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IN VITRO EXPLORATION OF ENTOMOPATHOGENIC NEMATODES AS POTENTIAL BIOCONTROL AGENTS OF BRINJAL BORER *LEUCINODES ORBONALIS* GUENÉE (LEPIDOPTERA: CRAMBIDAE)

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ABSTRACT

Leucinodes orbonalis is a potential pest of eggplant, commonly known as fruit and shoot borer. Owing to its devastating nature, chemical pesticides are frequently applied and insecticide pressure is significantly higher in developing nations. Keeping the current situation in mind the most effective and practical strategy for managing insect pests found in humid and subtropical environments is the bio-control. Entomopathogenic nematodes, has been utilized against many pests for their effective management. Keeping in view the biocidal potential of entomopathogenic nematodes, the current study was planned to explore the biocidal potential of *Steinernema* and *Heterorhabditis*, two significant genera of entomopathogenic nematodes against *L. orbonalis*. White trap extraction technique was used to isolate nematodes from several insect larvae. Four discrete concentrations of entomopathogenic nematodes viz. 60, 90, 120, and 150 infective juveniles per larvae were applied on larval stages. The virulence trial was followed by completely randomized block design. Multiple comparisons showed that among both the tested nematodes *H. bacteriophora* induced significant 81.33% mortality corresponding to 150 IJs/larva after 72 h of exposure as compared to *S. glaseri*. In the view of current research, entomopathogenic nematodes are recommended to be used in integrated pest management program for the management of insect pests.

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INTRODUCTION

The use of highly hazardous pesticides has negative consequences on not just the environment but also the health of individuals as well as wild animals. Insect pests have evolved resistance to a variety of pesticides (Shehzad et al., 2023). Similarly, *Leucinodes orbonalis* Guenee, has also developed insecticide resistance toward several insecticides (Shirale et al., 2017). *L. orbonalis* also known as Brinjal fruit and shoot borer is a devastating pest of eggplants which reduces the yield and inflicts colossal losses in production and may reduce the crop yield up to 60-70-70 % (Javed et al., 2011; Javed et al., 2017; Kassi et al., 2019; Muhammad et al., 2021). The resistance has been documented against

organophosphates, carbamates and marginal synthetic pyrethroids (Visnupriya and Muthukrishnan, 2017). By using various bio-control agents, such as predators, antagonistic fungi, bacteria, viruses, and entomopathogenic nematodes and entomopathogenic fungi, many pests of various crops have been effectively managed (Reddy and Devakar, 1997; Javed et al., 2019; Rahoo et al., 2019a,b; Gulzar et al., 2020; Shehzad et al., 2021, 2022). Entomopathogenic nematodes have existed all over the world and have a variety of ecologically distinct environments. They are incredibly intricate, specialized, and diversified (Friedman, 1990). *Heterorhabditis* and *Steinernema,* two distinct groups of entomopathogenic nematodes, are found in a variety of soil types and habitats all over the world (Bhat et al., 2019; Khanum and Javed, 2020). Lethal parasites of insect pests, *Steinernematidae* and *Heterorhabditidae* are safe for humans, other vertebrates, and non-target creatures. They are also easy to apply and have no negative environmental effects (Askary and Abd-Elgawad, 2017). EPNs get access through the body of the insect's natural holes. By releasing bacteria into the insect's hemocoel, they cause septicemia and ultimately death (Kaya and Gaugler, 1993).

The thick peritrophic membrane prevents *H. bacteriophora* from entering the host through the gut; as a consequence, they enter through the cuticle intersegmental membranes. Due to their need on insects as hosts, entomopathogenic nematodes are not facultative parasites of insects (Goudarzi et al., 2015). The use of EPNs is widely adopted throughout the world owing to their effectiveness and may be produced in huge quantities experimentally for a relatively cheaper price (Mutegi et al., 2018). Therefore, these parasites are considered the most effective and secure agents for the management of various insect pests (Ehlers, 2001). Consequently, the purpose of the current investigation was to assess, in a lab setting, the effectiveness of the two native EPNs *Steinernema glaseri* and *Heterorhabditis bacteriophora*, against different developmental stages of *L. orbonalis*.

MATERIALS AND METHODS

Survey and soil sampling

The survey was carried out in several locations, and field areas of University Agriculture Faisalabad having geographic coordinates latitude 31.411930, longitude 73.108050 respectively. The soil samples were taken from the rhizosphere of brinjal plants. The samples were carefully tagged, stored in plastic bags, and brought into the lab for further study.

