

RESEARCH

Open Access



Storage and efficacy of entomopathogenic nematode species as a biocontrol agent against the armyworm, *Spodoptera litura* (FABRICIUS) (Lepidoptera: Noctuidae)

Salma Javed*, Tabassum Ara Khanum and Ashraf Ali

Abstract

Background: The armyworm, *Spodoptera litura* (FABRICIUS) (Lepidoptera: Noctuidae) is a widely distributed polyphagous pest that is seriously affecting more than 100 different hosts. Globally, biopesticides are becoming the mainstream as chemicals and biocontrol agents gain more of the market share every year, because some of these biocontrol measures prevent the economic disruption of food crops.

Results: For storage, 3 substrates were tested for entomopathogenic nematodes (EPNs), viz., polyether polyurethane sponge, distilled water and soil. Best storage medium was found to be polyether polyurethane sponge for 2–12 months of storage at 15–18 °C. For biocontrol evaluation four species of Steinernematidae; *Steinernema pakistanense*, *S. siamkayai*, *S. ceratophorum* and *S. bifurcatum*, and one species of Heterorhabditidae; *Heterorhabditis indica*, were used against the armyworm. At 350 IJs/ml *S. pakistanense*; *S. siamkayai*, *S. ceratophorum*, *S. bifurcatum* and *H. indica*, showed 95, 78, 74, 90 and 87% mortality, respectively.

Conclusions: Nematode species, long-term storage and shelf life are important considerations for the success of EPNs against target insect pests which in turn are a prerequisite for successful commercialization and this can be achieved by maximum survival of IJs in a formulation for a long period before their utilization in the field.

Keywords: Steinernematidae, Heterorhabditidae, Storage, Biopesticides, Substrates

Background

The armyworm, *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae), has a wide geographical distribution and it is a serious pest of many ornamentals, vegetables and crops (Ahmad and Mehmood 2015). Under favorable conditions, it has a great potential to cause outbreaks and inflict a massive yield loss to the growers. Globally, it has been reported as a major pest for 120 cultivated and uncultivated plant species distributed in 44 families (Gao et al. 2014). Heavy resistance to all conventional

and some new chemistry insecticides have also been observed which need a proper rotation and alternatives for long-term benefits in pest management (Saleem et al. 2008). The exploit of alternative methods in a pest management has a number of profits and has proved their efficiency in integrated pest management in some rural crops. These benefits are: drop off in usage of chemicals; lesser manufacturing expenses; avoidance of extreme deposits levels and minimum hazard of pests mounting resistance, reduce the opportunity of insects recurrence (Shahina et al. 2017).

The nematodes of the family Steinernematidae Travassos, 1927, and Heterorhabditidae Poinar, 1975 along with symbiotic bacteria *Xenorhabdus* (Thomas and Poinar

*Correspondence: sajaved@uok.edu.pk
National Nematological Research Centre, University of Karachi,
Karachi 75270, Pakistan

1979) and *Photorhabdus* Boemare et al. 1993 have come to be regarded as optimistic biological control agent of a broad range of farming crop pests and the key constituent in IPM (Tabassum and Salma 2020). Their virulence mechanism and the infection process are based on the free-living infective juveniles (IJs), which are highly mobile and responsible for the dispersal of the nematodes (Ciche et al. 2008). Globally many researchers are now investigating the possible exploit of EPNs as biological controls to convene the innovative values, require approximately residue free fruits and vegetables (Shahina et al. 2017). Successful commercialization can be achieved by a maximum survival of IJs in a formulation for a long period before their utilization in the field. Nematode mortality may vary from 70 to 100%, if storage life of the formulated product has expired (Grewal 2002). The approval of beneficial nematode grounded products influenced on their permanency during shipment, healthy shelf life and their ease of practice and reliable enactment in field conditions (Piggot and Wardlow 2002).

Therefore, the present study was designed to evaluate different substrates for storage of EPNs and their efficacy against *S.litura* under laboratory conditions.

Methods

Storage of entomopathogenic nematodes on different substrates

Entomopathogenic nematodes species, viz., *S. pakistanense*; *S. siamkayai*, *S. ceratophorum*, *S. bifurcatum* and *H. indica*, were stored in incubating chamber on polyether polyurethane foam, distilled water and soil medium for 2, 4, 8, 10 and 12 months duration at 15–18 °C and tested out their survival rates. The average number of live IJs was calculated under stereo microscope (40 x) on counting chamber. Experiment was completely randomized block design, with three replicates of each EPNs species and months.

