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Relationship Between the Size of *Galleria mellonella* Larvae and the Production of *Steinernema feltiae* and *Heterorhabditis bacteriophora*

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, PMAS-Arid Agriculture University, Rawalpindi*
[3]*Department of Zoology, University of Sindh, Jamshoro*
[4]*Institute of Plant Sciences, University of Sindh, Jamshoro*

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

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ABSTRACT

The fitness of entomopathogenic nematodes (EPN) as biological control agents of specific target insects depends on their level of infectivity and reproductive capacity. 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 production of infective juveniles (IJ) from *Galleria mellonella* cadavers may differ among different species of EPN and hosts of various sizes. Therefore, the objective of conducting the present study was to compare the productivity of two EPN (*Steinernema feltiae* and *Heterorhabditis bacteriophora*) in *G. mellonella* larvae of different sizes. The size of *G. mellonella* had significant effect on the emergence of IJ. Significantly greater numbers of IJ both of *H. bacteriophora* and *S. feltiae* emerged from large sized *G. mellonella* than medium and small sized and the emergence was found to be positively correlated with the size of the host. It was also observed that emergence of IJ of *H. bacteriophora* was significantly greater than that of *S. feltiae* from the three different sized *G. mellonella*. The emergence of IJ started from the 4th day and continued till the 37th day. The maximum numbers of IJ were recorded on the 13th day in case of small and medium sized *G. mellonella* larvae while the maximum number of IJ from large sized *G. mellonella* was observed on the 10th day.

INTRODUCTION

Features of entomopathogenic nematodes (EPN) that affect their fitness 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 and has been shown to vary among nematodes within specific target hosts (Molyneux *et al*., 1983; Morris *et al*., 1990; Mannion, 1992) and among hosts for a given nematode species or strain (Morris *et al*., 1990). The reproductive capacity of nematodes has also been found to vary among nematodes within target insects 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 essential for 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*. (1993) found *Heterorhabditis* spp. to be more efficacious against the sweet potato weevil *Cylas formicarius*. Jansson *et al*. (1993) also found that heterorhabditids persisted longer than steinernematids in the field.

The technique of *in vivo* mass production of EPN has been used largely for laboratory use and small scale field testing and is appropriate for commercial uses where markets are so small or undeveloped that the *in vitro* (industrial technology) is not available or is not economically feasible (Ehlers and Shapiro-Ilan, 2005a). Production costs are higher with *in vivo* production because of the labour and for the supply of host insects.

* Corresponding author: drtmukhtar@uaar.edu.pk
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Arguably in a country like Pakistan where the labour costs may be relatively low, the value and benefit of these nematodes may justify their use on some crops (Ehlers and Shapiro-Ilan, 2005b). Until their use has been validated in Pakistan, there will remain scepticism among farmers that this method of biological control has a role. In Pakistan, some preliminary field evaluations of EPN were done with *in vivo* produced nematodes in hosts like *Galleria mellonella* (Rahoo *et al*., 2011, 2017, 2018a, b). The EPN produced thus can be used for the management of insect pests and root-knot nematodes (Hussain *et al*., 2016; Fateh *et al*., 2017; Javed *et al*., 2017a, b; Kayani *et al*., 2017, 2018; Khan *et al*., 2017; Mukhtar *et al*., 2017a, b, 2018; Mukhtar, 2018; Tariq-Khan *et al*., 2017; Kassi *et al*., 2018; Nabeel *et al*., 2018). As biological control becomes more prevalent in pest management, it will become increasingly important to anticipate interactions between biological control agents (Rosenheim *et al*., 1995; Iftikhar *et al*., 2018; Kayani and Mukhtar, 2018).

It was hypothesized that the production of IJ from *G. mellonella* cadavers would not differ among different species of EPN and hosts of various sizes. Therefore, the objective of the present study was to compare the productivity of two entomopathogenic nematodes (*Steinernema feltiae* and *Heterorhabditis bacteriophora*) in *Galleria mellonella* larvae of different sizes.

MATERIALS AND METHODS

EPNs (*Steinernema feltiae and Heterorhabditis bacteriophora* used in the study 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 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 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.

After incubation at 20°C for 10 days, the infected *G. mellonella* were taken from the Petri dishes and placed on modified white traps. 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 2 weeks. IJ of the nematode species were acclimatized at room temperature (21-23~) for an hour and their viability was tested under a stereomicroscope before use.

A consignment of *G. mellonella* larvae was sorted into three size categories, small, medium and large. The weights of twenty larvae from each category were taken and ranged as follows: small 0.18-0.22 g; medium 0.28-0.32 g and large 0.38-0.42 g. Each larva was placed on a filter paper in a 30 mm Petri dish and inoculated with 0.10 ml suspension of either *S. feltiae* or *H. bacteriophora* containing a mean of 63 infective juveniles. After leaving in an incubator at 20ºC for 4 days all larvae succumbed to nematode infection.

