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Comparing the Productivity of Five Entomopathogenic Nematodes in *Galleria mellonella*

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Authors' Contribution
AMR and TM designed the study, executed experimental work and analysed the data. SIA, BAB and RKR assisted in writing the manuscript.

Key words
Steinernematid, Heterorhabditid, Emergence, Greater wax moth, Infective juveniles.

ABSTRACT

The suitability of entomopathogenic nematodes as biological control agents of specific target insects is affected by their level of infectivity and reproductive capacity. Therefore, in the present study the productivity of five entomopathogenic nematodes (*Steinernema feltiae, S. kraussei, S. carpocapsae, Heterorhabditis bacteriophora* and *H. indica*) were compared in *Galleria mellonella* larvae. The production of infective juveniles (IJ) in *G. mellonella* was significantly affected by nematode species. Significantly higher numbers of IJ were produced by Heterorhabditid species than Steinernematid species in the cadaver. The production of IJ was the maximum in the case of *H. bacteriophora* which was not statistically different form *H. indica*. Minimum IJ were produced by *S. feltiae*. The IJ produced by *S. kraussei* and *S. carpocapsae* were statistically similar. The emergence of Steinernematids started from the 14th day and that of Heterorhabditids from the 17th day. In case of Heterorhabditids, the maximum emergence of *H. bacteriophora* IJ (199,894) was recorded on the 23rd day and that of *H. indica* on the 20th day (99,495). On the other hand, in case of Steinernematids, the maximum emergence of IJ of *S. feltiae* and *S. kraussei* was recorded on the 17th day (36,180 and 45,225 respectively) and that of *S. carpocapsae* on the 20th day (21,407). It is concluded that there was greater emergence of IJ from the Heterorhabditid species than those from the Steinernematid species and hence can be used for the management of insect pests.

INTRODUCTION

The biological control potential of entomopathogenic nematodes (EPN) has now become well established because the ability to mass produce them has allowed the development techniques for their inundative application (Griffin *et al*., 2005). Production of EPN on large scale involving techniques based on fermentation technology is an industrial process (Gaugler and Han, 2002; Ehlers and Shapiro-Ilan, 2005a). In developing countries like Pakistan, such technologies are not yet available and *in vivo* mass production of EPN is done in host insects (Ehlers and Shapiro-Ilan, 2005b). These techniques are laborious and are only feasible where labour costs are low. In Pakistan, preliminary field evaluation of EPN is done with *in vivo* produced nematodes in hosts like *Galleria mellonella* (Rahoo *et al*., 2011, 2017a). As biological control becomes more prevalent in pest management, it will become increasingly important to anticipate interactions between biological control agents (Kaya, 1990; Kaya *et al*., 1995; Rosenheim *et al*., 1995).

Features of EPN that affect their suitability as biological control agents of specific target insects are their level of infectivity and reproductive capacity. Infectivity refers to the ability of nematodes to cause infection in a target insect (Tanda and Fuxa, 1989) and has been shown to vary among nematodes within specific target hosts (Bedding *et al*., 1983; Molyneux *et al*., 1983; Morris *et al*., 1990; Mannion, 1992) and among hosts for a given nematode species or strain (Bedding *et al*., 1983; Morris *et al*., 1990). The reproductive capacity of nematodes has also been shown to differ among nematodes within target

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insects (Morris *et al*., 1990; Mannion and Jansson, 1992) and among hosts within specific nematode species or strains (Morris *et al*., 1990). Nematodes with higher levels of infectivity and reproduction within a specific target host may be more effective in controlling a particular insect under field conditions. The reproductive capacity is also central to long-term persistence. Morris *et al*. (1990) noted that a high infection rate of soil insects followed by a high rate of reproduction is critical to ensure re-infestation of the habitat by nematode progeny.

There can be differences in the production of infective juveniles (IJ) among different nematode genera. Mannion (1992) found that *Heterorhabditis* spp. had the lowest LC_{50} and LC_{90} values, produced more progeny per cadaver, had higher levels of infectivity in sand, soil and Petri plates, killed more hosts within sweet potato storage roots and had a greater ability to exit infected weevil cadavers within storage roots and infect new hosts in the soil than *Steinernema* spp. Jansson *et al*. (1990, 1991, 1993) found *Heterorhabditis* spp. to be more efficacious against sweet potato weevil *Cylas formicarius*. Jansson *et al*. (1993) also found that Heterorhabditids persisted longer than Steinernematids in the field. It was hypothesized that the production of IJ from *G. mellonella* cadavers would not differ among different species of EPN. Therefore, the objective of conducting the present study was to compare the productivity of five entomopathogenic nematodes (*Steinernema carpocapsae, S. feltiae, S. kraussei, Heterorhabditis bacteriophora* and *H. indica*) in *Galleria mellonella* larvae.

