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Pathogenicity of Pakistani isolates of *Steinernema bifurcatum* and *S. affine* (Rhabditida: Steinernematidae) in management of stored grain pests *Lasioderma serricorne* and *Tribolium castaneum* (Coleoptera: Ptinidae, Tenebrionidae)

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Abstract

Background: Stored grain products suffer a considerable economic loss due to insect infestations. The resistance of pests to insecticide, and residues on crops are produced by the application of insecticide. Nowadays, incremental necessity has been occurred to find out alternatives to chemicals. As biological control agents, entomopathogenic nematodes (EPNs) attract attention increasingly in the research area recently.

Results: The aim of the study was to evaluate the pathogenicity of Pakistani isolates *Steinernema bifurcatum* and *S. affine* against the stored grain pests, *Tribolium castaneum* and *Lasioderma serricorne*, under laboratory conditions. Suspensions of nematodes were applied at 4 different concentrations 0, 50, 100, and 200 IJs/ml and 3 variable temperatures 15, 30, and 45°C. Pathogenicity rate was recorded after 48 h of application. The larval stage is found more susceptible than the adult. At 200 IJs, *S. bifurcatum* showed high mortality of *L. serricorne* larva (92%) and *T. castaneum* larva (93%), whereas *S. affine* showed 90 and 95% mortality of *L. serricorne* and *T. castaneum* larva, respectively, at 200 IJs. The results revealed that both species of EPN were able to control and reproduce on *L. serricorne* and *T. castaneum*. Maximum mortality was recorded at 45 °C from *S. bifurcatum* whereas *S. affine* at 15 °C.

Conclusions: Therefore, *S. bifurcatum* and *S. affine* could be suggested as a biological control agent for hot and cold climatic zones, respectively.

Keywords: Entomopathogenic nematodes, Stored grain pests, *Tribolium castaneum*, *Lasioderma serricorne*, Pathogenicity

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Background

Grain protectants and fumigants are widely used in warehouses and storage facilities for stored grain protection, especially in the case of insects and mites (Zettler and Arthur, 2000). However, several stored product insect species have developed a significant level of resistance to chemical residual insecticides and fumigants (Nguyen *et al*. 2015). The cigarette beetle (*Lasioderma serricorne* [Fabricius], Coleoptera, Ptinidae) is a damaging pest and known to effectively grow and infest the stored product mainly tobacco (Dimetry *et al*. 2004). In the international market, the production of chemical residue-free tobacco was in demanded that increase the studies of *L. serricorne* (Silva *et al*. 2009). The red flour beetle (*Tribolium castaneum*) [Herbst] (Coleoptera: Tenebrionidae) is a significant worldwide agricultural problem. It is classified as a secondary pest because adults and immature stages of red flour beetle feed on grains previously cracked, broken, or damaged by primary pests the insect's ability to survive in undamaged grains. Stored product insects can survive on small amount of food that accumulate in inaccessible places such as cracks and crevices, under perforated floors, and inside machinery and may move from these refugees into packed and bulk stored products (Campbell *et al*. 2004). In addition to direct consumption of the product, stored insect pests inflict their damages on stored products through excretion, exuviations, dead bodies, and their own existence in the product, which is not commercially desirable.

Entomopathogenic nematodes (EPNs) possess incredible potential as a biological control agent of economically important insect pests (Shapiro-Ilan et al. 2016). EPNs have been recognized as one of the most effective, safe, and non-polluting bio-control agents for the control of insect pests that cause serious damage to major crops and fruit trees. These nematodes have already been effectively used to manage various insect pests of economically important crops (Campos-Herrera, 2015). Salma et al. (2020) reported *S. bifurcatum* for the control of against lesser grain borer and confused flour beetle under laboratory conditions.

In this study, the pathogenicity of the Pakistani isolates of *Steinernema bifurcatum* and *S. affine* was evaluated at different concentrations and temperatures in the management of stored grain pests *Lasioderma serricorne* and *Tribolium castaneum* under laboratory conditions.

Methods

Target pests

Tribolium castaneum and *L. serricorne* (larvae and adults) were provided by the Pakistan Agriculture Research Council, University of Karachi, Karachi, Pakistan, and maintained in a 1000-ml glass jar containing 500 g fresh wheat flour and coarsely ground wheat, respectively, and then incubated in a rearing cage (200 × 120 × 350 cm) at 35±2°C with 12:12-h day to night cycle.

