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Survey and Distribution of Plant Parasitic Nematodes on Tomato at Baruasagar in Jhansi (India)

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Abstract: Tomato (*Lycopersicon esculentum*) is an important high-value vegetable crop and acts as a natural medicine that alleviates the risk of a condition such as cancer and cardiovascular disease. Plant parasitic nematodes are obligate biotrophic feeders that cause the most devastating phytoeconomic damage to tomato cultivation across the globe. Their infection may lead to a spectrum of disorders like stunting, necrosis and wilting followed by lessening production. The present investigation embraces an extensive survey work (from October to December 2021) of three tomato crop production sites at Baruasagar in Jhansi and throws light on the presence, distribution and general divergence of parasitic nematodes in soil and root samples. The morphological identification of six genera *Meloidogyne*, *Rotylenchulus*, *Hoplolaimus*, *Pratylenchus*, *Helicotylenchus and Criconema* was also undertaken in the study. The root-knot nematodes (*Meloidogyne* genus) and lesion nematodes (*Pratylenchus* genus) were the most abundant PPNs in tomato crops. The study also highlighted the varying physicochemical properties of soil played a significant role in the abundance of nematode genera.

Keywords: Genera Diversity, Necrosis, Physico-Chemical, Plant Parasitic Nematodes, Tomato

Introduction

Nematodes are non-segmented thread-like triploblastic pseudocoelomate animals and survive in extreme ends of the environment including soil and roots. Nematodes feed on a variety of prey e.g., fungi, bacteria, protozoan, other nematodes and plants. Plant Parasitic Nematodes (PPNs) feed mostly on the softer tissues of plant roots. A few less common ones feed on aboveground plant parts. Among the root feeders, certain nematodes are ectoparasites that feed from outside of the root and others are endoparasites that reside inside the root to attain nutrients. The nematodes are vermiform, limbless, cylindrical, elongated animals. Their body is covered with transparent epidermis and tapered at the ends. The inner hypodermis secretes the cuticle and is adhered to with longitudinal muscles that allow only sinusoidal (snake-like) movement. Nematodes lack circulatory and respiratory systems but they obtain water and key metabolites from plants through their semi-permeable membrane. During parasitic adaptation, the absence of two vital systems allows them to grow in length and not in cross-section. PPNs range from 250 µm to 12 mm in length and about 15-35 µm in width (Lambert and Bekal, 2002). The mouth is located at the anterior part of the body and has a spear-shaped stylet which works in a similar way as the hypodermic needle does. The pulsating movement of stylet punctures the cell wall to withdraw key metabolites from the plant tissues and secretes cytokinin which mediates cell cycle regulation and regulates feeding site formation in the infected plants (Siddique *et al*., 2015). It also provides an excellent opportunity for the invasion of secondary pathogens like viruses, bacteria and fungi.

According to Panthee and Chen (2010), Tomato (*Solanum lycopersicum*) is one of the world's most consumed vegetables, after potatoes in terms of consumption. In India, it is grown on nearly 80,000 hectares each year, accounting for 1.5% of the world's total area. Di Mascio *et al*. (1989) emphasized that tomato is the key source of carotenoid (Lycopene) that prevents cardiac disease and minimizes the impact of cancer. Tomato has ample amounts of vitamins, valuable ions and the lowest level of bad cholesterol (Wener, 2000). Processing of

tomato increases the absorption of lycopene and the breakdown of the cell wall. The massive qualitative and quantitative loss of tomato crops occurs due to pests and diseases. Plant parasitic nematodes are the key menace in tomato production. They cause high levels of economic loss all over the world in a multitude of tomato crops. The PPNs infect the tomato crop by causing root necrosis, formation of root galls, stunted growth, yellowness of leaves, nutrient deficiency symptoms and secondary infections. The current submission is the first report on the occurrence of different PPN genera on tomato crops at Baruasagar. It also highlights the correlation of the physicochemical properties of soil with the distribution of PPNs.

Materials and Methods

The Survey of Nematodes

Extensive survey work was carried out from October to December 2021 to confirm the occurrence and distribution of plant pathogenic nematodes in three sites at Baruasagar in Jhansi. The samples were collected from different sampling sites of villages Harpura (S-1), Basvan (S-2) and Chitavar (S-3). Harpura is located at latitude 25.3851729 and longitude 78.7442586. Basvan is located at latitude 25.237912 and longitude 79.135156. Chitavar is located at latitude 25.444132 and longitude 78.567593. A collection of fifteen typical fields sampled having a common variety known as cherry tomato (desi), was chosen for examination. From every examination site, the samples of root and soil were gathered from the rhizospheric region of diseased desi tomato plants. The samples of soil were obtained from 15-20 cm below ground in a zigzag pattern of 10-12 plants/spots at random with the help of a scoop. Each sample consisted of 50 g of soil and 1 g of root. These soil samples were properly blended to obtain a homogenous sample of 200 cc soil. The samples of root and soil were immediately transferred to a polybag and sealed tightly with the help of a rubber band to minimize the loss of moisture in the soil and then brought to the laboratory. A questionnaire was filled out in the survey field to obtain data like as the farmer's name, crop variety, crop history, diseases and management of crops.

The Extraction and Identification of Nematodes

The soil samples collected from fields were blended manually to make them homogenous. The modified Baerman extraction tray technique, suggested by Hooper *et al.* (2005) applied to extract nematodes from two hundred cubic centigrams of soil and five grams of roots. The infected roots of every sample were cleaned in running water to remove particles of soil. Then roots were cut into small fragments transversely and placed in a glass flask filled with 100 mL water. The suspension of nematodes obtained from roots and soil was investigated with the help of a stereomicroscope after incubation of 48 h. 1 mL sample of nematode pipetted into a nematode counting plate and placed under a stereomicroscope for calculation. The event repeated for trice and the mean value ascertained. For optical microscopic analysis nematodes were killed with hot water and normal tap water in a 1:1 ratio, then fixed in 4% warm formaldehyde (Courtney *et al*., 1955) and kept in the cavity blocks for 48 h. A slow dehydration procedure was adopted for the light microscopic study suggested by in Seinhorst (1959). The morphological identification of mounted PPNs up to the genus level was undertaken with the help of three dichotomous keys (Mai *et al*., 1996; Siddiqi, 2000; Mekete *et al*., 2012; Mai, 1996). Morphological and morphometric analysis was carried out to ascertain the features such as length and width of the body, presence of cone, size of the cone, the shape of the anterior and posterior part and presence of genital organ i.e., vulval position (Cornejo-Condori *et al*., 2021). The images were captured with the trinocular microscope and dig eye camera (Dewinter capture pro software).

