of these chemicals. Therefore, in the present study, 4 strains of entomopathogenic nematodes (EPN) belong to the genus *Steinernema* were assessed against *Coptotermes heimi* (Wasmann). These EPN included *Steinernema pakistanense* NNRC-AS.04, *S. siamkayai* NNRC-As.12, *S. bifurcatum* NNRC-As.65, and *S. maqbooli* NNRC-As.88. Virulence of all strains was determined at 3 different EPN inocula in plastic containers layered with sand. A significant nematode inoculum effect was detected for all the tested EPN species. NNRC-AS.04 and NNRC-As.65 showed the highest virulence effects of 95 and 100%, respectively at 150 IJs/ml.

Keywords: Entomopathogenic nematode, *Coptotermes heimi*, Virulence, Biocontrol, *Steinernema* spp

Background

Worldwide, termites are a massive dilemma in both urban and agricultural areas, as they are the source for considerable devastation to plants, agricultural crops, wood structures, and account for financial loss. High-quality wood products are often preferred by customers, but physical or biological damages reduce their worth (Uzunovic et al. 2008). The incidence of termites is habitually not eagerly observed as of concealed behavior. They act as decomposers as well as herbivores feeding on a spacious variety of dead, rotten, or fresh plant material (Traniello and Leuthold 2000 and Bignell and Eggleton 2000). *Coptotermes heimi* (Wasmann) (Isoptera: Rhinotermitidae) has been reported from urban and agricultural fields of Pakistan as a serious pest (Manzoor and Mir 2010 and Manzoor et al. 2011). Successful management for termite colonies needs many particular skills depending on the species origin invasion. Knowledge of termite ecology and its identification can help to spot damage and ways of control (Khan et al. 2016). Chemical troubles made a big impact on the agricultural society and drew attention to the use of biocontrol agents as a safe and effective biopesticide alternative. It can be used in many diverse agricultural systems rather than an immediate solution. Therefore, biocontrol should be considered as a long-term research aim. The two important nematode families falling in the group of entomopathogenic nematodes (EPNs) are Steinernematidae Travassos, 1927, and Heterorhabditidae Poinar, 1975, which are considered as one of the most successful examples of biological tools used to control

* Correspondence: sajaved@uok.edu.pk
National Nematological Research Centre, University of Karachi, -75270, Karachi, Pakistan

soil dwelling insect pests. They possess virtually all the attributes of an ideal biological control agent. They enter the host through the natural openings such as mouth, anus or spiracles, or sometimes by abrasing the intersegmental membranes of the insect cuticle (e.g., *Heterorhabditis* spp.) (Grewal et al. 2005), reach the hemocoel, release there the cells of symbiotic bacteria from their intestine, which ultimately results in killing the host within 48 h (Askary 2010; Askary 2012). Because the nematode symbiotic bacterium kills insects, so quickly, there is no intimate host parasite relationship as characteristic for other insect parasitic nematodes (Shapiro-Ilan et al. 2012).

The aim of this research was to assess the virulence of EPNs, *Steinernema* species against *C. heimi*, under laboratory conditions.

Materials and methods

Target pest

Alive colonies of *C. heimi* were collected from infested fallen wooden logs of *Mangifera indica* (Sapindales: Anacardiaceae) from main campus University of Karachi, Karachi, Pakistan (24.9418° N, 67.1207° E) and identified with a help of the key of (Akhtar 1983). The termite colonies were bought to the culturing room of National Nematological Research Center (NNRC), University of Karachi (Uok), Karachi, Pakistan and maintained in 1000 ml plastic containers with wooden logs at 28 °C and 75–80% RH till the experiment was executed.

Entomopathogenic nematodes

Four species of EPN, *Steinernema pakistanense* NNRC-AS.04 (Shahina et al. 2001), *S. siamkayai* NNRC-As.12 (Stock et al. 1998), *S. bifurcatum* NNRC-As.65 (Shahina et al. 2014), and *S. maqbooli* NNRC-As.88 (Shahina et al. 2013), were reproduced on last larval instar of the greater wax moth, *Galleria mellonella* L. (Lepidoptera: Pyralidae), using the method of Dutky (1964). White traps (White 1927) were used for the collection of infective juveniles (IJs) of each nematode species and stock up separately in a flask (Pyrex) of a 100 ml with 70 ml distilled water at 10 °C. Infective juveniles were stored for 15 days to employ for experiment.

