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EVALUATION OF PATHOGENIC POTENTIAL OF ENTOMOPATHOGENIC NEMATODE *HETERORHABDITIS INDICA* AGAINST *HELICOVERPA ARMIGERA*

N V Lavhe*, V J Tambe, S L Borkar, P R Panchbhai and V K Biradar

Entomology Section, College of Agriculture, Nagpur, Maharashtra, India
*Email: nvlavhe@gmail.com (corresponding author): https://orcid.org/0000-0002-2224-5557

ABSTRACT

The isolation and identification studies revealed that the EPN isolates from the Sorghum cropping system was *Heterorhabditis indica* and showed pathogenicity to the third instar larvae of *Corcyra cephalonica* in laboratory. The pathogenicity studies revealed that mortality in 3rd, 4th and 5th instar larvae of *C. cephalonica* and *H. armigera* was highest at 30 IJs/100µl concentration of EPN suspension. The reproduction and multiplication studies revealed that recovery of EPN Infective Juveniles (IJs) was highest in the 3rd and 4th instar larvae of *C. cephalonica* and *H. armigera* when infected with EPN isolates from sorghum cropping system.

Key words: Entomopathogenic nematode, *Heterorhabditids indica, Corcyra cephalonica, Helicoverpa armigera,* infective juveniles, pathogenicity, sorghum, juveniles (IJs), EPN

Lethal pathogens of insects are entomopathogenic nematodes (EPNs) belonging to Steinernematidae and Heterorhabditidae. These pathogens contribute towards regulation of natural populations of insects. The success relies on the unique partnership between a host seeking nematode and lethal insect pathogenic bacterium. Considerable attention has been given over the past few decades to genus *Heterorhabditis* and *Steinernema* and their respective partners, *Photorhabdus* and *Xenorhabdus* for their bio-control potential.

Currently the species known of *Steinernema* was about 32, 08 from *Heterorhabditis* and 01 from *Neosteinernema*. Amongst these 10 species were reported from the USA, four each from China, Vietnam and India, three from Argentina, two from Pakistan and one each from other countries. Commercial use of entomopathogenic nematodes was attempted with the use of DD-136 against insect pest of paddy, sugarcane and apple in field condition. Also, the life cycle, field efficacy and compatibility with insecticides and fertilizers was evaluated.

High mortality of cutworm, *Pseudalatia seperata* Walker and *Spodoptera compta* Walker was noted with *S. capocapsae* and in tobacco the mortality was recorded 66% for cutworm (Gupta, et al., 1987). Further the effect of *S. feltiae* was studied on pre-pupa, pupae and adults of *Spodoptera litura*. *S. carpocapsae* and *H. bacteriophora* were tested against red headed hairy caterpillar, *Amsacta albistriga* infield in Groundnut. While *Agrotis ipsilon* and *A. segatum* were found parasitized by *S. carpocapsae* in potato. All the larval stages and pupal stage of bollworms were found susceptible to Heterorhabditid EPNs. With the identified potential of EPNs and the added advantage of their use in bio-control development of EPNs as a component in pest management will be economically beneficial to farmers.

MATERIAL AND METHODS

The investigations for pathogenecity of Entomopathogenic Nematode *Heterorhabditis indica* in Sorghum crop ecosystem was undertaken at Entomology section, College of Agriculture, Nagpur. The material required and the methodologies adopted for conducting the research work in the laboratory. The larvae of lepidopterous insects viz*., Corcyra cephalonica* [Pyralidae, Lepidoptera] was used as laboratory host and *Helicoverpa armigera* [Noctuidae, Lepidoptera] was used as test insect in the present investigations.

Healthy larvae of *Helicoverpa armigera* was collected from the field and reared in the laboratory of Entomology section with natural food as in the field. *C. cephalonica* larvae was supplied by the Bio-control laboratory of the Entomology Department. The 3rd, 4th and 5th instar larvae of these insects was used as host for EPN in the present studies.

