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

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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...
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 abrading 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 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 (IJJs) 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 (Whatmann 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.

\[
\text{Mortality} = \frac{\% \text{ mortality in treatment} - \% \text{ mortality in control}}{\% \text{ mortality in control}}
\]

**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
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 Tabasum (2010). Razia and Sivaramakrishnan (2016) evaluated 3 species of EPN, S. siamkayai, S. pakistanense, and H. indica against subterranean termites, Reticulitermes flavipes and Odontotermis horniei 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

![Fig. 1 Coptotermes heimi mean mortality treated with four different species of entomopathogenic nematodes in plastic containers depending on their concentration.](image)

| 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

Acknowledgements
Not applicable

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.

Funding
No funding

Availability of data and materials
All data and materials mentioned in the manuscript

Ethics approval and consent to participate
Not applicable

Consent for publication
Not applicable

Competing interests
The authors declare that they have no competing interests.

Received: 27 January 2020 Accepted: 4 March 2020
Published online: 17 March 2020

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