Entomopathogenic nematodes

Entomopathogenic nematodes (EPNs), *Steinernema bifurcatum* (Shahina *et al.* 2014) and *S. affine* (Bovien 1937), were obtained from stock cultures of NNRC, University of Karachi, Karachi, Pakistan. The nematodes were cultured in the larvae of the greater wax moth, *Galleria mellonella* L. Two batches of 15 wax moth larvae were placed on each Petri dishes lined with a filter paper. The larvae in dishes were individually inoculated with approximately 1000 infective juveniles (IJs) of the 2 EPN species in 1 ml of distilled water. Sealed Petri dishes with Parafilm were placed in an incubator at 15 and 45°C for *S. affine* and *S. bifurcatum*, respectively. After incubation for 48 h, the infected wax moth larvae were collected from the Petri dishes and placed on modified white traps at respected temperatures (White, 1927). After 2 days, the nematodes moved from the *G. mellonella* cadavers to the water. Harvested juveniles were kept at 16°C for less than 2 weeks before the tests.

Pathogenicity test

Batches of 25 insects from each of *T. castaneum* and *L. serricorne* (larvae and adults) were placed in a 9-cm plastic Petri dish bottomed with moistened filter paper (Whatman No.1) with each batch separately with nematode suspension 100, 150, and 200 IJs in 1 ml with few drops of 2% Tween 80 as an emulsifier and sealed with Parafilm incubated at the temperatures 15, 30, and 45°C. Mortality was determined after 48 h and control with 1 ml of distilled water without nematodes. Each treatment was replicated 5 times and the entire experiments were repeated 3 times. The cause of mortality was confirmed by emergence of IJs from dead cadaver.

Statistical analysis

The pathogenicity of EPNs was statistically validated by a one-way ANOVA (SAS Institute, 2002). Differences at the $P < 0.05$ level were considered statistically significant under Duncan's multiple range test (Duncan, 1955).

Results

Pakistani isolates of *S. bifurcatum* and *S. affine* were evaluated for pathogenicity of *T. castaneum* and *L. serricorne* (larvae and adults each) at different concentrations (Fig. 1) and temperatures (Fig. 2) by filter assay in laboratory conditions.

Pathogenicity of *Steinernema bifurcatum*

Significant differences in mortality rates were detected among the insect tested (ANOVA: $F = 6.56$; df = 3,

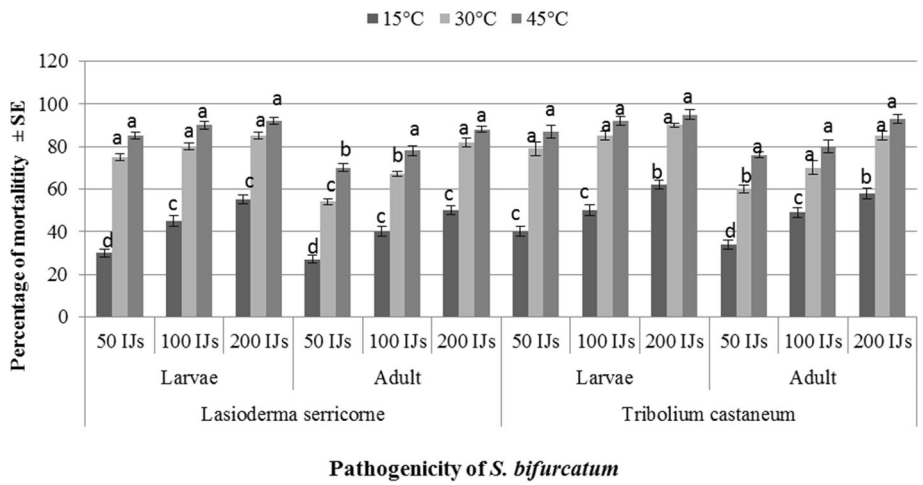

Fig. 1 Percentage mortality of insects at three different concentrations and temperatures inoculated by *Steinernema bifurcatum*

