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Studies of indigenous isolates of entomopathogenic nematodes in Hisar, Haryana ecosystem

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Abstract
Entomopathogenic nematodes (EPNs) of the genus *Steinernema* Travassos, 1927 and *Heterorhabditis* Poinar, 1976 are effective bio control agents for the wide variety of soil dwelling insect pests. They show better performances over chemical and microbial insecticides. But for the better performances EPNs should be adapted to the local environmental conditions. Hence the isolation and proper recognition of EPNs are decisive for the success of their use as bio pesticides. The populations of *Steinernema* and *Heterorhabditis* were isolated by '*Galleria* trap' method from the soils around the roots of various crops from Hisar, Haryana, India. Their morphological characters and morphometrics were studied to identify whether they are new or already known species. The species identified were, *S. pakistanense* Shahina *et al*. 2001 (three populations), S. *bicornutum* Tallosi *et al*. 1995, *S. siamkayai* Stock *et al*. 1998 with some intraspecific variations from the original descriptions. Hence it was concluded that in southern Haryana soils where texture is mostly sandy or sandy loam, the occurrence of *Steinernema* was more compared to *Heterorhabditis* species.

Keywords: Identificationm *Steinernema*, *Heterorhabditis*, entomopathogenic nematodes, species

Introduction
Entomopathogenic nematodes (EPNs) have a global distribution in the broadest geographic sense and exhibit differences in suitability of host range, virulence and environmental tolerance. Soil surveys have been conducted in different areas of the world extensively and has observed variability in abundance among the species across seasons, habitats and geographic regions. Factors such as soil texture, moisture content, temperature and host availability are thought to be important in determining distribution of entomopathogenic nematodes [8]. Compared with human modified areas, natural habitats offer increased opportunities for finding native nematode species [15]. Generally, EPNs have been trapped from soil samples using insect baiting technique [4]. The isolation of native species provides a valuable resource not only from a biodiversity perspective but also from a more applicable stand point in commercial aspects. Indigenous EPNs may be more suitable for inundative release against local insect pests because of adaptation to local climate and other population regulators. In addition many countries are concerned about the introduction of exotic EPNs because they may have negative impact on non target organisms [3].

There is a more considerable restriction in field use because some species have narrow host range. If more progress is done in field research, improved insect-nematode matches may become established. Regrettably nematodes have yet to be discovered which are effective against several of the most important soil insects, including wireworms, grape-vine Phylloxera, fire ants or corn root worms. The EPNs are constraint to specific conditions like other biocontrol agents as they are effective within a narrower temperature ranges than chemicals and more impacted by sub-optimal soil type, depth and irrigation frequency [7]. Hence there is now a tremendous need for the discovery of new nematode strains adapted to the local environmental conditions and pests especially in India, in association with growth and reproduction of nematodes that are affected by ecological parameters. The early identifications were carried out by insect pathologists or nematologists and insight view of taxonomists is lacking. In fact, most of the workers engaged in EPN research in India find it hard to identify the species due to lack of adequate expertise in the extraction of adults from insects, processing techniques, and non availability of compiled literature.

The taxonomic information on EPNs were earlier compiled by Poinar (1990) [12] and Homnick *et al*. (1997) [10]. In 2007, Nguyen and Hunt have brought out a monograph useful for fellow taxonomists. Also much of the work of EPNs has been done in temperate regions and comparative efforts are lacking in investigating tropical and sub-tropical soils. Most of the Steinernematids were thought to be found only in low temperature environment however, since the discovery of *S. riobravis* Cabanillas *et al*. 1994 [5] from USA, there are reports of this species from the hot countries like India (6). The collection of indigenous species or strains may provide more suitable biocontrol agents for the inundative release against local insect pests because of their adaptation to local climate and population regulators.

Hominick *et al*. (1997) [10] chartered out certain norms at an international conference to describe new species which involved studies on morphology, morphometrics, cross breeding and molecular characterization. For a biocontrol agent to be successful it should be amenable for mass production on a large scale, the ready availability of the organism in required quantity and at competitive cost makes them acceptable among entrepreneurs and farmers. Over the years many isolates of EPNs have been collected from Haryana soils. Though Haryana, is a small state it represents diverse agro-ecological zones. The north eastern zone comprises of Shivalik hilly region and alluvial plains and the western zone consists mainly of alluvial plain. The major pests are rice root weevil, leaf folder, brown plant hopper, stem borer, sugarcane pyrilla, cotton boll worms, mustard aphids, cabbage diamond back moths, etc. Hence for the successful control of these pests the local isolates have to be collected, identified up to species level and their mass multiplication methods are to be standardized to use them in field against insect pests. Hence, the present studies have conducted to describe the already existing species of EPNs in Hisar district of Haryana state of India.

About Hisar: Hisar is west central most district of Haryana State of India with the geographical area of 3983 sq. Km. and lies between the north latitudes 28° 56' 00'': 29° 38' 30'' and East longitudes 75° 21' 12'': 76° 18' 12''. It falls in Yamuna sub basin of Ganga basin having no natural drainage with the normal annual rainfall of about 330nm. Coming to the soil part, it is of three types *i.e.* arid brown solonized, sierozem and desert soils.
(http://cgwb.gov.in/District_Profile/Haryana/Hissar.pdf)

Materials and methodology

Morphological and Morphometric studies

Preparation of artificial diet

The artificial diet for culturing of *Galleria mellonella* larvae was prepared using the following constituents:

Part A: Corn flour- 4 parts
Wheat flour- 2 parts
Wheat bran – 2 parts
Milk powder- 2 parts
Yeast extract- 1 part

Part B: Honey- 2 part
Glycerine- 2 part

The ingredients of Part A were mixed thoroughly in a pan. The ingredients of Part B were mixed separately in a beaker. The mixture was slowly and continuously added to Part A and mixed thoroughly taking care that no lumps are formed as well the mixture is not too wet. The prepared diet was stored for at least 24 hours before use.

Culturing of *Galleria mellonella*

G. mellonella was collected from the stock cultures maintained in bio-control laboratory, Deptt. of Nematology, CCS HAU, Hisar. Freshly laid eggs of the moth were replaced in a glass jar along with some diet. The mouth of the jar was covered with cloth which was held in position by the rubber bands. These jars were placed in an incubator at 25±1°C. Once the eggs hatched, the larvae were given diet regularly. The adults were collected everyday and transferred into another glass jar with folded papers in a zigzag manner along with 10% sucrose/honey solution given in small Petri dishes. The jars were again kept in a BOD incubator at 25±1°C. These papers with laid eggs were cut and placed in fresh jars with diet. Staggering cultures of the larvae were maintained for use during the course of entire studies.

