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EVALUATION OF ENTOMOPATHOGENIC NEMATODE *STEINERNEMA GLASERI* AN EFFECTIVE BIOLOGICAL ENTITY AGAINST PINK BOLLWORM AND ARMYWORM IN LABORATORY

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ABSTRACT

Entomopathogenic nematodes belong to family Steinernematidae and Heterorhabditidae. They are obligate parasites of different insect pests and are used as natural control agents. The aim of this work was to check the virulence of *Steinernema glaseri* against two insect larvae belongs to genus lepidoptra. Three hundred infective juveniles at 27±2ºC were used in each treatment. *S. glaseri* was more effective against armyworm as compared to pink bollworm larvae. Effects of different concentrations and storage time of *S. glaseri* were also evaluated on these larvae. At 500 IJs concentration high virulence was recorded followed by 200, 100 and 50 IJs at 27±2ºC. High virulence of *S. glaseri* was recorded in 2 weeks followed by 4 and 6 weeks old culture. Maximum population of *S. glaseri* was recorded on 0.5g as compared to 0.25g larval weight. These findings will hopefully be helpful for researchers and farmers for batter management against both insect pests.

Keywords: EPNs, *Steinernema glaseri*, *Heterorhabditis, Xenorhabdus* and *Photorhabdus*

INTRODUCTION

Entomopathogenic nematodes (EPNs) are obligate parasites of insect pests and used as an alternative to synthetic insecticides in agriculture for insect pest management. Their juveniles have capability to infect insects and cause mortality within 24-48 hours (Poinar, 1979). The pathogenicity of EPNs depends on the ability of infective juveniles to find their host for penetration and toxins produced by bacteria (*Xenorhabdus* and *Photorhabdus*) (Akhurst, 1982; Griffin *et al.,* 2005). EPNs have great potential for biological control (Turlings *et al.,* 2012). They can be applied directly and conjugation with other pesticides, fertilizers and other biological amendments. EPNs used as foliar spray against leaf eating caterpillar on different crops (Somvanshi and Ganguly, 2007). EPNs enter the host insect through its natural openings oral cavity, anus and spiracles or in few cases through the cuticle (Dowds and Peters, 2002). After entering insect body EPNs release bacteria having symbiotic relationship. Symbiotic bacteria (*Xenorhabdus* and *Photorhabdus*) are primary agents that cause host death and provide nutrition to the nematodes and protect from secondary invaders (Poinar, 1990). Host insect provide shelter for their development and EPNs inhabit for two or three generations until food is depleted then IJs search for new hosts (Grewal and Georgis, 1999).

Cotton is a cash crop famous as white gold and lifeline of textile industry of Pakistan and many other developing countries of the world (Shabbir *et al.,* 2012). It is pest loving crop and pink bollworm (*Pectinophora gossypiella*) is a destructive pest (Khan *et al.,* 2015). Berseem (*Trifolium alexandrinum* L.) is an important leguminous forage crop in Pakistan (Karishnamurthi, 1959). It is highly nutritious and a high-yielding fodder crop that is well adopted in Pakistan (Ahmad *et al.,* 1998). *T. alexandrinum* is afflicted by various pests including the important armyworm (*Spodoptera litura*) (Perveen, 2000; Zalucki *et al.,* 1986). The Greater wax moth, *Galleria mellonella* L. is a major pest of bee keeping industry (Anwar *et al.,* 2014) but valued mainly for its dominant role as a fictitious host owing to the susceptibility to various biological control agents (Hendrichs *et al.,* 2009) for reproduction of many bio-control agents (Kulkarni *et al.,* 2012; Singh, 1997; Vashisth *et al.,* 2013) including EPNs, which are obligate parasites of a wide range of insect pests (Hussaini *et al.,* 2010).

Management of insect pests by biological control is an alternative strategy that results in pesticide-free produce with no hazard to the environment. Among the different agents for biological control, EPNs are gaining importance, because they possess many positive attributes of an effective biological control agent. EPNs often have broad-spectrum effectiveness, short life cycles, amenability to mass production, recycling ability, persistence etc. (Gaugler *et al.,* 1980; Kaya and Gaugler, 1993). The objective of this research was to access the virulence of *Steinernema glaseri* against two most destructive insects.

