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Occurrence of Entomopathogenic nematodes (EPNs) from fields of Assam Agricultural University, Jorhat, Assam

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Abstract
A survey was conducted for isolation of the entomopathogenic nematodes from the fields of Assam Agricultural University, Jorhat, Assam, India. During the survey, a total of 200 soil samples were collected from different locations of cultivated as well as uncultivated fields. Out of the total collection, 8 numbers of soil samples were positive for entomopathogenic nematodes with 4% frequency of occurrence. Soil samples recorded *Steinernema spp.* (2%), *Heterorhabditis spp.* (1%) and *Oscheius spp.*(1%). Based on morphological and morphometric studies, the *Steinernema* isolates were identified as *Steinernema aciari* and this species was recovered from diverse habitats. The soil of Jorhat may be the reservoir of many more species of EPNs which needs further study.

Keywords: Entomopathogenic nematodes, morphological and morphometric identification, *Steinernema* sp., *Heterorhabditis* sp.

Introduction
Entomopathogenic Nematodes (EPNs) are one of the biological control agents against many economically important insect pests. Species of *Steinernema* and *Heterorhabditis* are considered as potential biocontrol agents because of their association with parasitic symbiotic bacteria. These EPNs are environmentally safe and there is no problem of insect resistance by their application. Indigenous entomopathogenic nematodes may be more suitable for innundative release against insect pests because of their adaptability to local climate [1]. Steinernematids and heterorhabditids have been isolated from Indian soil by various workers [2]. Considering the viewpoint, a random survey was conducted to isolate and identify EPN species from cultivated as well as uncultivated fields of Assam Agricultural University, Jorhat, Assam. Findings of this research will be helpful for occurrence of the predominant species of EPNs and to develop environmentally safe and effective management strategies for insect pest management.

Materials and Methods
The district Jorhat falls under subtropical climatic condition with warm humid summer and cool dry winter. Assam Agricultural University has a wide diversity of crops, such as fruit trees, cereals, vegetables, tea and natural habitats. The Instructional Cum Research (ICR) Farm, Experimental farm of Department of Horticulture, and Experimental farm for plantation crops of Assam Agricultural University, Jorhat, has total area of 69.61 hectares, 16.43 hectares, and 52 hectares, respectively. These habitats are subject to various insect pests. The recorded soil temperature was ranged from 20-30ºC and the average rainfall was 135.77 mm per month. The soils are sandy or sandy loam with a good amount of organic matter. The organic matter content of soils ranged from 0.58% to 0.60% and pH 5.87.

Sample collection
A systematic survey was undertaken in Assam Agricultural University, Jorhat for the presence of entomopathogenic nematodes. Two hundred numbers of soil samples were collected randomly during the period from November 2014 to March 2017 from four habitats (vegetation type) *viz*., Instructional Cum Research (ICR) Farm, Experimental farm of the Department of Horticulture, Experimental farm for plantation crops and Fallow land.

From each habitat 50 numbers of soil samples were collected. Soil collected from non cultivated area that was covered by long grass, shrubs and weeds. Each soil sample was a composite of 5-20 random sub-samples taken in the same location and at least 10 m away from each other and to a depth of 20 cm, using a small shovel. Between samples, the shovel was thoroughly ringed with water and air dried to prevent contamination of the next sampling unit. Information regarding date of sampling, standing crop in the field and soil type along with GPS (Global Positioning System) location was recorded. The soil was thoroughly mixed on a plastic sheet and half of each sample was used for extraction of entomopathogenic nematodes (EPNs).

Isolation of entomopathogenic nematodes (EPNs) from soil samples

Entomopathogenic nematodes were isolated from the soil samples by 'nematode bait' method [3] with larvae of the greater wax moth (*Galleria Mellonella* L, Lepidoptera: Pyralidae). As *G. mellonella* was used as the laboratory bait insect, it was reared on artificial diet as per the procedure [4]. Ten last instar larvae of *G. mellonella* were released into the plastic container containing 200g of soil sample. Collected *G. mellonella* larvae were transferred to White traps [5] and infected cadavers were placed on a 9 cm Whatman No.1 filter paper over a small Petri dish (50 mm × 17 mm) which was then placed in bigger Petri dish (100 mm × 20 mm) containing water. IJ recovered for the 5-12 following days. IJ were stored in distilled water at 10 ^{0}C. To establish new cultures, emerging nematodes were pooled for each sample and used to infect new *G. mellonella* larvae. Only IJ collected during the week after the first emergence from the insect cadavers were used to establish new cultures. The colour of *G. mellonella* cadavers, which ranges from cream to brown (*Steinernema* spp.) or red (*Heterorhabditis* spp.) within 24-48 h after nematode penetration, was used for preliminary determination of EPN genera.

