Research Article

Isolation, Identification and Culturing of Native Strains of Entomopathogenic Nematodes (Epns), *Steinernema* spp. and *Heterorhabditis* spp. From Different Parts of Haryana

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Abstract

A total of 120 soil samples were collected from different habitats, 9 samples (7.5%) were found to be positive for EPN. The highest frequency of occurrence of EPNs (58.3%) was recorded in Ber orchard with 7 samples infected for EPNs followed by citrus and cactus (8.3%). Amongst them, the frequency of occurrence of Steinernematid and Heterorhabditid nematodes were 41.7 and 16.7 per cent, respectively. *G. mellonella* larvae infected with EPN of the genus *Heterorhabditidae* may have reddish-orange coloring while those infected with *Steinernema* spp. show gray-brown coloring. As the inoculum level increased, there was an increase in progeny production of both *S. abaasi* and *H. indica.*

Keywords: Entomopathogenic nematodes, *Galleria mellonella*, Culturing, *Heterorhabditis* spp., isolation, *Steinernema* spp

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Introduction

Entomopathogenic nematodes (EPNs) are obligate parasites in nature, which gives them the possibility of being used as bio-control agents and therefore represent a good alternative to chemical insecticides [1]. EPNs are found in a variety of habitats, and the various species and isolates exhibit considerable variation in their host range, reproduction, infectivity, and conditions for survival [2-3]. Occurrence and distribution of EPNs has been recorded in different local geographical locations [4]. In the last three decades, many EPNs have been isolated in various habitats all over the world, revealing hundreds of new isolates and many new species [5]. Currently, over 80 species of Steinernema and 20 species of Heterorhabditis have been described [6]. At present, about 10 species of EPNs are commercially available and used worldwide for the insect pest management of crops. Most of these EPNs were isolated from either North America or Europe [7]. In India too, the EPNs have been studied in all ecological zones [8]. Since then, various species of *Steinernema* have been recorded, of which some are new to the science [9-10]. These nematodes can easily be mass multiplied on *in vivo* and *in vitro* production systems.

The present study was aimed to isolate EPNs from soil samples collected in 10 locations of the CCS HAU, Hisar, Haryana. However, introduction of EPN as biocontrol agents in one particular site requires prior knowledge of their occurrence and proper identification of native species. In this study, an extensive survey was conducted to isolate and identify EPNs from disturbed habitats in Haryana.

Material and Methods

Soil samples were taken from field area of CCS HAU, Hisar and other parts of Haryana during 2016-17 for isolation of EPNs. A total of 120 soil samples were collected from different locations with various microclimate, soil types, moisture regime and plant combinations i.e. ber, guava, citrus, cotton, groundnut, cabbage, cactus, seasum, mung bean and rose. Sampling was done by taking soil samples from five to six random sites at the depth of 8 to 10 cm and all the five subsamples were bulked to make composite sample of about 500 g.

Nematodes were recovered from the soil samples by using the insect-baiting technique. The presence of insect pathogenicity of nematodes was tested by baiting with wax moth larvae, *Galleria mellonella* [11]. The dead larvae were transferred individually to modified White traps [12]. The samples were periodically checked for the presence of dead insects. Nematodes were harvested on 2^{nd}, 4^{th}, 6^{th}, 8^{th} and 10^{th} day from bottles and EPNs were isolated by white trap method [13] which consisted of a tissue culture flask with distilled water to a depth of 1 cm. The larvae were placed on the filter paper and incubated at 25 ± 1^{0}C until all nematode progeny had emerged and moved down into the water in the container.

For identification, at generic level (on the basis of change of colour of dead insect larva: *Heterorhabditis* spp.-red/ brownish pink and *Steinernema* spp.-grey/white) and at species level, identification was done using morphological characters.Pure cultures of indigenous entomopathogenic nematodes, *Steinernema abbasi* and *Heterorhabditis indica* were prepared and maintained separately in late instar larvae of *G. mellonella*. Nematodes were multiplied using the methods of [14] and extracted with white traps and stored in a thin layer of distilled water at room temperature.