The dead larvae from the bait traps were separated out and mounted on White traps for separation of EPN In vivo production uses a White trap (White,

1927). In order to get pure culture of the respective EPN isolates they were re-infected to the larvae of *C. cephalonica* at ambient laboratory conditions. Initially a measured amount of suspension with standard count of IJs/100 µl from the respective EPN isolates from sorghum cropping systems was taken and 10 larvae of *C. cephalonica* were inoculated by direct contact method. After the interval of 10-12 days the nematode suspension with infective juveniles was collected and re-infected to fresh *C. cephalonica* larvae and this process of inoculation and re-infection of nematode suspension to larvae of *C. cephalonica* repeated until the pure culture of nematode populations with infective juveniles obtained. These pure cultures were used for preparation of different doses/ concentration for finalising and deciding the treatments for further studies. The standard IJ counts were prepared by adopting a serial dilution method using double distilled water.

Ten 3rd, 4th and 5th instar larvae of *C. cephalonica* and *H. armigera* from laboratory culture were inoculated with a pure measured amount of EPN suspensions having standard count (IJs/100 µl) from sorghum cropping systems to study pathogenicity. The observations on larval mortalities were recorded up to 72 hours at regular intervals. The mortalities were expressed as % mortalities against each dose for the respective instar. There were seven treatments replicated thrice under completely randomized design.

Ten each from 3rd, 4th and 5th instar larvae of *C. cephalonica* from and *H. armigera larvae* from laboratory culture were inoculated with pure measured amount of EPN suspensions from okra cropping systems having standard count (IJs/100 µl) to study reproduction. The EPN suspension in the White trap along with infective juveniles was collected and infective juvenile count was recorded per 100 µl of EPN suspension and expressed as (IJs/100 µl) for okra cropping systems.

RESULTS AND DISCUSSION

Survey of EPN and installation of bait traps and collection of dead larvae in sorghum cropping system in Nagpur region

Out of the 27 traps installed in sorghum cropping system the *C. cephalonica* dead larvae observed was 35%.

Isolation of EPN from dead larvae

The results of White trapping revealed that the presence of EPN was noticed in sorghum. The isolated traps were tested and multiplied with the help of laboratory host *C. cephalonica*. EPN isolate was obtained from the infected *C. cephalonica* larvae from the sorghum cropping system. The EPN pure culture from the re-infected *C. cephalonica* larvae from sorghum cropping systems was used for studies.

Pathogenicity of EPN Isolates

The pathogenicity of various EPN isolate *H. indica* from sorghum cropping system against the 3rd, 4th and 5th instar larvae of *C. cephalonica* and *H. armigera* were tested in laboratory under ambient conditions and the results were depicted as follows.

Pathogenicity of EPN isolate from sorghum cropping system

Pathogenicity against *Corcyra cephalonica* larvae

The results depicted in Table 1 revealed that the EPN isolates from sorghum cropping system showed remarkable pathogenicity against 3rd, 4th and 5th instar larvae of *C. cephalonica* in laboratory. All the treatment concentrations prepared showed significantly higher mortality than control against the 3rd, 4th and 5th instar larvae. The highest mortalities, however, were recorded in the treatment concentration of 30 IJs/100µL which were 90.00, 86.67, 90.00, respectively for 3rd, 4th and 5th instar larvae of *C. cephalonica.* However, it was found at par with the treatment concentrations of 25 IJs/100µl and 20 IJs/100µl, in 3rd, 4th and 5th instar larvae of *C. cephalonica.*

Pathogenicity against *H. armigera* larvae

The results depicted in Table 1 revealed that the EPN isolates from sorghum cropping system showed remarkable pathogenicity against 3rd, 4th and 5th instar larvae of *H. armigera* in laboratory. All the treatment concentrations prepared showed significantly higher mortality than control against the 3rd, 4th and 5th instar larvae. The highest mortalities were, however, recorded in the treatment concentration of 30 IJs/100µl which were 86.67, 90.00, 83.33%, respectively for 3rd, 4th and 5th larvae of *H. armigera.* However, it was found at par with the treatment concentrations of 25 IJs/100µl in 3rd and 5th instar larvae, while it was found significantly superior overall the treatments in 4th instar larvae of *H. armigera.*

