EVALUATION OF SIX INDIGENOUS ENTOMOPATHOGENIC NEMATODES AGAINST TERMITE, *ANACANTHOTERMES OCHRACEUS* (ISOPTERA: HODOTERMITIDAE) UNDER LABORATORY CONDITIONS

ALGHAMDI, A. S.

Department of Biology, College of Science, Taif University, Taif 21944, Saudi Arabia (e-mail: a.alghamdii@tu.edu.sa; phone: +966-500-621-159)

(Received 21st Nov 2024; accepted 27th Feb 2025)

Abstract. Subterranean termites, *Anacanthotermes ochraceus*, are a widely distributed and mainly recognized in Saudi Arabia as agricultural pests and economically serious insects causing damage to wood structures. Because termites have a cryptic feeding habit and have developed resistance to several insecticides, the effectiveness of most synthetic insecticides against them has been diminished. This study was designed in laboratory using sawdust and sand bioassay to confirm six native entomopathogenic nematode (EPN) isolates' effectiveness including *Steinernema feltiae* NEM-29, *S. feltiae* AHN, *Heterorhabditis indica* NEM-19, *H. indica* NEM-18, *H. bacteriophora* NEM-26 and *H. bacteriophora* AHN22 against workers of *A. ochraceus*. Results revealed that termite worker mortality was higher in the sawdust bioassay than in sand bioassay. At both tested assay methods, *S. feltiae* had a significantly greater mortality rate, followed by *H. indica* and *H. bacteriophora*. The maximum mortalities (100% and 79.0%) was recorded for *S. feltiae* AHN at 1000 IJs/termite with lower LC_{50} values of 7.3 and 73.8 IJs/termites at 16 d-post exposure in sawdust and sand assay, respectively. All tested EPN strains reproduced successfully and emerged from dead *A. ochraceus* workers in 8–14 days with higher reproduction rate (22,193 IJs/termite) for *S. feltiae* AHN in sawdust bioassay. Conclusively, it has been discovered that native EPNs can control termites more successfully, presumably due to they have the ability to spread further infections via infected dead individuals and can directly interact with termite pests in the soil.
Keywords: *termites, entomopathogenic nematodes, biological control, reproduction, in-vitro*

Introduction

Termites belong to the Isoptera order and are recognized by their social behavior. A termite colony usually consists of workers, soldiers, and a reproductive caste (a queen and a king). Due to their symbiotic hindgut protozoa, termites are eusocial detritophagous feeders that consume wood, causing significant damage worldwide (Novita et al., 2020; Kim and Chung, 2022). Approximately 300 of the 2650 termite species known worldwide are dangerous pests that severely damage homes and other wooden structures (Abe et al., 2000). Most termite research in the Arabian Peninsula, particularly in Saudi Arabia, was documented by Badawi et al. (1986) and Husain et al. (2023). Temperature has a major impact on termite activity, and as a result of climate change, more and more invasive termite species are being discovered in new environments (Buczkowski and Bertelsmeier, 2017; Shim et al., 2021). The increasing global temperature is clearly a contributing cause to the rise in termite activity and damage levels worldwide (Pareek et al., 2017). The existing reports indicate an undeniable increasing pattern in termite activity as a consequence of global climate change, despite the challenges in accurately estimating the economic damages caused by termite infestations (Lee et al., 2021).

Termite damage includes the expensive cost of pest control and the unexpected side effects of pesticides, in addition to irreversibly reducing the stability and beauty of

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2303_43954416

timber structures (Verma et al., 2009). Five imported wood species that are frequently found in Saudi Arabia's local market were previously examined for their inherent resistance to termites that prefer one host over another. *Microcerotermes* were attracted to beech wood, and termites of the genus *Microtermes* were found on some spruce and pine stakes that had been damaged. Additionally, one of the damaged stakes of the latter species, along with the one apitong stake that was infested, displayed indications of *Anacanthotermes attacks* (Badawi et al., 1984). Where desert and marginal lands are irrigated for cultivation in the United Arab Emirates (UAE) and other Arab Gulf nations, termites are increasing in relevance as pests of structures and crops (Kaakeh, 2005).

Anacanthotermes ochraceus (Hodotermitidae), *Microcerotermes diversus* (Termitidae), and *Psammotermes hypostoma* (Rhinotermititidae) were the most common termite species. *Anacanthotermes* termites are found in North Africa along the northern Sahara from Algeria to Egypt, and southwest Asia, expanding to Turkestan and the Indian and Pakistani desert borders (Sands, 1998). There have been numerous reports on termites in the Persian Gulf, particularly in Saudi Arabia (Badawi et al., 1986; Chhotani and Bose, 1991). Wood from homes and historic structures, forest and fruit trees, vegetables, noncellulose items, agricultural weeds, and paper were all shown to have been harmed by *A. ochraceus*.

While there are a number of efficient termite management strategies in use worldwide, the most of them rely regarding chemical termiticides use (Lee et al., 2021). In arid regions as Saudi Arabia, the most common termite control methods, according to a number of studies, include fumigants, termite baits, and the application of synthetic termiticides as preservation agents for wood (Kaakeh, 2005; Gordon et al., 2022; Togaev et al., 2024; Alotaibi, 2024). However, the ongoing use of chemical pesticides has been repeatedly associated with the possibility of insecticide resistance and devastating impacts on non-target organisms and human life. Thus, biodiversity is reduced, environmental health is endangered, and pest outbreaks worsen (Meftaul et al., 2020; Rezende-Teixeira et al., 2022). On the other hand, termite-killing or repelling semiochemicals can be applied alone or integrated with biological control agents for better and more effective control of termites affecting palm trees in Saudi Arabia. Moreover, alarming or defensive semiochemicals of termites can be counteracted to suppress their defense mechanisms (Alotaibi, 2024).

As a safer and more effective alternative to synthetic chemical-based termiticides, entomopathogenic nematodes (EPNs) are becoming increasingly prominent (Bhat et al., 2020; Aslam et al., 2023). According to de Oliveira Giannasi et al. (2018) and Viteri et al. (2018), EPNs are members of the soil biota that occur naturally and provide advantageous biocontrol solutions for a number of economically important arthropod pests from several orders of insects. There are about 116 identified nematode species in the two EPN families, Heterorhabditidae and Steinernematidae to date; some of these have been successfully marketed as insect pest biocontrol agents in several of global locations (Acharya et al., 2020; Bhat et al., 2022). After actively searching the soil profile for a suitable host, the infective juveniles (IJs) of EPNs enter the host's body through wounds or naturally occurring openings like the anus, mouth, and spiracles. The IJs release bacterial symbionts into the host's body once they have reached the hemocoel. These symbionts proliferate and produce a variety of toxins and metabolites, which ultimately cause the host to die from toxemia or septicemia (Stock et al., 2017; Haq et al., 2021).

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2303_43954416

EPNs can undergo multiple generations within the insect body, dependent on the availability of nutrients, before emerging as IJs from the host cadaver to begin their biological cycle once more (Dillman et al., 2012). Steinernematid and Heterorhabditid nematodes survive in the moist, cold, and dark conditions of termites' preferred habitat (Glazer et al., 2001). According to preliminary research, certain termites are vulnerable to *Steinernema* species (Chouvenc et al., 2011). It is known that entomophillic nematodes exist in a number of termite species. They interact phoretically, saprophytically, parasitically, and pathogenically with termite hosts. The termite's head capsule, thorax, abdomen, and stomach are residence to these nematodes, especially those belonging to the genus *Rhabditis*. As infected termites look more lethargic than uninfected ones, they change the termite's behavior even if they do not always result in the host's death (Carta and Osbrink, 2006).

Based on earlier research, EPNs' effectiveness against subterranean termites varies. For instance, in laboratory and field experiments, *Steinernema carpocapsae* has demonstrated encouraging promise for managing *Reticulitermes tibialis* (Epsky and Capinera, 1988). However, depending on the insect species and stage of development, EPNs' pathogenicity against insect pests differs significantly (Platt et al., 2020; Yan et al., 2020). In order to prevent any negative effects on nontarget organisms and to accelerate the adoption and importation of novel species, indigenous biocontrol agents are strongly advised. In many cases, local isolates of EPNs have registered greater potential in managing significant pests of the region because of their compatibility to their native habitats (Griffin et al., 2005). In the same context, the isolation of native EPN as biological control agents against mosquitoes that may spread serious human diseases has been the subject of numerous investigations (Kovendan et al., 2018). Similarly, all indigenous EPN isolates at different concentrations had good potentials in the management of *Agrotis ipsilon* larvae in two different experimental setup including filter papers in Petri dishes and soil in plastic containers (Yuksel and Canhilal, 2018).

There is currently little use of biological control agents to combat termites, and more investigation is required to identify the best biocontrol agent for an instance, even though the fact that nematode application is generally considered a safe and efficient management strategy for a variety of insect pests (Yu, 2009). Through certain social interactions including trophollaxis, caring, and grooming, infected termite workers can also spread the inoculum to naive colony members due to the slow acting mechanism of EPNs (Hu and Dhang, 2011). Furthermore, infection of queen nests, which frequently exist but far from the intended site, is made more likely by such social connections (Quarcoo et al., 2010).

In Saudi Arabia, termite biological control is still unknown, and there is no recent evidence of an effective integrated pest management approach in the literature. Consequently, in the current work the termiticidal activity of six indigenous entomopathogenic nematodes against subterranean termite, *A. ochraceus* workers have been evaluated in-vitro.

Materials and methods

Termite collection and maintenance

Alive colony of *Anacanthotermes ochraceus* (Burmeister, 1839) was collected directly by hand picking from their nests in infested agricultural farms located at the southern area of Taif Governate, Saudi Arabia. After being brought to the Animal

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2303_43954416

Research Laboratory, Faculty of Science, Taif Univ., KSA, the individuals received sorting, mounting, identifying, and conserving. With the guidance of the Chhotani and Bose (1991) key, termite species were taxonomically identified. Wooden pieces and moist cardboard were used to sustain the termite colony in plastic boxes measuring 20 × 20 × 40 cm, which were then placed in an incubator (ES-20, Biosan, Dubai, U.A.E) at 27 ± 2°C, 75%–80% relative humidity, and 24 h of darkness. Active and non-injured adult termite workers with a uniform size with an average weight of 3 ± 0.5 mg were carefully removed from rearing containers the day before testing, counted, and switched to plastic cups with moist filter paper lining to provide humidity and cellulose feeding. Energetic termites were thereafter selected for the bioassay investigation.