Polyether polyurethane sponge

Polyurethane sponge was cut into small pieces (1.25 × 1.25 cm) cleanse with 75% ethanol and sterilized at 60 °C for 10 min. Freshly emerged from infected *Galleria mellonella* larvae, 1000 infective juveniles of each species were evenly poured on 240 gm pieces of sponge separately. Sponge-coated pieces were stored in a plastic bag sealed with tips and tied with rubber band. To check viability of nematodes, sponge was soaked separately (each species and treatment) in 20 ml water filled in a 100-ml sterilized beaker for half an hour and squeezed to release the nematodes.

Distilled water

Freshly harvested IJs of the five species of EPNs were stored in 80 ml distilled water in 250-ml conical flask separately at the concentration of 200 IJs/ml.

Soil medium

A 100-ml sterilized beakers was filled up to 80 ml with sterilized sand. Newly propagated 1000 IJs of mentioned nematodes species were pipetted in a beaker with 5 ml water suspension separately. For incubation beakers were covered with aluminum foil tied with elastic band. After completion of time survival of IJs was calculated by Cobb's sieving and decanting method using 150 mesh sieves over a 500 mesh sieve. Nematodes were retained on the bottom sieve and then collected to estimate the survival percentage.

Efficacy of EPNs against *Spodoptera litura*

Batches of 25 *S. litura* last stage larvae were placed in each 6-cm plastic Petri dish bottomed with moist filter paper and treated with nematode concentrations @ 150, 250 & 350 IJs in 1 ml distilled water with few drops of 2% Tween 80 as emulsifier. Plates were sealed with Parafilm and incubated at 28–30 °C. Mortality was determined after 48 h and untreated as a control, with 1 ml of distilled water without nematodes. Each treatment had four replicates and the experiments were repeated 3 times on different dates. The cause of mortality of insect was confirmed by dissecting the insect and observing the presence of nematodes.

Statistical analysis

Statistical data were analyzed by multifactor analysis of variance (ANOVA, followed by Duncan's multiple range test ($P < 0.05$) for separation of means (Duncan 1955).

Results

Polyether polyurethane sponge

In sponge after 2 months of storage, the average survival rates of *S. ceratophorum*, *S. pakistanense* were 72% and *S. siamkayai* 71%, *S. bifurcatum* 77% while *H. indica* showed 75% survival rate. As the time duration increased, the survival rates of *H. indica* declined up to 50%, while *S. ceratophorum*, *S. pakistanense* showed 45%, *S. bifurcatum* 42% and *S. siamkayai* 35% (Fig. 1). *S. pakistanense* showed a high survival during 2–6 months in sponge medium (ANOVA $F = 3.6$; $df = 3, 15$; $P < 0.05$), whereas *S. bifurcatum* showed a maximum survival in sponge during 2–6 months (ANOVA $F = 3.2$; $df = 3, 15$; $P < 0.05$), while *H. indica* showed a minimum survival in sponge during 2–6 months (ANOVA $F = 0.2$; $df = 3, 15$; $P < 0.05$),

