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Research Article

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Exploring Intra-specific Morphometric Variations of *Aphelenchoides besseyi* in Tuberose, a Key Ingredient for Essential Oils

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Abstract

An experiment was conducted for the study of nematode infested flowers of tuberose from the tuberose growing areas of Gangetic plains of West Bengal to extract the population of *Aphelenchoides besseyi*. Surveys were conducted at Nokari (Ranaghat –II block of Nadia district); Gayeshpur (Chakdah block of Nadia district); Raghunathpur (Bhangar I block of South 24-Parganas district) and Deulia (Panshkura II block of Purba Medinipur district). For studying morphometric variability, the nematode specimens from each population were processed by glycerol-ethanol method and permanently mounted in pure anhydrous glycerol. Some variations in morphometric characters among populations were observed. In all the four populations of *A. besseyi* the 'b' ratio (7.8-11.5), stylet length (8.8-11.9 µm), head height (2.1-2.9 µm), head width (5.6-6.9 µm), anal body width (8.4-10.1 µm) and V% (70.2-75.9) were observed as the least variable morphometric characters. Ratios like a, b′, c and c′ and distance between vulva and anus were either least or moderately variable. Body length of *A. besseyi* was found least variable character except for the Deulia population where the mentioned character was moderately variable. Greatest body width was found to be very consistent character of *A. besseyi* in all the population except for the Nokari population. The study is important for formulating integrated management modules of foliar nematode. Tuberose bulbs are coated with turmeric and butter and pasted on infants' red blemishes.

Keywords

Nematode, Population count, Morphometric characteristics, *Aphelenchoides besseyi*, Life on land

Introduction

Tuberose is one of the important ornamental flowers in global commerce. It is grown worldwide for its numerous uses. The bulbs are coated with turmeric and butter and pasted on infants' red blemishes. Powdered dried tuberose bulbs treat gonorrhea. In Java, flowers are eaten with vegetable juices. Tuberose oil can treat the onset of sickness to maintain a strategic distance from the awkward sensation. It is considered a compelling cure for nasal clog. Tuberose oil is a successful sexual enhancer. It makes a difference to avoid contamination of the skin. Its antispasmodic property is additionally useful for fitful hacks, shakings, as well as for decreasing muscle tension. Skincare-It has anti-fungal and anti-bacterial properties that aid treat skin problems like skin break out. It is additionally a great cure for split heels due to its mending properties. It smoothens fine lines and wrinkles as well as expanding the dampness official capability of the skin. As a result, skin looks more youthful and suppler. The main components of Tuberose oil are ben-

zyl acetate, butyric acid, eugenol, farnesol, geraniol, menthyl benzoate, menthyl anthranilate and nerol. In West Bengal, commercial cultivation of tuberose is observed in the areas of Ranaghat, Haringhata, Kolaghat, Panskura, Debra, Rajarhat and Bhangar. Both single and double flowered varieties are equally popular here but foliar nematode *A. besseyi* is a problem of concern. For the confirmation of the foliar nematode symptoms observed in the fields of tuberose an experimental trail has been conducted to study the morphological characteristics and the variations in the intra-specific populations of *A. besseyi* from the tuberose growing areas of plains of West Bengal.

Widespread occurrence of foliar nematode on rice, tuberose, onion and gladiolus was also reported from West Bengal [1-3]. Foliar nematode infestation is not only a pest in tuberose but also in rice, chrysanthemum, gladiolus and onion, and there is a lot of confusion between the different species of *Aphelenchoides*, so to confirm the species present in these areas and to see the variations in their morphometric characteristics we have to follow the keys of identification to confirm the species and note down the variations present among these population collected during our survey from different districts of tuberose growing areas. However, these identifications require a molecular study as well for validation, but this extensive morphometric study is one of method for morphometric diagnosis of the species. *A. besseyi* is amphimictic in nature, so both male and female population are present in the foliar parts (flowers and leaves). The idea was to collect the population and study both males and females for better understanding of the variations. These nematodes get disseminated by remaining in the bulbs, so the primary source of inoculation and spread is also a problem. The symptoms start to occur after a very late phase of infestation, so we have collected the infested flowers along with stalks and have extracted the nematodes for our study.

Materials and Methods

Experimental site

Laboratory of the Department of Agricultural Entomology, BCKV, Mohanpur, Nadia, West Bengal.

Survey and collection of sample

Nematode infested flowers of tuberose (*Pueraria tuberosa*) were collected from the different tuberose growing areas of Gangetic plains of West Bengal during 2014-16 to extract the population of *A. besseyi*. Survey was conducted at Nokari area (23°10.58′ N latitude and 88°35.41′ E longitude, 10 m elevation) of Ranaghat –II block of Nadia district; Gayeshpur Central Research Farm (22°58.296′ N latitude and 88°29.757′E longitude) of BCKV (Chakdah block), Nadia; Raghunathpur areas (22°30.33′ N latitude and 88°36.90′ E longitude) of Bhangar I block of South 24-Parganas district and Deulia areas (22°24.23′ N latitude and 87°45.57′ E longitude) of Panshkura II block of Purba Medinipur district. The samples comprised of infested flowers and leaves of tuberose weighing about 50 g were collected in a zip polythene bag, labelled and brought to the laboratory for extraction and processing of the nematode specimens for detail study.