Entomopathogenic nematodes (EPNs)

In this work, six native nematode isolates were taken from the live nematode culture of Prof. Dr. Ahmed Noureldeen of the Biology Department of the Faculty of Sciences at Taif University in Taif, Saudi Arabia. They were *Heterorhabditis indica* NEM-18, *H. indica* NEM-19, *H. bacteriophora* NEM-26, *H. bacteriophora* AHN22, *Steinernema feltiae* NEM-29, and *S. feltiae* AHN. With little modification, the greater wax moth, *Galleria mellonella* L. (Lepidoptera: Pyralidae), was used to cultivate all nematode strains in its last instar larvae, using the methods outlined by Kaya and Stock (1997). In brief, 10 *G. mellonella* larvae were released in a 9×1.5 cm petri dish over two Whatman No. 1 filter papers delivered with infective juveniles (IJs) kept in distilled water (1 ml suspension containing approximately 500 IJs) Following sealing, the petri plates were incubated at room temperature (25 ± 2°C). Up to 15 d following inoculation, the cadavers' emerged IJs were gathered in a white trap (White, 1927), retained in a tissue culture jar with distilled sterilized water at 15°C, and utilized for the experiments within two days. A dissecting microscope was used to check the movement viability of the IJs following 2 h of warming the EPN culture at 25 ± 2°C (Al-Zaidawi et al., 2020).

Bioassays

The effectiveness of six native strains of EPNs against *A. och*raceus workers using two application methods (sand and sawdust) was determined in laboratory experiments. The first experiment was executed to prevent termites from avoiding nematodes and to measure termite mortality in simulated natural settings. Additional experiment was conducted in a medium of sand because nematodes may behave differently against termites in the presence or absence of sand.

Sawdust bioassay

Active *A. ochraceus* termite workers were carefully transferred to sawdust (15 g) in muslin cloth-covered perforated lids in small plastic containers that measure 4 cm in diameter and height to permit airflow separately for each nematode strain and concentration (Razia et al., 2012). Each nematode strain was dispersed in 3 mL of deionized, sterile water, and six concentrations (100, 200, 400, 600, 800, and 1000 IJs/termite) were then distributed to various containers. Following that, the containers were kept in a desiccator for 2 h, which allowed the sawdust to dry until it reached a constant moisture content of 10% (w/w) that measured using sawdust moisture meter GI100W (Gaby Instruments, India). Plastic containers that contained only sterilized deionized water lacking nematode inoculum served as untreated controls.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2303_43954416

Ten termite workers were released into each container 5 min after the nematode inoculum was added. In an incubator (ES-20, Biosan) set at 25 ± 2°C with relative humidity of 70 ± 5%, the containers holding the test insects and nematode treatments were placed in a large plastic container wrapped with aluminum foil to create a dark atmosphere. The experimental trial involving three factors, i.e., nematode strains (S), nematode concentrations (C), and exposure times (T), was conducted twice, and a completely randomized design (CRD) was employed, with five repetitions per treatment (10 termites/replicate). The termite's mortality was checked at 2, 4, 8, and 16 days. Dead termite workers were characterized as immobile termites whose color had altered due to infection. Every day, dead worker cadavers were taken out and put in a white trap (one worker/dish) at 25 ± 2°C until the appearance of IJs verified that nematode infection was the cause of death. Under a dissecting microscope, the IJs that emerged from the deceased termites were gathered and counted.

Sand bioassay

In this trial, methods were similar to topic 2.3.1 except that the individuals were added to Petri dishes layered with 15-g sterilized dry sand (particle size of 0.2 to 0.4 mm), as described by Yu et al. (2008). In separate Petri plates, the equal concentrations for each EPN strain were added. Sterilized deionized water was the only treatment given to untreated control plates; no nematode inoculum was applied. Each Petri dish received ten worker termites after 5 min, which was then wrapped with Parafilm to allow the EPN IJs to move around while keeping the sand lining wet and incubated at the same previous laboratory conditions and their mortality was monitored daily up to 16 days. IJs that produced in the termites were also collected and determined.

Statistical analysis

All data expressed as percentages underwent arcsine square root transformation in order fulfill normality requirements. Furthermore, the mortality percentages were corrected using the Abbott formula (Abbott, 1925) as follows: Corrected mortality (%) = % mortality in treatment – % mortality in control × 100 / 100 – % mortality in control. The mortality data were then subjected to factorial analysis of variance (ANOVA) to examine the effects of nematode strains, nematode concentrations and exposure durations. Treatment means ± standard errors (M ± SE) were compared using Duncan's multiple range (DMR) test with a probability of $P \leq 0.05$. The statistical analyses for all experiments were performed using GraphPad Prism 8 (GraphPad Software, La Jolla, CA, USA). The PROBIT routine (Finney, 1971) of SPSS program (Version 23) was employed to analyze the percent mortality data following corrections and calculate mean lethal concentration (LC_{50} and LC_{90}) and lethal time (LT_{50}), intercept and slope, X^2, heterogeneity significance (P), and 95% fiduciary confidence level.

Results

Sawdust bioassay

In sawdust bioassay with 10 termites per plastic containers, the corrected mortalities of *A. ochraceus* differed substantially amongst EPN isolates (F = 2,988, df = 5, $P \leq 0.0001$), EPN concentrations (F = 1,001, df = 5, $P \leq 0.0001$), and exposure times

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2303_43954416

(F = 1,037, df = 3, $P \leq 0.0001$), with the three factors interacting significantly (F = 4.68, df = 75, $P \leq 0.000$). *S. feltiae* AHN caused the highest significant mortality percentage up 97.4% to termite workers among the six EPNs, followed by *S. feltiae* NEM-29 (93.8%), and *H. indica* NEM-18 (90.5%), whereas, *H. bacteriophora* NEM-26 showed the lowest mortality rate (55.3%) (*Table 1*). *A. ochraceus*'s mortality response was likewise influenced by the nematode strains' concentration. While the lower concentration (100 IJs/termite) recorded 65.7% mortality, the higher concentration of nematode (1000 IJs/termite) recorded 91.8% death for workers (*Table 1*). The findings also showed a positive lethality reaction dependent on exposure time was also noted. With the increase in the exposure period to 16 d, mortality percentage of the tested termites could significantly induce up to 88.1%, however, exposing the individuals to EPNs for 2 d achieved mortality percentage value of 69.2% (*Table 1*). The mortality of *A. ochraceus* was shown to be significantly impacted by the interaction of S × C ($P \leq 0.05$) and S × T ($P \leq 0.05$) in relation to the interactions of nematode strains (S), nematode concentrations (C), and exposure times (S × C × T) (*Table 1*).

Table 1. *Effect of nematode strains (S), concentrations (C), and exposure times (T) on mortality (%) of A. ochraceus worker in sawdust bioassay method*

Factors	Mortality (%) (mean ± SE)
Nematode strains (S)	
S. feltiae AHN	97.4 ± 0.95 a
S. feltiae NEM-29	93.8 ± 1.82 b
H. indica NEM-18	90.5 ± 1.86 c
H. indica NEM-19	77.6 ± 3.12 d
H. bacteriophora AHN22	64.1 ± 3.72 e
H. bacteriophora NEM-26	55.3 ± 1.19 f
Nematode concentrations (IJs/termite) (C)	
1000	91.8 ± 0.63 a
800	88.7 ± 2.10 b
600	82.6 ± 2.77 c
400	77.8 ± 2.33 d
200	72.0 ± 2.77 e
100	65.7 ± 2.05 f
Exposure times (d) (T)	
2	69.2 ± 3.21 a
4	77.7 ± 2.24 b
8	84.1 ± 2.01 c
16	88.1 ± 0.97 d
F-value (S)	2,988 ***
F-value (C)	1,001 ***
F-value (T)	1,037 ***
F-value (S × C)	72.6 ***
F-value (S × T)	31.5 ***
F-value (C × T)	37.6 ***
F-value (S × C × T)	4.7 ***

Within a single column with different lowercase letters, significant differences in the treatment averages were seen at $P \leq 0.05$ (DMR test), *** significant at $P \leq 0.0001$

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2303_43954416

The effectiveness of each EPN strain (S) was considerably affected by the nematode concentrations (C) employed in the current trial (*Fig. 1*). *A. ochraceus* mortality was higher in the cases of *S. feltiae* AHN and *S. feltiae* NEM-29 (100%) when the nematode isolates were employed at 1000 IJs/termite. The two nematode strains, *H. indica* NEM-18 and *H. indica* NEM-19, also produced higher mortality rates (97.4% and 92.2%, respectively) than *H. bacteriophora* AHN22 (87.0%) and *H. bacteriophora* NEM-26 (74.0%), respectively (*Fig. 1*). Regarding the nematode strains, at the remaining nematode concentrations that were examined (800, 600, 400, 200, and 100 IJs/termite), the same mortality pattern was also noticed. The impact of each nematode isolate on *A. ochraceus* mortality varied considerably based on the exposure times utilized, according to the S × T interaction. At the 8 and 16-d exposition, greatest mortality occurred for the *S. feltiae* AHN and *S. feltiae* NEM-29, and at 16-d for the *H. indica* NEM-18 (100%), followed by the 8-d for *H. indica* NEM-18 (96.9%), then 4 and 2-d post-treatment with values 99.0% and 90.6%; 91.0% and 84.4%; 89.6% and 75.3%; 74.0% and 64.9% for the *S. feltiae* AHN; *S. feltiae* NEM-29; *H. indica* NEM-18; and *H. indica* NEM-19, respectively. Moreover, the least mortalities (50.3% and 42.4%) were recorded at 4 and 2-d post-application of *H. bacteriophora* NEM-26 (*Fig. 2*).

