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# Potential of Entomopathogenic Nematode (*Steinernema kraussei)* against Last Instar Larvae of Different Lepidopteran Insect Pests

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**BK performed the experiments and wrote the manuscript. NJ, SAK, NAR and MA supervised the project. AR, AM, AJ and MAA helped in writing the manuscript, analysed results.**

**Key words**
***Galleria mellonella*, Infective juveniles, Fruit borer, Armyworm, Insect larvae**

## ABSTRACT

Entomopathogenic nematodes (EPNs) are considered as very effective biological agents against several soil dwelling pests. The following research demonstrates the reproductive potential of EPN specie *Steinernema kraussei* against last instar larvae of four lepidopteran insects, wax moth (*Galleria mellonella*), pink bollworm (*Pectinophora gossypiella*), eggplant fruit borer (*Leucinodes orbonalis*) and armyworm (*Spodoptera litura*) at 27±2°C under laboratory conditions. The results indicated that the larvae of *G. mellonella* and *S. litura* were better host as compared to *P. gossypiella* and *L. orbonalis* for multiplication of infective juveniles (IJs) of the *S. kraussei.* Four different concentrations (50, 100, 200 and 500 IJs) of tested EPN specie were used against insect's larvae. Reproduction rate of *S. kraussei* was highest at concentration of 500 IJs as compared to 50, 100 and 200 IJs. Similarly, the effect of different temperatures was also studied to evaluate the efficacy of *S. kraussei* on insect larvae. At 25°C, *S. kraussei* showed significantly higher larval mortality of insect larvae followed by 15°C and 10°C. Effect of storage time of EPN culture was studied on insect's larvae and results showed that 2-week-old culture was more efficient as compared to 4- and 6-week-old cultures in reproduction. The larvae weight of 0.5 g was best option in comparison to 0.25 g for the reproduction of *S. kraussei*. The results conclude that *S. kraussei* is a potential biological control candidate to suppress the larval populations of a number of Lepidopteran insects in the soil.

## INTRODUCTION

Insects are most important and wide group with diversity prevailing in the world. Insects are classified in different groups. Among these groups, lepidopteran insects are one of the most widely distributed insect pests in the world. In this order, about 180,000 species are described with 126 families and 46 superfamilies (Heppner, 2008; Jim, 2011). Several lepidopteran species are major destructive pests in agriculture. The larvae of the Noctuidae genus *Spodoptera* (armyworms), Gelechiidae genus *Pectinophora* (pink bollworms) and Crambidae genus *Leucinodes* (eggplant fruit borers) can cause extensive damage to a variety of crop plants (Scoble and Donahue, 1995). Similarly, the greater wax moth (*Galleria mellonella* L.) is a most important pest of beekeeping industry (Anwar *et al*., 2014) but valued mainly for its leading role as an invented host due to the susceptibility to several biological control agents (Hendrichs *et al*., 2009) and for reproduction of various bio-control agents (Kulkarni *et al*., 2012) including EPNs (Hussaini *et al*., 2010). In many lepidopteran species, the female may produce eggs from 200 to 600, while in some species, it may go as high as 30,000 eggs per day and create substantial problems for agricultural crops (Denlinger, 2009). This necessitates the development of management strategies to control these insect pests through biological means as chemical way of control is costly and hazardous to health and environment.

Entomopathogenic nematodes, mainly from genera *Steinernema* and *Heterorhabditis*, are obligate parasite of different insect pests (Poinar, 1979). EPNs frequently have an obvious ability to find and kill their insect hosts rapidly. EPNs show some specific characteristics including high reproductive potential, virulence and protection for non-target organisms (Kaya, 1985; Ehlers and Peters, 1995). The potential of EPNs as biocontrol agents and their biology and behavior has been studied extensively (Kaya, 1985;

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Ehlers and Shapiro-Ilan, 2005; Chaudhary *et al*., 2017; Rahoo *et al*., 2018). Steinernematids and Heterorhabditids have been described to infect many species of insect pests from several orders (Poinar, 1975). In soil, infective juveniles (IJs) can find larval hosts with variable degrees of efficacy. The pathogenicity of EPNs depends on the toxins produced by specific bacterial species (*Xenorhabdus* and *Photorhabdus*) (Boemare *et al*., 1993). Once the nematodes have entered into the host, they release bacteria, and multiplication of bacteria inside the host body produces proteolytic enzymes that kill the insect host within 24-48 h (Akhurst, 1980). Host insect provides shelter and nematodes inhabit there for 2 or 3 generations until food is completely depleted, then nematodes move into the soil in search of a new insect host (Grewal and Georgis, 1999).