Isolation of nematodes from soil

Fifteen isolates of *Steinernema* and *Heterorhabditis* were collected from the soil around the roots of different crops in various districts of Haryana by the insect trap method [4]. Fifth instar larvae of *G. mellonella* (5- 10) cultured on the artificial diet were placed at the bottom of the 500 cc glass container and filled with 200 cc soil sample. The glass containers were kept covered with the lid and placed at 25±1°C. The containers were examined for the dead larvae for 1-2 days. The cadavers were washed with distilled water and placed on filter paper lined in a Petri dish. The Petri dishes were covered with lids with proper labeling and placed in the polythene bags to conserve moisture and kept in an incubator at 25±1°C for 2- 7 days. Infective juveniles were extracted from the cadaver by 'White Trap' method [19]. In this method the watch glass was kept in an inverted position and was placed in the Petri dishes. Then a small filter paper was cut to the size of watch glass and placed on it. It was moistened with distilled water and the cadavars were placed on moistened filter paper. The Petri dishes were sealed with parafilm and kept at 25±1°C in an incubator for 2- 7 days, stored inside the polythene bags to preserve the moisture. The emerged IJs were collected in the sterile distilled water and stored in tissue cultured flasks at 15±1°C in a BOD incubator. For the prolonged storage, the flasks with perforated lids were used.

Isolation of 1st and 2nd generation adult nematodes from greater wax moth larvae

Nematode suspension containing 500 IJs in 2 ml sterile water was used to infect *G. mellonella* larvae placed in the Petri dish lined with a double layered filter paper. Dead cadavers were dissected in Ringer's solution 2 days after mortality to extract mature first generation males and females. Some of the cadavers were kept further for extraction of 2nd generation nematodes. These cadavers were dissected as above to extract 2nd generation males and females.

Preparation of nematode mounts on slides

For light microscope observations, IJs and adults of two generations of *Steinernema* and *Heterorhabditis* of all the collected populations were extracted as explained above and fixed in hot TAF (Triethanolamine-2 ml + 37% Formalin- 8 ml + sterile distilled water - 90 ml). The fixed nematodes were processed to dehydrated glycerine by Seinhorst's (1959)

method. Cleared specimens were mounted in dehydrated glycerine on the glass slides along with appropriate glass support. The cover slip was secured in position after sealing with nail polish.

Following morphometric characters were recorded:

L = Body length
MBD = Maximum body diameter
EP = Distance from anterior end to excretory pore
ES = Pharynx length
T = Tail length
H = Hyaline length
ABD = Anal body diameter
a = L/MBD
b = L/ES
c = L/T
c' = ABD/ T
V = Distance from anterior end to vagina
SL = Spicule length
GL = Gubernaculum length
D % = EP/ES ×100
E % = EP/T × 100
SW % = SL/ABD × 100
GS % = GL/SL × 100
H % = Hyaline length/ T × 100

Results

***Steinernema bicornutum* tallosi *et al*. 1995 (Fig. 1.)**

Measurements (Table 1.)

First generation male

Body curved ventrally at posterior end, tapering toward lip region. Cuticle with fine annulations. Cephalic extremity rounded bearing six labial and four cephalic papillae. Excretory pore located anterior to nerve ring. Stoma short, shallow and triangular at base, *ca* 4 µm long and 5 µm wide. Cheilorhabdions distinct, sclerotized, other rhabdions not distinguishable, forming a funnel-shaped structure below cheilorhabdions. Procorpus extending into a slightly swollen nonvalvated metacarpus, isthmus narrow, basal bulb valvated. Cardia present, intestine with a wide lumen. Genital system monorchic and reflexed. Spicules paired, symmetrical, moderately curved, light yellowish brown in colour under light microscope, spicule tip pointed; manubrium elongated, broad, ventrally projected, rostrum prominent, calomus distinctly separating manubrium from lamina, lamina well curved anteriorly and dorsally with two internal ribs of unequal size, straight posteriorly; velum distinct, extending from base part of rostrum to distal part of lamina. In lateral view, gubernaculum boat shaped, anterior part ventrally curved with hook like structure and enlarging to form corpus with two wings. In ventral view, cuneus long, needle-shaped. Tail bluntly conoid, usually concave at ventral side, mucron absent. Genital papillae, 25 in number comprising 12 pairs and a single ventral papilla, arranged as follows: five pairs located precloacal subventral, one pair at spicule end, two pairs postcloacal sub-ventral side, three pairs at tail end (one subventral, one ventral subterminal and one dorsal subterminal) and one at the level of gubernaculum head region.

Second generation male

Similar to the first generation males in other characters except shorter length and body width including spicules and gubernaculum. Tail short but slightly elongated, dorsally convex, mucron absent.

First generation female

Body spiralled coiled when heat-relaxed. Head rounded, continuous with body. Cuticle smooth. Cephalic extremity rounded, continous with the body. Cheilorhabdions prominent, well sclerotized, posterior part funnel-shaped. Procorpus cylindrical, muscular; metacorpus swollen; isthmus distinct; basal bulb enlarged, valvate. Nerve ring surrounding isthmus, just anterior to basal bulb. Pharyngo-intestinal valve present. Excretory pore located near metacorpus. Genital tract amphidelphic with reflexed ovaries; vulva symmetrical and transverse slit slightly protruding from the body contour. Vulval opening at mid-body with small vulval lips; epitygma absent. Postanal swelling absent. Tail shorter than anal body diameter, dorsally convex with bluntly rounded terminus, mucron present.

Second generation female

Body an open C shape when heat-relaxed, tapering posteriorly. Similar to first generation female but smaller in length and width. Vulva with symmetrical protuberance and situated at midbody; epiptygma not observed. Postanal swelling slightly present. Tail longer than anal body diameter, conoid and with fine terminus.

