Pakistan J. Zool., vol. 51(2), pp 717-724, 2019. **DOI**: http://dx.doi.org/10.17582/journal.pjz/2019.51.2.717.724

Comparison of Infectivity and Productivity of *Steinernema feltiae* and *Heterorhabditis bacteriophora* in *Galleria mellonella* and *Tenebrio molitor*

Ali Murad Rahoo[1,*], Tariq Mukhtar[2], Barkat Ali Bughio[3] and Rehana Kanwal Rahoo[4]

[1]*Wheat Research Institute, Sakrand, Sindh*
[2]*Department of Plant Pathology, Pir Mehr Ali Shah-Arid Agriculture University, Rawalpindi*
[3]*Department of Zoology, University of Sindh, Jamshoro*
[4]*Institute of Plant Sciences, University of Sindh, Jamshoro*

Article Information
Received 06 October 2018
Revised 15 November 2018
Accepted 22 November 2018
Available online 01 March 2019

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

Key words
Entomopathogenic nematodes, Greater wax moth, Yellow mealworm, Infectivity, Productivity.

ABSTRACT

The conditions for deploying entomopathogenic nematodes (EPN) in Pakistan can be harsh and the survival of infective juveniles (IJ) following inundative applications would be quite short. The application of EPN in cadavers may be appropriate because of the non-availability of industrially produced isolates. Therefore, in the present studies, *Galleria mellonella* and *Tenebrio molitor* were compared for invasion and production of IJ of *Steinernema feltiae* and *Heterorhabditis bacteriophora*. Both the nematodes caused 100% mortality of the test insects within 6 days. The mean numbers of IJ of *S. feltiae* invading each *G. mellonella*, *T. molitor* medium and small were significantly greater (11.2, 15.2 and 11.4 IJ, respectively) than those of *H. bacteriophora* (2.8 IJ each per *G. mellonella* and *T. molitor* medium and 3 IJ per *T. molitor* small). Contrarily, there was greater emergence of IJ of *H. bacteriophora* than *S. feltiae* in all the treatments. The mean numbers of *H. bacteriophora* emerging from *G. mellonella* larvae were 272,600 from *T. molitor* medium were 194,600 and in *T. molitor* small were 21,900. Whereas, emergence of the mean numbers of *S. feltiae* IJ emerging from *G. mellonella* were 136,000 from *T. molitor* medium were 51,200 and in *T. molitor* small were 12,940, respectively. *G. mellonella* was found to be more susceptible host than *T. molitor*. Likewise, *S. feltiae* proved to be more aggressive than *H. bacteriophora*. The results of this study showed that greater numbers of EPN could be produced in *G. mellonella* than in *T. molitor.*

INTRODUCTION

Entomopathogenic nematodes (EPN) are now established as potent biological control agents because the ability to mass produce them has allowed the development techniques of inundative application (Griffin *et al*., 2005). The mass production technique based on fermentation technology is an industrial process (Ehlers and Shapiro-Ilan, 2005). Such technologies are not yet available in countries like Pakistan where the use of EPN is in its infancy. In these countries, the development and use of EPN will depend initially on low technology mass production techniques such as use of host insects for *in vivo* production (Ehlers and Shapiro-Ilan 2005; Rahoo *et al*., 2011, 2017, 2018a, b, 2019). These techniques are labour intensive but are feasible where labour costs are low. In Pakistan initial field evaluation of EPN is likely to be done with *in vivo* produced nematodes in hosts such as greater wax moth (*Galleria mellonella*). Since *G. mellonella* may not always be available, therefore, yellow mealworm (*Tenebrio molitor*) could be an alternative host. One of the advantages of production of EPN in *T. molitor* is that it does not produce cocoons and retains structural integrity while infected by nematodes and is being commercially produced on large scale in many countries of the world. Use of *T. molitor* as a host for *in vivo* production of EPN in biological control has been reported by Shapiro-Ilan *et al*. (2002).

EPN are presently used against soil-dwelling insects attacking citrus, cranberries, turf and ornamentals (Georgis, 1990) and has potential for many others (Javed *et al*., 2017a, b; Iftikhar *et al*., 2018; Kassi *et al*., 2018a, b; Nabeel *et al*., 2018; Aslam *et al*., 2019a, b). On the other hand, entomopathogenic nematodes can reduce the incidence and severity of root-knot nematodes (Hussain *et al*., 2016; Kayani and Mukhtar, 2018; Kayani *et al*., 2017, 2018; Khan *et al*., 2017; Mukhtar, 2018; Mukhtar *et al*., 2017a, b, 2018; Tariq-Khan *et al*., 2017). More than a dozen companies are presently producing and selling nematodes in the USA, Australia, Japan, and Europe

* Corresponding author: alirahoo@gmail.com
0030-9923/2019/0002-0717 $ 9.00/0

(Georgis and Manweiler, 1994) but these companies are not yet producing EPN for use in the warmer countries of the tropics and sub-tropics. Despite the increasing commercial and scientific interest in Steinernematids and Heterorhabditids, a universal standard infectivity assay has not been established. The need to evaluate nematode insecticidal activity in the laboratory has resulted in the development of a variety of assays that measure nematode infectivity by recording host mortality.