MATERIALS AND METHODS

Nematode culture

The nematode specie *Steinernema glaseri* was used in this study to assess their infectivity against the pink bollworms (*Pectinophora gossypiella*) and armyworm (*Spodoptera litura*). EPNs were reared in the laboratory at 25 ± 2 °C on the last instars/larvae of the greater wax moth (Woodring & Kaya, 1988). The nematodes were stored in sterilized water at 5 °C before use and kept at room temperature (18-22 °C) for 24 hours before use.

Mass rearing

Mass rearing of nematodes were performed on solid culture 80 g chicken offal medium on a porous foam substrate was used (Tabassum and Shahina, 2004) which provides the largest surface-volume ratio and adequate interstitial space in 500 mL conical flasks. After two weeks of incubation, approximately 5-7 million infective juveniles were produced in a single flask and stored in sterilized water at 20-25 °C for 3-4 months.

Collection of insect larvae

Larvae were collected from experimental area of Department of Plant Pathology, University of Agriculture Faisalabad Pakistan. Armyworm larvae collected from fodder crop berseem in the month of February and March, 2015 for lab experiment. Pink bollworm larvae were collected from cotton crop in the month of August and September, 2015. Wax moth collected from infected honey cobs. Larvae's were separated according to their size and weight.

Efficacy of *S. glaseri* against two insect larvae

EPNs suspensions were used to check out efficacy against two insect larvae. Fresh 300 IJs were used to attack on two different insect's larvae to check the virulence of the nematodes by using insect baiting technique (Xuejuan and Hominick, 1991). Each treatment replicated 10 times for the confirmation of results.

Efficacy of *S. glaseri* at different concentrations

EPNs were applied on pink bollworm and armyworms following treatments (T1:50, T2:100, T3:200 and T4:500 infective juveniles/3 larvae). Each treatment replicated 10 times for the confirmation of results and dead larvae were shifted to white trap to check the reproductive potential. EPNs progeny was start after seven days and data was collected after 2, 4, 6, 8 and 10 days by using nematode counting dish method.

Efficacy of *S. glaseri* at different larval weight

Insect larvae were collected from different crops and larvae weights were taken (0.25g and 0.5g). Fresh 300 IJs were used to attack on different insect's larvae to check the virulence of nematodes by using insect baiting technique. Each treatment replicated 10 times for the confirmation of results.

Effect of storage time on the virulence of *S. glaseri*

EPNs suspensions were stored for 2, 4 and 6 weeks at 9ºC to check out the virulence of *S. glaseri* on two different insect larvae. 300 IJs were taken from 2 weeks old nematode suspension and allow to attack on two different insect's larvae. This procedure was repeated for 4 to 6 weeks old nematodes suspension to check the virulence of nematodes. Each treatment replicated 10 times for the confirmation of results.

Statistical Analysis

The data was analyzed by using completely randomized design with factorial arrangement for laboratory studies according to Fisher's analysis of variance (Steel *et al*., 1997). Means were compared by Least Significant Difference (LSD) test at 0.05 probability test.

RESULTS

Results showed that high virulence of the *S. glaseri* was recorded on armyworm (*S. litura*) larvae as compared to pink bollworm (*P. gossipyilla*) (Fig. **1**). The inoculations of *S. glaseri* on different concentration also differ from each other. Results indicate that the progeny of EPNs on both larvae was higher at 500 IJs that were 30000/larvae and minimum at 50IJs and progeny count was 5000 IJs/ larvae (Fig. **2**). Population of *S. glaseri* decreased with decreasing the concentration of IJs. The weight of host larvae significantly affects the reproduction potential and maximum population of *S. glaseri* at 0.5 g as compared to 0.25 g larvae weight. Recovered progeny decreased with

decreasing weight of larvae. The maximum count of IJs was 23000IJs/larvae at 0.5 g weight and 15000IJs/larvae at 0.25 g weight of both larvae (Fig. **3**).

The age of EPNs effect on their viability to reproduce and 2 weeks old *S. glaseri* was more efficient as compared to 4 weeks and 6 weeks (Fig. **4**). Reproductive potential decreased with increasing storage time of EPNs. Total count of IJs was 15000 IJs/larvae maximum at the age of 2 weeks and minimum 8000 IJs/larvae for 6 weeks old culture. The emergence of juveniles from insect body is lower in pink bollworm as compare to armyworm in all parameters.