Infective juvenile

Heat relaxed specimens, straight; tapering towards anterior and posterior ends. Cuticular striations very fine under light microscope. Labial region with two horn like structures, continuous with body. Lateral field having 9 incisures (8 ridges). Head region rounded to slightly truncate and usually slightly offset from the body contour, not annulated. Stoma funnel shaped. Pharynx with slightly expanded procorpus, narrower isthmus and subpyriform basal bulb. Excretory pore in mid-pharynx region anterior to nerve ring, cuticularized. Cardia present. Intestine filled with numerous fat globules, lumen not visible. Rectum straight, anus sickle-shaped. Tail conoid, terminus pointed.

Host and Locality

Host is unknown and collected by the *Galleria* bait method from around the roots of tomato *Solanum lycopersicum* Dunal plants CCS HAU, Hisar, Haryana, India.

***Steinernema pakistanense* shahina *et al*. 2001 (figs. 2.1, 2.2, 2.3)**

Measurements (Table 2.)

Description

First generation males

Body curved ventrally, J-shaped when fixed. Cuticle smooth. Head slightly truncate and continuous with the body. Stoma shallow. Oesophagus muscular; procorpus cylindrical; metacorpus slightly swollen non-valvate; isthmus distinct; basal bulb pyriform; pharyngo-intestinal valve distinct. Nerve-ring located just anterior to basal bulb. Cardia prominent and protruding into intestinal lumen. Excretory pore little above the nerve ring *i.e.* behind middle of oesophagus. Testis single, reflexed on ventral side. Spicules paired, yellowish-brown in colour, large, strongly curved; manubrium small rounded; blade arcuate, with straight tip, rostrum prominent, internal ribs unequal, velum large, not covering spicule tip. Gubernaculum *ca* 60% of spicule length, boat-shaped in lateral view, swollen in middle, with ventrally

curved knob at proximal end; in ventral view, cuneus long, bifurcate, reaching end of corpus; corpus separated posteriorly. Single ventral precloacal papilla and 11 pairs of genital papillae, latter arranged as follows: six pairs precloacal subventral, one pair subventral at spicule end, one post cloacal immediately below the cloacal opening, three at tail end- one pair subdorsal, one subterminal at the tail tip, one subventral. Tail conoid, without mucron. Phasmids inconspicuous.

Second generation males

Similar to first generation males but have lesser body dimensions. Spicule and gubernaculum similar in shape but are smaller than first generation males. Tail terminus conoid and elongated.

First generation female

Body spirally coiled when relaxed by heating. Cuticle thin and smooth. Cephalic extremity truncate to slightly round, not set-off. Lip region with 6 labial and 4 cephalic papillae. Amphids inconspicuous. Stoma shallow well developed with sclerotized cheilorhabdions. Oesophagus with anterior cylindrical portion, metacorpus slightly swollen followed by isthmus, basal bulb pyriform valvate. Excretory pore at the level of midbulb but anterior to isthmus. Cardia prominent, protruding into intestinal lumen. Didelphic, amphidelphic ovaries reflexed and filled with eggs. Vulva as a transverse slit, with small vulval lips, in middle of body. Vagina short, oblique with muscular walls. Tail with rounded terminus, shorter than anal body width, with peg like structure present.

Second generation female

Similar to first generation females in general morphology, but smaller. Tail pointed.

Infective juvenile

When heat killed, body straight. Mouth and anus closed. Two horn like structures present on the labial region. Lateral fields at mid-body with uniform 7 ridges (8 ridges). Oesophagus long, narrow; isthmus distinct, surrounded by nerve ring; basal bulb elongate, with valve. Cardia prominent. Excretory pore near middle of oesophagus. Tail long, narrowed in hyaline region. Hyaline region distinct, and occupying about half of the tail length.

Table 1: Morphometrics of first generation males and infective juviniles of *Steinernema bicornutum* (tomato population) and *Steinernema bicornutum* Tallosi *et al*. 1995. Measurements are in μm (mean ± SD)

Steinernema bicornutum				
Character	**Tomato population**		**Serbia population**	
	First generation male *	**Infective juvenile ***	**First generation male ***	**Infective juvenile ***
n	20	20	20	20
L	1414.7 ± 108.7 (1204.3- 1598)	772 ± 50 (691.5- 880.6)	1353 ±149 (945- 1539)	770 ± 52 (648- 873)
a	12.3 ± 1.4 (10.4- 15.2)	26.6 ± 1 (24.1- 28.9)	-	26.5 ± 1.5 (23- 29)
b	9.5 ± 0.9 (8.1- 11.4)	7.2 ± 0.4 (6.5- 8.6)	-	6.2 ± 0.3 (5.6- 6.9)
c	46.2 ± 5.7 (34.4- 58.3)	11.7 ± 0.5 (10.7- 12.6)	-	10.7 ± 0.06
c’	0.6 ± 0.1 (0.4- 0.9)	4 ± 0.8 (2.6- 6.5)	-	-
Body diameter	115.4 ±10.8 (100- 140)	29 ± 1.9 (26- 32)	108 ± 11 (80- 128)	-
EP	90 ± 7.2 (80- 107)	52 ± 2.4 (49- 57)	82±7.8 (67- 98)	-
ES	148 ± 7.6 (133- 162)	108.1 ± 10.9 (80- 126)	156 ± 7 (138- 167)	-
Tail length	30.8 ± 2.9 (26- 37)	65.9 ± 4.4 (59- 72)	32 ± 2.5 (25- 35)	72.5 ± 5 (63- 78)
Hyaline length	-	31.05 ± 1.2 (29-33)	-	-
Anal body diameter (ABD)	47.7 ± 5.3 (35- 57)	16.8 ± 3.2 (11- 26)	-	-
Spicule length	71.5 ±6.2 (60- 82)	-	65 ± 4.3 (53- 70)	-
Gubernaculum length	36.9 ± 2.7 (33- 43)	-	47 ± 3.5 (38- 50)	-
D%	61.2 ± 5.6 (51.9- 75.2)	48.6 ± 5.9 (39.6- 65)	50 ± 3 (50- 60)	50 ± 3 (40- 60)
E%	295.7 ± 38.2 (228.5- 384.6)	79.3 ± 7.2 (70- 93.3)	-	80 ± 6 (80- 100)
SW%	152.2 ± 25.4 (105.2- 222.8)	-	-	-
GS%	52 ± 6.6 (43.5- 69.3)	-	72	-
H%	-	47.2 ± 2.7 (42- 51.6)	-	45 (37- 55)