MATERIALS AND METHODS

Nematode cultures

Entomopathogenic nematodes (*Steinernema carpocapsae, S. feltiae, S. kraussei, Heterorhabditis bacteriophora* and *H. indica*) used in the study were obtained from stock cultures supplied by CABI Bioscience and were maintained in the laboratory at the Department of Agriculture, University of Reading, United Kingdom. The nematodes were cultured in the last instar larvae of greater wax moth, *Galleria mellonella* (Lepidoptera: Pyralidae) (Livefoods Direct Ltd. Sheffield, UK) at 25°C. Ten *G. mellonella* larvae were placed on each 9 cm Petri dishes lined with a Whatman® No. 1 filter paper. The larvae in dishes were individually inoculated with approximately 2000 infective juveniles (IJ) of abovementioned five EPN contained in 1 ml of tap water. The Petri dishes were sealed with Nescofilm® sealing film (Azwell Inc., Osaka, Japan) and placed in an incubator at 20°C (Dutky *et al*., 1964).

After incubation at 20°C for 10 days, the infected *G. mellonella* larvae were taken from the Petri dishes and placed on modified white traps (White, 1927). After some days, nematodes moved from the *G. mellonella* cadavers to the water. Water containing the IJ was transferred to a clean beaker filled with fresh tap water and the IJ were allowed to settle for 30 min. The supernatant was decanted, the beaker was refilled with fresh tap water and the process was repeated three times until a clean suspension was obtained. Excess water was discarded and nematodes were kept at 10°C and used within two weeks (Kaya and Stock, 1997). IJ of the nematode species were acclimatized at room temperature (21-23°C) for an hour and their viability was tested under a stereomicroscope before use.

Productivity of five EPN species in G. mellonella *larvae*

Fifty late instar larvae of *G. mellonella* weighing between 0.25-0.35 g were selected and individual weights recorded. Each larva was inoculated with 0.15 ml of suspension of *S. carpocapsae, S. feltiae, S. kraussei, H. bacteriophora* and *H. indica* containing a mean of 50, 67, 73, 55 and 47 IJ, respectively. This was done in 30 mm Petri dishes as previously described. The dishes were stored in an incubator at 20ºC for four days in which time all larvae succumbed to nematode infection. Fifty 30 mm Petri dishes containing 5 g of dry silver sand were prepared to which 1 ml of tap water was added. An infected larva (cadaver) killed by one of the above mentioned species was added to each dish which was sealed and then kept in an incubator at 20ºC. To facilitate counting, the nematodes were divided into two groups (Steinernematids and Heterorhabditids) and were evaluated on different days. One week after inoculation each cadaver was moved on the supporting Netlon and transferred to new Petri dish containing 5 g silver sand plus 1 ml water. The Petri dishes were then re-sealed and returned to the incubator. The sand from the original dish was moved to a modified miniature Baermann extraction tray made from a 50 mm Petri dish, to recover any nematodes that may have emerged from the cadavers. This procedure was repeated after every three days until no more nematodes were recovered. Each Petri dish was monitored daily to observe when nematodes first emerged from cadavers.

Statistical analysis

All the data were found normally distributed and did not require transformation. The data were subjected to Analysis of Variance (ANOVA) using GenStat package 2009, (12^{th} edition) version 12.1.0.3278 (www.vsni.co.uk). The differences among means were compared by Fisher's protected least significant difference test at ($P \leq 0.05$). Standard errors of means were calculated in Microsoft Excel 2007.

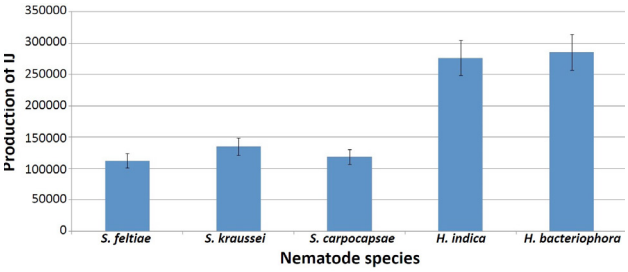

Fig. 1. Production of infective juveniles of five EPN in *G. mellonella.*

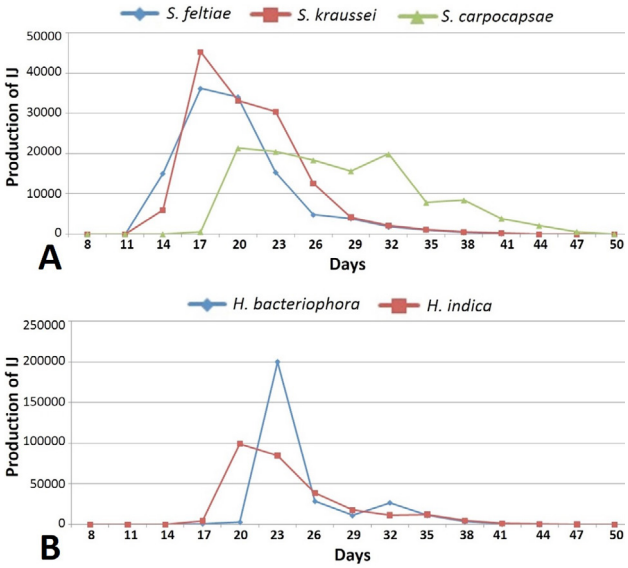

Fig. 2. Days-wise production of infective juveniles of three Steinernematids (A) and two Heterorhabditids (B) in *G. mellonella.*

RESULTS

Productivity of EPN species in G. mellonella *larvae*

The production of IJ in *G. mellonella* was significantly affected by nematode species. Significantly higher numbers of IJ were produced by Heterorhabditid species than Steinernematid species in the cadaver. The production of IJ was the maximum in the case of *H. bacteriophora* which was not statistically different form *H. indica*. The minimum IJ were produced by *S. feltiae*. The IJ produced by *S. kraussei* and *S. carpocapsae* were statistically similar (Fig. 1).