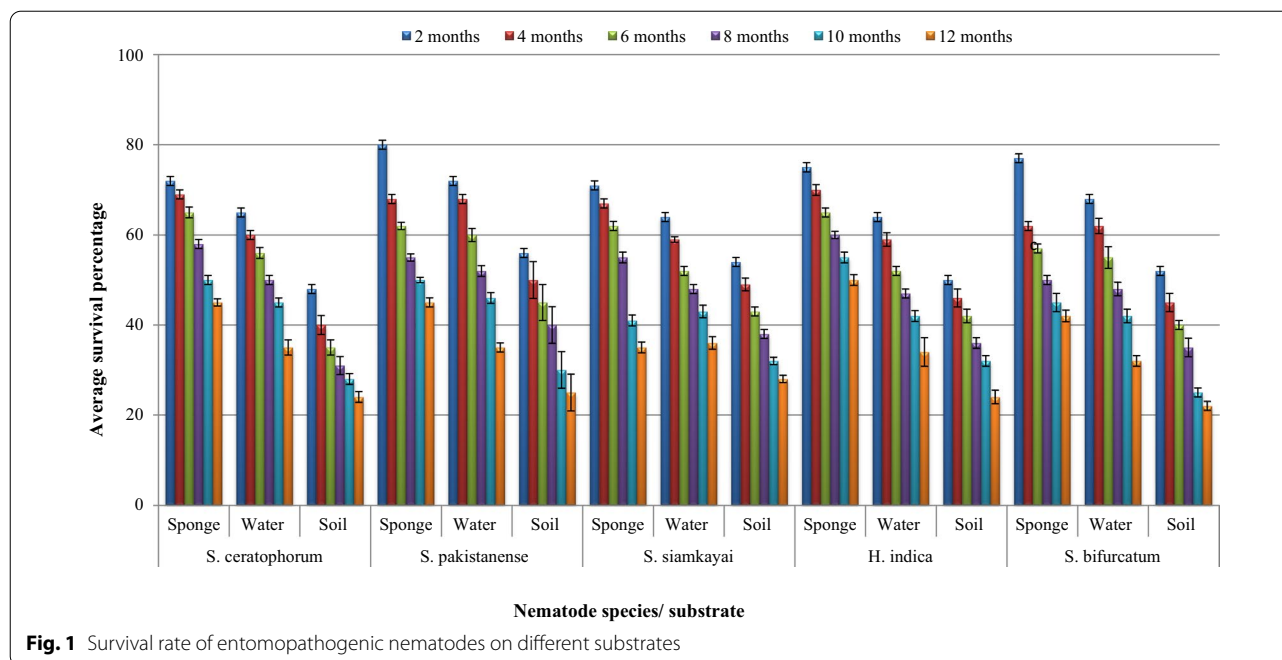


Fig. 1 Survival rate of entomopathogenic nematodes on different substrates

whereas *S. ceratophorum*, *S. siamkayai* showed less significant survival rates in sponge during 2–6 months (ANOVA $F=0.135$; $df=3, 15$; $P<0.05$).

Distilled water

After 2 months of storage in distilled water, the average survival rates of *S. ceratophorum* were 65%, *S. pakistanense* 72%, *S. bifurcatum* 68% and *S. siamkayai* and *H. indica* showed 64% of viability. After half year of storage the survival percentage of *S. ceratophorum* and *S. pakistanense* was 56 and 60%, respectively. *S. siamkayai* and *H. indica* showed 52% and *S. bifurcatum* 42%. After completion of one year of storage all species survival rates in distilled water were found to be 32–36% (Fig. 1). In water, *S. pakistanense* showed a significant high survival during 2–6 months (ANOVA $F=8.4$; $df=4, 15$; $P<0.05$), while *S. ceratophorum*, *S. bifurcatum*, *S. siamkayai* and *H. indica* showed a moderate significance during 2–6 months (ANOVA $F=4.0$; $df=3,15$; $P<0.05$).

Soil medium

In soil medium, viability of nematodes is shown in (Fig. 1) from 2 to 12 months of storage. In soil medium after 2 months of storage less number of nematodes survived of all tested species as compared to other substrate. The survival rates ranged from 35 to 60% at 6 months of storage, declined up to 22–30%. In soil, all type of nematodes showed nonsignificant survival rates during 2–6 months (ANOVA $F=0.1$; $df=4, 15$; $P<0.05$).

Efficacy of EPNs against *Spodoptera litura*

Isolates of *S. bifurcatum*, *S. ceratophorum*, *S. pakistanense*, *S. siamkayai* and *H. indica* were evaluated to find, infectivity and mortality *S.litura* last stage larvae at different concentrations at 28–30 °C by filter assay in laboratory experiments (Fig. 2). The ANOVA showed significant differences among effectiveness of nematode species on army worm (ANOVA $F=24$; $df=4$; $P=0.0005$). Concentration of nematodes also diverged significantly (RCBD-one-way ANOVA $F=23$; $df=4$; $P<0.05$) and impact of the three concentrations with five nematode species also had the remarkable results on army worm (ANOVA $F=30$; $df=4$; $P<0.05$). Outcome verified that the EPNs could restrain the frequency of *S.litura*. *S. pakistanense*, *S. bifurcatum* and *H. indica* showed higher mortality rates (87–95%) at high application concentrations than *S. ceratophorum* and *S. Siamkayai* (74–78%). Maximum mortality rates were attained at the concentrations of 350 IJs/ml (Fig. 2) after 48 h. At the lowest concentration (150 IJs/ml) 60% of army worms were killed by *S. bifurcatum*, after 48 h of exposure time showing significant differences with 50% against *H. indica* and *S. ceratophorum*. The mortality rates of *S. litura* increased depends upon the concentrations. At 350 IJs/ml, *S. pakistanense* showed the highest mortality rates (95%), while the lowest one was (74%) by *S. ceratophorum*. Nematode juveniles reproduced in the larvae of *S. litura*, which were clearly seen when dead larvae were transferred to vacant cavity block.

