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EFFICACY OF ENTHOMOPATHOGENIC NEMATODES AGAINST LARVAL STAGE OF *RHIZOPERTHA DOMINICA* (COLEOPTERA: BOSTRICHIDAE), UNDER LABORATORY CONDITIONS

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ABSTRACT

Rhizopertha dominica Fab. (Coleoptera: Bostrichidae) lesser grain borer is one of the most serious pest of stored grains. The present study carried out to investigate the efficacy of four species of enthomopathogenic nematodes against the last fourth instar larval stage of *R. dominica* under laboratory conditions. The four species of entomopathogenic nematodes (EPNs) belonged to the genus *Steinernema* and were isolated from different plantations of Pakistan Council of Scientific and Industrial Research Laboratories Complex Karachi, Pakistan (PCSIR). They were evaluated against last larval stage of *Rhizopertha dominica* Fab. These EPN included *S. pakistanense* PCSIR-10, *S. bifurcatum* PCSIR-39, *S. saimkayai* PCSIR-6 and *S. abbasi* PCSIR-17. Efficacy of all species was tested at three different concentrations in Petri dishes at laboratory conditions. A significant nematode concentration and species effect was achieved. PCSIR-10 and PCSIR-39 showed the highest percentages of mortality, 90 and 100%, respectively at 250 IJs/larva.

Keywords: Biological control, surveys, entomopathogenic nematodes, *Steinernema*, grain pest, larvae

ABBREVIATION

EPNs: Entomopathogenic nematodes; IJs: Infective juveniles

INTRODUCTION

Rhyzopertha dominica Fab. Lesser grain borer (Coleoptera: Bostrichidae), is a very destructive pest of stored grain established in worm and dry environment. It is a significantly distributed worldwide pest (Mahroof and Phillip, 2007). It may spread from one location to another since it is an excellent flyer, which might result in infestation of stored grain pest (Stejskal *et al.*, 2015). The most harmful stages of *R. domonica* are the larvae which develop in the kernels their eating on grain, causes webbing and faces pollution, lowering the grain's quality (Pheloug and Macbeth, 2002). At first time *R. domonica* was observed in, *Sorghum drummondii* (Nees ex Steud. (sudangrass seed), and the damage demonstrated that this insect is decreasing the quantity and quality of seed due to its rapid population growth (Teixeira *et al*., 2021). The incidence of insect attack was found to vary from locality to locality and from commodity to commodity depending upon the type of storage in godowns, ecological condition and storage period (Usman *et al*., 2018).

The most popular method of controlling insect and 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 hazardous effect on the environment. Biological control is now frequently utilized as an alternative to synthetic pesticides (Quarcoo *et al.*, 2014). Entomopathogenic nematodes genera *Steinernema* and *Heterorhabditis* are obligatory pathogens in nature and are distinguished by their connection with mutualistic bacteria of the genera *Xenorhabdus* and *Photorhabdus*. They have been discovered in a variety of biological environments, including farmed fields, woodlands, grasslands, deserts and ocean beaches (Hominick *et al.*, 1996) because of their connection with bacteria, entomopathogenic nematodes infect and kill a vast number of insects. They can be effective biological control agents for a variety of insect pests. The non-feeding infective third stage juveniles find the insect host and parasitize it through natural holes and inter-segmental membranes. They are quick acting, eliminating target insect pests in 24-48 hours. Entomopathoginic nematodes are the parasite of some species of insect like coleoptera, diptera, lepidoptera (Burnell and Stock, 2000) and they have been successfully used to control various insect and pest of stored grain pest (Campos-Herra 2015).The efficacy of EPNs against the targeted pest is species specific (Nurashikin–Khairuddin *et al*., 2022).

The current study was conducted to determine the occurrence of EPNs from different plantation of Pakistan Council of Scientific and Industrial Research (PCSIR) Laboratories Complex Karachi, Pakistan and the main goal of

the surveys was to determine the efficacy of indigenous enthomopathogenic nematodes against the larval stage of *R. dominica* in laboratory conditions.

