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Evaluation of two native entomopathogenic nematodes against *Odontotermes obesus* (Rambur) (Isoptera: Termitidae) and *Agrotis ipsilon* (Hufnagel) (Lepidoptera: Noctuidae)

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Abstract

Background: Entomopathogenic nematodes (EPNs) are one of the widely studied biological control agents. The present study was conducted to evaluate the efficacy of two EPNs species, *Heterorhabditis bacteriophora* (Poinar) (Rhabditida: Heterorhabditidae) and *Steinernema aciari* (Qui, Yan, Zhou, Nguyen and Pang) (Rhabditida: Steinernematidae), isolated locally from soils of Majuli river island, Assam, India against two important subterrenean pests; *Odontotermes obesus* (Rambur) (Isoptera: Termitidae) and *Agrotis ipsilon* (Hufnagel) (Lepidoptera: Noctuidae) under laboratory conditions.

Results: In case of *O. obesus,* mortality percent was recorded by *H. bacteriophora* after 72 h. at 300 IJs/termite and by *S. aciari* at 250 and 300 IJs/termite after 96 h. The lowest LD_{50} and LT_{50} values obtained for *H. bacteriophora* were 13.054 IJs/termite and 26.639 h., respectively, while those of *S. aciari* were 42.040 IJs/termite and 31.761 h., respectively. With respect to *A. ipsilon, H. bacteriophora* registered a highest mortality rate at 300 IJs/larvae after 144 h. *S. aciari* showed 100 percent mortality at 300 IJs/larva after 168 h. The lowest values of LD_{50} and LT_{50} for *H. bacteriophora* were 35.711 IJs/larva and 83.050 h., respectively. The lowest values of LD_{50} and LT_{50} for *S. aciari* were 71.192 IJs/larvae and 97.921 h., respectively. Overall, *H. bacteriophora* displayed more virulence toward *O. obesus* and *A. ipsilon* than *S. aciari.*

Conclusion: Both native EPNs were found effective against *O. obesus* and *A. ipsilon.* However, *H. bacteriophora* was more virulent toward *O. obesus* and *A. ipsilon* than *S. aciari* under the laboratory conditions.

Keywords: Biological control, Entomopathogenic nematodes, *Heterorhabditis bacteriophora*, *Steinernema aciari*, *Odontotermes obesus*, *Agrotis ipsilon*

Background

Out of all the soil insects, termites, being eusocial insect pests quite often are known as "silent destroyers." Many ecologists consider termites as one of the most important ecological service providers because of their dominant role in soil ecosystem, in many cases, they have severely disrupted the ecological systems and caused significant economic damage in their natural habitats. Beside termites, the cutworm, *Agrotis ipsilon* (Hufnagel) (Lepidoptera: Noctuidae) is also serious polyphagous pest, which feeds on a number of vegetable and grain crops (Capinera 2009). Termites were reported to be more predominant on poorly shaded hot slopes of the hillocks and their infestation was reported to be 90% in the old tea plantations of Barak Valley, Assam (Choudhury 1999), as well as 50% a threat to sugarcane fields (Bhagawati et al.

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2017). Similarly, *A. ipsilon* along with four other species of cutworms viz. *A. interacta* Walk, *A. flammatra* Schiff., *A. spinnifra* Hb. and *A. segetum* Schiff. were reported to cause 12–40% losses to potato tuber yield in India (Bhattacharyya et al. 2014).

Owing to the complex hiding behavior of the larvae of *A. ipsilon* and subterranean nature of termites, the available chemo-centric management options are found ineffective as they have already developed resistance to most of the synthetic insecticides. Considering the current pest management scenario, there is an urgent need to embrace effective eco-friendly pest management strategies. Entomopathogenic nematodes (EPNs) have drawn a global attention in the last few decades because of their high host specificity, good host searching capacity and high pathogenicity (Kaya 1990). The favorable features exhibited by EPNs make them extremely suitable as biocontrol agents and ideal for IPM programes. The nematode-bacteria complex formed by the association of nematodes with mutualistic bacteria, *Xenorhabdus* and *Photorhabdus* in case of Steinernematidae and Heterorhabditidae, respectively, works together to kill the host (Boemare 2002). Over the years, EPNs have been successfully explored against a variety of insect pests such as the termites (Epsky and Capinera 1988) and the cutworms (Kaya et al. 2006). 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).

