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Short Communication

Reproduction Potential of Entomopathogenic Nematodes on Armyworm (*Spodoptera litura*)

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ABSTRACT

Biocontrol potential of entomopathogenic nematodes (*Heterorhabditis bacteriophora* and *Steinernema glaseri*) against different larval instars (2nd, 3rd, 4th and 5th) of armyworm (*Spodoptera litura* F.) at different exposure times were evaluated. Entomopathogenic nematodes were applied at 1000 IJs/ml and larvae maintained at 25°C. Mortality was recorded upto four days. Both species of entomopathogenic nematodes proved effective against all larval instars. Maximum mortality was observed in 2nd and 5th larval instar. After the fourth day 100% mortality was observed in all larval instar. Mortality was increased with an increase in exposure time. Nematodes were harvested using White Traps. Multiplication of entomopathogenic nematodes was also recorded in all larval instar. The maximum number of *H. bacteriophora* was harvested from 5th larval instar which was 25,786 followed by 17,500, 12,642 and 9,652 from 4th, 3rd and 2nd larval instar, respectively.

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Authors' Contributions
HS and NJ designed the study. HS conducted the study with the help of SAK. MA analyzed the data.

Key words
Heterorhabditis indica, Steinernema glaseri, Spodoptera litura.

In Pakistan, army worm (*Spodoptera litura*) is the major vegetable pest. Army worm is widely distributed and considered as the most destructive and economically important polyphagous pest with host range of more than 120 plants (Singh and Jalali, 1997). It severely affects the crop production and cause huge crop losses.

For many years, insecticides remained the primary means for management of insects (Syed, 1992). Pesticides have harmful effects on environment therefore alternative control strategies are necessary for their management due to increasing concern over human safety and environment (Gaugler, 1988; Villani and Wright, 1988). Every year farmers spend $300 million on insecticides, of which 80% applied for control of chewing insects (Rao, 2007). Chemical pesticides provide only short term solution for pest control. Moreover, the random use of insecticides has posed many problems such as increased resistance in this insect against all groups of pesticides (Lohar *et al*., 1995; Kerns *et al*., 1998; Whalon *et al*., 2007), insect resurgence, bio accumulation and health hazards. Excessive use of pesticides had a negative impact in the environment and agriculture sustainability (Purwar, 2002). It has threatened biocontrol agents such as parasites and predators. The risk of using insecticides varies in several ways depending upon application coverage (Cilgi *et al*., 1988), their exposure (Kennedy, 1988), as well as upon the intrinsic toxicity of chemicals employed (Hassan, 1987). Pest populations in nature are regulated by a wide range of parasites, predators and pathogens. Biological plant protection is alternatives to chemical pesticides. Use of pathogens in biological control can be integrated with other insect pest management tactics. To maintain the pest population below the damage threshold level, crops can be protected by microbial control agents when parasitoids and predators fail to maintain this level. Biological control is an alternative control tactic for the management of insects (Brixey, 1997).

Entomopathogenic nematodes (EPN) as bioinsecticides against soil pests are extra ordinarily lethal to many insect pests (Klein, 1990; Georgis and Manweiler, 1994). EPN can kill insects within 24-48 h working with their symbiotic bacteria, while most of the biocontrol agents require longer time periods for such action. EPN are important biological control agents due to their various habitats, excellent host searching ability, wide insect host range and ease of mass culture (Kaya and Gaugler, 1993; Yu and Park, 2000).

The present study investigated the efficacies of *Steinernema glaseri* and *Heterorhabditis bacteriophora* against different larval stages of *Spodoptera litura* (F.)

Materials and methods

Heterorhabditis bacteriophora and *Steinernema glaseri* were obtained from Reading University, UK. These

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were reconfirmed on the basis of associated bacterium and symptoms produced by the bacteria in the cadaver of the insect. The EPN were cultured and multiplied on larvae of *Galleria* spp. (Wiesner, 1993). *In vivo* production of entomopathogenic nematodes was conducted by the methods described by Poinar (1979) and summarized by Woodring and Kaya (1988). Insect larvae infested with EPN (1000 IJ/ml) were kept at 15°C for further experiments.

Larvae of *Spodoptera litura* (F.) (Lepidoptera: Noctuidae) were collected from a Department of Entomology field at the University of Agriculture Faisalabad and reared on artificial diet. Larvae were regularly fed on the prepared diets and the adult stages were fed with sucrose solution (10%) for egg laying at room temperature 25° C±5. The diet consisted of chickpea powder (200g), yeast powder (30g), ascorbic acid (3.5g), methyl-p-hydroxybenzoate (2g), sorbic acid 171 (1g), formaldehyde solution (2.5ml), agar (14g) and 500ml distilled water. The entire quantity of agar was suspended in the water and brought to a boil. Gram flour was added to the boiled agar. Then, all remaining ingredients were added to the mixture. The prepared diets were then poured into the desired number of sterilized plastic boxes ($3ft^3$), allowed to cool, and harden (Shorey and Hale, 1965).

Table I.- Effect of EPN on different larval instars of *Spodoptera litura.*

EPN	Larval instars	Mortality (%)			
		After 1st day	After 2nd day	After 3rd day	After 4th day
H. bacteriophora	2nd	52.80a	93.20a	100.00a	100.00a
	3rd	6.60b	59.40bc	86.40ab	100.00a
	4th	6.60b	59.40bc	72.80ab	100.00a
	5th	6.60b	86.60ab	93.20b	100.00a
S. glaseri	2nd	19.80b	52.80c	100.00a	100.00a
	3rd	6.60b	46.20c	93.20a	100.00a
	4th	6.60b	39.60c	86.40ab	100.00a
	5th	13.20b	52.80c	97.80b	100.00a
Control	2nd	0.00c	0.00d	0.00c	0.00b
	3rd	0.00c	0.00d	0.00c	0.00b
	4th	0.00c	0.00d	0.00c	0.00b
	5th	0.00c	0.00d	0.00c	0.00b
LSD	-	28.198	27.575	19.796	-

*Means followed by the same letter are not significant from each other at *P = 0.01* according to least significant difference test.

