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SCIENTIFIC (SHORT) NOTE

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Biocontrol potential of entomopathogenic nematodes against the Khapra beetle *Trogoderma granarium* (Everts) (Coleoptera: Dermestidae)

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Abstract

Background The Khapra beetle, *Trogoderma granarium* (Everts) (Coleoptera: Dermestidae) is one of the most destructive pests of stored wheat and barley worldwide. The broad practice of insecticides has been connected with insect resistance development coupled with the renaissance treated primary insects, environmental contamination, and toxicity to animals, man and other non-target organisms. These harms have invigorated the usage of alternative methods of managing this insect pest.

Results For biocontrol potential, four species of entomopathogenic nematodes (EPN) were tested: *Steinernema pakistanense* PCSIR-10, *S. bifurcatum* PCSIR-39, *S. saimkayai* PCSIR-6 and *S. abbasi* PCSIR-17, against adult and larval stages of Khapra beetle under laboratory conditions. At 250 IJs/ml, *S. pakistanense* and *S. bifurcatum* caused 100 and 90% at larval stages and 92 and 89% against adult beetles, respectively.

Conclusion This study documented that EPNs had a potential to control stored grain pest and could be utilized as alternatives of insecticides, which provide an adequate control of insect pest at postharvest stage, but in future more experiment will be required in commercial storage conditions.

Keywords The Khapra beetle, *Trogoderma granarium*, Entomopathogenic nematodes, Biocontrol agent, Potential

Background

Trogoderma granarium (Everts) (Coleoptera: Dermestidae) (Khapra beetle) is one of the most destructive pests of stored wheat and barley worldwide. It ranked as one of the 100 worst invasive species worldwide (Lowe et al. 2000). Adults and larvae of *T. granarium* both caused damage, but the larvae are voracious feeder (Athanassiou et al. 2019). They have potential to survive for a lengthier period (Honey et al. 2017). All types of stored grains' pests have effect the quality and quantitative loss of grains. The most popular method of controlling the pest is the application of synthetic pesticides, which are pricy, harmful to non-targeted organisms due to the direct influence on the targeted organism and have hazardous effect on the environment. Biological control is now frequently utilized as an alternative tool to synthetic pesticides (Quarcoo et al. 2014). There is a need to develop safe and sustainable control measures in the pest management. Hence, biological control is growing more than three times faster than synthetic pesticides (Van Lantern et al. 2018).

Entomopathogenic nematodes (EPNs) of the Phylum Nematoda (Kanzaki et al. 2017) belong to genera *Steinernema* and *Heterorhabditis* are obligatory pathogens in

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nature and are distinguished by their connection with mutualistic bacteria of the genera *Xenorhabdus* (Bhat et al. 2020). They have been discovered in a variety of biological environments, including farmed fields, woodlands, grasslands, deserts and ocean beaches (Hominick et al. 1996). They release mutualistic bacteria carried in their intestine and survive in the soil (Morris et al. 2020). They complete 2–3 generations within the host and have ability to seek their host actively (Campbell and Lewis 2002). The infective juveniles release their symbiotic bacteria which infect the host and then kill it within 48 h (Gulcu et al. 2017). The large number of nematodes release, invade the pest population and obtain pest suppression (Gaugler 2018). They have potential to control economically important pests of insect orders: Coleoptera, Diptera, Lepidoptera, Hemiptera and Orthoptera (Hatting et al. 2019). Most of EPNs species have a wide range of hosts, but the susceptibility of pest depending on the EPNs species (Labaude and Griffin 2018). They are distributed all over the world with different habitation and growing as biological control agents against insect pest (Cranshaw and Zimmerman 2013).

Keeping in view the above constraints, the present study was conducted to determine the biocontrol potential of four species of EPNs against the adult and larval stages of the Khapra beetle, *T. granarium* under laboratory conditions.