Virulence assay

Active *C. heimi* termite individuals were collected from a rearing container for virulence assay of 4 EPN species in a plastic container (280 × 160 × 80 mm) separately for each nematode species and concentration. Containers were layered with 45-g sterilized moist soil. Twenty termites were added in each container exposed to 3 different numbers of nematodes viz., 50, 100, and 150 IJs/ml in 2.5 ml distilled water suspension covered with a plastic lid. Concentrations were dropped evenly in containers by a 1000 μl pipette, to evade mingle sterile pipette tips that were changed after each conduct. Simple distilled water 2.5 ml was dispensed in a control treatment. Mortality rate was calculated after 48 h of exposure and the containers were kept at 28 ± 2 °C. Dead termites were transferred in a plastic cavity block (4.5 × 4.5 cm) layered with moist filter paper disk (Whattmann No. 1) to record a nematode emergence. Experiment was carried out twice with 3 replicates at each concentration and EPN species.

Statistical analysis

Data were subjected to analysis of variance in SAS (ver. 9.1, SAS Institute, Cary, NC). If the interaction in EPN species and numbers was significant, it was used to explain results. If the interaction was non-significant ($P < 0.05$), means were separated with DMRT Duncan's multiple range test (Duncan 1955). Lethal concentration 50 and 90% (LC_{50} and LC_{90}) values, intercept, and chi-square values were analyzed by PROC PROBIT routine of SAS, 2000. Abbott (1925) formula was used to correct mortality percentages as follows.

Mortality (%) = % mortality in treatment–% mortality in control × 100% mortality in control

Results and discussion

The comparative virulence assay of the 4 EPN species against *C. heimi* termite was investigated plastic container layered with 45-g sterilized moist soil at 28 ± 2 °C in laboratory of NNRC conditions. The analysis of variance showed significant differences among nematode species efficacy on termites (ANOVA $F = 201.5$; df = 3; $P = 0.05$). Nematode inocula also differed significantly (ANOVA $F = 6.6$; df = 3; $P = 0.05$) and interaction of the 3 inocula with 4 nematode species, also had marked effect on the pest (ANOVA $F = 0.8$; df = 3; $P = 0.05$). Results demonstrated that the nematode could suppress the populations of *C. heimi* termite. *S. pakistanense* NNRC-AS.04 and *S. bifurcatum* NNRC-As.65 showed higher effects at all application concentrations than *S. siamkayai* NNRC-As.12 and *S. maqbooli* NNRC-As.88. The highest mortality rate was achieved when EPNs were applied at the concentrations of 150 IJs/ml after 48 h (Fig. 1). At the lowest concentration of 50 IJs/ml after 48 h of exposure time, at least 50% of termite were killed by *S. pakistanense* NNRC-AS.04, showing significant differences with *S. siamkayai* NNRC-As.12 30% and *S. maqbooli* NNRC-As.88 25%. Concentrations had a great impact on the

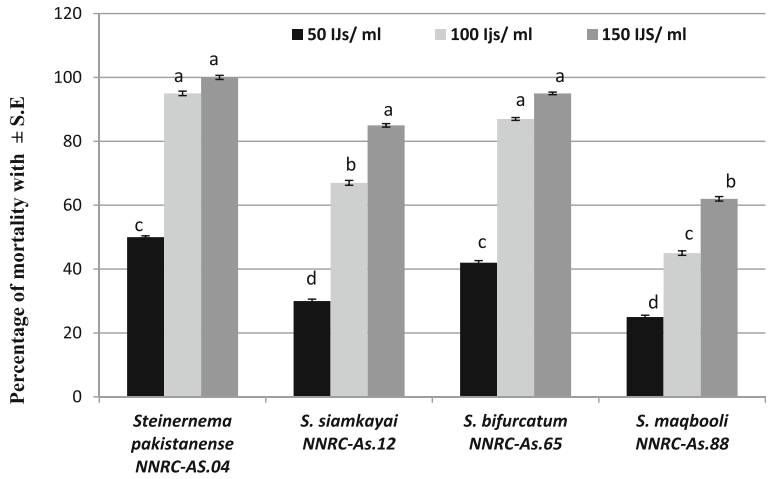

Fig. 1 *Coptotermes heimi* mean mortality treated with four different species of entomopathogenic nematodes in plastic containers depending on their concentration.