The enduring effect of EPNs treatment was described to be higher than that of typical chemical pesticide (Bari and Kaya, 1984). Control of destructive insect pests through biocontrol is an alternate strategy that helps to provide pesticide free foodstuffs without any environmental risk. Among the different biological control agents, EPNs have significant importance, because they have many positive characteristics of an efficacious bio-control agent. Moreover, EPNs often have broad spectrum effectiveness with short life cycles, easy mass production, salvaging ability and persistence etc. (Gaugler *et al*., 1980; Kaya and Gaugler, 1993). By keeping these facts in mind, the present study was designed with the objective to evaluate the reproductive potential of EPN (*Steinernema kraussei*) on different insect larvae using different concentrations, temperature and storage time.

## MATERIALS AND METHODS

*Nematode culture*

The stock culture of *S. kraussei* Steiner, species was reared on chicken offal solid culture 80 g by using substrate of porous foam, which provides maximum surface volume ratio and enough interstitial space in 500 ml conical flask (Tabassum and Shahina, 2004) in the laboratory of Plant Nematology, Department of Plant Pathology, University of Agriculture, Faisalabad, Pakistan. After 2 weeks of incubation, around 5-7 million IJs were produced in a single flask and stored in distilled water at 20-25°C for 3 to 4 months.

*Collection of insect larvae*

Last instar larvae of four lepidopteran insects, wax moth (*Galleria mellonella* Linnaeus), pink bollworm (*Pectinophora gossypiella* Saunders), eggplant fruit borer (*Leucinodes orbonalis* Guenée) and armyworm (*Spodoptera litura* Fabricius) were collected from infested field of host crop. Armyworm collected from fodder crop berseem and brinjal borer from infected brinjal crop in the month of February, March, and April, 2015 for lab experiment. Pink bollworm larvae were collected from cotton crop in the month of August and September, 2015. Wax moth were collected from infected honey cobs. Larvae were separated according to their size and weight.

*Efficacy of* S. kraussei *against different insect larvae*

EPN suspension was used to evaluate their efficacy against insect larvae of different species. Fresh 500 IJs were inoculated on larvae to check their reproduction potential by using insect baiting technique (Xuejuan and Hominick, 1991). Each treatment was replicated ten times. The experiment was repeated three times on different dates.

*Efficacy of* S. kraussei *at different population levels*

EPN infective juveniles were inoculated on brinjal borer, pink bollworm, wax moth, and armyworms at different concentrations like T1: 50, T2: 100, T3: 200, and T4: 500 infective juveniles. The treatments were replicated 10 times for accuracy of results and after death larvae were transfesrred on white trap to test out the progeny. The emergence of juveniles started after seven days and data were collected after 2, 4, 6, 8 and 10 d after emergence of juveniles by using nematode counting dish method. The experiment was repeated three times on different dates.

*Effect of temperature on larval mortality*

Larval mortality was evaluated on different temperature by using 500 IJs concentration. Plastic cups (6.5 cm in diameter and 6 cm in depth) were used for this experiment. Approximately 145 g autoclaved and air-dried sandy soil (pH 6) was placed into each cup. Six last instar larvae were placed at the bottom of each cup and soil moisture level was adjusted to 10% (w/w) by adding distilled water. Control cups were prepared, and water only was added to these cups. Five hundred of IJs were applied to the cups, which were then placed in incubator at 10, 15, and 25°C. The soil in each cup was poured out after 10 d of nematode treatment and larval mortality was assessed. Each treatment repeated ten times with three biological replicates.

*Efficacy of* S. kraussei *at larval weight*

The weight of larvae was taken after collection of larvae and those larvae having 0.5 g and 0.25 g weight were separated for assessment. Fresh 500 IJs were inoculated to check their reproduction potential on larval weight by using insect baiting technique with ten technical and three biological replicates.

