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Biocontrol Potential of Entomopathogenic Nematodes against *Odontotermes obesus* (Blattodea: Termitidae) under Laboratory and Field Conditions

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Abstract: *Odontotermes obesus* (Blattodea: Termitidae) is a prevalent subterranean wood-eating termite species that causes damage to mature trees, saplings and seedlings. The efficacy of most synthetic insecticides against this notorious pest has been compromised primarily because of its enigmatic feeding behavior and development of resistance to a number of insecticides. It has therefore become necessary to explore other alternative biologically sound and low-impact termite control methods, particularly for use in forests. Hence, this study was designed to verify the efficacy of different indigenous EPN isolates (*Steinernema carpocapsae*, *Heterorhabditis bacteriophora* and *Heterorhabditis indica*) against workers of *Odontotermes obesus*. The pathogenicity of each nematode isolate was assessed in laboratory conditions using filter paper and sawdust bioassay at two different temperatures (16 ± 1 and 26 ± 1 °C). Additionally, the efficacy of the nematode species was also assessed in field conditions. The results of the experiments revealed that the mortality of termite workers was more pronounced in sawdust bioassay in comparison with filter paper bioassay at both the tested temperatures. The mortality response in both bioassays was more pronounced at the higher temperature. A significantly higher mortality was recorded at both tested temperatures for *S. carpocapsae* followed by *H. bacteriophora* and *H. indica*. A dose-dependent positive mortality response was also recorded at both tested temperatures. Similar to the laboratory trials, the field applications of the three tested nematode species showed that maximum mortality was recorded for *S. carpocapsae* followed by *H. bacteriophora* and *H. indica*. It was therefore concluded that indigenous EPNs can provide more effective control of termites, possibly because of their direct interaction with pest species in the soil and the possibility of causing secondary infection through infected cadavers.

Keywords: bioassay; entomopathogenic nematode; mortality; *Odontotermes obesus*; temperature

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1. Introduction

Termites are eusocial small insects which belong to the order Blattodea, and there are 3106 known species, 363 of which are considered pests of significant importance to humans [1–4]. Although termites provide many important ecological services, such as dung removal, recycling and breakdown of organic matter, enhancement of soil fertility and maintenance of biodiversity, they nonetheless inflict huge economic losses (0.5–11 billion USD per annum) to our forests, housing and agroecosystems [5–7].

Members of the genus *Odontotermes* are voracious feeders which consume a number of economically important plants including poplar, eucalyptus, shisham, pine and wattle tree [8]. Of these, *Odontotermes obesus* (Blattodea: Termitidae) is a prevalent wood-eating, mound-building, subterranean termite species found in Pakistan which inflicts damage on ground timber and standing trees [9,10]. In forest plantations, mature trees are most vulnerable to termite attack, but damage has also been caused to saplings and seedlings in the

form of root debarking and ring-barking, and has also been observed in rangelands [11,12]. However, the extent of damage to standing trees in forests is poorly understood [13].

The efficacy of most synthetic insecticides against this notorious pest has been compromised primarily because of its enigmatic feeding behavior and development of resistance to a number of insecticides such as bifenthrin, fipronil, cypermethrin and deltamethrin [14–16]. Furthermore, most conventional termite control tactics are abortive, are ecologically incompatible and do not verify the underground termite incursion. Therefore, it is necessary to explore other alternative biologically sound and low-impact termite control approaches, particularly for use in forests [17–20].

Entomopathogenic nematodes (EPNs) are naturally occurring members of the soil biota which provide useful biocontrol services in relation to a number of economically important arthropod pests belonging to different insect orders [21,22]. To date, approximately 116 nematode species belonging to two EPN families, Steinernematidae and Heterorhabditidae, have been reported, some of which have been successfully commercialized in different parts of the globe as biocontrol agents for insect pests [23–27]. The infective juveniles (IJs) of EPNs are widely distributed from high mountains to sea level, from agricultural to natural ecosystems and even in highly polluted soils in which arthropod pests can live and serve as hosts [28–31]. The IJs actively move within the soil profile in search of a suitable host and, once located, they penetrate its body via injuries or natural body openings such as the spiracles, mouth and anus. The IJs eventually reach the hemocoel and release bacterial symbionts within the host body, which then reproduce and generate various toxins and metabolites that kill the host through toxemia or septicemia [32–35]. Depending on nutrient availability, EPNs can complete various generations in the insect body before finally emerging from the host cadaver in the form of IJs to start their biological cycle over again [36,37].

Previous studies have reported the variable efficacy of entomopathogenic nematodes against subterranean termites. These variations in the efficacy of nematodes may be due to their varying pathogenic potential, the resistance potential of the target pest and the prevailing ecological conditions [38–40]. For example, Epsky and Capinera [41] concluded that *Steinernema carpocapsae* showed promising potential for the control of *Reticulitermes tibialis* in laboratory and field trials. However, the pathogenicity of EPNs against insect pests varies considerably depending on the insect species and its developmental stage [42–46]. Temperature is also an important abiotic factor which significantly affects the survival, pathogenicity and host-searching ability of various EPN species [47–52]. Low temperature usually diminishes the mobility and enzymatic activities of EPNs and thus significantly hinders the performance of IJs [53].