efficacy of species of nematode (Trdan et al. 2009). The increased mortality of termite caused was concentration dependent. At 150 IJs/ml, the highest mortality rate was induced by NNRC-AS.04 (95 and 100%) by NNRC-As.65, while the lowest one was (62%) by NNRC-As.88. Nematode progenies can reproduce in termites, they were clearly seen when dead termites were transferred to vacant cavity block. The LC_{50}, LC_{90} values with *P* value are shown in Table 1. The overall result showed that *S. pakistanense* and *S. bifurcatum* were highly virulent against the target subterranean termite, *C. heimi* 48 h after application. If nematode reproduction can occur in the target insect, long-term management might be achievable. Similar results of maximum mortality response against termite species *Macrotermes* in a sand and filter paper assay caused by *S. pakistanense* were also stated by Shahina and Tabassum (2010). Razia and Sivaramakrishnan (2016) evaluated 3 species of EPN, *S. siamkayai*, *S. pakistanense*, and *H. indica* against subterranean termites, *Reticulitermes flavipes* and *Odontotermis hornei* under laboratory conditions. In sand assay method, *S. pakistanense* showed significant results, causing 100% mortality of both pests within 24 h, followed by *S. siamkayai* and *Heterorhabditis indica*, which were applied at 250 IJs/ml at 48 h. Wilson-Rich et al. (2007) reported that *Zootermopsis angusticollis* termite of wet timber showed a susceptibility to concentration-dependent by *S. carpocapsae* (Mexican strain). Under field conditions, few research studies have been accomplished, using EPNs as a biocontrol agent for termites (Dolinski and Lacey 2007). For the pathogenicity against the termite, *Macrotermes bellicosus* one strain of *H. indica* and 29 strains of *H. sonorensis* (Beninese) were tested by Zadji et al. (2014) and reported that 73% of the isolates parasitized more than 80% of the termite and was influenced by a grouping of biotic and abiotic factors, nematode strain and species. Divya and Sankar (2009) reported 50% mortality of the termite species, *Odontotermes obesus* after 36 h of post *H. indica* application. Termites, strained by chemical and pathogen sub-lethal doses, were more vulnerable by EPNs (San-blass and Gowen 2008). Termites exist and feed in environment that are cool, damp, and with no direct sunlight such as wood materials or soil. These ecological surroundings are perfect for the endurance and association of EPNs, providing the source for the concerned pest management. Investigations suggested that the nematodes are effective to control termite colonies as environmentally secure approach (Askari et al. 2012). During the last 30 years of research, EPNs gain common acceptance, and their

Table 1 Median lethal numbers (LC_{50}, LC_{90}) of four entomopathogenic nematodes against *Coptotermes heimi*

EPN species	LC_{50}	LC_{90}	Intercept	Chi-square	***P*** value
Steinernema pakistanense NNRC-AS.04	48.32	96.2	3.706	0.0034	0.9533
S. siamkayai NNRC-As.12	77.67	158	3.767	0.0162	0.0162
S. bifurcatum NNRC-As.65	54.4	117.94	3.906	0.0859	0.7695
S. maqbooli NNRC-As.88	116.08	245.53	3.85	0.05	0.8225

commercial applications are being developed as environmentally alternatives to chemical pesticides further noticed in decision of nematodes as better application methods against subterranean termites (Khan et al. 2016). In the fields, species of EPNs that are noted as heat tolerant or environmental stress are requisite for management of termites.

Conclusion

Obtained results are evidence that EPNs can be efficient tactics to control the termites but further studies under field conditions are required.

Abbreviations

EPNs: Entomopathogenic nematodes; IJs: Infective juveniles; LC: Lethal concentration; NNRC: National Nematological Research Center; Uok: University of Karachi

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Authors' contributions

SJ and TAK performed the experiment. SJ designed and wrote the text. TAK analyzed the data.
Both authors read and approved the final manuscript.