MATERIAL AND METHODS

Nematode collection

Surveys of Pakistan Council of Scientific and Industrial Research Laboratories Complex Karachi, Pakistan (PCSIR) conducted in the month of January - June 2021. The 50 soil samples (500 g each) from different plantations were collected with sterilized hand spade at the depth of 1×1m. (Table 1) Soil samples kept in polyethene bag and tagged with the relevant information and brought to the Entomopathogenic Research Laboratory at National Nematological Research Centre, University of Karachi, Pakistan and stored at 15°C.

Each soil samples were analyzed by *Galleria* trap method (Bedding and Akhurst, 1975) and incubated for one week. Approximately 500g soil were transferred in plastic containers (28×16×8cm) then four *Galleria mellonella* L. larva placed on soil in each plastic container. Each container were closed with plastic caps , placed upside down and incubated for one week at room temperature 28 ± 2ºC after that observation were taken on daily basis. The dead larvae were removed and washed with distilled water and placed in white trap method (White, 1927). The white trap based on plastic container (28×16×8cm) was filled with distilled water to a depth of 1cm, sealed with a lid, and incubated at 28 ± 2°C. The infective juveniles (IJs) that emerged from the dead larvae were migrated to the surrounding water. They were collected every day until the nematodes recovered. IJs were kept in a storage chamber at 28 ± 2°C in a 100 mL flask filled with distilled water. Approximately, 20 IJs, male and female of each species were morphologically identified according to Nguyen and Hunt (2007) and subsequently measured under compound microscope.

Nematode identification and biological cycle

For morphological characterization male, female and infective juveniles of isolated nematode species were heat killed preserved in tri ethanol amine formalin (TAF) solution for 24 h. After that precede with solution I (3-4 mL) which contains distilled water (80 mL), 95% ethanol (20 mL) and glycerin (1 mL), placed at 38-40°C in an incubator. After 12 h the specimens kept in solution II (3-4 mL) which contain 5ml glycerin, 95 mL ethanol and then kept for a second time in an incubator for 3 h at 40°C (Seinhorst, 1959). For permanent mounting the processed nematode were placed to microscopic glass slide (25.4 76.2 1mm) having a drop of glycerin.

To study the biological cycle of entomopathogenic nematodes, four *G. mollonella* larvae were infected by IJs of each species separately in 20 mL glass beaker filled with humid sterilized soil. On the top of the soil100 IJs in 1 mL water suspension were applied. Beaker was covered with plastic foil and placed at 28 ± 2°C. The dead larvae were rinsed with distilled water and placed in petri dish to assess the time period of each stage emergence.

Experimental pest

Initially fresh cultures of *Rhizopertha dominica* commonly known as lesser grain borer were obtained from the Pakistan Agriculture Research Council (PARC) University of Karachi, Pakistan. They were reared to obtain the last larval stage of *R. dominica* on the medium of wheat grains in the 1000 mL plastic jar at the temperature of 28 ± 2°C at 12:12 h day and night cycle. Jars covered with muslin cloths and kept in rearing chamber in the National Nematological Research Center, University of Karachi. After 15-17 days larvae were collected for experiment.

Laboratory bioassay

Larvae of lesser grain borer were collected from rearing jars. Ten larvae placed in petri dishes (9 cm diam.) on the filter paper (Whattmann No.1) placed at the bottom of each petri dish. Petri dish inoculated with the infected juveniles of enthomopathogenic nematodes *S. pakistanense* PCSIR-10, *S. bifutcatum* PCSIR-39, *S. siamkayai*. PCSIR-6 and *S. abbasi* PCSIR-17 with three different concentrations of 50,150 and 250IJs/larvae in distill water. There were three replicates of each treatment. The each petri dish sealed with Parafilm. Control treatments kept with distill water with the targeted insect. Data were collected after 48 hours of contact period. The dead larvae were separated and transferred to petri plates to determine the mortality and emergence of the nematodes.

