Indian Journal of Entomology Online published Ref. No. e23285 DoI. No.: 10.55446/IJE.2023.1285

PATHOGENICITY OF NATIVE STRAINS OF ENTOMOPATHOGENIC NEMATODES AGAINST APHIDS AND PSEUDOSTEM WEEVIL

S Sooraj[1], M S Nisha[1]*, R Narayana[1] and H Kesava Kumar[2]

[1]Department of Nematology, College of Agriculture, Kerala Agricultural University, Vellayani, Thiruvananthapuram 695522, Kerala, India
[2]Division of Crop Protection, ICAR-Central Tuber Crops Research Institute, Sreekaryam, Thiruvananthapuram 695017, Kerala, India
*Email: nisha.das@kau.in (corresponding author): ORCID ID 0000-0001-9768-3327

ABSTRACT

The pathogenicity of three native strains of entomopathogenic nematodes isolated from different locations of Kerala were evaluated against aphids, *Aphis craccivora* Koch and 4th instar grubs of banana pseudostem weevil, *Odoiporous longicollis* Olivier under laboratory condition. Based on the morphological characters and morphometric measurements, they were identified as *Steinernema* sp., *Metarhabditis* sp. and *Rhabditis* sp. *Metarhabditis* sp. @ 100 IJs (infective juveniles) recorded 100% mortality of aphids at 48 HAT (hours after treatment) and it was statistically on par with dimethoate 30EC. *Metarhabditis* sp.@ 100 and 200 IJs/ insect showed 47.49 and 62.66% mortality of pseudostem weevil grubs at 72 HAT. Molecular characterization of the most potent isolate effective against both the pests was done and it was identified as *Metarhabditis rainai.*

Key words: *Aphis craccivora*, *Odoiporous longicollis*, native strains, *Steinernema* sp., *Metarhabditis* sp., *Rhabditis* sp., infective juveniles, mortality, morphological characters, morphometrics, emergence, molecular characterization, *Metarhabditis rainai*

Pest management in agriculture is a challenging task in the context of increasing productivity without deteriorating the environment. Crop yield losses due to insect pests, diseases, weeds, nematodes and rodents range from 15-25% in India, among which 60-70% loss is due to insect pests which are of great concern. The consumption of pesticides in India during 2021-22 accounts to 58720 mt (https://ppqs.gov.in). Even though agrochemicals are very useful against crop pests, they are posing problems like environmental pollution, pesticide resistance, pest resurgence, residue in food, soil and water and some socioeconomic problems. The demand for biocontrol agents has thus increased to overcome these problems. Biological control of crop pest is successfully used in IPM and also as a component in organic farming due to their target specificity, eco safety, non-development of resistance, reduced number of applications, yield and quality improvement. Entomopathogenic nematodes (EPNs) belonging to families Steinernematidae and Heterorhabditidae are excellent biocontrol agents as they kill insect pests within days after infection. They possess many attributes such as wide host range, active host seeking, rapid killing of host within 48 hr, high multiplication rate, easiness in application, compatibility with other IPM strategies, environmental safety and are exempted from registration (Denno et al*.,* 2008). EPNs are symbiotically associated with bacteria (*Steinernema* with *Xenorhabdus* and *Heterorhabditis* with *Photorhabdus*) and the infective juveniles carry these bacteria in their gut and release inside insect haemocoel causing septicemia and death of the insect within 24-48 hr. EPNs have received little attention by researchers though they have a great potential in reducing pest population. Information pertaining to indigenous species of entomopathogenic nematodes effective against different pests in Kerala is meager. Hence the present study to identify new isolates of indigenous EPNs prevalent in Kerala and to evaluate their role as biocontrol agents.