*Figures in parentheses are in ranges

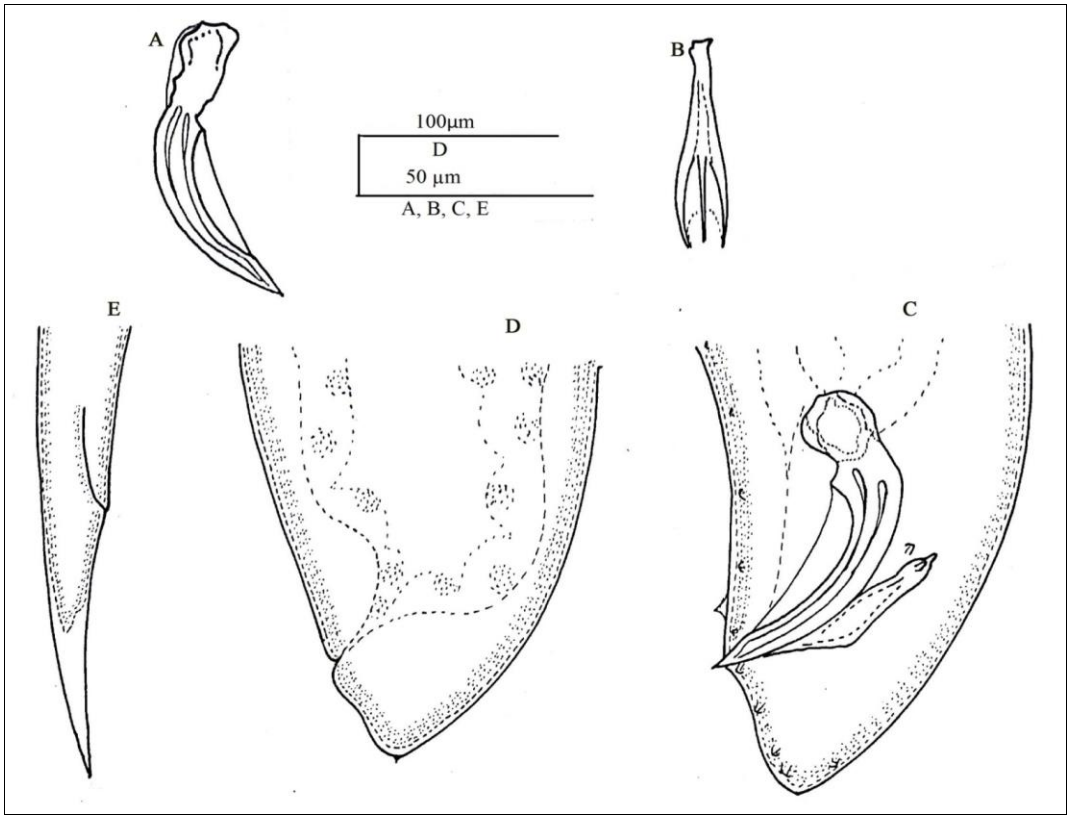

Fig 1: *Steinernema bicornutum* (tomato population): A-C: First generation male. A: Spicule; B: Gubernaculum; C: Tail. D: Tail of first generation female. E: Tail of infective juvenile

Table 2: Morphometrics of first generation male and infective juvenile of three Indian populations and *Steinernema pakistanense* Shahina *et al.*, 2001. Measurements are in µm (mean ± SD) *Steinernema pakistanense*

Character	Okra population		Neem population		Jujube population		Pakistan population	
	First generation male *	Infective juvenile *	First generation male *	Infective juvenile *	First generation male *	Infective juvenile *	First generation male *	Infective juvenile *
N	20	20	20	20	20	20	20	20
L	1216.2 ± 104.8 (1046.3- 1385.6)	660 ± 30.9 (611.2- 740.7)	1835.2 ± 132.2 (1600.6 - 2072)	615.2 ± 33.2 (556.8 - 678.5)	1614.3 ± 140.1 (1320 - 1813)	656.3 ± 32 (595.7 - 720)	1357 ± 89 (1163- 1505)	683 ± 21 (649- 716)
A	12.3 ± 1.3 (10.4- 14.7)	24.7 ± 2.4 (20.2- 30.6)	14.2 ±12- 1.1 (12 - 15.9)	24 ± 0.9 (22.3 - 26.1)	13.7 ± 0.7 (12.4 - 14.9)	25.4 ± 1.8 (22 - 29.2)	13.4 ± 1.8 (11- 16)	24 ± 1.5 (21- 27)
B	9.4 ± 0.5 (8.7- 10.2)	6.5 ± 0.4 (5.9- 7.5)	12.2 ± 0.9 (10.8 - 13.7)	5.8 ± 0.3 (5.3 - 6.3)	11.9 ± 1 (9.3 - 13.3)	6 ± 0.3 (5.3 - 6.6)	10.4 ± 0.6 (8.7- 11)	6 ± 0.3 (5- 6)
C	47.4 ± 4.6 (38.7- 52.6)	12.8 ± 1.2 (11.2- 15.1)	56.3 ± 8 (42.3 - 75.8)	11.1 ± 0.7 (9.7 - 12.4)	50 ± 4.9 (41 - 61.6)	11.4 ± 0.7 (9.69 - 13)	55 ± 3.3 (52- 62)	11 ± 0.5 (10- 12)
c’	0.6 ± 0.06 (0.53- 0.71)	3.4 ± 0.3 (2.7- 3.7)	0.6 ± 0.09 (0.5 - 0.8)	3.4 ± 0.3 (3 - 4.2)	0.6 ± 0.09 (0.5 - 0.9)	3.4 ± 0.2 (3 - 4)	-	-
Body diameter	99.2 ± 7.8 (87- 110)	26.8 ± 2.2 (23- 52)	129.3 ± 8 (117 - 145)	25.6 ± 1.4 (23 - 28)	117.5 ± 12.2 (100 - 136)	25.9 ± 1.6 (23 - 28)	102 ± 10.2 (80- 128)	27 ± 1.2 (24- 29)
EP	79.3 ± 9.2 (62- 88)	41.5 ± 2.2 (40- 52)	105.3 ± 5.8 (91 - 116)	48.9 ± 3.4 (44 - 55)	102.6 ± 10.3 (77 - 125)	51.2 ± 1.7 (48 - 55)	81 ± 4.8 (72- 92)	54 ± 2.2 (49- 58)
ES	128.5 ± 5.3 (119- 136)	101.3 ± 4 (94- 107)	151.1 ± 7.6 (135 - 165)	104.9 ± 4.1 (97 - 112)	140.8 ± 8.3 (125 - 154)	108.8 ± 4.7 (100 - 117)	132 ± 5.8 (126- 146)	113 ± 4.2 (108- 122)
Tail length	25.5 ± 1.9 (22- 29)	51.8 ± 3.8 (44- 62)	33.1 ± 4.6	55.6 ± 4.9 (47 - 66)	32.4 ± 2.8 (28 - 37)	57.6 ± 2.5 (52 - 62)	25 ± 0.8 (24- 27)	58 ± 2.1 (53- 62)
Hyaline length	-	27 ± 2.8 (22- 33)	-	26.5 ± 2.4 (21 - 32)	-	28.7 ± 2.7 (23 - 36)	-	-
Anal body diameter (ABD)	41.3 ± 3.6 (37- 47)	15.2 ± 1.2 (13- 17)	50.9 ± 4.9 (40 - 59)	16.2 ± 1.4 (14 - 20)	46.8 ± 4.3 (38 - 52)	16.5 ± 0.8 (15 - 18)	36 ± 2.3 (32- 40)	-
Spicule length	64.9 ± 1.02 (63- 67)	-	78 ± 4.7 (70 - 87)	-	70.6 ± 2.2 (65 - 75)	-	68 ± 3.6 (62- 73)	-
Gubernaculum length	39.9 ± 2.4 (34- 43)	-	40.8 ± 2.3 (36 - 46)	-	40.3 ± 1.6 (36- 44)	-	41 ± 3.2 (36- 45)	-
D%	62.3 ± 6.5 (50- 70.8)	44.9 ± 2.4 (42- 52)	69.8 ± 5.1 (63.6 - 84.6)	46.7 ± 3.9 (40.1 - 56.1)	73 ± 8 (54.2 - 88.8)	47.2 ± 2.6 (42.4 - 52)	60 ± 3 (50- 60)	47 ± 2.7 (42- 35)
E%	314.8 ± 49.2	88 ± 5.2	324.2 ± 50.4	88.3 ± 7	317.8 ± 37.2	89 ± 4.9	310 ± 40	91 ± 5