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). Lethal concentration 50 % (LC_{50}) values were analyzed by PROC PROBIT routine of SAS, 2000.

RESULTS AND DISCUSSION

Nematode collection

Total 50 soil samples were collected from the different plantation of PCSIR Karachi, Pakistan. Samples were collected from *Saccharu mofficinarum, Hibiscus rosasinensis, Mangifera indica, Lycopersicon esculentum, Musa paradisiacal, Rosa indica, Citrus aurantiifolia, Cynodon dactylon, Punica granatum, Psidium guava, Cocos mucifera* and *Solanum melongena*. Surveys provide the occurrence of entomopathogenic nematodes in twelve soil samples with 24% recovery rate. During the surveys only species belong to genus *Steinernema* recovered which comprising four nematode species; *S. bifurcatum* Shahina *et al*., 2014 (50%), *S. pakistanense* Shahina *et al*., 2001 (30%), *S. siamkayai* Stock *et al*.,1998 (12%) and *S. abbasi* Elawad *et al*.,1997 (8%). It is resulted that *S. bifurcatum* recovery rate is greater than other species (Fig. 1).

Nematode identification and biological cycle

Morphological characterization was done by 3rd stage infective juveniles followed by (Nguyen and Hunt 2007). After measurements nematodes species PCSIR-10; PCSIR-17; PCSIR-39; PCSIR-6 were identified as *S. pakistanense* (638 µm), *S. abbasi* (595 µm), *S. bifurcatum* with short body length (470 µm), *S. siamkayai* (568 µm), respectively.

The entire biological life lengths of *S. pakistanense*, *S. abbasi*, *S. siamkaya*, *S. bifurcatum* were studied in *G. mellonella* larvae at 28 ± 2°C (Table 1).

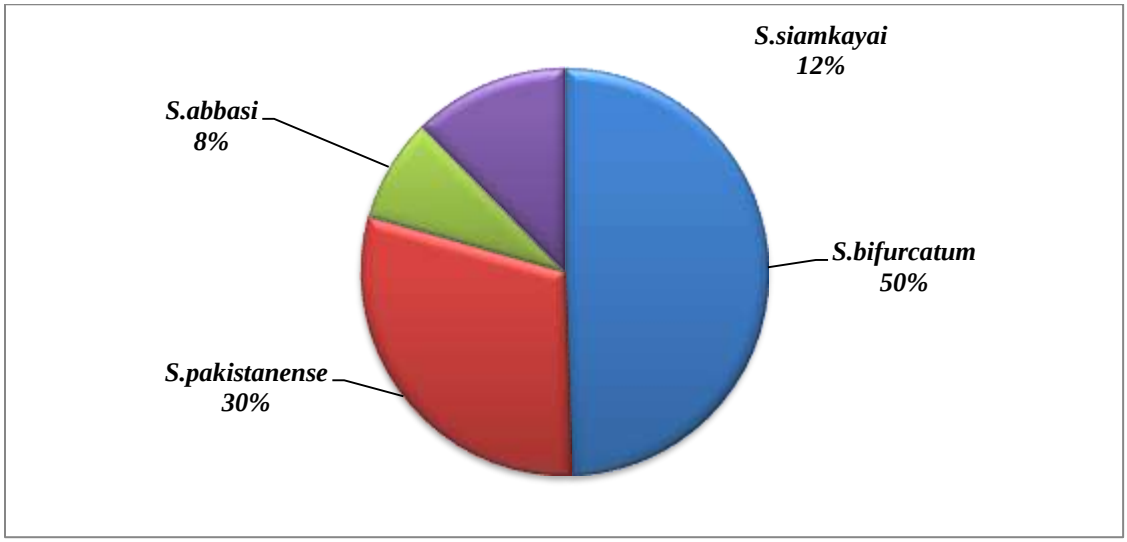

Fig. 1. Occurrence percentage of *Steinernema* species from PCSIR.

Table 1. Occurrence of entomopathogenic nematodes (*Steinernema* species) collected from different plantation of Pakistan Council of Scientific and Industrial Research Laboratories Complex Karachi.

