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Isolation of entomopathogenic nematodes from fruit orchards in mid-hills of Himachal Pradesh

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ABSTRACT

The studies were conducted to isolate and identify entomopathogenic nematodes from fruit orchards in Solan and Sirmour districts of Himachal Pradesh. Fifty six soil samples were collected from different localities and accessed for the presence of entomopathogenic nematodes using the soil baiting technique. Eleven soil samples (19.64%) collected from four locations were found to be positive for the presence of entomopathogenic nematodes. Six samples (10.71%) contained species of *Heterorhabditis* which was identified as *Heterorhabditis bacteriophora*. Five samples (8.93%) yielded *Steinernema* spp. One of the species was identified as *S feltiae*. One of the sites surveyed was found positive for both *Heterorhabditis* and *Steinerenema*.

Keywords: Fruit orchards; entomopathogenic nematodes; *Heterorhabditis*; *Steinernema*

INTRODUCTION

The use of nematodes in biological pest control has increased exponentially over the past few decades. Nematodes that parasitize insects have been described from 27 nematode families but the members of the families Steinernematidae and Heterorhabditidae also called as entomopathogenic nematodes (EPNs) have received the most attention because of their potential as inundatively applied biological control agents (Kaya and Gaugler 1993, Grewal et al 2005, Koppenhofer 2007). Entomopathogenic nematodes offer a non-toxic, environmentally safe and IPM compatible alternative to synthetic insecticides. Steinernematid and heterorhabditid nematodes carry symbiotic bacteria namely *Xenorhabdus* spp and *Photorhabdus* spp respectively in their gut (Boemare 2002) which are released in the insect haemoceol wherein the bacteria multiply and cause septicemia thus killing the host within 24-48 hours (Poinar 1990, Webster et al 2002). The developing nematodes feed on the bacteria and host tissues digested by the bacteria and develop through up to three

generations depending upon the nutritional status of host cadaver. The new infective juveniles emerge from the depleted host cadaver to search new hosts. Entomopathogenic nematodes occur in natural and agricultural soils around the world and are used for biological control of insects primarily soil dwelling insects. The entomopathogenic nematodes are important biocontrol agents against soil inhabiting insect pests as the nematodes and the insects share the common habitat. These nematodes have high searching ability, ease in mass production and application, can cause rapid host death and are safer to mammals and plants (Gaugler 1988). Worldwide over 85 species of entomopathogenic nematodes have been reported (Nguyen and Buss 2011, Lewis and Clarke 2012). The effectiveness of the entomopathogenic nematodes (EPNs) as biocontrol agents of crop pests can be enhanced by isolation or selection of better strains. The advantageous qualities of EPNs as biocontrol agents may include environmental tolerance, virulence, reproductive capacity etc (Shapiro-Ilan et al 2012). The local strains of entomopathogenic nematodes may be more effective as biocontrol agents against local pests because of their adaptability to climatic conditions of that area. Therefore the studies were conducted on isolation and identification of entomopathogenic nematodes from fruit orchards in mid-hills of Himachal Pradesh.

MATERIAL and METHODS

The entomopathogenic nematodes were isolated by using soil baiting technique (Bedding and Akhurst 1975). Soil samples were collected from different habitats for isolating entomopathogenic nematodes. About 500-1000 g soil samples were collected from the target sites. The samples so collected were put in polythene bags, labeled, tied with rubber band and brought to the laboratory for isolation of nematodes. Five to seven full grown larvae of *Corcyra cephalonica* were placed at the bottom of the plastic container (7.0 cm in diameter). After thoroughly mixing a soil sample about 250 g soil was taken and put over the *Corcyra* larvae and the plastic container was covered with muslin cloth. The plastic containers containing the soil samples and the trap host were incubated at 25 ± 1°C temperature. After 5-7 days the soil of the containers was checked for dead *Corcyra* larvae. These were collected and examined for the presence of nematodes.

The isolated nematode species were reared in *Corcyra* larvae to collect various development stages for identification of the same. These stages were killed in hot Ringer's solution, fixed in FA (4:1) and processed in glycerine by using the method given by Poinar (1975). The processed specimens were mounted in dehydrated glycerine and measured with the help of ocular micrometer calibrated with stage micrometer. The standard ratios used for identification were calculated as:

$$\text{Ratio a} = \frac{\text{Total body length}}{\text{Greatest body width}}$$

$$\text{Ratio b} = \frac{\text{Total body length}}{\text{Distance from head to the base of pharynx}}$$

$$\text{Ratio c} = \frac{\text{Total body length}}{\text{Tail length}}$$

$$\text{D (\%)} = \frac{\text{Distance from head to the excretory pore}}{\text{Distance from head to the base of pharynx}} \times 100$$

$$\text{E (\%)} = \frac{\text{Distance from head to the excretory pore}}{\text{Tail length}} \times 100$$

$$\text{Vulva (\%)} = \frac{\text{Distance of vulva from anterior end}}{\text{Total body length}} \times 100$$

$$\text{GS (\%)} = \frac{\text{Gubernaculum length}}{\text{Spicule length}} \times 100$$

$$\text{SW} = \frac{\text{Spicule length}}{\text{Anal body width}}$$

The specimens were identified as per keys given by Nguyen and Smart (1996) and Ganguly (2003).