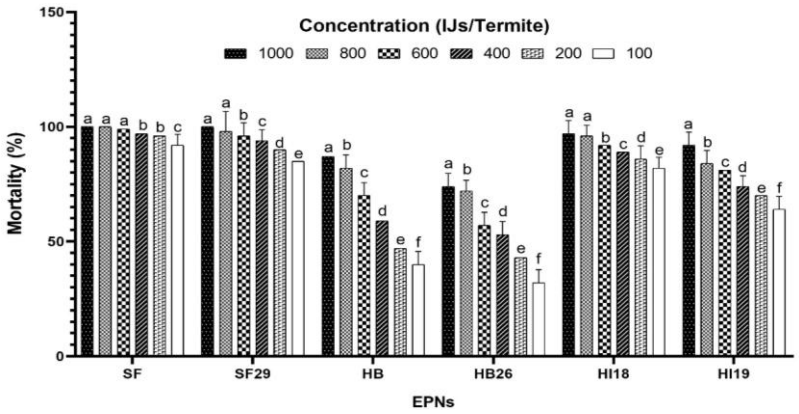

Figure 1. *Termiticidal activity of EPNs on subterranean termite, A. ochraceus workers as affected by EPN strains (S), and concentrations (C) in sawdust bioassay method. According to the DMR test, bars with distinct lowercase letters were substantially different at P ≤ 0.05. Values for standard error (SE) are shown by vertical bars. SF: Steinernema feltiae AHN; SF29: S. feltiae NEM-29; HB: Heterorhabditis bacteriophora AHN22; HB26: H. bacteriophora NEM-26; HI18: Heterorhabditis indica NEM-18; and HI19: H. indica NEM-19*

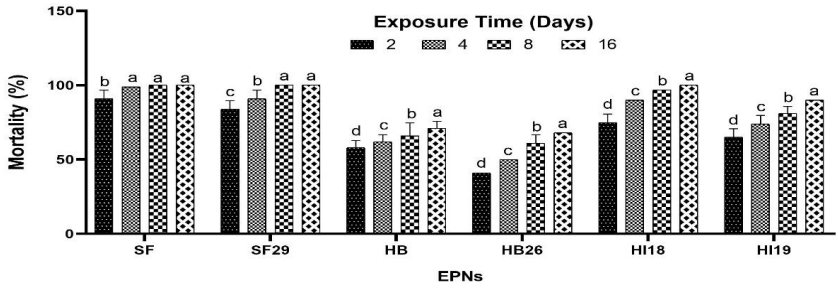

Figure 2. *Termiticidal activity of EPNs on subterranean termite, A. ochraceus workers as affected by EPN strains (S), and exposure times (T) in sawdust bioassay method. At P ≤ 0.05 (DMR test), bars with different lowercase characters were substantially differ. Standard error (SE) data are displayed by vertical bars*

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2303_43954416

Lethal concentrations (LC_{50} and LC_{90}) and lethal time (LT_{50}) were determined by means of a concentration response and time exposure assay implemented to investigate the pathogenicity of six native EPNs (*Tables 2* and 3). The values of LC_{50} and LC_{90} during the exposure periods showed that *S. feltiae* AHN; and *S. feltiae* NEM-29 were the most virulent against *A. ochraceus* workers, with LC_{50} values of 45.3, 12.6, 8.9, and 7.3; and 61.6, 20.1, 15.5, and 10.2 IJs/termite at 2, 4, 8 and 16 d of exposures, respectively, whereas, it exhibited 163.9, 62.3, 36.3, and 31.5; and 487.5, 253.1, 105.9, and 80.1 IJs/termite, respectively for the LC_{90} (*Table 2*). *H. bacteriophora* NEM-26 had the lowest efficacy against *A. ochraceus* following varying exposure durations, with LC_{50} and LC_{90} values of 617.4 and 2,499, 348.3 and 2,149, 195.6 and 1,926, and 131.0 and 1,719 IJs/termite, respectively at 2, 4, 8 and 16 d-exposures. With slope values of 1.85, 1.87, 1.95, and 2.12 after 2, 4, 8, and 16 d of exposure, respectively, *S. feltiae* AHN and *S. feltiae* NEM-29 exhibited optimum level of similarity for the population of *A. ochraceus*. In contrast, as viewed by the low slope values, the termites' reaction to the remaining four EPN isolates varied. The time needed to kill 50% of *A. ochraceus* workers showed a similar pattern, as indicated in *Table 3*. Termite workers were more susceptible to *S. feltiae* AHN and *S. feltiae* NEM-29, as each recorded lower LT_{50} (0.8 d), however, *H. bacteriophora* NEM-26 had higher LT_{50} (3.7 d). Additionally, *Table 3* showed that after exposure to *S. feltiae* AHN, *S. feltiae* NEM-29, and *H. in*dica NEM-18, the individual response showed the highest degree of uniformity, with slope values of 3.41, 2.29, and 2.19, respectively. Conversely, the other nematode isolates that were evaluated had low slope values, indicating that the responses of termite individuals to these nematodes were consistent (*Table 3*).

Table 2. *EPNs' virulence to A. ochraceus worker in sawdust bioassay method*

EPNs	Exposure time (d)	LC_{50} IJs/termite (95% LCL–UCL)	LC_{90} IJs/termite (95% LCL–UCL)	Slope ± SE	Intercept	X^2	*P*-value
S. feltiae AHN	2	45.3 (6.6-87.5)	163.9 (69.9-352.3)	1.85 ± 0.24	-2.77	7.22	0.13
	4	12.6 (0.4-36.9)	62.3 (5.5-101.1)	1.87 ± 0.69	-2.03	2.09	0.72
	8	8.9 (0.3-32.5)	36.3 (3.2-80.7)	1.95 ± 1.49	-1.71	0.56	0.97
	16	7.3 (0.1-29.6)	31.5 (2.4-76.9)	2.12 ± 1.61	-1.78	0.55	0.97
S. feltiae NEM-29	2	61.6 (26.5-104.3)	487.5 (313.4-1,178)	1.37 ± 0.19	-2.41	8.08	0.09
	4	20.1 (0.0-83.9)	253.1 (177-382.1)	1.74 ± 0.24	-2.89	5.15	0.27
	8	15.5 (0.0-50.7)	105.9 (9.5-186.2)	1.78 ± 0.22	-2.27	0.85	0.88
	16	10.2 (0.0-43.8)	80.1 (5.6-121.4)	1.80 ± 0.13	-2.17	0.81	0.86
H. indica NEM-18	2	65.9 (7.5-111.8)	1,313 (913.6-2,9934)	0.96 ± 0.16	-1.74	1.75	0.78
	4	46.2 (12.1-81.3)	325.4 (53.7-860.9)	1.26 ± 0.21	-1.38	12.77	0.01
	8	28.4 (0.0-86.1)	265.2 (121.5-411.7)	1.44 ± 0.36	-1.83	5.72	0.22
	16	17.7 (0.0-56.8)	188.5 (91.4-333.1)	1.54 ± 0.18	-1.70	0.78	0.86
H. indica NEM-19	2	90.6 (43.0-201.3)	1,395 (1,091-1,738)	0.65 ± 0.15	-1.86	3.76	0.44
	4	67.4 (9.0-132.9)	1,020 (859.9-1,543)	0.86 ± 0.16	-1.69	4.94	0.29
	8	49.0 (2.6-110.4)	751.1 (441.9-1,212)	0.97 ± 0.17	-1.83	7.27	0.12
	16	25.6 (3.5-77.1)	245.7 (189.8-311.6)	1.28 ± 0.21	0.18	5.63	0.23
H. bacteriophora AHN22	2	246.9 (167.9-324.9)	1,684 (853.0-3,685)	0.44 ± 0.16	-2.96	3.80	0.43
	4	205.2 (88.1-313.0)	1,438 (758.3-2,633)	0.58 ± 0.16	-3.11	10.24	0.04
	8	170.5 (73.7-257.2)	1,292 (891.2-4,808)	0.85 ± 0.16	-2.95	8.14	0.09
	16	101.3 (61.3-193.1)	1,195 (759.0-3,021)	0.92 ± 0.16	-2.82	5.56	0.24
H. bacteriophora NEM-26	2	617.4 (545.3-699.5)	2,499 (1,306-3,803)	0.31 ± 0.16	-3.55	6.68	0.15
	4	348.3 (271.7-436.2)	2,149 (945.7-3,675)	0.46 ± 0.15	-2.69	3.07	0.55
	8	195.6 (107.6-276.5)	1,926 (1,124-2,834)	0.79 ± 0.15	-2.50	4.26	0.37
	16	131.0 (47.1-152.8)	1,719 (1,247-3,543)	0.87 ± 0.15	-1.75	0.26	0.99

The lethal concentrations that kill 50% of termites and 90% of termites, respectively, are known as LC_{50} and LC_{90}. Chi-square value (X^2), standard error (SE), lower confidence limit (LCL), upper confidence limit (UCL), and probability (*P*-value)

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2303_43954416

Table 3. *EPNs' LT_{50} against workers of A. ochraceus in sawdust bioassay method*

EPNs	LT_{50} (d) (95% LCL–UCL)	Slope ± SE	Intercept	X^2	*P*-value
S. feltiae AHN	0.8 (0.0-1.9)	3.41 ± 3.95	0.31	3.91	0.14
S. feltiae NEM-29	0.8 (0.0-2.1)	2.29 ± 0.51	0.22	0.62	0.73
H. indica NEM-18	1.0 (0.5-1.4)	2.19 ± 0.39	0.08	0.31	0.86
H. indica NEM-19	1.1 (0.0-2.5)	0.95 ± 0.22	0.08	0.22	0.99
H. bacteriophora AHN22	1.3 (0.0-3.3)	0.75 ± 0.19	-0.01	0.01	0.98
H. bacteriophora NEM-26	3.7 (1.9-5.4)	0.39 ± 0.19	-0.42	0.05	0.98

LT_{50} = lethal time needed to kill 50% of insects, *P*-value = probability, SE = standard error, X^2 = chi-square value, LCL = lower confidence limit, and UCL = upper confidence limit

Within 8–14 d, all investigated EPN strains propagated and emerged from *A. ochraceus* dead workers (*Fig. 3*). Results of IJs that emerged from cadavers in total were were statistically significantly different among the nematode isolates (F = 839.8, df = 5, $P \leq 0.0001$), concentrations of EPNs (F = 1068, df = 5, $P \leq 0.0001$), and the interaction between IJs concentrations and EPN strains (F = 21.3, df = 25, $P \leq 0.0001$). Among EPN strains, the highest reproduction rate was recorded for *S. feltiae* AHN (16,540 IJs/termite), followed by *S. feltiae* NEM-29 (15,085 IJs/termite), whereas, the lowest emergency (9,433 IJs/termite) were obtained from *H. bacteriophora* NEM-26. However, at 1000 IJs/termite, the highest inoculation level, the average number of IJs of EPNs produced from *A. ochraceus* was 16,450, while the lower concentration (100 IJs/termite) resulted in 9,356/termite (*Fig. 3*). When comparing the IJs that emerged from *S. feltiae* NEM-29 (19,102 IJs/termite) and *H. indica* NEM-18 (16,915 IJs/termite), the maximum reproduction rate (22,193 IJs/termite) was substantially observed for *S. feltiae* AHN at 1000 IJs, whereas, *H. bacteriophora* NEM-26 was the least emerged nematode strain (11,923 IJs/termite) at the same concentration.