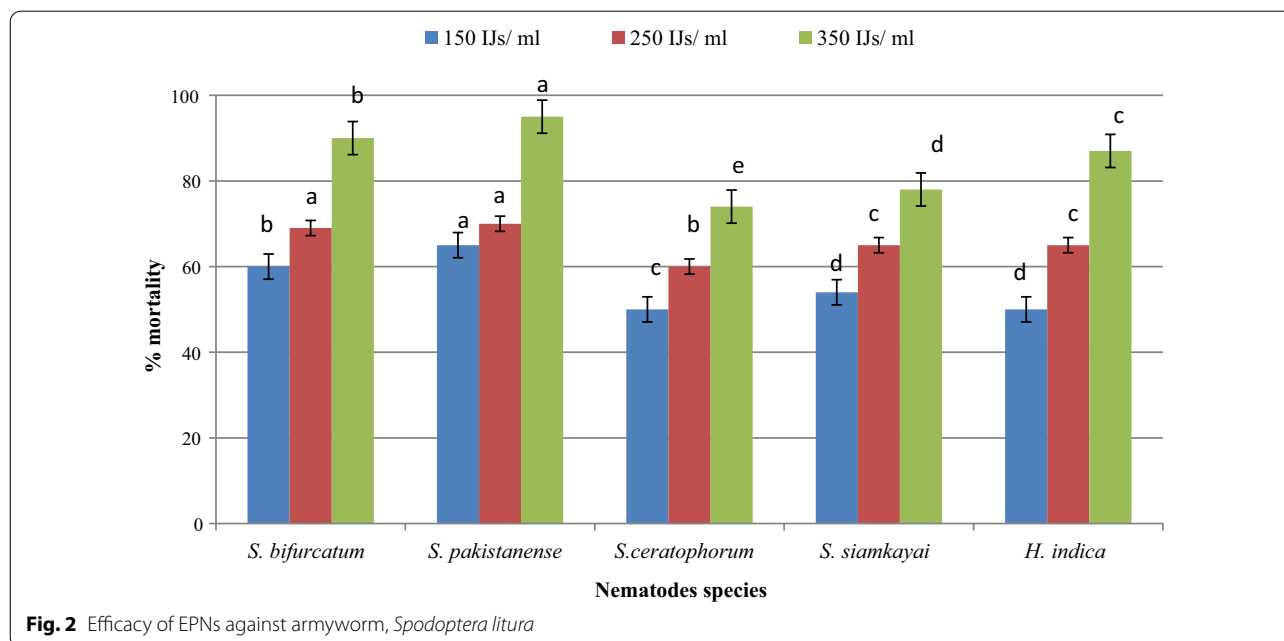


Fig. 2 Efficacy of EPNs against armyworm, *Spodoptera litura*

Discussion

For field application of EPNs as biocontrol agents against agricultural pests, key necessities are the suitable packing of IJs at optimum temperature according to species requirement, so that infective juvenile can persist with maximum shelf life (Lalramliana and Yadav 2009). In the present study, five species of EPNs, viz., *S. bifurcatum*, *S. ceratophorum*, *S. pakistanense*, *S. siamkayai* and *H. indica*, were stored in three different substrates: sponge, water and soil medium to check the longevity for 2–12 months. The best storage medium was sponge where the maximum number of infective juveniles remained alive, followed by water and then soil medium. Lewis and Shapiro-Ilan (2002) used sand, starch substrate and soil (high clay content) to store *S. carpocapsae* IJs. It was observed that, depending on the substrate used for storage, the IJs moved and obtained oxygen more or less easily and were able to increase or decrease their energy expenditures. The starch substrate, in which the space between particles is small, was not suitable to extend IJs survival. The soil used in the experiment was considered poorer for storage than sand; this can be explained by the fact that nematode movement is more difficult in that substrate, increasing energy expenditure and decreasing oxygen diffusion. Andalo et al. (2011) evaluated substrates to extend the survival of entomopathogenic nematodes, suspensions of 3000 IJ ml⁻¹ of *Heterorhabditis* sp. JPM4 and *Steinernema carpocapsae*. All were added to dirt, fine sand, coarse sand, foam, expanded clay, phenolic foam, agar, corn starch, Plantmax®, and water. *S. carpocapsae* IJs were still alive after 180 days