Methods

Collection of *Trogoderma granarium*

Fresh cultures of adult and larval stages of *T. granarium* were obtained from Pakistan Agriculture Research Council, University of Karachi, Pakistan. Wheat seeds (500 gm) were used as rearing medium in the 1000 ml glass jar covered with muslin cloths. Jars were kept in a rearing chamber 28 ± 2 °C and 65 ± 5 relative humidity in the rearing laboratory of NNRC, University of Karachi, Karachi, Pakistan.

Stock culture of entomopathogenic nematodes

The EPNs used in this study were isolated from soil samples collected from different plantation sites of Pakistan Council of Scientific and Industrial Research Laboratories Complex, Karachi, Pakistan. Four species of EPNs: *Steinernema pakistanense* PCSIR-10 (Shahina et al. 2001), *S. bifurcatum* PCSIR-39 (Shahina et al. 2014), *S. saimkayai* PCSIR-6 (Stock et al. 1998) and *S. abbasi* PCSIR-17 (Elawad et al. 1997), were maintained in the laboratory and identified byNNRC, University of Karachi, Pakistan. All the nematodes species were produced on *Galleria mellonella* L larvae of the greater wax moth at 28 ± 2 °C as described by the Duky et al. (1964). Insects killed by nematodes turned grayish. The infective juveniles were harvested by the white trap (White 1927), collected and stored in 50 ml beaker having distilled water at 10 °C for two weeks for their applications in the experiments.

Bioassay experiment

Batches of 20 larvae and adults were kept in a sterilized glass Petri dish (9-cm) bottomed with moistened filter paper (Whatman No. 1) separately. Four species of nematodes were applied at the concentrations 50, 150, and 250 IJs/ml with few drops of 2% Tween 80 as an emulsifier and wrapped with Parafilm incubated at 25 °C. Mortality rate was analyzed after 48 h, and control treatment had 1 ml of aqueous solution with few drops of 2% Tween 80 without nematodes. The entire experiments were repeated 3 times with five replications. The cause of mortality was confirmed by emergence of IJs from dead cadaver of larvae and adult beetle (Figs. 1, 2).

Data analysis

Data were subjected to analysis of variance (ANOVA) in SAS (ver.9.1, SAS Institute, Cary, NC). Abbott (1925) formula was used to correct mortality percentages. Mortality means were separated with DMRT Duncan's multiple range test (Duncan 1955).

Fig. 1 *Trogoderma granarium* larva infected with entomopathogenic nematodes after treatment (40× magnifications)

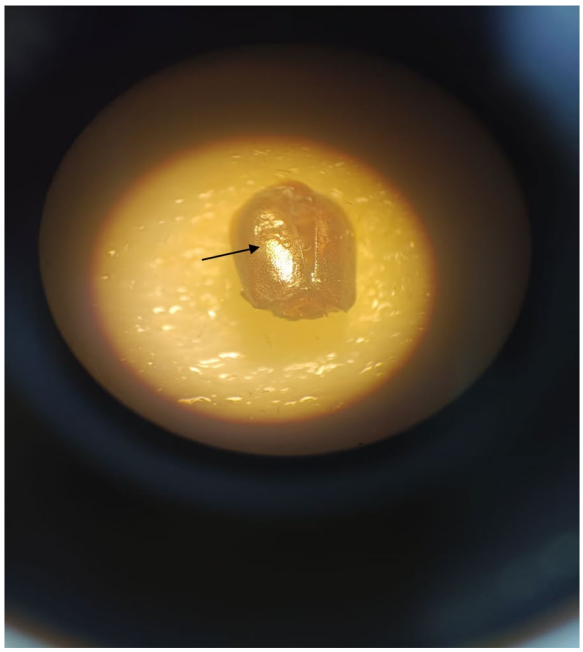

Fig. 2 *Trogoderma granarium* adult infected with entomopathogenic nematodes after treatment (40× magnifications)

