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Identification of entomopathogenic nematodes occurring in Tamil Nadu

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Abstract
A study was taken up to identify entomopathogenic nematodes (EPN) species occurring in Tamilnadu from three districts *viz.,* Erode, Coimbatore and Nilgiris during September to December 2014. One hundred and twenty six soil samples collected from 3 districts of Western Tamil Nadu were analysed for the presence of EPN. The survey revealed the occurrence of EPN in undisturbed lands through the use of standard insect baiting bioassay technique. EPN were recovered in two samples in two of the districts *viz*., Erode and Coimbatore. First population was identified as *Steinernema carpocapsae* from Bhavanisagar, Erode and second population was identified as *Steinernema abbasi* from Anamalais, Coimbatore.

Keywords: Entomopathogenic nematode, *Steinernema carpocapsae, Steinernema abbasi*

1. Introduction
Insecticides play a major role in the management of insect pests for crop protection activities. Several insecticides are considered as rapid and effective for control of different insect pests. The indiscriminate use of insecticides however, has resulted in problems such as insecticide resistance, pest resurgence and residues on crops [10]. This has led to the adoption of alternate strategies of pest management, such as biocontrol. EPN are one of the biocontrol agents for controlling most of the crop pests. EPN commonly *Steinernema, Neosteinernema* and *Heterorhabditis* are associated with mutualistic bacteria belonging to the genera *Xenorhabdus* for *Steinernema* and *Photorhabdus* for *Heterorhabditis* [18]. An understanding of the factors governing the natural occurrence and abundance of the nematodes is important in formulating a rational approach for their utilization as biocontrol agents. Totally 73 species of EPN have been identified so far, among which 64 species belongs to *Steinernema*, 8 species to *Heterorhabditis* and 1 species to *Neosteinernema* [6]. Several survey in India have revealed natural occurrence of several species of *Steinernema* and *Heterorhabditis* in Tamilnadu [3], New Delhi [5] and Gujarat [22]. More utilization of agricultural practices related to extreme use of agro chemicals without any concern about the environmental perpetuation. The present study was aimed to focus on occurrence of EPNs in Western Tamil Nadu.

2. Materials and Methods
2.1 Occurrence and distribution of entomopathogenic nematodes in Tamil Nadu
A random survey was undertaken in Tamil Nadu for the entomopathogenic nematodes. Totally 126 soil samples were collected in 3 districts during September to December 2014. Samples were collected from cultivated, undisturbed lands and perennial crops at a depth of 10-15cm. Five to ten representative samples were taken at random representing an area of $10m^2$ approximately. The samples were mixed thoroughly and an aliquot of 250 g taken for baiting EPN. Information on locality, soil type and standing crop in the field were noted and the samples were packed in plastic polythene bags and transported to laboratory. The bioassay of samples was carried out by soil baiting technique [2] using *Corcyra cephalonica* larvae as bait. The samples were baited two times to attract maximum number of nematodes.

2.2 Identification of Entomopathogenic nematodes
The isolates of *Steinernema* sp. encountered in the survey were identified based on the taxonomic keys proposed by Lucskai [14]. De Man's formulae were used to identify the nematodes.

2.3 Taxonomy
The required nematodes for study were killed by gentle heat, fixed in TAF and stored in glass vials. The fixed specimens were processed by glycerol ethanol method [20] and stored in desiccator which contains calcium chloride until mounting them in anhydrous glycerine. Drawings and measurements of adults and infective juveniles were made with a mirror type camera lucida. In addition to the de Man's formulae, the following characters such as D%, E%, SW, GS, Ratio D and E were worked out.

3. Results and Discussion
3.1 Survey for entomopathogenic nematodes
Survey for entomopathogenic nematodes was conducted in Tamil Nadu of three districts viz., Erode, Coimbatore and Nilgiris. EPNs were isolated only from two
samples out of 126 samples collected. Those two EPN populations were named as S1 and S2. S1
population was collected from Bhavanisagar in a perennial tree crop, Moringa (*Moringa oleifera* L.) and S2 population was collected from undisturbed land of Anamalais.