in the foam treatment as compared to other treatments, while expanded clay, Plantmax® and phenolic foam were not effective in maintaining the survival rate. Foam, coarse sand and fine sand provided greater *Heterorhabditis* species IJs survival at 180 days. Agar, phenolic foam and Plantmax® had lower survival indices than the control. The polythene sponges used as carriers for infective juvenile storage have been successful in many studies (Ley and Mundo-Campos 2004). The physical carriers can reduce the stress as compared to water suspension by simulating a natural environment because it provided a large surface area for oxygenation through perforation (San-Blas 2013). The use of storage substrates can reduce stress by creating an environment more similar to natural conditions than storage in water alone, as those substrates provide better oxygenation and conditions for IJ movement (Andalo et al. 2010).

In the present investigation, five species of EPNs, viz., were also evaluated against the last stage larvae of *S. litura*. As nematode concentration increases the mortality response increases. Radhakrishnan and Shanmugam (2017) also reported that the percentage mortality increased with increase in the concentration of *H. indica* and *S. glaseri*. *H. indica* proved to be more effective. Rashad et al. (2018) found that the highest mortality rates of the second instar larvae were observed by using the mixtures of *S. carpocapsae* or *H. indica*. Similar result was reported by Acharya et al. (2020) with *H. bacteriophora*, *H. indica*, *S. carpocapsae* and *S. longicaudum* against fall armyworm. Yan et al. (2020) screened 15 EPN isolates against *S. litura* larvae and stated that EPN are effective against *S. litura* and can reduce the

overuse of chemical insecticides. Other researchers were also reported that *H. indica* and *S. carpocapsae* are highly effective against *S. litura* and other lepidopteran pests, viz., (Holajjer et al. 2014). Park et al. (2001) studied that the last instar larvae of *S. litura* were more sensitive than younger one of *S. litura* upon infection of EPN because the size of the last larva is larger to provide more chance for IJ penetration.

Conclusions

It was concluded that *S. bifurcatum*, *S. pakistanense* and *H. indica* have higher virulence, penetration and reproduction rates than *S. ceratophorum* against *S. litura*. For sustainable agriculture farming, EPNs as a biocontrol agent is vital implement in pest management approach. One of the significant desires in employing beneficial nematodes is the appropriate storing of infective juveniles of EPNs, in a given inhabitants and also at optimal temperature, so that maximum IJs can persist for extensive times for application in field conditions. The current study showed that the best storage medium was sponge where the maximum number of infective juveniles remains alive followed by water and soil medium. Long-term storage and persistence is the need of the hour for successful applications.

Abbreviations

ANOVA: Analysis of variance; RCBD: Randomized completely block design; EPNs: Entomopathogenic nematodes; IJs: Infective juveniles.

Acknowledgements

Not applicable

Authors' contributions

SM performed storage; TAK analyzed the data. AA carried out the biocontrol experiment. All authors read and approved the final manuscript.

Funding

Not applicable.

Availability of data and materials

Not applicable.

Declarations

Ethics approval and contents to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

We have no potential conflicts of interest.