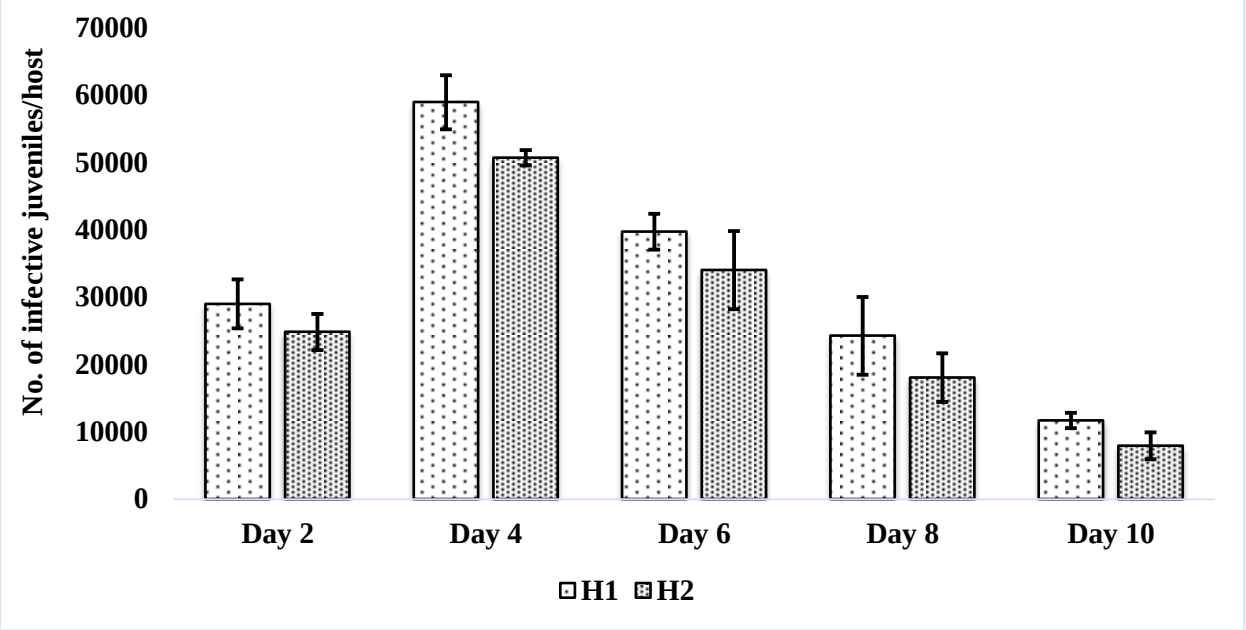

Fig. 1. Virulence of *S. glaseri* on two different insect larvae. H1 (Host) = armyworm, H2 = pink bollworm larvae, Day 2 = 1st counting after emergence, Day 4 = 2nd counting after emergence, Day 6 = 3rd counting after emergence, Day 8 = 4th counting after emergence and Day 10 = 5th counting of nematodes after emergence of nematodes.

