A NEW ISOLATE OF *STEINERNEMA ABBASI* 1997 (NEMATODA: STEINERNEMATIDAE) AS A FIRST RECORD FROM EGYPT

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ABSTRACT

Steinernema abbasi was isolated from soil in alfalfa fields in Sultanate of Oman, while the Egyptian population of S. abbasi was isolated from a soil sample from Ismailia Governorate. The occurrence of this isolate is reported for the first time in Egypt The distinctness of Egyptian S. abbasi in comparison with populations of S. scapterisci, S. carpocapsae, S. riobravis, S. kushidai and original S. abbas diagnostic characters include the length of the infective stage juveniles, the ratio E (distance from the head to the excretory pore divided by the tail length). The Shape and the length of spicules and gubernaculum and absence of a tail projection in the male. Morphological examination indicateded that third-stage infective juveniles, males and females of this isolate have almost the same morphological and morphometric characters as those of the original description.

Key Words: *Steinernema abbasi* ,Steinernematidae, Nematoda, description, Egypt.

INTRODUCTION

Steinernematid and heterorhabditid nematodes have received increasing alertness because of their potential as biological control agents of insect pests found in soil (Klein, 1990). Much of this alertness has been centralized in the temperate regions. The demand for biopesticides as alternatives to chemical pesticides is increasing due to the concern about pesticide residues in crops and hazards to the environment. Nematodes of the family Steinernematidae are parasites of insects. They have worldwide distribution and inhabit mainly various types of soil (Weiser and Mracek, 1988). Many indigenous entomopathogenic nematodes have been found naturally suppressing insect pest populations in Egypt (Shamseldean *et al.* 1990; Abd El-Rahman, 1997&2001).

The genus *Steinernema*, created by Travassos (1927) comprises at present more than twenty species worldwide. Among these species is *S. abbasi* which was isolated from Sultanate of Oman (Elawad *et al.*, 1997). The present paper deals with the morphological description of a new isolate from this species, isolated from Ismailia Governorate as a first record for this nematode from Egypt.

MATERIALS AND METHODS

Nine localities in the newly reclaimed areas were chosen for sampling collection. These localities were in most of the Egyptian governorates. Each soil sample was carefully processed for entomopathogenic nematodes extraction. About 15% of the collected samples were positive for entomopathogenic nematodes. The nematodes were detected using *Galleria* baiting technique (Bedding and Akhurst, 1975). Dead larvae were placed into White traps and infective juveniles were collected and used to infect live *G. mellonella* larvae (Woodring and Kaya, 1988). The infective juveniles (IJs) emerged from the host cadavers 10-12 days after initial exposure to the nematode infection. Insect cadavers were dissected on day 3 to recover the first giant and normal female generations, while the second generation was obtained by dissecting the insects on days 5-6.

Morphological observations

For light microscope study, all stages of the nematode life cycle were killed in hot water (60°C), fixed in (TAF) fixative (Hoopper, 1986), and processed via the glycerol-ethanol evaporation method (Seinhorst, 1959), slowly dehydrated, then mounted on glass slides in an hydrous glycerol, using a clear nail polish ring technique. The cover slip was supported by glass wool to avoid flattening of the nematodes. Quantitative measurements were made using a Zeiss light microscope with an ocular micrometer. All measurements are given in microns in the following order: mean ± standard deviation (range). Taxonomic characters were also recorded (Poinar, 1990), the drawings were made using a drawing tube mounted on a light microscope.

RESULTS

The Egyptian isolate of *Steinernema abbasi* (Figs. 1&2).

Description and measurements

Males (first generation): Body slender, ventrally, curved, J-shaped upon fixation. Lip region continuous, with distinct labial papillae. Oesophagus muscular with cylindrical procorpus;

slightly narrow isthmus and round basal bulb. Nerve ring surrounding isthmus above the basal bulb. Excretory pore always above the nerve ring near the base of metacorpus. Distance from the anterior end to the excretory pore always more than body width at excretory pore. Gonads monorchid. testis usually reflexed consisting of a germinal growth zone leading tto seminal vesicle. Spicules paired arcuate, symmetrical nd istinct golden dark yellow in coloration, urved entrally. ead monubrium) usually swollen with many cavities and bulges; shaft (calomus) is very short or almost absent; shaft and blade angle averages between 100-120 m); lade (lamina) tapering to form a cylindrical part and then tapering smoothly toward the distal ortion; velum present. Each spicule with two internal ibs. Gubernaculum about 71% of the spicule length, boat-shaped in lateral view, and ventrally curved, slightly swollen in the middle and gradually narrowing distally; proximal end with or without knob or hook. Bursa absent. Twenty-three genital papillae present. Tail short and conoid with rounded terminus; terminal mucron absent. Thin flap of sclerotized cuticle occurring over the cloaca, closing he cloacal opening when spicules in a resting position. Cloacal opening slit like with, on both sides, two small lateral papillae-like structures (Table 1).