The number of nematodes that penetrate a host is a measure that has often been used to evaluate infectivity in bioassays. Fan and Hominick (1991) initially showed a significant linear correlation between nematode dose and number of infective juveniles (IJ) invading larvae of *G. mellonella*. Therefore, Hominick and Reid (1990) proposed the use of invasion efficiency (measured as the slope resulting from the linear regression of the number of established nematodes against the dose) as a direct measure of nematode infectivity. The assumption was that the nematode with the greatest efficacy against a target insect would have shown the highest invasion efficiency. However, when invasion efficiency was measured in insect hosts different from *G. mellonella,* the regression coefficients were low and the results were not reproducible (Epsky and Capinera, 1993; Mannion and Jansson, 1993). Hay *et al*. (1993) suggested that a linear model might be adequate to describe all nematode-host combinations.

There are possibilities that Heterorhabditids (such as *Heterorhabditis bacteriophora*) may not behave in a similar way to Steinernematids (such as *Steinernema. feltiae*) in which case it would seem appropriate to evaluate both species on different hosts and at different host densities to establish if such considerations need to be addressed when seeking methods of using EPN in new locations (such as Pakistan). Therefore, the rationale for conducting the following experiments was to test the principle of applying EPN in cadavers.

The first objective of the study was to compare the invasion and mortality of larvae of *G. mellonella* and *T. molitor* by a single dose of 1000 IJ of *S. feltiae* and *H. bacteriophora* and the production of IJ from the cadavers. Additionally, the infection and IJ production was compared between two sizes of *T. molitor*. The second objective was to evaluate whether invasion and mortality of larvae of *G. mellonella* and *T. molitor* by the two EPN is affected by number of hosts when the former were exposed to a dose of 5000 IJ of the latter.

MATERIALS AND METHODS

Nematode cultures

Entomopathogenic nematodes *Steinernema feltiae* and *Heterorhabditis bacteriophora* used in the studies were taken 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 sixth 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 *S. feltiae* and *H. bacteriophora* 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 *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 2 weeks (Kaya and Stock, 1997). IJ of both the nematode species were acclimatized at room temperature (21-23~) for an hour and their viability was tested under a stereomicroscope before use.

Invasion and mortality of larvae of G. mellonella *and* T. molitor *by* S. feltiae *and* H. bacteriophora *and the production of IJ from the cadavers*

Twenty last instar (sixth instar) larvae of *G. mellonella* ranging 0.25-0.35 g in weight and twenty larvae each of *T. molitor* of medium size with weights ranging 0.14-0.20 g and those of small size (< 0.14 g) were taken. Each larva was placed on filter paper in a 30 mm Petri-dish and larvae from each category of host were divided into two groups of ten. Each larva from one group was inoculated with 0.1 mL suspension containing a mean of 1,000 IJ of *S. feltiae* while larvae of other group were inoculated individually with a mean of 1,000 IJ of *H. bacteriophora*. The dishes were sealed and kept in an incubator at 20°C for 24 h. Mortalities of *G. mellonella* and *T. molitor* were recorded after 24 h from each treatment. After 24 h, five insect larvae from each group were shifted to a freezer while the remaining five larvae were transferred to clean filter papers in Petri dishes and 0.1 mL of tap water was added to make the filter papers moist and labelled 1, 2, 3, 4, and 5. The Petri-dishes of each group placed in the freezer were also labelled as 1, 2, 3, 4, and 5 for each treatment.

The mortality of the insects placed in incubator was recorded after 12 h till all the insect larvae were dead. The dead larvae were processed in White traps for nematode recovery (White, 1927). The insect cadavers that died with *S. feltiae* were placed on White traps after 10 days and those by *H. bacteriophora* after 16 days. Two weeks after incubation in the White traps the nematodes were harvested and quantified for both nematodes species. The insect carcasses in the freezer were enzymatically digested in pepsin solution for 2 h and were homogenized and the invading IJ were counted.

Invasion and mortality of larvae of G. mellonella *and* T. molitor *as affected by number of hosts*

Three hundred and twenty late instar larvae of *G. mellonella* and 320 late instar larvae of *T. molitor* were taken and divided into two groups of 160. The larvae of each group were placed on filter papers in 90 mm Petri dishes and arranged in three treatments, which are: T1, one larva per Petri-dish; T2, five larvae per Petri-dish and T3, ten larvae per Petri-dish. There were ten replications for each treatment.

The larvae of first group of *G. mellonella* and *T. mollitor* arranged in three treatments were inoculated with 5000 IJ of *S. feltiae* contained in 1 mL of water while those of second group were inoculated with *H. bacteriophora*. The dishes were sealed with Nescofilm and kept in an incubator at 20°C. The mortality of *G. mellonella* and *T. molitor* was recorded after 24 h and all larvae from each treatment were rinsed with water to remove any nematodes from the surface of the larvae. After rinsing, all Petri-dishes were cleaned and the filter papers changed, 0.1 mL of tap water was added and the same larvae were returned in the same Petri-dishes and labelled properly. The Petri-dishes were placed again in the incubator set at 20°C and mortalities of *G. mellonella* and *T. molitor* were recorded after every 24 h till 72 h. The dead larvae at the time of rinsing and those died on subsequent days were recorded and appropriately labelled before placing in a freezer. The cadavers were removed from the freezer and dissected in pepsin solution and homogenised for 30 s with a laboratory homogeniser.