Received: 2 November 2021 Accepted: 9 January 2022

Published online: 18 January 2022

References

Acharya R, Hwang H-S, Mostafiz MM, Yu YS, Lee KY (2020) Susceptibility of various developmental stages of the Fall Armyworm, *Spodoptera frugiperda*, to entomopathogenic Nematodes. *Insects* 11:1–13

- Ahmad M, Mehmood R (2015) Monitoring of resistance to new chemistry insecticides in *Spodoptera litura* (Lepidoptera: Noctuidae) in Pakistan. *J Econ Entomol* 108:1279–1288
- Andalo V, Cavalcanti RS, Molina JP, Moino A Jr (2010) Substrates for storing entomopathogenic nematodes (Rhabditida: Steinernematidae, Heterorhabditidae). *Sci Agric* 67:342–347
- Andalo V, Moino A Jr, Maximiniamo C, Campos VP, Mendonca LA (2011) Influence of temperature and duration of storage on the lipid reserves of entomopathogenic nematodes. *Rev Colomb Entomol* 37:203–209
- Boemare NE, Akhurst RJ, Mourant RG (1993) Deoxyribonucleic acid relatedness between *Xenorhabdus* spp. (Enterobacteriaceae), symbiotic bacteria of entomopathogenic nematodes, with a proposal to transfer *Xenorhabdus luminescens* to a new genus, *Photorhabdus* gen.nov. *Int J Syst Bacteriol* 43:249–255
- Ciche TA, Kim KS, Kaufmann-Daszczuk B, Nguyen KCQ, Hall DH (2008) Cell invasion and matricide during *Photorhabdus luminescens* transmission by *Heterorhabditis bacteriophora* nematodes. *App Environ Microbiol* 74:2275–2287
- Duncan DB (1955) Multiple range and multiple F-test. *Biometrics* 11:1–41
- Gao M, Mu W, Wang W ZC, Li X (2014) Resistance mechanisms and risk assessment regarding indoxacarb in the beet armyworm, *Spodoptera exigua*. *Phytoparasitica* 42:585–594
- Grewal PS (2002) Formulation and application technology. In: Gaugler R (ed) Entomopathogenic nematology. CAB International, Wallingford, pp 265–287
- Holajjer P, Patil JB, Harish G, Nataraja MV (2014) Evaluation of entomopathogenic nematodes, *Steinernema carpocapsae* and *Heterorhabditis indica* for their virulence against *Spodoptera litura*. *Ann Plant Prot Sci* 22:163–165
- Lalramliana Yadav AK (2009) Effect of relative humidity on the emergence and reproduction of three entomopathogenic nematode species (Rhabditida: Steinernematidae and Heterorhabditidae) from Meghalaya, India. *Sci vis* 9:92–96
- Lewis E, Shapiro-Ilan S (2002) Host cadavers protect entomopathogenic nematodes during freezing. *J Invert Pathol* 81:25–32
- Ley P, Mundo-Campos M (2004) Cultivation of nematodes. In: Chen ZX, Chen SY, Dickson DW (eds) Nematology advances and perspectives. CABI Publishing, Wallingford, pp 541–619
- Park SH, Yu Y S, Park JS, Choo HY, Bae S D, Nam M H (2001) Biological control of tobacco cutworm, *Spodoptera litura* Fabricius with entomopathogenic nematodes. *Biotechnol Bioproc Eng* 6:139–143
- Piggot S, Wardlow L (2002) A fresh solution for the control of western flower thrips: dramatic results in trials. *Commer Greenh Grower* 5:20–23
- 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
- Rashad RK, Rameesha A, Ali AA, Muhammad A, Shahid M, Sohail A, Sajid AK, Muhammad A (2018) Compatibility of entomopathogenic nematodes (Nematoda: Rhabditida) and thebiocide, spinosad for mitigation of the armyworm, *Spodoptera litura* (F) (Lepidoptera: Noctuidae). *Egypt J Biol Pest Control* 28:58
- Saleem MA, Ahmad M, Ahmad M, Aslam M, Sayyed AH (2008) Resistance to selected organochlorine, organophosphates, carbamates and pyrethroids in *Spodoptera litura* (Lepidoptera: Noctuidae) from Pakistan. *J Econ Entomol* 101:1667–1675
- San-Blas E (2013) Progress on entomopathogenic nematology research: a bibliometric study of the last three decades: 1980–2010. *Biol Control* 66:102–124
- Shahina F, Salma J, Christos I R, Christos GA (2017) Control of stored grain pests by entomopathogenic nematodes. In: Biocontrol agents, entomopathogenic and slug parasitic nematodes. Elagawad, Tariq and James, p 629
- Tabassum AK, Salma J (2020) Virulence of four Steinernema species as abiological control agent in controlling the termite, *Coptotermes heimi* (Wasmann) (Isoptera: Rhinotermitidae). *Egypt J Biol Pest Control* 30:26
- 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

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.