

Morphological and morphometric variation studies within and among populations of *Helicotylenchus* sp. From banana rhizosphere of Nilambur and Kalikavu blocks, Malappuram dt., Kerala

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Abstract

Thirty populations of *Helicotylenchus* sp. from banana rhizosphere of fourteen Panchayaths of Nilambur and Kalikavu blocks of Malappuram District, Kerala were subjected for morphological and morphometric characterization and they were compared within and among different populations for their similarities and differences. The total population comprises10 juveniles, 7 males and 13 females. Among these thirteen females majority were identified as *Helicotylenchus multicinctus* (10 out of 13) and less were *H. dihystera* using their morphological and morphometric characters. Both in morphological and morphometric data, the helicotylenchus from various panchayaths and from the same panchayth show both similarities and differences. The study is a first report of *H. multicinctus* and *H. dihystera* from these block panchayaths of Malappuram district. The present findings were compared with other workers and subjected for an analysis.

Keywords: population, various panchayaths, juveniles, identified, analysis

Introduction

Banana production in India is constrained by several factors including erratic weather conditions, unavailability of quality planting material, assured source of irrigation water, scarce supply of fertilizers and infestation of pests and disease (Roy et al. 2014) ^[9]. Important biotic constrains against successful banana cultivation includes diseases, insect pests, mite and plant parasitic nematodes (Roy et al., 2014). Among variously reported nematodes, spiral nematode Helicotylenchus sp. seems to be highly destructive to various crops and dozens of Helicotylenchus sp. have been reported with various, agronomic, horticultural crops including banana, Cotton, vegetables like Cabbage, bitter gourd, chilies, cucumber, eggplant, cow pea, carrot, Cauliflower, tomato etc.. (Anwar and Mckenry 2012; Roy et al. 2014; Singh and khanna 2015; Narkhedkar 2006) ^[1, 9, 12, 7]. Including banana several species parasitize diverse group of agricultural importance by Helicotylenchus and are globally distributed (Subbotin et al., 2011)^[13].

Helicotylenchus spp. is an important pest in India causing significant yields loss (Nath *et al.* 1998; Sahu *et al.* 2011; Chanu and Meitei 2016) ^[8, 10, 2]. It is the richest genus in the family Hopolaimidae and infraorder tylenchomorpha with about 2509 species (Siddiqi 2000; De Ley and Blaxter 2004; Decraemer and Hunt 2006; Andrassy 2007; Kashi and Karegar 2014) ^[5]. Morphological and morphometric characters have been found to be altered among this nematode, may be due to geographical variation or may be due to many biotic and abiotic influences. The present studies thus focus on a comparative study of morphological and morphometric characters between *Helicotylenchus* sp. Within and among populations collected from Nilambur and Kalikavu blocks of Malappuram district, Kerala. The work

here thus aims on proper and accurate identification of spiral nematode by considering the importance of applying management strategies.

Methodology

Soil samples from the rhizosphere of banana were collected from fourteen panchayaths of kalikavu and Nilambur blocks in Malappuram district of Kerala, India include Amarambalam, Karuvarakundu, Kalikavu, Karulai, Chokkad, Edapatta, Tuvvur, Chaliyar, Chungathara, Edakkara, Moothedam, Nilambur, Vazhikadavu, and Pothukallu during July- November 2018. Soil samples were collected from depth of 15-20 cm and each composite soil was a representative of five such samples at each field.

The collected samples were then labeled with date, locality and variety of host and brought to the laboratory and stored in refrigerator at 5 ⁰c for further extraction process and processing of nematode specimens. Nematodes were extracted using Cobb's decanting and sieving method. For fixing the nematodes eight percent of formalin was poured to an equal amount of nematode suspension. By using binocular stereomicroscope (Magnus MSZ-TR) the nematodes were counted and observed. In order to observe under Research microscope (Olympus CX21i) temporary slides are prepared and photographs of the nematodes were taken using the camera attached on the microscope (Magnus). Identification up to generic level was done by using different keys (Vovlas 1983; Fortuner 1984; Mekete *et al.* 2008; Roy *et al.* 2014) ^[14, 6, 9].

Magnus pro software is used to measure nematodes after calibrating the camera.