Physico-Chemical Analysis of Soil

The 500 g of soil was collected from each field and processed to find out its physical and chemical properties suggested by Juma *et al*. (2020). The soil analysis was carried out in R.S.T. Laboratory in Jhansi, India using standard methods according to Anderson and Ingram (1994). The following soil parameters were analyzed i.e., pH, organic carbon, nitrogen, phosphorous and potassium and Electrical Conductivity (EC) (µS/cm) where the ratio of soil and water is 1:2.5 (Richards, 1954).

Results

The present investigation was undertaken on the cherry tomato (desi) at three sites Harpura (S-1), Basvan (S-2) and Chitavar (S-3) and every experimental site had five different fields (R1-R5). During the investigation, the six genera of nematodes were observed and their systematic classification, feeding strategies and infective stages have been mentioned in Table 1.

Table 1: Taxonomic position and feeding strategies of plant parasitic nematodes found in tomato plants

Class	Order	Family	Feeding strategy	Genera example	Infective stage	Symbol
Secernentea		*Heteroderidae*	Sedentary endoparasites	*Meloidogyne*	J2	Mel
		Hoplolaimidae	Semi-endoparasites	*Helicotylenchus*	J2	Hel
	Tylenchida	*Pratylenchidae*	Migratory endoparasites	*Pratylenchus*	J2	Pra
		Hoplolaimidae	Semi-endoparasites	*Rotylenchulus*	J4	Rot
		Criconematidae	Sedentary ectoparasite	*Criconema*	J2	Cri
		Hoplolaimidae	Semi-endoparasites	*Hoplolaimus*	J2	Hop

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Structural Analysis of Different Genera of Nematodes Observed

Meloidogyne

The root-knot nematode was discerned as majorly prevalent in three sites and their respective fields. The appearance of gall in response to the presence of the *Meloidogyne* was observed in the diseased root samples (Fig. 1). The J2 stage of nematode entered the rhizospheric root, modified the giant cells close to their head and started feeding. Later, Juveniles developed into elliptical-shaped females that were embedded completely or fragmentarily in root tissues. Figure 2A illustrated a vermiform male which was approximately 1-2 mm in length. The male had a circular lip region, cone and nodules at the base were clearly visible (Fig. 2B). Juvenile (J2) was observed straight in general and slightly curved when relaxed. The lip framework was lightly sclerotized. The intestine as a part of the alimentary canal could be visualized in (Fig. 2C). The excretory pore was observed at the posterior end. The spikes were present at the hyaline area of the tail and the circulatory organ (CP) at its extreme end (Fig. 2D-E).

Fig. 1: Root-knot nematode (*Meloidogyne* spp.) induced galling of plant roots. Enlarged, tuberous-type galls formation in roots

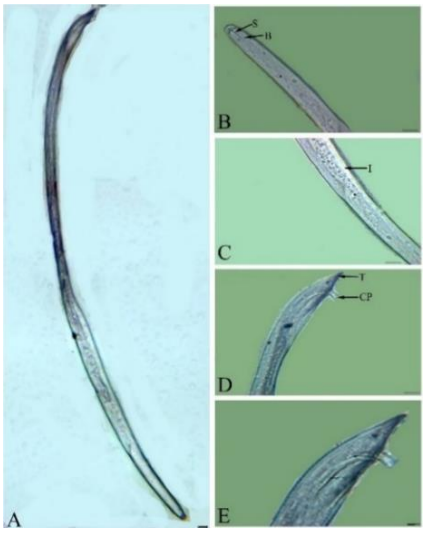

Fig. 2: Genus *Meloidogyne* spp; (A) Complete body structure; (magnification 100X; (B) Anterior region; rounded labial disc, stylet and basal nodules (magnification 400X); (C) Arrow shows intestine (magnification 400X); (D-E) Posterior region showing tail and copulatory organs (magnification of D-400X magnification of E-1000X)

Helicotylenchus

Helicotylenchus commonly known as spiral nematodes observed with key characteristic C-shaped in the relaxed stage (Fig. 3A). The stylet was large with strong basal nodules, robust, well-developed approximately 20-50 µm in length (Fig. 3B). The oesophageal gland lacked clearness in permanent specimen in comparison of hoplolamids, overlapped ventrally. The vulva is situated at around 60% (Fig. 3C). The tail is usually rounded (Fig. 3D).

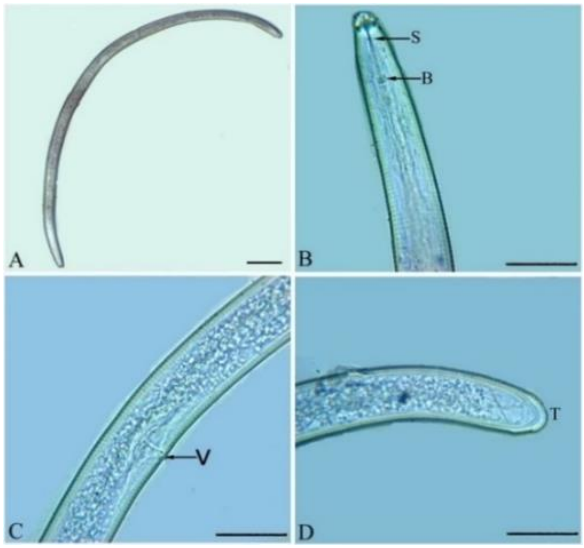

Fig. 3: Genus *Helicotylenchus*spp. (A) Body length (Magnification 100X); (B) Anterior region showing the head, stylet with strong basal nodules (magnification 400X); (C) Arrow shows vulva (magnification 400X); (D) Tail (magnification 400X)