Statistical analysis

The data for production of *S. feltiae* and *H. bacteriophora* from larvae of *G. mellonella* and T. *molitor* were not found normally distributed and transformed to log 10 while data for all the other parameters were found normally distributed and did not require transformation. All the data were subjected to analysis of variance (ANOVA) using GenStat Package 2009 (12th edition) version 12.1.0.3278 (www.vsni.co.uk). The means were compared by Fisher's Protected Least Significant Difference Test at 5%.

RESULTS

Mortality of larvae of G. mellonella *and* T. Molitor

The entomopathogenic nematode *S. feltiae* caused 100% mortality of the tested insects *G. mellonella* and *T. molitor* within 4 days, whereas *H. bacteriophora* caused 100% mortality within 6 days (Fig. 1).

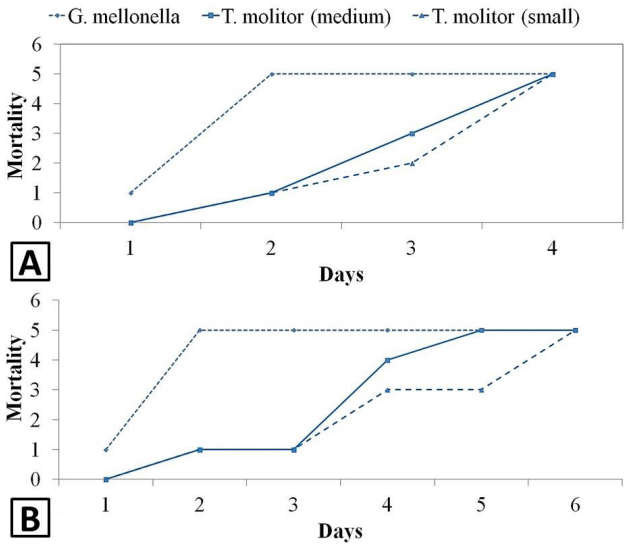

Fig. 1. Mortality of *G. mellonella* and *T. molitor* medium and small inoculated with 1000 IJ of *S. feltiae* (A) and *H. bacteriophora* (B).

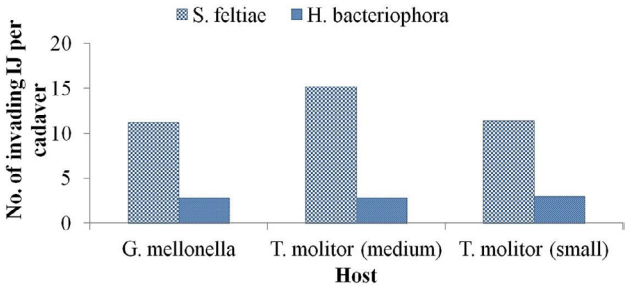

Fig. 2. Numbers of IJ of *S. feltiae* and *H. bacteriophora* invading larvae of *G. mellonella* and *T. molitor* 24 h following inoculation with 1000 IJ.

Invasion of larvae of G. mellonella *and* T. molitor *by nematodes*

The difference in invasion of larvae of *G. mellonella* and *T. molitor* by the two nematode species was highly significant ($P<0.001$). Different nematode ranking was observed for penetration rate in both insect hosts. The

mean number of invading IJ of *S. feltiae* invading per *G. mellonella* was 11.2, *T. molitor* medium was 15.2 and those of small were 11.4. Whereas, in case of *H. bacteriophora* the mean number of IJ that invaded per *G. mellonella* and *T. molitor* medium was 2.8 each and a mean of 3 IJ were found invading *T. molitor* small size larvae (Fig. 2).

Production of IJ from the insect cadavers

Production of IJ in the insect cadavers was also found significant between the nematode species while the interaction between host and species was not significant. There was greater emergence of IJ of *H. bacteriophora* than *S. feltiae* in all the treatments. The mean numbers of *H. bacteriophora* emerging from *G. mellonella* larvae was 272,600, from the *T. molitor* medium size was 194,600 and in small size *T. molitor* was 21,900. Whereas, emergence of the mean numbers of *S. feltiae* IJ emerging from *G. mellonella* was 136,000, from the *T. molitor* medium size was 51,200 and in *T. molitor* small was 12,940, respectively (Fig. 3).

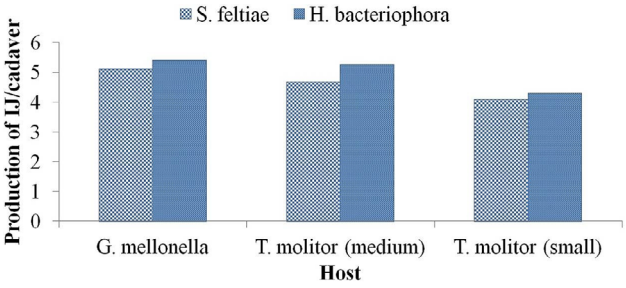

Fig. 3. Production of *S. feltiae* and *H. bacteriophora* from larvae of *G. mellonella* and *T. molitor* following inoculation with 1000 IJs for 24 h (data transformed to log 10).

