Vashisth et al. Egyptian Journal of Biological Pest Control (2018) 28:37
https://doi.org/10.1186/s41938-018-0040-5

Egyptian Journal of Biological Pest Control

RESEARCH Open Access

Biological control potential of North West Himalayan strains of heterorhabditid nematodes against the turnip moth, *Agrotis segetum* (Denis & Schiffermuller) (Lepidoptera: Noctuidae)

Sumit Vashisth[1,2*], Y. S. Chandel[1] and R. S. Chandel[1]

Abstract

Himachal Pradesh is a northern state of India and is situated to the southern of the mighty Himalaya. The agro-climatic conditions are conducive for the production of off-season vegetables. Biological control by entomopathogenic nematodes (EPNs) is one of the alternatives to manage the turnip moth, *Agrotis segetum* (Denis & Schiffermuller) (Lepidoptera: Noctuidae). The present study was, therefore, undertaken with the objective to assess the virulence of local EPN isolates from Himachal Pradesh, *Heterorhabditis* sp., against pests to minimize the use of insecticides during crop protection. Against L3–L5 of *A. segetum*, *Heterorhabditis indica* was found highly effective, resulting in 33.33–93.33% mortality at 40 infective juveniles (IJs)/larva after 96 h of treatment. Among local isolates, *Heterorhabditis* sp. (HSG) influenced maximum mortality of L3 and L4, whereas in L5, *H. bacteriophora* (HRJ) influenced maximum mortality. The sensitivity of L3 to different EPNs was (7.0–16.6%) higher than that of L4. In soil bioassay carried out against L4 of *A. segetum*, *H. bacteriophora* (HRJ) was found highly effective, followed by *Heterorhabditis* sp. (HKM) and *Heterorhabditis* sp. (HSG) at 10,000 IJs/kg of soil. The mortality varied from 78.33 to 81.67% with local isolates after 7 days of treatment. The results suggested that EPNs can be used as one of the components for managing *A. segetum* under field and greenhouse conditions to reduce over dependence on insecticides.

Keywords: *Agrotis segetum*, Biological control, Entomopathogenic nematodes, Potential, Vegetable crops

Background

The turnip moths are polyphagous and cosmopolitan pests attacking a large number of crops worldwide (Napiorkowska and Gawowska 2004). The genus *Agrotis* includes many species of turnip moths which cause extensive damage to several vegetable and field crops in India. Five species of cutworm viz., *Agrotis ipsilon* (Hufnagel), *A. segetum* (Denis & Schiffermuller), *A. flammatra* Denis & Schiffermuller, *A. interacta* Walker, and *A. spinifera* Hubner have been reported damaging potato and other crops in India (Chandel et al. 2007). Verma and Verma (2002) reported that *A. segetum* and *A. ipsilon* as predominant species attack various crops (3–18% infestation) in Himachal Pradesh, India. *A. segetum* is a leading species causing extensive damage in vegetables, ornamentals, and field crops. *A. ipsilon* is prevalent in low and mid hills, whereas *A. segetum* is more abundant in higher elevations (Anonymous 2003). Lv et al. (2006) and Esbjerg and Sigsgaard (2014) reported that *A. segetum* is distributed throughout the temperate regions of Europe, Africa, and Asia. Turnip moth larvae which become photonegative by the fourth instar hide in the soil during the daylight hours. In these latter instars, they also tend to severe plants at the soil surface, pulling the plant tissue below ground (Sharma et al. 2012). However, application of soil insecticides is generally ineffective because of their difficulty in translocation through the soil into the root

* Correspondence: sumitvashisth_hpau@yahoo.co.in
[1]Department of Entomology, CSK Himachal Pradesh Agriculture University, Palampur, Himachal Pradesh 176062, India
[2]International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Telengana State 502324, India

zone. In early stages, the crops suffer severely and irreparably and the crop stand is considerably reduced. This is particularly true for cole crops like cauliflower and cabbage, the important cash crops in north western Himalaya. Farmers used large quantity of farm yard manure (FYM) to increase their yields but the excessive of using FYM enhances the severity of cutworm incidence.

The control of *Agrotis* spp. through chemicals has been reported by various researchers in India (Chandel and Chandla 2003 and Sharma et al. 2012). Because of the difficulty in managing turnip moth through the use of insecticides, special attention has been focused on entomopathogens, especially nematodes (EPNs). The EPNs have been used as biological control agents to effectively impress a large number of economically important insect pests (Grewal et al. 2005 and Laznik et al. 2010). Hassan et al. (2009) have observed steinernematids and heterorhabditids to infect over 200 species of insects belonging to different orders. EPNs have been applied successfully against soil-inhabiting insects (as soil application) as well as aboveground insects (foliar spray) in cryptic habitats (Arthurs et al. 2004; Trdan et al. 2007; and Laznik et al. 2011). According to Sharma et al. (2011), the performance of EPNs has got more success in controlling soil insects when compared to foliar pests. They are mobile in soil environment and have a potential in inundative and inoculative releases with persistence for years (Bathon 1996). However, the efficacy of most biological control agents including EPNs declines with advancement of larvae into higher instar stages (Laznik and Trdan 2015). Recently, Vashisth et al. (2015) surveyed the EPNs in temperate areas of Himachal Pradesh and identified virulent strains of *Heterorhabditis* spp.