Extraction of nematodes

Twenty grams of infested plant samples from each of the surveyed area were cut into small pieces of 2-3 cm and kept on the double layer facial tissue paper to follow the modified Baermann process [4] for extraction of all the vermiform stages of *A. besseyi*.

Killing and fixing of nematodes

The nematode specimens were killed by hot-water-bath method. The concentrated nematode suspension was plunged into a beaker containing hot water at about 60-65 C and shaken the suspension continuously inside the beaker for about 2-3 minutes. Then the nematodes were killed and suspension of nematode was kept out of hot water to bring the suspension at room temperature as well as to allow the nematode for settling at the bottom of suspension [5]. To concentrate the nematode suspension, excess amount of water was removed gently from the top of suspension by means of dropper without disturbing the whole. Killed nematodes were then fixed in 4:1 formalin glacial acetic acid and kept separately in the labelled vial for further study.

Processing of nematodes

For studying morphometric variability, the nematode specimens from each population of *A. besseyi* were processed by glycerol-ethanol method [6].

Mounting of nematodes

The processed specimens were permanently mounted in pure anhydrous glycerol. A small drop of glycerol was placed on the center of clean glass slide measuring 3″×1″. About 7-8 specimens of processed nematodes were picked up and placed in the center of glycerol drop so that their head pointing at same direction, making sure that they were resting on the surface of glass slide and not floating on the surface of drop. Microscopic cover glass of 18 mm was placed over the specimen and sealed by paraffin wax-ring method [7].

Observation on intra-specific morphometric variability

Important morphometric features of taxonomic significance were studied in detail for each population of *A. besseyi* obtained from tuberose crop adopting standard diagnostic protocol for *A. besseyi* [8]. Four populations of the mentioned nematode species of tuberose were examined for morphometric characterization for the generation of information on intra-specific variations. This description was modified from Hunt [9].

The following set of parameters used to characterize nematode species were developed are often known as the de Man Formula or the de Man Indices.

n = number of specimens on which measurements are taken

L = total body length

Tail = portion of body from anus or cloaca to posterior terminus

Ratios

a = body length/greatest body width

b = body length/distance from anterior end to junction of oesophagus and intestine

b′= body length/distance from anterior end to end of oesophageal glands

c = body length/tail length

c′= tail length/body width at anus or cloaca

Percentage

V% = (Distance of vulva from anterior/body length) x 100

T% = (Distance from cloaca to anterior part of testis/total body length) x 100

The measurement of all the specimens was taken under trinocular compound microscope (Nikon CiS) taking the support of image capturing device Nikon DS- Ri2 and image analyzing device NIS-Elements BR 4.30.00.

Statistical analysis

To compare morphometric data of four different population *A. besseyi* arithmetic mean, standard deviation (SD), and co-efficient of variation (CV) were computed. Considering CV values the taxonomic characters were rated as least variable, moderately variable and highly variable, using scale <12%, 12-20% and >20%, respectively both for female and male. The data thus obtained was compared with the earlier descriptions of this species [9-12].

Results and Discussion

Comparative morphometric and morphological observations on the females of *A. besseyi*

Observations pertaining to the comparative morphometric and morphological variability of the females of *A. besseyi* Christie has been presented in the table 1. All the populations of *Aphelenchoides* species studied from West Bengal were found to be *A. besseyi* when taxonomic characters were compared with the morphometric descriptions cited in the OEPP/EPPO Standard PM 7/39. Some variations in morphometric characters of the tuberose populations of *A. besseyi* collected from the different areas of West Bengal had been observed.

Descriptions of *A. besseyi* associated with tuberose in the *Gangetic* plains of West Bengal

Female

Data on morphometric variability of *A. besseyi* has been presented in the table 2, table 3, table 4, and table 5. The body length of *A. besseyi* varied from 444-737 µm among females (Figure 1a and 1b).

Body was straight to ventrally arcuate when heat relaxed, body cuticle thin and finely annulated, head low, flat, slightly offset or continuous with the body contour, cephalic region slightly sclerotized and unstriated, lateral field with four to six incisures (Figure 4a and 4b), stylet with weakly developed basal knobs (Figure 1c), procorpus narrow and cylindrical (Figure 1d), strongly developed, offset, ovoid to rectangular and spherical median oesophageal bulb (Figure 1d, 1e, 1f and 1g) measuring 7.5-10.5 µm median width with crescentic valve plate. Pharyngeal gland invariably overlapped the intestine dorsally. Excretory pore was present near the anterior edge of the nerve ring (Figure 2a and 2b). Ovary single, prodelphic and outstretched with narrow post vulval sac (post uterine sac) which varies (34.0-40.7 µm) in length. Length of post vulval sac was 3.5-4.3 times of anal body width but less than 33% of distance between vulva and anus. Vulva post-median in position, usually at 72-74% of the total body length. Vulval opening was transverse slit like (Figure 2c) and raised slightly

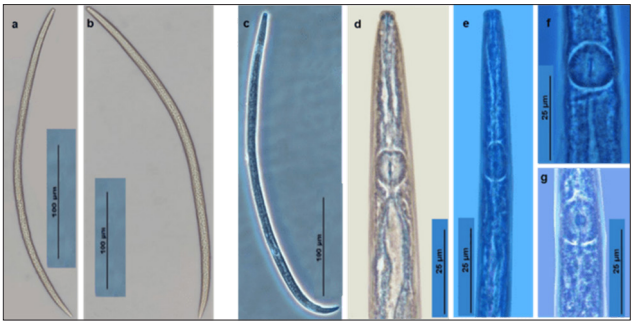

Figure 1: Photomicrographs of *A. besseyi* Christie from West Bengal. (**a, b)** entire female **(c)** entire male **(d)** anterior part of female **(e)** anterior part of male and **(f, g)** variously shaped median bulb.