3.2 Isolation and identification of nematodes
The entomopathogenic nematodes encountered from the two samples were identified as *S. carpocapsae* (S1) and *S. abbasi* (S2).

3.3 Identification of *S. carpocapsae*
3.3.1 Females (2nd generation)
All females were variable in size, with smooth cuticle, head truncate to slightly rounded, lips united, stoma partially collapsed with only an anterior vestibule remaining. Oesophageal tissues were close to mouth opening, reaching to base of the vestibule. Oesophagus muscular, the anterior portion of the procarpus slightly expanded just behind the stoma, then extending into a slightly enlarged metacarpus, followed by an isthmus and a basal bulb containing a small valve. Nerve ring surrounding isthmus just anterior to the basal bulb. Excretory pore anterior to nerve ring. Gonads didelphic, amphidelphic and reflexed. Vulva a transverse slit situated on a protruberance. Tail was conical to dome-shaped (Fig. 1 F).

3.3.2 Males (2nd generation)
Anterior region of males were similar to females. Average length of male was 949.10µm.
Testis single and reflexed, spicules paired, symmetrical, moderately or slightly curved, length 66.02µm. Gubernaculum tapering anteriorly to form a short narrow part. Tail tip with mucron, bursa absent (Fig. 1 E, D; Table 1).

3.3.3 Infective juveniles (3rd stage juvenile)
Parasitic juveniles were narrow, mouth and anal openings closed, esophagus and intestine collapsed. Average length of juvenile was 556.59µm. Excretory pore always above the nerve ring and42.14µm from anterior end. Tail pointed. Tail length of juvenile was 50.12µm (Fig. 1 A, B, C; Table 1).

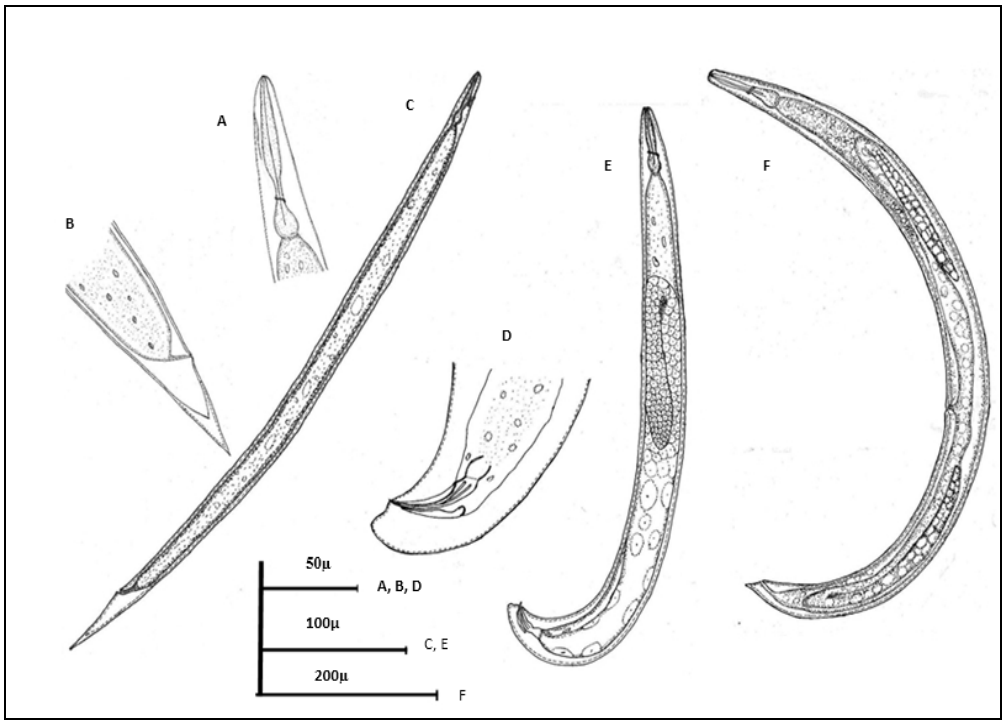

Fig 1: Diagrams of Steinernema carpocapsae. A- Infective juvenile(IJ) head region, B- tail portion of IJ, C-Infective juvenile, D-tail of male, E-entire male, F- entire female