The present study aimed to evaluate the potential of the species/strains of *Heterorhabditis* spp. against *A. segetum*, native to north western Himalaya.

Materials and methods

Soil sampling and nematode extraction

Soil samples were collected during 2011–2013 from different parts of Himachal Pradesh, India. The samples were transported to the laboratory at the Himachal Pradesh Agriculture University (HPAU), Palampur, Himachal Pradesh, India, and kept in a cool place until processing. Information on sampling date, location, soil type, habitat, longitude, latitude, and altitude was recorded for each sample (Vashisth et al. 2015). EPNs were recovered from soil samples, using an insect baiting method described by Bedding and Akhurst (1975). Greater wax moth, *Galleria mellonella* (Linnaeus), and rice moth, *Corcyra cephalonica* (Stainton), were used as baiting agents. Twenty last-instar larvae of either of these insects were placed at the bottom of a jar containing 250 g moistened soil each sample and kept at room temperature (25 ± 2 °C) for 1 week. Dead larvae from the container were examined for the presence of nematodes and placed in White traps (White (1927)) to collect the emerging infective juveniles (IJs). To verify the pathogenicity of collected nematodes and to establish new cultures, the emerging nematodes were pooled in each sample and used to infect fresh *A. grisella* and *C. cephalonica* larvae. For identification, the nematodes were placed in Ringer's solution (Kaya and Stock 1997) on an object glass, covered with a ring glass. Adams and Nguyen (2002) methodologies were used for the nematode identification. For further studies, mass production of the nematode population was carried out, using chicken offal medium (Bedding 1984). After about 20 days of inoculation, harvesting of IJs was done following the method of Tabassum and Shahina (2004). The pathogenicity of the harvested IJs was tested three times against the last-instar larvae of *A. grisella* and *C. cephalonica.* All the experiments were performed within 15 days of emergence.

Insect culture of turnip moth, *Agrotis segetum*

The turnip moth culture was maintained according to the procedure standardized by Verma (1996). First-instar larvae were maintained on soft cabbage leaves grown in small plastic containers (5.5 × 7 cm). The petiole end of each leaf was pressed into a piece of wet cotton to maintain the turgidity of the leaf for a longer period. The larvae were cultured *en masse* up to the third-instar larvae (L3); however, after L3, the larvae showed cannibalism against each other; therefore, subsequent culturing was undertaken in glass jars (15 × 18 cm), filled with mixture of soil + sand up to 10 cm. Fresh cabbage leaves were provided regularly in jars as food for developing larvae, and the leaves were changed frequently. The full-grown larvae pupated in the soil, and the adult moths emerged out in about 15 days. The adults were sexed on the basis of their antennae and were transferred to glass chimneys for mating. A crumpled paper was placed in each chimney to support easy repose of moths on these sites. In each glass chimney, 10% honey solution was kept in a small Petri plate and three pairs of *A. segetum* were released in it. The eggs were collected by a camel hair brush and placed on the moist filter paper in the Petri plate for hatching.

Evaluation of local EPN isolates against *Agrotis segetum*

Petri plate bioassay

Efficacy of the three local EPNs (*Heterorhabditis* sp. (HSG), *Heterorhabditis* sp. (HKM), and *H. bacteriophora* (HRJ)), obtained during the survey from Sangla, Kamand, and Rajgarh areas of Himachal Pradesh, India, along with commercially available formulation of *Heterorhabditis indica* (National Bureau of Agricultural Insect Resources, Bengaluru, India), were tested against L3–L5 of *A. segetum*, following the Petri plate bioassay. For Petri plate

bioassay, a Whatman No. 1 filter paper (9.5 cm in diameter) was placed in a sterile Petri dish (9 cm in diameter). The EPN concentrations were adjusted to 10, 20, 30, and 40 IJs/larva. Healthy and uniform size laboratory-reared larvae of test insects were used for bioassay studies. The larvae were exposed to 1 ml nematode suspension at 10, 20, 30, and 40 IJs/larva on to a moist filter paper lined in a Petri dish (10 larvae per Petri plate), and each set of treatment was replicated three times. In the control, only 1 ml of distilled water was used. The insects were provided with their natural food. Inoculated plates were incubated at 25 ± 1 °C. The insect mortalities were checked, 24, 48, 72, and 96 h post inoculation. Mortality data were corrected using Abbott's formula (Abbott 1925). The corrected mortality data were analyzed on the pattern of a completely randomized design, using three-way tables.

Soil inoculation bioassay

This method was used only against L4 of *A. segetum* against all species of EPNs (Fig. 2). For testing, 1 kg sterilized moist soil was placed into a plastic/glass jar. Different concentrations of nematodes (1000, 5000, and 10,000 IJs) were applied in 10 ml distilled water per kilogram of soil. In each jar, 10 turnip moth larvae (L4) were released, and tender cabbage leaves were also placed in the jars as food for the larvae. There were three replications for each treatment. The glass jars were covered with muslin cloth and kept at room temperature. The insect mortalities were checked 3, 5, and 7 days post inoculation. For data recording, the soil was tipped out and the larvae were gently searched in soil. The healthy and dead larvae were counted, and the soil was filled again in the same jars along with the healthy larvae. Mortality data were corrected using Abbott's formula (Abbott 1925), which were used for further analysis, using a completely randomized design.