12; $P < 0.05$), and the nematode concentration showed significant differences (ANOVA: $F= 4.5$; df = 3, 12; $P < 0.05$). *S. bifurcatum* caused a high mortality at 200 IJs of *L. serricorne* larva (ANOVA: $F =0.051$; df = 3, 8; $P < 0.05$), *L. serricorne* adult (ANOVA: $F= 0.069$; df = 3, 5; $P < 0.05$), *T. castaneum* larva (ANOVA: $F= 0.188$; df = 3, 8; $P < 0.05$), and *T. castaneum* adult (ANOVA: $F= 0.034$; df = 3, 8; $P < 0.05$) as compared to 50 and 150 IJs. All insects showed significant mortality rates with 200 IJs of *S. bifurcatum*. Both insects' larvae showed above 90% mortality with the concentration @ 200IJs (Fig. 1). The nematode concentration showed a significant variance (ANOVA: $F= 0.311$; df = 3, 5; $P < 0.05$) as compared to control treatment. Insect pests, *L. serricorne* and *T. castaneum* (larvae and adult each), were also exposed to 3 different temperatures 15, 30, and 45°C. Significant differences in mortality were detected among the insects tested at different temperatures. *S. bifurcatum* showed the highest mortality that was 92 and 88% of *L. serricorne* larvae and adult, respectively, at 45°C (ANOVA: $F= 9.2$; df = 3, 20; $P < 0.05$) and *T. castaneum* larvae and adult showed 95 and 93%, respectively (ANOVA: $F= 9.2$; df = 3, 20; $P < 0.05$). *S. bifurcatum* showed less significant at 15°C where 55% and 50% larval mortality of *L. serricorne* and *T. castaneum*, respectively, and 62% and 58% mortality of *L. serricorne* and *T. castaneum* adults, respectively, were recorded at 200 IJs (Fig. 1).

Pathogenicity of *Steinernema affine*

Significant differences in mortality were detected among the insects tested (ANOVA: $F= 0.015$; df = 3, 8; $P < 0.05$) and among concentrations (ANOVA: $F = 0.011$; df = 3, 5; $P < 0.05$). *S. affine* significant difference showed a high

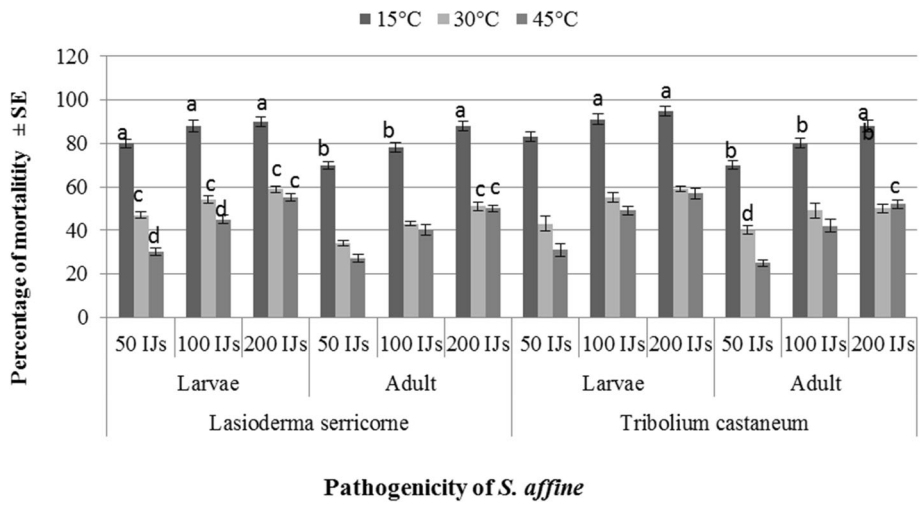

Fig. 2 Percentage mortality of insects at three different concentrations and temperatures inoculated by *S. affine*