RESULTS and DISCUSSION

Isolation of nematodes

The frequency of occurrence of entomopathogenic nematodes in the localities surveyed remained low (Table 1). Out of nine localities surveyed entomopathogenic nematodes were encountered in only four localities. *H bacteriophora* was isolated from orchard soil of Rajgarh and Bhuira (Sirmour district) with 20.00 and 66.67 per cent frequency of occurrence respectively. *S feltiae* was obtained only from Rajgarh with very low (10%) frequency of occurrence.

An unidentified species of *Steinernema* was isolated from apricot orchards at Nauni and Bagar (Solan district) with 16.67 and 75.00 per cent frequency of occurrence respectively. *H bacteriophora* and *S feltiae* were isolated from the orchards situated at higher altitudes (above 1500 m amsl) whereas the unidentified species of *Steinernema* was isolated from localities situated at altitudes of 1225 to 1385 m amsl. The occurrence of isolated entomopathogenic nematodes in present studies at different altitudes may be related to the climatic preferences of the nematode species. All the isolated species preferred mild climatic conditions for their survival, development and host infectivity. *H bacteriophora* and *S feltiae* preferred slightly cooler climate as compared to unidentified species of *Steinernema* sp as these species were isolated from higher altitudes.

Climatic preference of entomopathogenic nematodes has also been reported by Ganguly and Singh (2000) where they have isolated *S thermophilum* from soils of Delhi which required warm climate for its survival, development and hosts infectivity.

All the nematode species were isolated from habitats with least soil disturbance ie fruit orchards, surveyed in the present studies. The entomopathogenic nematodes have also been isolated by other workers from places with least soil disturbance like fruit orchards (Singh 1990, Chandel et al 2009) and forest soil (Sivakumar et al 1989, Lalramliana and Yadav 2010) from India. By using the soil baiting technique entomopathogenic nematodes have been isolated from various countries (Lewis and Clarke 2012).

$$\text{Frequency} = \frac{\text{Number of samples containing nematodes}}{\text{Total number of samples collected}} \times 100$$

Table 1. Occurrence of entomopathogenic nematodes in Solan and Sirmour districts of Himachal Pradesh

District	Locality	Altitude (m amsl)	Habitat/fruit tree orchard	# soil samples collected	# samples containing nematodes		
					Hb	Sf	*Steinernema* sp
Solan	Bagar	1385	Apricot	4	-	-	3 (75.00)
	Kandaghat	1450	Apricot, peach	8	-	-	-
	Kotla	1392	Peach	6	-	-	-
	Majhgaon	1539	Peach	3	-	-	-
	Nauni	1225	Apricot	6	-	-	1 (16.67)
	Ner	1389	Apricot, peach	5	-	-	-
Sirmour	Bhuira	1650	Apple	6	4 (66.67)	-	-
	Kheradhar	2032	Apple, pear	8	-	-	-
	Rajgarh	1500	Apple, apricot, peach	10	2 (20.00)	1 (10.00)	-

Hb= *Heterorhabditis bacteriophora*, Sf= *Steinernema feltiae*, Figures in parentheses indicate frequency of occurrence (%)

Identification of entomopathogenic nematodes

The population of *Heterorhabditis* from Rajgarh and Bhuira (Sirmour district) when compared with the described species (Poinar 1976, Nguyen and Smart 1996, Singh and Gupta 2006) resembled *H bacteriophora* (Poinar 1976). The diagnostic morphometric characters as presented in Table 2 include IJs: average body length of 542.44 (515.04- 589.04) µm, tail length of 91.59 (84.36-97.68) µm and E (%) 108.24 (106.06-115.87), male spicule length 39.08 (35.00-42.92) µm and GS (%) 50.00. This species differ from *H indica* (Poinar et al 1992) in having higher body length (542.44 µm) and E (%) (108.24) as compared to *H indica* (528 µm and 94% respectively). The population of *Steinernema* isolate from Rajgarh was identified as *S feltiae* (Filipjev 1934, Poinar 1990) as it shared most of the characters used for identification of this species (Nguyen and Smart 1996, Singh and Gupta 2006). The diagnostic characters included IJs: average body length 808.05 (740.00-890.96) µm, distance from anterior end to excretory pore 63.92 (55.00-69.59) µm and without a double horn like structure on labial region and in male: D (%) 58.08 (50.00-72.73), spicule length 69.08 (65.00-75.00) µm and presence of mucron. This population can be separated from *S neocurtillis* (Nguyen and Smart 1996) on the basis of its greater distance of excretory pore from anterior end ie 63.92 µm in present population against 18.00 µm in *S neocurtillis*. The species of *Steinernema* collected from Nauni and Bagar (Solan district) could not be identified.