Results

Bioassay experiment on *T. granarium* larvae

All the four EPNs biocontrol potential significantly affected corrected mortalities against larval stage of Khapra beetle *T. granarium* (ANOVA $F=49.012$, $df=3$, $P<0.001$) with three concentrations (ANOVA $F=7.247$, $df=2$, $P=0.001$). At 250 IJs/ml *S. pakistanense* PCSIR-10 gave the 100% mortality rate, while the *S. bifurcatum* PCSIR-39, *S. saimkayai* PCSIR-6 and *S. abbasi* PCSIR-17showed 95, 90 and 75% mortality rates, respectively (Fig. 3). They were the highest mortality effect among other concentrations of EPN species. At 150 IJs/ml *S. pakistanense,* mortality rates were as follows: PCSIR-10 (90%), *S. bifurcatum* PCSIR-39 (82%), *S. saimkayai* PCSIR-6 (66%) and *S. abbasi* PCSIR-17 (58%) at the same concentration (Fig. 3). The mortality of targeted pest reduced by two nematodes species *S. saimkayai* PCSIR-6 and *S. abbasi* PCSIR-17, while mortality increased by *S. pakistanense* PCSIR-10, which caused the highest mortality ratio (100%) at 250 IJs/ml. At the 50 IJs concentration, *S. pakistanense* PCSIR-10 showed (72%), whereas *S. bifurcatum* PCSIR-39, *S. saimkayai* PCSIR-6 and *S. abbasi* PCSIR-17 showed 48, 68 and 34%, respectively.

Bioassay experiment on *T. granarium* adult beetles

Corrected mortality rates of *T. granarium* adults significantly varied by concentrations (ANOVA $F=9.2567$, $df=2$, $P=0.001$) and nematode species (ANOVA $F=51.452$, $df=3$, $P<0.001$) as in larval stage after 48 h. The highest mortality ratio was observed in *S. pakistanense* PCSIR-10 (92%), while *S. bifurcatum* PCSIR-39, *S. saimkayai* PCSIR-6 and *S. abbasi* PCSIR-17 showed 89, 80 and 78% mortality rates at the same concentration of nematodes, respectively. The mortality rate of *S. pakistanense* PCSIR-10 (72%), *S. bifurcatum* PCSIR-39 (68%), *S. saimkayai* PCSIR-6 (58%) and *S. abbasi* PCSIR-17 (52%) was at 150 IJs/ml (Fig. 4). The lowest mortality rate was observed (30%) in *S. abbasi* PCSIR-17 at 50 IJs/ml, while it was 38% in *S. saimkayai* PCSIR-6, 40% in *S. bifurcatum* PCSIR-39 and 45% in *S. pakistanense* PCSIR-10.

The results revealed that all the four species of EPNs had the potential to kill the *T. granarium* at adults and larvae with the increase in concentration of EPNs and with increase in time.

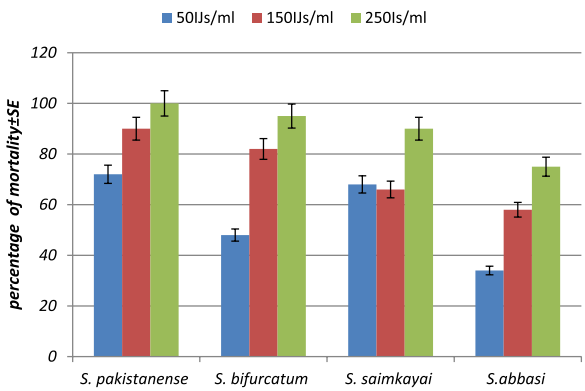

Fig. 3 *Trogoderma granarium* larval mortality treated with four different species of Entomopathogenic nematodes in Petridishes depending on their concentrations