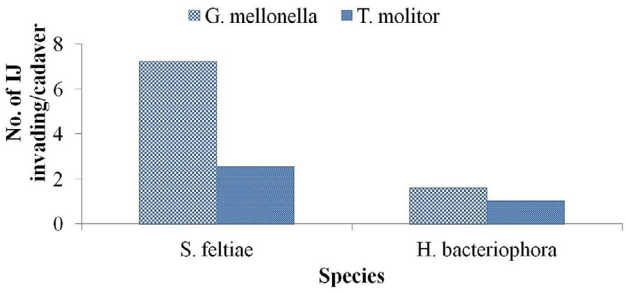

Fig. 4. Invasion of *G. mellonella* and *T. molitor* larvae by IJ of *S. feltiae* and *H. bacteriophora* following exposure to 5000 nematodes over a 24 h period.

Invasion and mortality of G. mellonella *and* T. molitor *larvae as affected by number of hosts*

The effects of host, nematode species and treatments were highly significant (P<0.001). Similarly, the interaction between host and nematode species was also highly significant. *G. mellonella* was found to be more susceptible host than *T. molitor*. Likewise, *S. feltiae* proved to be more aggressive than *H. bacteriophora* (Fig. 4). As regards treatments, maximum average number of IJ penetrated the treatment where single host was exposed to the nematodes followed by the treatment with ten hosts. Minimum number of average IJs penetrated the treatment with five hosts per Petri plate (Fig. 5).

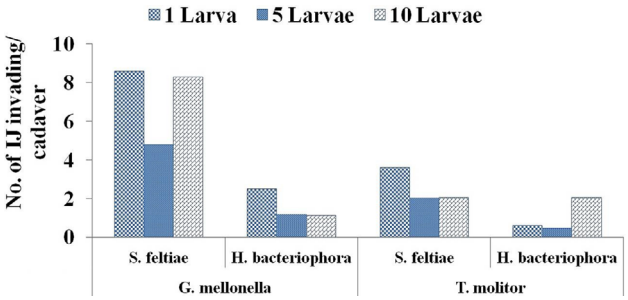

Fig. 5. Individual invasion of *G. mellonella* and *T. molitor* larvae by IJ of *S. feltiae* and *H. bacteriophora* following exposure to 5000 IJ in cohorts of 1, 5 and 10 larvae per dish.

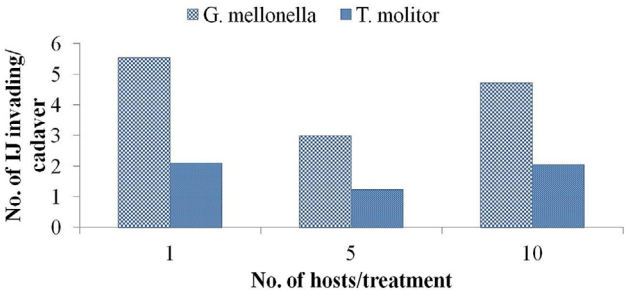

Fig. 6. Mean invasion of *G. mellonella* and *T. molitor* larvae by IJ of *S. feltiae* and *H. bacteriophora* following exposure to 5000 IJ in cohorts of 1, 5 and 10 larvae per dish.

The interaction among host, species and treatments was significant (P<0.005). The invasion of *S. feltiae* on *G. mellonella* was higher than that of *T. molitor* as compared to *H. bacteriophora* particularly in treatment where 1 *G. mellonella* and *T. molitor* were exposed to IJ excepting *T. molitor* with *H. bacteriophora*. The individual average penetration in each treatment in each host with both the nematode species is given in Figure 6.

DISCUSSION

The objective of firs part of the study was to determine the invasion of nematodes into two different hosts and the

numbers of IJ recovered. Additionally, the infection and IJ production was compared between two sizes of *T. molitor*. *S. feltiae* caused 100% mortality of the smaller *T. molitor* larvae within two days as compared to *G. mellonella* and medium sized *T. molitor* larvae. Possibly this was due to the fact that the small larvae of *T. molitor* had less resistance than the *G. mellonella* and medium sized larvae since their mortality was delayed up to 4th day. On the other hand, 100% mortality of *G. mellonella* was achieved by *H. bacteriophora* after two days which suggested high susceptibility to that species. The small larvae of *T. molitor* showed 100% mortality up to the 6th day. The possible reason was that the IJ of *H. bacteriophora* took more time to locate and penetrate the larvae of *T. molitor*. In case of medium sized *T. molitor,* the larvae exhibited 100% mortality after the 5th day due to easy location and penetration of the IJ of *H. bacteriophora.* More *S. feltiae* IJ penetrated into the hosts as compared to *H. bacteriophora*. Perhaps because being larger in size, *S. feltiae* IJ are more active and possess more energy and hence succeed to penetrate into the host. Contrarily, the production of *H. bacteriophora* was comparatively more than *S. feltiae*. This was due to the fact that the life cycle of *H. bacteriophora* is hermaphroditic (Selvan *et al*., 1993; Woodring and Kaya, 1988). The production of *H. bacteriophora* differed among different hosts with *G. mellonella* showing the highest production while in case of small *T. molitor* the production was less (Fig. 3). It was also observed that the reproduction of IJ from *H. bacteriophora* was greater than *S. feltiae* in all the three host larvae (Fig. 3). The reason of more production may be the life cycle of *H. bacteriophora*. According to Poinar, entomopathogenic nematodes can be reared by *in vivo* methods, with yields of 100,000-200,000 infective juveniles per *G. mellonella* larva (Poinar, 1979). According to Woodring and Kaya (1988), up to 350,000 *H. bacteriophora* infective juveniles have been harvested from one last instar *G. mellonella* larva. Average production is much less, in the order of 30,000 to 50,000 IJ per insect. In this experiment approximately similar numbers of IJ were obtained for *H. bacteriophora*; however, such great numbers were not attained for *S. feltiae* IJ (Fig. 3). The body size of the host definitely affects the total number of IJ developing inside the cadavers. The hosts used in the Experiment 1 differed in their weights which significantly affected the production.