	(50- 70.8)	(81.2- 100)	(245.2 - 460)	(74.6 - 100)	(274.2 - 446.4)	(80 - 101.9)	(210- 370)	(87- 102)
SW%	158.4 ± 14.8 (134- 178.3)	-	154 ± 18.9 (125 - 192.5)	-	152 ±14.5 (130 - 184.2)	-	189	-
GS%	60.6 ± 3.8 (51.5- 66.2)	-	52.5 ± 4.5 (41.3 - 60)	-	57.2 ± 3.2 (49.3 - 62.8)	-	60.3	-
H%	-	52 ± 3.4 (46- 60)	-	47.8 ± 4.5 (39.3 - 56)	-	49.8 ± 4.5 (41.8 - 63.2)	-	50

* Figures in the parentheses are ranges

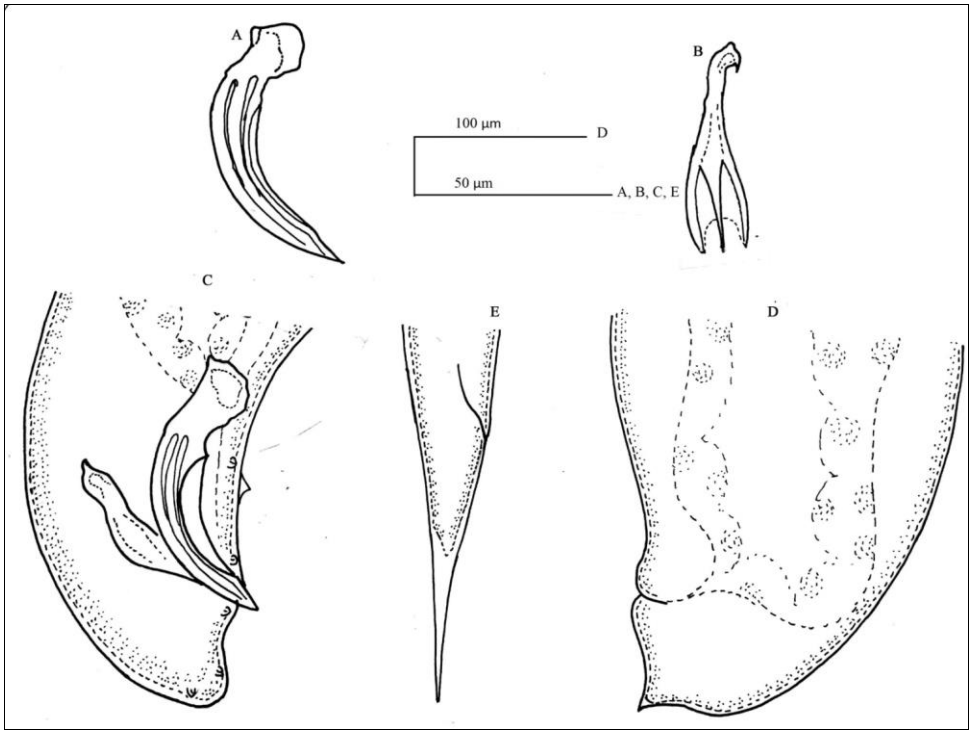

Fig 2.1: *Steinernema pakistanense* (okra population): First generation male (A-C): A. Spicule, B. Gubernaculum, C. Tail, D. Tail of first-generation female, E. Tail of infective juvenile

