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EVALUATION OF VIRULENCE OF ENTOMOPATHOGENIC NEMATODE ISOLATES AGAINST *HELICOVERPA ARMIGERA* AND *SPODEPTERA LITURA*

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ABSTRACT

A trial was laid down to test the efficacy and mass production of the 15 isolates of the two important Entomopathogenic nematodes viz. *Steinernema* and *Heterorhabditis* sp. using three of the important pests i.e. *Galleria mellonella*, *Helocoverpa armigera* and *Spodeptera litura* under laboratory condition. Different inoculants *Viz*. 10, 20, 40, 60, 80 Infective Juveniles/insect were used for the study. Observations were taken at 6 hrs intervals on the mortality of insects (6–72hrs). A dose of 10-30 Infective Juveniles (second generation) of all the 5 tested entomopathogenic nematode isolates were found significant (P < 0 05) causing mortality (60-80%) within 50-94 hrs. *S. littura* was found more susceptible as compared to *G. mellonella* and *H. armigera.* The values of LC_{50} and LT_{50} indicated that nematodes of both genera gave significant results in virulence and time required for killing the pest larvae.

Key words: Entomopathogenic nematodes, *Helicoverpa armigera, Spodeptera litura, Galleria mellonella, Steinernema*, *Heterorhabditis*, efficacy

INTRODUCTION

Biological control occurs in nature when populations are limited through the action of parasites, predators, and pathogens. As an applied science, biological control often involves release of exotic natural enemies in an attempt to suppress introduced pest species in agriculture, forestry, and human health (classical biological control), but it is also implemented through the augmentation or conservation of native natural enemies. Natural and applied biological control tactics are important in successful management of pest populations, below those causing economic injury. Most of the synthetic chemicals decimate the beneficial parasitoids and predators. The value of biocontrol is now well recognized particularly in the context of environmental protection as well as stable pest management strategy. The role of biopesticides particularly microbial pathogens in biorational pest management has been well documented and the efficacy of microbial pathogens can be enhanced further by genetic improvement.

The modern history of biological control dates from the remarkable success that was achieved in controlling the cottony cushion scale (*Icerya purchasi*) on California citrus in 1888 through introduction from Australia of the predatory vedalia beetle (*Rodolia cardinalis*). Biological control stands today as a corner stone of integrated pest management (IPM) and is the foremost alternative to the use of chemical pesticides (Greathead 1986, De-Bach and Rosen 1991). Much work has been done on biological control of the different insect pests using EPNs (Grewal 2002).

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Research Career with major achievements:

1. Awards/Honours: **08**
2. Publications:

i. Full Paper- **40;** ii. Book (edited) – **02**; **iii**. Abstract- 115

3. Training/Workshop Completed: **06**
4. Research project:

i. Completed- **03**; ii. On going- **01**

5. Human Resource Development: **28**

i. Ph. D.- **04**; ii. M. Phil. - **25**

6. Visited Abroad: Australia

Highlights of the Research Work:

1. Diversity and infection incidence of *Meloidogyne* {*M. incognita* (Race 1, 2, 3, 4); *M. javanica* (Race 1); *M. arenaria* (Race 1, 2); *M. hapla* (Race 1)} from some districts of Western Uttar Pradesh have been studied.
2. Two species *M. arenaria, M. hapla* reported first time from this region. The presence of all these species was also confirmed on the molecular basis.
3. Biochemical estimation viz. carbohydrate, protein, cholesterol, lipid and free amino acids and karyological study of root knot nematodes have been done.
4. Two major Entomopathogenic Nematodes (EPN) have been isolated viz. *Steinernema* & *Heterorhabditis*.
5. Monoculture of 66 Isolates of both the genera (*Steinernema*-49, *Heterorhabditis*-17) and monoxenic culture of each of the isolates is maintained in the laboratory.
6. Nematicidal Screening of Fungi (12 spp.) and Bacteria (16 isolates of *Bacillus subtilis*) have been done and mass production of these bio-agents are in progress.
7. Culture filtrates of all these bio-agent isolates have been tested for the nematicidal properties that showed positive effect against *Meloidogyne* spp.
8. Properties of antagonistic fungi vs pathogenic fungi; antagonist bacteria vs pathogenic fungi have also been studied.
9. Cost effective mass production technology of EPN has been developed and maintained in the laboratory
10. Four formulations have been develop for storing the EPN for longer duration and all the four formulations have been tried for their best efficacy.
11. All the products are ready for commercial production and for distribution to the farmers.