Pertinent to above, an attempt was made to evaluate the efficacy of two EPNs species, *Heterorhabditis bacteriophora* (Poinar) (Rhabditida: Heterorhabditidae) and *Steinenrnema aciari* (Qui, Yan, Zhou, Nguyen and Pang) (Rhabditida: Steinernematidae) locally isolated from soils of Majuli river island, Assam, India against both the termite and the cutworm under laboratory conditions.

Methods

Two EPNs native species were collected from the Department of Nematology, AAU, Jorhat, Assam, India. The EPNs species *Steinernema aciari* and *Heterorhabditis bacteriophora* were isolated from dead grubs of *Lepidiota mansueta* (Burmeister) (Coleoptera: Scarabaeidae) and soil samples from different cultivated and uncultivated fields of Majuli river island (26°45 N–27°12 N latitude and 93°39^{E}–94°35E longitude) of Assam. The EPNs species were reared and maintained by both *in vivo* and *in vitro* methods.

In vivo method

Rearing of bait insect (Galleria mellonella)

In order to maintain the virulence of the EPNs species, both species were routinely cultured on *Galleria mellonella* (Linnaeus) (Lepidoptera: Pyralidae) larvae, which was maintained on a semi-synthetic diet based on the procedure as described by David and Kurup (1988). The solid ingredients mainly consisted of corn flour (400 g), wheat flour (200 g), wheat bran (150 g), wheat germ (50 g), yeast and milk powder (200 g) which were then added to a mixture of honey (200 g) and glycerin (200 g), dissolved in lukewarm water (200 ml). This was followed by addition of streptomycin sulfate (100 g). The diet was then transferred to a 2 l capacity wide mouth jars and filled up to 1/4th of its volume. The jars comprising the diet were then inoculated by 20–25 egg masses (each containing 500 eggs) of *G. mellonella*.

Multiplication and isolation of EPNs from bait insect

Larvae of *G. mellonella* were then exposed to EPNs, using the method as described by Woodring and Kaya (1988). About five larvae of *G. mellonella* were released in a petri dish (100 × 15 mm) over two Whatman No. 1 filter papers inoculated with infective juveniles (IJs) stored in distilled water (1 ml suspension containing 200 IJs). The petri dishes were then sealed and incubated at room temperature. Emerged IJs from the cadavers were then collected in a white trap (White 1927) up to 12 days after inoculation and used under laboratory conditions at 15 °C.

In vitro method

Multiplication and isolation from culture medium

For mass multiplication, both the EPNs species were reared on a pre-defined media consisting of dog food and distilled water mixed thoroughly in equal amounts (House et al. 1965). The prepared media was coated thinly on polyurethane foam chips (0.4 g) of 1 cm^3, transferred to 100 ml conical flasks and autoclaved at 121 °C and 15 lb pressure per square inch for 15 min. Colonies of nematodes were observed on the walls of the flasks and test tubes 20 days post-inoculation. In order to be used in the infectivity test, the EPNs were surface sterilized by 0.1% hyamine for 15 min. The EPNs produced through *in vitro* method were used in the infectivity assay against the test insect species.

Collection and preparation of test insects

The most commonly available termite species, *Odontotermes obesus* (Rambur) (Isoptera: Termitidae), were collected from the mounds located in tea (*Camellia sinensis*) plantation site and kept in large plastic containers (29 × 15 cm) in laboratory at room temperature (25–30 °C). The adult termite workers with an average weight of 3.2 ± 0.6 mg were carefully separated from the mound soil and used in the infectivity test.