Table 1: Comparative morphometric data of females of *A. besseyi.*

Taxonomic characters	Present findings	Data as described in OEPP/EPPO, *Bulletin*
	Range	Range
Shape of mucro	diverse shape with three to four processes	diverse shape with three to four processes
Length of post vulval sac	< 33% of distance between vulva to anus	< 33% of distance between vulva to anus
Tail shape	Conoid	Conoid
Position of excretory pore	Anterior or at level of nerve ring	Usually near the anterior edge of nerve ring
a	25.7-54.4	32-42/26.6-58
b	7.8-11.5	10.2-11.4/9.0-13.1
Body length (mm)	0.44-0.74	0.66-0.75/0.57-0.88
Stylet length (mm)	8.8-11.9	10.0-12.5
Lateral lines	04-06	4
c	12.3-21.3	17.0-21/13.8-21

Table 2: Morphometric and morphological variability of *A. besseyi* associated with tuberose in Gayeshpur Central Research Farm of BCKV, West Bengal (all measurements are in µm).

Taxonomic characters	Female (n=19)	CV (%)	Male (n=6)	CV (%)
	Mean ± SD		Mean ± SD	
	(Range)		(Range)	
L	623.8 ± 67.5	10.8	556.5 ± 45.8	8.2
	(477.4-715.1)		(503.2-613.0)	
A	46.2 ± 5.5	12	42.7 ± 0.7	1.7
	(35.6-54.4)		(42.1-43.8)	
B	9.6 ± 0.9	8.8	8.8 ± 0.6	6.4
	(7.9-11.5)		(8.3-9.8)	
b′	4.8 ± 0.6	11.6	4.7 ± 0.4	7.5
	(3.4-5.5)		(4.3-5.1)	
C	17.6 ± 2.1	12.1	18.1 ± 1.7	9.6
	(13.6-21.3)		(16.1-21.2)	
c′	3.7 ± 0.4	9.6	2.8 ± 0.2	7
	(3.2-4.5)		(2.6-3.2)	
V%	72.5 ± 1.7	2.3	-	-
	(70.3-75.9)			

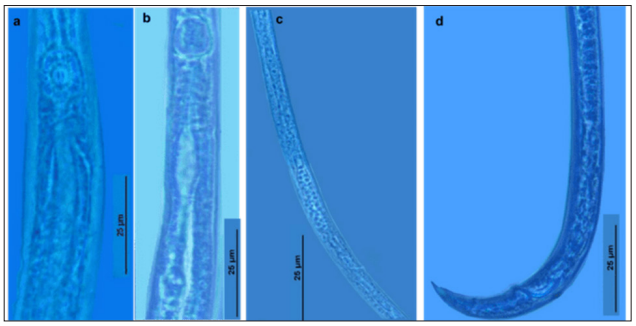

Figure 2: Photomicrographs of *A. besseyi* Christie from West Bengal. **(a, b)** oesophagous region showing nerve ring and excretory duct **(c)** female gonad, vulva, and post uterine sac and **(d)** male genital tract, rose thorn shaped spicule, cloacal aperture, and tail with mucro.

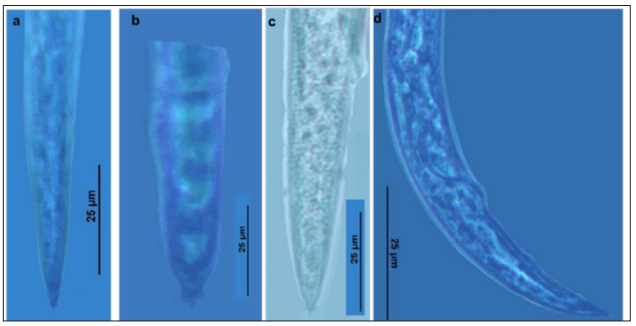

Figure 3: Photomicrographs of *A. besseyi* Christie from West Bengal. **(a, c)** female tail showing anal opening and mucro **(b)** close view of mucro and **(d)** male tail showing spicule, cloacal aperture with mucro.

at the exterior. Tail conoid, 3.7-3.8 anal body width long, the terminus bearing a mucro of diverse shape with three to four processes (Figure 3a, 3b and 3c).

The above description of females of *A. besseyi* was similar with the earlier descriptions made by several workers [8, 9].

Male

The body length of males of *A. besseyi* varies from 386-640 µm. They were slightly smaller than females. The habitus of male body when heat relaxed was of typical walking stick shape or 'J' shape (Figure 1c), which is very distinctive feature of this species. The 'T' % ranged between 32.8-39.3. Cloacal opening was transverse slit like and raised slightly (Figure 2d and figure 3d). The spicule was rose thorn shaped with prominent apex and rostrum, paired and separate, gubernaculums and bursa absent, tail terminus bears star shaped mucro (Figure 2d and figure 3d). Remaining morphometric features were similar with that of females.