The use of indigenous biocontrol agents to target a specific pest is highly recommended because of their negligible ill effects on nontarget organisms. The slow action mechanism of EPNs also allows infected termite workers to transfer inoculum to naive colony members via certain social connections such as trophollaxis, caregiving and grooming [54]. These social connections also enhance the possibility of infecting queen nests that are usually present but away from the point of application [55].

Bearing in mind the above realities, this study was designed to verify the efficacy of different indigenous EPN isolates (*Steinernema carpocapsae*, *Heterorhabditis bacteriophora* and *Heterorhabditis indica*) against workers of *Odontotermes obesus*. The pathogenicity of each nematode isolate was assessed using filter paper and sawdust bioassays at two different temperatures (16 ± 1 and 26 ± 1 °C). Additionally, the efficacy of the nematode species was also assessed in field conditions. This study is novel because the potential of these Pakistani EPN isolates against *O. obesus* at two temperature ranges (16 ± 1 and 26 ± 1 °C) has not been previously assessed.

2. Materials and Methods

2.1. Insect Culture

2.1.1. Termites

A living colony of termites (*Odontotermes obesus*) was collected from infested fallen and standing timber logs from the *Dalbergia sissoo* plant in the Chichawatni Reserved Forest (30.5311° N, 72.6329° E), Punjab, Pakistan. The collected termite colony was brought to the Insect Biodiversity and Biosystematics Laboratory (IBBL), Department of Entomology, University of Agriculture, Faisalabad, Pakistan for culturing. The colony was maintained in plastic containers (80 cm × 70 cm × 70 cm) and offered pieces of wood in a dark chamber at 25 ± 1 °C and 65–70% relative humidity. Termite workers used in the experimental trials were collected using a modified aspirator.

2.1.2. Entomopathogenic Nematodes

Initially, infective juveniles (IJs) of *Steinernema carpocapsae, Heterorhabditis bacteriophora* and *Heterorhabditis indica* were obtained from the IBBL and then cultured on the final instar larva of the wax moth, *Galleria mellonella* L. (Lepidoptera: Pyralidae), following the procedures described by Woodring and Kaya [56]. The emerging IJs (progeny) were collected on a daily basis from the dead bodies of the wax moths and then stored at 18 °C in distilled sterilized water until further use. A culture of EPNs that were less than 5 days old was used to verify their pathogenicity against workers of *O. obesus.* The culture was allowed to warm up for 2 h (25 ± 1 °C) before use, and the motion viability of the EPNs was assessed under a dissecting microscope [16].

2.2. Laboratory Bioassays

Filter Paper Bioassay

O. obesus workers were collected from the laboratory-maintained colony and carefully transferred to Petri dishes with filter papers (Whatman No. 1) using a modified aspirator. Different concentrations (200, 400, 600, 800 and 1000 IJs/termite) of each nematode species (*S. carpocapsae, H. bacteriophora* and *H. indica*) suspended in 1 mL of deionized water were added to separate Petri dishes. Petri dishes containing only deionized water without nematode inoculum served as untreated controls. Ten minutes after addition of the nematode inoculum, 20 termite workers were released in each Petri plate. The Petri dishes containing nematode treatments and test insects were kept under laboratory conditions at two different temperatures (16 ± 1 and 26 ± 1 °C) in a large plastic container covered with aluminum foil to maintain a dark environment.

2.3. Sawdust Bioassay

In this experiment, sawdust (15 g) was added to small plastic containers (4 cm diameter, 4 cm high) with perforated lids covered with muslin cloth to allow aeration [57,58]. The same concentrations (200, 400, 600, 800 and 1000 IJs/termite) of each EPN species were added to different containers and the moisture was adjusted to 10% (*w*/*w*). Untreated control containers received only deionized water without nematode inoculum. After 10 min, 20 worker termites were released into each container, which were then incubated at two different temperatures, 16 ± 1 and 26 ± 1 °C, in closed plastic containers covered with aluminum foil.

After 3 days of treatment, the mortality dates of the termite workers in both laboratory trials were carefully recorded. The laboratory experimental trials which involved three factors, i.e., bioassays (B), nematode species (Sp.) and nematode concentrations (C), were laid out in a completely randomized design (CRD) with five replications/treatment (20 larvae/replication). Immobile infected termite workers which had changed color were considered to be dead. However, the worker cadavers were also dissected under a microscope to confirm the presence of nematodes inside the insect body.

2.4. Field Bioassay

The field experiment was executed in the Gutwala forest plantation located at Faisalabad (33°17′43.5″ N 44°25′30.9″ E), Punjab, Pakistan. The plantation was selected because of the presence of a large proportion of trees infested with *O. obesus*. Naturally infested *D. sissoo* plants which had not received any pesticidal or microbial treatment during the current and preceding year were selected.