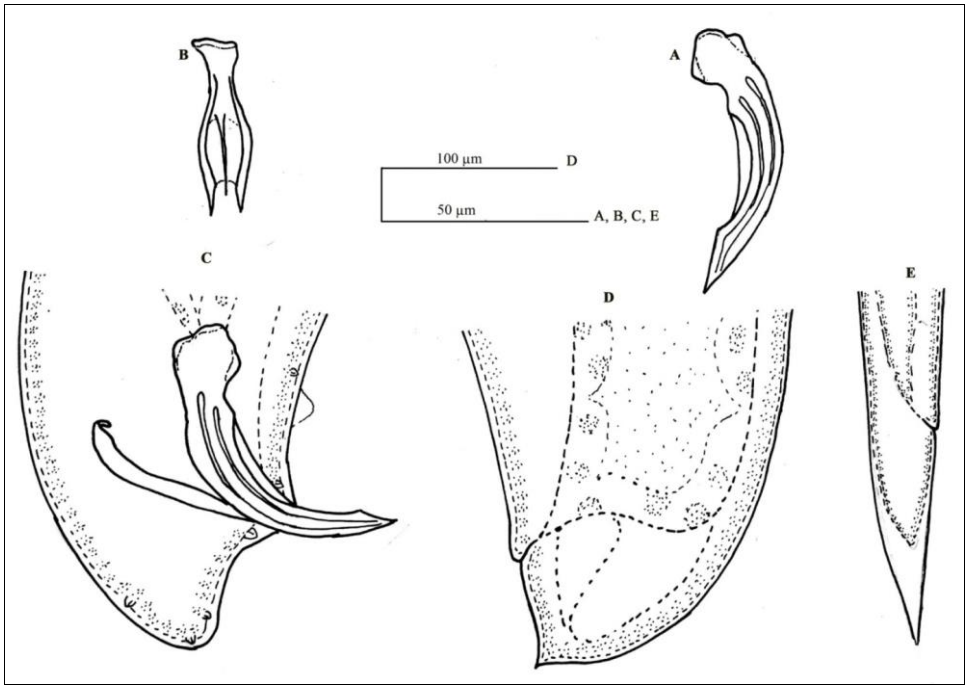

Fig 2.2: *Steinernema pakistanense* (neem population): A-C: First generation male. A: Spicule; B: Gubernaculum; C: Tail. D: Tail of first generation female. E: Tail of infective juvenile.

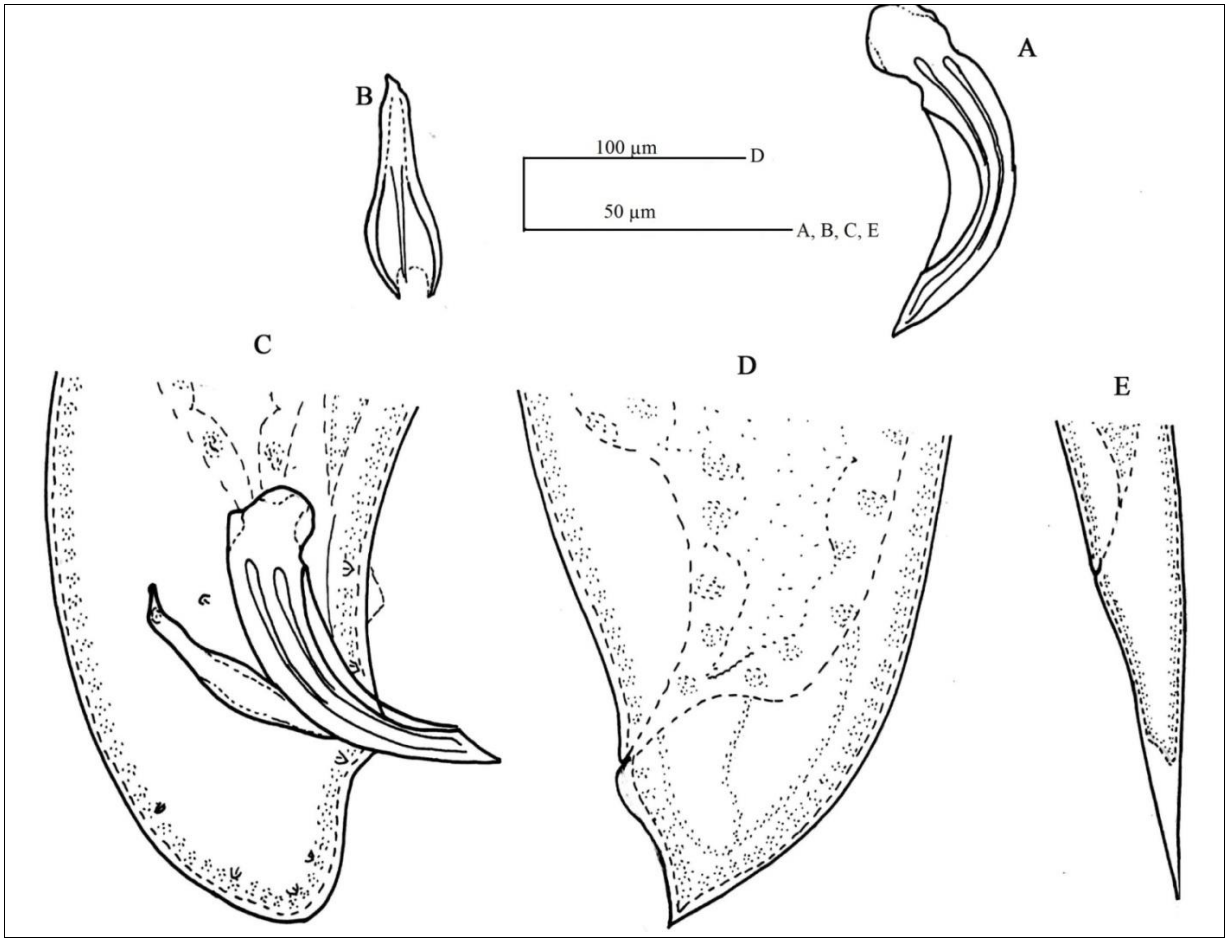

Fig. 2.3: *Steinernema pakistanense* (jujube population): A-C: First generation male. A: Spicule; B: Gubernaculum; C: Tail. D: Tail of first generation female. E: Tail of infective juvenile.

Host and locality

S. pakistanense has been isolated by the Galleria bait method from the soils around the roots of neem tree *Azadirachta indica*, okra plant *Abelmoschus esculentus* and ber tree *Ziziphus jujube* from CCS HAU, Hisar, India. Host is unknown.

***Steinernema siamkayai* stock *et al*., 1998 (Fig. 3)**

Measurements (Table 3.)