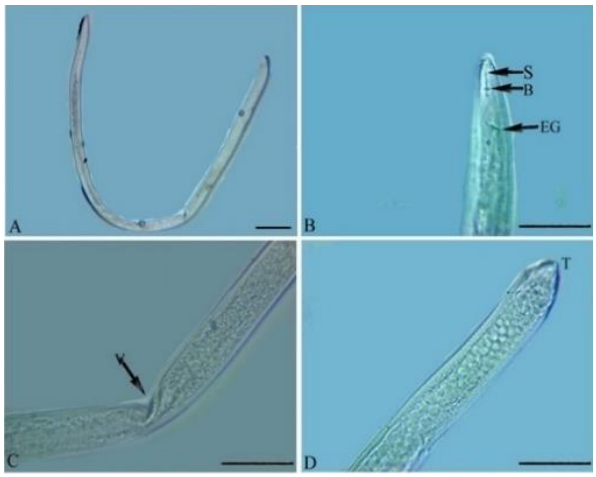

Fig. 4: Genus *Pratylenchus*spp; (A) Body length (magnification 100X); (B) Anterior region of the body. The arrow shows stylet with basal nodules and esophageal gland (magnification 400X); (C) Vulva (magnification 400X); (D) Posterior region showing tail (magnification 400X)

Pratylenchus

Pratylenchus commonly known as lesion nematodes noticed with a cylindrical body (Fig. 4A). The stylet was a well-developed and basal nodule with an oesophageal gland (Fig. 4B). The esophagus overlapped the intestine ventrally. The vulva region was located in 65-85% (Fig. 3C). The tail shape was rounded and conical (Fig. 4D).

Rotylenchulus

Rotylenchulus commonly known as reniform nematodes had a vermiform body and cephalic region (Fig. 5A). The immature female had a well-developed stylet and basal nodules with esophageal gland (Fig. 5B-C). The vulva region was located at 65-70% (Fig. 5D-E). The tail shape was noticed as bluntly rounded (Fig. 5F).

Criconema

Criconema nematode was observed with a tiny, strong and elliptical body at the ventral surface (Fig. 6A). The annules around the lip area were with 6 pseudo lips and the lobes of the submedian region were absent (Fig. 6B). Anterior vulval lip was overhanging vulva. The juveniles were found with rows of scales and annulus (Fig. 6C-D). The tail was found conoid and pointed (Fig. 6E).

Hoplolaimus

Female *Hoplolaimus* were noticed as spiral and C-shaped and 1-2 mm in length (Fig. 7A). The stylet (20-50 µm) was large, robust and well developed (Fig. 7B). The Labial region close to the body, was broader, flat at the anterior end, with visible annuli and stria were longitudinal. The esophagus was found overlapping in the intestine (Fig. 7C). The lateral field was observed indistinct (Fig. 7D). The vulva region was situated at a total length of 60% (Fig. 7E). The tail was observed broadly rounded (Fig. 7F).

Distribution of PPNs in the Study Sites in Tomato Soil Rhizosphere

Data was organized on MS Excel sheets and analyzed in the R programming language with the Analysis Of Variance (ANOVA) technique at a 5% level of significance. This means significant separated by Tukey's HSD method at a 5% level of significance. Table 2, the p-value (0.016) <0.05 indicated that all three sites differ significantly at a 5% level of significance and site S-3 had a maximum mean of 2200 with a standard deviation of 495 followed by site S-2 which had a mean value of 1360 with standard deviation 365 and site S-1 found with a minimum value of mean 1240 and standard deviation 559. The error bar diagram with groups (Fig. 8A) mentioned that different letters on bars S-1 and S-3 made significant differences, whereas the same letters on bars S-1 and S-3 as well as on S-2 and S-3 depicted no considerable differences regarding the mean values of PPNs.

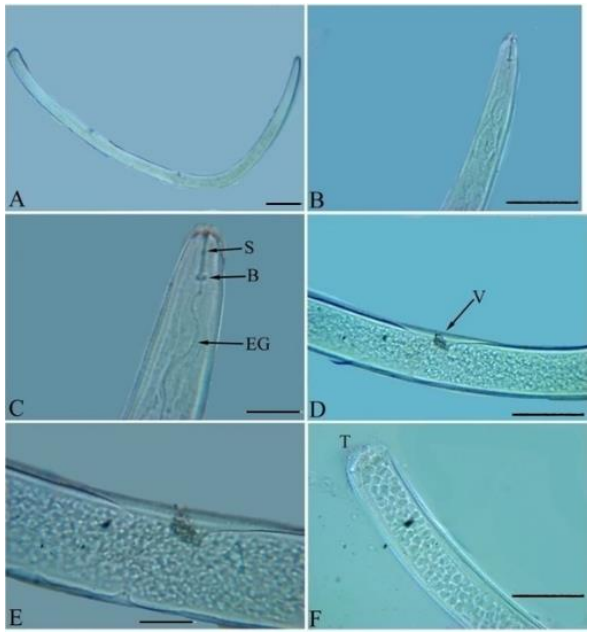

Fig. 5: Genus *Rotylenchulus*; (A) Body length of immature female (Magnification 100X); (B) Head of immature female (Magnification 400X); (C) Anterior region of the body. The arrow shows stylet and basal nodules with esophageal gland (Magnification 1000X); (D, E) Vulva (Magnification of D-400X, Magnification of E-1000X); (F) Tail of immature female (Magnification 400X)

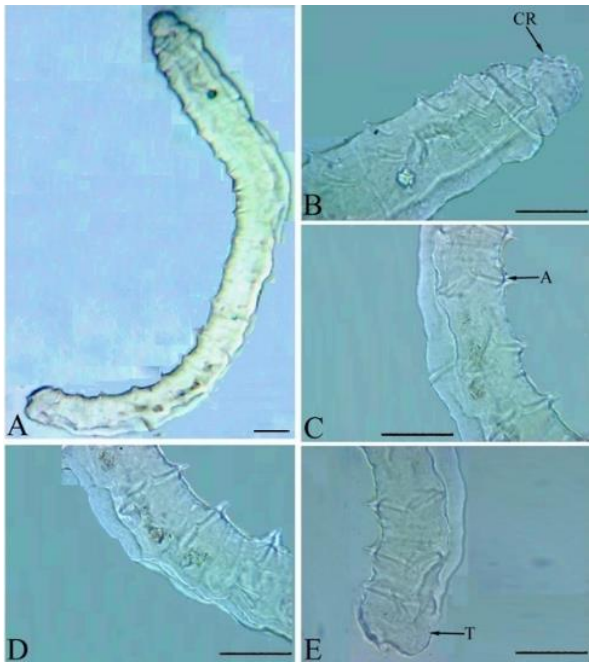

Fig. 6: Genus *Criconema* spp. (A) Body length (magnification 100X); (B) Anterior region of the body. (magnification 400X) Arrow showing disc-shaped cephalic ring; (C-D) Arrow shows annulus (magnification 400X); (E) Tail end (magnification 400X)