Likewise, *A. ipsilon* larvae were hand collected from the infested potato fields of Instructional-cum-Research farm of AAU, Jorhat and Majuli river island, Assam in a plastic container (9.2 × 8 cm) along with soil and a piece of potato. The containers were kept at a room temperature (25–30 °C) and were held for not more than one day until testing. Only the full-grown fourth instar larvae were used for the infectivity tests. The larval instar was determined by measuring the width of the head capsule of the larvae as described by Satterthwait (1933).

Infectivity assay

The infectivity tests were carried out in cavity blocks (55 mm × 55 mm). One larva/worker of the test insect was introduced per treatment to the cavity block. Infective juveniles of *H. bacteriophora* and *S. aciari* were inoculated at 0 (control), 10, 50, 100, 150, 200, 250 and 300 IJs/insect with 10 replications, each using 1 ml insulin syringe. In control, the test insect was not treated with EPNs but occasional distilled water was sprayed on it. Termite mortality was observed after 24, 48, 72 and 96 h. of exposure. In case of cutworm, the mortality was estimated after 24, 48, 72, 96, 120, 144 and 168 h. of exposure. The insect cadavers were then dissected and observed under a Stereo zoom Microscope (Model: Zeiss Stemi 2000-C) to check for emerging nematode progeny.

Statistical analysis

The data obtained during the course of investigation were analyzed using standard statistical procedure. The mortality data in different doses were adjusted for the observed mortality in control using Abbott's formula (Abbott 1925) and then subjected to Probit analysis (Finney 1964) by using IBM *SPSS* statistical 21 software for computation of Median Lethal Dose (LD_{50}) and Median Lethal Time (LT_{50}) for each nematode species (Hewlett and Placket 1979).

Results

Infectivity of *H. bacteriophora* and *S. aciari* against *O. obesus* workers

The mortality observed in *O. obesus* was subjective to the dose as well time of exposure and varies accordingly. Perusal of data showed a considerable difference between the mortality induced by both EPNs species. In both cases, the lowest mortality rates were registered at 24 h, which tended to increase with the increase in concentration, as well as exposure time. At 24 h, *H. bacteriophora* reported mortality rates of 10, 30 and 40% at 200, 250 and 300 IJs/termite, respectively, while *S. aciari* showed 10 and 30% mortality rates at 250 and 300 IJs/termite, respectively. Both *H. bacteriophora* and *S. aciari* were able to achieve a mortality of more than 50% within 48 h., followed by a gradual increase. As expected, both nematode species registered highest mortality rates after prolonged periods of exposure (72 and 96 h) by *H. bacteriophora* and*S. aciari,* respectively, at the highest IJ dosage of 300 IJs/termite. In case of *H. bacteriophora*, 100% mortality rate was observed at a time interval of 72 h., while *S. aciari* lagged behind at a time interval of 96 h. In case of control, due to absence of any sustenance, except water, the mortality observed was 10 and 30% after 72 and 96 h., respectively.

The LD_{50} and LT_{50} values of both the EPNs species differed significantly. The LD_{50} values of *H. bacteriophora* were 693.194, 105.691, 23.237 and 13.054 IJs/termite at 24, 48, 72 and 96 h, respectively. The LD_{50} of *S. aciari* was considerably higher at 2997.000, 215.737, 84.431 and 42.040 IJs/termite at 24, 48, 72 and 96 h, respectively. In the case of LD_{50} values, the LT_{50} values of *H. bacteriophora* viz. 72.817, 66.431, 57.595, 52.708, 43.113, 33.541 and 26.639 h at inoculation rates 50, 100, 150, 200, 250 and 300 IJs/termite, respectively, were lower than those *S. aciari* (Fig. 1). The LT_{50} values being 99.616, 85.040, 72.817, 65.957, 55.271, 43.951 and 31.761 h at 10, 50, 100, 150, 200, 250 and 300 IJs/termite, respectively (Fig. 2). Emergence of IJs was observed after 5–6 days from the cuticle of the dead worker termites in case of both EPNs species (Fig. 5a, b). At the highest level of inoculation (300 IJs/ termite), the average amount of IJs of *H. bacteriophora* and *S. aciari* produced from *O. obesus* was 521 and 320 worker^{-1}, respectively.