MATERIALS AND METHODS

Survey was conducted in Thiruvanathapuram, Kollam, Pathanamthitta and Alappuzha districts during 2017-2019 and a total of forty samples were collected from the rhizosphere of vegetables, banana and coconut by random sampling. Indigenous strains were isolated from soil samples using *Corcyra cephalonica* larvae as bait by insect baiting method (Bedding and Akhrust, 1975); *C. cephalonica* were reared in the Department of Nematology, College of Agriculture, Vellayani on diet prepared with crushed maize (sterilized in an

oven at 100°C for 30 min), broken ground nut kernel, dry yeast, and wettable sulfur. Fifth instar larvae of *C. cephalonica* (10 no's) were released into plastic containers containing 200 g soil sample, covered with a lid, turned upside down and kept at room temperature (25 ± 2°C) for baiting. Larvae were checked daily for mortality and fifth day after baiting dead larvae from each sample were rinsed thrice in sterilized distilled water (Chandler et al., 1997) and placed in white trap (White, 1927). The emerged IJs were collected in tissue culture flasks and kept in BOD incubator at 15°C. The adults and first generation of EPNs were extracted by dissecting the infected cadavers in Ringer's solution. Morphological characters of the isolated strains were studied by preparing permanent mounts (Seinhorst, 1959). Twenty specimens of IJs, females, males and hermaphrodites were observed for their detailed morphological characters viz. shape of head, shape and size of spicules and gubernaculum, presence, or absence of post anal swelling in adult females, shape of tail in both adult and IJs, presence or absence of mucron in adults of both sexes. Linear body dimensions viz. body length (L), body width (W), oesophageal length (ES), distance from anterior end to excretory pore (EP), spicule length (SL), gubernaculum length (GL), anal body width (ABW), tail length (T) were recorded, ratios were calculated and compared with known species to establish their taxonomic identity.

Pathogenicity of native isolates was evaluated by inoculating IJs at different concentrations viz. 10, 50, 100 and 200 on to petri plates lined with moist filter paper (Whatman No.1) and releasing non alate adults of *A. craccivora* (50 no's). Nematode suspension was inoculated to 5 cm long fresh banana pseudostem and 4th instar grubs of *O. longicollis* (10 no's) were introduced to treated pseudostem and kept under room temperature. Experiment was laid out in CRD with five replications. Dimethoate 30EC 0.2% and chlorpyriphos 20EC 0.2% were used as chemical checks for experiment with *A. craccivora* and *O. longicollis*, respectively. Mortality was recorded at 24, 48 and 72 HAT (hours after treatment). Dead insects were placed in white trap. Colour change and number of IJs emerged out of each insect cadaver was counted under stereozoom microscope. The corrected mortality in % was worked out using Abbott's formula (Abott,1925). The data generated were subjected to ANOVA (Cochran and Cox, 1965). The variables which did not satisfy the basic assumptions of ANOVA were subjected to appropriate transformation. DNA from potent EPN strain was isolated from sterilized IJs (2000no's) using DNA extraction method developed by Kary et al. (2009). PCR amplification of ITS region of the DNA was done according to the protocol of Williams et al. (1990) using TTGATTACGTCCCTGCCCTTT as forward primer and TTTCACTCGCCGTTACTAAGG as reverse primer (Vrain et al., 1992). Purified PCR products were sequenced using DNA sequencing servicer provided by SciGenom Labs, Cochin, Kerala. Sequence analysis of 18S rRNA was done in NCBI website using BLAST.

RESULTS AND DISCUSSION

In the present study, three native strains of EPNs were obtained from Vellayani in Thiruvanathapuram (8.4410°N, 76.9891°E), Mylom in Kollam (8.5490°N, 76.3877°E) and Kainidi in Alappuzha districts (9.4925°N, 76.4703°E) with 10% frequency of occurrence. Anes et al. (2018) reported frequency of occurrence of 13.5% in soil samples collected from Kollam, Pathanamthitta and Alappuzha districts. They reported a new strain of *Steinernema hermophroditism* from Alappuzha district in Kerala. Presence of EPN's in soil samples collected from Thiruvananthapuram, Kollam and Alappuzha districts in Kerala highlights natural occurrence of EPN's in soils of Kerala and importance of more intensive surveys for recovering native isolates which can be utilized without introducing new strains.