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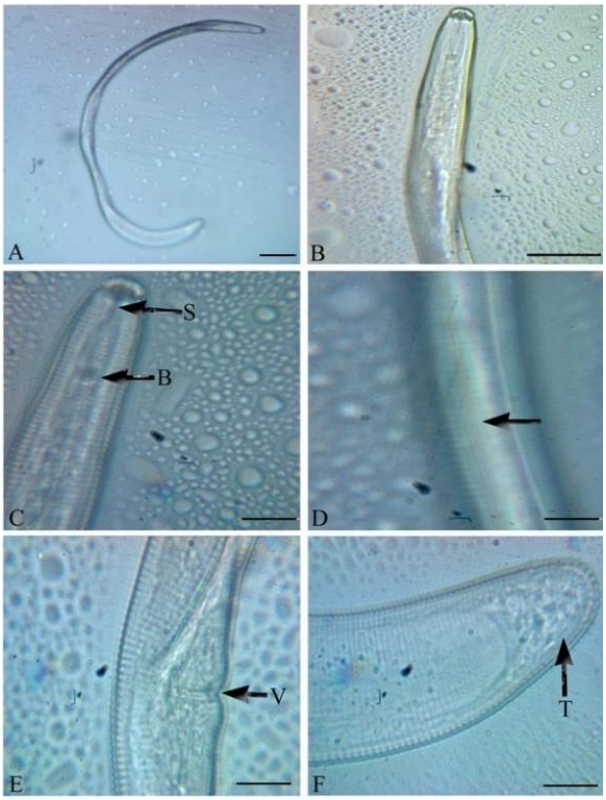
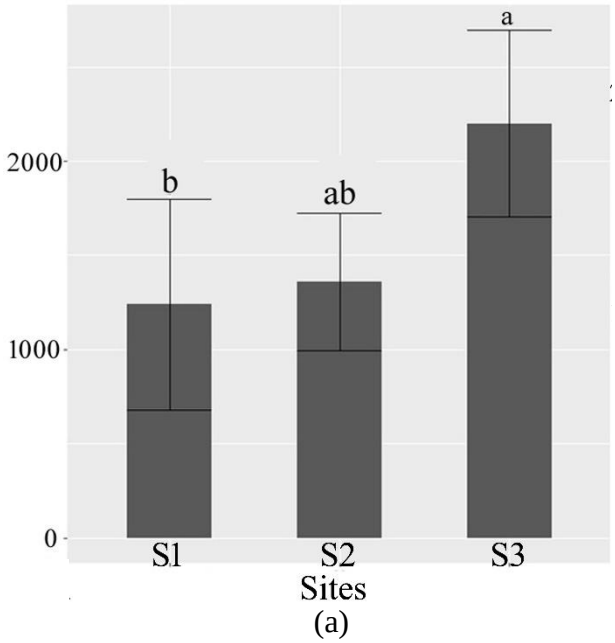
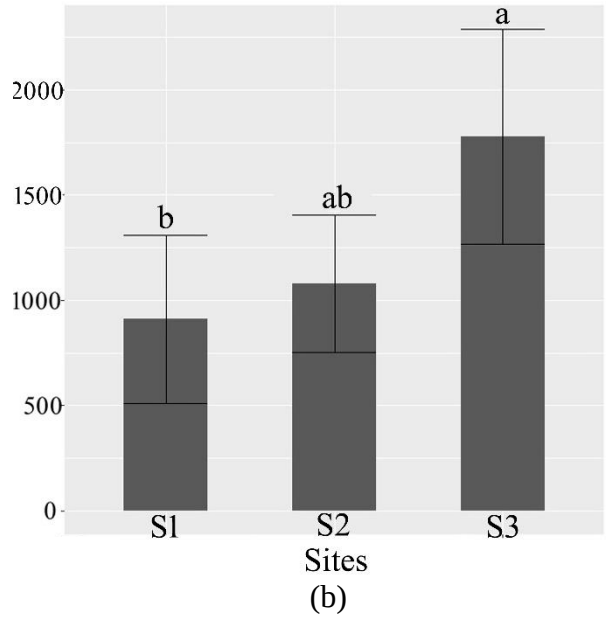


Fig. 7: Genus *Hoplolaimus* spp. (A) Body length (magnification 100X); (B) Anterior region of the body (magnification 400X); (C) Arrow shows stylet and basal nodules (magnification 1000X); (D) Indistinct lateral line (magnification 1000X); (E) Vulva (magnification 1000X); (F) Posterior region shows tail (magnification 1000X)





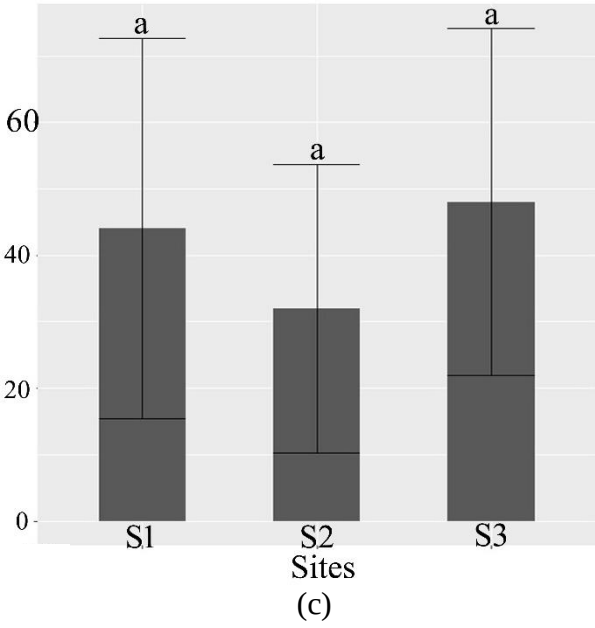


Fig. 8: (A) Error bar with grouping demonstrated the total number of plant parasitic nematodes in three sites S1, S2 and S3; (B) Error bar with grouping indicated the distribution of Genus *Meloidogyne* spp. in three sites S1, S2 and S3; (C) Genus *Helicotylenchus* spp. in three sites S1, S2 and S3. Study areas Bars with the same letter are not significantly different ($p \geq 0.05$) from Turkey's HSD test