Table 1: Comparison of morphological characters of Steinernema carpocapsae isolate from Bhavanisagar with original description of Steinernema carpocapsae [23]

S. No	Character	Bhavanisagar isolate		Original description of *S. carpocapsae* [23]	
		IJ	Male	IJ	Male
1	Total length(L) in µm	556.59 (520-577)	949.10 (921-1468)	558 (438-650)	1450 (1090-1710)
2	Width(W) in µm	28 (20-39)	79.334 (77-91)	25 (20-30)	102 (77-131)
3	Excretory pore (EP) in µm	42.142 (34-55)	38.9 (36-42)	38 (30-60)	61 (47-74)
4	Oesophagus length(ES) in µm	134.45 (122-145)	136.59 (128-149)	120 (103-190)	155 (136-167)
5	Tail length(T) in µm	50.14 (47-54)	31.46 (22-41)	53 (46-61)	30 (23.4-39)
6	Spicule length(SPL) in µm	-	66.02 (57-77)	-	64.6 (58.5-71.5)
7	Maximum body width at cloaca in µm	-	40.10 (36-45)	-	42.6 (32.5-54.6)
8	a=L/W	19.87	13.85	21	-
9	b=L/ES	4.13	8.04	4.4	-
10	c=L/T	11.10	34.92	10	-
11	D%=EP/ES*100	31.34	28	26	-
12	E%=EP/T*100	84.04	123.63	60	-
13	SW=SPL/maximum body width at cloaca in µm	-	1.6	-	1.7

Values in parentheses depict maximum and minimum values

3.4 Identification of *S. abbasi*

3.4.1 Females (2nd generation)

All females were varied in shape. Body robust strongly curved and often 'C' or spiral shaped. Lip region rounded, continuous with the body. Oesophagus muscular with cylindrical procorpus, slightly swollen, narrow isthumus and rounded basal bulb. Nerve ring just above the basal bulb. Excretory pore at the level of metacarpus. Gonads didelphic, amphidelphic and reflexed, often containing many eggs. Vulva a transverse slit. Tail short and conoid with a pointed tip (Fig. 2 A; Table 2).

3.4.2 Male (2nd generation)

The 2nd generation male body was slender, ventrally curved, J-shaped after fixation (total length of male was 799.38µm). Lip region continuous, with distinct labial papillae, stoma shallow, partially collapsed. Esophagus muscular with cylindrical procarpus. narrow isthumus and round basal bulb and three esophageal glands. Nerve ring encircle esophagus just above the basal bulb. Excretory pore just above the nerve ring near the base of metacarpus (excretory pore position 70.48µm). Testis reflexed, spicule paired (length of spicule was 61.50µm) shaft almost absent, gubernaculum boat shaped, ventrally curved, slightly swollen in the middle. Bursa absent. Terminal mucron absent (Fig. 2 B, C; Table 2).

3.4.3 Infective juveniles (2nd generation)

Length of juvenile was 627.60µm, body thin, elongate, lip region continuous, excretory pore (45.40µm) weak, near the base of the metacarpus. Esophagus with cylindrical procorpus and slightly swollen median bulb (length of esophagus was 95.82µm). Nerve ring just above the basal bulb, tail elongate, attenuated and gradually tapering (tail length was 52.15µm) (Fig. 2 D, E, F; Table 2).