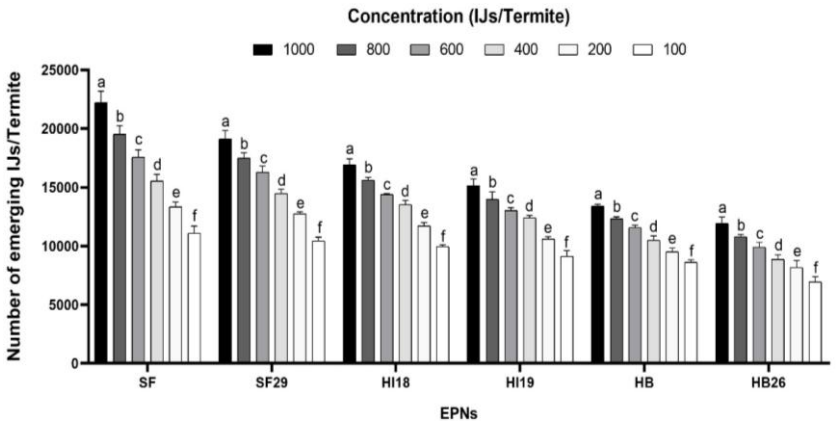

Figure 3. *Infective juvenile production of EPNs in A. ochraceus workers at different nematode concentrations in sawdust bioassay method. Significant differences were observed between bars with different lowercase characters at P ≤ 0.05 (DMR test). Vertical bars show the values of the standard error (SE)*

Sand bioassay

The results regarding the mortalities of *A. ochraceus* workers in sand bioassay method were also varied significantly among EPN strains (F = 387.7, df = 5,

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2303_43954416

$P \leq 0.0001$), EPN concentrations (F = 822.0, df = 5, $P \leq 0.0001$), and exposure times (F = 114.5, df = 3, $P \leq 0.0001$), with a non-significant interaction between the three factors (F = 0.47, df = 75, $P = 1.0$) (*Table 4*). Among the six EPNs tested, *S. feltiae* AHN did significantly higher in terms of the mortality rate of *A. ochraceus* (64.9%), followed by *S. feltiae* NEM-29 (62.2%), *H. indica* NEM-18 (58.9%), and *H. indica* NEM-19 (54.2%), while, *H. bacteriophora* AHN22 recorded the least mortality percentage (39.3%). The mortality response of *A. ochraceus* was similarly influenced by exposure durations and nematode strain concentrations, as *Table 4* demonstrates. Individual mortality peaked at the longest exposure (16 h, 59.0%) and the highest nematode concentration (1000 IJs/termite, 71.6%). Except for the S × C ($P \leq 0.0001$) interacting, all potential interactions between S × C × T revealed an insignificant impact ($P > 0.05$) on the mortality of *A. ochraceus* (*Table 4*).

Table 4. *Effect of nematode strains (S), concentrations (C), and exposure times (T) on mortality (%) of A. ochraceus worker in sand bioassay method*

Factors	Mortality (%) (Mean ± SE)
Nematode strains (S)	
S. feltiae AHN	64.9 ± 3.94 a
S. feltiae NEM-29	62.2 ± 4.14 b
H. indica NEM-18	58.9 ± 3.47 c
H. indica NEM-19	54.2 ± 4.15 d
H. bacteriophora AHN22	39.3 ± 3.74 f
H. bacteriophora NEM-26	48.0 ± 6.76 e
Nematode concentrations (IJs/termite) (C)	
1000	71.6 ± 3.66 a
800	66.1 ± 4.57 b
600	58.9 ± 4.54 c
400	52.3 ± 3.79 d
200	44.5 ± 4.77 e
100	33.9 ± 5.08 f
Exposure times (d) (T)	
2	49.2 ± 4.50 a
4	53.4 ± 4.13 b
8	56.7 ± 4.58 c
16	59.0 ± 4.27 d
F-value (S)	387.7 ***
F-value (C)	822.0 ***
F-value (T)	114.5 ***
F-value (S × C)	28.5 ***
F-value (S × T)	0.8 ns
F-value (C × T)	1.18 ns
F-value (S × C × T)	0.47 ns

The DMR test revealed that the treatment means with different lowercase letters within a single column were significantly different at $P \leq 0.05$, *** significant at $P \leq 0.0001$, and ns non-significant

A. ochraceus mortality was greater when *S. feltiae* AHN and *S. feltiae* NEM-29 were employed at 1000 IJs/termite (79.0% and 77.0%, respectively), according to the interactive impact of S × C. Similar mortality percentages occurred in the other two

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2303_43954416

nematode strains, *H. indica* NEM-18 and *H. indica* NEM-19 (75.0%, and 70%, respectively) at the same concentration (*Fig. 4*). Similarly, *S. feltiae* AHN showed the highest mortality (73.0%) at the concentration (800 IJs/termite), followed by *S. feltiae* NEM-29 (71.0%). In accordance with the S × T interaction, the impact of each nematode strain on *A. ochraceus* mortality varied insignificantly based on the duration of exposure (*Fig. 5*). At the longest exposition (16 d), *S. feltiae* AHN and *S. feltiae* NEM-29 further exhibited highest mortality rates (68.0%, and 66.0%, respectively), *H. indica* NEM-18 came next (63.0%) and *H. indica* NEM-19 (60.0%), while the lowest mortality (34.0%) was obtained from the *H. bacteriophora* AHN22 at 2-d post treatment (*Fig. 5*).

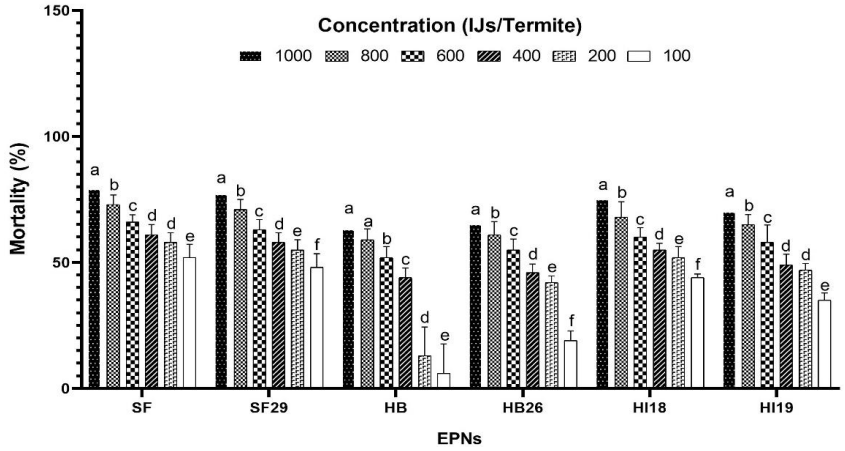

Figure 4. *Termiticidal activity of EPNs on subterranean termite, A. ochraceus workers as affected by EPN strains (S), and concentrations (C) in sand bioassay method. At P ≤ 0.05 (DMR test), bars with different lowercase characters were substantially differ. Standard error (SE) data are displayed by vertical bars*

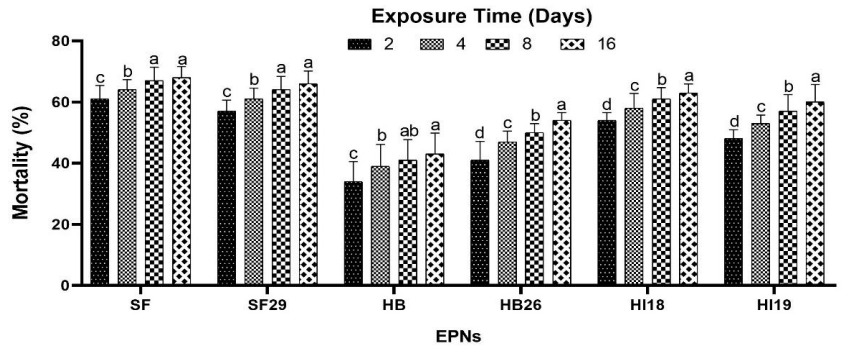

Figure 5. *Termiticidal activity of EPNs on subterranean termite, A. ochraceus as affected by EPN strains (S), and exposure times (T) in sand bioassay method. According to the DMR test, bars with distinct lowercase letters were substantially different at P ≤ 0.05. Values for standard error (SE) are shown by vertical bars*

The calculated LC_{50} and LC_{90} values of the EPNs strains during the exposure periods differed significantly (*Table 5*). Probit analysis revealed that *S. feltiae* AHN required less numbers (124.9, 114.3, 88.1, and 73.8 IJs/termites), and (1,682, 1,432, 1,301, and 1,240 IJs/termites) for desired mortality of 50 and 90% of *A. ochraceus*

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2303_43954416

individuals at 2, 4, 8, and 16 d, respectively. However, *S. feltiae* NEM-29 ranked second with values (194.8, 157.6, 11.4, and 109.6 IJs/termites), and (1,812, 1,611, 1,442, and 1,341 IJs/termites), whereas *H. bacteriophora* AHN22 required more numbers (729.3, 610.4, 558.9, and 531.5 IJs/termites), and (2,689, 2,316, 2,274, and 2,165 IJs/termites) for LC_{50} and LC_{90} at the same exposure times, respectively (*Table 5*). Likewise, the population of *A. ochraceus* workers exhibited the greatest degree of homogeneity for *S. feltiae* AHN and *S. feltiae* NEM-29 treatments with slope values of 1.87, 1.98, 2.21, and 2.04; and 1.28, 1.25, 1.08, and 0.99 at 2, 4, 8, and 16 d post-exposures, respectively, whereas, all of the other treatments had low slope values, indicating heterogeneity in the termite reaction to these treatments (*Table 5*). Regarding the LT_{50}, data in *Table 6* confirmed also that the IJs of *S. feltiae* AHN were the most potent to *A. ochraceus* workers, with LT_{50} value being 0.1 d, followed by *S. feltiae* NEM-29 (0.4 d), *H. indica* NEM-18 (0.7 d), and *H. indica* NEM-19 (2.6 d), while *H. bacteriophora* AHN22 took more time (26.9 d) to kill *A. ochraceus* individuals (*Table 6*). For the *A. ochraceus* population, the greatest level of homogeneity was recorded by *S. feltiae* AHN (0.35) and S. feltiae NEM-29 (0.34); however, it recorded 0.26 for *H. indica* NEM-18 and *H. indica* NEM-19 each. Conversely, the termites' low slope values for the other two EPN isolates showed that their response to them was heterogeneous (*Table 6*).