Bait stations were installed around the infested trees well in advance of the execution of the actual field experiment. The bait stations were monitored regularly for almost three months to verify the termite invasion. Twenty bait stations were prepared. Each bait station consisted of a plastic container with four holes 8 × 4 cm in diameter located opposite each other and eight holes in the base of container to facilitate the entrance of termite workers. Five naturally infested *D. sissoo* plants were selected and four holes (25 cm depth) were made around each tree, in which four bait stations containing three nematode species (*S. carpocapsae*, *H. bacteriophora* and *H. indica*) and an untreated control were installed. The bait stations were buried around the infested trees and filled with soil up to the level of their lids. Fresh palm fronds were placed in the bait stations to attract and trap termite workers. However, before the palm fronds were inserted in each bait station, they were cut into small pieces, packed together and sterilized in an electric oven at 120 °C for 48 h. The tested nematode species were applied to new palm fronds using a manual sprayer at a dose rate of 1000 IJs/cm^2 and these were then inserted into each bait station, bearing in mind the infection time of termites. Sterilized water alone was added to the untreated control stations. After treatment, the bait stations were covered with lids and the soil inside each bait station was removed. Data regarding mortality were recorded one week after treatment.

2.5. Statistical Analysis

To meet normality assumptions, arcsine square root transformation was performed for all data expressed as percentages. In addition, the Abbott formula was used to correct the mortality in the controls [59]. The mortality data were later subjected to factorial analysis of variance (ANOVA) to test the effect of the bioassays, nematode species and nematode concentrations. Treatment means with significant differences were separated using Fisher's protected least significant difference (LSD) test at $p \leq 0.05$ [60,61]. The statistical analyses were carried out using Statistics 8.1 software (Analytical Software, Tallahassee, FL, USA).

3. Results

3.1. Mortality in Laboratory Trials

3.1.1. Bioassays (B)

The mortality of *O. obesus* varied significantly ($p \leq 0.001$) among the tested bioassays (B) under both temperature regimes (16 ± 1 and 26 ± 1 °C). At both tested temperatures, mortality was more pronounced in the sawdust bioassay than the filter paper bioassay. In addition, the mortality response in both bioassays was more pronounced at the higher temperature (26 ± 1 °C) than at the lower temperature (16 ± 1 °C). The maximum mortality at both temperatures was recorded for the sawdust bioassay (16 °C: 47.02% and 26 °C: 61.37%) followed by the filter paper bioassay (16 °C: 39.33% and 26 °C: 53.86%) (Tables 1 and 2).

3.1.2. Nematode Species (S)

Similarly, the efficacy of the different nematode species (*S. carpocapsae*, *H. bacteriophora* and *H. indica*) also varied significantly ($p \leq 0.001$) with regard to the mortality of *O. obesus* at both tested temperatures (16 ± 1 and 26 ± 1 °C). The efficacy of each nematode species was more pronounced at the higher temperature than at the lower temperature. A significantly higher mortality was recorded in the case of *S. carpocapsae* (16 °C: 48.28% and 26 °C: 66.17%), followed by *H. bacteriophora* (16 °C: 43.17% and 26 °C: 56.34%) and *H. indica* (16 °C: 38.08% and 26 °C: 50.34%) (Tables 1 and 2).

Table 1. Effect of bioassay methods (B), nematode species (S) and their concentrations (C) on mortality (%) of *Odontotermes obesus* workers kept under laboratory conditions at 16 ± 1 °C.

Factors	Mortality (%) (Mean ± SE)
Bioassays (B)	
Sawdust	47.02 ± 2.79 a
Filter paper	39.33 ± 2.34 b
Nematode species (S)	
Steinernema carpocapsae	48.28 ± 3.12 a
Heterorhabditis bacteriophora	43.17 ± 2.63 b
Heterorhabditis indica	38.08 ± 2.19 c
Nematode concentrations (IJs/termite) (C)	
200	23.06 ± 2.06 e
400	32.62 ± 2.25 d
600	41.89 ± 1.95 c
800	53.05 ± 3.54 b
1000	65.27 ± 2.89 a
F-value (B)	47.20 **
F-value (S)	27.65 **
F-value (C)	175.91 **
F-value (B × S)	0.54 *
F-value (B × C)	0.12 *
F-value (S × C)	0.80 ^{NS}
F-value (B × S × C)	0.02 ^{NS}

Treatment means within a single column with different lowercase letters were significantly different at $p \leq 0.05$; (LSD test); * significant at $p \leq 0.05$; ** significant at $p \leq 0.01$; NS nonsignificant.

Table 2. Effect of bioassay methods (B), nematode species (S) and their concentrations (C) on mortality (%) of *Odontotermes obesus* workers kept under laboratory conditions at 26 ± 1 °C.