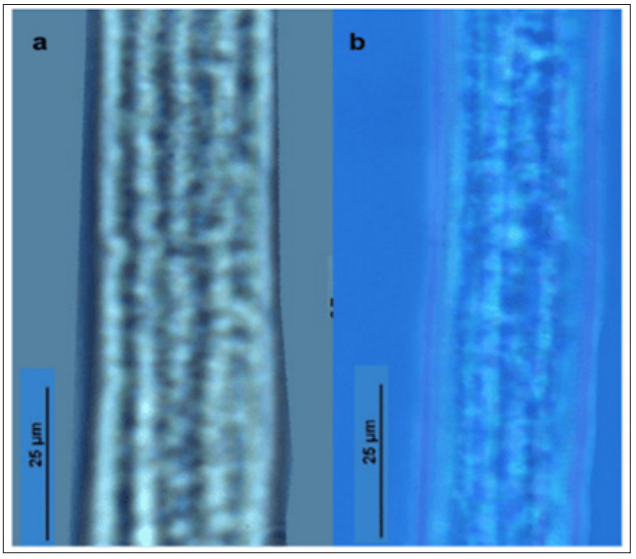

Figure 4: Photomicrographs of *A. besseyi* Christie from West Bengal. **(a, b)** showing lateral lines.

T%	-	-	32.8 ± 4.9 (27.4-40.7)	15.1
Habitus	straight to ventrally arcuate	-	walking stick/J shaped	-
Head height	2.5 ± 0.2 (2.1-2.9)	9	2.7 ± 0.2 (2.3-2.9)	8.4
Head width	6.5 ± 0.3 (5.6-6.9)	4.8	6.4 ± 0.4 (5.9-6.8)	5.8
Stylet length	10.5 ± 0.7 (9.4-11.7)	7	10.3 ± 0.4 (9.8-10.7)	3.3
Basal swellings/knobs	weakly developed	-	weakly developed	-
Median bulb (shape)	oval-rectangular/spherical	-	oval-rectangular	-
Width of median bulb	8.1 ± 1.0 (6.7-10.2)	12.6	7.8 ± 1.0 (6.4-8.5)	13
Greatest body width	13.6 ± 1.3 (11.9-17.3)	9.7	13.0 ± 1.0 (12.0-14.5)	7.6
Isthmus (present/absent)	Present	-	Present	-
Distance from head to oesophagus	130.7 ± 10.2 (117.4-153.2)	7.8	119.1 ± 7.1 (109.9-126.9)	6
Distance from head to junction of oesophagus and intestine	65.2 ± 3.9 (55.0-70.2)	6	63.2 ± 2.8 (60.1-68.0)	4.4
Relative position of excretory pore	near anterior edge of nerve ring	-	near anterior edge of nerve ring	-
Distance of excretory pore from anterior end	74.3 ± 6.9 (60.1-89.2)	9.4	72.5 ± 6.1 (64.3-81.1)	8.4
Ovary	single, prodelphic outstretched	-	-	-
Length of post vulval sac	34.6 ± 6.6 (20.9-46.8)	19	-	-
Length of pvs > or < 33% distance between vulva and anus	< 33% distance between vulva and anus	-	-	-
Tail shape	conoid	-	Conoid	-
Tail length	35.6 ± 3.4 (31.8-42.6)	9.5	30.9 ± 2.2 (28.6-34.2)	7.2
Anal body width	9.6 ± 0.4 (8.4-10.1)	4.1	10.9 ± 0.7 (9.9-11.8)	6.3
Tail terminus	with mucro		with mucro	-
Shape of mucro	diverse shape with three to four processes	-	diverse shape with two to three processes	-
Shape of spicules	-	-	rose thorn	-
Length of spicule	-	-	14.8 ± 1.2 (13.8-17.0)	7.9
Bursa	-	-	Absent	-
Distance between vulva and anus	146.1 ± 24.1 (99.4-193.3)	16.5	-	-
Distance of vulva from anterior end	451.6 ± 44.6 (345.9-503.0)	9.9	-	-
Distance of cloaca from testis	-	-	181.4 ± 19.2 (168.1-219.9)	10.6
Number of lateral lines	4.4 ± 0.8 (4-6)	19	4.0 ± 0.0	0

Hunt [9] also described male as numerous as females. Posterior end of body curved by about 180° in relaxed specimens. Tail conoid, having terminal mucro with two to four pointed processes. Spicules typical of the genus except that the proximal end lacks an apex and has only a moderately developed rostrum.

The study confirmed that all the populations of *Aphelenchoides* species investigated from the major tuberose growing tracts of West Bengal were of *A. besseyi*.

Morphometric and morphological variability of *A. besseyi* associated with tuberose in the *Gangetic* plains of West Bengal

The population of *A. besseyi* from the Gayeshpur Central Research Farm of BCKV, West Bengal revealed least variability (CV<12) in body length among females and males (Table 2). Usually, females were longer in size than males. The stylet length (9.4-11.7 µm) showed least variability among females and males. In females, ratios like a and c were moderately variable while b, b′ and c′ were least variable. All these mentioned features of male were least variable. Number of lateral lines ranged from 4-6 (Table 2). Morphometric features of female such as head width, head height, greatest body width, and distance of excretory pore from anterior end, tail length, anal body width and V% were found least variable (CV<12) in the population of *A. besseyi* recovered from the Gayeshpur Central Research Farm of BCKV. Length of post vulval sac and distance between vulva and anus in females were observed moderately variable in the population. Width of median bulb was observed moderately variable among females and males here (Table 2). Length of spicule in male was found least variable while width of median bulb and T% were moderately variable (Table 2).