**Table I. Reproductive potential of *S. kraussei* on different larvae.**

| Treatment | Day 2 | Day 4 | Day 6 | Day 8 | Day 10 | Mean |
|---|---|---|---|---|---|---|
| *G. mellonella* | 36844.33$^{g}$ | 70983$^{a}$ | 50055.67$^{d}$ | 32058.33$^{i}$ | 19933.33$^{n}$ | 41974.93$^{A}$ |
| *P. gossypiella* | 24840$^{l}$ | 50725.67$^{c}$ | 34047.33$^{h}$ | 18066.33$^{o}$ | 7929$^{r}$ | 27121.67$^{C}$ |
| *L. orbonalis* | 21011.33$^{m}$ | 45624$^{e}$ | 26842.33$^{k}$ | 13875.67$^{p}$ | 6110$^{s}$ | 22692.67$^{D}$ |
| *S. litura* | 29006.33$^{j}$ | 58968$^{b}$ | 39742$^{f}$ | 24262$^{l}$ | 11695.67$^{q}$ | 32734.80$^{B}$ |
| Mean | 27925.50$^{C}$ | 56575.17$^{A}$ | 37671.83$^{B}$ | 22065.58$^{D}$ | 11417$^{E}$ | |

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. Different superscript letters indicate significant differences within treatments and references.

**Table II. Reproduction of *S. kruassei* at different concentrations.**

| Treatment | *G. mellonella* | *P. gossypiella* | *L. orbonalis* | *S. litura* | Mean |
|---|---|---|---|---|---|
| 50 IJs | 94831.32$^{f}$ | 44162.7$^{i}$ | 37389.33$^{i}$ | 84896$^{gh}$ | 2474.92$^{D}$ |
| 100 IJs | 122183.66$^{d}$ | 66552.33$^{h}$ | 57262.33$^{i}$ | 108118.66$^{e}$ | 4117$^{C}$ |
| 200 IJs | 168784.33$^{c}$ | 92010$^{fg}$ | 79495.03$^{h}$ | 158525$^{d}$ | 5273.42$^{B}$ |
| 500 IJs | 279241.03$^{a}$ | 163154.66$^{c}$ | 139445.67$^{e}$ | 254447.37$^{b}$ | 11059.17$^{A}$ |
| Mean | 9066.92$^{A}$ | 3952.83$^{C}$ | 2787.17$^{D}$ | 7117.58$^{B}$ | |

Different superscript letters indicate significant differences within treatments and references.

*Effect of storage time on reproductive potential of* S. kraussei

Juvenile's suspension was stored for 2, 4, and 6 weeks in incubator at 9°C to evaluate the effect of storage time on reproduction rate of *S. kraussei*. The juveniles of 2, 4 and 6-week-old were inoculated on different larvae to evaluate their reproduction potential. The whole procedure was replicated ten times with three experiments on different dates.

*Statistical analysis*

The results were analyzed by using completely randomized design under factorial arrangement for lab experiments (Steel *et al*., 1997). Least significance difference test (LSD) at 95% level of confidence ($P \leq 0.05$) was applied for mean comparison.

## RESULTS

*Potential of* S. kraussei *against larvae of different insect species*

The virulence of EPN specie against last instar larvae of different insect species was evaluated in plate assay. The results revealed that all larvae of insects were susceptible to EPN, but wax moth and armyworm were highly susceptible. The highest reproductive potential of *S. kraussei* was recorded on *G. mellonella* (wax moth) and *S. litura* (armyworm) while lowest rate of EPN was observed on *P. gossipyilla* (pink bollworm) and *L. orbinalis* (brinjal borer) (Table I). However, statistical differences were detected among the time intervals (2, 4, 6, 8, and 10 d) after the emergence of nematodes ($P \leq 0.05$). It was observed that the maximum progeny of EPN was harvested at 4th and 6th days as compared to 3rd and 8th and 10th days. Results indicated that wax moth and armyworm are favorable hosts for EPN reproduction.

*Population levels of IJs influenced reproduction rate of EPNs on insect larvae*

The maximum reproduction rate of EPN was recorded at 500 IJs host$^{-1}$ while minimum was shown at 50 IJs (Table II). Test revealed significant differences among the IJs concentrations used against different host larvae ($P \leq 0.05$). EPN population decreased as concentration of IJs decreased and vice versa. The maximum juveniles were counted in wax moth and armyworm larvae while lowest amount of nematodes was detected in pink bollworm and brinjal borer larvae at different concentrations 50, 100, 200 and 500 IJs/host after emergence of nematodes.