Code	Original collection locations	Host plant
Fcu-N2	Nilambur-Chungathara	Musa X paradisiaca L. (AAB) 'Poovan'
Fe-M	Nilambur- Edakkara	Musa X paradisiaca L. (AAB) 'Poovan'
Fm-Po	Nilambur-Moothedam	Musa X paradisiaca L. (AAB) 'Monthan'
Fm-M	Nilambur-Moothedam	Musa X paradisiaca L. (AAB) 'Poovan'
Fv-P	Nilambur-Vazhikadavu	Musa X paradisiaca L. (AAB) 'Rasthali'
Fca-N	Nilambur-Chaliyar	Musa X paradisiaca L. (AAB) 'Poovan'
Oa-M	Kalikavu- Amarambalam	Musa X paradisiaca L. (AAB) 'Poovan'
Oa-N	Kalikavu- Amarambalam	Musa X paradisiaca L. (AAB) 'Poovan'
Ok-M	Kalikavu-Karuvarakundu	Musa X paradisiaca L. (AAB) 'Poovan'
Ot-R	Kalikavu- Thuvvur	Musa acuminate Colla (AAA) 'Robusta'
Oka-M	Kalikavu- Karulai	Musa X paradisiaca L. (AAB) 'Poovan'

Table 1: Origin of Helicotylenchus sp. Populations used in this study

NB: Nilambur and Kalikavu blocks were denoted using the alphabets F and O respectively. Female, male and juveniles were denoted by f, m and j respectively and the numbers were given as 1, 2, 3 for more than one members in the same population (for e.g.: Oa-Mf1, Oa-Mf2 denotes first and second *Helicotylenchus* female members in the Amarambalam panchayats of Nilambur block)

Thirty populations of *Helicotylenchus* from the study area were subjected for morphological as well as morphometric characterization in order to analyze variations and similarities within and among populations of Nilambur block and Kalikavu. Different parameters were used to measure the

Measurements of Nematodes

Measurement mostly was taken using Magnus pro software after calibration at 40X. The measurements used in the different stages of *Helicotylenchus* sp. include

n=number of specimens on which measurements are taken

L=total body length (μ m)

Mbw = maximum body width

b.w.a=body width at anus

Stylet length, lip height, lip width, tail length, spicule length etc.

Ratios

a=ratio of total body length to mbw

b=ratio of L to distance from anterior end to junction of esophagus and intestine

b'= ratio of L to distance from anterior end to posterior end of esophageal glands

c=ratio of body length to tail length

 \mathbf{c} '=tail length to anal body width ratio

Percentage

V%= distance from head to vulva given as the percentage of the body length.

Result and Discussion Morphology

Adult female(n=13): Heat relaxed specimens were spiral or open- C shaped, annules distinct, cephalic surface moderate to heavily sclerotized, stylet well developed usually 3- 4 times lip region diameter with rounded or cup shaped stylet knobs, lip region hemispherical, slightly offset with 4-5 annuli, esophagus with round metacorpus and oesophageal gland overlap intestine ventrally. Female reproductive structure composed of two ovaries with posterior one sometimes reduced, vulva, depressed transverse slit located ventrally at about 70% from the anterior end. Tail short, usually sub or hemispherical with convex-conoid in shape with more curving in dorsal margin than ventral, or sometimes with dorsal and ventral sides joining at an angle with terminal projection.

Adult male (N=7): Similar to female except genital features, Testis single, outstretched. Caudal alae crenate, enveloping tail. Tail short, Spicule well developed cephalated, bursa reaching tail tip gubernaculums simple.

Juveniles (N=10): juveniles were similar to that of their adult except the presence of reproductive structures. Heat relaxed specimens were spiral, short and stout when compared to adults.

Morphometrics

Adult female

Table I and II showed the morphometric data of all female *Helicotylenchus* sp. from Nilambur and Kalikavu block, respectively. There are no distinct variations except in some populations. In the Nilambur block Longest body was collected from Chungathara (0.55 mm) from Nendran variety while shortest was from Moothedam Panchayat (0.52 mm) observed in Poovan variety of banana whereas in Kalikavu block the longest one was reported from Poovan variety of Karuvarakundu Panchayat and shortest one from Robusta variety from Thuvvur panchayat with an average value of 0.56 mm while in the Nilambur Block average length of all members gives 0.52mm which is somewhat close to the shortest member in the Kalikavu Block (0.49 mm). More members of *Helicotylenchus* females were reported from Amarambalam panchayath.