Table (1): Comparative measurements of adult males of the Egyptian and original populations of *steinernema abbasi* (all measurements in µm).

Character	First generation		Second generation	
	Egyptian *S. abbasi*	Original *S. abbasi*	Egyptian *S. abbasi*	Original *S. abbasi*
Total body Length	1670 ± 110 (1360 - 1880)	1252 ± 189 (999-1534)	1220±76 (728 - 1516)	861 ± 121 (606-1035)
Greatest body width	145 ± 30 (119.5 - 195)	87 ± 6.7 (82 - 98)	112.3 ± 37.6 (92 -158)	70 ±4.6 (64 - 80)
Length of stoma	5.1 ± 1.3 (3.8 - 8.3)	4.50 ± 0.64 (4 - 6)	4.1 ± 1.8 (2.6 - 6.24)	4 ± 0.26 (4 - 5)
Width of stoma	6 ± 1 (5 - 7.5)	7.20 ± 0.41 (7 - 8)	5.8 ± 0.95 (4.3 - 7)	7 ± 0.93 (6 - 7)
Head to excretory pore	68.4 ± 9.8 (56.4 - 78.4)	80 ± 7.8 (68 - 89)	60.1 ± 6.7 (46.4 - 70.6)	66 ± 5 (62-79)
Head to nerve ring	147.2 ± 13.6 (130 - 164.6)	103.20 ± 6.48 (99 - 123)	97.8 ± 10.5 (76.2 - 123.6)	99 ± 3.90 (93 -106)
Head to pharynx base	165.6 ± 15 (143-183)	133 ± 6 (121 - 144)	139.2 ± 12.5 126.7 -153,3)	121 ± 4.90 (112 -130)
Tail length	34 ± 3.8 (30.6 - 42.0)	26 ± 3 (20 -31)	26.9 ± 4.4 (20.6 - 36.9)	21 ± 1.70 (17-24)
Length reflex of testis	593 ± 71 (215.7 - 703)	274 ± 33 (234 - 319)	573.6 ± 60 (432 - 722)	241 ± 20
Body width at cloaca	54.2 ± 7.4 (48.2 - 62.4)	43 ± 4.90 (37 - 55)	45.2 ± 6.2 (38.8 - 51.6)	39 ± 1.60 (36 -42)
Length of spicule	69.5 ± 4.5 (61.8 - 86)	65 ± 5.70 (57 - 74)	56.5 ± 5.8 (54 - 72)	61 ± 4.80 (51 -69)
Width of spicule	11.8 ± 1.3 (10.2 - 13.9)	12 ± 1.30 (10 - 14)	9.6 ± 1.5 (9.1 - 11.8)	10.27 ± 1.62 (8 - 13)
Length of gubernaculum	49.2 ± 5.6 (42.1 - 57.7)	45 ± 4.30 (33 - 50)	41.2 ± 4.0 (37 - 51)	43 ± 3.30 (35 - 48)
Width of gubernaculum	5.4 ± 0.85 (4.6 - 7.5)	7 ± 0.10 (6 - 8.5)	4.2 ± 0.63 (4.3 - 6)	7 ± 0.65 (6 - 8)

Males (second generation): Similar to first generation, except smaller and thinner. Spicules and gubernaculums smaller than first generation. Tail short, lacking a mucron (Table 1).

Female (first generation): Heat-killed specimens usually spiral or C-shaped. Cuticle with fine striae. Lateral ield nd phasmids in conspicuous. Lip region rounded, continuous with the body; bearing

two circles with ten labial papillae and amphids small pore-like. Stoma shallow, triangluar at base. Oseophagus extending to the oral opening. Cheilorhabdions prominent, well sclerotized, oesophagus muscular, cylindrical; meta-corpus slightly swollen; non-valvate metacorpus; isthmus slightly narrowing, terminating in a muscular basal bulb with small, distinct, valve. Cardia short. Nerve ring at level of isthmus just above the basal bulb. Excretory pore opening usually anterior to nerve ring at level of metacorpus. Genital tract amphidelphic, reflexed ovaries filled with containing eggs. Vulva median in position, a transverse slit, vulva lips usually protruding slightly from body surface with well developed double flapped, epiptygmal.Vagina sclerotized. Tail short, not constricted, dorsally convex with bluntly rounded terminus, lacking mucron. Tail shorter than anal body width (Table 2).

Female (second generation): Similar in general aspects to first generation female except smaller length and width, length ranging from 50-68% of the first generation emales, idth anging rom 75-87% of the first generation females. Vulva a transverse slit, generally protruding from the body surface; vulval lips are weakly developed ouble flapped, epiptygma; and less bulger than those in first generation. Tail more sharply pointed; anal body width about 1.52 of the tail length (Table 2).