Isolation of adults

The adults of first and second generation were found in the haemocoel of cadaver; hence they were extracted by dissection in Ringer's solution. The dissection was done at 2-4 and 4-5 days after inoculation (DAI) for recovering the first generation and second generation adults of *Steinernema* and *Heterorhabditis*, respectively. The recovered nematodes were kept in clean ringer's solution for further processing.

Processing of nematodes

Third stage infective juveniles in sterile distilled water and freshly dissected out first and second generation adults in Ringer's solution were killed and fixed [6]. Killed and fixed nematodes were further processed with slow glycerol dehydration method [7]. Permanent mounts were prepared by transferring the nematodes to a drop of anhydrous glycerine on a clean glass slide supported by radially placed 3 small pieces of glass wool supports.

Light microscopic studies

The morphological identification was performed on the basis of characters of third stage infective juveniles, and first and second generation adults [8] using a compound microscope (Magnus) equipped with an ocular micrometer. In addition to the deMan formula, the other characters studied were: D% (Distance from head to Excretory pore/oesophageal length x 100), E% (Distance from Head to Excretory pore/tail length x 100), F% (Body width/tail length x 100), SW, GS. Morphological and morphometrical data of the isolates were compared with the original description of the type species.

Results and discussions

Survey data revealed that out of 200 soil samples, eight soil samples were positive for entomopathogenic nematodes with 4% frequency of occurrence, Among the extracted populations, 4 isolates were identified as the genus *Steinernema* (2%), 2 isolates were identified as the genus *Heterorhabditis* (1%) and 2 isolates were identified as the genus *Oscheius* (1%). Steinernematid isolates were designated as EPN-S-J-1, EPN-S-J-2, EPN-S-J-3.The isolates were found from rhizosphere of mung bean, arahar and cowpea respectively from ICR Farm, AAU, Jorhat. Another isolate of steinernematid (designated as EPN-S-J-4) was found from rhizosphere of citrus from the Experimental farm of the Department of Horticulture. One heterorhabditid isolate (designated as EPN-H-J-1) was isolated from rhizosphere of citrus and one isolate of *Oscheius* (designated as EPN-O-J-1) was found from rhizosphere of coconut in the Experimental farm of the Department of Horticulture. One heterorhabditid isolate (designated as EPN-H-J-2) and another isolate of *Oscheius* (designated as EPN-O-J-2) were found from Experimental farm for plantation crops of AAU, Jorhat. Entomopathogenic nematodes were not recovered from the fallow land (Table 1). The nematode presence and abundance were varied from four different habitats of most of the sampling sites. Although EPNs were recovered at a low rate in present study, three different species were isolated from three different habitat (vegetation) *viz*., ICR field, Experimental farm of Department of Horticulture, Experimental farm for plantation crops. It may be resulted due to condition of the crop land in terms of irrigation of the field, where the temperature and the soil moisture was suitable for their persistence. One reason for the low recovery rate obtained in the present study, could be that only one insect, *G. mellonella*, was used as bait insect and it may not be the appropriate host for all EPN species [9]. EPN distribution depends on temperature, precipitation and soil type and is closely related to vegetation type and presence of insect hosts [10-14]. Furthermore, the choice of sampling sites may contribute to differences in EPN recovery percentage [15]. Lower percentage of EPNs probably also due to chemical control of insect pests in experimental fields which partially reduces the abundance of natural biocontrol agents. However, this low recovery has already been reported from other surveys also [16]. Rosa *et al*. [17] reported that most of the surveys showed their recovery rate from soil varies from 6% to 35% in Northern Ireland. Raj Kumar *et al*. [18] reported that out of 105 soil samples collected from Rajasthan, 5(4.76%) were found to be positive for EPNs. Recovery frequency of EPNs may vary from 0.7% to 70.1% [19-20]. Entomopathogenic nematodes were not recovered from the fallow land that was covered by long grass, shrubs and weeds. Akhurst and Brooks [21] and Griffin *et al.*, [22] observed that entomopathogenic nematodes were more prevalent in agricultural fields than in natural habitats. All the eight EPN positive soil samples were from sandy loam soil and this finding was in agreement with the findings of the surveys conducted by Ambika and Sivakumar [23] which revealed that the occurrence of EPNs was more in light soils like sandy loam, sandy, loamy sand, loam soils rather than in heavy soils. However EPNs are