Stylet length ranging from 18.29-22.30 µm shows slight variation in which the shortest was from Moothedam Panchayat, host as Monthan variety and the longest stylet were found from Edakkara panchayat. Lip height and lip width were in same range with 3-3.94 and 5.46-6.96 respectively in the Nilambur block. Average value of 'a'ratio is 26.08 and 26.88 in the range of 24.19-28.40 and 24.27-29.6 in Nilambur and Kalikavu block respectively and it thus shows not much difference among the populations. Tail is short in both the populations and vulva is located in about 65-77% of the total body length of female.

Helicotylenchus multicinctus shows variations within the same population itself through various characters like tail features like symmetrical and asymmetrical tail terminus sometimes with a hemispherical end and sometimes with a rounded end. Body length of the members also varies along with the shape of lip annules, stylet knob (Fig. 2) but shows similarities in the body habitus as most of the members with c shaped or open c shaped habitus except few. All the members of *H. dihystera* from both Kalikavu and Nilambur blocks shows similar characters from body habitus to tail features except slight variations in length of the body.

Adult male

Differing from females, male *Helicotylenchus* were short and stout, with longest one being from Pothukal (490.69) and Karuvarakundu (466.09) and the shortest one from Chaliyar (418.54) panchayat. 'a' ratio ranges from, 25.07-30.12 in Nilambur block in which both lowest and highest was from Moothedam panchayat in Poovan and Monthan variety respectively. Stylet length value ranges in between 14.46 to 20.43, having an average value of 17.50.only two male members were reported from Kalikavu Panchayat and there is not much differences among the members except a slight difference in 'b.w.a' of Amarambalam Poovan (9.10) and Karuvarakundu Poovan (15.60).

Comparative studies within populations can be conduct within Moothedam and Chaliyar panchayaths in case of male members. Individuals from Moothedam panchayat show similarities in some characters (like L, a, b, b', c etc.) and difference in some others (c', stylet length etc.).

Juveniles

Five juveniles each from Nilambur and Kalikavu blocks were studied. Table III and IV shows morphometric data of all *Helicotylenchus* juveniles collected from these block panchayats. Body length of juveniles from Nilambur shows somewhat similar measurement ranges from 331.27(from Chaliyar) - 395.9 (Moothedam) In case of Kalikavu block there is huge difference in between the shortest and longest juveniles.

Both the shortest (290.64) and longest (418.42) were reported from the Karuvarakundu panchayath from the same host i.e. the Poovan variety of banana. Comparative studies can be easily done with juvenile as two individuals were reported from most of the study sites. From Chaliyar panchayath both samples shows somewhat similar morphometric data. In other members there are some differences in the data including length, ratios like a and b' etc. and in lip width.

In Nilambur block 'a' ratio ranges from, 20.66-26.89 in which lowest was from Chaliyar (Nendran) and Moothedam (Poovan). Stylet length ranging from 16.14 to 18.77 with an average value of 17.47, mid body width is in between 14.72 and 16.36. Highest lip height and lip width was reported from Vazhikadavu (Rasthali).

Comparative studies of present findings with other workers

Morphometric data of all *Helicotylenchus* sp. (except juveniles) studied in Nilambur and Kalikavu block were compared with that of after with after (Siddiqi 1973/ Ganguly *et al.* 2003 and Roy *et al.* 2014) ^[11, 4, 9]. and the data were given in table IX. Morphological characters including habitus, body length, a, b, b', c, c' stylet length, lip region, tail shape and terminus were compared with that of previous workers.

The present study indicates the presence of substantial number of Helicotylenchus sp. from all the fourteen panchayaths of Nilambur and Kalikavu block and it also shows considerable variations within and among the different populations. The morphometric data shows somewhat similar to that of after (Siddiqi 1973/ Ganguly et al. 2003) [11, 4]. although it shows considerable variations among the body length of the nematode, in our study the length range from 493-597 with an average value of 544.39 somewhat shorter but, Ganguly et al. 2003 reported a body length of 470-680 µm. and (Roy et al. (2014)^[9]. reported the Helicotylenchus population from banana rhizosphere whose length ranges from 475-740 µm. the maximum body length of our study population hardly reaching 600 µm. characters like a, b and b' shows similarities with that of previous studies showing similar ratios but 'c' value of Helicotylenchus male population is lesser than that of (Roy et al. (2014)^[9]. and (Ganguly et al. (2003)^[4]. Shortest stylet length was also reported from the present study both in female and male populations. Lip region, tail terminus and Vulva % were also shows similar values and characters.