Description

First generation males

Body curved posteriorly ventrally when relaxed by heat. Cuticle smooth. Cephalic extremity rounded and continuous with body contour. Four cephalic and six labial papillae prominent. Stoma short and broad forming funnel shape. Excretory pore at the level of metacorpus and anterior to the nerve ring. Pharynx muscular with cylindrical procorpus, extending into a slightly swollen non-valvated metacorpus and a large valvated basal bulb. Cardia prominent. Testis reflection ventral in position. Spicules are paired, symmetrical, curved with small rounded rhomboid shaped manubrium, rostrum less prominent and ribs of equal length. Velum present, gubernaculum long having neck with ventral projection. 11+ 1 single genital papillae present and arranged as follows: six pairs located subventral precloacal, one pair postcloacal near spicule end, one pair postcloacal subdorsal, two pairs subventral near tail tip and one lateral pair near spicule manubrium. Tail conoid without mucron.

Second generation males

Similar in morphology to the first generation males, but smaller in body length and other characters. Tail filamentous and longer than first generation males.

First generation females

Body long and coiled when heat killed and fixed. Cuticle smooth and lateral field not observed. Head rounded and slightly tapering anteriorly. Excretory pore located anterior to nerve ring and genital tract amphidelphic with reflexed ovaries, symmetrical vulva with transverse slit. Double flapped epiptygma present. Rectum narrow with distinct anal opening. Tail conoid with mucron. Post anal swelling present.

Second generation females

Similar to first generation females, but smaller.

Infective juveniles

Body straight in heat relaxed specimens, small (av. 459.3 (422.2- 489.5) µm) and slender, tapering towards anterior and posterior ends. Cuticular striations vague under light microscope, horn like papillae are absent on the cephalic extremity. Lateral field distinct with 6 ridges. Oral aperture closed. Pharynx slender and long with nerve ring located at isthmus level. Excretory pore located above the metacorpus. Cardia prominent. Rectum straight and anus distinct. Tail conoid with pointed terminus.

Table 3: Morphometrics of first generation males and infective juveniles of vetiver population and *Steinernema siamkayai* Stock *et al*. 1998. Measurements are in µm (mean ± SD)

Steinernema siamkayai				
Character	**Vetiver population**		**Thailand population**	
	First generation male*	**Infective juvenile***	**First generation male***	**Infective juvenile***
n	20	20	20	20
L	1324.6 ± 138 (989.3- 1554)	459.3 ± 18.6 (422.2- 489.5)	1135 (1035- 1278)	446 (398- 495)
a	12.4 ± 1.1 (9.9- 14.8)	18.7 ± 1.02 (16.2- 20.8)	-	21 (19- 23)
b	10 ± 1.4 (7.2- 13.6)	4.5 ± 0.3 (3.8- 5.4)	-	4.7 (4- 6.1)
c	48 ± 8.5 (32.9- 64.7)	10.4 ± 0.7 (9.3- 12)	-	11.3 (10.3- 14.8)
c’	0.7 ± 0.1 (0.5- 1)	2.3 ± 0.1 (1.9- 2.6)	-	-
Body diameter	107 ± 10.8 (90- 134)	24.6 ± 1.8 (22- 30)	140 (107- 159)	21 (18 - 24)
EP	74.1 ± 8.4 (55- 91)	36.9 ± 1.43 (35- 40)	57 (47- 67)	35 (29- 38)
ES	133.4 ± 11.9 (110- 168)	101.9 ± 6.9 (85- 118)	134 (123- 141)	95 (80- 107)
Tail length	28.2 ± 4.5 (18- 36)	44.2 ± 3.3 (38- 50)	28 (22- 32)	36 (31- 41)
Hyaline length	-	18.8 ± 1.3 (17- 22)	-	-
Anal body diameter (ABD)	39.7 ± 5.2 (30- 48)	12.9 ± 0.9 (12- 15)	45 (37- 54)	11.5 (9- 15.5)
Spicule length	73.5 ± 4.4 (66- 82)	-	78 (75- 80)	-
Gubernaculum length	52 ± 5.2 (40- 62)	-	54 (47-65)	-
D%	56.2 ± 9.7 (39.2- 82.7)	36.3 ± 2.65 (30.7- 43.5)	42 (35- 49)	37 (31- 43)
E%	267.9 ± 45 (183.3- 388)	83.9 ± 6.07 (74- 100)	207 (166- 257)	96 (85- 112)
SW%	188.2 ± 28.3 (139.5- 250)	-	170 (140- 220)	-
GS%	71 ± 8.34 (53.2- 90.9)	-	70 (60- 80)	-
H%	-	42.6 ± 3.6 (37.5- 52.6)	-	