Infectivity of *H. bacteriophora* and *S. aciari* against *A. ipsilon* larvae

The results showed that none of the EPNs species were able to cause any mortality the first 2 days, but on the 3rd day, *H. bacteriophora* registered mortality rates of 10, 10, 20 and 30% at 150, 200, 250 and 300 IJs/larva, respectively. In case of *S. aciari*, mortality rates of 10, 20 and 20% were observed at 200, 250 and 300 IJs/larva, respectively. EPNs had a faster invasion rate and subsequent mortality with respect to smaller insects for all EPNs strains as noticed in the case of *O. obesus*. Hence, the longer time required by the EPNs to cause mortality in *A. ipsilon.* At 96 h, *H. bacteriophora* was able to cause at least 60 and 70% mortality rate at 250 and 300 IJs/larva, respectively, whereas *S. aciari* caused mortality rates of 50% at inoculation rates of 250 and 300 IJs/larva, respectively. The highest mortality rate (100%) was recorded by *H. bacteriophora* at 144 h at an inoculation rate of 300 IJs/larva, while *S. aciari* reported 100% mortality rate at 168 h at inoculation rate of 300 IJs/larva. In control, 10, 20 and 40% mortality rates were observed at 120, 144 and 168 h, respectively.

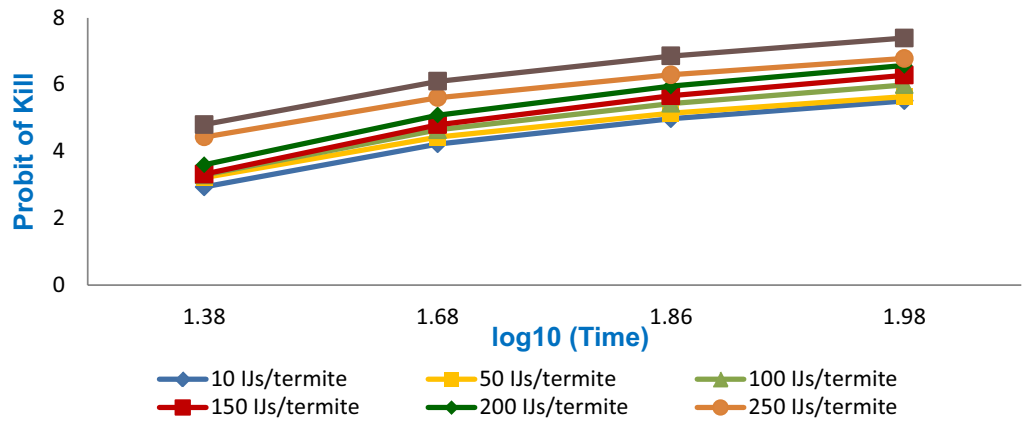

Fig. 1 Time response curve of *O. obesus* workers exposed to *H. bacteriophora* during different time intervals and concentrations