Collection of *L. orbonalis*

Infested eggplant fruits were collected from infested brinjal field plots. The infested fruits were handpicked and sliced to obtain 3rd and 4th instar larvae of *L. orbonalis* for laboratory bioassay study. Identification of collected larval instars was carried out based on head capsule width and body coloration as described by Rostaee et al. (2021).

Rearing of wax moth (*Galleria mellonella*)

Galleria mellonella larvae were removed from honeybee hives that were gathered from various locations. The larvae were kept under ideal regulated conditions (25 ± 2°C, 60% RH) and honey comb were provided as a natural diet for immatures until adult emergence. The adults were moved to plastic jars covered with muslin fabric to provide sufficient air circulation. The newly laid eggs were retrieved and placed in plastic jars with paper folds hatched after 3-4 days under ideal circumstances while receiving a natural diet. Fifth instar larvae were processed for rearing of entomopathogenic nematodes.

Culture of entomopathogenic nematodes

Entomopathogenic nematode species, *Steinernema glaseri* and *Heterorhabditis bacteriophora*, were utilized in the current study as described by Woodring and Kaya (1988). Fifty *G. mellonella* larvae in their last instar were arranged in Petri plates, each of which had two filter papers moistened with a nematode water suspension at a concentration of 10,000 IJs/ml of distilled water. Following a period of 10 to 15 days of infection, the host cadavers were placed on white traps (White 1927) in order to gather the infectious juveniles. Harvesting of juveniles was performed every day after 10 days of inoculation, kept in plastic bottles (15 × 15 × 10 cm) filled with distilled water at 10°C until they were needed. For confirmation of species collected juvenile stages were examined under a stereomicroscope (Olympus BX50). The nematodes culture was collected in a little plastic container and refrigerated at 10°C for virulence trial.

Identification of EPNs

The color of the dead insect larvae revealed the existence of EPNs. EPN vaccination results in color changes in insect larvae. *Heterorhabditis* spp. has brick red color on dead larvae, while *Steinernema* spp. exhibits grey color (Flores et al., 2021). Leica DM750 compound

microscope with Leica Application Suite (LAS) 4.12 was used for the measurements. The morphological analyses were performed using the taxonomy keys (Hominick et al., 1997).

Bioassay procedure

The insecticidal activity of the newly isolated EPNs (*H. bacteriophora* and *S. glaseri*) was evaluated by subjecting individual 3rd and 4th instar larvae of brinjal borer under laboratory conditions. The larvae were introduced into 6-well plate. Each well plate was lined with Whatman no.1 filter paper. Four discrete concentrations of Entomopathogenic Nematodes, namely 60, 90, 120, and 150 infective juveniles/ml of water were prepared and applied on filter paper. A total of five replicates were employed for each concentration, while the control treatment involved the utilization of distilled water. There were 10 larvae in each replicate making 50 immatures/treatment (10 larvae × 5 replicates). The plates after placing larvae were covered and subjected to incubation within a growth chamber devoid of light, maintaining a constant temperature of 26 ± 2°C. The mortality rate was assessed at 24, 48 and 72 h following the administration of the treatment. To confirm nematode infection, the cadavers of the dead larvae were collected and dissected under a stereomicroscope.

Statistical analysis

The virulence trial was followed by completely randomized block design. The obtained mortality data was subjected to analysis of variance and means were compared through Tukey's statistic at $P \leq 0.05$.

RESULTS

The data obtained demonstrated that, unlike the control group, each tested entomopathogenic nematode was capable of effectively eliminating the brinjal shoot and fruit borer. Compared to the controls, both tested instars experienced substantial mortality when exposed to all entomopathogenic nematodes. The mortality rate was positively correlated with the number of nematodes per insect and the duration of treatment after application. Notably, there was a significant difference between the applied dose and the post-treatment time interval.

The results clearly indicated that the maximum mortality was caused by 150 infective juveniles per larva, resulting in 85% virulence after 72 h of Entomopathogenic nematodes application. However, when the same dose was applied after 48 h, it led to a 57% control of the Brinjal shoot and fruit borer. During the initial time interval (24 h), the dosage of 150 infective juveniles per larva of *H. bacteriophora* exhibited only 15% pathogenicity. Subsequent dilutions of the nematodes resulted in a significant decline in the mortality rate of the tested insects. Notably, the lowest mortality occurred in the control treatment, which consisted of water only, as evident from Figure 1.