Factors	Mortality (%) (Mean ± SE)
Bioassays (B)	
Sawdust	61.37 ± 3.12 a
Filter paper	53.86 ± 2.51 b
Nematode species (S)	
Steinernema carpocapsae	66.17 ± 2.73 a
Heterorhabditis bacteriophora	56.34 ± 1.74 b
Heterorhabditis indica	50.34 ± 1.34 c
Nematode concentrations (IJs/termite) (C)	
200	35.45 ± 1.82 e
400	45.84 ± 3.48 d
600	56.92 ± 2.52 c
800	67.08 ± 3.30 b
1000	82.79 ± 2.79 a
F-value (B)	60.43 **
F-value (S)	91.10 **
F-value (C)	289.68 **
F-value (B × S)	0.19 *
F-value (B × C)	0.02 *
F-value (S × C)	3.27 *
F-value (B × S × C)	0.31 ^{NS}

Treatment means within a single column with different lowercase letters were significantly different at $p \leq 0.05$; (LSD test); * significant at $p \leq 0.05$; ** significant at $p \leq 0.01$; NS nonsignificant.

3.1.3. Nematode Concentrations (C)

The results further revealed that the mortality response of *O. obesus* was also dependent on the concentration of the nematode species. A dose-dependent positive mortality response was recorded at both tested temperatures. However, the mortality response was more pronounced at the higher (26 ± 1 °C) than at the lower (16 ± 1 °C) temperature. The maximum mortality of *O. obesus* was recorded at the highest nematode concentration (1000 IJs/termite) (16 °C: 65.27% and 26 °C: 82.79%) followed by the lower concentrations (800 IJs/termite) (16 °C: 53.05% and 26 °C: 67.08%) (Tables 1 and 2).

3.1.4. Interactions of Bioassay (B), Nematode Species (S) and Their Concentrations (C)

The results with regard to the interactions of bioassay (B), nematode species (S) and their concentrations (C) (B × S × C) at 16 ± 1 °C revealed that the mortality of *O. obesus* was significantly influenced by the interaction of B × S ($p \leq 0.05$) and B × C ($p \leq 0.05$) (Table 1). The bioassays (B) used in the current trial significantly affected the efficacy of each nematode species (S) (Table 1). In the case of *S. carpocapsae*, the mortality of *O. obesus* was more pronounced when the nematode species was used in the sawdust (51.69%) bioassay compared with the filter paper bioassay (44.87%). Similarly, both the other nematode species, i.e., *H. bacteriophora* and *H. indica*, caused greater mortality in the sawdust assay than in the filter paper assay (Figure 1).

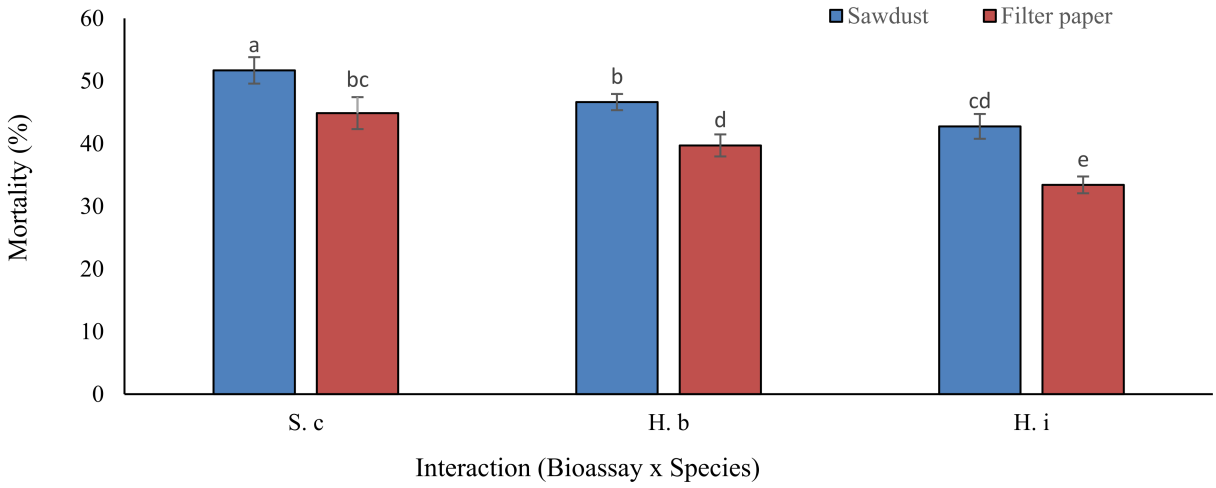

Figure 1. Interactive effect of bioassays × nematode species (B × S) on percentage mortality of *Odontotermes obesus* workers kept under laboratory conditions (16 ± 1 °C). Bars with different lowercase letters are significantly different at $p \leq 0.05$ (LSD test). Vertical bars indicate standard error (SE) values. *S. c: Steinernema carpocapsae*; *H. b: Heterorhabditis bacteriophora*; and *H. i: Heterorhabditis indica*.

The B × C interaction also revealed that the effect of each nematode concentration on the mortality of *O. obesus* varied significantly depending on whether the filter paper or sawdust bioassay was used. At the maximum nematode concentration (1000 IJs/termite), maximum mortality was recorded for the sawdust bioassay (68.99%) followed by the filter paper bioassay (61.54%). A similar trend of mortality with regard to the sawdust and filter paper bioassays was also recorded at the other tested nematode concentrations (800, 600, 400 and 200 IJs/termite) (Figure 2).