Statistical analysis

The insect mortality was corrected using Abbott's formula (Abbott 1925). Data were subjected to ANOVA using Genstat Version 14.0. Significance of differences between the isolates was tested by *F* test, while the treatment means were compared by LSD at $P \leq 0.05$.

Results and discussion

Petri plate bioassay

There were significant differences in mortality of L3 of *A. segetum* across EPN isolates. *H. indica* caused the maximum larval mortality (93.3%) 96 h post treatment, at the concentration of 40 IJs/larva (Fig. 1a). Among the locally extracted EPN isolates, *Heterorhabditis* spp. (HKM) caused a maximum mortality (81.3%) of *A. segetum* in comparison to the other two isolates. The data revealed significant differences in the percent larval mortality of *A. segetum* for each nematode species, population species (S) wise $F = 43.2$, $df = 3$, $p = < 0.001$; exposure (E) period wise $F = 421.4$, $df = 3$, $p = < 0.001$; and population (P) number wise $F = 228.52$, $df = 3$, $p = < 0.001$.

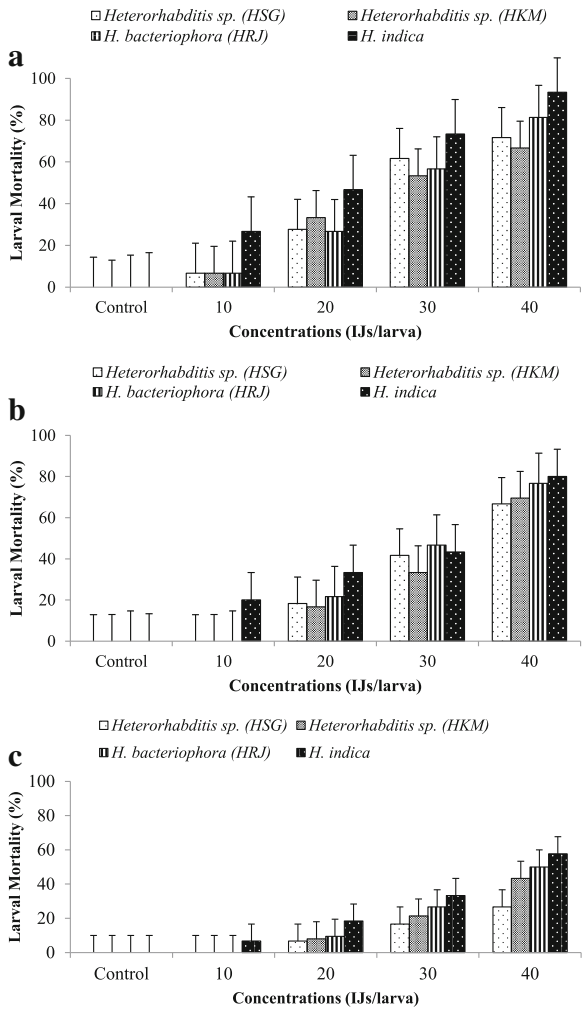

Fig. 1 The percentage mortality of different instar larvae of *Agrotis segetum*, following exposure to different concentrations of infective juveniles (IJs) of nematodes under laboratory conditions (Petri plate bioassay). **a** Third instar. **b** Fourth instar. **c** Fifth instar

The differences in potential of the different isolates of EPNs against L4 of *A. segetum* were insignificant but differences across the concentrations were significant. All EPN isolates caused equivalent amount rates of larval mortality 96 h post inoculation, at the concentration of 40 IJs/larva (Fig. 1b). The data revealed significant differences in percent larval mortality of *A. segetum* for each nematode species, population S wise $F = 30.36$, $df = 3$, $p = < 0.001$; E period wise $F = 323.19$, $df = 3$, $p = < 0.001$; and P number wise $F = 183.31$, $df = 3$, $p = < 0.001$.

Among local EPN isolates, *H. bacteriophora* (HRJ) caused the maximum larval mortality in L5 of *A. segetum* 96 h post inoculation, at the concentration of 40 IJs/larva (Fig. 1c), whereas the commercial isolate *H. indica* caused the maximum larval mortality (56.6%). The data revealed significant differences in larval mortality of *A. segetum* for each nematode species, population S wise $F = 0.7$, $df = 3$, $p = < 0.001$; E period wise $F = 112.78$, $df = 3$, $p = < 0.001$; and P number wise $F = 70.07$, $df = 3$, $p = < 0.001$. Comparative data pertaining to efficacy of *Heterorhabditis* sp. (HSG), *Heterorhabditis* sp. (HKM), *H. bacteriophora* (HRJ) and *H. indica* 96 h post inoculation, at the concentration of 40 IJs/larva against L3-L5 of turnip moth were illustrated in Fig. 2.