Based on taxonomic keys, morphological observations and morphometrics, isolates obtained from Vellayani, Mylom and Kainidi were identified as *Steinernema* sp., *Metarhabditis* sp. and *Rhabditis* sp. respectively (Table 1). Infective juveniles of *Steinernema* sp. had slender body, closed mouth, and retained the second stage cuticle. The adults were characterized by short stoma, excretory pore anterior to the nerve ring and muscular oesophagus without a well-defined butterfly valve in the basal bulb. Females have a sub median protruding vulva.The males had symmetrical, slightly curved, and paired spicules (Fig. 1). The shape of spicule and gubernaculum was different from that of *S. abbasi* (Elawad et al., 1997). The spicule length was like that of *S. abbasi* (44.10 vs 45 µm) but shorter than *S. akhursti* (90 µm), *S. feltiae* (70 µm), *S. masoodi* (53 µm) and *S. sangi* (63 µm). Beneath the spicule, a boat shaped gubernaculum was present. Gubernaculum was found shorter (23.70 vs 38.8 µm) than *S. abbasi* but similarity was seen only with *S. kraussei* (29 µm). The females had a short and conoid tail with a pointed tip. The male tail was conoid with a mucron and without bursa. Isolate obtained from Mylom was identified as *Metarhabditis* sp. with hermaphroditic condition

Table 1. Morphometrics of native EPN isolates

Characters	Measurement (µm)						
	Steinernema sp.		*Metarhabditis* sp.			*Rhabditis* sp.	
	Female	Male	Hermaphrodite	Female	Male	Female	Male
L	2133.88± 87.11 (1866-2466)	859.10± 42.43 (801-901)	1374.26± 63.00 (1236-1547)	1308.70± 95.22 (1280-1634)	696.60± 73.89 (644-860)	1337.14± 47.18 (1256-1394)	804.67± 46.34 (741-862)
W	114.80± 4.21 (104-136)	46.70± 4.00 (40-52)	86.13± 6.55 (79-98)	54.90± 4.84 (48-64)	54.30± 6.72 (48-74)	53± 6.34 (43-59)	36.43± 5.09 (29-39)
EP	154.00± 5.57 (148-159)	-	132.33± 6.51 (126-148)	146.50± 15.18 (138-176)	109.00± 6.35 (98-135)	139.25± 5.50 (122-154)	71.67± 4.72 (68-77)
ES	165.60± 6.74 (158-175)	144.40± 4.14 (140-151)	198.50± 8.44 (162-208)	215.78± 10.60 (206-234)	138.00± 5.33 (134-156)	208.14± 5.64 (202-216)	84.71± 5.35 (78-94)
V%	48.40± 2.17 (45-51)	-	46.17± 2.32 (43-49)	66.50± 5.26 (62-74)	-	67.38± 5.38 (59-75)	-
ABW	23.90± 2.85 (20-27)	-	40.30± 4.08 (34-48)	31.20± 6.03 (25-45)	-	29.43± 4.43 (24-35)	-
SL	-	44.10± 4.46 (40-51)	-	-	39.63± 3.59 (34-46)	-	33.83± 4.36 (29-39)
GL	-	23.70± 2.98 (20-29)	-	-	28.00± 3.86 (24-37)	-	28.88± 4.26 (22-37)
T	54.10± 5.26 (48-61)	35.80± 5.20 (28-41)	98.70± 9.03 (86-106)	166.50± 12.11 (146-180)	28.60± 5.10 (21-37)	112.5± 4.64 (106-121)	29.71± 4.86 (24-38)
a	17.40± 1.06 (15.82-18.51)	18.52± 1.77 (15.40-20.60)	12.51± 0.69 (11.48-13.45)	25.91± 3.65 (21.16-34.04)	54.51± 8.25 (42.86-66.67)	12.60± 2.08 (10.95-14.07)	22.80± 2.31 (18.76-26.38)
b	7.13± 0.60 (6.23-8.15)	5.95± 0.33 (5.56-6.43)	8.63± 0.66 (7.37-9.63)	6.73± 0.74 (5.83-8.71)	10.80± 1.93 (9.6-15.4)	7.35± 0.48 (6.54-8.05)	9.72± 0.70 (9.00-10.96)
c	7.10± 0.56 (6.03-7.63)	24.36± 2.89 (20.61-28.60)	11.86± 1.85 (9.59-12.77)	8.43± 2.89 (7.33-9.01)	3.90± 0.50 (3.4-5.7)	10.73± 2.21 (9.08-12.85)	28.66± 4.26 (21.29-35.91)
D%	95.90± 3.42 (91-97)	-	64.81± 5.26 (58.74-68.10)	64.61± 7.96 (60.55-76.24)	19.16± 3.07 (18.8-26.6)	67.02± 1.94 (58.93-76.12)	85.91± 3.92 (58.93-76.12)
E%	278.14± 9.12 (246-288)	-	198.25± 6.69 (182.25-208.50)	70.48± 6.97 (61.03-80.50)	85.29± 0.92 (84.44-86.36)	248.18± 6.27 (228.12-264.72)	272.25± 32.37 (248.38-309.09)