Results and Discussion

A total of 120 samples were collected during 2016-17 for isolation of EPNs. The total frequencies of occurrence of EPNs encountered in the surveyed crops were 7.5 per cent. The highest frequency of occurrence of EPNs (58.3%) was recorded in ber orchard with 7 samples positive for EPNs followed by citrus and cactus (8.3%). Several samples viz., guava, cotton, groundnut, cabbage, seasum, mung bean, rose and sapota, collected from the fields showed no results on the presence of the EPNs (**Table 1**). The percentage of positive samples for nematodes obtained in this study was 7.5 per cent, are comparable with other studies, i.e. [15] recorded a single nematode isolate at each of the 2.03 per cent positive sites, of which 15 were Steinernematid isolates and 7 were Heterorhabditid isolates representing a total of 4 species. Similar results were achieved by [16] who collected 415 soil samples from 15 localities of Sindh and Balochistan. EPNs were detected in 51 (12.2%) sites, 20 isolates (40%) were identified as Steinernema and 31 (60%) as Heterorhabditis, of which 23 were identified as *H. indica.*

Table 1 Crop based distribution of entomopathogenic nematodes in different localities of CCS HAU, Hisar

Sr. No.	No. of samples collected	Crops sampled	No. of samples positive for entomopathogenic nematodes						Frequency of occurrence
			EPNs		Steinernematids		Heterorhabditids		
			No.	%	No.	%	No.	%	
1.	Ber	12	7	58.3	5	41.7	2	16.7	58.3
2.	Guava	12	0	0	0	0	0	0	0
3.	Citrus	12	1	8.3	0	0	1	8.3	8.3
4.	Cotton	12	0	0	0	0	0	0	0
5.	Groundnut	12	0	0	0	0	0	0	0
6.	Cabbage	12	0	0	0	0	0	0	0
7.	Cactus	12	1	8.3	0	0	1	8.3	8.3
8.	Seasum	12	0	0	0	0	0	0	0
9.	Mung bean	12	0	0	0	0	0	0	0
10.	Rose	12	0	0	0	0	0	0	0
	Total	120	9	7.5	5	4.2	4	3.3	-

The probable reason for low or no infestation of EPNs in sample collected from fallow and other perineal crop is prevailing high temperature at the time of survey. This is supported by the studies of [17] who reported that temperature is one of the major climatic factors regulating temporal and spatial distribution of EPNs. The results indicated that the absence of nematodes in any of the soil samples, which might be due to the prevalence of high atmospheric temperature, low relative humidity and long dry spell, resulting in the suppression of growth and development of the EPNs in the soil. These results were further supported by [18] who studied that IJs of EPNs are very sensitive to environmental extremes such as temperature and relative humidity. It was observed that optimum level of temperature for development of IJs was 25°C, No development and reproduction of IJs could be observed when the atmospheric temperature goes more than 33°C and relative humidity was below 50 per cent.

The present study concluded that the occurrence of EPNs was more in perennial crops than in annual crops. This may due to the presence of insect pest buildup and undisturbed soil environment areas. [15] also reported the same finding in different ecosystems. In perennial crops, where the ecosystem remains largely undisturbed, provide substantial and constant food material for continuous breeding of insect populations, which are hosts for EPNs. All the nematode species were isolated from habitats with least soil disturbance i.e. fruit orchards. According to [19] the abundance of native EPNs can also be high in ecosystems where human impact is substantial, such as agricultural fields. The EPNs have been recorded by other workers from places with least soil disturbance like fruit orchards by [20] and [21] from India.

In the present study the infestation in ber orchard was more where the possibilities of soil born insects are more. This also is supported by [22] who observed that the highest percentages of nematode in soil samples were found where the grubs were present. In terms of percentage of positive samples by genera, 4.2 per cent of EPNs in the genus

Steinernema and 3.3 per cent in *Heterorhabditis*, which concurs with [22] who also found 67 per cent in *Steinernema* and 33 per cent in *Heterorhabditis*. Results of the present study revealed that the timing of EPN sampling contributed towards the level of EPN recovery. Surveys carried out during the heavy rain season, that is, end July-August, yielded more EPN compared to the surveys carried out at other periods of time.