Table (3) reveals Comparative measurements of infective juveniles of the Egyptian and the original populations of *Steinernema abbasi* (all measurements in µm). Females (first generation; giant forms): body specimens spiralled, length of body about three times longer than the normal female; other structures as described or ormal emales ut width at anus level more than as first generation while the vulval edges lower than those in normal form (Table 2).

Infective stage juvenile: Body cylindroid, tapering smoothly at both extremities; narrow, elongate. Lip region ontinuous. outh nd nus closed. Excretory pore always eak, lightly anterior to nerve ring. Distance from anterior end to excretory pore always more than body width at the same level. Oesophogus with cylindrical procorpus and slightly swollen median bulb. Nerve ring just above the basal bulb. Tail elongate, attenuated, gradually tapering, dorsally curved at tip (Table 3).

DISCUSSION

Egyptian isolate of *Steinernema abbasi* could be separated from the most similar species of the genus, *i.e.*, *S. scapterisci* (Nguyen & Smart), *S. carpocapsae* (Weiser), *S. riobravis* (Cabanillas, Poinar & Raulston) *S. kushidai* (Mamiya) and original *S. abbasi* (Elawad, Ahmed eid) by morphological characters.

Morphologically, the average length of Egyptian isolate of *S. abbasi*. (535 µm) infective juveniles is different from that of *S. scapterisci* (572 µm), *S. riobraavis* (622 µm), *S. Kushidai* (589 µm), however, it approached *S. carpocapsae* (558 µm) and closer to the original *S. abbasi* (541 µm) (Table 4). The length range of Egyptian *S. abbasi* infective juveniles (464-582 µm) somewhat overlaps with those of original *S. abbasi* (496-579 µm) and *S. carpocapsae* (438-650 µm) and *S. Scapterisci* (511-609 µm) more than that of *S. kushidi* (524-662 µm) or *S. riobravis* (561-701 µm).

The ratio E (distance from the head to the excretory pore divided by the tail length) of the infective juveniles of Egyptian *S. abbasi* (0.76-0.93) does not overlap with that of *S. carpocapsae* (0.54-0:66) and *S. riobravis* (0.93-1.11), but it somewhat overlaps with that of *S. scapterisci* (0.60-0.80) and *S. kushidai* (0.84-0.95), while it nearly matches that of the original *S. abbasi* (0.79-0.94) (Table 4).

The first generation male of Egyptian *S. abbasi* can be separated from other species *S. scapterisci* and *S. carpocapsae* by the absence of terminal mucron. However, his erminal mucron is absent also in *S. riobravis*, *S. kushidai* and in the original *S. abbasi*. The male of Egyptian solate uperficially appears similar to that of *S. riobravis* in having golden dark yellow spicules, like the original isolate, has shorter body length and less curvature n the head/blade angle of spicule; ranging from 100-120° for Egyptian *S. abbasi* and 107-120° for the original *S. abbasi* compared to 90-100° for *S riobravis*.

The females of Egyptian *S. abbasi* are nearly similar to those of original isolate especially at head to excretory pore distance, vulva % and width of body at the anus level (Table 4). Finally, it was obvious that the diagnostic morphological characters of the third-stage infective juveniles, ·ales and females of Egyptian isolate of *S. abbasi* were mostly similar to those of the original isolate.

Table (2): Comparative measurements of the adult females of the Egyptian and original populations of *Steinernema abbasi* (all measurements in µm).

Character	First generation		Second generation		Giant form	
	Egyptian *S. abbasi*	Original *S. abbasi*	Egyptian *S. abbasi*	Original *S. abbasi*	Egyptian *S. abassi*	Original *S. abbasi*
Total body length	4180±335 (3122-5150)	3510±638.1 (2453-4477)	2863±635 (1563.4-3951)	2609±622 (1897-3917)	6247±1138 (4192-7227)	10730±2197 (8055-13735)
Greatest body width	146±27.6 (120-183)	159±11.5 (143-181)	128.5±30 (90.5-145.5)	130±5 (123-138)	228.8±40 (195.7-297.5)	225±42 (186-303)
Length of stoma	5.8±0.48 (5.1-9.5)	7.8±0.42 (7-8)	5.2±0.76 (4.6-802)	6±0.32 (6-7)	7.1±0.91 (6.3-10)	7.5±0.53 (7-8)
Width of stoma	8.5±1.4 (6.5-12.2)	8.6±0.52 (8-9)	7.1±1.3 (6.8-10.4)	8±0.42 (8-9)	9.8±1.2 (9.2-14.2)	9.4±0.52 (9-10)
Head to excretory pore	72±4.9 (54-85.6)	71±10.9 (58-79)	63.4±10.6 (46.1-81.0)	66±3.94 (61-73)	80.1±7.8 (56.1-97)	84±13 (51-99)
Head to nerve ring	131.6±10.5 (97.6-188.7)	125±5.8 (120-137)	122±15.5 (85.6-184)	114±2.5 (110-118)	167±14.5 (156-220)	149±13.5 (131-182)
Head to pharynx base	172±41 (139.5-204)	165±7.48 (155-176)	165±42.4 (115-191.6)	146±6.7 (136-157)	230±46.7 (190.3-342)	193±26 (125-224)
% Vulva	53.1±3.2 (50.4-58.5)	55±2.4 (52-60)	53.1±3.6 (49-58)	55±2.2 (50-77)	52.3±2.9 (44.4-58.2)	50±5.5 (43-57)
Tail length	47.5±3 (37.2-65.3)	37±3.1 (31-40)	42.4±4.6 (31.4-53.6)	36±2.5 (32-39)	52.1±3.7 (46.7-70)	46±4.7 (40-55)
Width at anus level	73.4±8.6 (51.6-97.8)	62±6.8 (51-70)	51±3.8 (35.6-60.2)	50±2 (47-54)	99.5±7.5 (70.4 -130)	99±16.8 (73-129)