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References

Abbott WS (1925) A method of computing the effectiveness of insecticide. J Econ Entomol 18:265–267
Akhtar M (1983) Wood destroys termites (Isoptera) of Pakistan: key to the most important species, their distribution and pattern of attack. Material U–Organismen 18:278–291
Askary TH (2010) Nematodes as biocontrol agents. In: Lichtfouse E (ed) *Sociology, Organic Farming*. Climate Change and Soil Science. Springer, The Netherlands, pp 347–378
Askary TH, Khan AA, Waliullah MIS, Banday SA, Iqbal U, Mir MM (2012) Slug pest management through nematodes in agricultural and horticultural crops. In: Boeri F, Chung JA (eds) Nematodes: Morphology. Functions and Management Strategies. Nova Publishers, New York, pp 197–211
Bignell DE, Eggleton P (2000) Termites in ecosystems. Termites: Evolution, Sociality, Symbioses, Ecology (eds T. Abe, D.E. Bignell & M. Higashi), pp. 363-387. Kluwer Academic Press, Dordrecht, the Netherlands
Divya K, Sankar M (2009) Entomopathogenic nematodes in pest management. Indian Journal of Science and Technology 2:53–60
Dolinski C, Lacey LA (2007) Microbial control of arthropod pests of tropical tree fruits. Neotropical Entomology 36:161–179
Duncan DB (1955) Multiple range and multiple F-test. Biometrics 11:1–41
Dutky SR, Thompson JV, Cantwell GE (1964) A technique for the mass production of the DD-136 nematode. J Insect Pathology 6:417–422
Grewal PS, Ehlers RU, Shapiro-Ilan DI (2005) Nematodes as biocontrol agents. CAB International, Wallingford, UK
Khan MA, Ahmad W, Paul B, Paul S, Khan Z, Aggarwal C (2016) Entomopathogenic nematodes for the management of subterranean termites. In: Hakeem KR, Akhtar MS, Abdullah SNA (eds) Plant, Soil and Microbes, vol 1. Implications in Crop Science. Springer International Publishing, Switzerland
Manzoor F, Beena W, Malik S, Naz N, Naz S, Syed WH (2011) Preliminary evaluation of *Ocimum Sanctum* as toxicant and repellent against termite, *Heterotermes indicola* (Wasmann) (Isoptera: Rhinotermitidae). Pak J of Sci 63: 59–62
Manzoor F, Mir N (2010) Survey of termite infested houses, indigenous building materials and construction techniques in Pakistan. Pak J of Zool 42:693–696
Poinar GO Jr (1975) Entomogenous nematodes. A manual and host list of insect-nematode associations. E.J. Brill, Leiden, The Netherlands, 317 pp
Razia, Sivaramakrishnan (2016) Evaluation of entomopathogenic nematodes against termites J Entomol and Zool Stud 4:324–327
Shahina F, Anis M, Reid AP, Rowe J, Maqbool MA (2001) *Steinernema pakistanense* sp. n. (Rhabditida: Steinernematidae) from Pakistan. Int J Nematol 11:124–133
Shahina F, Tabassum KA (2010) Virulence of *Steinernema pakistanense* against different insect species in laboratory condition. Pak J Nematol 28:279–284
Shahina F, Tabassum KA, Mehreen G, Salma J (2013) *Steinernema maqbooli* n. sp. (Rhabditida: Steinernematidae) a species of the 'bicornutum' group from Pakistan. Int J Nematol 23:59–72
Shahina F, Xun Y, Lihong Q, Richou H, Mehreen G, Tabassum AK, Salma J (2014) A new entomopathogenic nematode, *Steinernema bifurcatum* n. sp. (Rhabditida: Steinernematidae) from Punjab, Pakistan. Nematol 16:821–836
Shapiro-Ilan DI, Han R, Dolinksi C (2012) Entomopathogenic nematode production and application technology. J Nematol 44:206–217
Stock SP, Somsook V, Reid AP (1998) *Steinernema siamkayai* n. sp. (Rhabditida: Steinernematidae) an entomopathogenic nematode from Thailand. Syst Parasitol 41:105–113
Traniello IFA, Leuthold RH (2000) Behavior and ecology of foraging in termites. In: Abe T, Bignell DE, Higashi M (eds) *Termites evaluation*, *Sociality*, *Symbioses*, *Ecology*. Kluwer Academic, Dordrecht, pp 36–409
Travassos L (1927) Sobre o genera *Oxysomatium*. *Boletín Biologico* 5:20–21
Trdan S, Vidrih M, Andjus L, Laznik Ž (2009) Activity of four entomopathogenic nematode species against different developmental stages of Colorado potato beetle, *Leptinotarsa decemlineata* (Coleoptera, Chrysomelidae). Helminthologia 1:14–20
Uzunovic A, Byrne T, Gignac M, Yang DQ (2008) Wood discolorations and their prevention, with an *Ephasis* on *Bluestain*. In: Special Publication SP-50. Canada, FP Innovations, Vancouver, British Columbia
White GF (1927) A method for obtaining infective nematode larvae from cultures. Science 66:302–303
Wilson-Rich N, Stuart RJ, Rosengaus RB (2007) Susceptibility and behavioral responses of the dampwood termite *Zootermopsis angusticollis* to the entomopathogenic nematode Steinernema carpocapsae. J Invert Pathol 95:17–25
Zadji L, Baimey H, Afouda L, Moens M, Decraemer W (2014) Characterization of biocontrol traits of heterorhabditid entomopathogenic nematode isolates from South Benin targeting the termite pest *Macrotermes bellicosus*. Bio Control 59:333–344

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