* Figures in the parentheses are ranges

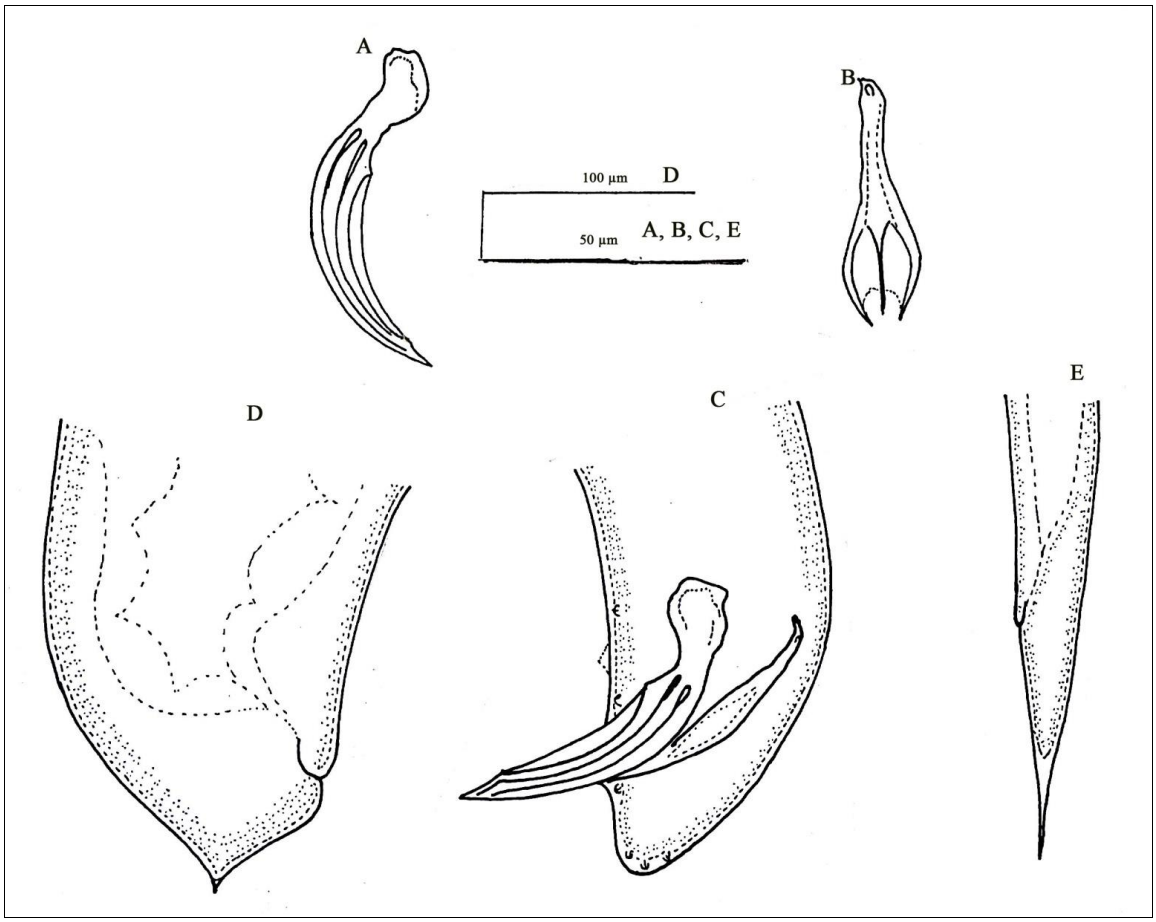

Fig 3. *Steinernema siamkayai* (tomato population): A-C: First generation male. A: Spicule; B: Gubernaculum; C: Tail. D: Tail of first generation female. E: Tail of infective juvenile

Host and locality

The present population collected from the soils around roots of vetiver *Chrysopogon zizanioides* (L.) Roberty at CCS HAU, Hisar, Haryana, India by the *Galleria* bait method and the host is unknown.

Results and Discussion

The species *S. bicornutum* Tallosi *et al.* 1995 was described from mountain soil in Serbia. The species with horn like structures have now been placed in ‘*bicornutum*’-group and has since been described from Czeck Republic, Denmark, Germany, Slovak Republic, Switzerland, Jamaica and the

Canary Islands [9]. The present population resembles with Tallosi *et al.* 1995 description of *S. bicornutum* in having horn like structures, nine incisures at lateral field and similar body length of IJs. The variations from the type specimens were recorded in IJs having smaller tail length (av. 65.9 (59- 72) vs. 72.5 (63- 78) µm) and first generation males being longer (av. 1414.7 (1204.3- 1598) vs 1353 (945- 1539) µm), with longer spicules (av. 71.5 (60- 82) vs 65 (53- 70) µm) and smaller gubernaculum (av. 36.9 (33- 43) vs 47 (38- 50) µm).

S. pakistanense was first described from Sindh, Pakistan from soils around roots of vegetables and is the member of the group having horn like structures on the head region ('bicornutum' group). *S. pakistanense* is characterised by the IJs length less than 700 µm and more than 500 µm and 7 ridges in lateral field. The species has also been isolated from grass and ornamental plants collected at Sonmiani bench and Mubarak village, Balochisthan, Pakistan [14], soils around the roots of Ziziphus jujube from Hisar, Haryana, India (Bajaj and Walia, 2005 unpublished).

S. pakistanense has been isolated from okra, neem and jujube plantations at CCS HAU, Hisar campus and Farm area. However, some intraspecific variations were recorded in the three populations. The IJs of the three populations were smaller (av. 660 (611.2- 740.7) µm, 615.2 (556.8- 678.5) µm and 656.3 (595.7- 720) µm in okra, neem and jujube plantations) compared to original description (av. 683(649- 716) µm). First generation males were longer (av. 1835.2 (1600.6- 2027) µm and 1614.3 (1320- 1813) µm in neem and jujube populations) compared to Pakistan population (av. 1357 (1163- 1505) µm). Also excretory pore was anteriorly placed (av. 41.5 (40-52) µm vs 54 (49- 58) µm) in okra population, first generation females possessed a mucron in the tail region of all the three populations and jujube population IJs had 8 ridges in the lateral field.

S. siamkayai Stock *et al*. 1998 described from sandy clayey loam soils under sweet tamarind from Petchabun Province, northern Thailand. The species was characterized by the IJs having small body length av. 445 µm (398- 495), short tail length av. 35.5 µm (31- 41) and lateral field with 6-8 lateral ridges. The present population too resembled *S. siamkayai* in morphological and morphometrical characters. However, some variations were recorded in pharynx length in IJs (av. 101.9 (85- 118) vs 95 (80- 107) µm), longer tail (av. 44.2 (38- 50) vs 36 (31- 41) µm) and shorter E% (av. 83.9 (74- 100) vs 96 (85- 112)). In case of first generation males the present population differs from the original description in having longer body length (av. 1324.6 (989.3- 1554) vs 1135 (1035- 1278) µm), excretory pore located posteriorly (av. 74.1 (55- 91) vs 57 (47- 67)) and greater SW% (av. 188.2 (139.5- 250) vs 170 (140- 220)) and mucronated tail. The species has been described from India [1, 2].

Conclusions

The entomopathogenic nematodes are efficient bio-control agents of insect pests. For a successful control of a pest it is advisable to search for indigenous isolates of the bio-control agents which are adapted to similar environmental conditions. The first objective was intended to fulfill this aim. In an earlier study 13 % of the soil samples collected from CCS HAU, Hisar campus a farm contained either *Steinernema* or *Heterorhabtidis* (17). Fifteen such isolates were processed for identification since different species behave differently and correct identification is mandatory for further experimentation. From India, very few species have been identified. *S. meghalayensis*, *S. thermophilum*, *S. seemae*, *S. qazii*, *S. masoodi* and *S. solanense* have been reported from various parts of India. Of these *S. thermophilum* is synomised with *S. abbasi* (11) while *S. seemae*, *S. qazii* and *S. masoodi* are species *inquirendae*. *S. solanense* though a valid species was put in species *inquirenda* due to incomplete description since the material was lost during culturing (Walia and Bajaj, 2005 unpublished).

The diagnosis and relationship of new species is discussed along with the description of the respective species according to the taxonomic rules. The variations and relationship of the already known species is also discussed along with the details of the species.

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