Table 5. *Pathogenicity of EPNs against A. ochraceus worker in sand bioassay method*

EPNs	Exposure time (d)	LC_{50} IJs/termite (95% LCL–UCL)	LC_{90} IJs/termite (95% LCL–UCL)	Slope ± SE	Intercept	X^2	*P*-value
S. feltiae AHN	2	124.9 (30.2-212.7)	1,682 (590.7-2,443)	1.87 ± 0.18	-1.15	1.59	0.81
	4	114.3 (40.2-183.9)	1,432 (311.5-2,145)	1.98 ± 0.20	-1.39	3.06	0.55
	8	88.1 (26.3-149.9)	1,301 (255.9-1,987)	2.21 ± 0.20	-1.33	3.04	0.55
	16	73.8 (36.0-150.1)	1,240 (221.5-1,890)	2.04 ± 0.19	-1.53	4.36	0.35
S. feltiae NEM-29	2	194.8 (86.6-292.3)	1,812 (614.1-2,551)	1.28 ± 0.16	-1.40	2.08	0.72
	4	157.6 (74.8-232.2)	1,611 (534.9-2,334)	1.25 ± 0.16	-1.58	2.78	0.60
	8	111.4 (38.7-179.9)	1,442 (354.7-2,114)	1.08 ± 0.16	-1.39	3.47	0.48
	16	109.6 (44.7-170.8)	1,341 (225.8-1,998)	0.99 ± 0.15	-1.54	4.59	0.33
H. indica NEM-18	2	286.9 (180.2-400.3)	1,915 (756.6-2,777)	0.70 ± 0.15	-1.73	2.01	0.73
	4	208.7 (114.9-295.4)	1,776 (755.8-2,322)	0.71 ± 0.15	-1.66	3.89	0.42
	8	155.0 (68.4-232.9)	1,624 (534.6-1,886)	0.68 ± 0.15	-1.49	3.99	0.41
	16	145.8 (74.9-210.5)	1,543 (515.4-1,777)	0.80 ± 0.14	-1.74	3.82	0.43
H. indica NEM-19	2	463.3 (359.8-614.2)	2,012 (822.4-2,888)	0.94 ± 0.15	-2.51	0.95	0.92
	4	326.4 (230.0-436.8)	1,865 (700.3-2,566)	0.81 ± 0.15	-2.04	2.79	0.59
	8	244.1 (159.2-326.9)	1,745 (667.5-2,023)	0.83 ± 0.16	-1.97	2.31	0.68
	16	196.8 (119.5-268.4)	1,701 (612.8-1,996)	0.84 ± 0.13	-1.92	3.41	0.49
H. bacteriophora AHN22	2	729.3 (545.8-1,157)	2,689 (1,755-3,434)	0.67 ± 0.15	-5.66	9.76	0.04
	4	610.4 (542.7-695.4)	2,316 (1,794-3,305)	0.69 ± 0.15	-6.16	5.09	0.27
	8	558.9 (493.5-639.8)	2,274 (1,811-3,467)	0.77 ± 0.14	-5.60	3.52	0.48
	16	531.5 (465.0-614.1)	2,165 (1,914-3,943)	0.55 ± 0.15	-5.09	2.87	0.58
H. bacteriophora NEM-26	2	626.5 (516.3-798.3)	2,432 (1,557-3,412)	0.61 ± 0.15	-3.57	5.88	0.21
	4	475.2 (392.3-585.5)	2,112 (1,588-3,333)	0.72 ± 0.13	-3.35	2.60	0.63
	8	392.7 (311.2-492.8)	1,911 (1,446-3,221)	0.68 ± 0.15	-2.79	7.02	0.14
	16	315.4 (237.7-400.1)	1,844 (1,324-3,010)	0.76 ± 0.17	-2.48	3.87	0.43

50% of termites that killed by a lethal concentration (LC_{50}), 90% that killed by a lethal concentration (LC_{90}), Lower confidence limit (LCL), upper confidence limit (UCL), chi-squared value (X^2), standard error (SE), and probability (*P*-value)

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2303_43954416

Table 6. *LT_{50} of EPNs against workers of A. ochraceus in sand bioassay method*

EPNs	LT_{50} (d) (95% LCL–UCL)	Slope ± SE	Intercept	X^2	*P*-value
S. feltiae AHN	0.1 (0.05-0.4)	0.35 ± 0.19	0.29	0.28	0.87
S. feltiae NEM-29	0.4 (0.1-1.1)	0.34 ± 0.19	0.11	0.33	0.85
H. indica NEM-18	0.7 (0.2-1.3)	0.26 ± 0.17	0.12	0.33	0.85
*H. in*dica NEM-19	2.6 (1.4-3.8)	0.26 ± 0.19	-0.14	0.48	0.79
H. bacteriophora AHN22	26.9 (12.5-45.9)	0.22 ± 0.19	-0.46	0.47	0.79
H. bacteriophora NEM-26	7.9 (4.1-13.6)	0.25 ± 0.18	-0.32	0.54	0.77

LT_{50}—lethal time needed to kill 50% of individuals, LCL—lower confidence limit, UCL—upper confidence limit, X^2—Chi-square value, SE—standard error and *P*-value—probability

Emergence of IJs was noticed after 10–15 days in the workers *A. ochraceus* (*Fig. 6*). A statistically significant difference existed among the EPN strains (F = 1,504, df = 5, $P \leq 0.0001$), its concentrations (F = 1,423, df = 5, $P \leq 0.0001$), and the interaction between the concentrations of IJs and EPN isolates (F = 18.5, df = 25, $P \leq 0.0001$). The reproduction rates ranged within 10,279–18,926, 9,801–17,754, 8,891–15,877, 8,399-14,099, 7,544-12,495, and 5,890-10,871 IJs/termite for *S. feltiae* AHN, *S. feltiae* NEM-29, *H. indica* NEM-18, *H. indica* NEM-19, *H. bacteriophora* NEM-26, and *H. bacteriophora* AHN22, respectively (*Fig. 6*). The largest average number of IJs (per worker) generated from *A. ochraceus* was *S. feltiae* AHN (15,340 IJs), and lowest *H. bacteriophora* AHN22 (8,467 IJs). At the higher concentrations (1000 and 800 IJs/termite), the average number of IJs emerged from *A. ochraceus* was 15,004, and 13,993/worker, respectively, whereas at the lower concentrations (200 and 100 IJs/termite), it recorded 9,931, and 8,501, respectively. The highest emergency of IJs was significantly observed from *S. feltiae* AHN and *S. feltiae* NEM-29 at 1000 IJs (18,926 and 17,754 IJs/termite), respectively, comparing to *H. bacteriophora* AHN22 which at the same concentration had lower production (10,871 IJs/termite).

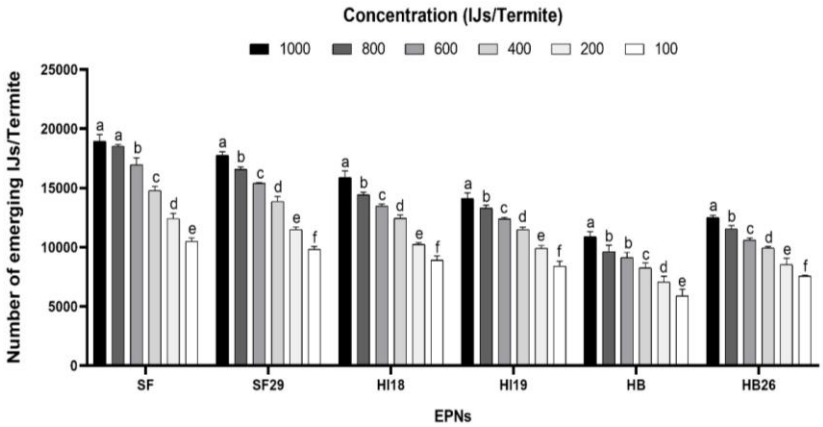

Figure 6. *Infective juvenile production of EPNs in A. ochraceus workers at different nematode concentrations in sand bioassay method. Bars with different lowercase letters were significantly different at P ≤ 0.05 (DMR test). Vertical bars indicate standard error (SE) values*

Discussion

The effectiveness of conventional insecticides against termites has been decreased due to their underground habitat and unusual eating patterns. Additionally, strong and

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2303_43954416

eco-friendly pest management strategies must be implemented urgently given the current state of insect resistance development. The ability of entomopathogenic nematodes (EPNs) to transmit secondary inoculum across infected bodies, their high virulence, their effective host-searching ability, and their target-specific activity have made them a viable alternative to several traditional methods for controlling pests (Bhairavi et al., 2021). The nematode infectivity test in sand or sawdust in the current investigation revealed that the various nematode isolates exhibited dramatically varying levels of termite-repelling activity. Our findings showed that *Steinernema* spp. were more efficient than *Heterorhabditis* spp. against A. ochraceus, and in contrast to the sand assay, the pathogenicity among every investigated EPN isolates was more pronounced in the sawdust bioassay. These findings are consistent with those of Aslam et al. (2023), who found that the mortality rate *Odontotermes obesus* workers was higher in the sawdust bioassay than in the filter paper bioassay. However, the present findings are particularly similar to those of Mostafa et al. (2022), who demonstrated that EPNs increased the mortality of *A. ochraceus* and *Psammotermes hypostoma* in Petri dishes as versus containers with sand/clay/peatmos medium, and that *Heterorhabditis* spp. were less effective than *Steinernema* spp. In contrast, (Changlu et al., 2002) proved that *Steinernema* spp. were less effective than *Heterorhabditis* spp. against two subterranean termites, *R. flavipes* and *Coptotermes formosanus*, and that the LD_{50} of *H. bacteriophora* against *R. flavipes* in containers with vermiculite/sand medium was higher than in Petri dishes. The symbiotic bacterium *Xenorhabdus nematophila*, which is found in the alimentary tract of *S. carpocapsae*, was also more virulent against *G. mellonella* in a sand medium than in a filter paper media (Mahar et al., 2005). Eppendorf tubes, packed cell volume (PCV) tubes, containers, Petri plates with sterile sand at the bottom, or wet filter paper were typically used for termite and EPN laboratory testing. According to Baïmey et al. (2017), termites often consume straw pieces of filter paper and corrugated wood blocks as food in all situations. Since the moist, cool, and dark environment of EPNs is similar to that of termites and is generally best suited for Heterorhabditid and Steinernematid nematode motility and survival, it may also be the reason for its superior efficacy in the sawdust bioassay (Glazer et al., 2001; Askari et al., 2012). In addition, termites in our sand assay had a tendency to feed sparingly and to constantly patrol the Petri dish's perimeter. On the other hand, termites in the sawdust assay began chewing the sawdust right away, which raised the chance that nematodes would infect the termites. Consequently, there was an inverse relationship between termite activity level and infection rate. When compared to other species, two steinernematid and two heterorhabditid species showed the best biocontrol capability in the current study. Against *Coptotermes heimi*, the same species showed similar outcomes (Tabassum and Salma, 2020). Compared to the other EPN isolates studied, *S. feltiae* AHN showed greater virulence against termite workers in the ongoing experimental trials. Regression analysis between termite mortality and nematode level slope, as well as exposure durations, the nematode species' virulence hierarchy was *S. feltiae* > *H. indica* > *H. bacteriophora*. *S. feltiae* AHN and *S. feltiae* NEM-29 were significantly more virulent against *A. ochraceus* than *H. indica* NEM-18 and *H. indica* NEM-19, whereas *H. bacteriophora* NEM-26 and *H. bacteriophora* AHN22 were lower effective against termite workers. Their LC_{50}, LC_{90} and LT_{50} values are very low compared with those of *H. indica* and *H. bacteriophora* nematodes species against *A. ochraceus*. The present results are in accordance with the previous findings of our group who stated that the EPNs *Steinernema* spp. were more virulent than *Heterorhabditis*