All possible interactions occurring between B × S × C at 26 ± 1 °C had a significant effect ($p \leq 0.05$) on the mortality of *O. obesus* with the exception of the interaction between B × S × C ($p > 0.05$) (Table 2). The interactive effect of B × S revealed that *O. obesus* mortality was more pronounced when *S. carpocapsae* was used in the sawdust bioassay (69.90%) compared with the filter paper bioassay (62.44%). The other two nematode species, *H. bacteriophora* and *H. indica*, also showed similar mortality trends in sawdust and filter paper bioassays (Figure 3).

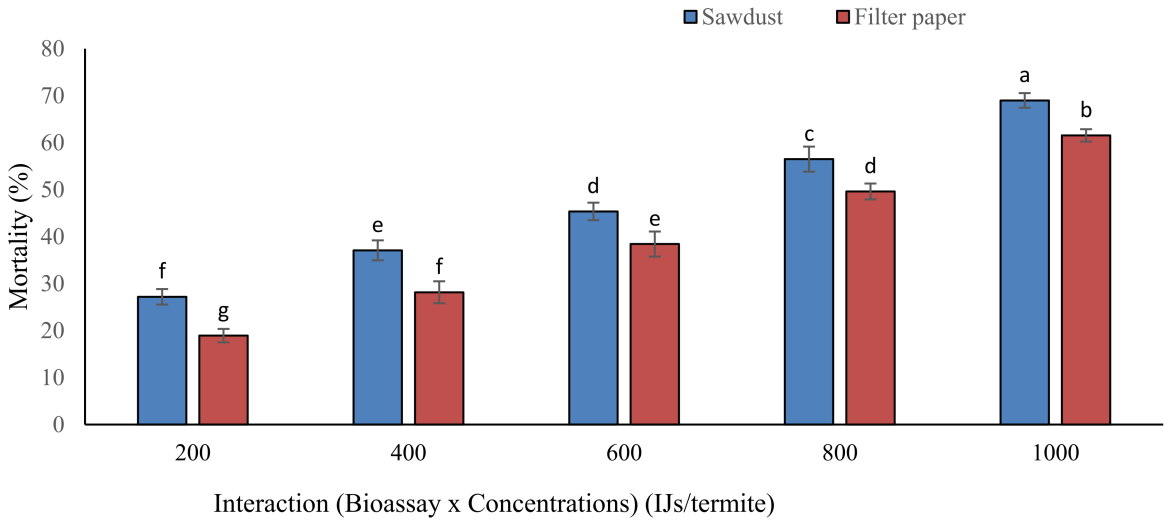

Figure 2. Interactive effect of bioassays × nematode concentrations (B × C) on percentage mortality of *Odontotermes obesus* workers kept under laboratory conditions (16 ± 1 °C). Bars with different lowercase letters were significantly different at $p \leq 0.05$ (LSD test). Vertical bars indicate standard error (SE) values.

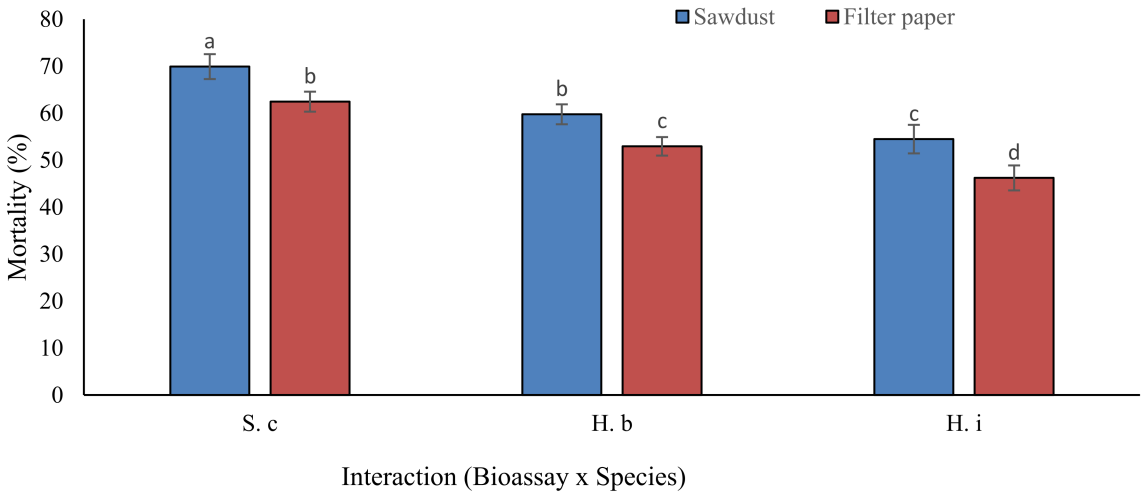

Figure 3. Interactive effect of bioassays × nematode species (B × S) on percentage mortality of *Odontotermes obesus* workers kept under laboratory conditions (26 ± 1 °C). Bars with different lowercase letters were significantly different at $p \leq 0.05$ (LSD test). Vertical bars indicate standard error (SE) values. *S. c: Steinernema carpocapsae; H. b: Heterorhabditis bacteriophora;* and *H. i: Heterorhabditis indica*.