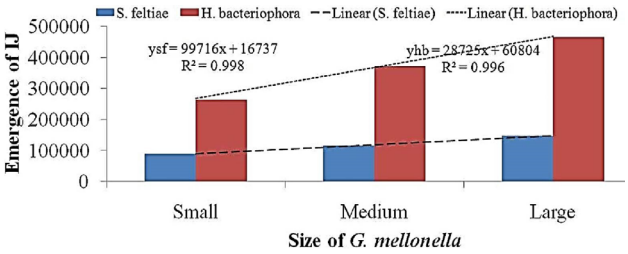

Fig. 1. Comparison of emergence of IJ of *Steinernema feltiae* and *Heterorhabditis bacteriophora* from *Galleria mellonella* larvae.

Sixty 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. One week after inoculation each cadaver was moved on the supporting Netlon and transferred to new Petri dish containing 5 g silver sand plus 1.0 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 every 3 days until no more nematodes were recovered. Each Petri dish was monitored daily to observe when nematodes first emerged from cadavers.

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

The size of *G. mellonella* had significant effect on the emergence of IJ. Significantly greater numbers of IJ both of *H. bacteriophora* and *S. feltiae* emerged from large sized *G. mellonella* than medium and small sized and the emergence was found to be positively correlated with the size of the host (Fig. 1). It was also observed that emergence of IJ of *H. bacteriophora* was significantly greater than *S. feltiae* from the three different sized *G. mellonella* (Fig. 1).

In case of *S. feltiae*, the emergence of IJ started from the 4th day and continued till the 37th day. The maximum numbers of IJ were recorded on the 13th day in case of small and medium sized *G. mellonella* larvae while the maximum number of IJ from large sized *G. mellonella* was observed on the 10th day and the numbers of emerging IJ declined gradually reaching to almost nil on the 37th day as shown in Figure 2A. On the other hand, the emergence of IJ was the maximum on the 13th day in case of *H. bacteriophora* and followed the same pattern as in the case of *S. feltiae* (Fig. 2B). The comparison of emergence of IJ between *S. feltiae* and *H. bacteriophora* from the three sizes of *G. mellonella* has been given in Figure 3.

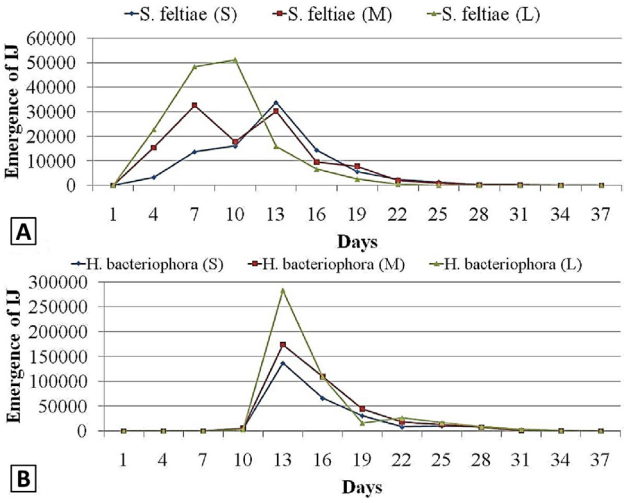

Fig. 2. Comparison of emergence of IJ of *Steinernema feltiae* (A) and *Heterorhabditis bacteriophora* (B) from small, medium and large sized *Galleria melonella.*

DISCUSSION

In the present study differences were observed in the reproduction between *S. feltiae* and *H. bacteriophora* and in the time of emergence of the infective juveniles from the host cadaver. The reproduction of *H. bacteriophora* was higher than that of *S. feltiae* in all three sizes of *G. mellonella* larvae. On the other hand, *S. feltiae* IJ emerged from cadavers sooner than those of *H. bacteriophora.* The reason may be that the size of IJ of *S. feltiae* is greater than that of *H. bacteriophora*. The larger size of *S. feltiae* 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, 1987) and possibly build up of ammonia (San-Blas *et al*., 2008). Patterns in total reproduction of nematodes differed among the two species. *H. bacteriophora* consistently produced more progeny than *S. feltiae*. *H. bacteriophora* is hermaphroditic in its first generation of the life cycle. It is possible that this has an effect on the numbers of IJ produced as there is no dependence on the encounter of male and female.