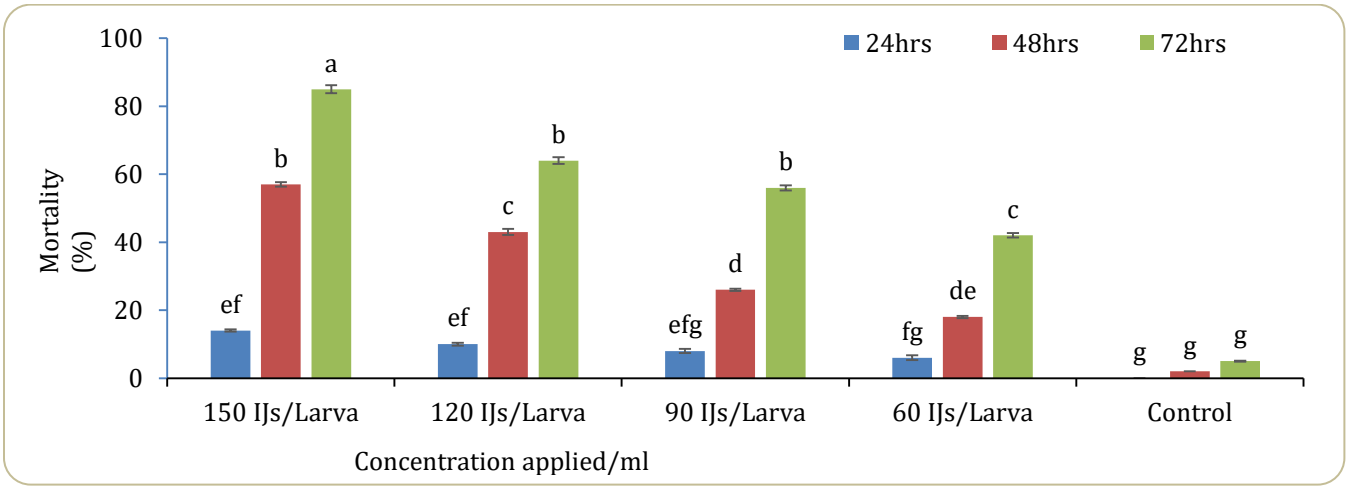

Figure 1: *H. bacteriophora* induced mortality against 3rd instar larvae of *L. orbonalis* after different time intervals (α=0.05).

The data from Figure 2 revealed that larval mortality was initially lower after 24 hours of treatment but increased over time. The maximum mortality percentage, reaching 72%, occurred when 150 IJs per

larva were applied after 72 h. In contrast, when the same dosage of *S. glaseri* was administered after 48 h, it resulted in a 40% control of *L. orbonalis*. During the initial time interval (24 h), the dosage of 150 IJs per larva /7of *H. bacteriophora* exhibited only 12% pathogenicity. This suggested that *H. bacteriophora* demonstrated greater virulence compared to *S. glaseri*.