present in heavy soils like clay soil also as recorded by Shyamprasad *et al*. [24] and Sosamma and Rasmi [25] in the South Andamans and Kerala, respectively. In Sri Lanka, *Heterorhabditis sp*. was reported to be restricted to sandy soils within 100 m of the sea [26, 27]. Since our samples were collected from the upper soil layer, this could explain the low recovery of *Heterorhabditis sp.*

Morphological studies of all the four steinernematid isolates were undertaken and it was found to be similar to each other. Morphological and morphometrical studies of each isolates *viz*., EPN-S-J-1, EPN-S-J-2, EPN-S-J-3, EPN-S-J-4 different life stages (infective juveniles and adults of both the generations) revealed it to closely resemble with *Steinernema aciari* (Table 2-Table 6) in most of the characters. Third stage infective juvenile heat killed specimens are almost straight, slender, slightly tapering towards anterior and posterior ends. Lip region is smooth and rounded. Oral aperture and anus closed. Oesophagus is long and slender. Tail is wide and straight. First generation males body are slender, smaller than females, curved ventrally posteriorly, J-shaped in heat-relaxed specimens. Head is truncate and slightly swollen anteriorly. Gonad is monorchic, testis reflexes one time. Spicules are paired, curved, separate. Spicule head is elongated, broad and somewhat angular-shaped manubrium. Distal tip of spicule is blunt. Gubernaculum is boat shaped in lateral view, and ventrally curved, with a proximal knob or hook. Tail is straight. Second generation males are similar to the first-generation males, but slightly smaller in the body length and other measurements. Excretory pore located more anterior than in the first generation. Tail mucron is absent in both the generations. First generation female body is long, little C-shaped on heat relaxation. Head is bluntly rounded, slightly tapering anteriorly. Basal bulb slightly enlarged. Excretory pore opening anterior to nerve ring. Gonads are didelphic, amphidelphic with reflexed ovaries. Vulva is a transverse slit, protruding slightly from the body surface. Eggs are deposited initially, but later hatching inside the female bodies. First generation tail is usually straight and wide with a prominent postanal swelling. Second generation females are similar to first generation females in general morphology but smaller in dimension. Excretory pore is located more anterior than in the first generation females. Vulva is distinctly asymmetrical and oblique slit in all observed specimens. Tail is not mucronated, tail longer than body width at anus.

The morphometrics of the infective juvenile of all the isolates (EPN-S-J-1, EPN-S-J-2, EPN-S-J-3, EPN-S-J-4) were almost in similarity with the type specimen of *Steinernema aciari* Qiu, Yan, Zhou Nguyen & Pang, 2004 (Table2), however differed from the type isolate in some characters such as body length and tail length. The morphometrics of the first generation males of all the isolates were almost in agreement with those of the original isolate (Table 3), however differed from the type isolate in some characters such as, body length, position of excretory pore, SW, GS. The morphometrics of the second generation males of all the isolates were almost in agreement with those of the original isolate (Table 4), however differed from the type isolate in some characters such as body length, tail length and SW%. The morphometrics of the first generation and second generation females of all the isolates were differed from the type isolate in some characters such as body length, body width, tail length and anal body width (Table 5, Table 6). The isolates were thus identified as *Steinernema aciari*, which is a new record of this species from Assam, India. The nematode *S. aciari* was isolated and described from Shantou district on the eastern coast of Guangdong province, People's Republic of China [28]. Nguyen and Smart, [29] observed variations in body length, position of excretory pore, tail length and value of E% of *Steinernema glaseri* in relation to time of harvest. It was observed that body length of infective juvenile was 1464µm (1256µm-1610µm) on 3rd day of harvest where as body length 1306µm (726 µm -1530 µm) on 12th day of harvest. Shishiniova *et al*. [30] observed that the infective juveniles of Bulgarian strains of *S. carpocapsae* differed in some morphometric characters and indexes from the known strains from Europe, USA and South America. Morphometric variations were observed in adult stage of both male and female generations in some characters like pharynx, excretory pore, tail length, spicule length and gubernaculum length, etc. which were considered as intraspecific variations of *S. aciari*. Bamel and Waghmare [31] recorded *S. siamkayai* first time from India. The isolate, *S. siamkayai* showed variation with respect to adult stage of both male and female generations in some characters like pharynx, excretory pore, tail length, spicule length and gubernaculum length etc from the original description.