The results of second part of study suggested that *T. molitor* may not be a good host for the infection by entomopathogenic nematodes. Mealworms are not natural hosts of entomopathogenic nematodes since they live in very different habitats and it is unlikely that they come in contact in the environment. Mealworms have a hard, smooth cuticle with shallow segments (relative to some soil dwelling insect larvae) which could be a barrier to infection, impeding penetration by both nematode species. Secondly, mealworms are comparatively more active than *G. mellonella* and thus could avoid infection by entomopathogenic nematodes.

The infectivity of *S. feltiae* was better than *H. bacteriophora* in the insect hosts, extremely low penetration rates of *H. bacteriophora* were detected (Fig. 4). Similar low rates were recorded by Grewal *et al*. (1994b) with *G. mellonella* on filter paper in dishes. The lower penetration into *G. mellonella* and *T. molitor* larvae can be partly explained by nematode behaviour. *S. feltiae* invaded both insect hosts with higher numbers than *H. bacteriophora.* The observed levels of penetration are similar to the ones reported by other investigators (Fan and Hominick, 1991; Epsky and Capinera, 1993; Selvan *et al*., 1993; Grewal *et al*., 1994a, b). As a sample of 1000 nematodes was exposed to only one insect for a rather long time, it is possible that, following the invasion of the first nematodes, many avoided penetration into host that had already been infected. The results of first part of the study support the use of penetration rate to compare the infectivity of entomopathogenic nematodes. Further studies are necessary to clarify the relationship between nematode penetration and host mortality. At present, invasion measures are useful tools in infectivity studies.

Invasion of *G. mellonella* was greater than that of *T. molitor* suggesting that *G. mellonella* is more susceptible than *T. molitor* possibly because it has a softer cuticle enabling easier penetration of the body (Fig. 4). Also, the larvae of *G. mellonella* are not as active as those of *T. molitor*, so there is an opportunity for the nematodes to have a longer period of time to gain entry when the larvae are motionless. The invasion by *S. feltiae* was greater than *H. bacteriophora*. This might be due to the relatively larger size of *S. feltiae* which confers an advantage in invading a host. When two species of insects of the same biomass (consisting of different numbers of individuals *i.e.* larvae of *G. mellonella* and *T. molitor*) are available in the same environment, the greatest number of the IJ of *S. feltiae* penetrated the *G. mellonella* larvae as compared to *T. molitor*. In the same experimental conditions, the lowest numbers of IJ of *H. bacteriophora* penetrated *T. molitor* as compared to *G. mellonella*. Infection of a particular insect species by *H. bacteriophora* was the greatest in the case of *G. mellonella* caterpillars, smaller for *T. molitor*.

In the earlier studies, it was found that infection of particular insects by entomopathogenic nematodes is influenced by the host's individual resistance (Bednarek, 1986), the size of the insects and their natural openings (Mracek and Ruzicka, 1990) or the host biomass (Wojcik, 1986). The results show a slight difference in the intensity

of infection of *T. molitor* by both species of nematodes. The intensity of infection of those small *T. molitor* with small natural openings by *S. feltiae*, whose invasive larvae are bigger than *H. bacteriophora*, was lower; on the other hand, the biomass of the hosts of different insect species does not have a crucial importance in the intensity of infection of particular individuals. *H. bacteriophora* infected *G. mellonella* with greater intensity than *T. molitor*. The studies conducted so far point out differentiated susceptibility of particular insect species to infection by entomopathogenic nematodes, butterflies being more susceptible, cockchafers less, while the Diptera, Homopterons and Orthopterans the least (Bedding *et al*., 1983; Dutky, 1959; Laumond *et al*., 1979; Molyneux *et al*., 1983; Morris, 1985; Kreft and Skrypek, 2002), ladybird larvae, earth-worms and snails are resistant to infection by *Steinernema* spp. and *Heterorhabditis* spp. (Capinera *et al*., 1982; Poinar, 1979).