Body length of females and males of *A. besseyi* from the Nokari areas of Ranaghat, Nadia, West Bengal was found least variable (CV<12) (Table 3). Number of lateral lines (4) was found very consistent. The stylet length (10.0-11.9 µm) and ratios like a, b and c were all least variable in the females and males. Ratios b′ and c′ were either least or moderately variable among females and males.

Table 3: Morphometric and morphological variability of *A. besseyi* associated with tuberose in Nokari areas of Ranaghat, Nadia, West Bengal (all measurements are in µm).

Taxonomic characters	Female (n=19)	CV (%)	Male (n=5)	CV (%)
	Mean ± SD		Mean ± SD	
	(Range)		(Range)	
L	625.3 ± 57.9	9.3	560.8 ± 46.8	8.3
	(489.5-736.5)		(523.4-639.5)	
A	44.4 ± 4.3	9.7	47.8 ± 2.9	6.1
	(40.7-51.5)		(40.5-52.0)	
B	9.2 ± 0.8	8.8	8.6 ± 0.8	9
	(7.8-11.4)		(8.1-10.0)	
b′	4.7 ± 0.6	13.1	4.3 ± 0.2	4.9
	(3.8-6.1)		(4.0-4.5)	
C	17.6 ± 1.4	8	17.0 ± 1.4	8
	(15.2-19.8)		(15.3-18.8)	
c′	3.8 ± 0.4	10.3	3.0 ± 0.4	12.9
	(3.2-4.6)		(2.5-3.6)	
V%	72.8 ± 1.6	2.3	-	-
	(70.4-75.9)			
T%	-	-	32.8 ± 5.7	17.2
			(27.2-42.0)	
Habitus	straight to ventrally arcuate	-	walking stick/J shaped	-
Head height	2.5 ± 0.2	9.1	2.6 ± 0.3	12.1
	(2.1-2.9)		(2.1-2.9)	
Head width	6.5 ± 0.3	5.1	6.5 ± 0.3	4.9
	(5.6-6.9)		(6.1-6.8)	
Stylet length	11.0 ± 0.5	4.8	11.2 ± 0.5	4.7
	(10.0-11.9)		(10.5-11.9)	
Basal swellings/knobs	weakly developed	-	weakly developed	-
Median bulb (shape)	oval-rectangular	-	oval-rectangular	-

Width of median bulb	7.3 ± 0.9 (6.3-9.4)	11.7	7.2 ± 0.7 (6.4-7.9)	9.7
Greatest body width	13.1 ± 1.2 (11.3-15.3)	15	12.7 ± 1.5 (10.2-14.1)	12.1
Isthmus (present/absent)	Present	-	Present	-
Distance from head to oesophagus	134.9 ± 17.7 (104.5-173.2)	13.1	129.0 ± 8.7 (119.1-141.9)	6.7
Distance from head to junction of oesophagus and intestine	68.1 ± 4.6 (62.4-79.7)	6.7	65.0 ± 3.0 (61.9-69.0)	4.6
Distance of excretory pore from anterior end	76.9 ± 9.7 (62.6-94.1)	12.6	73.9 ± 2.3 (71.3-76.3)	3.1
Relative position of excretory pore	near anterior edge of nerve ring	-	near anterior edge of nerve ring	-
Ovary	single, prodelphic outstretched	-	-	-
Length of post vulval sac	40.7 ± 6.5 (25.2-50.0)	15.9	-	
Length of pvs > or < 33% distance between vulva and anus	< 33% distance between vulva and anus	-	-	-
Tail shape	conoid	-	Conoid	-
Tail length	33.2 ± 4.1 (29.3-39.2)	12.2	35.7 ± 3.3 (30.8-41.1)	9.3
Anal body width	9.5 ± 0.4 (8.4-9.9)	4	10.9 ± 0.7 (9.9-11.8)	6.7
Tail terminus	with mucro	-	with mucro	-
Shape of mucro	diverse shape with three to four processes	-	diverse shape with two to three processes	-
Shape of spicules	-	-	rose thorn	
Length of spicule	-	-	16.1 ± 1.9 (13.6-18.4)	12
Bursa	-	-	Absent	-
Distance between vulva and anus	146.5 ± 16.9 (112.1-173.8)	11.5	-	-
Distance of vulva from anterior end	454.7 ± 38.5 (359.8-521.8)	8.5	-	-
Distance from cloaca to testis	-	-	182.5 ± 21.2 (168.1-219.9)	11.6
Number of lateral lines	4.0 ± 0.0	0	4.0 ± 0.0	0

Morphometric features of female like head width, head height, width of median bulb, anal body width, distance between vulva and anus, distance of vulva from anterior end and V% were observed least variable (CV<12) in the population of *A. besseyi* recovered from the Nokari areas of Ranaghat (Nadia, West Bengal).

The greatest body width, distance of excretory pore from anterior end, length of post vulval sac and tail length of females of *A. besseyi* were found moderately variable in the population. Length of post vulval sac of Nokari population of *A. besseyi* was comparatively larger than the remaining three populations. In males, length of spicule and T% were found moderately variable here.