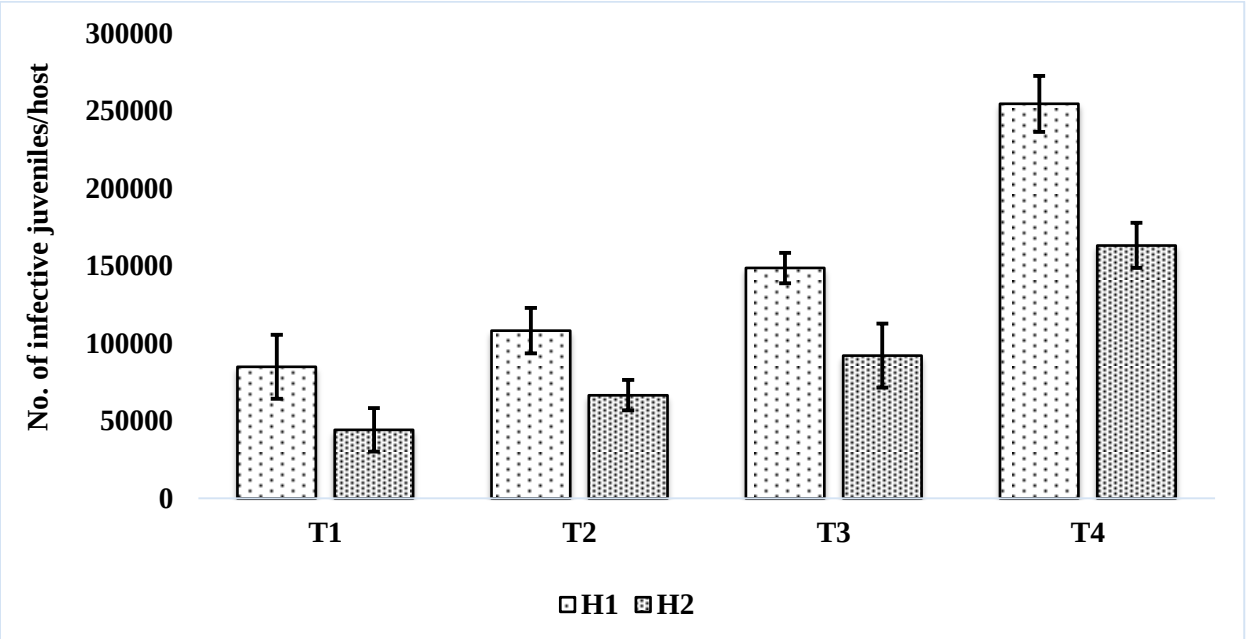

Fig. 2. Efficacy of *S. glaseri* at different concentrations. T1 = 50 infective juveniles, T2 = 100 infective juveniles, T3 = 200 infective juveniles, T4 = 500 infective juveniles, H1 = armyworm and H2 = pink bollworm larvae.

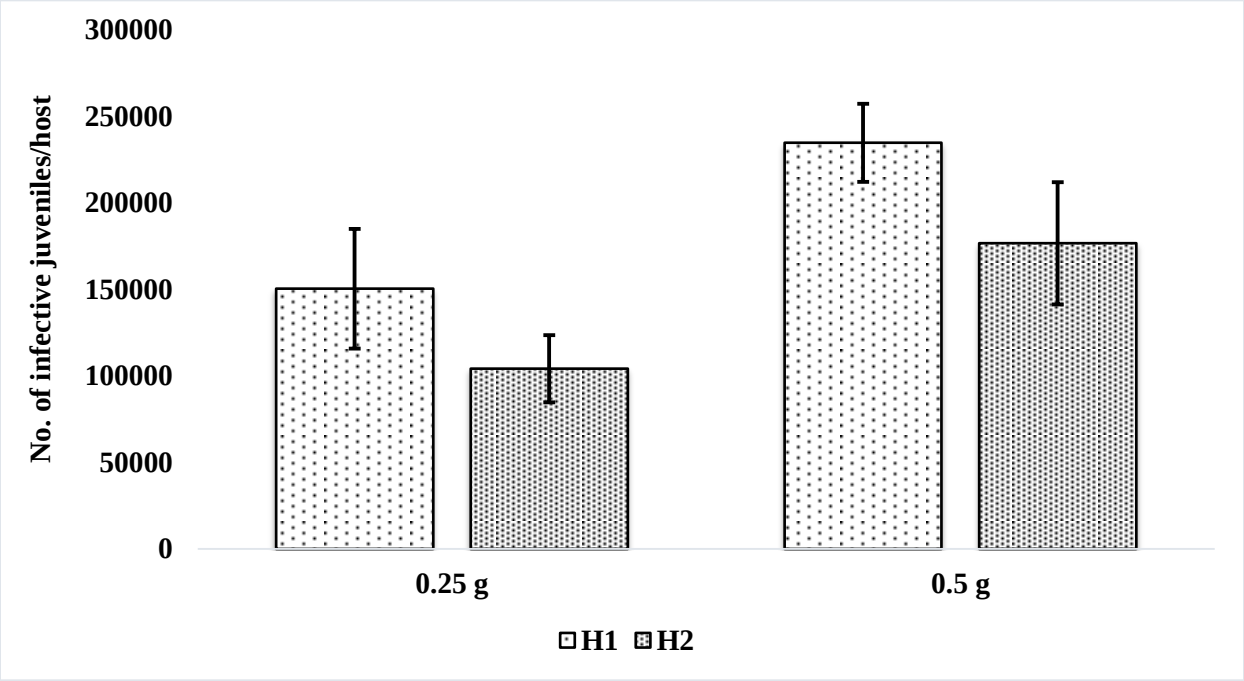

Fig. 3. Impact of larvae weight on *S. glaseri* reproduction. H1 = armyworm, H2 = pink bollworm larvae, 0.25 g and 0.5 g = two different larvae weight in grams.