Soil inoculation bioassay

Heterorhabditis sp. (HSG) achieved 28.33% mortality of *A. segetum* L4 at 10,000 IJs/kg soil, whereas at 1000 IJs/kg of soil, only 3.33% mortality was recorded 3 days post treatment. After 5 days of treatment, 8.33, 30.10, and 53.33% mortality were recorded at 1000, 5000, and 10,000 IJs/kg soil, respectively. The highest mortality rate (78.33%) was obtained at 10,000 IJs/kg of soil 7 days post treatment (Fig. 3a). The data revealed significant differences in larval mortality rates of *A. segetum* for each nematode species, population S wise $F = 16.67$, $df = 3$, $p = < 0.001$; E period wise $F = 462.68$, $df = 2$, $p = < 0.001$; and P number wise $F = 772.24$, $df = 2$, $p = < 0.001$.

Heterorhabditis sp. (HKM) showed a slightly higher mortality against L4 of *A. segetum*, compared to *Heterorhabditis* sp. (HSG) at the tested concentrations. There was 31.67% mortality with this EPN isolate, at 10,000 IJs/kg of soil 3 days post treatment. After 5 and 7 days of treatment, the concentration of 10,000 IJs/kg soil caused 58.33 and 80.00% mortality rates, respectively. At the population level of 5000 IJs/kg soil, the mortality varied from 18.33 to 58.33%, 3 to 7 days post treatment. The minimum mortality rate (21.67%) was recorded 7 days post treatment, at 1000 IJs/kg of soil (Fig. 3b).

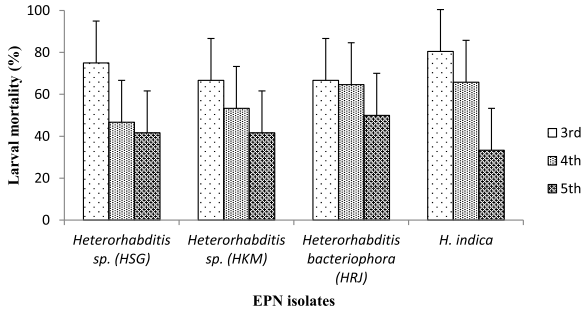

Fig. 2 Relative susceptibility of *Agrotis segetum* against different EPN isolates

H. bacteriophora (HRJ) was comparatively more effective than *Heterorhabditis* spp. (HSG) and *Heterorhabditis* sp. (HKM). There achieved 20.00 and 33.33% mortality rates, at 5000 and 10,000 IJs/kg soil, 3 days post treatment. The lowest concentration of 1000 IJs/kg of soil recorded 3.33–26.67% mortality, 3 to 7 days post treatment. At a concentration of 5000 IJs/kg of soil, the mortality rate varied from 36.67 to 60.00%, 5 to 7 days post treatment. The maximum mortality rate (81.67%) was recorded at 10,000 IJs/kg soil 7 days post treatment (Fig. 3c).

Comparative data pertaining to efficacy of *Heterorhabditis* sp. (HSG), *Heterorhabditis* sp. (HKM), and *H. bacteriophora* (HRJ) were illustrated in Fig. 4. All the three local isolates were statistically at par with each other, at the concentration of 1000 IJs/kg soil. The mortality rate varied from 11.66 to 13.89% at this concentration. At 5000 IJs/kg of soil, *H. bacteriophora* (HRJ) and *Heterorhabditis* sp. (HKM) showed 38.89 and 37.22% mortality and both were statistically at par with each other. *Heterorhabditis* sp. (HSG) recorded 33.33% mortality rate, which differed significantly than *Heterorhabditis* sp. (HKM) and *H. bacteriophora* (HRJ). At the highest population level of 10,000 IJs/kg of soil, *Heterorhabditis* sp. (HKM) was statistically at par with *Heterorhabditis* sp. (HSG) and *H. bacteriophora* (HRJ), whereas *Heterorhabditis* sp. (HSG) differed significantly than *H. bacteriophora* (HRJ). The highest mortality rate (57.78%) was recorded by *H. bacteriophora* (HRJ) at this concentration. The mortality rate varied from 16.11–18.89%, 3 days post treatment, and the differences among isolates were insignificant (Fig. 4).

In order to study the reproduction of EPNs, the *A. segetum* larvae were exposed to 10, 20, 30, and 40 IJs/larva of each nematode species. The host mortality rate and the emerging IJs from host cadavers were collected and counted. The data revealed that all four test nematodes were successfully invaded and propagated in the insect larvae and produced IJs (Figs. 5a–d). It was also evident that all nematode species exhibited a linear relationship between the concentrations of IJs applied and the total number of IJs produced per infected larva. In this study, *H. indica* and *H. bacteriophora* (HRJ) produced significantly more number of IJs per insect larva than the other two nematode species (Fig. 2c, d). For *H. bacteriophora* (HRJ) and *H. indica*, the maximum production of IJs per larva ($14.23 \pm 1.34 \times 10^3$ IJs/larva and $11.09 \pm 1.14 \times 10^3$ IJs/larva) was obtained at 40 IJs/larva concentration. Among the four EPNs studied, the least progeny production was recorded for *Heterorhabditis* sp. (HKM). It increased linearly with the increase of IJ concentration where it reached its maximum of $7.62 \pm 1.04 \times 10^3$ IJs/larva, at the concentration of 40 IJs/larva.