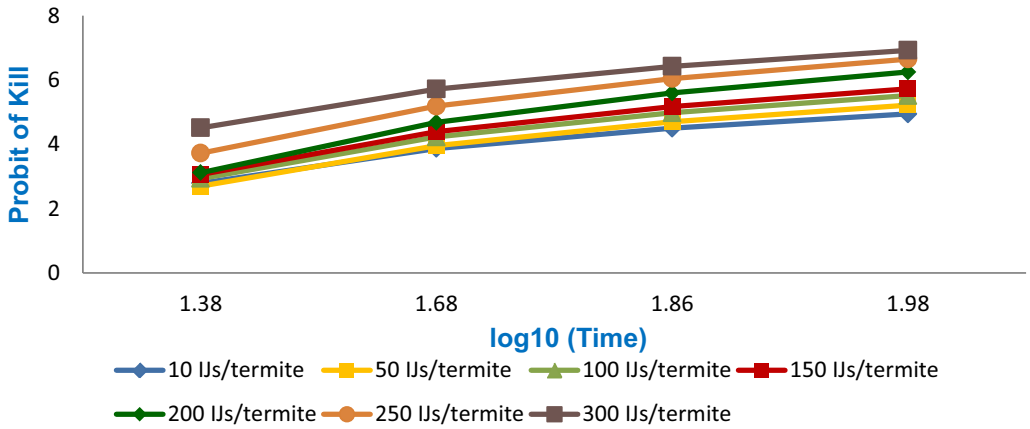

Fig. 2 Time response curve of *O. obesus* workers exposed to *S. aciari* during different time intervals and concentrations

Perusal of the mortality data obtained by both the EPNs species indicated that at all concentrations and exposure periods attempted, *H. bacteriophora* caused a significantly greater mortality rate in the larvae of *A. ipsilon* than *S. aciari.* This trend is further established when the LD_{50} and LT_{50} values were taken into consideration. The LD_{50} values of *H. bacteriophora* were 1314.790, 200.752, 131.532, 56.107 and 35.711 IJs/larva at 72, 96, 120, 144 and 168 h, respectively, while those of *S. aciari* 2649.610, 308.319, 232.993, 168.329 and 71.192 IJs/larva at 72, 96, 120, 144 and 168 h, respectively. With respect to *H.* bacteriophora, the LT_{50} values were 156.655, 153.592, 127.233, 110.660, 104.691, 93.697 and 83.050 h at 10, 50, 100, 150, 200, 250 and 300 IJs/larva, respectively (Fig. 3), while the LT_{50} values of *S. aciari* were 173.144, 161.743, 150.077, 136.084, 115.928, 103.283 and 97.921 h, at inoculation rates of 10, 50, 100, 150, 200, 250 and 300 IJs/larva, respectively (Fig. 4). Emergence of IJs was observed after 10–12 days in the larval *A. ipsilon* (Fig. 5c, d). At the highest level of inoculation (300 IJs/larva), the average amount of IJs of *H. bacteriophora* and *S. aciari* produced from *A. ipsilon* was 18,450 and 15,320 larvae^{-1}, respectively, 3 days after the first IJ emergence.

Discussion

An increase in the exposure time is linked to a rise in mortality rate as it allocates more time for the penetration of the insect by the IJs (Ebssa and Koppenhöfer 2012). The mortality rates also tended to increase with an increase in the rate of inoculation (El-Bassiouny and El-Rahman 2011). Razia and Sivaramakrishnan (2016) observed a positive relationship between the concentration and time of exposure, as well as mortality and variation between the nematodes and termite species for Lethal Time and Lethal Dose.

Over the years, a series of laboratory bioassays for different EPNs species belonging to *Heterorhabditis* and *Steinernema* have indicated varied responses against