The EPNs encountered during the survey were identified on the basis of the colour of dead cadver. It was observed that dead insect larvae turned to brick red/brown for heterorhabditid nematode infections and remained with natural colour or grey cream for steinernematid nematode infections in generic level and at species level, identification were done using morphological characters. Based upon the morphological parameters different strains of Steinernema and Heterorhabitis were identified as *S. abaasi* and *H. indica.* But the studies were carried out on different strains of Steinernema and Heterorhabitis. These results conformity with those of finding by [23] mentioned that the *G. mellonella* larvae infected with EPN of the genus *Heterorhabditidae* may have reddish-orange coloring while those infected with *Steinernema* spp. show gray-brown coloring.

Mass culturing of EPNs strain was done *in vivo* using *G. mellonella*. Individual strain of EPNs was maintained in lab. Studies were carried out on multiplication of the EPNs, *Steinernema* and *Heterorhabditis* using *G. mellonella* as the host insect, by inoculating with 5, 10, 20 and 40IJs per larva. The present studies revealed that as the inoculum level increased, there was an increase in progeny production of both *S. abaasi* and *H. indica*. This might be due to the reason that individual *G. mellonella* larva could accommodate more number of IJs that yielded maximum of 289800IJs of *H. indica* as compared to *S. abaasi* where a single larva of *G. mellonella* yielded only 137600IJs (**Figure 1**). A report of [24] where they have observed that the highest IJs ranging from *G. mellonella* by inoculating IJs ranging from 50 to 75 and they have recorded highest yield of *S. carpocapsae* at 50IJs inoculum level and of *H. bacteriophora* at 75IJs inoculum level.

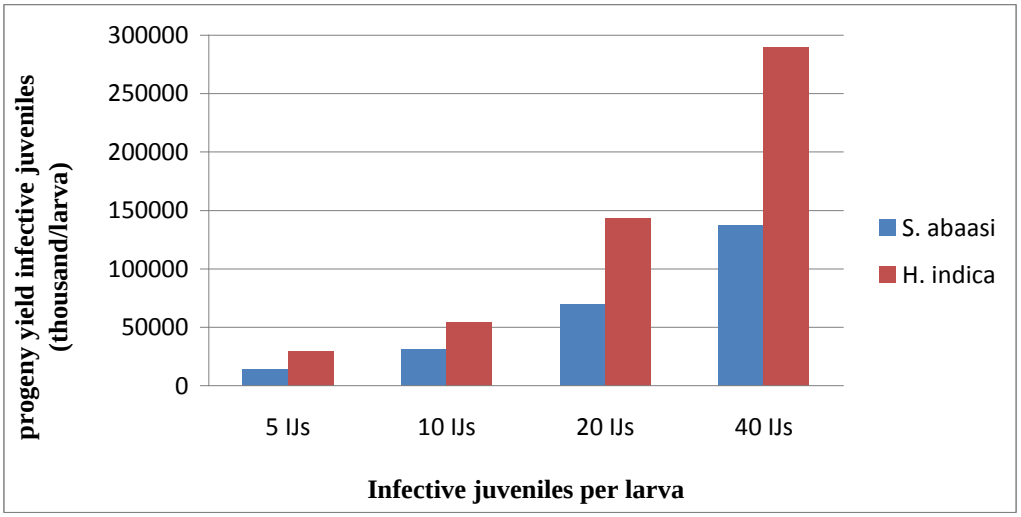

Figure 1 Mass multiplication of entomopathogenic nematodes on *Galleria mellonella*

Conclusion

On the basis of results obtained in present investigation, it may be concluded that, native isolates of biological control agents are better adapted to the local agro-climatic conditions. The search for local EPN isolates though systematic survey is the first critical step in building an effective biological management program for insect pests.

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