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2303_43954416

spp. against the two pomegranate insect pests, *Virachola livia* and *Ectomyelois ceratoniae*. The LC_{50} values of 13.6 and 32.4 IJs/larva of *V. livia* were recorded for *Steinernema* spp. and *Heterorhabditis* spp. at 72 h post-treatment, respectively, whereas it recorded 6.6 IJs (*Steinernema* spp.)/larva and 26.6 IJs (*Heterorhabditis* spp.)/larva for *E. ceratoniae* at the same exposure (Alotaibi et al., 2022). These findings are also consistent with those of Memari et al. (2016), who observed that mortality percentages of *E. ceratoniae* larvae in laboratory tests corresponded to LC_{50} values of 2.02 IJ/larva for *S. feltiae*, while *H. bacteriophora* showed low pathogenicity against the larvae, with an LC_{50} of 426.92 IJ/larva. The effectiveness of three concentrations (100, 300, and 900 IJs/larva) of *S. feltiae*, for instance, to suppress the Mediterranean flour moth, *Ephestia kuehniella*, in stored wheat was also examined (Athanassiou et al., 2008). Although most of the Steinernematids have ambush behaviours (Kaya and Koppenhoffer, 1996), the success of our *S. feltiae* tested isolates could be attributed to the highly mobile behaviours of termite workers, the easiest and most efficient host, which may increase the distribution patterns of EPNs and can thus, increase the pest's mortality. Furthermore, the capacity of nematodes to infect and reproduce is contingent upon the behavioral, morphological, and physiological defense mechanisms employed by insects (Razia et al., 2012). According to Kamali et al. (2013), *H. bacteriophora* showed a lower LC_{50} value in a natural soil medium (45.89 IJs/cm^2) in contrast to a filter paper medium (325.68 IJs/cm^2). Javed et al. (2021) confirmed these findings, stating that the mortality rate of *Microtermes obesi* was significantly higher when exposed to *S. bifurcatum* (100%), *S. pakistanense* (100%), *S. siamkayai* (85%–87%), and *S. ceratophorum* (77%–80%) comparing to *H. indica* that gave lower mortality (70%–77%). Maketon et al. (2010) showed the higher potency of *S. carpocapsae*, reporting 100% German cockroach mortality following exposure to this exact nematode species as compared to other EPNs. The higher lethality of *S. carpocapsae* to German cockroaches in comparison to the *H. bacteriophora* was also noted by Baker et al. (2012). According to Al-Zaidawi et al. (2020), *S. carpocapsae* is the most effective EPN species for infecting termite workers. This may be because it has a greater capacity to infect insect pests in their adult stages. Interestingly, the present study recorded the greater mortality response of termite workers to most of the indigenous EPNs tested. Because native EPN strains are compatible with their local environment, they frequently offer efficient control of insect pests that are economically important (Griffin et al., 2005). A noteworthy finding of the current investigation was the potential of native EPN species to affect and kill termites under both test methods (sand and sawdust). These findings, however, contradict those published by Mostafa et al. (2022). They found that imported nematodes were more virulent against *P. hypostoma* than *A. ochraceus* and more injurious than indigenous isolates of *H. bacteriophora*. Additionally, at greater nematode concentrations, mortality% was more apparent. The aforementioned results align with the findings of Wang et al. (2002), who documented increased virulence of *H. bacteriophora* and *S. carpocapsae* against workers of *R. flavipes* in a lab setting. The higher mortality response of termites and other insect pests with increased dosage rates (IJs) of EPNs species, including *S. carpocapsae*, *S. glaseri*, *H. indica*, and *H. bacteriophora*, has been previously verified by numerous researchers (Baker et al., 2012; Razia et al., 2012; Radhakrishan and Shanmugam, 2017; Aslam et al., 2023). The pathogenicity of four native EPN species—*S. feltiae*, *S. carpocapsae*, *H. marelatus*, and *H. bacteriophora*—against the last instar of *Rhagoletis cerasi* was also documented by Kepenek et al. (2015). According to the findings, *S. feltiae* caused 95%

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2303_43954416

mortality at a concentration of 1000 IJs/larva, followed by *H. marelatus* (82%) and *H. bacteriophora* (76%). Based on this research and our own, insects parasitized with a larger concentration of nematodes typically die away faster. Increasing infection levels cause the developing nematodes to create more toxins (Burman, 1982), which in turn causes their symbiotic bacteria to worsen septicemia and eventually kill the host more quickly. Another crucial trait for the EPN population that improves their chances of effectively establishing and surviving in the host environment is reproduction. Furthermore, the persistence and efficacy of EPN species in the host habitat—which is regarded as an effective factor for insect control—are largely dependent on their ability to reproduce and recycle (Phan et al., 2005). Both sawdust and sand conditions were favorable for the reproduction of the six EPN isolates employed in this investigation in A. ochraceus workers. The emergence rate, however, differed between species; in both plate and container bioassays, *S. feltiae* produced the most IJs at high IJs concentrations (1000 IJs/termite). The outcomes presented by (Salari et al., 2014) are incompatible with the results of the present research. When used against the Leopard moth borer, *Zeuzera pyrina* larvae, in a laboratory setting, they found that the reproduction rate of H. bacteriophora was higher than that of *S. carpocapsae*. This could be because of different methods. The amount of IJs that penetrate the host determines the rate of nematode development. According to Lewis et al. (2006), the lower production of IJs by *Heterorhabditis* can likely be attributed to the host's fraction of the available food material. Numerous in-vitro bioassays for multiple EPNs strains that belong to *Steinernema* and *Heterorhabditis* have revealed varying reactions against various termite species over the years (Khan et al., 2016). The type of interactions that occur between the host insects and the bacteria (*Photorhabdus* spp. and *Xenorhabdus* spp. symbiotically associated with *Heterorhabditis* spp. and *Steinernema* spp.) may be the cause, as these variables primarily affect the many innate virulence factors (Yu et al., 2010). Because of the ideal environmental conditions and lack of any ecological or behavioral restrictions, EPN species may generally be more virulent in the lab than in the field (Gaugler et al., 1997; Yee and Lacey, 2003). Furthermore, compared to populations maintained for an extended period of time in laboratory settings, outdoor populations of insect pests typically show higher resistance against various control tactics, maybe as a result of their ongoing exposure to those pest management techniques. Temperature changes have a major impact on the infectious ability of EPNs (Foelkel et al., 2016). However, due to their capacity to adapt to the current ecological conditions, each EPN reacts to these temperature fluctuations in a unique way (Pervez et al., 2008). It should be noted that there are few field research employing EPN species to control termite species, not only in Saudi Arabia but also in other nations worldwide (Baimey et al., 2015). However, the findings of the present study are contradict those found by Koppenhofer et al. (2000) who documented that nematodes have less ability to control subterranean species because of their behavior. Meanwhile, the repellence may be the key factor influencing the potency of EPN species in the field (Wang et al., 2002). Also, the outcomes of the current investigation are in contrast with those of Mauldin and Beal (1989) who discovered that *Reticulitermes flavipes* was resistant to *H. bacteriophora* when IJs of these nematodes were applied on infested wood under field conditions. Termites are more active in their natural habitat, which could be a reason of this outcome. However, Lezama-Gutiérrez et al. (2006) suggest that the foraging strategy of EPNs may have an influence on their capability to control insect pests. The moisture percentage might provide suitable conditions to investigate how nematodes

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2303_43954416

locate their host with ease. Thus, the sawdust could be importance for improving the field efficacy of EPNs formulation which provides a better wetting matrix for nematode activity. This would also be important for further studies on this topic. Integrated management of termite is still an urgent issue. The results of the current preliminary study indicated that applying EPNs promise to provide good efficacy against termite, but further experiments targeting other economically important insect species are currently ongoing to assess the compatibility of indigenous EPN species with other bio-agents or selective pesticides that have been registered.

Conclusions

This study concluded that when indigenous EPNs are directly administered to the inoculating media, like sawdust and sand, they have a positive environmentally friendly influence on termite workers, making them a good choice for controlling termites in their various consuming settings. More trials are required in the laboratory and field to provide accurate recommendations on ideal dosages, treatment timings, environmental persistence, and formulation development to confirm the potentiality of various native EPN species either alone or in integrated pest management programs for termites and other cryptic feeding insects.

Acknowledgements. The author would like to acknowledge the Deanship of Graduate Studies and Scientific Research, Taif University for funding this work.