There were significant differences in the efficacy of different isolates of EPNs against the L3 of *A. segetum* across concentrations and the observation periods. The

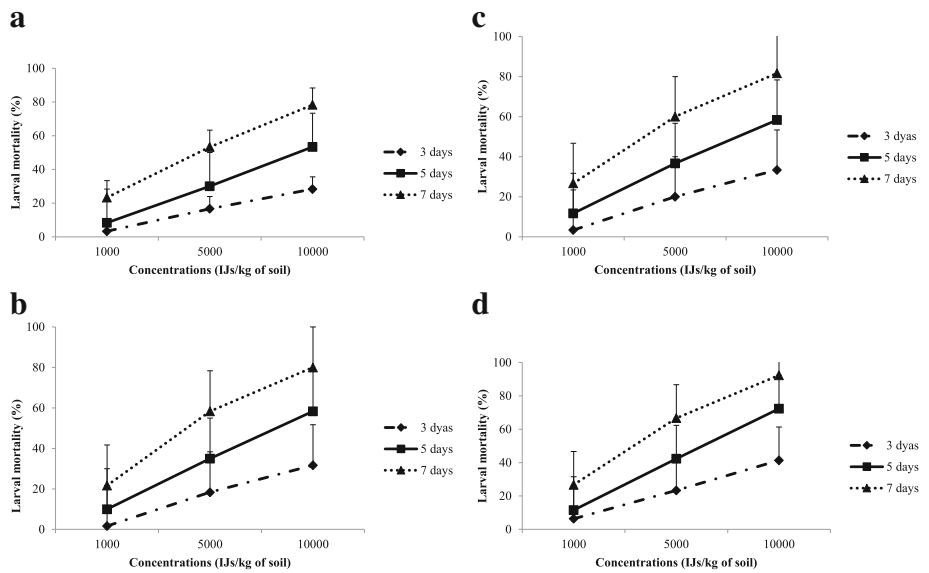

Fig. 3 The percentage mortality of fourth-instar larvae of *Agrotis segetum* following exposure to different concentrations of infective juveniles (IJs) of nematodes under laboratory conditions (soil inoculation method). **a** *Heterorhabditis* sp.(HSG). **b** *Heterorhabditis* sp.(HKM). **c** *H. bacteriophora* (HRJ). **d** *H. indica*

efficiency of EPNs against a given host partly depends on the host-finding, ability, and penetration capability of the IJs (Peters & Ehlers, 1994). EPNs have been tested against a large number of insect pest species, with results varying from poor to excellent control (Laznik and Trdan 2015). Many factors can influence the successful use of nematodes as biological agents, but matching the biology and ecology of both the nematode and the target pest is a crucial step towards successful application. The *H. bacteriophora* (HRJ) resulted in a significantly greater mortality of *A. segetum* than the isolates from Sangla and Kamand across the isolates and observation periods, though the differences in larval mortality between the isolates were much smaller. These findings are in partial agreement to the findings of Chandel et al. (2009) who reported 100% mortality of L3 and L4 of *A. segetum* in a Petri plate bioassay, at 10–40 IJs/cm^2, due to infection with *H. bacteriophora.* Fetoh et al. (2009) tested an Egyptian strain of *H. bacteriophora* against L4 of *A. ipsilon* under laboratory conditions and recorded 80 ± 4.0 to 100 ± 0.0% mortality rate, at 25–100 IJs/ml and reported that *H. bacteriophora* was highly virulent against *A. ipsilon.* Larval mortality may be presumed to be related to the number of viable nematodes ingested by the insect during feeding, or infection could take place by invasion of the nematodes through the natural openings/cuticle of the insect with an undetermined minimum number required for mortality to occur. The maximum mortality (93.3%) was caused by *H. indica* (commercially available isolate during studies) against L3 of *A. segetum.* Hussaini et al. (2005) studied the infectivity of *H. indica* PDBC EN6.71 along with other EPNs against the last-instar larvae of *A. ipsilon* and obtained absolute mortality after 48 and 72 h at 25 and 32 °C. Against *A. segetum*, there was a 66.70% mortality rate, with *H. indica* PDBC EN 6.71 72 h post inoculation (Hussaini et al. 2000). Yan et al. (2014) also reported that *H. indica* LN2 was the most virulent and promising species, causing 83.3% mortality to the L3 72 h post infection.

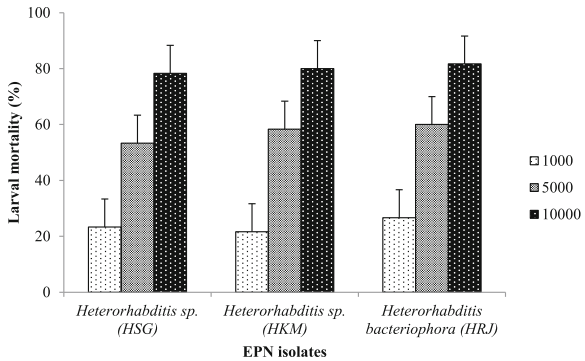

Fig. 4 Relative susceptibility of *Agrotis segetum* against different EPN isolates

In the soil bioassay carried out against L4 of *A. segetum*, *H. bacteriophora* (HRJ) was found highly effective, followed by *Heterorhabditis* sp. (HKM) and then *Heterorhabditis* sp. (HSG) at10,000 IJs/kg of soil. The mortality rate varied from 78.33 to 81.67% 7 days post treatment, at local isolates. Chandel et al. (2009) found that the concentration