Table (3): Comparative measurements of infective juveniles of the Egyptian and the original populations of *Steinernema abbasi* (all measurements in µm).

Character	Egyptian isolate	Original isolate
Body Length	535 ± 19 (464 - 582)	541 ± 24 (496 - 579)
Maximum body diameter	27.7 ± 1.2 (23.6 - 28)	29 ± 0.99 (27 - 30)
Head to excretory pore	46.9 ± 2.4 (34.1 - 50.7)	48 ± 1.5 (46-51)
Head to nerve ring	63.2 ± 4.2 (41.8 - 72)	68 ± 2.4 (64 - 72)
Head to pharynx base	86.8 ± 3.8 (77.6 - 100.8)	89 ± 1.8 (85 - 92)
Tail length	56.5 ± 3.2 (46.5 - 56.9)	56 ± 3.2 (52 - 61)
Ratio A	20.0±1.3 (21.1 - 23.6)	18 ± 0.91 (17-20)
Ratio B	6.3 ± 1.5 (5.4 - 7.3)	6 ± 0.32 (5.5 - 6.6)
Ratio C	10.2 ± 1.2 (8.4 - 12.2)	9.8 ± 0.83 (8.1 -10.8)
Ratio D	0.5 ± 0.03 (0.49 - 0.57)	0.53 ± 0.02 (0.51 - 0.58)
Ratio E	0.87 ± 0.13 (0.76 - 0.92)	0.86 ± 0.05 (0.79 - 0.94

Table (4): Selective differentiating data between species of *Steinernema* most similar to Egyptian population of *S. abbasi* (all measurements in µm).

Character	Nematode species and measurements					
	S-scapterisci	***S.carpocapsae***	***S.riobravis***	***S.kushidai***	***Original S.abbasi***	***Egyptian S.abbassi***
			Juvenile			
Body length	572 (517-609)	558 (438-650)	622.5 (561.0-701.0)	589 (524-662)	541 (496-579)	535 (464-582)
Head to excretory pore	39 (36-48)	38 (30-56)	56.2 (51.2-63.7)	46 (42-50)	48 (46-51)	46.9 (43.1-50.7)
Tail Length	54 (48-60)	53 (47-59)	53.5 (46.2-58.7)	50 (44-59)	56 (52-61)	56.5 (46.5-56.9)
Ratio D	0.31 (0.27-0.40)	0.26 (0.23-0.28)	0.49 (0.45-0.55)	0.41 (0.38-0.44)	0.53 (0.51-0.58)	0.50 (0.49-0.57)
Ratio E	0.73 (0.60-0.80)	0.60 (0.54-0.66)	1.05 (0.93-1.11)	0.92 (0.84-0.95)	0.86 (0.79-0.94)	0.87 (0.76-0.92)
			Male (first generation)			
Mucron	present	Present	Absent	Absent	Absent	Absent
Length of spicule	83 (72-92)	66 (58-77)	66.9 (62.5-75)	63 (48-72)	65 (57-74)	69.5 (61.8-86)
Length of gubernaculum	65 (59-75)	47 (39-55)	51 (47.5-56)	44 (39-60)	45 (33-50)	49.2 (42.1-57.7)
			Female (first generation normal form)			
Tail Length	46 (34-59)	36 (28-47)	45.1 (41.3-50.0)	38 (30-45)	37 (31-40)	47.5 (37.2-65.3)
Anal body width	58 (41-72)	69 (50-87)	92.7 (62.5-115.0)	64 (54-84)	62 (51-70)	73.4 (51.6-97.8)

D= head to excretory pore distance / head to pharynx base distance

E = head to excretory pore distance/ tail length

All measurements in microns, and range is given in parentheses following the average value (N=25)

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