L-Length, W-Width, EP-Distance from anterior end to excretory pore, ES-Oesophageal length,V%-Distance of vulva from anterior end /L*100,ABW- Anal body width, SL-Spicule length, GL-Gubernaculam length, T-Tail length, a- Body length/greatest body width, b=Body length/oesophageal length, c-Body length/tail length, D%-Distance from anterior end to excretory pore/oesophageal length x100, E%-Distance from anterior end to excretory pore/tail lengthx100; (Mean±SD), n=15

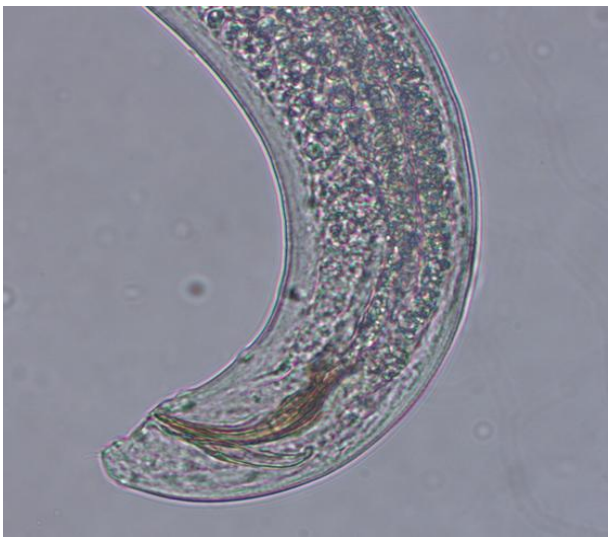

Fig. 1. Tail of *Steinernema* sp. (male)

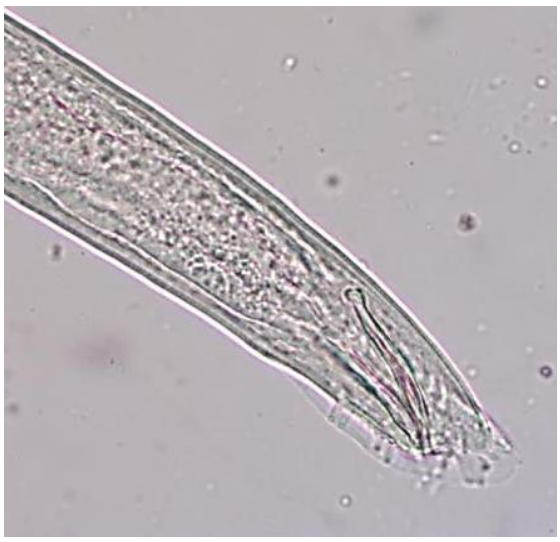

Fig. 2. Tail of *Metarhabditis* sp. (male)

in the first generation followed by males and females in the next generations. Hermaphrodites had well cuticularized cheilorhabdions unlike males and females in which it is absent. Hermaphrodites had a cylindrical corpus throughout whereas the males and females had rhabditoid oesophagus with well-developed butterfly valve in basal bulb. The eosophageal glands were lobed protruding into the intestine. Hermaphrodites had a conoid tail with post anal swelling and pointed terminus. Females had a filiform tail whereas males had a short, conoid and pointed tail. Vulva was transverse slit like with slightly protruding vulval lips and was seen near to anal slit. The spicules were paired and asymmetrical and slightly curved ventrally.