S. No.	Vegetation	Nematodes species	Occurrence percentage	Life cycle (days)
1	A	*S. bifurcatum*	50	3-7
2	B	*S. pakistanense*	30	2-5
3	C	*S. siamkayai*	12	9-10
4	D	*S. abbasi*	8	2-4

A = *Saccharum officinarum, Hibiscus rosasinensis, Mangifera indica, Lycopersicon esculentum*
B = *Musa paradisiacal, Rosa indica, Citrus aurantiifolia;* C = *Cynodondactylon, Punica granatum*
D = *Psidium guava, Cocosnucifera, Solanummelongena*

Bioassay

Bioassay results showed that all the four species of enthomopathogenic nematodes were able to kill the larvae of *R. dominica*. There was no mortality observed in control. The analysis of variance (ANOVA) on efficacy of larval stage showed significant differences among nematode species (ANOVA $F = 4.90$; $df = 3$; $P < 0.05$). Nematode concentration also differed significantly (ANOVA $F = 2.21$; $df = 2$; $P < 0.05$). *S. bifurcatum* PCSIR-39 and *S. pakistanense* PCSIR-10 showed the highest mortality rate after 48 h of contact period 100 % and 90 %, respectively against the fourth instar larvae of *R. dominica* at the concentration of 250IJs/larva (Fig. 2).Whereas the *S. saimkayai* PCSIR-6 showed 75% and *S. abbasi* PCSIR-17 55% at the same concentration and time. At the 150 IJs /larva the mortality rate was 90% in *S. pakistanense*, 77% in *S. bifurcatum*, 62% in *S. saimkayai* and 30% in *S. abbasi*. The mortality effect of the targeted insect larvae increased as concentration increased, while the lowest mortality achieved in *S. abbasi* 10% at 50 IJs/larva whereas, *S. pakistanense* showed 70%, *S. bifurcatum* showed 55% and *S. saimkayai* resulted in 40% of mortality on same concentration. It is apparent that all the four species of EPNs have potential to kill the larvae of *R. dominica* dependent on concentrations. The LC_{50} value of *S. pakistanense* and *S. bifurcatum* was 65.9 (84.3-47.5) and 42.23 (39.8-14.8), respectively while *S. saimkayai* and *S. abbasi* showed LC_{50} value 97.7 (132.2-63.06) and 229.2 (252.03-206.3), respectively.