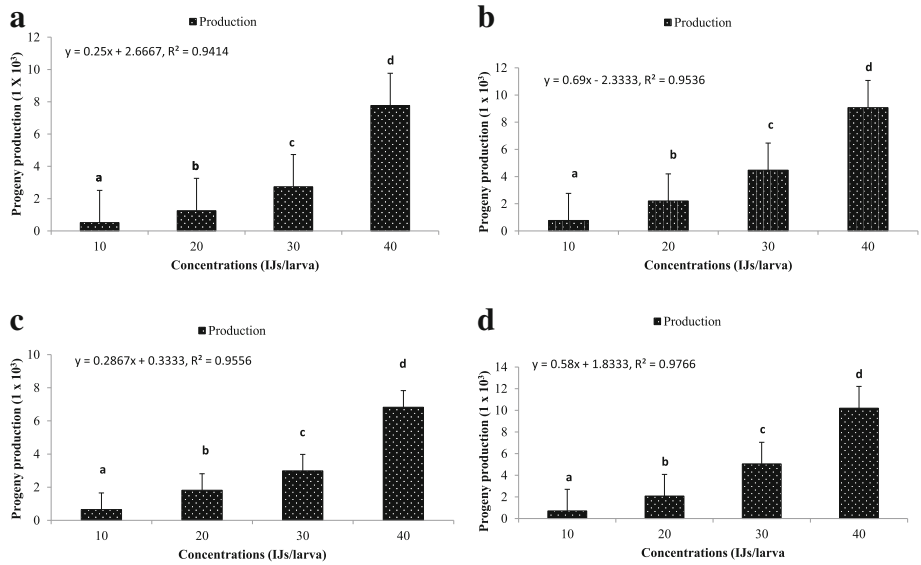

Fig. 5 Production of infective juveniles in *Agrotis segetum* proxima larvae at different dosages of infective juveniles. **a** *Heterorhabditis* sp. (HSG). **b** *Heterorhabditis* sp. (HKM). **c** *H. bacteriophora* (HRJ). **d** *H. indica*. **$p < 0.01$%; means shown by the same letter are not significantly different ($p > 0.05$)

of 1000 IJs/kg of soil of *H. bacteriophora* was sufficient to initiate infection in the larvae of *A. segetum*. They reported 61.3–91.6% mortality rate in L4 at 1000–10,000 IJs/kg of soil. Obtained data support the findings of Hussaini et al. (2001) who recorded that *Heterorhabditis* was virulent against *A. ipsilon* larvae in sand column assay. Gupta (2003) studied the efficacy of EPNs against *A. ipsilon* and found that the nematodes applied as foliar spray (50–100 IJs/larva), paper wrapping method (25–75 IJs/larva), and food dip (25–75 IJs/larva) caused 40–80, 100, and 40–60% pest mortality rates, respectively.

When *A. segetum* were exposed to *Heterorhabditis* spp. in sand, higher inoculation concentrations were required as compared to inoculation concentrations on filter paper. The differences in the two inoculation methods may be attributed to the differences in the inoculation substrates and bioassay arenas. In the sand bioassay technique, *A. segetum* was exposed to *Heterorhabditis* spp. in three-dimensional substrates, where the nematode has to search the insect host. In the Petri plate bioassay method, *A. segetum* was exposed to *Heterorhabditis* spp. in two-dimensional substrates, where the nematode and the host were in direct and close contact with each other. Obtained results are in agreement with that of Grewal et al. (1994) who reported that the substrate had a profound effect on host finding by EPNs. A major factor that restricts the EPNs' host range was the foraging behavior of the IJs. These nematodes employed different foraging strategies to locate and infect hosts that range from one extreme of sit-and-wait (ambush) to the other of widely foraging strategy (cruise) (Lewis 2002; Laznik and Trdan 2013).

Timing of nematode applications is also an important consideration. Different turnip moths may arrive to the root feeding zone near the soil surface at varying times during the growing season. Nematodes applied too early may provide poor insect control and may not reach deep in the soil before their upward seasonal migration. To overcome the dispersal behavior of the EPNs, *Heterorhabditis* spp., so that it can infect its host, the application of high dosages to the soil surface may increase the infection rate. However, it is also important to note that results from the laboratory tests are not always comparable to field testing (Cantelo and Nickle 1992) as the functioning of EPNs in the open is influenced by an extensive list of factors. In one relevant study, the 100% efficacy rate of *S. carpocapsae* in controlling Colorado potato beetle adults, pupae, and larvae in the laboratory manifested as only a 31% reduction rate in this pest population when the test was repeated outdoors (Stewart et al. 1998).