Similarly, the B × C interaction also revealed a significantly higher mortality of *O. obesus* in the sawdust bioassay compared with the filter paper bioassay at all tested nematode concentrations. At the highest nematode concentration (1000 IJs/termite), the maximum mortality was recorded in the sawdust bioassay (86.50%) followed by the filter paper bioassay (79.08) (Figure 4).

The results regarding the S × C interaction further revealed that at all tested nematode concentrations, *S. carpocapsae* performed significantly better with regard to the mortality of *O. obesus*, followed by *H. bacteriophora* and *H. indica. S. carpocapsae* exhibited maximum mortality (95.39%) at its highest concentration (1000 IJs/termite) followed by its lower concentration (800 IJs/termite) (76.94%) (Figure 5).

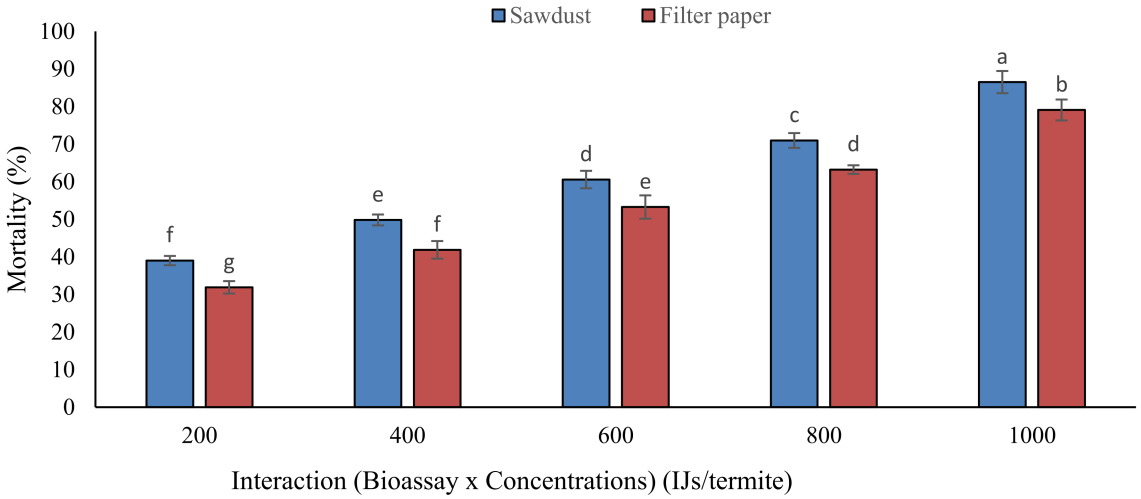

Figure 4. Interactive effect of bioassays × nematode concentrations (B × C) on percentage mortality of *Odontotermes obesus* workers kept under laboratory conditions (26 ± 1 °C). Bars with different lowercase letters were significantly different at $p \leq 0.05$ (LSD test). Vertical bars indicate standard error (SE) values.

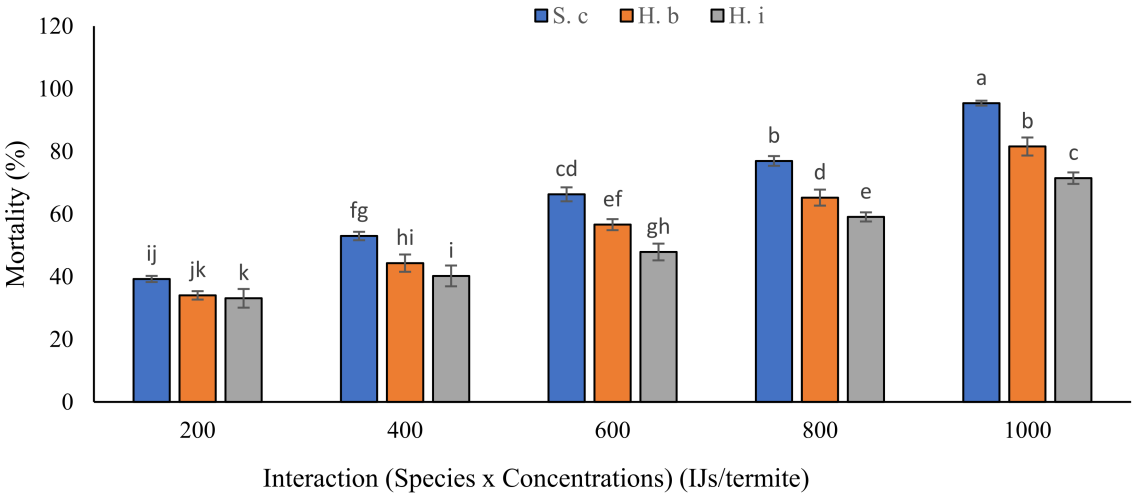

Figure 5. Interactive effect of nematode species × nematode concentrations (S × C) on percentage mortality of *Odontotermes obesus* workers kept under laboratory conditions (26 ± 1 °C). Bars with different lowercase letters were significantly different at $p \leq 0.05$ (LSD test). Vertical bars indicate standard error (SE) values. *S. c: Steinernema carpocapsae; H. b: Heterorhabditis bacteriophora;* and *H. i: Heterorhabditis indica*.

