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Biocontrol potential of entomopathogenic nematode species against *Tribolium confusum* (Jac.) (Coleoptera: Tenebrionidae) and *Rhyzopertha dominica* (Fab.) (Coleoptera: Bostrichidae) under laboratory conditions

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Abstract

Grain commodities, like cereals and legumes, are subject to insect infestation during postharvest processing and storage. Public concerns about the potential risks that derive from the consumption of pesticide-treated commodities have created interest for the development and integration in stored product protection of alternative, ecologically safe methods for the disinfestation of stored commodities. The present study was conducted to evaluate the biocontrol potential of four entomopathogenic nematodes (EPNs): *Steinernema pakistanense* (LM-07), *S. bifurcatum* (LM-30), *S. affinae* (GB-14), and *S. cholashanense* (GB-22) against adult beetles of *Tribolium confusum* (Jac.) (Coleoptera: Tenebrionidae) and *Rhyzopertha dominica* (Fab.) (Coleoptera: Bostrichidae) under laboratory conditions. Suspensions of nematodes were applied at three different concentrations, 50, 100, and 150 IJs/beetle in 1 ml of distilled water at three different temperatures, 20, 25, and 30 °C. Mortality was recorded after 3 days of application. The concentration of 150 IJs/beetle achieved a maximum mortality of 100% for *S. pakistanense* at 30 °C. The same concentrations revealed that all four species of EPN were able to cause mortal effects depended on temperature and concentrations. *S. pakistanense* (LM-07) and *S. bifurcatum* (LM-30) were the most effective at 150 IJs/beetle at 30 °C and *S. affinae* (GB-14) and *S. cholashanense* (GB-22) at the same concentration at 20 °C.

Keywords: Entomopathogenic nematode, *Tribolium confusum*, *Rhyzopertha dominica*, Potential, Biocontrol

Background

Entomopathogenic nematodes (EPNs) such as *Steinernema* and *Heterorhabditis* are multicellular organisms that harbor bacteria in their intestine. They have the potential to provide effective control of some economically important insect pests belonging to orders Lepidoptera, Coleoptera, and Diptera (Burnell and Stock 2000). They are established all over the world in diverse ecological habitats and are being developed as one of the biological control agents against insect pests (Cranshaw and Zimmerman 2013). In Pakistan, six new EPN species were described: *S. pakistanense (*Shahina et al. 2001), *S. asiaticum* (Anis et al. 2002), *S. maqbooli* (Shahina et al. 2013), *S. bifurcatum* (Shahina et al. 2014), *S. balochiense* (Shahina et al. 2015), and *Heterorhabditis pakistanense* (Shahina et al. 2017), whereas nine species were reported as new records from Pakistan: *S. siamkayai* (Stock et al. 1998), *S. abbasi* (Elawad et al. 1997), *S.carpocapsae* (Wouts et al. 1982), *S. feltiae* (Filipjev 1934) *S. litorale* (Yoshida 2004), *S. affine* (Mracek et al. 2005), *S. cholashanense* (Nguyen et al. 2008), *Heterorhabditis bacteriophora* (Poinar Jr 1976), and *H. indica* (Poinar et al. 1992).

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The lesser grain borer, *Rhyzopertha dominica* Fabricius (Coleoptera: Bostrichidae), is a destructive, internal grain feeder of stored cereals throughout the world (Mahroof and Phillips 2007). The larvae and adults are more dangerous so that the damage to stored cereal grains is greater than other pests (Vardeman et al. 2007). The confused flour beetle, *Tribolium confusum* Jacquelin du Val (Coleoptera: Tenebrionidae), is another stored-product pest that contaminates a wide range of food products, from flour and cereals to spices. It effectively exploits food patches of varying sizes and qualities that are produced during various milling operations (Ming and Cheng 2012).

The present study aimed to investigate the biocontrol potential of four *Steinernema* species against adult beetles of *T. confusum* and *R. dominica* under laboratory conditions.

Materials and methods

Stock cultures of target pests and entomopathogenic nematodes

The bulk populations of the stored grain pests, *T. confusum* and *R. dominica*, were obtained from Pakistan Agricultural Research Council (PARC), University of Karachi, Karachi, Pakistan, and further reared in a 1000-ml glass jar at 30 ± 2 °C, 60 ± 5% RH, and a photoperiod of 12:12 (L:D), covered with the muslin cloths contained fresh whole wheat grain (*Triticum aestivum* L.) as a diet in a rearing laboratory of National Nematological Research Centre (NNRC), University of Karachi, Karachi, Pakistan. Four species of EPNs, LM-07, LM-30, GB-14, and GB-22, obtained from the stock culture maintained in a storage unit of NNRC, were further propagated in the last instar larvae of *Galleria mellonella* L. (Pyralidae), following the method of Dutky et al. (1964). The nematodes' infective juveniles (IJs) were harvested from White traps (White 1927) and stored in a 100-ml beaker containing 50 ml distilled water at 10 °C for 15 days before they were employed for biocontrol potential.