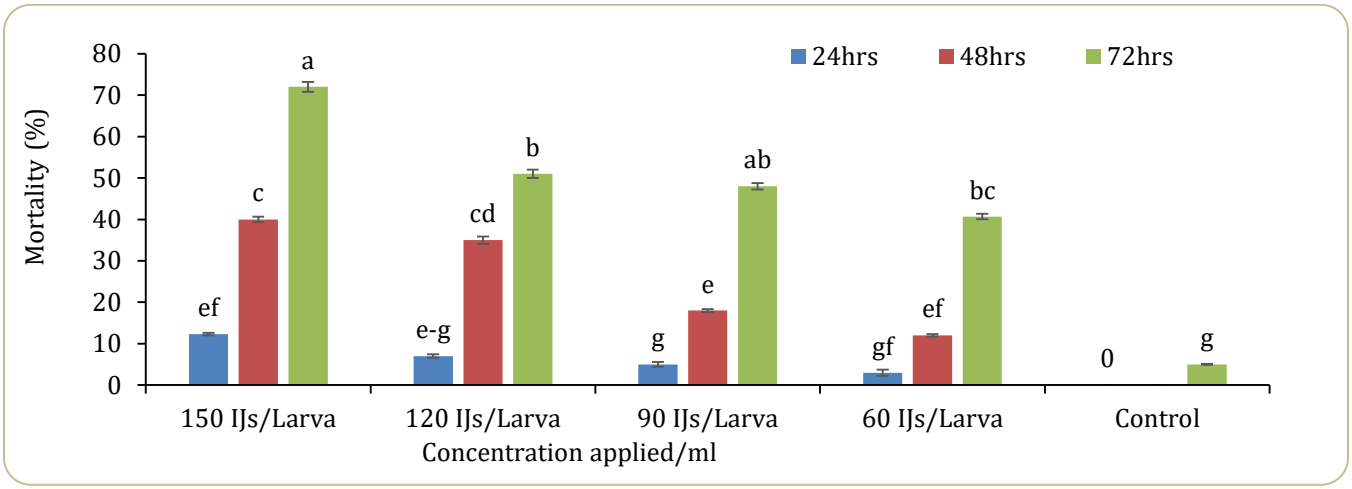

Figure 2: *S. glaseri* induced mortality after different time intervals against *L. orbonalis* (α=0.05).

Infective juvenile concentration 150, 120, 90, and 60 of *H. bacteriophora* displayed 92-50% mortality against larvae of brinjal fruit and shoot borer after 72hrs of post-treatment (Fig 3). However same doses grasped 71,56,32 and 25 % mortality after 48hrs of post-treatment. Obvious from the results the mortality percentage was lower after 48hrs while showed climbed values after 72hrs of juvenile application.

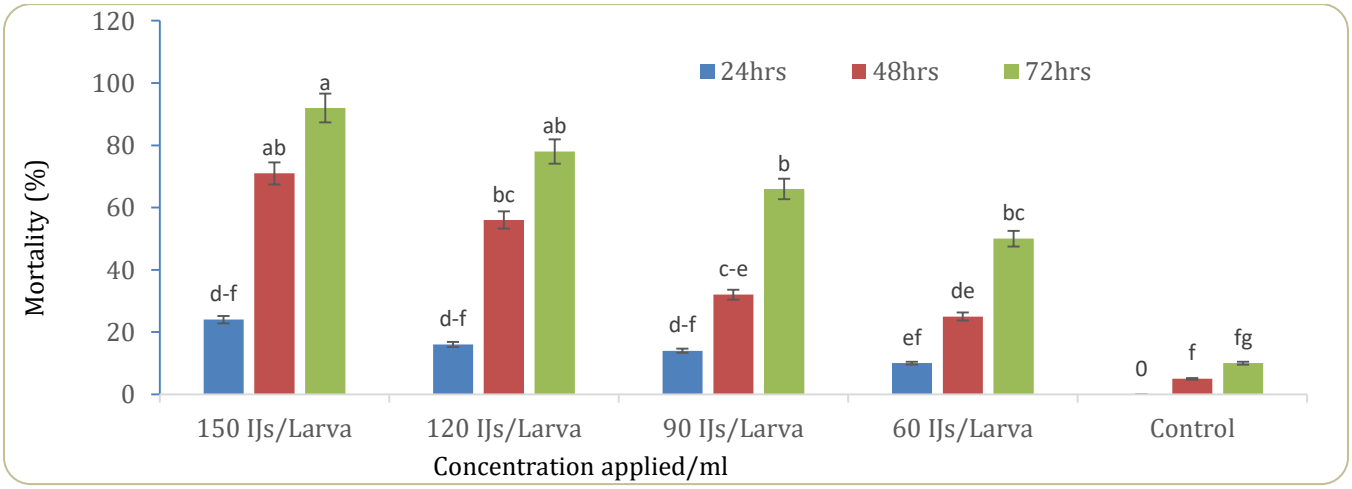

Figure 3: *H. bacteriophora* induced mortality against 4th instar larvae of *L. orbonalis* after different time intervals (α=0.05).

According to Figure 4, the trial results illustrated that the greatest insect mortality induced by *S. glaseri* against 4th instar larvae occurred with a concentration of 150 IJs per larvae after 72 h of application. In comparison, a mortality rate ranking second was observed with 120 IJs per larvae after the same time interval.

DISCUSSION

According to Kaya and Gaugler (1993), species of EPNs from the families Steinernematidae and Heterorhabditidae are the most effective biocontrol agents for managing insect pests. EPNs are utilized as biocontrol agents to manage various insect pests such as earthworms, borers, cutworms,

and pink bollworm (Poinar, 1990; Rahoo et al., 2011, 2017, 2018a,b). The J2 stage of nematodes, often referred to as infective juveniles, enters the host insect through natural openings such as its mouth, as well as through wounds caused by various environmental conditions (Dowds and Peters, 2002).