CONCLUSIONS

The conditions for deploying EPN in Pakistan can be harsh and it is likely that the survival of IJ following inundative applications would be quite short. The concept of applying EPN while still in the insect host cadaver has an attraction, particularly as the *in vivo* system of mass production is likely to be the principal means of production at least in the short-term. The application of EPN in cadavers may be appropriate in Pakistan because of the non-availability of industrially produced isolates. The selection of host insect for *in vivo* mass production depends on a number of factors. Ease of production is the one and *G. mellonella* can be readily cultured on artificial diet which has long been used by nematologists for laboratory rearing of EPN species. Size of larvae is also a factor as the *G. mellonella* larvae are relatively large weighing 0.2-0.4g. The results of the present study show that greater numbers of EPN could be produced in *G. mellonella* than *Tenebrio molitor.*

Statement of conflict of interest

Authors have declared no conflict of interest.

REFERENCES

Aslam, M.A., Javed, K., Javed, H., Mukhtar, T. and Bashir, M.S., 2019a. Infestation of *Helicoverpa armigera* Hübner (Noctuidae: Lepidoptera) on soybean cultivars in Pothwar region and relationship with physico-morphic characters. *Pak. J. Agric. Sci.,* (in press) https://doi.org/10.21162/PAKJAS/19.6979

Aslam, M.N., Mukhtar, T., Jamil, M. and Nafees, M., 2019b. Analysis of aubergine germplasm for resistance sources to bacterial wilt incited by *Ralstonia solanacearum. Pak. J. Agric. Sci.,* **56**: 119-122. https://doi.org/10.21162/PAKJAS/18.6082Bedding, R.A., Molyneux, A.S. and Akhurst, R.J., 1983. *Heterorhabditis* spp., *Neoaplectana spp.,* and *Steinernema kraussei*: Interspecific and intraspecific difference in the infectivity for insect hosts. *Exp. Parasitol*., **55**: 249-257. https://doi.org/10.1016/0014-4894(83)90019-X

Bednarek, A., 1986. Development of the *Steinernema feltiae* (Fil.) entomogenous nematode (Steinernematidae) in the condition of occurrence in the insect's body cavity of other pathogens. *Annls. Warsaw Agric. Univ. SGGW-AR. Anim. Sci*., **20**: 69-74.

Capinera, J.L., Blue, S.L. and Wheeler, G.S., 1982. Survival of earthworms exposed to *Neoaplectana carpocapsae* nematodes. *J. Inverteb. Pathol*., **39**: 419-2421. https://doi.org/10.1016/0022-2011(82)90072-6

Dutky, S.R., 1959. Insect microbiology. *Adv. appl. Microbiol*., **1**: 175-200. https://doi.org/10.1016/S0065-2164(08)70479-9

Dutky, S.R., Thompson, J.V. and Cantwel, G.E., 1964. A technique for the mass propagation of the DD-136 nematode. *J. Insect Pathol*., **6**: 417-422.

Ehlers, R.U. and Shapiro-Ilan, D., 2005. Mass production. In: *Nematodes as biocontrol agents* (eds. P. Grewal, R.U. Ehlers and D. Shapiro-Ilan). CABI, Wallingford, UK, pp. 65-78. https://doi.org/10.1079/9780851990170.0065

Epsky, N.D. and Capinera, J.L., 1993. Quantification of invasion of two strains of *Steinernema carpocapsae* (weiser) into three lepidopteran larvae. *J. Nematol*., **25**: 173-180.

Fan, X. and Hominick, W.M., 1991. Efficiency of the *Galleria* (wax moth) baiting technique for recovering infective stages of entomopathogenic rhabditids (Heterorhabditis and Steinernematidae) from sand and soil. *Rev. Nematol*., **14**: 381-387.

Georgis, R., 1990. Formulation and application technology. In: *Entomopathogenic nematodes in biological control* (ed. R. Gaugler and H.K. Kaya). CRC Press, Boca Raton, Florida, USA, pp. 173-191.

Georgis, R. and Manweiler, S.A., 1994. Entomopathogenic nematodes: A developing biological control technology. *Agric. Zool. Rev*., **6**: 63-94.

Grewal, P.S., Lewis, E.E. and Gaugler, R., 1994a. Host

finding behaviour as a predictor of foraging strategy in entomopathogenic nematodes. *Parasitology*, **108**: 207-215. https://doi.org/10.1017/S003118200006830X

Grewal, P.S., Selvan, S. and Gaugler, R., 1994b. Thermal adaptation of entomopathogenic nematodes: Niche breadth for infection, establishment and reproduction. *J. Therm. Biol*., **19**: 245-253. https://doi.org/10.1016/0306-4565(94)90047-7

Griffin, C.T., Boemare, N.E. and Lewis, E.E., 2005. Biology and behaviour. In: *Nematodes as biocontrol agents* (eds. P. Grewal, R.U. Ehlers and D. Shapiro-Ilan). CABI, Wallingford, UK, pp. 47-64. https://doi.org/10.1079/9780851990170.0047

Hay, D.B., Fenlon, J.S. and Richardson, P.N., 1993. The analysis of *in vivo* bioassay in entomopathogenic nematode research: A behavioural approach. *IOBC/WPRS Bull.*, **17**: 136-139.

Hominick, W.M. and Reid, A.P., 1990. Perspective in entomopathogenic nematology. In: *Entomopathogenic nematodes in biological control* (eds. R. Gaugler and H.K. Kaya). CRC Press, Boca Raton, Florida, pp. 327-345.