Petri dish bioassay

Freshly emerged adult beetles of each insect species were collected as a requirement for bioassay from the rearing jars 1 day prior to the application of nematodes. Ten beetles of *T. confusum* and *R. dominica* with 10 fresh grains of wheat (*T. aestivum*) were placed in Petri dishes (90 mm diameter) lined with filter paper disk (Whattmann No. 1) in the bottom of each nematode species, concentrations, and temperatures separately. The biocontrol potential of

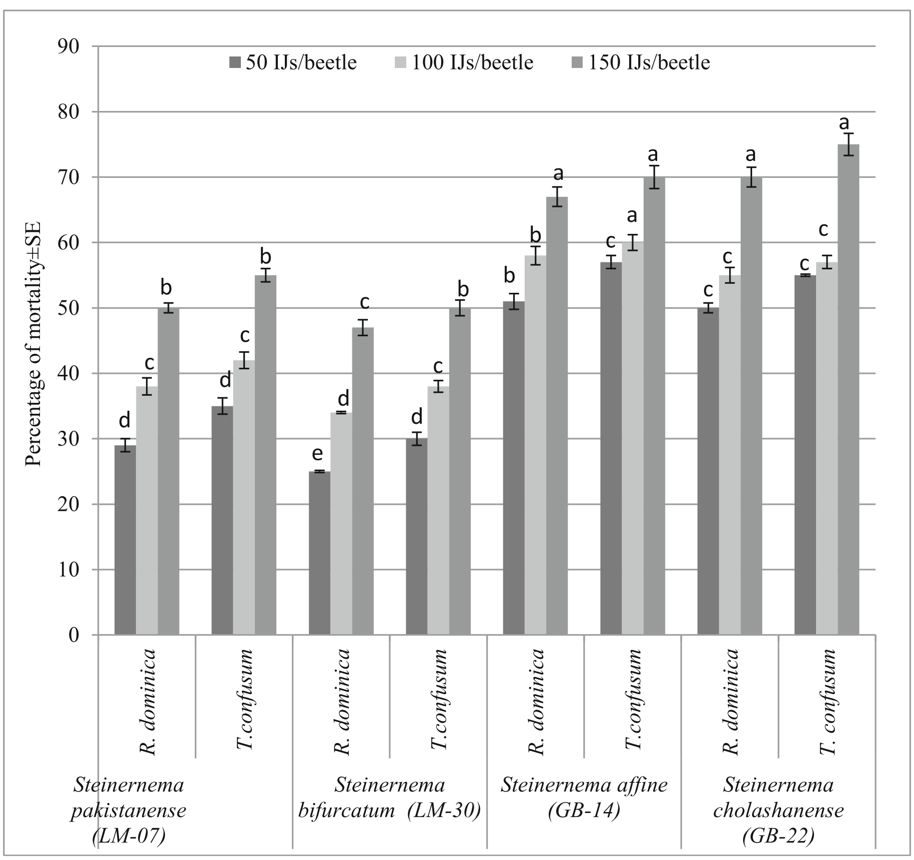

Fig. 1 Adult beetle mean mortality treated with four different species of EPNs in a Petri dish depending on nematode concentrations at 20 °C

EPNs was tested at three different concentrations: 50, 100, and 150 IJs/beetle in 1 ml of water. Concentrations were dispensed in a Petri dish with a 100–1000-μl micropipette to avoid mixing, Eppendorf tips were replaced after each conduct. For the control treatment, 1 ml distilled water was applied. The Petri dishes were sealed by parafilm (PM-996). Efficacy was tested at three different temperatures (20, 25, and 30 °C). After 3 days, the number of dead beetles was transferred to White trap in a new Petri dish lined with filter paper disk in the bottom to determine the emergence of the nematode under a stereomicroscope. There were four replicates for each treatment combination with concentration, insect, and nematode species, and the entire experiment was carried out twice.

Statistical analysis

Statistical data were analyzed by multifactor analysis of variance (ANOVA followed by Duncan's multiple range test ($P < 0.05$) for the separation of means (Duncan 1955). LC_{50} values were analyzed with probit analysis by using the PROC PROBIT routine of SAS, 2000. Control mortality was corrected as suggested by Abbott (1925).