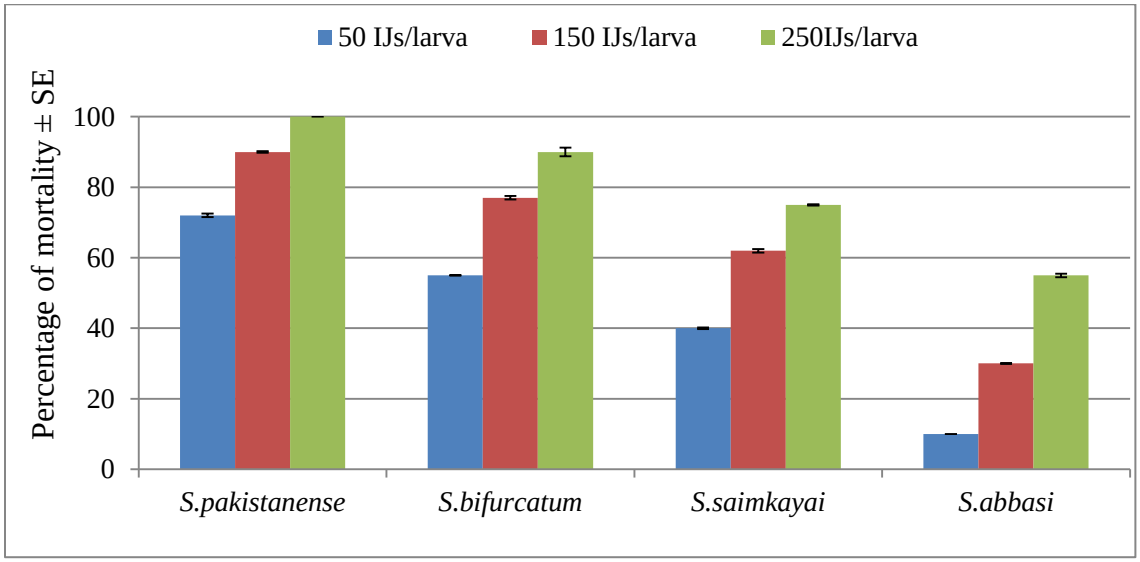

Fig. 2. *Rhizoperthado minica* larval mean mortality treated with four different species of Entomopathogenic nematodes (*Steinernema* species) in Petridishes depending on their concentration.

Research on the entomopathogenic nematodes has provides the evidence that EPNs used as biological control against the stored grain pest (Lacey and Georgis, 2012). In this study four EPNs species *S. pakistanense*, *S. bifurcatum*, *S. saimkayai* and *S.abbasi* used at three different concentrations, were highly effective against the *R.dominica* at larval stage. *S. pakistanense* and *S. bifurcatum* were the most effective at high concentration, whereas *S. saimkayai* and *S. abbasi* were the lowest one at the 48 h contact period.

Various studies have been carried out to assess the pathogenicity of EPNs against the stored grains pest (Romos-Rodriguez *et al.*, 2006). Athanassiou *et al*. (2010) investigated the efficacy of three EPNs species *H. bacteriophora*, *S. carpocapsae* and *S. feltiae* against the *R. dominica* adult with the concentration 20,000 IJs/mL ,his result showed that the *S. feltiae* and *S. carpocapsae* give the mortality 23.3% and 41.7%, respectively. In another study, Romos-Rodriguez *et al*. (2006) studied the pathogenicity of three strains of EPNs *S. carpocapsae*, *S. feltiae* and *S. riobrave* against eight insect species in which *R. dominica* adult was tested by *S. carpocaspae* which give the 70% mortality when exposed to 20 IJs among other species. according to the research of Tulek *et al*. (2015) applying *S. feltiae*, *S. carpocaspae* and *H. bacteriophora* at the two concentration of IJs (1000 and 2000IJs/ml) against the *R. dominica* adult beetles *S. carpocaspae* and *H. bacteriophora* showed the mortality 37.9 and 54.06% respectively at 25ºC.In this study the virulence of EPNs studied against the *R. dominica* forth instar larvae different mortality rates were observed. Salma *et al.* (2020) investigated the efficacy of four *Steinernema* species *S. pakistanense*, *S. bifurcatum*, *S. affinae* and *S. cholashanese* against the adult of *Tribolium castaneum* and *R. dominica* it was determined that concentration of 150 IJs/beetle gave the maximum mortality 100% for *S.*

pakistanense at 30ºC, but in current study *S. pakistanense* give the 100% mortality of *R. dominica* forth instar larvae at the concentration of 250IJs/larva. In another study, Yuksel *et al.* (2019) studied four native isolates of EPNs *S. feltiae* (UTP-5), *H. bacteriophora* (UMK-7), *S. feltiae* (DDKY-11) and *H. bacteriophora* (AVB-15) tested against the *R. dominica* adult beetles with the 250, 500 and 1000IJs / adult at the 15, 20 and 25ºC resulted that the all isolates showed the infectivity not more than 44% at all concentration and temperature. Most of the studies agree with that the increasing the mortality related to the increase in concentration of IJs and time. Several studies reported that the entomopathogenic nematodes were most effective for larvae of beetle species in comparison with adult (Kakouli-Duarte *et al.,* 1997; Ramos-Rodriguez *et al.,* 2006; Athanassiou *et al*., 2008).

Conclusions

This study evaluated that EPNs have potential to control the stored grain pest and may be an alternatives of chemical pesticides and provide the adequate control of insect pest at postharvest but in future more experiment required for pest management as bio control agent.

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