3.2. Mortality in Field Trials

As for the laboratory trials, the results of the field applications of the three tested nematode species varied significantly ($p \leq 0.001$) in comparison with the untreated control with regard to the mortality of termite workers. The maximum mortality was recorded for *S. carpocapsae* (58.46%), followed by *H. bacteriophora* (45.45%) and *H. indica* (32.39%) (Figure 6). The results also showed that the efficacy of all the tested nematode species was more pronounced in the laboratory trials than in the field experiments.

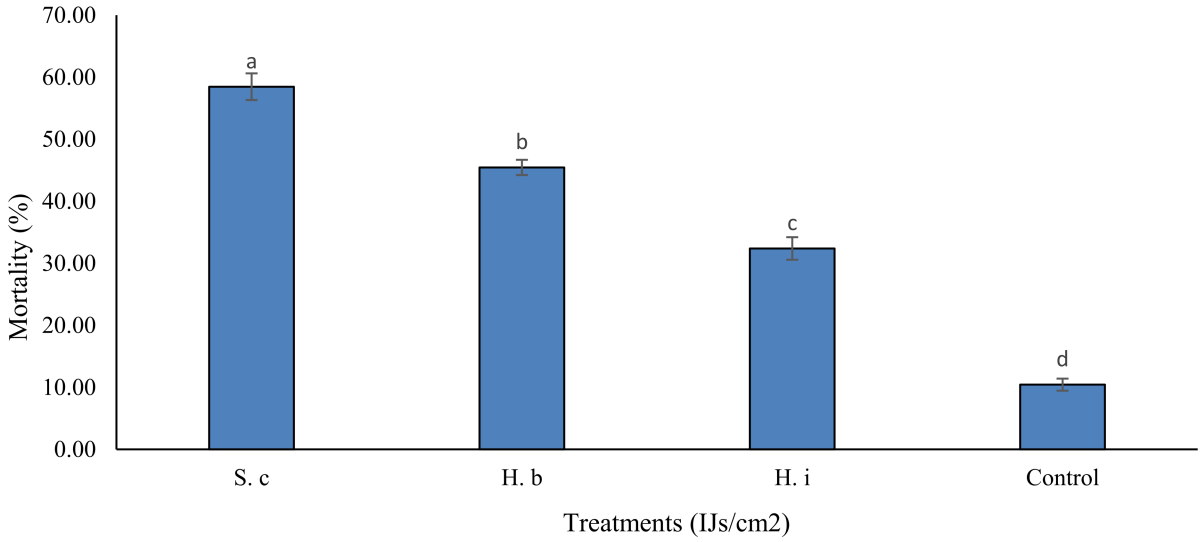

Figure 6. Effect of different nematodes on the control of *Odontotermes obesus* workers in field conditions. Bars with different lowercase letters were significantly different at $p \leq 0.05$ (LSD test). Vertical bars indicate standard error (SE) values. *S. c: Steinernema carpocapsae; H. b: Heterorhabditis bacteriophora;* and *H. i: Heterorhabditis indica.* Treatments were applied at a dose rate of 1000 IJs/cm^2.

4. Discussion

The efficacy of chemical pesticides against termites has been compromised because of their subterranean nature and enigmatic feeding habits. Furthermore, in view of the current situation with regard to resistance development in insects, there is a pressing need to embrace robust and eco-friendly pest management approaches. Entomopathogenic nematodes (EPNs) have emerged as a suitable replacement for some conventional pest management strategies, possibly because of their efficient host-searching ability, high virulence, target-specific action and ability to spread secondary inoculum via infected bodies [33,62,63]. In many cases, indigenous EPN strains provide good control of economically important insect pests because of compatibility with their native area [33].

The results of this study revealed that, under laboratory conditions, all the tested nematode strains significantly affected the mortality of *O. obesus* compared with the untreated control. Furthermore, the mortality rate was more pronounced at higher nematode concentrations. The above findings are compatible with those of Wang et al. [39], who reported higher pathogenicity of *H. bacteriophora* and *S. carpocapsae* against *Reticulitermes flavipes* workers under laboratory conditions. Similarly, the efficacy of different EPN species, such as *S. glaseri*, *S. carpocapsae* and *H. bacteriophora,* against termites has been proven in a number of other studies [38,40,41,64]. Many researchers have previously reported the greater mortality response of insect pests with increased dose rates (IJs) of EPNs [58,65,66]. Radhakrishan and Shanmugam [67] also reported that the percentage mortality of *Spodoptera litura* increased in line with increases in the concentrations of *H. indica* and *S. glaseri*. Similarly, Kepenek et al. [24] reported the pathogenic potential of four indigenous entomopathogenic nematode species, *S. carpocapsae, S. feltiae, H. bacteriophora* and *H. marelatus,* against the last instar of *Rhagoletis cerasi.* They reported that nematode concentration had a significant effect on the efficacy of each nematode species tested. The results showed that, at a concentration of 1000 IJs/larva, *S. feltiae* caused 95% mortality, followed by *H. marelatus* (82%) and *H. bacteriophora* (76%). These studies and our findings suggest that insects parasitized with a higher nematode dose usually die within a shorter period. High infection rates increase the toxins produced by the developing nematodes [68], and their symbiotic bacteria therefore enhance septicemia and ultimately kill the host more rapidly.