Results and discussion

The adult stage of the lesser grain borer and confused flour beetle was found to be susceptible to all the four tested nematode species, but the degree of susceptibility and nematode infection varied according to exposure temperatures and concentrations. The data revealed that at 20 °C, *S. affine* (GB-14) showed 70, 67% and *S. cholashanense* (GB-22) 75, and 70% at 150 IJs/beetle against *T. confusum* and *R. dominica*, respectively, which was the highest mortality effect among other concentrations and nematode species (Fig. 1). At 25 °C, the mortality effect of the targeted pest reduced by these two nematode species (Fig. 2) and increased by *S. pakistanense* (LM-07), which caused the highest mortality rate 100, 95% at 100 IJs/beetle at 30 °C, followed by *S. bifurcatum* (LM-30) 80, 92, and 85% at the concentrations of 50,100, and 150 IJs/beetle, respectively (Fig. 3). Significant differences in mortality rates were detected between *S. pakistanense* (LM-07) and *S. bifurcatum* (LM-30) (ANOVA, $F = 4.4$; df = 7; $P < 0.05$) at 25 °C, less significant (ANOVA, $F = 4.1$; df = 7; $P < 0.05$) at 20 °C, and non-significant at 12 °C (ANOVA, $F = 0.48$; df = 7; $P < 0.8$). *S. cholashanense* (GB-22) and *S. affine* (GB-14) showed a high mortality rate and more significant (ANOVA, $F = 4.0$; df = 7; $P < 0.05$) at 20 °C, whereas non-significant (ANOVA, $F = 0.76$; df = 7; $P < 0.62$) at 30 °C. The nematode concentrations (50,100, and 150 IJs/ml) also differed significantly (ANOVA, $F = 4.2$; df = 3; $P < 0.05$). *R. dominica* was highly susceptible against *S. pakistanense*

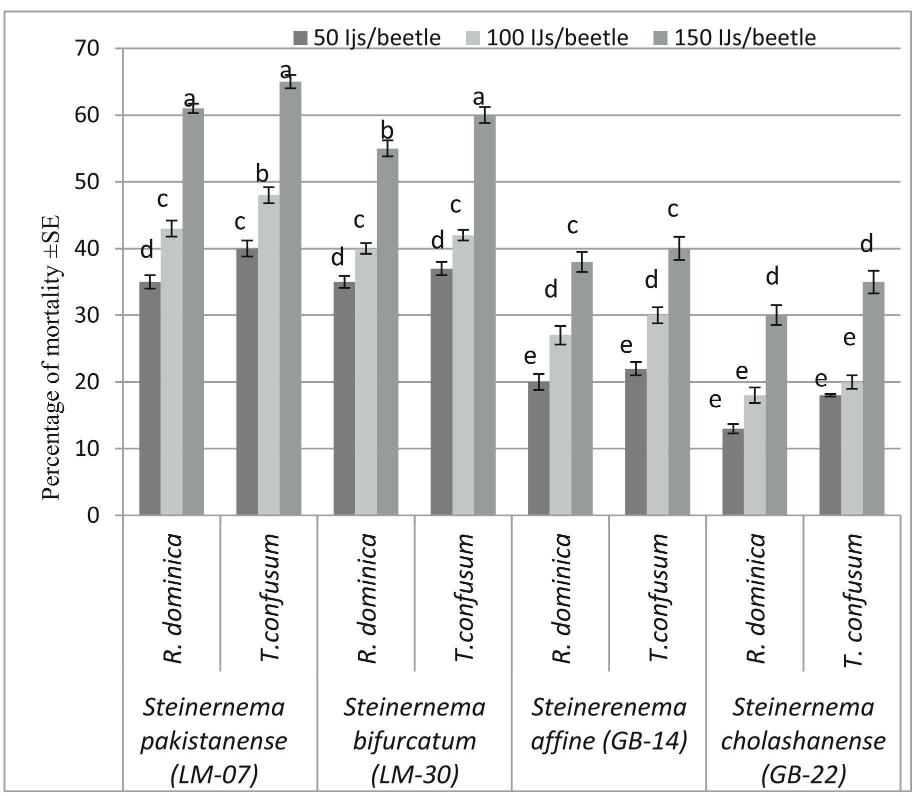

Fig. 2 Adult beetle mean mortality treated with four different species of EPNs in a Petri dish depending on nematode concentrations at 25 °C.