Metarhabditis sp. differed from species in the *Rhabditis* (*Oscheius*) *dolichura* group (Sudhaus and Hooper, 1994) which have nine bursal rays and spicules with swollen, uncurved distal tip (Fig. 2). Gubernaculum was slightly curved ventrally and had a length more than half the spicule length. Compared to *R. adenobia, Metarhabditis* sp. obtained in the study had a wider hermaphrodite body (86.13 µm *vs* 55 µm), and lower hermaphrodite 'a' value (12.51 *vs* 20.8). The males had a shorter body length than *R. adenobia* (696.60 µm *vs* 926 µm). The morphometric characters of *Metarhabditis* sp. isolated from Mylom compared with *M. rainai* (Fiji population) showed similarities in hermaphrodite (1374.26± 63.00 µm vs 1245± 233µm) and male (696.60± 73.89 µm vs739± 37 µm) body lengths. The oesophageal length of males (138.00± 5.33 µm vs 144± 4 µm) of both strains were also similar. Morphological features of spicules (39.63± 3.59 µm vs 45± 3.6 µm) varied in both strains though morphologically they were similar. Male tail of *Metarhabditis* sp. obtained in this study was shorter (28.60± 5.10 µm vs 36± 2 µm), compared to *R. rainai* (Fiji population). Both strains had same range of values of ratios a, b and c. Isolate obtained from Kainadi was identified as *Rhabditis* sp. Most of morphological characters were like the males and females of *Metarhabditis* sp. with some differing characters viz. ventrally curved tail, leptoderan bursa with nine papillae (Fig. 3), spicules with a manubrium which was not round and oesophageal glands were not lobed.

Data on pathogenicity of indigenous EPN isolates showed significant difference in mortality of *A. craccivora* and grubs of *O. longicollis* at different concentration levels of IJs. Mortality of test insects increased with concentration and exposure time. *Metarhabditis* sp. @50 IJs recorded 86.71% mortality of

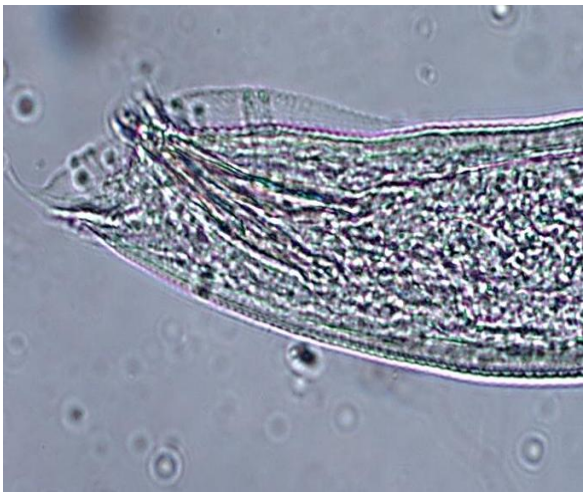

Fig. 3. Tail of *Rhabditis* sp. (male)