The success of any insect biological control programme by entomopathogenic nematodes depends on the nematode species, the target insect pest and environmental conditions. During the recent past, greater attention has been given to entomopathogenic nematodes to explore their biocontrol potential for the management of insect pests. Nematodes belonging to more than 30 families are known to parasitize insects but potential entomopathogenic nematodes belong to only two families, Steinernematidae and Heterorhabditidae.Entomopathogenic nematodes from these families possess wide host range and have been widely exploited for their biocontrol potential (Lacey *et. al*. 2001, Kaya & Gaugler 1990). The family Steinernematidae comprises two genera Steinernema with known 56 species and Neosteinernema with only one species, whereas Heterorhabditidae has one genus *Heterorhabditis* with 10 species (Ganguly 2006). Entomopathogenic nematodes have been recovered from soil of a wide variety of climatic regions in India (Karunakar *et. al*. 1999, Ganguly & Singh 2001, Hussaini *et. al.* 2004).

The nematode belonging to family Steinernematidae and Heterorhabditidae carry symbiotic bacteria in their gut. These bacteria are got released through faecal matter of the nematode in the haemocoel of the insect and cause rapid death of the insect host within 24-72 hrs due to septicemia (Kaya & Gaugler, 1993). The biocontrol potential of these nematodes is well established in laboratory and also in field under favorable conditions. Performance of Entomopathogenic nematodes, however, varies greatly with the isolate, in addition to insect host and climatic conditions. Therefore it is need of day to explore the native strains of Entomopathogenic nematodes which could perform well under given set of environmental conditions and could provide a better and long lasting control.

In the present study we have tried to find out the most efficacious locally available entomopathogenic nematode isolate against the important insect pests viz. *Gallaria mellonella*, *Helicoverpa armigera* and *Spodoptera litura.*

MATERIALS AND METHODS

1. Target insects and their rearing in the laboratory

The original stock culture of target insects, *Galleria mellonella*, *Helicoverpa armigera* and *Spodeptera litura* were reared in the laboratory on semi-synthetic diet in plastic-jars until reached the desired larval stage.

i. *G. mellonella* was reared on the artificial feed composed of Glycerol - 125ml; Maize flour - 200g; Wheat brawn - 100g, Milk Powder - 100g; and Yeast powder - 30g.

ii. *H. armigera* was also reared on artificial diets which was prepared in three fractions *viz*. Fraction-A, Fraction-B and Fraction-C. The preparation of all fractions is as follows:

a. **Fraction-A** : Chick-pea flour - 94g and Wheat germ - 13g mixed in Luke warm water (500ml).

b. **Fraction B** : Agar-agar - 15g added in to 325ml distilled water. Fraction B was then added in to fraction A and shake well for 1 minute.

c. **Fraction C** : Yeast powder - 24.5g; Casein - 15g; Ascorbic acid - 6g; Methyle-para-hydroxy benzoate - 2g; Asorbic acid - 1.2g; Streptomycin sulphate - 0.2g; Cholesterol - 0.6g; Formaldehyde (40%) - 1ml; Multivitamin solution (vitamins ABCDE) - 1.5ml; Multivitamin capsule - 2 and á-tacopherol - 400mg. Fraction C then added in to A and B and mixed for 30 seconds. This warm homogenous mixture poured in to sterilized petriplates and allowed to cool.

iii. *S. litura* (larvae and adults) were reared on castor leaves and on 1% sucrose solution respectively.

2. Tested Nematode Isolates

Fifteen entomopathogenic nematode isolates were screened and isolated collected from different agriculture fields of Meerut region (Table 1). The nematodes were baited out using *Gallaria* on white trap (Bedding and Akhurst, 1975). One-week-old cultures were used in the experiments. All the isolates were produced in large number using the 5th instar of *G. mellonella* larvae adopting the procedure as described by Woodring & Kaya (1988).