Reproducing and recycling of EPNs in a host play an important role in their persistence in the soil and also in their overall effectiveness in pest control (Georgis and Hague 1991). A prior knowledge about reproducing and recycling nematodes is considered important in determining the time and concentration of subsequent EPN application, which may be useful in reducing the cost of application. The data in the present study suggested that following application, all the tested species of nematodes were able to infect and propagate within the insect host and produce IJs. The evidence obtained in this study suggests that all three tested indigenous species of EPNs were virulent enough to produce 100% mortality to the larvae of

A. segetum. Furthermore, all EPNs could also propagate in the infected larva and produce F1 generation IJs.

Conclusions

The results showed that *H. indica* could offer a great potential and a higher virulence than other species/strains of EPNs against the larvae of *A. segetum*. EPNs (*Heterorhabditis* sp. (HSG), *Heterorhabditis* sp. (HKM), and *H. bacteriophora* (HRJ)) had a potential to be used as biocontrol agents for the integrated management of *A. segetum* in vegetable crops and flowers under protected cultivations. Among the local isolates, *Heterorhabditis* sp. (HSG) recorded the maximum mortality rates during L3–L4, and the sensitivity of larva decreased with increasing the larval age. Hence, EPNs prudent to target young larvae as compared to older ones. In soil bioassay, local isolates gave very high mortality rates within a week's time. The EPNs can propagate in the infected larvae and produce IJs; hence, they hold a good potential to be used as inoculative agents in soil for biological control of turnip moth larvae. The soil provides the most congenial conditions where IJs can survive, persist, establish, recycle, and develop a long-term regulation of turnip moths and other soil pests.

Acknowledgements
No acknowledgements

Declaration statements
The responsibility of the author(s) is to obtain permission from the copyright holder to reproduce figures or tables that have previously been published elsewhere: No figures or tables are reproduced that have previously been published.

Funding
No funding

Availability of data and materials
The data and materials are available from https://doi.org/10.6084/m9.figshare.5882188.v1.

Authors' contributions
SV carried out the bioassay studies, statistical analysis and drafted the manuscript. YS participated in the design of the study and assisted in the statistical analysis. RS conceived of the study and participated in its design and coordination. All authors read and approved the final manuscript.

Ethics approval and consent to participate
Not applicable

Consent for publication
Not applicable

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

Publisher's Note

Received: 24 January 2018 Accepted: 4 March 2018
Published online: 05 April 2018

References

Abbott WS (1925) Methods for computing the effectiveness of an insecticide. J Econ Entomol 18:265–267
Adams BJ, Nguyen KB (2002) Taxonomy and systematics. In: Gaugler R (ed) Entomopathogenic nematology. CABI, New York, NY, pp 1–34
Anonymous (2003) Annual report—all India network project on white grubs and other soil arthropods. Department of Entomology, Chaudhary Sarwan Kumar Himachal Pradesh Krishi Vishvavidyalaya, Palampur, p 20
Arthurs S, Heinz KM, Prasifka JR (2004) An analysis of using entomopathogenic nematodes against above-ground pests. Bull Entomol Res 94:297–306
Bathon H (1996) Impact of entomopathogenic nematodes on non-target hosts. Biocontrol Sci Techn 6(3):421–434
Bedding RA (1984) Large scale production, storage and transport of the insect-parasitic nematodes *Neoaplectana* spp. and *Heterorhabditis* spp. Ann ApplBiol 104:117–120
Bedding RA, Akhurst RJ (1975) A simple technique for the detection of insect parasitic nematodes in soil. Nematology 21:109–110
Cantelo WW, Nickle WR (1992) Susceptibility of prepupae of the Colorado potato beetle (Coleoptera: Chrysomelidae) to entomopathogenic nematodes (Rhabditida: Steinernematidae, Hererorhabditidae). J Entomol Sci 27:37–43
Chandel RS, Chandla VK (2003) Managing tuber damaging pests of potato. Indian Hort 48:15–17
Chandel RS, Dhiman KR, Chandla VK, Kumar V (2007) Integrated pest management in potato. In: Jain PC, Bhargava MC (eds) Entomology: novel approaches in entomology. New India Publishing Agency, New Delhi, pp 377–398
Chandel YS, Kapoor S, Kumar S (2009) Virulence of *Heterorhabditis bacteriophora* (Poinar) against cutworm, *Agrotis segetum* (Denis and Schiff.) J Biol Control 23(4):409–415
Esbjerg P, Sigsgaard L (2014) Phenology and pest status of *Agrotis segetum* in a changing climate. Crop Prot 62:64–71
Fetoh BSA, Khaled AS, Elwagar TFK (2009) Combined effect of entomopathogenic nematodes and biopesticides to control the greasy cutworm, Agrotis ipsilion (Hufr.) in the strawberry fields. Egyptian Acad J Biol Sci 2(1):227–236
Georgis R, Hague NGM (1991) Nematodes as biological insecticides. Pesticide Outlook 2:29–32
Grewal PS, Ehlers RU, Shapiro-Ilan DI (2005) Nematodes as biological control agents. CABI Publishing, Wallingford
Grewal PS, Lewis EE, Gaugler R, Campbell JF (1994) Host finding behavior as a predictor of foraging strategy in entomopathogenic nematodes. Parasitology 108:207–215
Gupta P (2003) Entomopathogenic nematodes—work done at Allahabad agricultural institute, Allahabad. In: Hussaini SS, Rabindra RJ, Nagesh M (eds) Current status of research on entomopathogenic nematodes in India: workshop on the entomopathogenic nematodes in India held on 22 and 23rd January, 2003, pp 161–165
Hassan W, Singh CP, Askary TH (2009) Entomopathogenic nematodes—as a biocontrol agent for insect pests of various crops. Indian Farm Dig 3:15–18
Hussaini SS, Nagesh M, Shakeela V (2005) Survival of infective juveniles of entomopathogenic nematodes under storage and their infectivity against *Galleria mellonella* and *Spodoptera litura*. Indian J Plant Prot 33(1):68–71
Hussaini SS, Singh SP, Parthasarathy R, Shakeela V (2000) Virulence of native entomopathogenic nematodes against black turnip moth, *Agrotis ipsilon* (Hufnagel) and *A. segetum* (Noctuidae : Lepidoptera). Indian J Nematol 30(1): 103–105
Hussaini SS, Singh SP, Parthasarathy R, Shakeela V (2001) Comparison of bioassays to measure the infectivity of some isolates of entomopathogenic nematode against *Agrotis ipsilon* (Hufnagel) and *A. segetum* (Schiff.) Curr Nematol 12(1/2):63–68
Kaya HK, Stock SP (1997) Techniques in insect nematology. In: Lacey L (ed) Manual of techniques in insect pathology. Biological Technique Series, Academic Press Ltd, London, pp 281–324
Laznik Z, Toth T, Lakatos T, Vidrih M, Trdan S (2010) Control of the Colorado potato beetle (*Leptinotarsa decemlineata* [Say]) on potato under field conditions: a comparison of the efficacy of foliar application of two strains of *Steinernema feltiae* (Filipjev) and spraying with thiametoxam. J Plant Dis Protect 117(3):129–135
Laznik Z, Trdan S (2013) An investigation on the chemotactic responses of different entomopathogenic nematode strains to mechanically damaged maize root volatile compounds. Exp Parasitol 134(3):349–355