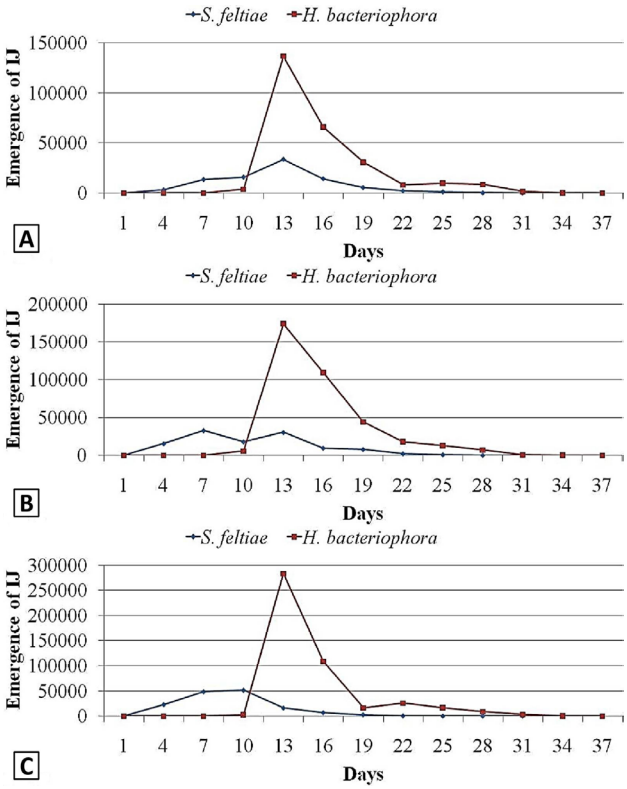

Fig. 3. Comparison of emergence of IJ of *Steinernema feltiae* and *Heterorhabditis bacteriophora* from small sized (A), medium sized (B) and large sized (C) *Galleria melonella.*

EPNs 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. However, average production was much less, in the order of 30,000 to 50,000 infective juveniles per insect. In the present study, approximately similar numbers of IJ were obtained for *H. bacteriophora*; however, such great numbers were not recovered for *S. feltiae*. The body size of the host almost certainly affected the total number of IJ developing inside the cadavers.

Differences between the reproduction potential of EPN may be related to the isolates, species, host susceptibility, number of bacteria per infective stage, invasion rate, temperature and humidity. It is possible that differences in virulence between species and isolates might be greater for less susceptible hosts. Prior to applications, bioassays should be performed against the target insect pest (Stirling, 1992).

Under *in vivo* conditions, *H. bacteriophora* IJ entering a host insect encounter a food signal that immediately induces the recovery of IJ. The hermaphrodite that develops from the IJ lays eggs until the egg production rate decreases. At that moment, the first stage juveniles hatch within the uterus. Owing to the low food concentration in the uterus, the development of infective juveniles is reduced. The juveniles develop and feed on the body of the hermaphrodite until all tissues are digested. After the hermaphrodite has died, the IJ emerge from the empty carcass. These IJ either develop into a second generation of hermaphrodites or are arrested in the infective juvenile stage, depending on the food signal concentration in the surrounding medium. Thus the bacterial food signal indicates whether food is abundant for another propagative life cycle or whether the resources have been used up (Strauch and Ehlers, 1998). If the nutrients are consumed, the IJ leave the insect cadaver in search of other hosts (Ehlers, 2001). An overview of the *H. bacteriophora* life cycle supports the findings about the early emergence of *H. bacteriophora*. Time of emergence also strongly depends on the host, especially on its size. It is known from *S. feltiae* that time of emergence can vary dramatically with the size of the host. In the small sciarid flies, nematodes emerged after 6-7 days whereas in *G. mellonella* it can take 2-3 weeks. Probably, the use of different sizes of *G. mellonella* larvae is responsible for such surprising results in this study. In a similar study conducted at 25°C with nematode isolates from Turkey, Oguzoglu Ünlu and Özer (2003) showed that infective juveniles of *H. bacteriophora* and *S. feltiae* respectively emerged from the *G. mellonella* hosts 6 and 9 days post infection. These data are contrary to other reports where steinernematids emerged earlier than heterorhabditids at the same temperature regime (Poinar, 1979). For example, the infective juveniles of *S. feltiae* emerged from cadavers between 5 and 7 days post infection at 25°C (Hazir *et al*., 2001) and heterorhabditid species emerged from cadavers 10 or more days post infection (Khan *et al*., 1976). Such differences may be due to experimental conditions such as temperature and extraction techniques.

CONCLUSION

It is concluded from the present study that the size of *G. mellonella* had significant effect on the emergence of IJ. Significantly greater numbers of IJ of *H. bacteriophora* and *S. feltiae* emerged from large sized *G. mellonella* compared to medium and small sized larva. It was also observed that emergence of IJ of *H. bacteriophora* was significantly greater than that of *S. feltiae* from the three different sized *G. mellonella*. As *H. bacteriophora* produced more IJs in large sized *G. mellonella*, hence can be used for the management of insect pests under controlled conditions.