*Effect of different temperatures on larval mortality*

Temperature had a significant effect on the larval mortality of different insects. Virulence of *S. kraussei* was correlated with rising temperature (Table III). At 25°C, *S. kraussei* showed significantly higher larval mortality followed by 15°C and 10°C. However, no significant

difference was observed between *G. mellonella* and *S. litura* larvae. Significant difference was observed between (*G. mellonella*, *S. litura*) and (*P. gossipyilla*, *L. orbinalis*) larvae ($P \leq 0.05$). Moreover, maximum larval mortality was observed in *G. mellonella* and *S. litura* as compared to *P. gossipyilla* and *L. orbinalis* larvae.

**Table III. Effect of temperature on larval mortality.**

| Treatments | 10 °C | 15 °C | 25 °C | Mean |
|---|---|---|---|---|
| *G. mellonella* | $50.67^{ef}$ | $72.21^{c}$ | $100^{a}$ | $74.29^{A}$ |
| *P. gossypiella* | $38.33^{g}$ | $55.67^{e}$ | $88.42^{b}$ | $60.80^{C}$ |
| *L. orbonalis* | $27.77^{h}$ | $50^{ef}$ | $77.69^{c}$ | $51.82^{D}$ |
| *S. litura* | $44.47^{f}$ | $67.37^{d}$ | $100^{a}$ | $70.61^{B}$ |
| Control | $0^{i}$ | $0^{i}$ | $0^{i}$ | $0.00^{E}$ |
| Mean | $32.24^{C}$ | $49.05^{B}$ | $73.22^{A}$ | |

Different superscript letters indicate significant differences within treatments.

*Reproduction rate of EPN on insect larvae with different weights*

The highest progeny of EPN was recorded at 0.5 g host$^{-1}$ larvae weight and lower progeny was at 0.25 g host$^{-1}$ larvae weight (Fig. 1). However, statistically significant difference was observed between the two values (0.25 g and 0.5 g) used for larval weight ($P \leq 0.05$). EPN population was decreased as larval weight decreased from 0.5 to 0.25 g and vice versa.

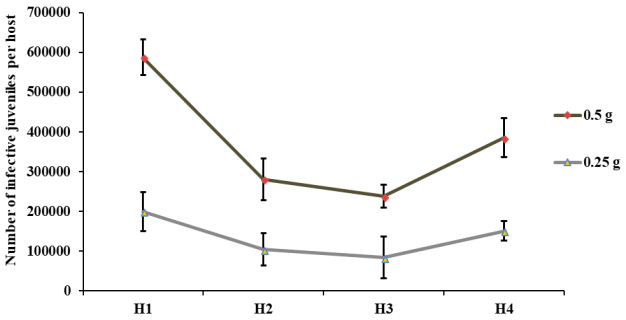


Fig. 1. Impact of larvae weight on *S. kraussei* reproduction. 0.25 gm and 0.5 gm = two different larvae weight in grams.

*Efficacy of EPN cultures with different storage times on insect larvae*

The result of EPN culture age test indicated that there were significant differences among the culture age (2, 4, and 6 weeks) used against insect larvae ($P \leq 0.05$). The highest progeny of EPN was recorded for 2-week-old culture as compared to 4- and 6-week old culture (Fig. 2). It means that 2-week old culture is more active and viable and reproduced maximum population of *S. kraussei* when fed on different insect hosts. The rate of reproduction of nematodes decreased as culture storage time increased.

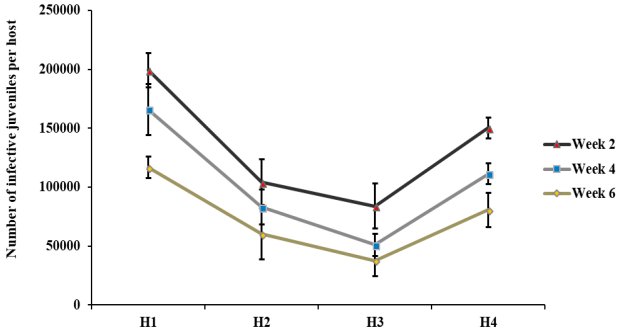


Fig. 2. Effect of storage time on reproductive potential of *S. kraussei*. 2 week: two-week old culture of nematode, 4 week: four-week old culture of nematode and 6 week: six-week old culture of nematode.