The mortality of *Heterorhabditis bacteriophora* and *S. glaseri* were evaluated against different larval instars (2nd, 3rd, 4th and 5th). Filter paper was placed in petri plates and three larvae were placed on filter paper. EPNs (1000IJ/ml) were sprayed on these larvae. A water control was also used in which simple water were applied on larvae. The petri plates were covered with their lid. These petri plates were placed on the laboratory bench and held at room temperature. Each treatment was replicated five times with three larvae in each petri plate. Petri plates were observed daily for 4 days to record the mortality of *Spodoptera litura* (F.) larvae. Data were recorded on mortality and subjected to statistical analysis.

EPN multiplication was recorded by using white trap method. In this method a small petri plate (20×15mm) was placed in a large cup in inverted position. A filter paper was placed on small petri plate and dead larvae were placed on this filter paper. A small amount of water was poured at the bottom of the large cup that the filter paper touched the water surface. EPNs emerged from dead larvae come into the water through filter paper. These EPNs were collected in a beaker upto 8 days until the emergence of last EPN and observed under light microscope.

Results

Spodoptera litura (F.) morality to *H. bacteriophora* and *S. glaseri* differed among instar stages. Table I shows that after Ist day, maximum mortality was observed in 2nd larval instar which was 52.80 % in case of *H. bacteriophora* and 19.80 % in case of *S. glaseri* as compared to control (0%). In case of 3rd and 4th larval instar of both species, mortality was non-significant between them (6.60%). Fifth instar of *Spodoptera litura* (F.), *S. glaseri* showed 13.20 % mortality and *H. bacteriophora* showed 6.6% mortality. After 2nd day mortality increased as time increased in each larval instar. *H. bacteriophora* showed 93.20 %, 59.40 %, 59.40 % and 86.60 % mortality against 2nd, 3rd, 4th and 5th larval instar of *Spodoptera litura* (F.). *S. glaseri* gave 52.80 %, 46.20 %, 39.60 % and 52.80 % against 2nd, 3rd, 4th and 5th larval instar of *Spodoptera litura* (F.). After 3rd day *H. bacteriophora* and *S. glaseri* produced 100 % mortality in the 2nd larval instar. In 3rd, 4th and 5th larval instar, *H. bacteriophora* showed 86.40 %, 72.80 % and 93.20 % mortality. *S. glaseri* showed 93.20 %, 86.40 % and 97.80 % mortality in 3rd, 4th and 5th larval instar. *H. bacteriophora* and *S. glaseri* showed 100 % mortality in all larval instars of *Spodoptera litura* (F.) after 4 days exposure time.

EPN multiplication in larvae of *Spodoptera litura* (F.) was examined by comparing the number of nematodes in each larval instar and number of nematode counted upto 7 days. More number of nematodes was observed in later instars as compared to earlier instars (Table II).

Table II.- Reproductive potential of entomopathogenic nematodes in different larval instars of *Spodoptera litura* (F.).

EPN	Larval instars	Multiplication
H. bacteriophora	2nd	9652 d
	3rd	12642.8 c
	4th	17500.8 b
	5th	25786.8 a
S. glaseri	2nd	458.4 g
	3rd	1291.2 f
	4th	1765.6 e
	5th	1944.2 e
Control	2nd	0.00 h
	3rd	0.00 h
	4th	0.00 h
	5th	0.00 h
LSD	-	455.03

*Means followed by the same letter are not significant from each other at *P = 0.01* according to least significant difference test.

Discussion

EPNs were evaluated against different larval instars of *Spodoptera litura*. Maximum mortality was recorded in 2nd and 5th larval instar. Because 2nd larval instra was sensitive (small in size) therefore highest mortality was recorded. In case of 5th larval instar, it was mature and maximum number of IJs invaded in its body and caused infection. Similar results were observed in a study of Park *et al*. (2001). Different species of *Steinernema* and *Heterorhabditis* were evaluated againt *S. litura* (F.). After 20 h *H. bacteriophora* caused 100% mortality against 2nd larval instar. Highest number of nematodes was harvested in 5th larval instar. Results were in confirmity with Park *et al*. (2001), maximum nematodes were harvested from 5-6th instar of *S. litura* (F.) by *H. bacteriophora*. Number of EPN depend upon size of larvae and ability of entomopathogenic nematodes species to multiply. King (1994) recorded that most susceptible larval instar was 2nd instar of *H. armigera* later instars decreasing in time to nematode infection. Salem *et al*. (2007) also recorded that the 200 IJs/larva of *Heterorhabditis* spp. against *S. litura* and *Plutella xylostella* caused 50% mortality of second instar. Sanker (2009) reported that after 24 h, 200 IJs of *H. indica* per larva showed significantly less time to cause 100% mortality against final instar of *Cnaphalocrosis medinalis* than at lower dose of 100 and 50 IJs per larva. Kaya and Hara (1981) found that the rate of nematodes infection against target host differs between life stages and species of host.

Conclusion

It was concluded from present study that entomopathogenic nematodes are successful biocontrol agents against controlling army worm.

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Statement of conflict of interest

Authors have declared no conflict of interest.

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