The population of *A. besseyi* from the Raghunathpur areas of Bhangar, South 24-Parganas, West Bengal revealed least

Table 4: Morphometric and morphological variability of *A. besseyi* associated with tuberose in Raghunathpur areas of Bhangar, South 24-Parganas, West Bengal (all measurements are in µm).

Taxonomic characters	Female (n=21)	CV (%)	Male (n=4)	CV (%)
	Mean ± SD		Mean ± SD	
	(Range)		(Range)	
L	624.0 ± 34.6	5.5	518.3 ± 33.5	6.5
	(562.3-705.3)		(470.2-545.0)	
A	41.5 ± 2.0	4.6	37.4 ± 3.2	8.7
	(38.2-45.7)		(40.7-51.5)	
B	9.1 ± 0.6	6.3	7.6 ± 0.2	3
	(8.0-10.0)		(7.5-8.0)	
b′	4.7 ± 0.6	13.1	4.3 ± 0.2	4.9
	(3.8-6.1)		(4.0-4.5)	
C	17.6 ± 1.5	8.6	16.4 ± 0.8	5.2
	(14.7-20.4)		(15.3-17.2)	
c′	3.7 ± 0.3	8.2	2.8 ± 0.1	3.2
	(3.2-4.3)		(2.7-2.9)	
V%	72.0 ± 1.1	1.6	-	-
	(70.2-75.1)			
T%	-	-	34.7 ± 5.8	16.8
			(29.4-42.1)	
Habitus	straight to ventrally arcuate	-	walking stick/J shaped	-
Head height	2.5 ± 0.2	8.6	2.7 ± 0.3	10.5
	(2.1-2.9)		(2.3-2.9)	
Head width	6.6 ± 0.3	4.9	6.2 ± 0.3	4
	(5.6-6.9)		(5.9-6.5)	
Stylet length	9.8 ± 0.4	4.6	9.6 ± 0.2	2.5
	(8.9-10.5)		(9.4-9.9)	
Basal swellings/knobs	weakly developed	-	weakly developed	-
Median bulb (shape)	oval-rectangular	-	oval-rectangular	-
Width of median bulb	8.8 ± 0.5	5.9	8.3 ± 0.4	4.3
	(7.8-9.8)		(8.0-8.8)	
Greatest body width	15.1 ± 1.1	7.1	13.9 ± 1.2	8.5
	(13.5-18.2)		(12.7-15.0)	
Isthmus (present/absent)	present	-	Present	-
Distance from head to oesophagus	133.8 ± 12.4	9.3	110.4 ± 17.4	15.8
	(114.1-155.6)		(90.1-132.3)	
Distance from head to junction of oesophagus and intestine	68.6 ± 4.8	7	67.9 ± 4.2	6.2
	(62.4-79.7)		(62.3-72.0)	
Relative position of excretory pore	near anterior edge of nerve ring	-	near anterior edge of nerve ring	-
Distance of excretory pore from anterior end	65.8 ± 3.4	12.6	62.3 ± 3.7	5.9
	(60.1-73.3)		(58.7-66.4)	
Ovary	single, prodelphic outstretched	-	-	-

Length of post vulval sac	36.2 ± 4.3 (29.0-46.0)	11.8	-	-
Length of pvs > or < 33% distance between vulva and anus	< 33% distance between vulva and anus	-	-	-
Tail shape	conoid	-	Conoid	-
Tail length	35.7 ± 3.3 (30.8-43.2)	10.2	31.6 ± 0.7 (30.8-32.2)	2.2
Anal body width	9.5 ± 0.4 (8.4-10.1)	4.1	11.3 ± 0.5 (10.6-11.8)	4.5
Tail terminus	with mucro	-	with mucro	-
Shape of mucro	diverse shape with three to four processes	-	diverse shape with two to three processes	-
Shape of spicules	-	-	rose thorn	-
Length of spicule	-	-	14.0 ± 0.8 (13.1-15.0)	5.7
Bursa	-	-	Absent	-
Distance between vulva and anus	140.5 ± 13.0 (118.7-171.7)	9.3	-	-
Distance of vulva from anterior end	449.0 ± 24.9 (409.7-507.1)	5.5	-	-
Distance of cloaca from testis	-	-	179.3 ± 27.7 (157.3-219.9)	15.5
Number of lateral lines	4.0 ± 0.0	0	4.0 ± 0.0	0

variability in body length among females and males (Table 4). The stylet length (8.9-10.5 µm) and ratios like a, b, c and c′ were least variable among females and males here. Ratio b′ was found least variable in males while moderately variable in females. In females, head height, head width, greatest body width, width of median bulb, length of post-vulval sac, tail length, anal body width, distance between vulva and anus, distance of vulva from anterior end and V% were found very consistent (least variable) morphometric features. Here number of lateral incisures was 4 both in the male and female. Among males, the length of spicule, tail length and anal body width were noticed as stable character (least variable) while T% was moderately variable.