Meloidogyne

The p-value (0.016) <0.05 revealed that the distribution of *Meloidogyne* genera in all three sites had significant differences. Site S-3 (Chitavar) had

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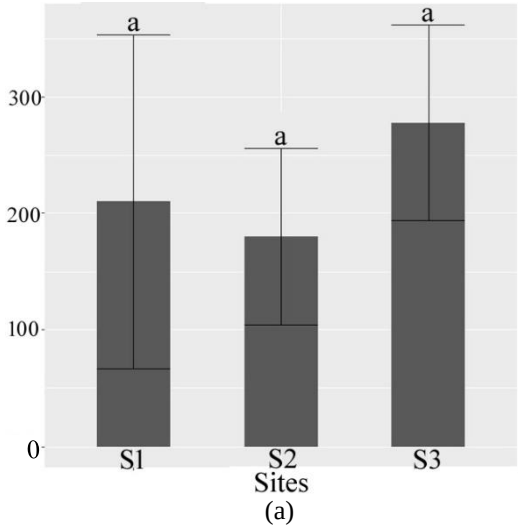
maximum *Meloidogyne* genera on average of 1777 with a standard deviation of 511, followed by S-2 (Basvan) mean of 1080 with a standard deviation of 327. Site S-1 (Harpura) was found with a minimum value of a mean of 910 and a standard deviation of 400 (Table 2). The error bar diagram with groups (Fig. 8B) stated that sites S-1 and S-3 had significant differences, but S-1 and S-2 as well as S-2 and S-3 did not have considerable differences.

Helicotylenchus

The p-value (0.603) >0.05 indicated that the distribution of *Helicotylenchus* genera in all three sites did not have significant differences on average, at a 5% level of significance. Sites S-3 had a maximum value of a mean of 48 and a standard deviation of 26, followed by S-1 a mean of 44 with a standard deviation of 28 and S-2 mean of 32 and a standard deviation of 21 (Table 2). The error bar diagram (Fig. 8C) with the same letter on the bar also indicated that there were no significant differences amongst S-1, S-2 and S-3 regarding the mean population of *Helicotylenchus* genera.

Pratylenchus

The p-value (0.356) >0.05 revealed that all three sites did not differ significantly at a 5% level of significance based on the mean population of Pratylenchus genera. Site S-3 had a maximum mean of 277 and a standard deviation of 84, followed by S-1 with a mean of 210 and a standard deviation of 143 and S-2 with a minimum mean of 180 and a standard deviation of 76 (Table 2). The error-bar diagram (Fig. 9A) also indicated that there were no considerable differences among all the sites S-1, S-2 and S-3.



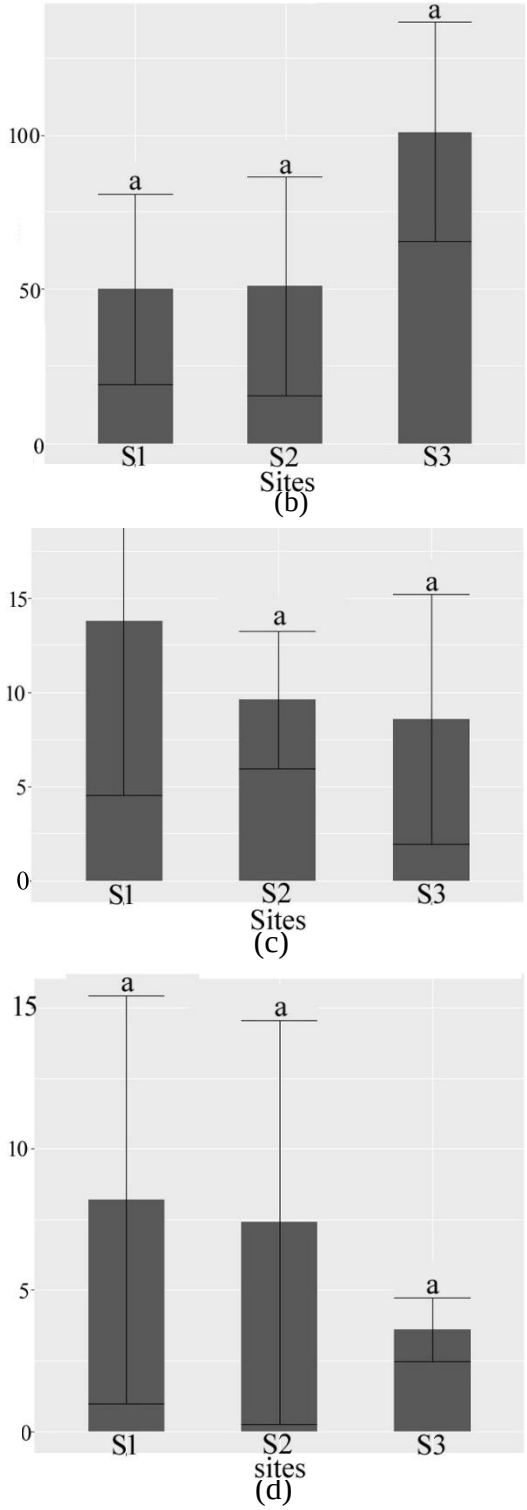


Fig. 9: (A) Error bar with grouping indicated the distribution of Genus *Pratylenchus* spp; in three sites S1, S2 and S3; (B) Genus *Rotylenchulus* spp. in three sites S1, S2 and S3; (C) Genus *Criconema* spp. in three sites S1, S2 and S3; (D) Genus *Hoplolaimus* spp. In three sites S1, S2 and S3. Study areas Bars with the same letter are not significantly different (p≥0.05) from Turkey's HSD test

Table 2: Mean and standard deviation of PPN genera from rhizosphere soils in the study sites. Keys: Site-1 = Harpura, Site-2 = Basvan, Site-3 = Chitavar, N = Number of fields, S. D. = Standard deviation, Mel = Meloidogyne, Hel = Helicotylenchus, Pra = Pr