Reproduction of EPN isolate on *C. cephalonica* and *H. armigera* larvae

The result depicted in Table 2 revealed that the highest reproduction and recovery of infective

Table 1. Larval mortality of *C. cephalonica* and *H. armigera* as influenced by various concentration of EPN isolates from sorghum cropping system

Sl. No.	Concentration of EPN isolate	Larval mortality of *C. cephalonica* (%)			Larval mortality of *H. armigera* (%)		
		3rd instar	4th instar	5th instar	3rd instar	4th instar	5th instar
1.	5 IJs/ 100µl	70.00 (56.79)	60.00 (50.85)	63.33 (52.78)	40.00 (39.15)	46.67 (43.08)	43.33 (41.07)
2.	10 IJs/ 100µl	73.33 (59.00)	66.67 (54.78)	70.00 (56.79)	56.67 (48.85)	60.00 (50.77)	60.00 (50.85)
3.	15 IJs/ 100µl	76.67 (61.22)	73.33 (59.00)	76.67 (61.22)	63.33 (52.78)	66.67 (54.78)	66.67 (54.78)
4.	20 IJs/ 100µl	83.33 (66.14)	83.33 (66.14)	80.00 (63.43)	73.33 (59.00)	70.00 (56.79)	70.00 (56.79)
5.	25 IJs/ 100µl	86.67 (68.86)	83.33 (66.14)	86.67 (68.86)	83.33 (66.14)	80.00 (63.43)	76.67 (61.22)
6.	30 IJs/ 100µl	90.00 (71.57)	86.67 (68.86)	90.00 (71.57)	86.67 (68.86)	90.00 (71.57)	83.33 (66.14)
7.	Control (distilled sterile water)	3.33 (6.75)	0.00 (0.025)	6.68 (12.59)	0.00 (0.025)	0.00 (0.025)	0.00 (0.025)
	SE(m)±	2.98	2.47	2.78	2.36	2.50	2.38
	CD at 5%	9.05	7.48	8.43	7.15	7.59	7.23
	CV(%)	9.28	8.17	8.71	8.54	8.90	8.34

*Figures in parenthesis are corresponding arc sine transformed values

Table 2. Reproduction of EPN isolate from Sorghum cropping system on *C. cephalonica* and *H. armigera* larvae

Sl. No.	Concentration of EPN isolate	EPN count (IJs/ 100 µl) obtained					
		C. cephalonica			*H. armigera*		
		3rd instar	4th instar	5th instar	3rd instar	4th instar	5th instar
1.	5 IJs/ 100µl	63.33± 20.81	101.00± 29.71	65.67± 7.09	115.67± 5.13	76.00± 7.93	90.67± 34.04
2.	10 IJs/ 100µl	95.67± 22.05	134.67± 7.76	105.33± 5.68	125.00± 5.29	103.00± 6.08	113.33± 15.2
3.	15 IJs/ 100µl	110.67± 12.01	155.67± 4.04	108.33± 25.65	138.30± 7.63	111.33± 4.04	124.33± 13.57
4.	20 IJs/ 100µl	120.00± 5.00	168.33± 12.74	115.00± 18.02	166.67± 11.01	121.00± 18.24	127.33± 12.50
5.	25 IJs/ 100µl	123.00± 21.37	178.33± 10.40	143.00± 24.24	168.33± 17.09	124.33± 9.29	158.67± 3.21
6.	30 IJs/ 100µl	154.67± 6.11	196.67± 15.27	146.67± 15.27	179.67± 9.60	142.67± 5.85	162.33± 16.86

juveniles obtained from 3rd, 4th and 5th instar were recorded 154.67 IJs, 196.67 IJs and 146.67 IJs for *C. cephalonica* and 179.67 IJs, 142.67 IJs, and 162.33 IJs for *H. armigera* per 100 µl of EPN suspension. when they were infected at 30 IJs/100 µl concentration of EPN isolate from the sorghum cropping system. The lowest recovery of infective juveniles per 100 µl of EPN suspension in treatment concentration of 5 IJs/ 100 µl was obtained for both insects *C. cephalonica* and *H. armigera*.