REFERENCES

[1] Abbott, W. S. (1925): A method of computing the effectiveness of an insecticide. – J Econ Entomol 18: 265-267.
[2] Abe, T., Bignell, D. E., Higashi, M., Higashi, T., Abe, Y. (2000): Termites: Evolution, Sociality, Symbioses, Ecology. – Springer Dordrecht.
[3] Acharya, R., Yu, Y. S., Shim, J. K., Lee, K. Y. (2020): Virulence of four entomopathogenic nematodes against the tobacco cutworm *Spodoptera litura* Fabricius. – Biol Control 150: 104348.
[4] Alotaibi, N. J. (2024): The potential use of termite's semiochemicals for safeguarding date palms against termite. – Applied Ecology and Environmental Research 22(6): 5383-5397.
[5] Alotaibi, S. S., Darwish, H., Zaynab, M., Alharthi, S., Alghamdi, A., Al-Barty, A., Asif, M., Wahdan, R. H., Baazeem, A., Noureldeen, A. (2022): Isolation, identification, and biocontrol potential of entomopathogenic nematodes and associated bacteria against *Virachola livia* (Lepidoptera: Lycaenidae) and *Ectomyelois ceratoniae* (Lepidoptera: Pyralidae). – Biology 11: 295.
[6] Al-Zaidawi, J. B., Karimi, J., Mahdikhani, E. (2020): Entomopathogenic nematodes as potential biological control agents of Subterranean termite, *Microcerotermes diversus* (Blattodea: Termitidae) in Iraq. – Environ Entomol 49: 412-421.
[7] Aslam, A., Chi, D. F., Abbasi, A., Arshad, M. (2023): Biocontrol potential of entomopathogenic nematodes against *Odontotermes obesus* (Blattodea: Termitidae) under laboratory and field conditions. – Forests 14: 580.
[8] Athanassiou, C. G., Palyvos, N. E., Kakouli-Duarte, T. (2008): Insecticidal effect of *Steinernema feltiae* (Filipjev) (Nematoda: Steinernematidae) against *Tribolium confusum*

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2303_43954416

du Val (Coleoptera: Tenebrionidae) and *Ephestia kuehniella* (Zeller) (Lepidoptera: Pyralidae) in stored wheat. – J Stored Prod Res 44: 52-57.
[9] Badawi, A., Faragalla, A. A., Dabbour, A. (1984): The natural resistance of some imported wood species to subterranean termites in Saudi Arabia. – Zeitschrift für Angewandte Entomologie 98: 500-504.
[10] Badawi, A., Al-Kady, H., Faragalla, A. (1986): Termites (Isoptera) of Saudi Arabia, their hosts and geographical distribution. – J Appl Entomol 101(1-5): 413-420.
[11] Baimey, H., Zadji, L., Afouda, L., Moens, M., Decraemer, W. (2015): Influence of pesticides, soil temperature and moisture on entomopathogenic nematodes from southern Benin and control of underground termite nest populations. – Nematology 17: 1057-1069.
[12] Baïmey, H., Lionel, Z., Léonard, A., André, F., Régina, K. W. (2017): Searching for Better Methodologies for Successful Control of Termites Using Entomopathogenic Nematodes. – In: Shah, M. M., Mahamood, M. (ed.) Nematology - Concepts, Diagnosis and Control. IntechOpen, London.
[13] Baker, N. R., Hydar, B., Gowen, S. (2012): Reproduction of entomopathogenic nematodes *Steinernema carpocapsae* and *Heterorhabditis bacteriophora* on the German cockroach *Blatella germanica* at different temperatures. – Iraqi J Sci 53: 505-512.
[14] Bhairavi, K. S., Bhattacharyya, B., Devi, G., Bhagawati, S., Das, P. G., Devi, E. B., Manpoong, N. S. (2021): Evaluation of two native entomopathogenic nematodes against *Odontotermes obesus* (Rambur) (Isoptera: Termitidae) and *Agrotis ipsilon* (Hufnagel) (Lepidoptera: Noctuidae). – Egypt J Biol Pest Control 31: 111.
[15] Bhat, A. H., Chaubey, A. K., Askary, T. H. (2020): Global distribution of entomopathogenic nematodes, *Steinernema* and *Heterorhabditis*. – Egypt J Biol Pest Control 30: 31.
[16] Buczkowski, G., Bertelsmeier, C. (2017): Invasive termites in a changing climate: a global perspective. – Ecol Evol 7: 974-985.
[17] Burman, M. (1982): *Neoaplectana carpocapsae*: toxin production by axenic insect parasitic nematodes. – Nematologica 28: 62-70.
[18] Carta, L. K., Osbrink, W. (2006): *Rhabditid rainai* n.sp. (Nematoda: Rhabditida) associated with the Formosan subterranean termite, *Coptotermes formosanus* (Isoptera: Rhinotermitidae). – Nematology 7(6): 863-879.
[19] Changlu, W., Powell, J. E., Nguyen, K. (2002): Laboratory evaluations of four entomopathogenic nematodes for control of subterranean termites (Isoptera: Rhinotermitidae). – Environmental Entomology 31(2): 381-387.
[20] Chhotani, O. B., Bose, G. (1991): Isoptera from Saudi Arabia and Kuwait, with a key to Arabian species. – Fauna Saudi Arabia 12: 256-265.
[21] Chouvenc, T., Su, N. Y., Grace, J. K. (2011): Fifty years of attempted biological control of termites—analysis of a failure. – Biol Control 59: 69-82.
[22] de Oliveira Giannasi, A., Roque Brambila, C., Zart, M., Guide, B. A., Alves, V. S. (2018): Assessment of entomopathogenic nematodes in *Agrotis ipsilon* (Lepidoptera: Noctuidae) under laboratory and greenhouse conditions. – Rev Colomb Entomol 44: 25-31.
[23] Dillman, A. R., Lima, S., Bandi, C., Wanji, S., Diagne, M., Casiraghi, M. (2012): An entomopathogenic nematode by any other name. – PLoS Pathog 223: 18.
[24] Epsky, N. D., Capinera, J. L. (1988): Efficacy of the entomogenous nematode *Steinernema feltiae* against a subterranean termite, *Reticulitermes tibialis* (Isoptera: Rhinotermitidae). – J Econ Entomol 81: 1313-1317.
[25] Finney, D. (1971): Probit Analysis. – Cambridge University Press, Cambridge.
[26] Foelkel, E., Monteiro, L. B., Voss, M. (2016): Virulence of nematodes against larvae of the south-American fruit fly in laboratory using soil from Porto Amazonas, Paraná, Brazil, as substrate. – Ciênc Rural 46: 405-410.
[27] Gaugler, R., Lewis, E., Stuart, R. J. (1997): Ecology in the service of biological control: the case of entomopathogenic nematodes. – Oecologia 109: 483-489.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2303_43954416

[28] Glazer, I., Alekseev, E., Samish, M. (2001): Factors affecting the virulence of entomopathogenic nematodes to engorged female *Boophilus annulatus* ticks. – J Parasitol 87: 808-813.
[29] Gordon, J. M., Velenovsky, J. F., Chouvenc, T. (2022): Subterranean termite colony elimination can be achieved even when only a small proportion of foragers feed upon a CSI bait. – J Pest Sci 95: 1207-1216.
[30] Griffin, C., Boemare, N., Lewis, E., Grewal, P., Ehlers, R., Shapiro-Ilan, D. (2005): Nematodes as Biocontrol Agents. – CABI, Wallingford.
[31] Haq, I. U., Ashraf, H. J., Abbasi, A., Majid, M., Inayat, R. (2021): Integration of silicon with entomopathogenic nematode against fall armyworm, *Spodoptera frugiperda* (Lepidoptera: Noctuidae). – In: Proceedings of the 4th International Conference on Applied Zoology-2021 (ICAZ-2021), Islamabad, Pakistan, 24-25 November.
[32] Hu, X. P., Dhang, P. (2011): Urban Pest Management: An Environmental Perspective. – CABI, Wallingford.
[33] Husain, M., Rasool, K. G., Sharaf, M. R., Tufail, M., Sutanto, K. D., Al-Waneen, W. S., Aldawood, A. S. (2023): Mitochondrial COI based molecular identification of harvester termite, *Anacanthotermes ochraceus* (Burmeister, 1839) in Riyadh Region, the Kingdom of Saudi Arabia. – Journal of King Saud University - Science 35: 102782.
[34] Javed, S., Ali, A., Khanum, T. A. (2021): Biocontrol potential of the entomopathogenic nematodes (Rhabditida: Steinernematidae and Heterorhabditidae) against the termite species, *Microtermes obesi* (Holmgren) (Blattodea: Termitidae). – Egypt J Biol Pest Control 31: 99.
[35] Kaakeh, W. (2005): Survival and feeding responses of *Anacanthotermes ochraceus* (Hodotermitidae: Isoptera) to local and imported wood. – Household and Structural Insects 98(6): 2137-2142.
[36] Kamali, S., Karimi, J., Hosseini, M., Campos-Herrera, R., Duncan, L. W. (2013): Biocontrol potential of the entomopathogenic nematodes *Heterorhabditis bacteriophora* and *Steinernema carpocapsae* on cucurbit fly, *Dacus ciliatus* (Diptera: Tephritidae). – Biocontrol Sci Technol 23: 1307-1323.
[37] Kaya, H. K., Koppenhoffer, A. M. (1996): Effects of microbial and other antagonistic organism and competition on entomopathogenic nematodes. – Biocontrol Sci Technol 6: 357-372.
[38] Kaya, H. K., Stock, S. P. (1997): Techniques in Insect Nematology. – In: Lacey, L. A. (ed.) Manual of Techniques in Insect Pathology. Elsevier, Amsterdam, pp. 281-324.
[39] Kepenekci, I., Hazir, S., Özdem, A. (2015): Evaluation of native entomopathogenic nematodes for the control of the European cherry fruit fly *Rhagoletis cerasi* L. (Diptera: Tephritidae) larvae in soil. – Turk J Agric For 39: 74-79.
[40] Khan, M. A., Ahmad, W., Paul, B., Paul, S., Khan, Z., Aggarwal, C. (2016): Entomopathogenic Nematodes in the Management of Subterranean Termites. – In: Hakeem, K. R., Akhtar, M. S., Abdullah, S. N. A. (eds.) Plant, Soil and Microbes. Springer International Publishing, Bern, pp. 317-352.
[41] Kim, S. H., Chung, Y. J. (2022): Analysis of factors affecting termite damage to wooden architectural heritage buildings in Korea. – Forests 13: 465.
[42] Koppenhöfer, A. M., Brown, I. M., Gaugler, R., Grewal, P. S., Kaya, H. K., Klein, M. G. (2000): Synergism of entomopathogenic nematodes and imidacloprid against white grubs: greenhouse and field evaluation. – Biol Control 19: 245-251.
[43] Kovendan, K., Chandramohan, B., Govindarajan, M., Jebanesan, A., Kamalakannan, S., Vincent, S., Benelli, G. (2018): Orchids as sources of novel nanoinsecticides? Efficacy of *Bacillus sphaericus* and *Zeuxine gracilis*-fabricated silver nanoparticles against dengue, malaria and filariasis mosquito vectors. – Journal of Cluster Science 29(2): 345-357.
[44] Lee, S. B., Tong, R. L., Kim, S. H., Im, I. G., Su, N. Y. (2021): Potential pest status of the Formosan subterranean termite, *Coptotermes formosanus* Shiraki (Blattodea: Isoptera:

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2303_43954416

Rhinotermitidae), in response to climate change in the Korean peninsula. – Fla Entomol 103: 431-437.
[45] Lewis, E. E., Campbell, J. F., Griffin, C. T., Kaya, H. K., Peters, A. (2006): Behavioral ecology of entomopathogenic nematodes. – Biological Control 38: 66-79.
[46] Lezama-Gutie´rrez, R., Molina-Ochoa, J., Pescador-Rubio, A., Galindo-Velasco, E. A., Angel-Sahagun, C. A., Michel-Aceves, A. C., Gonzalez-Reyes, E. (2006): Efficacy of steinernematid nematodes (Rhabditida: Steinernematidae) on the suppression of *Anastrepha ludens* (Diptera: Tephritidae) larvae in soil of differing textures: laboratory and field trials. – J Agr Urban Entomol 23: 41-49.
[47] Mahar, A. N., Munir, M., Elawad, S., Gowen, S. R., Hague, N. G. M. (2005): Pathogenicity of bacterium, *Xenorhabdus nematophila* isolated from entomopathogenic nematode (*Steinernema carpocapsae*) and its secretion against *Galleria mellonella* larvae. – J Zhejiang Univ Sci 6: 457-463.
[48] Maketon, M., Hominchan, A., Hotaka, D. (2010): Control of American cockroach (*Periplaneta americana*) and German cockroach (*Blattella germanica*) by entomopathogenic nematodes. – Rev Colomb Entomol 36: 249-253.
[49] Mauldin, J. K., Beal, R. H. (1989): Entomogenous nematodes for control of subterranean termites, *Reticulitermes* spp. (Isoptera: Rhinotermitidae). – J Econ Entomol 82: 1638-1642.
[50] Meftaul, I. M., Venkateswarlu, K., Dharmarajan, R., Annamalai, P., Megharaj, M. (2020): Pesticides in the urban environment: a potential threat that knocks at the door. – Sci Total Environ 711: 134612.
[51] Memari, Z., Karimi, J., Kamali, S., Goldansaz, S. H., Hosseini, M. (2016): Are entomopathogenic nematodes effective biological control agents against the carob moth, *Ectomyelois ceratoniae*? – J Nematol 48: 261-267.
[52] Mostafa, E., Arafa, O. E., Hegab, M., Awad, E. (2022): Joint actions between entomopathogenic nematodes and abamectin for controlling the termites, *Psammotermes hypostoma* (Desn.), and *Anacanthotermes ochraceus* (Burm.). – Egypt Acad J Biolog Sci 14(1): 9-22.
[53] Novita, N., Amiruddin, H., Ibrahim, H., Jamil, T. M., Syaukani, S., Oguri, E., Eguchi, K. (2020): Investigation of termite attack on cultural heritage buildings: a case study in Aceh province, Indonesia. – Insects 11: 385.
[54] Pareek, A., Meena, B. M., Sharma, S., Tetarwal, M. L., Kalyan, R. K., Meena, B. L. (2017): Impact of Climate Change on Insect Pests and Their Management Strategies. – In: Kumar, P. S., Kanwat, M., Meena, P. D., Kumar, V., Alone, R. A. (eds.) Climate Change and Sustainable Agriculture. New India Publishing Agency, New Delhi, pp. 253-286.
[55] Pervez, R., Ali, S., Ahmad, R. (2008): Effect of temperatures on the emergence of entomopathogenic nematodes. – Int J Nematol 18: 25-28.
[56] Phan, K., Tirry, L., Moens, M. (2005): Pathogenic potential of six isolates of entomopathogenic nematodes (Rhabditida: Steinernematidae) from Vietnam. – BioControl 50: 477-491.
[57] Platt, T., Stokwe, N., Malan, A. (2020): A review of the potential use of entomopathogenic nematodes to control above-ground insect pests in South Africa. – S Afr J Enol Vitic 41: 1-16.
[58] Quarcoo, F. Y., Appel, A. G., Ping Hu, X. (2010): Effects of indoxacarb concentration and exposure time on onset of abnormal behaviors, morbundity, and death in eastern subterranean termite (Isoptera: Rhinotermitidae). – J Econ Entomol 103: 762-769.
[59] Radhakrishnan, S., Shanmugam, S. (2017): Bioefficacy of entomopathogenic nematodes against *Spodoptera litura* (Lepidoptera: Noctuidae) in Bhendi. – Int J Curr Microbiol Appl Sci 6: 2314-2319.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2303_43954416

[60] Razia, M., Rajkuberan, C., Chellapandi, P., Sivarama Krishnan, S. (2012): Agriculture practices diminished the entomophilic nematode diversity in Karur district of Tamil Nadu, India. – Journal of Advances in Developmental Research 3: 45-48.
[61] Rezende-Teixeira, P., Dusi, R. G., Jimenez, P. C., Espindola, L. S., Costa-Lotufo, L. V. (2022): What can we learn from commercial insecticides? Efficacy, toxicity, environmental impacts, and future developments. – Environ Pollut 300: 118983.
[62] Salari, E., Karimi, J., Sadeghi-Nameghi, H., Hosseini, M. (2014): Efficacy of two entomopathogenic nematodes *Heterorhabditis bacteriophora* and *Steinernema carpocapsae* for control of the leopard moth borer *Zeuzera pyrina* (Lepidoptera: Cossidae) larvae under laboratory conditions. – Biocontrol Sci Technol 25: 260-275.
[63] Sands, W. A. (1998): The Identification of Worker Castes of Termite Genera from Soil of Africa and the Middle East. – CAB International, Wallingford.
[64] Shim, J., Park, H., Ju, H. J., Song, J. H. (2021): First record of the termite family Kalotermitidae (Blattodea: Termitoidea) in Korea. – J Asia Pac Entomol 24: 1266-1269.
[65] Stock, S. P., Kusakabe, A., Orozco, R. A. (2017): Secondary metabolites produced by symbionts and their application in agriculture: what we know and what to do next. – J Nematol 49: 373-383.
[66] Tabassum, A. K., Salma, J. (2020): Virulence of four *Steinernema* species as a biological control agent in controlling the termite, *Coptotermes heimi* (Wasmann) (Isoptera: Rhinotermitidae). – Egypt J Biol Pest Cont 30: 26.
[67] Thanwisai, A., Muangpat, P., Meesil, W., Janthu, P., Dumidae, A., Subkrasae, C., Ardpairin, J., Tandhavanant, S., Yoshino, T. P., Vitta, A. (2022): Entomopathogenic nematodes and their symbiotic bacteria from the national parks of Thailand and larvicidal property of symbiotic bacteria against *Aedes aegypti* and *Culex quinquefasciatus*. – Biology 11(11): 1658.
[68] Togaev, U., Turaev, A. S., Mathur, V., Tilyabaev, Z., Zhaloliddinov, F., Turageldiyev, S., Shakirzyanova, G., Khashimova, M., Rustamov, M., Matchanov, A. (2024): Innovative strategies for termite management: development and evaluation of effective baits against *Anacanthotermes turkestanicus*. – Biosciences Biotechnology Research Asia 21(4): 1429-1437.
[69] Verma, M., Sharma, S., Prasad, R. (2009): Biological alternatives for termite control: a review. – Int Biodeterior Biodegrad 63: 959-972.
[70] Viteri, D. M., Linares, A. M., Flores, L. (2018): Use of the entomopathogenic nematode *Steinernema carpocapsae* in combination with low-toxicity insecticides to control fall armyworm (Lepidoptera: Noctuidae) larvae. – Fla Entomol 101: 327-329.
[71] Wang, C., Powell, J. E., Nguyen, K. (2002): Laboratory evaluations of four entomopathogenic nematodes for control of subterranean termites (Isoptera: Rhinotermitidae). – Environ Entomol 31: 381-387.
[72] White, G. F. (1927): A method for obtaining infective nematode larvae from cultures. – Science 66(1709): 302-303.
[73] Yan, X., Shahid Arain, M., Lin, Y., Gu, X., Zhang, L., Li, J., Han, R. (2020): Efficacy of entomopathogenic nematodes against the tobacco cutworm, *Spodoptera litura* (Lepidoptera: Noctuidae). – J Econ Entomol 113: 64-72.
[74] Yee, W. L., Lacey, L. A. (2003): Stage-specific mortality of *Rhagoletis indifferens* (Diptera: Tephritidae) exposed to three species of *Steinernema nematodes*. – Biol Control 27: 349-356.
[75] Yu, H. (2009): Parasitism of subterranean termites (Isoptera: Rhinotermitidae: Termitidae) by entomopathogenic nematodes (Nematoda: Steinernematidae: Heterorhabditidae). – Ph.D. dissertation, Department of Entomology, The University of Arizona, Tucson, AZ.
[76] Yu, H., Gouge, D. H., Stock, S. P., Baker, P. B. (2008): Development of entomopathogenic nematodes (Rhabditida: Steinernematidae; Heterorhabditidae) in the

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2303_43954416

desert subterranean termite *Heterotermes aureus* (Isoptera: Rhinotermitidae). – Journal of Nematology 40: 311-317.

[77] Yu, H., Gouge, D. H., Shapiro-Ilan, D. I. (2010): A novel strain of *Steinernema riobrave* (Rhabditida: Steinernematidae) possesses superior virulence to subterranean termites (Isoptera: Rhinotermitidae). – J Nematol 42(2): 91-95.

[78] Yuksel, E., Canhilal, R. (2018): Evaluation of local isolates of entomopathogenic nematodes for the management of black cutworm, *Agrotis ipsilon* Hufnagel (Lepidoptera: Noctuidae). – Egyptian Journal of Biological Pest Control 28: 82.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2303_43954416