PPNs	Sites	N	Mean	S. D.	Minimum	Maximum	p-value
TN	Site-1	5	1240.00	559.464	500	2000	0.016
	Site-2	5	1360.00	364.692	800	1800	
	Site-3	5	2200.00	494.975	1500	2800	
	Total	15	1600.00	626.783	500	2800	
Mel	Site-1	5	910.00	400.625	400	1500	0.016
	Site-2	5	1080.00	327.109	600	1500	
	Site-3	5	1777.40	510.751	1000	2275	
	Total	15	1255.80	549.429	400	2275	
Hel	Site-1	5	44.00	28.592	20	80	0.603
	Site-2	5	32.00	21.679	20	70	
	Site-3	5	48.00	26.125	20	90	
	Total	15	41.33	24.746	20	90	
Pra	Site-1	5	210.00	143.178	50	400	0.356
	Site-2	5	180.00	75.829	100	250	
	Site-3	5	277.60	83.942	200	400	
	Total	15	222.53	106.294	50	400	
Rot	Site-1	5	50.00	30.822	20	100	0.056
	Site-2	5	51.00	35.426	15	100	
	Site-3	5	101.00	35.426	50	150	
	Total	15	67.33	39.949	15	150	
Cri	Site-1	5	13.80	9.257	3	25	0.472
	Site-2	5	9.60	3.647	5	15	
	Site-3	5	8.60	6.618	3	20	
	Total	15	10.67	6.800	3	25	
Hop	Site-1	5	8.20	7.225	2	20	0.446
	Site-2	5	7.40	7.162	2	20	
	Site-3	5	3.60	1.140	2	5	
	Total	15	6.40	5.853	2	20	

Rotylenchulus

The p-value (0.056) >0.05 revealed that all three sites did not differ significantly at a 5% level of significance based on the mean population of *Rotylenchulus* genera. Site S-3 had a maximum mean of 101 and standard deviation (S. D.) of 35, followed by S-2 with an average of 51 and S. D. value of 35. The S-1 was found with a minimum average of 50 and a standard deviation of 31 (Table 2). The error-bar diagram (Fig. 9B) also shows that there were no considerable differences between S-1, S-2 and S-3.

Criconema

The p-value (0.472) >0.05 enumerated that all three sites did not differ significantly at a 5% level of significance based on the mean population of genera *Criconema*. Site S-1 had a maximum mean of 14 and a standard deviation of 9 followed by site-2 with a mean of 9 and with standard deviation of 3. Site -3 was found with a minimum value of a mean of 8 and a standard deviation of 6 (Table 2). The error-bar diagram (Fig. 9C) also shows that there was no considerable variation among sites 1-3.

Hoplolaimus

The p-value (0.446) >0.05 for all three sites did not differ significantly at a 5% level of significance based on the mean population of genera *Hoplolaimus*. Site S-1 had a maximum mean of 8 and a standard deviation of 7 followed by site-2 which had a mean of 7 and a standard deviation of 7. Site S-3 was found with a minimum value of a mean of 3 and a standard deviation of 1 (Table 2). The error-7 bar diagram (Fig. 9D) shows that there was no considerable discrepancy among S-1, S-2 and S-3.

Distribution of FLNs in the Study Sites in Tomato Soil Rhizosphere

Free-living nematodes of the following genera were observed during investigation in desi tomato soil *viz Diplogaster* spp., *Dorylaimus* spp. and *Mononchus* spp. (Fig. 10). The statistical data was organized and arranged in Microsoft Excel sheets and observed using ANOVA techniques at a 5% level of significance ($p<0.05$) using R programming language for computing. Means significance was tabled separately using Tukey's HSD at a 5% level of significance ($p<0.05$). The p-value ($p>0.000$) indicated that all three sites differ significantly at a 5% level of significance and site-2 had a maximum mean of 18 with a standard deviation of 2 followed by site-1 which had a mean of 8 with a standard deviation of 1. Site-3 was found with a minimum value of a mean of 6 and a standard deviation of 2 (Table 3). Bar diagram (Fig. 11A) shows that S1 and S2 followed by S2 and S3 were found significant. There was no considerable discrepancy between S-1 and S-3.

Table 3: Mean and Standard deviation of FLNs genera from rhizosphere soils in the three sites. Keys: Site-1 = Harpura, Site-2 = Basvan, Site-3 = Chitavar, N = Number of fields, S. D. = Standard deviation, Dip = *Diplogaster*, Dor = *Dorylaimus*, Mon = *Mononchus*

FLNs	Sites	N	Mean	S. D.	Minimum	Maximum	p-value
TN	1	5	8.0000	1.58	6	10	0.000
	2	5	18.0000	2.00	16	20	
	3	5	6.4000	2.07	5	10	
	Total	15	10.8000	5.60	5	20	
Dip	1	5	4.2000	1.30	3	6	0.000
	2	5	12.0000	2.74	10	15	
	3	5	3.2000	1.10	2	5	
	Total	15	6.4667	4.42	2	15	
Dor	1	5	2.6000	0.55	2	3	0.005
	2	5	3.8000	0.84	3	5	
	3	5	2.0000	0.71	1	3	
	Total	15	2.8000	1.01	1	5	
Mon	1	5	1.2000	0.45	1	2	0.005
	2	5	2.2000	0.45	2	3	
	3	5	1.2000	0.45	1	2	
	Total	15	1.5333	0.64	1	3	

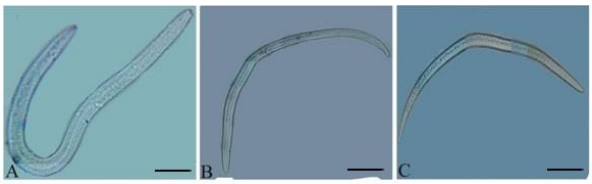


Fig. 10: Total body length of free-living nematodes; (A) Genus *Diplogaster* spp. (Magnification 100X); (B) Genus-*Dorylaimus* spp. (Magnification 100X); (C) Genus-*Mononchus* spp. (Magnification 100X)

Diplogaster

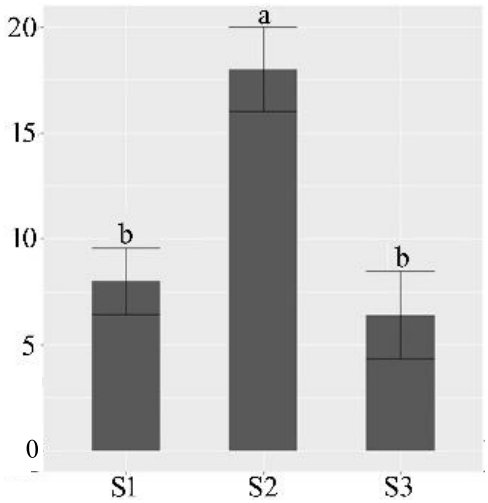
The p-value (0. 00) <0.05 revealed that all three sites differ significantly at a 5% level of significance considering the mean population of *Diplogaster* genera. Site-2 had a maximum mean of 12 and a standard deviation of 2 followed by site S-1 which had a mean of 4 with a standard deviation of 1. Site -3 was found with a minimum value of a mean of 3 and a standard deviation of 1 (Table 3). It was noticed in the error bar diagram (Fig. 11B) that S-1 and S-2 followed by S-2 and S-3 were significant. No considerable alteration was observed between the values of S1 and S3.