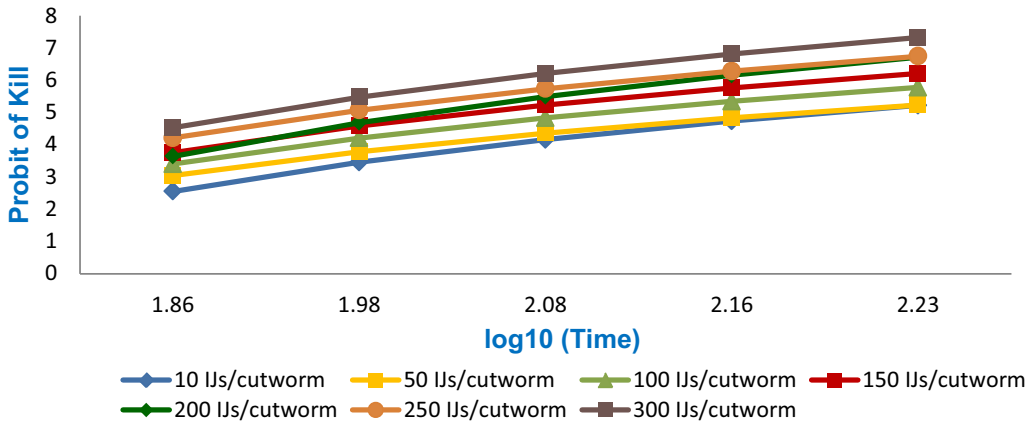

Fig. 3 Time response curve of *A. ipsilon* larvae exposed to *H. bacteriophora* during different time intervals and concentrations

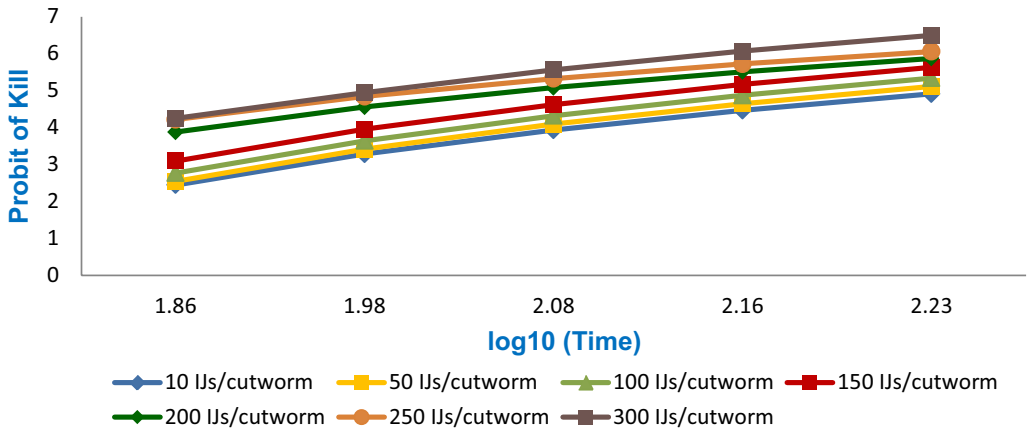

Fig. 4 Time response curve of *A. ipsilon* larvae exposed to *S. aciari* during different time intervals and concentrations

different termite species (Khan et al. 2016). It may be due to the different innate virulence factors which are mainly influenced by the nature of the interactions between the associated bacteria (*Xenorhabdus* spp. and *Photorhabdus* spp. in case of *Heterorhabditis* spp. and *Steinernema* spp., respectively) with the host insects (Yu et al. 2010). Similar to the mortality data obtained during the present study, Devi et al. (2018) reported occurrence of 50% mortality rate in *O. obesus* by *H. bacteriophora* and *Steinernema* spp. at 100 IJs/termite within 48 h. *S. aciari* was able to induce a complete mortality in workers of *Coptotermes formosanus* after 96 h. (Wagutu and Kan'gethe 2017). *H. indica* was reported as being more effective than *Steinernema* spp. against *Reticulitermes tibialis* with a LD_{50} value of 1.5×10^4 termite^{-1} (Epsky and Capinera 1988). In the filter paper bioassay of *Microtermes* spp., the LD_{50} of *H. indica* was 5.11 IJs alate^{-1} at 60 h, while for *S. abbasi*, it was attained at 72 h with 6.91 IJs alate^{-1} (Mohan et al. 2016). The LT_{50} value of the indigenous *H. indica* isolate against *M. bellicosus* was estimated to be 24.07 h *(*Zadji et al. 2014). The LT_{50} of *S. pakistanense, S. siamkayai and H. indica* against *O. hornei* was 15.5, 16.3 and 19.8 h (Razia and Sivaramakrishnan 2016). (Qodiriyah et al. 2015) reported that the EPNs belonging to *Heterorhabditis* spp. were more effective in controlling subterranean termites than *Steinernema* spp.