Table 1: Occurrence of Entomopathogenic nematodes in AAU, Jorhat, Assam

Agro- climatic regions	District	Habitat (vegetation)	No. of sample	Soil texture	No. of +ve sample	Crop	Species (Nematode Isolate)	Latitude, Longitude
Upper Brahmaputra Valley	Jorhat	ICR Farm, AAU, Jorhat	50(soil)	Sandy loam	1	Mungbean	*Steinernema* sp. (EPN-S-J-1)	26^{0}43'7.896"N 94^{0}11'40.098"E
					1	Arahar	*Steinernema* sp. (EPN-S-J-2)	26^{0}43'07.9"N 94^{0}11'40.1"E
					1	Cowpea	*Steinernema* sp. (EPN-S-J-3)	26^{0}43'27.3"N 94^{0}12'01.5"E
		Experimental farm, Deptt. of Horticulture, AAU, Jorhat	50(soil)	Sandy loam	1	Coconut	*Oscheius* sp. (EPN-O-J-1)	
					1	Citrus	*Steinernema* sp. (EPN-S-J-4)	26^{0}43.356´ N 94^{0}11.936´ E
					1	Citrus	*Heterorhabditis* sp. (EPN-H-J-1)	
		Experimental farm for plantation crops, AAU, Jorhat	50(soil)	Sandy loam	1	Tea	*Heterorhabditis* sp. (EPN-H-J-2)	
					1	Tea	*Oscheius* sp. (EPN-O-J-2)	
		Fallow land, AAU, Jorhat	50(soil)	Sandy loam	-	-	-	-

Table 2: Morphometrics of infective juvenile of *Steinernema aciari* isolate from fields of AAU, Jorhat, Assam in comparison with original description of *Steinernema aciari* Qiu, Yan, Zhou. Nguyen &Pang, 2004. Measurements in µm and in the form: mean± SD (range)

Character	***Steinernema* sp. (EPN-S-J-1) (n=20)**	***Steinernema* sp. (EPN-S-J-2) (n=20)**	***Steinernema* sp. (EPN-S-J-3) (n=20)**	***Steinernema* sp. (EPN-S-J-4) (n=20)**	**Original description of *Steinernema aciari* Qiu, Yan, Zhou. Nguyen & Pang, 2004 (n=20)**
Body length(L)	528.5±117.3 (350-700)	585±51.7 (530-640)	527.8±169.4 (300-730)	552±43.1 (450-680)	1113±68 (975-1250)
Body width(W)	31.4±9.6 (22-40)	34±4.2 (30-40)	30.9±10.2 (18-45)	26±3.7 (16-32)	47±2.5 (42-53)
Anterior end to excretory pore(EP)	88.6±22.6 (65-125)	80±15.4 (60-100)	83.7±21.4 (50-100)	91.7±12.1 (70-106)	95±3.7 (87-100)
Anterior end to nerve ring(NR)	94.5±19.4 (70-105)	98±8.4 (90-110)	98.0±27.6 (75-118)	95.3±11.4 (72-106)	114±5.3 (106-125)
Anterior end to esophagus base(ES)	122.1±19.3 (100-130)	138±8.4 (130-150)	125.4±26.2 (87-124)	107.6±8.5 (98-125)	146±5 (135-157)
Tail length(T)	33.5±5.2 (25-40)	36±4.2 (30-40)	31.4±8.0 (17-48)	38.4±2.2 (35-42)	78±5.2 (68-88)
Anal body width (ABW)	17.6±3.9 (15-20)	18.2±1.7 (16-20)	18.3±4.4 (15-22)	15.5±2.4 (12-19)	30±1.3 (28-33)
Ratio a=(L/W)	18±2.3 (12-20)	17.3±1.9 (15.1-20.1)	17.2±1.5 (12.6-20.0)	21.2±1.8 (17.1-24.2)	24±2.1 (20-27)
Ratio b=(L/ES)	4.1±4.8 (3.5-5.1)	4.2±0.2 (4.0-4.5)	3.8±.6.3 (3.4-4.5)	4.1±0.3 (3.5-4.5)	7.6±0.4 (7.0-8.3)
Ratio c = (L/Tail)	15.9±3.3 (11.6-20.4)	16.3±1.1 (15.1-17.6)	16.6±2.4 (11.4-19.7)	12.1±1.6 (10.1-13.6)	14.4±0.5 (14-16)
D%=(EP/ES)*100	54.5±9.5 (48-58.6)	57.9±13.3 (42-76)	54.8±10.9 (43.3-61.7)	52.2±10.1 (50.4-60.7)	65±3 (60-70)
E%=(EP/Tail)*100	1.15±15 (0.98-1.22)	2.2±0.8 (1.7-3.2)	2.2±0.8 (1.2-2.8)	116±0.5 (102-128)	123±7 (113-134)