aphids 48 HAT while *Steinernema* sp. and *Rhabditis* sp. recorded 80.02 and 52% mortality, respectively (Table 2). Exposure time to kill the test insects also varied with nematode species. *Metarhabditis* sp. @100 IJs recorded 100% mortality at 48 hr of exposure while *Steinernema* required more exposure time (72 hr) to attain same mortality. Kumar and Ganguly (2011) reported 66 to 83% mortality of *A. gossypii* in leaf disc assay with *S. thermophilum* at an inoculum level of 50 to 500 IJs/ml at 72 HAT. Present study highlights the potential of *Metarhabditis* sp. in causing highest mortality of aphids with minimum inoculum levels and exposure periods. Regarding the mortality of *O. longicollis* grubs also, *Metarhabditis* sp. @ 200 IJs/grub recorded highest mortality (62.66%) at 72 HAT, while *Steinernema* sp. and *Rhabditis* sp. recorded only 50 and 37.44% mortality at the same exposure period and inoculum level (Table 2). The result of this study corroborates with the findings of Padmanabhan et al. (2002) who reported that *O. longicollis* third-instar grubs treated with inoculum levels of 10 to 70 and 80 to 100 IJs/grub caused 33.3 and 66.6% mortality, respectively after 72 HAT. A similar work was undertaken by Mwaitulo et al. (2011) using native strains belonging to genera *Steinernema* and *Heterorhabditis* with inoculum levels viz., 100, 500 and 1000 IJs/ grub in a cylindrical piece of pseudostem and found that with increase in dosage of EPNs, the mortality of *O. longicollis* grubs increased. Giribabu et al. (2020) reported 100% mortality of *O. longicollis* adults at a dose of 20000 IJs of *H. indica* while *S. siamkayai* caused 80% mortality for the same concentration after 120 HAT. In this study, *Metarhabditis* sp. recorded maximum emergence of IJs from *A. craccivora* ($1.2x10^3$) and *O. longicollis* ($3.6x10^5$) compared to *Steinernema* sp. ($1x10^3$-$1.85x10^5$) and *Rhabditis* sp. ($0.3x10^3$-$0.5x10^5$). Progeny production was found maximum with *Metarhabditis* sp. compared to other two isolates and cadaver infected by *Metarhabditis* sp. was reddish brown while *Steinernema* sp. infected ones were creamish.

Based on pathogenicity studies, *Metarhabditis* sp. was identified as the most potent EPN isolate and molecular characterization was done and blast details of the most matching sequence homology in NCBI data base confirmed the identity of isolate as *M. rainai* and it was reported for the first time from Kerala. Based on the findings it can be stated that *M. rainai* can be exploited for the sustainable management of aphids and pseudostem weevil due to low inoculum requirement for killing the pests and high efficacy.

ACKNOWLEDGEMENTS

The authors thank the Head, Department of Entomology, College of Agriculture, Vellayani for facilitating the research work.

FINANCIAL SUPPORT

The authors thank Kerala Agricultural University for the financial support

AUTHOR CONTRIBUTION STATEMENT

MSN conceived and designed the research. SS conducted the experiments and analyzed the data. MSN wrote the manuscript, RN edited the manuscript and HKK identified the specimens. All authors read and approved the manuscript.

Table 2. Mortality of aphids by native isolates of EPN at different time intervals

Dose (IJ)	Mortality (%) at different exposure periods (hr)								
	Steinernema sp.			*Metarhabditis* sp.			*Rhabditis* sp.		
	24 hr	48 hr	72 hr	24 hr	48 hr	72 hr	24 hr	48 hr	72 hr
10	38.49^{e} (38.35)	59.00^{c} (50.19)	79.02^{b} (62.74)	21.89^{e} (27.90)	44.47^{c} (41.82)	71.26^{b} (57.58)	20.99^{e} (27.27)	37.49^{e} (37.75)	67.63^{c} (55.32)
50	50.00^{d} (45.00)	80.02^{b} (63.45)	100.00^{a} (90.00)	39.98^{d} (39.22)	86.71^{b} (68.62)	100.00^{a} (90.00)	29.99^{d} (33.20)	52.00^{d} (46.15)	82.58^{b} (65.33)
100	68.52^{c} (55.87)	98.54^{a} (83.05)	100.00^{a} (90.00)	52.00^{c} (46.15)	100.00^{a} (90.00)	100.00^{a} (90.00)	43.99^{c} (41.55)	76.05^{c} (60.69)	99.27^{a} (85.08)
200	76.01^{b} (60.68)	100.00^{a} (90.00)	100.00^{a} (90.00)	61.52^{b} (51.66)	100.00^{a} (90.00)	100.00^{a} (90.00)	57.50^{b} (49.32)	91.87^{b} (73.44)	100.00^{a} (90.00)
Dimethoate 30EC	100.00^{a} (90.00)	100.00^{a} (90.00)	100.00^{a} (90.00)	100.00^{a} (90.00)	100.00^{a} (90.00)	100.00^{a} (90.00)	100.00^{a} (90.00)	100.00^{a} (90.00)	100.00^{a} (90.00)
CD (p= 0.05)	(1.637)	(3.588)	(0.941)	(2.967)	(4.277)	(5.521)	(1.330)	(3.540)	(5.022)

Figures in parenthesis arc sine transformed values

CONFLICT OF INTEREST

No conflict of interest.