Dorylaimus

The p-value (0.005) <0.05 revealed that all three sites differ significantly at a 5% level of significance on the basis of the average population of genera Dorylaimus. Site S-2 had a maximum mean of 3.8 and a standard deviation of 0.84 followed by site S-1 which had a mean 2.6 and a standard deviation of 0.55. Site-3 was found with a minimum value of mean 2 and a standard deviation of 0.71 (Table 3). The error bar diagram (Fig. 11C) shows that S-2 and S-3 were significant. No remarkable difference was found between S-1 and S-3 followed by S-1 and S-2.

Mononchus

The p-value (0.005) <0.05 revealed that all three sites differ significantly at a 5% level of significance on the basis of the average population of genera Mononchus. Site S-2 had a maximum mean of 2.2 and S. D. of 0.45 followed by site-1 which had a mean 1.2 and standard deviation of 0.45. Site-3 was found with a minimum value of a mean of 1.2 and a standard deviation of 0.45 (Table 3). The error bar diagram (Fig. 11D) shows that S-1 and S-2 followed by S-2 and S-3 differ significantly. There was no considerable difference between S-1 and S-3.



(a)

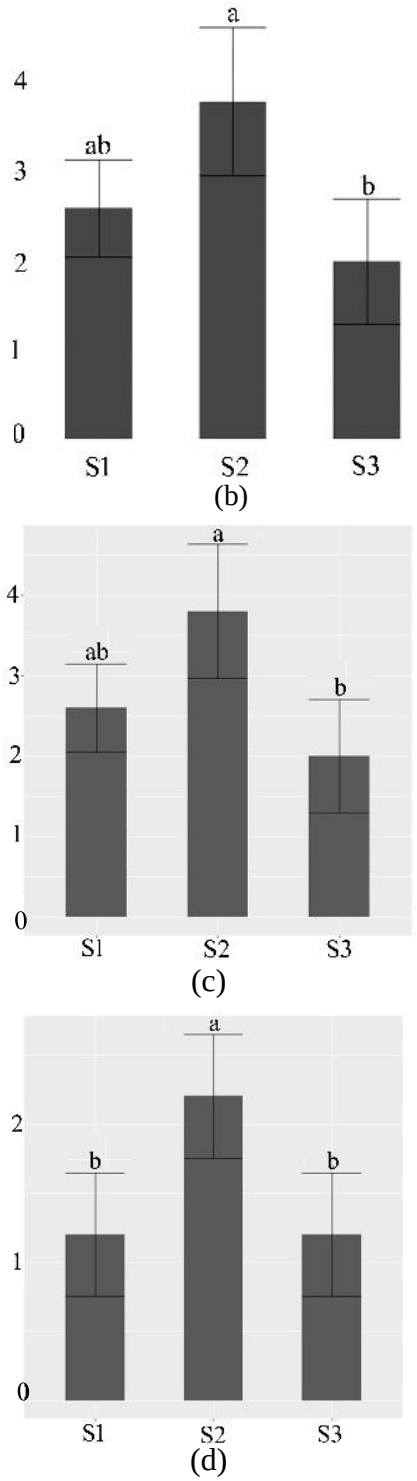


Fig. 11: (A) Error bar with grouping demonstrated total number of free living nematodes in three sites-S1, S2 and S3; (B) Error bar with grouping indicated the distribution of Genus *Diplogaster* spp; in three sites-S1, S2 and S3; (C) Genus *Dorylaimus* spp. in three sites-S1, S2 and S3; (C) Genus *Mononchus* spp. in three sites-S1, S2 and S3. In Study areas, Bars with the same letter are not significantly different ($p \geq 0.05$) with Turkey's HSD test

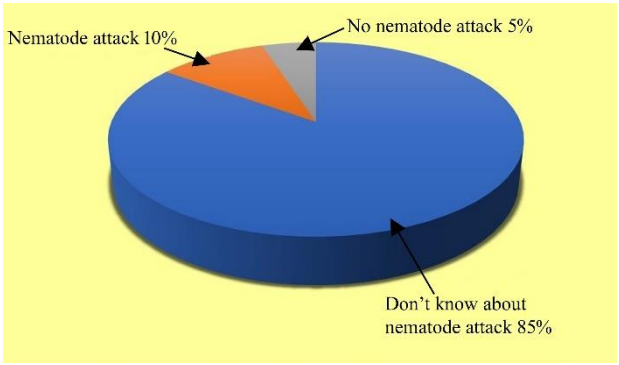


Fig. 12: The crops were grown three years prior to the plantation of tomato crops by cultivators in sites S1, S2 and S3

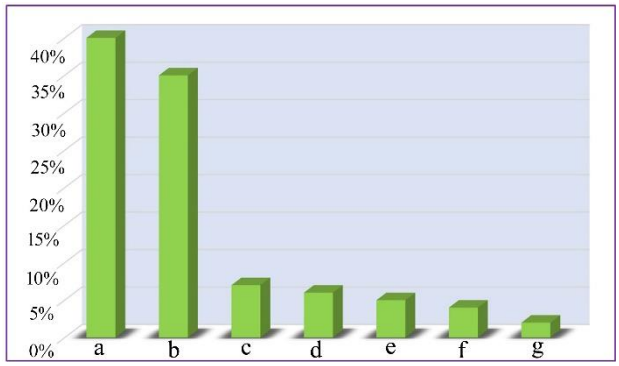


Fig. 13: Percentage of tomato plants cultivator's awareness of nematodes attack in sites S1, S2 and S3

Cultivation Practices of Tomato Plant Production in Study Sites

The cultivation of tomato plants was low in all experimental sites. 60% of the crop growers used to grow tomato plants in the area which was less than 0.35 acres. Only 40% of the farmers used between 0.35 and 1 acre of land.