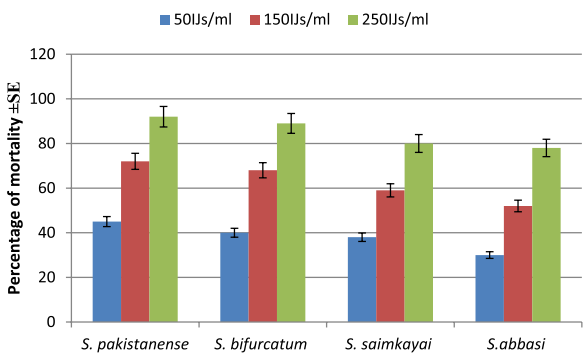

Fig. 4 *Trogoderma granarium* adult mortality treated with four different species of Entomopathogenic nematodes in Petridishes depending on their concentrations

Discussion

The EPNs provide the evidence that they can be used as a biological control agent against the stored grains pest (Lacy and Georgis 2012). The present study conducted to evaluate the efficacy of four EPNs species *S. pakistanense* PCSIR-10, *S. bifurcatum*PCSIR-1139, *S. saimkayai* PCSIR-6 and *S. abbasi* PCSIR-17 used highly effective against the adult and larval stages of *T. granarium*. The result revealed that the efficacy of the four EPNs species at high concentrations. Salma et al. (2020) studies on four species of EPNs *S. pakistanense* (LM-07) and *S. bifurcatum* (LM-30) were the most virulence at 150 IJs/beetle at 30 °C, while *S. affinae* and *S. cholashanense* (GB-22) at the same concentration at 20 °C give the maximum mortality rate of *T. confusium* and *R. dominica* adult beetles. Mortality rate of targeted insects varied by time, EPN species and their concentration were positively correlated with nematodes concentration and time (Caglayan et al. 2021). Karanastasi et al. (2020) observed that the efficacy of *S. feltiae*, *S. carpocapsae* and *H. bacteriophora* that killed the large larvae of *T. granarium* was 87.8, 63.3 and 60%, respectively, on stored wheat treated with the EPNs concentration of 50,000 IJs/ml after 8 days of exposure at 30 °C under laboratory conditions. The research studies on the efficacy of *T. granarium* are limited; many studies have been carried out to determine the virulence of EPNs against stored grain pest (Ramos-Rodríguez et al. 2006).Ali et al. (2011) studied the efficacy of *S. masoodi* Ali, Shaheen, Pervez and Hussain (Rhabditida: Steinernematidae) showed 100% mortality of *T. granarium* larvae on filter paper at the concentration of 500 IJs/individual after 2 days. Shahina and Salma (2010) reported the increased susceptibility of rice weevil *Sitophilus oryzae* adults to six Pakistani EPNs strains. Karanastasi et al. (2020) reported that complete mortality of small *T. granarium* larvae and 88% of largelarvae. This result co-relates along with the present study that *S. pakistanense* PCSIR-10 and *S. bifurcatum* PCSIR-39 gave 100 and 92% mortality rates, respectively, at the concentration of 250 IJs/ml after 48 h of treatment. Qader et al. (2021) reported that the highest mortality rate was recorded in *H. bacteriophora* of all concentration 500, 1000, 2000 and 3000 IJs/ml. Mortality was recorded between 24 and 72 h against the larvae of *T. granarium*. It was also observed that the efficacy of *S. riobrav* and *H. bacteriophora* showed the highest mortality of 16.67% at 2000 IJs/ml after 72 h of post-treatment of *C. maculates* and *T. granarium*. The result of the study provides the use of EPNs for controlling *T. granarium* larvae and adults in the future.

Conclusion

The present study was carried out to evaluate the virulence of four EPNs species against the *T. granarium* adult and larval stages under laboratory condition. Four species of EPN provided appropriate control of *T. granarium*. The utilization of EPNs against the stored grains insect provides at strong control at laboratory conditions, but in future more experiment will be required. All the four species of EPNs may be alternative to reduce the use of chemical pesticides in the pest management.

Abbreviation

EPNs	Entomopathogenic nematodes
IJs	Infective juveniles
PCSIR	Pakistan Council of Scientific and Industrial Research

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Author contributions

BN performed the experiment, BN and SJ designed and wrote the text, and SJ analyzed the data. Both authors read and approved the final manuscript.

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