REFERENCES

Anes K M, Babu M, Sivadasan J, Rajkumar J A. 2018. New distributional record of *Steinernema hermaphroditum* (Rhabditida: Steinernematidae) from Kerala, India. Indian Journal of Nematology 48(2): 169-177.

Bedding R A, Akhurst R J. 1975. Simple technique for the detection of insect parasitic rhabditid nematodes in soil. Nematologica 21: 109-110.

Chandler D, Hay D, Reid AP.1997. Sampling and occurrence of entomopathogenic fungi and nematodes in UK soils. Applied Soil Ecology 5(2): 133-141.

Cochran W G, Cox G M. 1965. Experimental design (2nd Ed.). John Wiley and Sons Inc, New York. 182 p.

Denno R F, Gruner D S, Kaplan I. 2008. Potential for entomopathogenic nematodes in biological control: a meta-analytical synthesis and insights from trophic cascade theory. Journal of Nematology 40: 61-72.

Elawad S, Ahmad W, Reid A P. 1997. *Steinernema abbasi* sp. n. (Nematoda: Steinernematidae) from the Sultanate of Oman. Fundamental and Applied Nematology 20(5): 435-442.

Giribabu P, Anuradha C, Anithasree T, Padmanaban, B. 2020. Isolation, identification, bioassay of native entomopathogenic nematodes, *Steinernema siamkayai* Stock, Somsook and Reid, 1998 (Rhabditida: Steinernematidae) and *Heterorhabditis indica* Poinar, Karunakar and David, 1992 (Rhabditida: Heterorhabditidae) and their bio-efficacy against banana stem weevil, *Odoiporus longicollis* (Olivier) (Coleoptera: Curculionidae). Indian Journal of Nematology 50: 117-125.

Kary N E, Niknam G, Mohammadi S A, Griffin C, Moghaddam M. 2009. A survey of entomopathogenic nematodes of the families Steinernematidae and Heterorhabditidae (Nematoda: Rhabditida) in the north-west of Iran. Nematology 11(1): 107-116.

Kumar H R, Ganguly S. 2011. Bio efficacy of Indian strains of entomopathogenic nematodes against different homopterans under laboratory conditions. Indian Journal of Nematology 41(2): 197-200.

Mwaitulo S, Haukeland S, Saethre M G, Laudisoit A, Maerere A P. 2011. First report of entomopathogenic nematodes from Tanzania and their virulence against larvae and adults of the banana weevil, *Cosmopolites sordidus* (Coleoptera: Curculionidae). International Journal of Tropical Insect Science 31(3): 154-161.

Padmanabhan B, Sundararaju P, Cannayane I, Hussaini S. S. 2002. Effect of entomopathogenic nematodes, *Heterorhabditis indica* (PDBCEN 13.3) on banana stem weevil, *Odoiporous longicollis in vitro*. Indian Journal of Nematology 32: 207.

Seinhorst J W. 1959. A rapid method for the transfer of nematodes from fixative to anhydrous glycerin. Nematologica 4(1): 67-69.

Sudhaus W, Hooper D J. 1994. *Rhabditis*(*Oscheius*) *guentheri* sp. n., an unusual species with reduced posterior ovary, with observations on the Dolichura and Insectivora groups (Nematoda: Rhabditidae). Nematologica 40: 508-533.

Vrain T C, Wakarchuk D A, Levesque A C, Hamilton R I. 1992. Intraspecific rDNA restriction fragment length polymorphism in the *Xiphinema americanum* group. Fundamental and Applied Nematology 15(6): 563-573.

White G F. 1927. A method for obtaining infective nematode larvae from cultures. Science 66 (1): 1709.

Williams J G, Kubelik A R, Livak K J, Rafalski J A, Tingey S V.1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Research 18(22): 6531-6535.

(Manuscript Received: May, 2023; Revised: September, 2023;
Accepted: September, 2023; Online Published: September, 2023)
Online First in www.entosocindia.org and indianentomology.org Ref. No. e23285