## DISCUSSION

Our findings provide an insight into efficacy of EPN specie against larvae of *G. mellonella*, *P. gossipyilla*, *L. orbinalis*, and *S. litura*. The laboratory investigation showed the highest virulence of *S. kraussei* against *G. mellonella* and *S. litura* larvae. However, both larvae were highly susceptible and the best hosts for *S. kraussei* that reproduced maximum number of IJs as compared to *P. gossipyilla* and *L. orbinalis*. The virulence of EPN is closely related to several factors, such as choice of host, penetration and multiplication (Kaya and Gaugler, 1993). In the current plate assay, EPN and insect larvae were very close to each other and the *S. kraussei* did not need to cover a great distance. Differences in reproduction potentials of EPN specie might be described by differences in their capability to penetrate the insect's larvae. Greater efficacy of *S. kraussei* against *G. mellonella* and *S. litura* could be correlated to the most preferred host finding behavior of this EPN specie. This output supports the statement of Lewis *et al.* (1992, 1993) that some EPN species respond less toward some insect larvae due to some volatile compounds produced by insects. Several studies have shown differences in $CO_2$ production by various insects which is used as chemical indicator for chemotaxis of nematodes towards their host (Gaugler *et al.*, 1991; Ramos-Rodríguez *et al.*, 2007; Ali *et al.*, 2017).

To be effective as an insect pathogen, the EPN species must have to penetrate and reproduce inside their host. Caroli *et al.* (1996) reported that penetration rates are different among different EPN species and primarily influenced by host specie. In the present work, *S. kraussei*

entered and reproduced in the last instar of all tested host larvae and IJs emerged from the host bodies. However, reproductive potential of *S. kraussei* was significantly greater for *G. mellonella* and *S. litura* insect's larvae and both could be considered as the most suitable hosts for reproduction of tested EPN. Similarly, two EPN species (*S. carpocapsae* and *H. downesi*) reproduced successfully in *Rhagium bifasciatum* (longhorn beetles) larvae, but the reproduction potential of *S. carpocapsae* was 50% more than that of *H. bacteriophora* (Harvey *et al*., 2012). The potential of insect larvae to help reproduction of EPNs progenies is an essential criterion for a promising biocontrol agent.

EPNs are efficient control agents of lepidopteran larvae and several reports in different studies confirmed this statement (Glazer and Navon, 1989; Navon *et al*., 2002; Shahina *et al*., 2014). Moreover, the invading EPN population into the insect larvae also affects the entomophagous efficacy of a particular EPN specie. Our findings revealed that 500 IJs gave significant highest progeny as compared to 50, 100 and 200 IJs host$^{-1}$. The progeny of *S. kraussei* depends on inoculum concentrations which directly affect the reproduction. The number of IJs used as an inoculum is the key factor of the final progeny of nematodes (Gouge *et al*., 1997).

It was determined that the infectivity of EPN against different insect larvae was correlated with temperature. We observed that the virulence of *S. kraussei* increased with increase in temperature (25°C), whereas deceased with decreasing temperatures (10 and 15°C). Similarly, the weight of larvae significantly affects the reproductive potential of EPN. Reproductive potential decreased with decreasing larva weight. Results indicate that *S. kraussei* reproduced more on 0.5 g as compared to 0.25 g larvae weight. The higher rate of reproduction might depend on the body size and weight of the larvae. Our results also endorse the previous reports that the weight of larvae provides mass for nematode reproduction and increase in weight means increase in progeny (Pervez *et al*., 2007; Pervez and Ali, 2009). Loya and Hower (2003) also proposed that host size and behavior of EPN species might be a cause for differences in reproduction of *Heterorhabditis bacteriophora* in different life stages of *Sitona hispidulus*.

Age of stored EPN IJs also affect the reproductive efficacy of EPNs. Our results confirmed that *S. kraussei* reproduction potential depends on storage time of IJs used for inoculation. Two-week old culture was more efficient than 4- and 6-week old cultures when tested against different insect larvae. Results confirmed that at all factors weight, age of culture and number of IJs applied on all tested insect larvae showed same effect as on wax moth and army worm larvae. Overall, this EPN specie has potential to be used against all tested insect larvae as a management tool but only two insect larvae (wax moth and armyworm) showed high susceptibility and are most appropriate host option for the reproduction of the tested EPN.

## CONCLUSION

Our findings confirmed that *G. mellonella* and *S. litura* are the appropriate option for the reproduction of *S. kraussei*. All factors, including concentration, temperature, larval weight and age of nematode culture clearly affect the reproductive potential of tested EPN specie. In future, our outcomes will be supportive for the researchers to choose an appropriate host for the reproduction of *S. kraussei*.

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*Statement of conflict of interest*

The authors declare there is no conflict of interest.

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