Table 3: Morphometrics of first generation male *of Steinernema aciari* isolate from fields of AAU, Jorhat, Assam in comparison with original description of *Steinernema aciari* Qiu, Yan, Zhou. Nguyen & Pang, 2004. Measurements in µm and in the form: mean± SD (range)

Character	***Steinernema* sp. (EPN-S-J-1) (n=12)**	***Steinernema* sp. (EPN-S-J-2) (n=12)**	***Steinernema* sp. (EPN-S-J-3) (n=12)**	***Steinernema* sp. (EPN-S-J-4) (n=12)**	**Original description of *Steinernema aciari* (1st gen male) Qiu, Yan, Zhou. Nguyen & Pang, 2004 (n=20)**
Body length(L)	1412.5±117.6 (1100-1525)	1359.66±191.0 (1170-1452)	1316.2±164.9 (1035-1425)	1361±134.1 (1050-1450)	1597±93 (1400-1750)
Body width(W)	85.0±6.5 (70-95)	91.7±12.5 (80-105)	94.2±7.5 (82-108)	96.3±1.7 (80-110)	104±7.7 (88-125)
Anterior end to excretory pore(EP)	98.5±6.5 (90-110)	96.3±11.5 (90-110)	104±14.1 (88-120)	108±14.4 (85-118)	135±10 (115-155)
Anterior end to nerve ring(NR)	120±11.7 (120-133)	143.3±20.8 (120-155)	135.1±8.1 (118-144)	134.4±10.5 (100-148)	144±13 (118-165)
Anterior end to esophagus base (ES)	155±2.5 (130-140)	175±18.02 (125-160)	162.5±6.2 (124-168)	158.5±1.4 (134-150)	178±16 (140-200)
Tail length(T)	40.5±1.5 (32-46)	46.3±5.7 (40-48)	35.0±4.6 (32-36)	36.6±2.3 (35-40)	40±4.4 (31-47)
Anal body width(ABW)	35±3.5 (28-40)	36.3±5.7 (30-42)	36.4±3.4 (30-40)	40.6±1.8 (36-48)	42±4.5 (33-50)
Spicule length(SL)	62.5±3.5 (50-65)	56.66±5.7 (50-60)	51.8±7.5 (45-55)	59.5±2.4 (56-61)	86±6.3 (75-95)
Gubernaculum length(GL)	42.5±3.5 (30-45)	38.33±2.8 (35-40)	41.4±3.7 (15-45)	40.3±2.0 (38-42)	56±5.2 (48-65)
SW%=(SL/ABW)*100	180±15 (178-200)	170±15 (166-200)	166±20 (150-180)	160±20 (155-180)	204±15 (180-240)
GS%=(GL/SL)*100	62± 6 (60-70)	65± 5 (70-76)	45±5 (35-50)	70±5 (67-78)	65±6 (57-77)
D%=(EP/ES)*100	0.73±2.1 (0.66-0.78)	0.58±2.03 (0.58-0.77)	0.60±8.8 (0.68-0.78)	0.62±3.8 (0.69-0.75)	76±5 (69-88)