Overall, *H. bacteriophora* recorded higher mortality than *S. aciari* in case of both *O. obesus* and *A. ipsilon.* Isolates of *S. carpocapsae* and *H. indica* were found to cause 80.0 and 83.3% mortality rates, respectively, to the 3rd instar larvae *A. ipsilon* after 72 h. of infection (Yan et al. 2014). 98 and 90% of the larvae of *A. segetum* were parasitized 5 days after being exposed to *H. bacteriophora* and *S. carpocapsae* (Goudarzi et al. 2015). Lankin et al. (2020) reported that the weight of *A. deprivata* larvae was proportional to the production of IJs/host, which explained

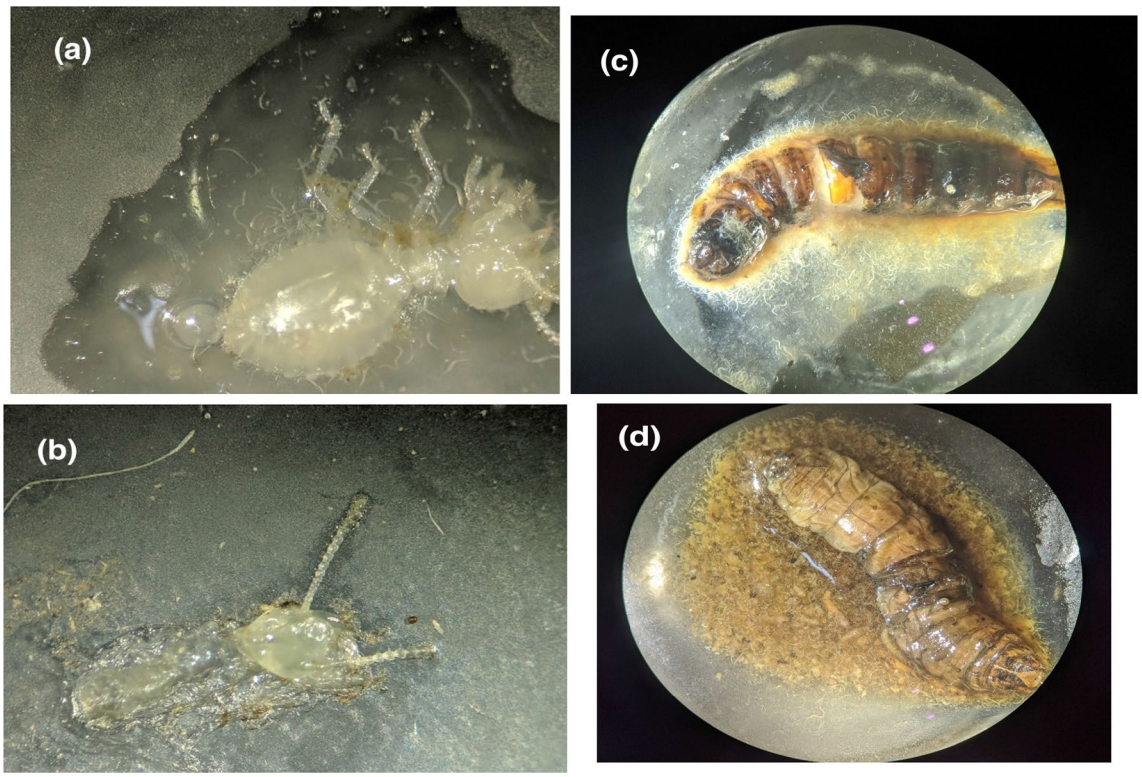

Fig. 5 Emergence of EPNs from cadaver**, a** from *O. obesus* worker due to *H. bacteriophora* infection, **b** from *O. obesus* worker due to *S. aciari* infection, **c** from *A. ipsilon* larva due to *H. bacteriophora* infection, and **d** from *A.ipsilon* larvae due to *S. aciari* infection

the longer time taken by the EPNs to cause mortality in the present experiment. Also, the LC_{50} and LT_{50} values often tend to increase in proportion to the size of insect (Bedding et al. 1983).