The aim of the study was to know the occurrence of EPN from the soils of various cropping systems in this locality and to study the pathogenicity and reproduction of indigenously isolated EPN against the two lepidopterous insects viz., *C. cephalonica* and *H. armigera.* Survey for EPN by many workers prominently revealed occurrences of several species of EPN from these two groups only. Some of them have been exploited and commercialized for use against insect pests of crops. Hominick et al. (1996) reported twenty-four species of *Steinernema* and eight of *Heterorhabditis* from various insects from the soil worldwide. The survey results in the present studies revealed occurrence of *Heterorhabditis* in Nagpur vicinity. These findings are in correspondence to the

earlier work by Meshram (2015) who reported the existence of *Heterorhabditis* spp. in Nagpur district from soybean and mungbean ecosystems. Trapping of EPN from soil by baiting with susceptible insects like *Galleria mellonella.* Woodring and Kaya (1988) or recently with *C. cephalonica* Meshram, (2015), both being non soil inhabitants, did not affect much on the pathogenicity of EPNs. The variation in pathogenicity of EPN to different stages of insects have been reported by different workers as well. The highest mortalities at 30 IJs/ 100µl of EPN suspensions from sorghum cropping system in the 3rd, 4th and 5th instrar larvae of these insects under study revealed a good deal of opportunities to exploit these EPN isolates for pest management. This observation corresponds to the previous work by Woodring and Kaya (1988), Banu et al. (2007), Divya et al. (2010), Kumar and Ganguly (2011) and Meshram (2015). It was observed that the 3rd and 4th instar larvae of *C. cephalonica* and *H. armigera* in present studies were more susceptible to EPN isolates from the sorghum cropping system.

The differential mortalities within larval instars of an insect can, however, be attributed to the doses of EPN suspension used for treatment. Kary et al. (2012) reported that mortality of *H. armigera* larvae inoculated with 50 and 100 IJs of *H. indica* ranged between 11% and 51% after 6 days. Similarly, the report from Hussain and Ahmad (2015) that the 3rd and 4th instar larvae with mortalities of 74 and 75%, respectively being more susceptible than 5th instar larvae with 66% mortality after 72 hrs post-exposure of various concentrations of EPN ranging from 25 to 150 IJs per larva which strongly supports the findings of the present studies.

Reproduction and recycling of EPN strain in hosts plays an important role in their persistence in soil, infectivity and overall effectiveness in pest control. A prior knowledge about reproduction and recovery of the EPN is considered important in determining the time and dose of subsequent application in the field. As far as reproduction and recovery of IJs from EPN isolate in the present studies is concern, the data on reproduction suggest that following treatment inoculation, the tested EPN isolate from the sorghum cropping system was able to infect and propagate within the body of the two host insects viz., *H. armigera* and *C. cephalonica* used in the present studies. The highest recovery of IJs was obtained from 5th instar larvae at a fixed concentration of 30 IJs/ 100 µl in *H. indica* under study. These results, although could not match the assumptions by Koppenhofer and Kaya (1995) who assumed that the final EPNs population varies with the number of IJs inoculated, they are resembling the results of the studies conducted by Meshram (2015) who obtained highest recovery of IJs from the 5th instar larvae of *H. armigera*, *Argyrogramma signata* (green semilooper) and *C. cephalonica* at fixed treatment concentration of 50 IJs/ 100µl of EPN suspension isolated from soybean and mungbean ecosystems. Observations on the similar line were also reported by Yadav and Lalramliana (2012) on indigenous EPNs from Meghalaya against Taro leaf beetle *Aplosonyx chalybaeus* (Hope).

The findings of the present investigations suggests that the indigenous EPN isolate from sorghum cropping system is virulent enough to cause satisfactory mortalities in 3rd, 4th and 5th instar larvae of *H. armigera* which is polyphagous pest of many crops. Similarly, as observed they are able to infect and multiply within the body of these two insects and produce infective juveniles in large numbers. From the studies conducted it can be concluded that EPN *H. indica* found in the soils of this region shown good potential as pest management agent against some polyphaghous pest like *H. armigera*.

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