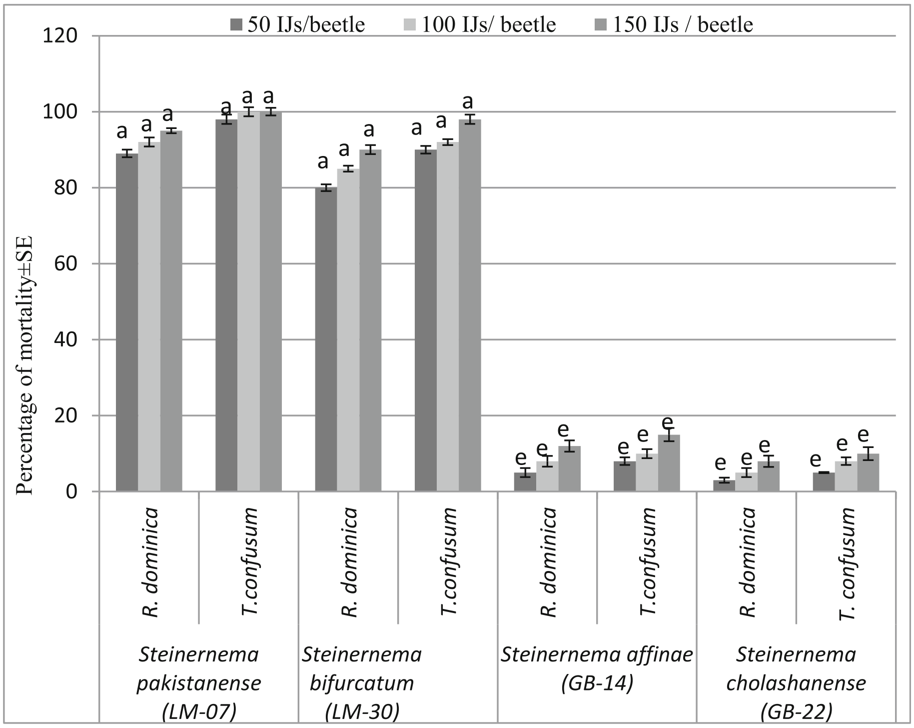

Fig. 3 Adult beetle mean mortality treated with four different species of EPNs in aPetri dish depending on nematode concentrations at 30 °C

(LM-07) and *S. bifurcatum* (LM-30) (ANOVA, F = 5, 25; df = 3; P < 0.05) at 25 °C, whereas in the case of 20 °C, *R. dominica* was highly susceptible against *S. cholashanense* (GB-22) and *S. affine* (GB-14) (ANOVA, F = 5.14; df = 3; P < 0.05). *T. confusum* was highly susceptible against *S. pakistanense* (LM-07) and *S. bifurcatum* (LM-30) (ANOVA, F = 5.25; df = 3; P < 0.05) at 25 and 30 °C, whereas in the case of 20 °C, it was highly susceptible against *S. cholashanense* (GB-22) and *S. affine* (GB-14) (ANOVA, F = 0.8; df = 3; P < 0.05). *S. pakistanense* (LM-07) showed non-significant differences in mortality rates of both insects at 20 °C (ANOVA, F = 0.48; df = 7; P < 0.05), whereas a more significant difference at 30 °C (ANOVA, F = 14; df = 5; P < 0.05). *S. bifurcatum* (LM-30) showed a high mortality rate and the most significant (ANOVA, F = 22; df = 5; P < 0.05) at 30 °C, while non-significant at 20 °C (ANOVA, F = 14; df = 1; P < 0.05). *S. cholashanense* (GB-22) showed significant differences in mortality rates of both insects at 20 °C (ANOVA, F = 31.6; df = 5; P < 0.05), while non-significant at 30 °C (ANOVA, F = 0.8; df = 1; P < 0.05). *S. affine* (GB-14) showed a high mortality rate and more significant (ANOVA, F = 14; df = 3; P < 0.05) at 20 °C, whereas non-significant at 30 °C (ANOVA, F = 1.2; df =

Table 1 Median lethal concentrations (LC_{50}) of four nematode species against adult stages of *Rhyzopertha dominica* and *Tribolium confusum*