Table 4: Morphometrics of second generation male of *Steinernema aciari* isolate from fields of AAU, Jorhat, Assam in comparison with original description of *Steinernema aciari* Qiu, Yan, Zhou. Nguyen & Pang, 2004. Measurements in µm and in the form: mean± SD (range)

Character	*Steinernema* sp. (EPN-S-J-1) (n=7)	*Steinernema* sp. (EPN-S-J-2) (n=7)	*Steinernema* sp. (EPN-S-J-3) (n=12)	*Steinernema* sp. (EPN-S-J-4) (n=10)	Original description of *Steinernema aciari* (2nd gen male) Qiu, Yan, Zhou. Nguyen & Pang, 2004 (n=20)
Body length(L)	812.5±17.67 (700-920)	959.6±91.1 (870-1052)	816.2±64.9 (735-925)	701±34.1 (650-750)	1113±98 (1000-1300)
Body width(W)	35±4.5 (30-40)	51.6±12.6 (40-65)	44.2±2.5 (42-48)	32.3±1.8 (30-35)	62±2.4 (58-65)
Anterior end to excretory pore(EP)	95.5±3.53 (90-110)	93.3±11.5 (80-100)	97.4±14.1 (80-119)	115.2±14.4 (97-144)	113±12 (93-135)
Anterior end to nerve ring(NR)	120±9.7 (118-126)	123.3±20.8 (110-140)	125.1±8.1 (114-140)	134.4±10.6 (120-143)	145±12 (113-165)
Anterior end to esophagus base (ES)	155±2.5 (150-160)	175±18.1 (155-190)	162.5±6.2 (154-174)	157.3±9.5 (143-170)	177±14 (148-205)
Tail length(T)	35.5±1.5 (30-40)	43.3±5.7 (40-50)	39.0±4.6 (35-48)	40.6±0.9 (39-42)	39±4 (33-48)
Anal body width(ABW)	32±3.5 (25-35)	33.3±5.7 (30-40)	26.4±3.4 (20-30)	29.1±2.6 (24-32)	40±2.7 (35-43)
Spicule length(SL)	42.5±3.5 (40-45)	46.6±5.7 (40-50)	37.8±7.5 (25-45)	48.5±1.4 (46-51)	79±4.3 (73-85)
Gubernaculum length(GL)	22.5±3.5 (20-25)	28.3±2.8 (25-30)	21.4±3.7 (15-25)	30.3±1.1 (28-32)	46±3.1 (42-51)
SW%= (SL/ABW)*100	170± 20 (160-180)	160± 20 (150-170)	150±22 (140-168)	195± 15 (190-210)	197±21 (184-247)
GS%= (GL/SL)*100	55± 2 (50-60)	65±2 (62-68)	65± 5 (60-68)	62± 4 (60-66)	59±8 (53-64)
D%= (EP/ES)*100	0.65±2.1 (0.60-0.68)	0.54±2.1 (0.44-0.64)	0.60±8.8 (0.48-0.68)	0.68±6.2 (0.66-0.72)	65±4 (61-72)

Table 5: Morphometrics of first generation female of *Steinernema aciari* isolate from fields of AAU, Jorhat, Assam in comparison with original description of *Steinernema aciari* Qiu, Yan, Zhou, Nguyen & Pang, 2004 Measurements in µm and in the form: mean± SD (range)