Statement of conflict of interest

Authors have declared no conflict of interest.

REFERENCES

Ehlers, R.U., 2001. Mass production of entomopathogenic nematodes for plant protection. *Appl. Microbiol. Biotechnol*., **56**: 523-633. https://doi.org/10.1007/s002530100711

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

Ehlers, R.U. and Shapiro-Ilan, D., 2005b. Forum on safety and regulation. In: *Nematodes as biocontrol agents* (eds. P.S. Grewal, R.U. Ehlers and D. Shapiro-Ilan). CABI Publishing, Wallingford, UK, pp. 107-114. https://doi.org/10.1079/9780851990170.0107

Fateh, F.S., Mukhtar, T., Kazmi, M.R., Abbassi, N.A. and Arif, A.M., 2017. Prevalence of citrus decline in district Sargodha. *Pak. J. agric. Sci*., **54**: 9-13. https://doi.org/10.21162/PAKJAS/17.5643

Hazir, S., Stock, S.P. and Kaya, H.K., 2001. Development temperature effects on five geographic isolates of the entomopathogenic nematode *Steinernema feltiae* (Nematoda: Steinernematidae). *J. Inverteb. Pathol*., **77**: 243-250. https://doi.org/10.1006/jipa.2001.5029

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. http://dx.doi.org/10.17582/journal.pjz/2018.50.5.1885.1893

Jansson, R.K., Lecrone, S.H. and Gaugler, R., 1993. Field efficacy and persistence of entomopathogenic nematodes (Nematoda*: Steinermatidae*, *Heterorhabditidae)* for the control of sweet potato weevil (Coleoptera: Apionidae) in southern Florida. *J. econ. Ent*., **86**: 1055-1063.

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. *Pakistan J. agric. Sci*., **54**: 763-768. https://doi.org/10.21162/PAKJAS/17.5282

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. *Pakistan J. agric. Sci*., **54**: 65-70.

Kassi, A.K., Javed, H. and Mukhtar, T., 2018. Screening of okra cultivars for resistance against *Helicoverpa armigera*. *Pakistan J. Zool*., **50**: 91-95.

Kaya, H.K., 1987. Diseases caused by nematodes. In: *Epizootiology of insect diseases* (eds. J.R. Fuxa and Y. Tanada). J Wiley, New York, pp. 453-470.

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

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

Khan, A., Brooks, W.M. and Hirschmann, H., 1976. *Chromonema heliothids* n. gen., n. sp. (Steinernematidae, Nematoda) a parasite of *Heliothis zea* (Noctuidae: Lepidoptera) and other insects. *J. Nematol*., **8**: 159-168.

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

Mannion, C.M., 1992. *Selection of suitable entomopathogenic nematodes for biological control of* Cylas formicarius *(Coleoptera: Apionidae)*. Ph. D. thesis, University of Florida, Gainesville, FL.

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., Converse, V. and Harding, J., 1990. Virulence of entomopathogenic nematode-bacteria complex for larvae of noctuids, a geometrid, and a pyralid. *Can. Entomol*., **122**: 309-319. https://doi.org/10.4039/Ent122309-3

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*, **75**: 108-112. https://doi.org/10.1590/1678-4499.005

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

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

Oguzoglu Ünlü, I. and Özer, N., 2003. Evaluation of the reproductive potential and competition between two entomopathogenic nematodes, *Steinernema feltiae* Filipjev, 1934 (Rahbditida: Steinernematidae) and *Heterorhabditiditis bacteriophora* Poinar 1976 (Rahbditida: Heterorhabditidae). *Turkish J. Biol*.,

27: 149-155.

Poinar, Jr. G.O., 1979. Nematodes for biological control of insects. CRC Inc. Press, 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

Rosenheim, J.A., Kaya, H.K., Ehlers, L.E., Marois, J.J. and Jaffee, B.A., 1995. Intraguild predation among biological control agents theory and evidence. *Biol. Contr.*, **5**: 303-335. https://doi.org/10.1006/bcon.1995.1038

San-Blas, E., Gowen, S.R. and Pembroke, B., 2008. *Steinernema feltiae*: Ammonia triggers the emergence of their infective juveniles. *Exp. Parasitol*., **119**: 180-185. https://doi.org/10.1016/j.exppara.2008.01.008

Stirling, P.D., 1992. *Introductory ecology.* Prentice Hall, Englewood Cliffs, NJ.

Strauch, O. and Ehlers, R.U., 1998. Food signal production of *Photorhabdus luminescens* inducing the recovery of entomopathogenic nematodes *Heterorhabditis* sp. in liquid culture. *Appl. Microbiol. Biotechnol*., **50**: 369-374. https://doi.org/10.1007/s002530051306

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

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.