mortality rate 90% of *L. serricorne* larvae at the concentration of 200IJs (ANOVA: F= 4.2, df = 3, 12; $P < 0.05$) and *L. serricorne* adults showed 88% mortality (ANOVA: F= 0.03, df= 3, 8; $P < 0.05$) (Fig. 2). There was a non-significant difference at concentrations of 50 and 100 IJs of *S. affine* which caused 30% and 45% larval mortality of *L. serricorne* and 27% and 40% adult mortality of *L. serricorne*, respectively. *S. affine* showed a significant difference against *T. castaneum* larvae at the concentration of 200 IJs (ANOVA: F= 0.39; df = 3, 8; $P < 0.05$); *T. castaneum* adult less significant at 50 and 100 IJs (ANOVA: F= 0.098; df = 3, 8; $P < 0.05$). *S. affine* was also tested at the 3 different temperature degrees 15, 30 and 45°C. Significant differences in mortality rates were also found among the tested temperature degrees (ANOVA: F = 0.011; df = 4, 10; $P < 0.05$) at 15, 30, and 45°C. *S. affine* showed 90% mortality rate of *L. serricorne* larvae at 15°C (ANOVA: F= 10.8; df = 3, 20; $P < 0.05$). There was a non-significant difference in case of adults of *L. serricorne* at 30 and 45°C (ANOVA: F= 10.8; df = 3, 20; $P < 0.05$), i.e., 59 and 55% mortality, respectively. Significant differences in mortality rates were detected among the *T. castaneum* larvae and adults (ANOVA: F= 15.8; df = 3, 8; $P < 0.05$). *S. affine* showed 95% mortality of *T. castaneum* larvae at 15°C (ANOVA: F= 10.8; df = 3, 20; $P < 0.05$) whereas 57 and 52% at 30 and 45°C, respectively. There was a non-significant difference (ANOVA: F= 15; df = 3, 8; $P < 0.05$) in case of adults of *T. castaneum* 50 and 52% at 30 and 45 °C, respectively.

Discussion

Obtained results indicated that the larval stage was more susceptible than the adults. Susceptibility of the 2 pests varied at different EPN species. Precisely, the pests' larvae were more susceptible to EPN infection than adults. In this regard, several researches had similar results and confirmed these findings (Shapiro-Ilan *et al*. 2002 and Shahina and Salma, 2010). This has been attributed to the structural and behavioral characteristics of the different life stages. For instance, adult beetles are protected by the hard, chitinous exoskeleton, which acts as a mechanical hurdle to the invasion of EPNs, in contrast to the soft body of the larvae, which is more easily penetrable (Shahina *et al*. 2017).

Bedding et al. (1983) suggested testing EPNs at a concentration of 100 IJs/insect as a preliminary assessment of host susceptibility and to begin the process of selecting nematode species or strains as a potential biological control agent. Shahina and Salma (2010) tested the susceptibility of *Tribolium castaneum* (Herbst) to 7 entomopathogenic isolates from Steinernematidae and Heterorhabditidae and found promising results. Shahina et al. (2017) also reported promising results for the control of stored grain pests by EPNs. Various studies have also been conducted for the evaluation of the virulence of EPNs against the confused flour beetle, *Tribolium confusum* (Rumbos and Athanassiou, 2012). Larvae of *T. castaneum* were highly susceptible to 3 steinernematid species, i.e., *S. feltiae*, *S. carpocapsae*, and *S. riobrave*, whereas pupae and adults' susceptibility was species dependent (Ramos-Rodríguez et al., 2006a, b). Different EPN species have also been evaluated against *S. oryzae*, with variable success (Shahina and Salma, 2010 and Barbosa-Negrisoli *et al*. 2013). Most studies suggest that *S. oryzae* adults are not highly susceptible to EPNs, with mortality levels of 9, 26, or 40% (Ramos-Rodríguez et al., 2006a, b). In contrast, Laznik et al. (2010) noted high mortalities (40–72%) at the highest nematode suspension concentration (2000 IJs/insect) of 3 *S. feltiae* strains, whereas Shahina and Salma (2010) reported increased susceptibility of *S. oryzae* adults (65–72%) to 6 Pakistani EPN strains. *S. bifurcatum* (Shahina *et al*. 2014) and *S. affine* isolated from Gilgit district Pakistan and reported by Tabassum et al. (2016).

Conclusion

The present study showed that the EPNs, *S. bifurcatum* and *S. affine*, had potentials against *L. serricorne* and *T. castaneum* (larvae and adult) under laboratory conditions. *S. bifurcatum* and *S. affine* showed potentials at hot and cold climates, respectively. Further investigations are required for the management of stored grain pests in stored product arenas.

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Authors' contributions

TAK and SJ carried out the experiment; TAK designed and wrote the manuscript, and SJ analyzed the data. Both authors read and approved the final manuscript.

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Declarations

Ethics approval and consent to participate

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Consent for publication

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Competing interests

The authors declare that they have no competing interests.

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