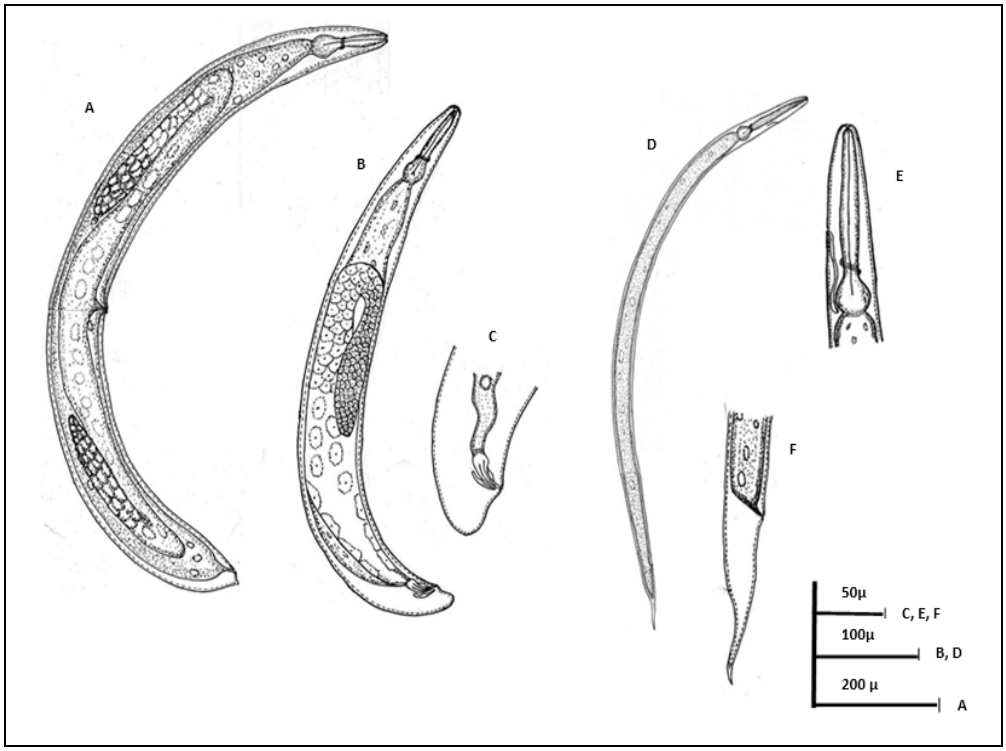

Fig 2: Diagrams of Steinernema abbasi. A- entire female, B- entire male, C- tail of male, D- Infective juvenile, E- Infective juvenile(IJ) head region, F- tail portion of IJ

Table 4: Comparison of morphological characters of the Steinernema abbasi isolate from Anamalais with original description of Steinernema abbasi [4]

S. No	Character	Anamalais isolate			Original description of *S. abbasi* [4]		
		IJ	Male	Female	IJ	Male	Female
1	Total length (L) in µm	627.60 (552-665)	799.38 (908-691)	2732.38 (987-3800)	541 (496-579)	861 (606-1035)	2609±622 (1897-3917)
2	Width (W) in µm	26.90 (22-29)	73.84 (64-88)	130.06 (121-139)	29 (27-30)	70 (64-80)	130±5 (123-138)
3	Excretory pore (EP) in µm	45.40 (39-52)	70.48 (61-80)	64.20 (58-70)	48 (46-51)	66 (62-79)	66±3.94 (61-73)
4	Esophagus Length (ES) in µm	95.82 (88-104)	128.61 (118-139)	141.9 (130-152)	89 (85-92)	121 (112-130)	146±6.7 (136-157)
5	Tail length (T) in µm	52.152 (42-62)	31.02 (24-31)	38.29 (34-41)	56 (52-61)	21 (17-24)	36±2.5 (32-39)
6	Spicule length (SPL) in µm	-	61.50 (49-64)	-	-	61 (51-69)	-
7	Maximum body width at cloaca in µm	-	40.998 (35-51)	-	-	39 (36-42)	-
8	Vulval length V			58.9 (50-75)			55±2.2 (50-77)
9	a=L/W	23.52	-	-	18 (17-20)	-	-
10	b=L/ES	6.3	-	-	6 (5.5-6.6)	-	-
11	c=L/T	10.09	-	-	9.8 (8.1-10.8)	-	-
12	D% = EP/ES*100	0.47	0.54	0.45 (0.39-0.52)	0.53 (0.51-0.58)	0.56 (0.50-0.70)	0.45±0.01 (0.43-0.47)
13	E% = EP/T*100	0.79	-	-	0.86 (0.79-0.94)		-
14	SW = SPL/maximum body width at cloaca in µm	-	1.5	-	-	1.59 (1.28-1.88)	-