EPN species	LC_{50} (95% CL)					
	20 °C		25 °C		30 °C	
	T. confusum	*R. dominica*	*T. confusum*	*R. dominica*	*T. confusum*	*R. dominica*
Steinernema pakistanense LM-07	128.46 (357.5–93.07)	166.9 (372.1–124.72)	86.71 (117.0–61.2)	208.7 (259.10–158.4)	1.671 (251.61–0.011)	1.671 (251.61–0.011)
Steinernema bifurcatum LM-30	137.6 (262.4–105.5)	164.40 (209.53–119.2)	109.88 (190.4–81.5)	133.33 (186.28–80.38)	5.1193 (19.52–0)	5.676 (89.72–0.359)
Steinernema affine GB-14	30.55 (109.8–8.49)	49.284 (75.39–0.68)	276.55 (251.5–147.14)	199.35 (251.55–147.14)	429.42 (501.46–357.3)	723.73 (915.2–532.1)
Steinernema cholashanense GB-22	39.267 (66.638–0)	55.756 (78.47–14.04)	230.62 (20.47–180.77)	441.99 (283.78–195.24)	506.94 (601.15–412.74)	445.15 (545.85–344.4)

LC_{50} expressed as no. of IJs per beetle
Confidence limit, CL, are given in parenthesis

1; $P < 0.05$). LC_{50} values calculated from probit analysis of all tested nematode strains are given in Table 1. *S. pakistanense* (LM-07) and *S. bifurcatum* (LM-30) were the most effective at high temperatures, whereas *S. affine* (GB-14) and *S. cholashanense* (GB-22) were the lowest one.

Research studies on the efficacy of EPNs against stored-product insects are restricted, and the research conducted in laboratory conditions so far provides strong evidence that EPNs are a promising tool for the control of postharvest insects. Early studies evaluated the virulence of *Steinernema feltiae* Filipjev (formerly *Neoplectana carpocapsae*) (Alikhan et al. 1985) and *S. carpocapsae* Weiser (Wójcik 1986) against adults of *S. granaries*. Various studies have also been conducted for the evaluation of the virulence of EPNs against the stored grain pest *T. castaneum* (Ramos-Rodríguez et al. 2006) and the confused flour beetle, *Tribolium confusum* (Alikhan et al. 1985; Athanassiou and Kavallieratos 2010; Rumbos and Athanassiou 2012). Larvae of *T. castaneum* were highly susceptible to three steinernematid species, i.e., *S. feltiae*, *S. carpocapsae*, and *S. riobrave*, whereas for pupae and adults, susceptibility was species-dependent (Ramos-Rodríguez et al. 2006). Six heat-tolerant Pakistani nematode strains also showed successful results against *T. castaneum* larvae and adults (Shahina and Salma 2010; 2011). For *T. confusum* larvae, different responses to EPNs have been demonstrated, varying from low (Alikhan et al. 1985; Rumbos and Athanassiou 2012) to high susceptibility (Athanassiou et al. 2008), depending mainly on the nematode species and strain. However, most studies agree on the poor infectivity of EPNs against *T. confusum* adults (Athanassiou et al. 2008; Rumbos and Athanassiou 2012). *R. dominica* larvae develop inside the kernel; hence, studies with EPNs have focused on the susceptibility of the free-living adult stage, which exhibited low to moderate susceptibility (Ramos-Rodríguez et al. 2006; Athanassiou and Kavallieratos 2010). Trdan et al. (2006) also studied the effect of two heterorhabditid species, *Heterorhabditis bacteriophora* Poinar and *H. megidis* Poinar, Jackson and Klein, with *H. megidis* being the least efficient. Laznik et al. (2010) noted high mortalities at the highest nematode suspension concentration (2000 IJs/insect) of three *S. feltiae* strains, whereas Shahina and Salma (2010) reported an increased susceptibility of the rice weevil *Sitophilus oryzae* adults to six Pakistani EPN strains. These contradicting results indicate that the various nematode and host strains used, as well as the different experimental protocols followed, may have an impact on the results. Low susceptibility to EPNs was shown also for the maize weevil *S. zeamais* (Barbosa-Negrisoli et al. 2013).

Therefore, there is a substantial gap in field studies, where the efficacy of the large-scale application of EPNs will be evaluated under "real world" conditions. The low humidity conditions that prevail in these facilities, especially in warm regions, could affect the survival and virulence of EPNs negatively and subsequently reduce their efficacy. To overcome these limitations, the development of EPN formulations and evaluation of heat-tolerant strains of EPNs needs further investigation.

Conclusion

The evaluation of EPNs against stored-product insects, providing evidence that EPNs can provide adequate control of postharvest insects but at certain conditions, could be included in future pest management strategies in storage facilities.

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Authors' contributions
SJ designed the study, wrote the manuscript, and inputted from all authors. TAK analyzed the data and SK carried out the experiment. All authors read and approved the final manuscript.

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