Previous studies have showed varied results with respect to the infectivity of the EPNs against *A. ipsilon.* In some cases, *Steinernema* spp. performed better than *Hterorhabditis* spp. *S. carpocapsae* was the best performing species against *A. ipsilon* with a high speed of kill of 68% after 4 days under golf course conditions (Ebssa and Koppenhofer 2011). *S. kraussei* registered highest mortality (98%) in the larvae of *A. segetum* at the rate of 500 IJs g^{-1} dry sand after 7 days (Gökçe et al. 2013). But perusal of data from other similar works attest to the fact that in most cases *Heterorhabditis* spp. has been found to be more virulent against *A. ipsilon* than *Steinernema* spp. In a study by Hussaini et al. (2005), the isolates of *H. indica* proved to be more virulent causing 100% mortality in the 4th day of exposure than the isolate of *S. carpocapsae* which was found as the least effective isolate against the *A. ipsilon* larvae. In a plastic container experiment by Yuksel and Canhilal (2018), the maximum mortality rate was reached within 2 days after inoculation by the two isolates *Heterorhabditis bacteriophora* FLH-4-H and *H. indica* 216-H at the concentrations of 50 and 100 IJs/cm^2, respectively. The lowest LC_{50} and LC_{90} values were found to be 17 IJs and 23 IJs for the isolate *H. bacteriophora* FLH-4-H. After 5 days of treatment, *Heterorhabditis* spp. were able to cause the percentage mortality ranging from 24 to100% in 3rd instar larvae and 16–80% in pupae of *A. ipsilon* at different concentrations. The lowest LC_{50} value of two strains of *Heterorhabditis* spp. TAN5 and PGN6 against *A. ipsilon* larvae was 1285.527 and 1560.747 IJs/cup, respectively (Nouh 2021). Under laboratory and glasshouse conditions, *H. indica* registered the highest LC_{50} of 16.39 IJs/larva and LT_{50} of 28.69 h/larva, while *S. glaseri* registered lowest LC_{50} of 18.03 IJs/larva and LT_{50} of 23.98 h/larva and against *A. ipsilon* (Radhakrishnan et al. 2017). The infectivity of an EPNs species against their host species may depend upon a variety of reasons including but not specific to the search tactics and dispersal pattern displayed by the EPNs (Griffin et al. 2005). This explains the different mortality rates obtained in case of different nematode species. However, it should be noted that there are certain similarities between the performances of EPNs species belonging to the same families.

Conclusions

The results showed that the native EPNs species were indubitably virulent and effective in causing mortality in *O. obesus* and *A. ipsilon,* while *H. bacteriophora* had a higher virulence than *S. aciari* against both tested insects. The latter was effective albeit less than *H. bacteriophora* under laboratory conditions. Although the EPNs species were able to cause effective mortality under laboratory conditions, it is extremely necessary that further experiments should be carried out under field conditions. More native potential strains of EPNs species need to be screened and isolated to explore their efficacy against various soil insect pests under both laboratory and field conditions.

Abbreviations

EPNs: Entomopathogenic nematodes; IBM: International Business Machines Corporation; IJs: Infective Juveniles; IPM: Integrated pest management; lb: Pound; LD_{50}: Median lethal dose; LT_{50}: Median lethal time; SPSS: Statistical product and service solutions.

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Authors' contributions

BB and GD designed and conceptualized the research. The research was carried out by KSB under the guidance and supervision of BB. EBD and NSM assisted with the practical work. SB helped in the analysis and interpretation of data. The first version of the manuscript was drafted by KSB with the assistance of PPGD. BB revised and finalized the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

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Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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