 Table 2: Morphometric of all Helicotylenchus sp. females collected from Nilambur block

code	L	a	B	b'	с	C'	Stylet length	Lip height	Lip width	mbw	b.w.a	Tail length	V%
^a F ^b cu- ^f N ₂ f ₁	554.44	26.22	6.35	4.09	38.13	1.21	20.31	3.47	5.62	21.14	11.96	14.54	65.69%
F ^c e- ^g Mf ₁	518.02	25.81	4.86	3.77	37.26	1.37	22.3	3.34	6.96	20.07	10.14	13.9	71.49%
F ^d m- ^g Pof ₁	520.41	26.48	6.32	4.73	37.55	1.23	18.58	3.49	6.66	19.65	11.21	13.86	67.41%
Fm- ^h Pof ₂	528.84	28.4	6.62	4.51	34.12	1.06	18.29	3.3	6.6	19.56	14.61	15.5	68.19
Fm-Mf ₁	502.62	25.42	5.53	4.15	34.42	1.04	21.51	3	5.46	19.77	13.91	14.6	76.14%
F ^e v- ⁱ Pf ₁	537.92	24.19	5.16	4.11	47.2	1.07	19.84	3.94	6.75	20.74	11.36	10.63	69.23%

NB: (^aF-Nilambur, ^bcu-hungathara, ^ce-Edakkara, ^dm-Moothedam ^ev-Vazhikadavu; ^fN-nendran, ^gM-Poovan, ^hPo-Monthan, ⁱP-Rasthali)

Table 3: Morphometric of all Helicotylenchus sp. females collected from Kalikavu block

code	L	Α	b	b'	с	С'	Stylet length	Lip height	Lip width	mbw	b.w.a	Tail length	V%
${}^{a}F^{b}cu-{}^{f}N_{2}f_{1}$	554.44	26.22	6.35	4.09	38.13	1.21	20.31	3.47	5.62	21.14	11.96	14.54	65.69%
F ^c e- ^g Mf ₁	518.02	25.81	4.86	3.77	37.26	1.37	22.3	3.34	6.96	20.07	10.14	13.9	71.49%
F ^d m- ^g Pof ₁	520.41	26.48	6.32	4.73	37.55	1.23	18.58	3.49	6.66	19.65	11.21	13.86	67.41%
Fm- ^h Pof ₂	528.84	28.4	6.62	4.51	34.12	1.06	18.29	3.3	6.6	19.56	14.61	15.5	68.19
Fm-Mf ₁	502.62	25.42	5.53	4.15	34.42	1.04	21.51	3	5.46	19.77	13.91	14.6	76.14%
F ^e v- ⁱ Pf ₁	537.92	24.19	5.16	4.11	47.2	1.07	19.84	3.94	6.75	20.74	11.36	10.63	69.23%

NB: (^aO- Kalikavu, ^ba- Amararambalam, ^ck- karuvarakundu, ^dt- Tuvvur; ^eM-Poovan, ^fN-Nendran, ^gR-Robusta)

Table 4: Body characters and species level identification of <i>Helicotylenchus</i> sp. from Nilambur and Kalikavu block

Code	Body characters	Identified as
Fcu-N ₂ f ₁	Habitus-Spiral, tail- with dorsal and ventral sides joining at an angle with terminal projection	H. dihystera
Fe-Mf ₁	Habitus- C shaped, tail- asymmetrical (more curved dorsally), with hemispherical end	H. multicinctus
Fm-Pof1	Habitus- C shaped, tail-asymmetrical and regularly rounded	H. multicinctus
Fm-Pof2	Habitus- C shaped, tail-asymmetrical and regularly rounded	H. multicinctus
$Fm-Mf_1$	Habitus-Spiral, tail-asymmetrical and regularly rounded	H. multicinctus
Oa-Mf ₁	Habitus-C shaped, tail-symmetrical and regularly rounded	H. multicinctus
Oa-Mf2-	Habitus-Spiral, tail- with dorsal and ventral sides joining at an angle with terminal projection	H. dihystera
Oa-Mf ₃	Habitus-C shaped, tail- asymmetrical (more curved dorsally), with rounded end	H. multicinctus
Oa-Nf ₁	Habitus- C shaped, tail- with dorsal and ventral sides joining at an angle with terminal projection	H. dihystera
Ok-Mf1	Habitus- C shaped, tail asymmetrical (more curved dorsally), with rounded end	H. multicinctus
Ot-Rf1	Habitus- C shaped, tail- asymmetrical (more curved dorsally), with hemispherical end	H. multicinctus
Ot-Rf2	Habitus-Spiral, tail-asymmetrical(more curved dorsally), and hemispherical end	H. multicinctus