Values in parentheses depict maximum and minimum values.

4. Discussion

The results of present study indicate the occurrence of two species of EPN in place, where there is evidence of continuous breeding of insect pests in cryptic habitat. A random survey conducted by Ambika and Sivakumar [1] in Coimbatore, Erode and Nilgiris districts of Tamil Nadu and also revealed the presence of *Steinernema* sp. The isolates of *Steinernema* (2.2%) and *Heterorhabditis* (0.3%) in Karur district of Tamil Nadu [17]. *S. carpocapsae* is widely distributed throughout the world. These species are found in many temperate and tropical regions of the world [7]. It has also adapted to temperature greater than 30°C by Karimi *et al* [10]. *S. carpocapsae* has been reported from Karnataka [8] Gujarat [21] and Tamil Nadu [19]. In the present study, *Steinernema* spp. was recovered from both undisturbed soil and perennial cropping systems. Midduturi *et al* [15] reported that samples collected from cultivated field did not yield EPN. Higher frequency of *Steinernema* occurred in hilly areas followed by coastal and plain areas. Similar findings were reported by Joeseph rajkumar and Sivakumar [9]. *H. indica, S. asiaticum* and *S. abbasi* have been reported from India [5] which was confirmed by Khan and Haque [12] who reported that *S. abbasi* was the predominant species in Western Uttar Pradesh, India. Lalramliana and Yadav [13] reported that *S. thermophilum, S. glaseri* and *H. indica* occur in Meghalaya, North Eastern India and frequency of *Steinernema* (73.03%) was more than *Heterorhabditis* (26.97%).

4.1 Identification of nematodes

4.1.1 *Steinernema carpocapsae*

The measurements of different stages of *Steinernema* sp. taken from Bhavanisagar, closely resembled the original description of *S. carpocapsae* [23]. The length of infective juvenile was less than 600µm, anterior end to excretory pore 42.142 µm (38µm (30-60) in the original description), E% was 84% (60 (55-66) in the description) and C ratio was 11.10(10 (9.1-11.2) in the present study), thus confirming the criteria given by Weiser [23] for diagnosing *S. carpocapsae.*

4.1.2 *Steinernema abbasi*

Steinernema sp. isolated from Anamalais was closely similar to the original description of *S. abbasi* [4]. Length of infective juvenile was 552-665µm (496-579µm in original description). Excretory pore was 39-52µm from anterior end (46-51µmin original description). E and D values of juveniles were 0.47 and 0.79 (in original description it was 0.51 and 0.81). Spicule length of male was 49-64µm (in original description it was51-69µm), thus confirming the criteria given by Elawad [4] for diagnosis of *S. abbasi.*

5. Conclusion

The study reveals that the two EPN species were recorded in three districts of Tamil Nadu and nematodes play a crucial role in relation to insect pest population in both cultivated and uncultivated areas and with changing abiotic factor conditions. Favorable conditions of these two Steinernema species are required that includes moderate temperature and humidity, for their survival. Recovery of EPN was more in undisturbed land and less in case of cultivable land because frequent use of agrochemical which leads to decrease the population of EPN. The recovered EPN species are potentially useful to develop new commercial strains adapted to the local area environment for biological control of insects. Extensive studies on the virulence of potential EPNs against insect pests will be needed in selective agro systems in the world.

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