Laznik Z, Trdan S (2015) Failure of entomopathogens to control white grubs (Coleoptera: Scarabaeidae). Acta agriculturæ Scandinavica. Section B, Soil and Plant Science 65(2):95–108
Laznik Z, Znidarcic D, Trdan S (2011) Control of *Trialeurodes vaporariorum* (Westwood) adults on glasshouse-grown cucumbers in four different growth substrates: an efficacy comparison of foliar application of *Steinernema feltiae* (Filipjev) and spraying with thiamethoxamn. Turk J Agric Forestry 35(6):631–640
Lewis EE (2002) Behavioural ecology. In: Gaugler R (ed) Entomopathogenic nematology. CABI Publishing, Wallingford, pp 205–223
Lv Z, Wang PL, Zhang QH, Gong ZZ, Ding H (2006) Relationships between overwintering *Agrotis segetum* population and snow. Chin J Ecol 25(12): 1532–1534 (In Chinese with English abstract)
Napiorkowska KJ, Gawowska J (2004) Increase of harmfulness of caterpillars (Hadeninae and Noctuinae, Lepidoptera: Noctuidae) on cabbage and other cole crops. Progress in Plant Protection 44:978–980
Peters A, Ehlers RU (1994) Susceptibility of leatherjackets (*Tipula paludosa* and *T. oleracea*; Tipulidae; Nematocera) to the entomopathogenic nematode *Steinernema feltiae*. J Invertebr Pathol 63:163–171
Sharma A, Chandel RS, Kumar V, Singh BP (2012) Potato cultivation in North Western Himalaya. CPRI technical bulletin no. 95 - potato cultivation in North Western Himalaya by Ashwani Sharma, RS Chandel, Vinod Kumar and BP Singh. 2012. 72p
Sharma MP, Sharma AN, Hussaini SS (2011) Entomopathogenic nematodes, a potential microbial biopesticide: mass production and commercialization status—a mini review. Arch Phytopathology Plant Protect 44(9):855–870
Stewart JG, Boiteau G, Kimpinski J (1998) Management of late-season adults of the Colorado potato beetle (Coleoptera: Chrysomelidae) with entomopathogenic nematodes. Can Entomol 130:509–514
Tabassum KA, Shahina F (2004) In vitro mass rearing of different species of entomopathogenic nematodes in monoxenic solid culture. Pakistan J Nematol 22(2):167–175
Trdan S, Znidarcic D, Vidrih M (2007) Control of *Frankliniella occidentalis* on greenhouse-grown cucumbers : an efficacy comparison of foliar application of *Steinernema feltiae* and spraying with abamectin. Russ J Nematol 15(1):25–34
Vashisth S, Chandel YS, Chandel RS (2015) Distribtuion and occurrence of entomopathogenic nematodes in Himachal Pradesh. J Entomol Res 39(1):71–76
Verma KS (1996) Bioecology and management of turnip moth (Lepidoptera, Noctidae) attacking vegetable crops. Ph.D. Thesis, Dr. Yashwant Singh Parmar University of Horticulture and Forestry, Solan, p 121
Verma KS, Verma AK (2002) Cutworm species associated with different crops in Himachal Pradesh. Insect Environ 8:23
White GF (1927) A method for obtaining infective nematode larvae from cultures. Sci 66:302–303
Yan X, Wang X, Han R, Qiu X (2014) Utilization of entomopathogenic nematodes, *Heterorhabditis* spp. and *Steinernema* spp., for the control of *Agrotis ipsilon* (Lepidoptera, Noctuidae) in China. Nematol 16(1):31–40