Table: 5: Morphometric of all Helicotylenchus sp. male collected from Nilambur block

code	L	a	b	b'	с	C'	Stylet length	Lip height	Lip width	mbw	b.w.a	Tail length	Spicule length
^a F ^b e- ^f Mm ₁	476.62	25.26	3.23	4.26	35.54	1.54	20.43	3.16	6.33	18.87	8.69	13.41	18.57
F ^c m-Mm ₁	454.11	25.07	5.84	4.91	34.09	2.77	13.98	3.23	4.26	12.61	14.98	13.32	20.5
Fm- ^g Pom ₁	456.09	30.12	5.67	4.91	35.88	1.46	17.98	2.91	5.83	15.14	8.69	12.71	15.95
F ^d ca- ^h Nm ₁	418.54	27.62	6.18	5.04	30.5	1.17	18.73	2.83	6.42	15.15	11.73	13.72	16.02
Fca-Nm ₂	460.07	28.24	5.99	4.78	35.06	1.55	19.47	3.32	5.43	16.29	8.42	13.12	16.24
F ^e p-Nm ₁	490.69	27.55	5.99	4.78	34.55	3.26	14.46	2.33	7.9	17.87	15.89	14.2	18.15

NB: (^aF-Nilambur, ^be-Edakkara, ^cm-Moothedam ^dca-Chaliyar, ^ep-Pothukallu; ^fM-Poovan, ^gPo-Monthan ^hN-nendran)

Table 6: Morphometric of all Helicotylenchus sp. male collected from Kalikavu block

code	L	a	b	b'	с	С'	Stylet length	Lip height	Lip width	mbw	b.w.a	Tail length	Spicule length
^a O ^b a-Mm ₁	440.18	27.3	4.36	3.87	24.84	1.95	19.84	3.39	4.16	16.12	9.1	17.72	18.44
O ^c k-Mm ₁	466.09	25.69	5.37	4.57	28.07	1.06	21.99	3.46	6.55	18.14	15.6	16.6	17.69
NB: (^a O- Kal	NB: (^a O- Kalikavu, ^b a- Amararambalam, ^c k- karuvarakundu)												

Table 7: Morphometric of all Helicotylenchus sp. juveniles collected from Nilambur block

code	L	a	b	b'	с	С'	Stylet length	Lip height	Lip width	mbw	b.w.a	Tail length
Fm-Mj ₁	395.9	26.89	4.61	4.07	49.11	1.11	16.61	2.59	4.79	14.72	7.26	8.06
Fm-Poj ₁	344.86	21.08	4.57	3.25	29.72	1.01	18.72	3.01	6.89	16.36	11.43	11.6
Fca-Nj ₁	331.27	20.66	4.56	3.77	28.38	1.09	16.14	3.52	6.22	16.03	10.69	11.67
Fca-Nj ₂	359.84	22.7	4.46	3.24	31.96	1.04	17.13	3.14	6.78	15.85	10.78	11.26
Fv-Pj ₁	338.85	21.09	4.56	3.73	33.45	1.11	18.77	3.94	6.92	16.06	9.07	10.13

Table 8: Morphometric of all Helicotylenchus sp. juveniles collected from Kalikavu block

code	L	a	b	b'	с	С'	Stylet length	Lip height	Lip width	mbw	b.w.a	Tail length
Oa-Mj ₁	385.24	27.49	4.36	3.99	37.62	1.32	17.37	3.38	6.09	14.01	7.74	10.24
Oa-Mj2	340.33	22.94	4.47	3.35	28.71	1.15	19.65	3.16	5.03	14.83	10.28	11.85
Oka-Mj ₁	418.42	22.02	4.99	3.76	27.29	1.12	18.34	3.15	6.87	19	13.67	15.33
Oka-Mj ₂	290.64	21.44	4.46	3.33	28.38	1.2	15.21	3.29	6.16	13.55	8.53	10.24
Ot-Rj ₁	344.45	22.75	4.77	3.58	22.03	1.24	14.58	2.94	6.25	15.14	12.2	15.63