The population of *A. besseyi* from Deulia areas of Panshkura, Purba Medinipur, West Bengal revealed moderate variability (CV=12-20) in body length among males and females (Table 5). The stylet length (8.8-11.8 µm) was least variable (CV<12) in females and moderately variable among males. In females, ratios like b, b′ and c were least variable but a and c′ were moderately variable. Among males, ratio c was least variable while remaining ratios like a, b, b′ and c′ were moderately variable. Among females, head height, head width, greatest body width, width of median bulb, anal body width and V% were found very consistent (least variable) morphometric features here. Distance of excretory pore from anterior end, distance between vulva and anus, distance of vulva from anterior end were moderately variable morphometric features of females. Tail length and length of post vulval sac were highly variable. Here number of lateral incisures was 4 both in the male and female. In males, except anal body width the length of spicule, tail length and T% were observed as moderately variable character.

In all the four populations of *A. besseyi* b, stylet length, head height, head width, anal body width and V% were the least variable morphometric characters. Ratios like a, b′, c and c′ and distance between vulva and anus were either least or moderately variable. Body length of *A. besseyi* was least variable character except Deulia population where the mentioned character was moderately variable. Greatest body width was found to be very consistent character of *A. besseyi* in all the population except from Nokari area. Width of median bulb and number of lateral lines were very consistent feature among population other than Gayeshpur population of *A. besseyi* where the aforementioned feature was moderately variable.

Distance of excretory pore from anterior end was observed as moderately variable morphometric attribute in the populations of *A. besseyi* except Gayeshpur population where the attribute was highly stable. Tail length was found as stable feature in the Gayeshpur and Raghunathpur population. Length of post vulval sac of Nokari population of *A. besseyi* was comparatively larger than the remaining three populations.

Khan et al. documented body length of females of *A. besseyi* ranged from 514 to 845 µm which was moderately to highly variable [12]. The ratios like a, b, b′, c and c′ were highly variable among females of tuberose population of *A. besseyi*. Stylet length (10-11 µm) and V% (71-79) were either

Table 5: Morphometric and morphological variability of *A. besseyi* associated with tuberose in Deulia areas of Panshkura, Purba Medinipur, West Bengal (all measurements are in µm).

Taxonomic characters	Female (n=13) Mean ± SD (Range)	CV (%)	Male (n=8) Mean ± SD (Range)	CV (%)
L	544.7 ± 87.2 (443.5-720.8)	16	488.8 ± 87.1 (386.2-631.5)	17.8
A	30.3 ± 3.7 (25.7-36.9)	12.3	32.7 ± 4.3 (27.6-39.5)	13.3
B	9.1 ± 1.0 (8.2-11.5)	11.1	7.8 ± 1.2 (6.4-9.6)	15.2
b′	4.4 ± 0.2 (4.1-4.7)	4.9	4.2 ± 0.6 (3.5-5.5)	13.7
C	15.4 ± 1.5 (12.3-17.1)	10	15.8 ± 1.2 (14.2-17.6)	7.6
c′	3.8 ± 0.7 (3.1-5.3)	18.6	2.8 ± 0.5 (2.2-3.6)	17.3
V%	73.6 ± 0.7 (70.6-75.9)	2.7	-	-
T%	-	-	39.3 ± 7.2 (35.1-56.9)	18.4
Habitus	straight to ventrally arcuate	-	walking stick/J shaped	-
Head height	2.5 ± 0.2 (2.1-2.9)	9.4	2.6 ± 0.3 (2.1-2.9)	11.4
Head width	6.5 ± 0.3 (5.6-6.9)	5.6	6.4 ± 0.3 (5.9-6.8)	5.3
Stylet length	9.7 ± 0.9 (8.8-11.8)	8.9	9.7 ± 1.4 (8.0-12.1)	14.2
Basal swellings/knobs	weakly developed	-	weakly developed	-
Median bulb (shape)	oval-rectangular	-	oval-rectangular	-
Width of median bulb	10.5 ± 1.2 (9.4-12.9)	11.4	8.5 ± 0.7 (7.2-9.4)	7.9
Greatest body width	18.0 ± 1.5 (15.5-19.5)	8.1	14.9 ± 1.7 (12.3-17.2)	11.1
Isthmus (present/absent)	Present	-	Present	-
Distance from head to oesophagus	124.1 ± 18.2 (104.9-167.1)	14.7	115.7 ± 15.5 (94.8-136.9)	13.4
Distance from head to junction of oesophagus and intestine	59.6 ± 5.3 (55.3-65.1)	8.8	63.2 ± 7.3 (50.7-72.0)	11.6
Relative position of excretory pore	near anterior edge of nerve ring	-	near anterior edge of nerve ring	-
Distance of excretory pore from anterior end	65.7 ± 8.7 (56.2-85.4)	13.3	61.0 ± 6.9 (51.5-68.9)	11.3
Ovary	single, prodelphic outstretched	-	-	-
Length of post vulval sac	34.0 ± 7.5 (20.9-46.8)	22	-	-