Hussain, M.A., Mukhtar, T. and Kayani, M.Z., 2016. Reproduction of *Meloidogyne incognita* on resistant and susceptible okra cultivars. *Pak. J. agric. Sci*., **53**: 371-375. https://doi.org/10.21162/PAKJAS/16.4175

Iftikhar, A., Aziz, M.A., Naeem, M., Ahmad, M. and Mukhtar, T., 2018. Effect of temperature on demography and predation rate of *Menochilus sexmaculatus* (Coleoptera: Coccinellidae) reared on *Phenacoccus solenopsis* (Hemiptera: Pseudococcidae). *Pakistan J. Zool*., **50**: 1885-1893. https://doi.org/10.17582/journal.pjz/2018.50.5.1885.1893

Javed, H., Hussain, S.S., Javed, K., Mukhtar, T. and Abbasi, N.A., 2017a. Comparative infestation of Brinjal stem borer (*Euzophera perticella*) on six aubergine cultivars and correlation with some morphological characters. *Pak. J. agric. Sci*., **54**: 753-758.

Javed, H., Mukhtar, T., Javed, K. and Ata ul Mohsin, 2017b. Management of eggplant shoot and fruit borer (*Leucinodes orbonalis* Guenee) by integrating different non-chemical approaches. *Pak. J. agric. Sci*., **54**: 65-70. https://doi.org/10.21162/PAKJAS/17.5282

Kassi, A.K., Javed, H. and Mukhtar, T., 2018a. Screening of okra cultivars for resistance against *Helicoverpa armigera*. *Pakistan J. Zool*., **50**: 91-95. http://dx.doi.org/10.17582/journal.pjz/2018.50.1.91.95

Kassi, A.K., Javed, H. and Mukhtar, T., 2018b. Screening of different aubergine cultivars against infestation of brinjal fruit and shoot borer (*Leucinodes orbonalis* Guenee). *Pakistan J. Zool*., **51**: 2191-2195. http://dx.doi.org/10.17582/journal.pjz/2019.51.2.603.609

Kaya, H.K. and Stock, S.P., 1997. Techniques in insect nematology. In: *Manual of techniques in insect pathology* (ed. L. Lacey). Academic Press, San Diego, Canada, pp. 281-324. https://doi.org/10.1016/B978-012432555-5/50016-6

Kayani, M.Z. and Mukhtar, T., 2018. Reproductivity of *Meloidogyne incognita* on fifteen cucumber cultivars. *Pakistan J. Zool*., **50**: 1717-1722. https://doi.org/10.17582/journal.pjz/2018.50.5.1717.1722

Kayani, M.Z., Mukhtar, T. and Hussain, M.A., 2017. Effects of southern root knot nematode population densities and plant age on growth and yield parameters of cucumber. *Crop Prot*., **92**: 207-212. https://doi.org/10.1016/j.cropro.2016.09.007

Kayani, M.Z., Mukhtar, T. and Hussain, M.A., 2018. Interaction between nematode inoculum density and plant age on growth and yield of cucumber and reproduction of *Meloidogyne incognita*. *Pakistan J. Zool*., **50**: 897-902. https://doi.org/10.17582/journal.pjz/2018.50.3.897.902

Khan, A.R., Javed, N., Sahi, S.T., Mukhtar, T., Khan, S.A. and Ashraf, W., 2017. *Glomus mosseae* (Gerd & Trappe) and neemex reduce invasion and development of *Meloidogyne incognita*. *Pakistan J. Zool*., **49**: 841-847. https://doi.org/10.17582/journal.pjz/2017.49.3.841.847

Kreft, A. and Skrypek, H., 2002. Insects infection by entomopathogenic nematodes (Rhabiditida: Steinernematidae and Heterorhabditidae) in the conditions of competition. *Ann. UMCS Sec. C*., **57**: 39-52.

Laumond, C., Mauleon, H. and Kermarrec, A., 1979. Donne'es novelles surle spectre o' hotes et le parasitisme du nematode entomophage *Neoaplectana carpocapsae*. *Entomophaga*, **24**: 13-27. https://doi.org/10.1007/BF02377505

Mannion, C.M. and Jansson, R.K., 1993. Infectivity of five entomopathogenic nematodes to the sweet potato weevil, *Cylas formicarius* (F.) (Coleoptera: Apoinnidae), in three experimental arenas. *J. Inverteb. Pathol*., **62**: 29-36. https://doi.org/10.1006/jipa.1993.1070

Molyneux, A.S., Bedding, R.A. and Akhurst, R.J., 1983. Suecsptiblity of larvae of the sheep blowfly *Lucinia cuprina* to various *Heterorhabditis* spp. *Neoaplectana* spp. and an undescribed

steinernematid (Nematoda). *J. Inverteb. Pathol*., **55**: 239-257.

Morris, O.N., 1985. Susceptibility of 31 species of agricultural insect pests to the entomogenous nematode *Steinernema feltiae* and *Heterorhabditis bacteriophora*. *Can. Entomol*., **117**: 401-407. https://doi.org/10.4039/Ent117401-4

Mracek, Z. and Ruzicka, Z., 1990. Infectivity and development of *Steinernema* sp. strain Hylobius Nematoda: steinernematidae) in aphids and *Aphidophagaous coccinellids*. *J. appl. Ent*., **110**: 92-95.