The present experiment was conducted to measure the mortality percentage of *S. glaseri* and *H. bacteriophora* on Lepidopteran insect larvae (specifically brinjal borers) at various time intervals using the same dosage of entomopathogenic nematodes. According to the research, *H. bacteriophora* had the highest percentage of insect larval mortality. Abd El Azim (2022) indicated that the native isolate of EPN has biocidal potential against *S. littoralis*. In a study conducted by Ibrahim et al. (2019), the pathogenicity of the entomopathogenic nematode *H. zealandica* was examined. Specifically, the researchers investigated the effects of this EPN on the final larval stage of the greater wax moth, *G. mellonella*. Ebubekir and Ramazan (2018) employed entomopathogenic nematodes as a means to counteract subterranean insect populations, specifically targeting cutworms. The researchers found that entomopathogenic nematodes proved to be effective biological control agents in managing *Agrotis ipsilon* larvae infestations.

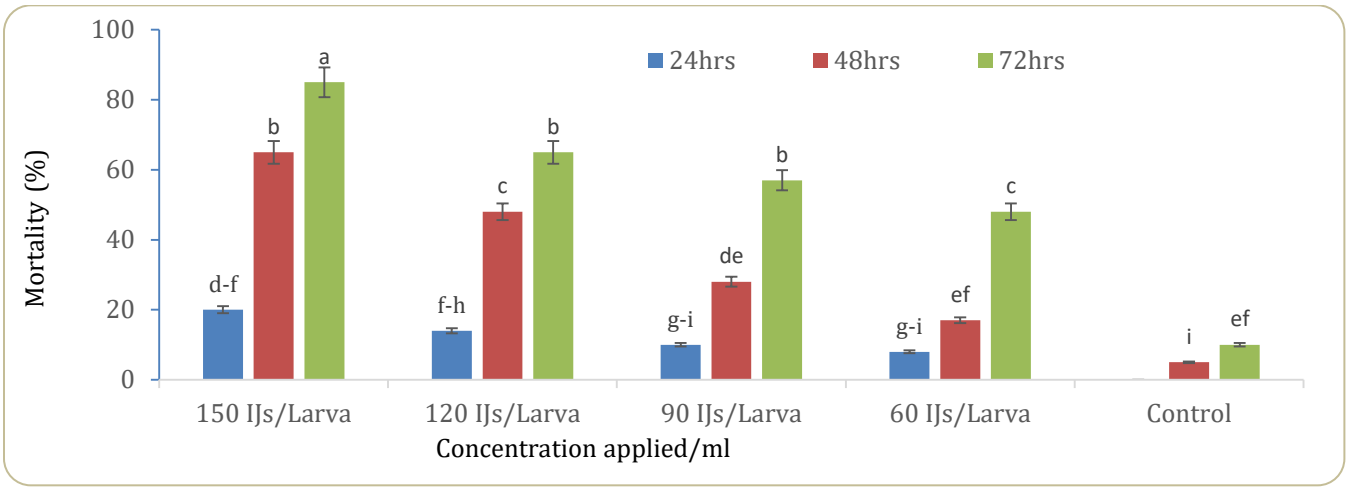

Figure 4: *S. glaseri* induced mortality after different time intervals against 4th instar larvae of *L. orbonalis* (α=0.05).

In laboratory experiments, it was observed that the application of *H. bacteriophora* (FLH-4-H) and *H. indica* (216-H) isolates at concentrations of 50 and 100 infective juveniles/cm² (IJs/cm²) resulted in a rapid and complete mortality rate of 100% within a span of 2 days. The findings of the study revealed a significant association between the concentration of the pathogen and the mortality rate observed in the host population. Similar to earlier studies which showed that the ability of entomopathogenic nematodes to reproduce is closely correlated with their concentration of infectious juveniles, suggesting that more infectious juveniles would grow from the culture if the concentration of infective juveniles is at its highest (Sankar et al., 2009; Xu et al., 2010). This study highlighted the biocidal potential of EPNs against insect pests.

CONCLUSION

The current investigation has revealed the identification and characterization of the Entomopathogenic nematodes species *Heterorhabditis bacteriophora* and *Steinernema glaseri*, which have demonstrated remarkable pathogenicity against the Brinjal fruit and shoot borer. Furthermore, the compatibility of these native EPNAs still needs to be addressed in future research.

AUTHORS' CONTRIBUTIONS

MA and SR conducted the trial; MT and AQ supervised the experiment; AA formatted the article according to the journal's guidelines, requirement and corresponded the article; AA provided necessary material to conduct the trial; MS reviewed the article and MFA statically analyzed the collected data.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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