The emergence of Steinernematids started from the 14th day and those of Heterorhabditids from the 17th day. In case of Heterorhabditids, the maximum emergence of *H. bacteriophora* IJ (199,894) was recorded on the 23rd day and that of *H. indica* on the 20th day (99,495). On the other hand, in case of Steinernematids, the maximum emergence of IJ of *S. feltiae* and *S. kraussei* was recorded on the 17th day (36,180 and 45,22, respectively) and that of S. *carpocapsae* on the 20th day (21,407). The number of *S. feltiae* emerging from the larvae was between 14,094 and 35,120 on the 14th and 17th days, respectively whereas the number of *S. kraussei* was 6,167 and 45,200 on the 14th and 17th days, respectively. Similarly, the number of *S. carpocapsae* was 4 on the 14th day and 21,570 on the 20th day (Fig. 2A). On the other hand, *H. bacteriophora* yielded 114 and 203,000 IJ on the 17th and 23rd days, respectively whereas *H. indica* gave 2,300 and 101,536 IJ on the 17th and 20th days, respectively (Fig. 2B).

DISCUSSION

In the present study that compared the productivity of different EPN species in the larvae of *G. mellonella,* greater numbers of *H. bacteriophora* IJ were recovered than other species. The smallest number of IJ was with *S. carpocapsae*. There was a difference in the time of emergence of the IJ from the host cadaver; Steinernematids emerged from cadavers sooner than Heterorhabditids. The reason may be that the size of Steinernematid IJ is greater than those of *Heterorhabditis* spp. The larger size of Steinernematids would occupy more space inside the cadavers and require more nutrient resources and so produce less progeny. It is known that emergence of IJ is related to depletion of food reserves and crowding within the host cadavers (Kaya, 1985, 1987) and possibly build-up of ammonia (San-Blas *et al*., 2008). Patterns in total reproduction of nematodes differed among the five species. *Heterorhabditis* species consistently produced more progeny than the *Steinernema* species. Patterns of emergence from cadavers of *G. mellonella* were consistent. As noted earlier, emergence of infective juveniles is related to depletion of food reserves and crowding (Kaya, 1985, 1987). These factors may have been less apparent to emerging infective juveniles from all species from *G. mellonella* larvae. It is recognized that a laboratory bioassay that predicts performance of EPN in the field is needed to facilitate selection of nematodes in biological control programmes (Hominick, 1990; Mannion, 1992). Mannion (1992) conducted Petri dish, sand, soil and simulated field bioassays to select suitable EPN for biological control of *C. formicarius* and consistently found that Heterorhabditids were superior to Steinernematids in all bioassay systems tested.

Differences between the reproduction potential of EPN may also be related to the isolates, species, and host susceptibility, number of bacteria per infective stage, invasion rate, temperature and humidity (Rahoo *et al*., 2016a, b, 2017b; Nabeel *et al*., 2018). The life cycles

of Steinernematid and Heterorhabditid nematodes are different. The mode of reproduction of the first generation adults is bisexual for *Steinernema* spp. (Kondo and Ishibashi, 1987; Wouts, 1984), while it is hermaphroditic for *Heterorhabditis* spp. which begins sexual reproduction from the second generation (Glazer *et al*., 1994; Zioni *et al*., 1992). In most of the previous studies, attention has been placed mainly on the production and/or pathogenicity of IJ (Dunphy *et al*., 1985; Mracek *et al*., 1988; Selvan *et al*., 1993; Glazer *et al*., 1994). Contrarily, not so much attention has been placed on the origin of juveniles via *endotokia matricida* (intrauterine larval development leading to the destruction of the female by the juveniles) which is generally considered as the failure of normally oviparous nematodes to deposit their eggs which may then accumulate and continue development within the female body. In the comparison between *H. bacteriophora* and *S. feltiae*, the former differed from the latter in the occurrence rate of *endotokia matricida* and the production of IJ. Generally the Heterorhabditids produced more IJ than the Steinernematids. It is concluded from the present study that *Heterorhabditis* species produced more IJ, hence can be used for the management of insect pests and root-knot nematodes (Fateh *et al*., 2017; Hussain *et al*., 2016; Javed *et al*., 2017a, b; Kassi *et al*., 2018; Kayani *et al*., 2017; Khan *et al*., 2017; Mukhtar *et al*., 2017a, b; Tariq-Khan *et al*., 2017).

CONCLUSION

As heterorhabditid species produced greater numbers of infective juveniles in *Galleria mellonella* larvae than steinernematid species and are recommended for use as biological control agents of insect pests in Pakistan.

Statement of conflict of interest

Authors have declared no conflict of interest.

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