Table 9: Comparison of morphometric	lata of <i>Helicotylenchus</i> sp	. with after siddigi, 1973	Ganguly et al., 20	03 and Roy <i>et al.</i> , 2014

Morphological	After Siddiqi, 1973/ G 2003	anguly <i>et al</i> .,	Roy et	al., 2014	Present findings			
features	Female	Male	Female(n=22)	Male(n=13)	female (n=13)	Male(n=78)		
Habitus	open c shaped	open c shaped	open c shaped	open c shaped	Open c shaped/spiral	Open c shaped/ spiral		
L	470-680 (540)/ 400-673	460-680 (540)	475-740 (596)	440-685 (527)	493-597 (544.39)	419-491 (458)		
a	24-30	23.8-28.5	24-37 (29)	25-34 (31)	24-30 (26)	25-30 (27)		
b	4.8-7.6 (5.9)	4.4-6.2 (5.4)	4.7-5.4	4.8-7.6 (5.9)	4.9-7.4(6.05)	3.2-5.9(5.32)		
b'	4.0-6.2 (4.8)	3.8-5.0 (4.4)	3.4-4.1		3.8-5.2(4.4)	3.9-5		
с		37-47 (41)	31-66		32-47(37.9)	25-36(32)		
Stylet length	21-24/20-28	20-24	22-25 (23)	21-22 (21)	18-22	14-22		
Vulva % (v)	61-76		62-77		64-76	0		

Lip region	hemispherical	hemispherical	hemispherical	hemispherical	hemispherical	hemispherical
Tail shape and terminus	sub-hemispherical, annulated & conoid	hemispherical	sub- hemispherical, annulated & conoid,	short-elongate conoid	Hemispherical annulated tail terminus/ rounded with terminal projection	Short-elongate conoid

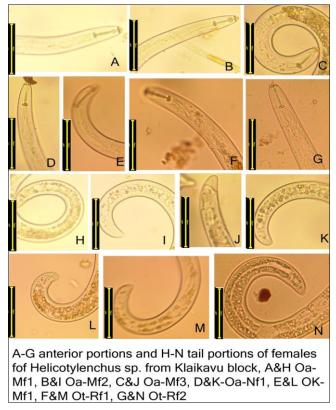


Fig 1: Showing anterior and tail portions of all Helicotylenchus sp. females collected from Kalikavu block

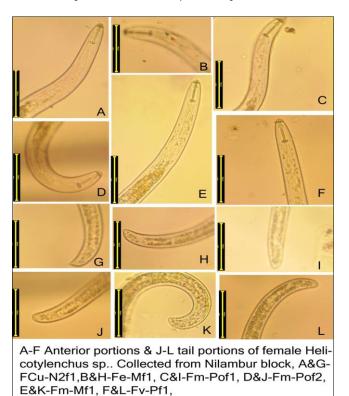


Fig 2: Showing anterior and tail portions of all Helicotylenchus sp. females collected from Nilambur block

Conclusions

The present study confirm the wide distribution of *H.multicinctus* as well as *H. dihystera* in these blocks also

as a concluding remark as it is the first report from our study area. Comparative studies on morphology and morphometrics of *Helicotylenchus sp.* Collected from various panchayaths of Nilambur and Kalikavu blocks of Malappuram district reveals the existence of similarities as well as differences among the same genus both morphologically and morphometrically. Among thirteen female members of *Helicotylenchus* sp. three were identified as *H. dihystera* and others were *H. multicinctus*. Within the same species there shows differences in their tail features, their body habitus and also in their morphometric data like body length, stylet length, mid body with etc.

While comparing with the morphological and morphometric data of other workers, most of our present findings show resemblances. For the proper management of nematodes in any crop acuurate and quick diagnosis of them and recognition of species and pathogenic variant is a must. The present study result will surely assist in selecting and applying proper management of *Helicotylenchus* as their similarities and differences are clearly drawn through their morphological and morphometric studies.

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