3. Bioassay

Fifteen nematode isolates were tested for their virulence on *G. mellonella* and same were tested on the larvae of *H. armigera* and *S. litura*. The tested nematodes were suspended in distilled water to obtain the desired concentrations, control (0), 10, 20, 40, 60 and 80 Infective Juveniles/ml/larva against the control using 1ml of distilled water/ Petridis. There were ten replicates for each concentration. Each concentration was sprayed on single larva placed in a Petridis (50 mm dia) lined with filter paper and left for 72 hrs at 28$\pm$2^{0}C in

Table 1. Geographical location of the Entomopathogenic Nematode ioslates in district Meerut (Western Uttar Pradesh).

S. No.	EPN isolate	Soil P^H	Crops	Geographical Origin
1	H_2	6.4	Pea Pegion	Meerut
2	H_4	6.8	Pea Pegion	Meerut
3	H_{11}	7.6	Wheat	Kaili
4	H_{17}	6.2	Sugarcane	Sevaya
5	H_{18}	6.2	Sugarcane	Sevaya
6	H_{19}	6.2	Sugarcane	Sevaya
7	H_{20}	6.2	Sugarcane	Sevaya
8	S_5	7.2	Rice	Meerut
9	S_7	7.3	Potato	Bajaut
10	S_8	7.5	Wheat	Bhatjevda
11	S_{12}	7.9	Cabbage	Kharkhauda
12	S_{13}	7.7	Radish	Lalsana
13	S_{14}	7.1	Jowar	Dorli
14	S_{21}	7.2	Jowar	Sevaya
15	S_9	6.9	Wheat	Kharkhauda

Table 2: LC_{50} and LT_{50} values of some of the Entomopathogenic Nematode isolates on *G. mellonella* larvae.

S.No.	Indigenous EPN Isolates	LC_{50} (IJs/ larvae)	LT_{50} (In hrs.)
1	H_{20}*	10.16	56.46
2	H_{19}*	18.90	61.22
3	S_{13}*	19.28	56.32
4	H_{17}*	23.74	51.52
5	S_{14}	27.24	55.57
6	S_{5}*	28.64	67.19
7	H_{11}	32.21	69.45
8	S_{9}	38.88	66.92
9	H_{2}	40.81	57.21
10	H_{18}	42.08	55.89
11	S_{7}	42.08	61.67
12	S_{21}	42.11	69.48
13	S_{8}	52.83	69.03
14	H_{4}	36.11	65.28
15	S_{12}	55.12	69.66

* Entomopathogenic Nematode isolates showing significance efficacy.

Table 3: Relative efficiency of 5 isolates of *Steinernema* sp. and *Heterorhabditis* sp. nematodes against *Helicoverpa armigera* larvae.

	***Steinernema* sp.**		***Heterorhabditis* sp.**		
	5S	13S	17H	19H	20H
LC_{50} (IJs/ml.)	30 P>0.05	20 P>0.01	25 P>0.05	20 P>0.005	10 P>0.001
LT_{50} (In hrs.)	94.22 P>0.05	74.86 P>0.05	80.32 P>0.05	53.24 P>0.05	48.45 P>0.01

Table 4: Relative efficiency of 5 best isolates of *Steinernema* sp. and *Heterorhabditis* sp. nematodes against *Spedoptera litura* larvae.

	***Steinernema* Sps.**		***Heterorhabditis* sps.**		
LC_{50} (IJs/ml.)	S_{5} 30	S_{13} 20	H_{17} 25	H_{19} 20	H_{20} 10
LT_{50} (In hrs.)	58.38	30.65	51.70	41.30	38.29

BOD incubator. Mortality counts were recorded daily for 72 hrs from the initiation of the experiment and percent mortality was calculated for each concentration level. Probit analysis was used for determining the LC_{50} values (IJs/larva), and time mortality relation LT_{50} (mortality/6 hrs) was calculated. Duration of 50% kill of the larvae was determined for each nematode on targeted insect pest (*G. mellonella*, *H. armigera* and *S. litura*).