Mukhtar, T., 2018. Management of root-knot nematode, *Meloidogyne incognita*, in tomato with two *Trichoderma* species. *Pakistan J. Zool*., **50**: 1589-1592. https://doi.org/10.17582/journal.pjz/2018.50.4.sc15

Mukhtar, T., Arooj, M., Ashfaq, M. and Gulzar, A., 2017a. Resistance evaluation and host status of selected green gram genotypes against *Meloidogyne incognita*. *Crop Prot*., **92**: 198-202. https://doi.org/10.1016/j.cropro.2016.10.004

Mukhtar, T., Hussain, M.A. and Kayani, M.Z., 2017b. Yield responses of 12 okra cultivars to southern root-knot nematode (*Meloidogyne incognita*). *Bragantia*, **76**: 108-112. https://doi.org/10.1590/1678-4499.005

Mukhtar, T., Jabbar, A., Raja, M.U. and Javed, H., 2018. Re-emergence of wheat seed gall nematode (*Anguina tritici*) in Punjab, Pakistan. *Pakistan J. Zool*., **50**: 1195-1198. https://doi.org/10.17582/journal.pjz/2018.50.3.sc4

Nabeel, M., Javed, H. and Mukhtar, T., 2018. Occurrence of *Chilo partellus* on maize in major maize growing areas of Punjab, Pakistan. *Pakistan J. Zool.*, **50**: 317-323. https://doi.org/10.17582/journal.pjz/2018.50.1.317.323

Pionar, Jr. G.O., 1979. *Nematodes for biological control of insects*. CRC Press Inc., Boca Raton, FL, USA, pp. 277.

Rahoo, A.M., Mukhtar, T., Gowen, S.R. and Pembroke, B., 2011. Virulence of entomopathogenic bacteria *Xenorhabdus bovienii* and *Photorhabdus luminescens* against *Galleria mellonella* larvae. *Pakistan J. Zool*., **43**: 543-548.

Rahoo, A.M., Mukhtar, T., Gowen, S.R., Rahoo, R.K. and Abro, S.I., 2017. Reproductive potential and host searching ability of entomopathogenic nematode, *Steinernema feltiae*. *Pakistan J. Zool*., **49**: 229-234. https://doi.org/10.17582/journal.pjz/2017.49.1.229.234

Rahoo, A.M., Mukhtar, T., Jakhar, A.M. and Rahoo, R.K., 2018a. Inoculum doses and exposure periods affect recovery of *Steinernema feltiae* and *Heterorhabditis bacteriophora* from *Tenebrio molitor*. *Pakistan J. Zool*., **50**: 983-987. https://doi.org/10.17582/journal.pjz/2018.50.3.983.987

Rahoo, A.M., Mukhtar, T., Abro, S.I., Bughio, B.A. and Rahoo, R.K., 2018b. Comparing the productivity of five entomopathogenic nematodes in *Galleria mellonella*. *Pakistan J. Zool*., **50**: 679-684. https://doi.org/10.17582/journal.pjz/2018.50.2.679.684

Rahoo, A.M., Mukhtar, T., Bughio, B.A. and Rahoo, R.K., 2019. Relationship between the size of *Galleria mellonella* larvae and the production of *Steinernema feltiae* and *Heterorhabditis bacteriophora*. *Pakistan J. Zool.,* **51**: 79-84. https://doi.org/10.17582/journal.pjz/2019.51.1.79.84

Selvan, S., Campbell, J.F. and Gaugler, R., 1993. Density-dependant effects on entomopathogenic nematodes (Heterorhabditis and Steinernematidae) within an insect host. *J. Inverteb. Pathol*., **62**: 278-284. https://doi.org/10.1006/jipa.1993.1113

Shapiro-Ilan, D.I., Gouge, D.H. and Koppenhöfer, A.M., 2002. Factors affecting commercial success: Case studies in cotton, turf, and citrus. In: *Entomopathogenic nematology* (ed. R. Gaugler). CAB International, Wallingford, UK, pp. 333-335. https://doi.org/10.1079/9780851995670.0333

Tariq-Khan, M., Munir, A., Mukhtar, T., Hallmann, J. and Heuer, H., 2017. Distribution of root-knot nematode species and their virulence on vegetables in northern temperate agro-ecosystems of the Pakistani-administered territories of Azad Jammu and Kashmir. *J. Pl. Dis. Prot*., **124**: 201-212. https://doi.org/10.1007/s41348-016-0045-9

White, G.F., 1927. A method for obtaining infective juveniles from cultures. *Science*, **66**: 302-303. https://doi.org/10.1126/science.66.1709.302-a

Wojcik, W.F., 1986. Influence of the size host on the growth of the *Neoaplectana carpocapsae* Weiser, 1955 nematodes. *Annls. Warsaw Agric. Univ. SGGW-AR Anim. Sci*., **20**: 75-85.

Woodring, J.L. and Kaya, H.K., 1988. *Steinernematid and heterorhabditid nematodes: A handbook of biology and techniques*. Southern Cooperative Series Bulletin 331, Arkansas Agricultural Experimental Station, Fayetteville, Arkansas, pp. 30.