In our experimental trials, *S. carpocapsae* demonstrated grater virulence against termite workers compared with the other EPN species tested. These results are validated by Javed et al. [69], who reported a significantly higher mortality of *Microtermes obesi* when treated with *S. pakistanense* (100%), *S. bifurcatum* (100%), *S. siamkayai* (85%–87%) and *S. ceratophorum* (77%–80%) compared with *H. indica*, which exhibited a lower mortality (70%–77%). The greater virulence of *S. carpocapsae* was also proven by Maketon et al. [70], who reported 100% mortality of German cockroaches when exposed to this particular nematode species compared with other EPNs. Similarly, Baker et al. [40] also highlighted the greater virulence of *S. carpocapsae* against German cockroaches compared with the other experimental nematode species (*H. bacteriophora*). Of all the EPN species, *S. carpocapsae* exhibits a better ability to infect the adult stages of insect pests, and this might account for its superiority in infecting termite workers in our trials [16].

Our results further revealed that the pathogenicity of all the tested EPN species was more profound in the sawdust bioassay compared with the filter paper assay. These results are in accordance with the findings of Kamali et al. [71], who reported that *H. bacteriophora* exhibited a lower LC_{50} value when used in a natural soil medium (45.89 IJs/cm^2) compared with a filter paper medium (325.68 IJs/cm^2). Similarly, the virulence of *Xenorhabdus nematophila*, a symbiotic bacterium in the gut of *S. carpocapsae*, was greater against *G. mellonella* under a sand medium compared with a filter paper medium [72]. The superior efficacy of EPNs in the sawdust bioassay might also be associated with its moist, cool and dark environment, which resembles the termite habitat, as this is also generally most suitable for the movement and survival of Steinernematid and Heterorhabditid nematodes [73–75].

The results of this study further revealed that the tested EPNs showed more pathogenicity against termite workers in laboratory conditions than in the field trial. Usman et al. [76] also reported that the virulence of *S. riobrave* and *S. carpocapsae* against *Rhagoletis pomonella* was more pronounced in short- and long-term laboratory assays than in pot experiments. Generally, the pathogenicity of most EPN species under laboratory conditions is higher than in field conditions because of the optimal environmental circumstances and absence of any ecological or behavioral constraints [77]. Furthermore, field populations of insect pests usually exhibit greater resistance against different control strategies compared with populations reared for a considerable time in laboratory conditions, possibly because of their continuous exposure to those pest management approaches.

Our study also revealed that the efficacy of all the tested EPNs against termite workers was enhanced at a higher (26 ± 1 °C) compared with a lower (16 ± 1 °C) temperature range. These results are compatible with the findings of Chen et al. [47], who investigated the susceptibility of the last instar larvae of *Delia radicum* to *S. feltiae*, *S. carpocapsae*, *S. arenarium*, *H. megidis* and *H. bacteriophora* in laboratory conditions at 10 °C, 15 °C and 20 °C. The results of their study demonstrated that the pathogenicity of all the tested EPNs was more profound at higher than at lower temperatures. Furthermore, limited mobility and host-searching ability was noted at lower temperatures. Aatif et al. [78] also reported that *H. bacteriophora*, *H. indica*, *S. carpocapsae* and *S. asiaticum* were more virulent against the immature stages of *Bactrocera dorsalis* at a higher temperature (35 °C) compared with the lower tested temperature (15 °C). Furthermore, Langford et al. [79] reported that insect mortality was significantly enhanced after exposure to the EPN *S. feltiae* at 20 and 25 °C compared with 15 °C. Although some EPNs demonstrated promising results even at low temperatures, an increase in the time required to penetrate the host body was observed, which compromised the effectiveness of EPNs in various IMP programs [47].

The infectious potential of EPNs is significantly influenced by temperature variations [80–82]. Significant temperature variations can alter the infectivity, invasion, adhesion and reproduction of various isolates of infective juveniles (IJs) of EPN species [49]. However, each EPN responds differently to these temperature variations because of their ability to acclimatize to the prevailing ecological conditions [51].

5. Conclusions

This study confirmed the beneficial eco-friendly role of EPNs against termite workers when applied directly to the soil or via other inoculating mediums such as sawdust and filter papers. The EPN species exhibited significant mortality at both tested temperatures, making them a viable option for termite management in their diverse feeding habitats. However, the potential of different indigenous EPN species, together with their optimal dosages and application times, require further elucidation in both laboratory and field experiments to identify potential biocontrol candidates for the management of enigmatic feeding pests such as termites.

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