Table 2. Morphometrics of different stages of indigenous strain of *Heterorhabditis bacteriophora*

Character	Males (n= 10)	Females (n= 10)	Infective juveniles (n= 30)
Total body length (µm)	772.45 (735.00-807.00)	1991.65 (1875-2112.50)	542.44 (515.04-589.04)
Greatest body width (µm)	36.85 (32.56-41.44)	97.75 (82.50-112.50)	27.74 (20.72-31.08)
Distance of the nerve ring from the anterior end (µm)	68.25 (65.00-72.50)	-	86.21 (81.40-93.24)
Distance of the excretory pore from the anterior end (µm)	113.00 (110.00-120.00)	-	102.46 (91.00-105.80)
Distance of the pharynx from the anterior end (µm)	99.85 (96.20-105.00)	135.15 (125.00-150.00)	119.21 (111.00-130.00)
Length of the tail (µm)	25.50 (22.50-30.00)	53.75 (50.00-60.00)	91.59 (84.36-97.68)
Ratio a	21.02 (19.47-22.57)	20.49 (18.78-22.84)	19.82 (18.57-24.86)
Ratio b	7.73 (7.64-7.80)	14.75 (13.55-15.70)	4.57 (4.13-4.67)
Ratio c	30.49 (29.00-33.20)	37.15 (34.86-39.86)	5.96 (5.82-6.11)
D%	-	-	82.93 (81.00-86.00)
E%	-	-	108.24 (106.06-115.87)
Length of spicule (µm)	39.08 (35.00-42.92)	-	-
Length of gubernaculum (µm)	19.45 (15.00-25.00)	-	-
Body width at anus (µm)	36.85 (32.56-41.44)	-	-
GS (%)	50.00 (43.00-59.00)	-	-
Vulva (%)	-	45.03 (43.24-48.82)	-
SW	1.06 (1.01-1.10)	-	-

Figures in parentheses represent the range

Table 3. Morphometrics of different stages of indigenous strain of *Steinernema feltiae*

Character	Males (n= 10)	Females (n= 10)	Infective juveniles (n= 30)
Total body length (µm)	854.70 (767.50-922.50)	3735.00 (2310.00-5060.00)	808.05 (740.00-890.96)
Greatest body width (µm)	70.38 (67.70- 73.33)	203.00 (162.50-237.50)	29.75 (26.64-32.56)
Distance of nerve ring from anterior end (µm)	98.00 (87.50-112.50)	114.25 (100.00-125.00)	96.31 (85.00-97.68)
Distance of excretory pore from anterior end (µm)	76.00 (62.50-100.00)	80.75 (70.00-87.50)	63.92 (55.00-69.59)
Distance of pharynx from anterior end (µm)	132.16 (125.00-137.50)	175.75 (150.00-212.50)	123.75 (120.00-137.50)
Length of tail (µm)	24.75 (22.50-27.50)	38.60 (37.50-52.50)	69.82 (66.60-74.00)
Ratio a	12.09 (11.10-13.47)	18.09 (14.22-21.90)	27.20 (26.30-28.10)
Ratio b	7.19 (6.02-7.32)	20.80 (15.40-25.86)	6.35 (6.17-6.48)
Ratio c	34.65 (30.70-40.76)	83.49 (61.60-107.78)	10.53 (9.46-11.64)
D%	58.08 (50.00-72.73)	-	49.44 (43.00-51.00)
E%	-	-	84.07 (81.56-89.80)
Length of spicule (µm)	69.08 (65.00-75.00)	-	-
Length of gubernaculum (µm)	42.45 (37.50-47.50)	-	-
Body width at anus (µm)	56.00 (55.00-57.00)	-	-
GS (%)	62.00 (50.00-75.00)	-	-
Vulva (%)	-	49.10 (46.75-58.50)	
Length of tail spine (µm)	4.83 (4.50-5.20)	-	-
SW	1.30 (1.16-1.36)	-	-

Figures in parentheses represent the range

CONCLUSION

The studies conducted on entomopathogenic nematodes from fruit orchards in Solan and Sirmour districts of Himachal Pradesh showed that out of fifty six soil samples collected using the soil baiting technique eleven (19.64%) were found to be positive for the presence of entomopathogenic nematodes. The indigenous strains of entomopathogenic nematodes isolated during present studies can be utilized for control of local insect pests like cut worms, white grubs etc of the horticultural crops in the mid-hills of Himachal Pradesh.

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