Length of pvs > or < 33% distance between vulva and anus	< 33% distance between vulva and anus	-	-	-
Tail shape	conoid	-	Conoid	-
Tail length	35.7 ± 7.2	20.2	31.0 ± 4.5	14.4
	(29.8-52.3)		(24.9-38.9)	
Anal body width	9.4 ± 0.5	4.9	10.9 ± 0.6	5.4
	(8.4-9.9)		(9.9-11.8)	
Tail terminus	with mucro	-	with mucro	-
Shape of mucro	diverse shape with three to four processes	-	diverse shape with two to three processes	-
Shape of spicules	-	-	rose thorn	-
Length of spicule	-	-	16.1 ± 1.9	12
			(14.2-20.0)	
Bursa	-	-	Absent	-
Distance between vulva and anus	126.2 ± 20.7	16.4	-	-
	(100.7-174.3)			
Distance of vulva from anterior end	399.7 ± 55.9	14	-	-
	(334.9-514.1)			
Distance from cloaca to testis	-	-	188.7 ± 26.8	14.2
			(157.3-226.9)	
Number of lateral lines	4.0 ± 0.0	0	4.0 ± 0.0	0

least or moderately variable. They also observed variations in the tail shape and terminal mucro where mucro was with 2-3 processes, shape of median bulb, cephalic regions and number of lateral lines ranging from 4-6. In the present study, data pertaining to morphometry of *A. besseyi* populations collected from four important tuberose growing tracts of West Bengal namely, Gayeshpur (Chakdah, Nadia), Nokari (Ranaghat –II, Nadia), Raghunathpur (Bhangar I, South 24-Parganas) and Deulia (Panshkura II, Purba Medinipur) was at par with the measurements as reported by many others [8-10, 13-17].

There were considerable variations in morphometric measurements among tuberose populations of *A. besseyi* recovered from West Bengal (India). Similar variations in morphometry of *A. besseyi* were earlier reported by several workers [8, 12]. These variations may be attributed due to difference in genetic constituents, adoption of different crop cultivars, existence of varied agro-ecological conditions etc. Based on morphometry and culturability on fungal hosts Rajan et al. also proposed two groups of *A. besseyi* [18].

The morphology of *A. besseyi* populations showed limited variation in the tail shape, medial bulb of oesophagus and head end of anterior regions. Furthermore, the occurrence of four as well as six lateral lines in the lateral field of the rice population was observed in scanning electron microscope as well as light microscope observations [12]. In the present investigation, number of lateral incisures varied from 4-6 only in the Gayeshpur population of *A. besseyi* recovered from tuberose crop. Hence, variation in number of lateral lines among tuberose population of *A. besseyi* can be evident.

Hooper & Ibrahim described *Aphelenchoides nechaleos*, a very closely related species of *A. besseyi* and viewed four lines with light microscope and six lines with scanning electron microscope [19]. The number of lateral lines was not considered as a stable character to differentiate *A. nechaleos* from remaining 14 species of *Aphelenchoides*.

Conclusion

Tuberoses contain cancer prevention agents and antibacterial properties. After rubbing on skin and hair it helps in treating various bacterial development, diseases, and exterior harms. so it is an important commercial crop. The body length of *A. besseyi* varied from 444-737 µm among females. Body was straight to ventrally arcuate when heat relaxed, body cuticle thin and finely annulated, head low, flat, slightly offset or continuous with the body contour, cephalic region slightly sclerotised and unstriated, lateral field with four to six incisures, stylet with weakly developed basal knobs, procorpus narrow and cylindrical, median oesophageal bulb strongly developed, offset ovoid to rectangular or spherical measuring 7.5-10.5 µm median width with crescentic valve plate. Pharyngeal gland invariably overlapped the intestine dorsally. Excretory pore was present near the anterior edge of the nerve ring. Ovary single, prodelphic and outstretched with narrow post vulval sac (post uterine sac) which varies (34.0-40.7 µm) in length. Length of post vulval sac was 3.5-4.3 times of anal body width but less than 33% of distance between vulva and anus. Vulva post-median in position, usually at 72-74% of the total body length. Vulval opening was transverse slit like and raised slightly at the exterior. Tail conoid, 3.7-3.8 anal body

width long, the terminus bearing a mucro of diverse shape with three to four processes.

The body length of males of *A. besseyi* varied from 386-640 µm. They were slightly smaller than females. The habitus of male body when heat relaxed was of typical walking stick shape or 'J' shape, which is very distinctive feature of this species. The 'T' % ranged between 32.8-39.3. Cloacal opening was transverse slit like and raised slightly. The spicule was rose thorn shaped with prominent apex and rostrum, paired and separate, gubernaculum and bursa absent, tail terminus bears star shaped mucro. Remaining morphometric features were similar with that of females.

In all the four populations of *A. besseyi* the 'b' ratio (7.8-11.5), stylet length (8.8-11.9 µm), head height (2.1-2.9 µm), head width (5.6-6.9 µm), anal body width (8.4-10.1 µm) and V% (70.2-75.9) were observed as the least variable morphometric characters. Ratios like a, b′, c and c′ and distance between vulva and anus were either least or moderately variable. Body length of *A. besseyi* was found least variable character except for the Deulia population where the mentioned character was moderately variable. Greatest body width was found to be very consistent character of *A. besseyi* in all the population except for the Nokari population. Width of median bulb and number of lateral lines were very consistent feature among population other than Gayeshpur population of *A. besseyi* where the aforementioned features were moderately variable.

Distance of excretory pore from anterior end was observed as moderately variable morphometric attribute in the populations of *A. besseyi* except Gayeshpur population where the attribute was highly stable. Tail length was found as stable feature in the Gayeshpur and Raghunathpur population. Length of post vulval sac of Nokari population of *A. besseyi* was comparatively more than the remaining three populations.

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Conflict of Interest

The authors declared that they have no conflict of interest with respect to this work.

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