Character	*Steinernema* sp. (EPN-S-J-1) (n=12)	*Steinernema* sp. (EPN-S-J-2) (n=8)	*Steinernema* sp. (EPN-S-J-3) (n=12)	*Steinernema* sp. (EPN-S-J-4) (n=8)	Original description of *Steinernema aciari* (1st gen female) Qiu, Yan, Zhou. Nguyen & Pang, 2004 (n=20)
Body length(L)	1657.5±188.4 (1325-1785)	1636.4±178.2 (1180-1710)	1783.1±188.7 (1600-1850)	1724.1±153.4 (1540-1880)	7233±1013 (5500-8850)
Body width(W)	160.5±14.8 (140-185)	158.8±13.8 (112-165)	158±12.9 (120-178)	158.3±13.7 (160-180)	231±23 (188-262)
Anterior end to excretory pore(EP)	111.2±9.6 (90-120)	115.0±2.5 (100-120)	112.5±11.9 (105-120)	122.5±5.2 (100-128)	179±11 (160-195)
Anterior end to nerve ring(NR)	126.0±8.4 (120-132)	131.2±14.2 (125-138)	134.7±16.9 (110-143)	130.6±5.6 (112-148)	195±18 (163-241)
Anterior end to esophagus base (ES)	170.2±17.0 (128-205)	180±5.0 (156-198)	183.2±21.9 (160-204)	184.2±7.3 (168-208)	262±24 (220-307)
Tail length(T)	40.0±3.9 (30-43)	34.0±1.4 (30-48)	38.8±2.7 (30-42)	44.1±2.2 (40-46)	75±12 (50-92)
Anal body width(ABW)	55.2±2.1 (42-68)	62.5±2.4 (40-65)	63.1±2.9 (40-64)	60±2.9 (43-65)	83±69 (64-91)
D%= (EP/ES)*100	0.58±2.6 (0.50-0.60)	0.56±3.2 (0.51-0.61)	0.58±7.7 (0.48-0.65)	0.60±2.94 (0.55-0.65)	0.54±13.2 (0.442-0.68)
V%= distance from anterior end to vulva as percentage of length	70.5±1.1 (68.1-72.5)	68.2±6.8 (62.9-72.3)	66.7±9.6 (62.8-70.9)	69.7±2.4 (65.6-70.7)	52±4.1 (47-57)

Table 6: Morphometrics of second generation female of *Steinernema aciari* isolate from fields of AAU, Jorhat, Assam in comparison with original description of *Steinernema aciari* Qiu, Yan, Zhou, Nguyen & Pang, 2004. Measurements in µm and in the form: mean± SD (range)

Character	*Steinernema* sp. (EPN-S-J-1) (n=12)	*Steinernema* sp. (EPN-S-J-2) (n=8)	*Steinernema* sp. (EPN-S-J-3) (n=12)	*Steinernema* sp. (EPN-S-J-4) (n=8)	Original description of *Steinernema aciari* (2nd gen female) Qiu, Yan, Zhou, Nguyen & Pang, 2004 (n=20)
Body length(L)	1057.5±88.4 (1025-1185)	1136.4±78.2 (980-1510)	1083.1±88.7 (700-1150)	1024.1±53.4 (740-1280)	2022±247 (1675-2425)
Body width(W)	120.5±14.8 (100-125)	118.8±13.8 (110-125)	110±9.9 (100-128)	88.3±13.7 (60-100)	102±4.2 (98-110)
Anterior end to excretory pore(EP)	95.2±9.6 (80-100)	95.0±2.5 (90-110)	99.5±11.9 (85-110)	102.5±5.2 (90-118)	124-7.9 (113-135)

Anterior end to nerve ring(NR)	121.0±8.4 (100-130)	129.2±14.2 (105-136)	124.7±16.9 (110-133)	109.6±5.6 (102-118)	164±8.3 (150-175)
Anterior end to esophagus base (ES)	157.2±17.0 (118-200)	175±5.0 (150-190)	163.2±21.9 (140-184)	174.2±7.3 (162-180)	219±6.7 (205-225)
Tail length(T)	47.0±7.9 (38-50)	54.0±0.4 (50-58)	40.8±6.7 (30-45)	45.1±5.2 (40-56)	74±4.6 (70-85)
Anal body width(ABW)	45.2±1.1 (42-48)	42.5±1.4 (40-45)	43.1±0.9 (40-44)	40±2.9 (33-45)	42±3.2 (38-48)
D%= (EP/ES)*100	0.58±2.60 (0.52-62)	0.56±3.28 (0.51-64)	0.56±7.7 (0.48-0.65)	0.60±2.94 (0.55-0.65)	
V% = distance from anterior end to vulva as percentage of length	70.5±1.1 (69.1-72.6)	68.2±6.8 (52.9-72.3)	66.7±9.6 (52.8-75.9)	69.7±2.4 (66.6-72.7)	53±5.8 (48-60)

Conclusion

Although, entomopathogenic nematodes were recovered only from 8 soil samples out of 200 soil samples, the recovery of *Steinernema aciari*, *Heterorhabditis spp.* and *Oscheius* spp. highlights the importance of conducting a more intensive survey in Assam. Further studies on characterization and host ranges of these EPN species are necessary to explore and ascertain their possible utilization in biological control programme of insect pests.

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