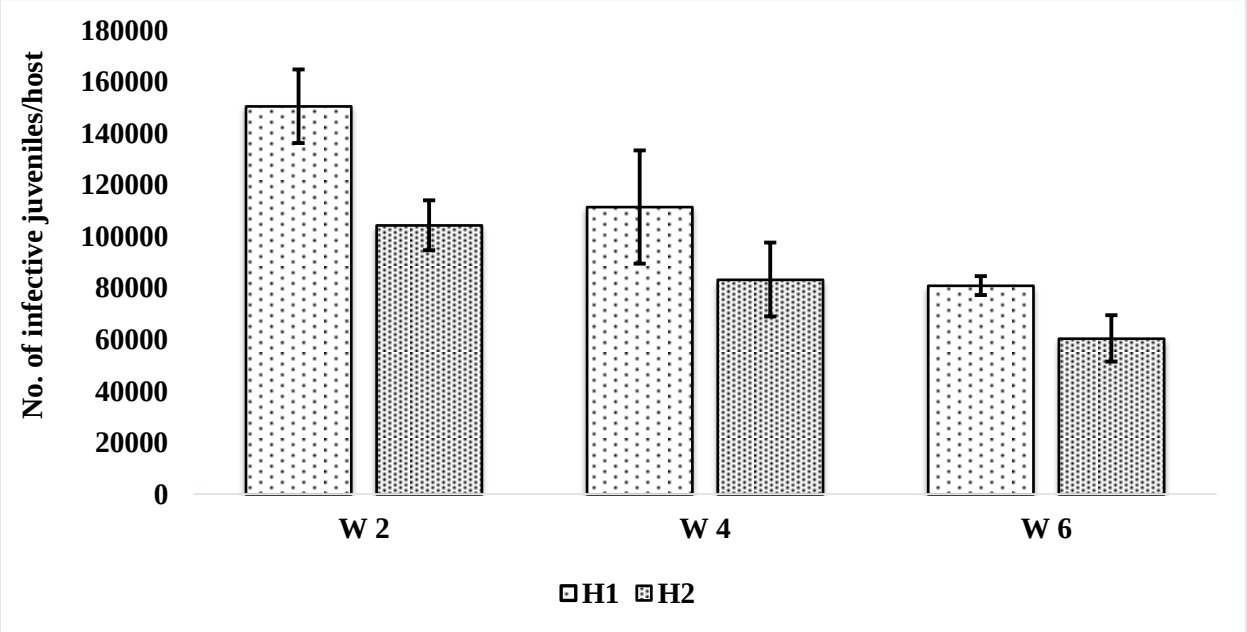

Fig. 4. Efficacy of *S. glaseri* at different storage time. H1 = armyworm, H2 = pink bollworm larvae, W2 = two-week old culture, W4 = four-week old culture and 6 weeks = six-week old culture of nematode.

DISCUSSION

EPNs are effective biological agents against many soil pests. Infectivity of EPNs is an important factor for developing them as biological control agents for insect pests (Ricci *et al*., 1996; Shahina *et al.*, 2014; Navon *et al.*, 2002). The efficacy of *S. glaseri* was evaluated against army worm and pink bollworms. Between the tested insects,

S. litura larvae showed significantly ($P \leq 0.05$) high virulence when they exposed for *S. glaseri.* However, the similar factors (Number of IJs, larval weight and age of IJs) were assessed against both insects' larvae. *S. glaseri* reproduced maximum at 500 IJs and minimum at 50 IJs. Population of *S. glaseri* significantly ($P \leq 0.05$) decreased with decreasing the concentration of IJs. The maximum progeny was obtained from armyworm (*S. litura*) larvae as compared to pink bollworm (*L. orbinalis*). Test larvae weight also affects the reproduction of EPNs as well as host mortality. Maximum progeny of *S. glaseri* was recorded on 0.5 g as compared to 0.25 g larval weight. Reproductive potential of *S. glaseri* significantly ($P \leq 0.05$) increased with decreasing the storage time/age of IJs vice versa. It might be due to high efficacy of 2 weeks old juveniles as compared to 4 and 6 weeks. Thus, results showed that the infectivity of *S. glaseri* differs between host species followed by number of IJs, larval weight and age of IJs. Our findings endorse the previous reports (Pervez *et al*., 2012; Ali *et al*., 2008). The failure of EPNs is the wrong selection of nematode specie against a particular pest (Georgis and Gaugler, 1991).

CONCLUSION

Our efforts confirmed that the armyworm larva is an efficient bioassay for *S. glaseri* as compared to pink bollworm. Results also confirmed that at all factors; e.g. weight, storage, and number of IJs which were used against these larvae clearly mention the efficiency of *S. glaseri* against armyworm. In future, our findings will be helpful for the researchers as well as farmers to choose an appropriate way of management against two evaluated insects.

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