Out of fifteen isolates only five isolates (two of *Steinernema*, S_{5} & S_{13} and three of *Heterorhabditis* H_{17}, H_{19} & H_{20}) have much potential to kill *G. mellonella* larvae. Therefore, in the present study, only these five isolates were used to check their potential against *H. armigera* and *S. litura*. The bioassay for target insect pests *viz. H. armigera* and *S. litura* was conducted same as *G. mellonella* but the dose of Ijs were applied according to the LC_{50} values of EPN isolates on *G. mellonella* and LT_{50} values were calculated for these isolates (S_{5}, S_{13}, H_{17}, H_{19} and H_{20}) separately on *H. armigera* and *S. litura*.

RESULTS AND DISCUSSION

Gallaria mellonella

Comparing the virulence of the native EPN isolates of *Steinernema* and *Heterorhabditis* species to *G. mellonella* larvae (5th instar), as indicated by values of lethal mortality concentrations and lethal time required (Table 2), it might be concluded that out of fifteen EPN isolates (Eight *Steinernema* sps. and Seven *Heterorhabditis* sps.), only five isolates (two of *Steinernema* and three of *Heterorhabditis isolates*) 5S, 13S, 17H, 19H and 20H were more virulent and faster in killing *G. mellonella* larvae, in which, isolate 20H was more virulent. These results are in accordance with those of dose concentrations.

Helicoverpa armigera

In case of comparing the virulence of above mentioned best 5 isolates on *G. mellonella* and on *H. armigera* larvae, as indicated by value of LC_{50} and LT_{50} (Table 3), it might be concluded that by using the same dose of EPNs on *G. mellonella* and on *H. armigera*, isolate 13S was more virulent and faster in action to the *G.*

mellonella larvae than *H. armigera* larvae followed by 5S to both the larval species. As well, comparing the virulence of 20H, 19H and 17H isolates to *H. armigera* larvae as indicated by the values of Lethal time required (Table 3), it could be concluded that the 20H and 19H was more virulent to the *H. armigera* than *G. mellonella*, while 17H was more virulent to the *G. mellonella* than *H. armigera*. We found that all 5 nematode isolates were pathogenic on the basis of $LT_{50.}$ It is worth mentioning here that during the isolation procedure, the larvae of *H. armigera* succeeded in trapping the nematode rather then the larvae of the common host *G. mellonella*.

Spodoptera litura

On the other hand comparing the virulence of the same isolates to *S. litura* larvae, as indicated by values of LT_{50} (Table 4), it could be concluded that 13S was the most virulent to the *S. litura* larvae followed by 20H, 19H, 17H and 5S on the basis of LT_{50}. The sharp rapid effect of the nematodes in controlling the *S. litura* as proved by these results could be due to the high susceptibility of the pest to nematode. We found, in the bioassay test, that *Steinernema* and *Heterorhabditis* species isolates at 10-30 IJs/larvae of all the insect larvae used in this study (viz. *G. mellonella*, *H. armigera*, *S. litura*) induced 60-80% mortality with in 50-70 hrs. in *G. mellonella*, 48-94 hrs. in *H. armigera* and 30-58 hrs. in *S. litura*.

General comparison among Steinernematid and Heterorhabditid tested species through values of LC_{50} and LT_{50} indicated that nematodes of both genera gave valuable results in virulence and time required for killing the pest larvae. *S. litura* tested larvae, appeared more susceptible than *H. armigera* and *G. mellonella* larvae to all tested nematodes. The result obtained can be corroborated with the findings of Morton *et al*. (2009). The result also revealed that the higher nematode inoculums levels, however, caused higher and faster mortality than the lower levels. The death of the treated insect larvae is caused mainly by the effect of the nematode associated bacteria. Larval death probably arises from the production of proteolytic enzymes which may explain the relative lack of resistance to bacteria. Thus, it could be suggested that the higher concentrations of nematodes will elaborate much more bacteria which in turn multiply rapidly producing huge numbers of bacterial cells which in tern kill the insect larvae more rapidly. Thus the higher concentrations caused faster and higher mortality than the lower ones.

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