Different varieties of crops were grown in the previous three years in the field where tomato plants are cultivated at present. The multiple crops were grown in 40%, vegetable crops 35%, cereal crops 8%, pulses 6%, fruit crops 5%, medicinal crops 4% and grasses 2% (Fig. 12). 85% of crop growers had no information on the invasion of nematodes whereas 10% of farmers had awareness of nematodes attack but 5% had no idea of it (Fig. 13).

Physicochemical Features of Soil at the Sites of Examination

The physicochemical characteristics of soil at investigation sites are presented in Table 4. The higher pH in site S-1 was obtained than that of S-2 and S-3. The nitrogen content was noticed quite high in site-1 in comparison to site-2 and site-3. The amount of phosphorous, potassium and Electrical Conductivity (EC) was high on site-2 and site-3 in comparison to site S-1. The value of Organic Carbon (OC) was ascertained at a minimum at site S-3 than S-2 and S-1.

Table 4: Physiochemical properties of soils from three study areas. Keys: BS = Black Soil, RS = Red Soil, OC = Organic Carbon, N = Nitrogen, P = Phosphorus, K = Potassium

	Areas		
Properties	Site 1	Site 2	Site 3
pH	7.00	7.30	7.10
EC	0.45	0.51	0.48
OC	0.40	0.55	0.35
N	180.00	213.00	168.00
P	8.00	4.50	9.50
K	305.00	237.00	310.00
Texture	BS	RS	BS

Discussion

In the present submission, the authors have highlighted the distribution and structure of some of the deleterious PPNs that prevailed in a major common variety known as cherry tomato (desi) in Jhansi India. The physicochemical properties of soil and its relationship with the abundance of PPN genera have also been explored. The six PPNs genera have been enumerated for the first time in the tomato crop grown in this region. The distribution of six genera of PPNs (*Meloidogyne*, *Hoplolaimus*, *Rotylynchulus*, *Pratylenchus*, *Helicotylenchus and Criconmea*) based on prominence value and the significance test was also verified with morphological analysis.

Site S-3 and S-2 were documented in a higher population of PPNs. The high quantity of PPNs in the two sites could be attributed to the coexistence of tomato plants with vulnerable crops like ginger, turmeric, fenugreek, red chili, bringles, papaya, coriander, carrot and cabbage spotted during the survey. Different varieties of crops were grown in the previous three years in the same field where tomato plants are cultivated at the present time, explaining the incident of the high number of nematodes in the rhizosphere region of roots. The infection on tomato plants after cropping was brought on by previously cultivated crops. Severe crop loss has been recorded due to the long-term planting of nematode infestation-prone crops, which may result in PPN assemblances (Bhan *et al*., 2010). The moderate amount of rain helped to maintain substantial moisture that ensured the mobility of nematodes (Juma *et al*., 2020). During the survey, it was noticed that site S-1 (Harpura), had a lesser number of total nematodes, this may be the reason that the farmers of that village applied inorganic fertilizer and decomposed cow and buffalo dung, which reduced the total number of nematodes significantly. Organic decomposed waste liberated toxic chemicals against PPNs and intensified the argumentation of antagonistic micro-organisms to tackle the menace of PPNs (Juma *et al*., 2020). It was observed that infection of PPNs on tomato crops declined significantly during the summer season because hot and dry climatic conditions did not allow them to grow. Tendering support to the above findings, Williamson and Hussey (1996) stated that hot and arid seasons reduce PPN infection in crops.

During the investigation, it was ascertained that all three sites of tomato plant cultivation had been predominantly affected by PPN genera *Meloidogyne* followed by *Pratylenchus*, *Rotylenchulus* and *Helicotylenchus*. Vásquez and Soria (1984) examined the similar distribution of PPNs in tomato crops and *Meloidogyne* spp. was noted as the most abundant genera. These PPNs are polyphagous and their huge number is associated with the plantation of tomato crops with other prone host crops. On the other hand, while preparing the questionnaire, the authors interacted with crop growers and found out that many of them had grown the crop in the past three years which was highly susceptible to PPNs. This could be the key cause of the high number of PPNs extracted from soil and roots. During the survey, the low population of *Hoplolaimus* and *Criconema* genera was observed in cultivated lands. Blake (1969) opined that it could be possible because of the occurrence of different offensive dominating genera and interspecific enmity from various PPNs such as *Pratylenchus* spp. and *Meloidogyne*. spp., *Helicotylenchus* spp. and *Rotylenchulus* spp.

Free-Living Nematodes (FLNs) are a very important group of nematodes that are bacteriovorus, algivores and detrivorous in nature. They recycle the nutrients for plant use and consolidate the structure of soil to retain water. During the present investigation, the count of FLNs, in all sites-1, site-2 and site-3 was quite low as compared to PPNs, like the observation of Juma *et al*. (2020). At the cultivation sites, the farmers were advised to use cow and buffalo dung in the soil because Organic Amendments (OAs) help enhance the FLN population and combat the population of PPNs as suggested by Hillocks and Waller (1997).

Soil physicochemical parameters enact a crucial value in the improvement of soil fertility for the cultivation practices of agricultural crops and tomatoes and have been found to influence the distribution of PPNs in all three sites of study. According to Kandji *et al*. (2001); Kimenju *et al*. (2009), the physio-chemical characteristics of soil regulate the abundance, distribution and community structure of nematodes. The minimum population of PPNs was recorded in S-1 (Harpura), it might be occurred due to a high level of Organic Carbon (OC), Organic Amendments (OAs) and nitrogen (referred to the Table 4) in comparison to that of S-2 (Basvan) and S-3 (Chitavar), which were recorded a high number of PPNs. Juma *et al*. (2020) stated that

Organic Carbon (OC) increases the number of nematophagous microbes in the soil which reduces the population of PPNs. On the other hand, Agbenin (2004) stated that soil Organic Amendments (OAs) increase the quantum of nematodes trapping fungi compared with inorganic Argo supplements. Nchore *et al*. (2012) have opined that animal excreta have been used in the reduction of root-knot nematodes and other PPNs, which is suggestive of the organic management of the PPNs population in tomato crops.

The Physico-chemical analysis of soil also revealed that S-2 and S-3 sites had greater Phosphorus (P) and Potassium (K) levels than S1. Phosphorus and potassium supplement the nutrition, multiplication and production of eggs in the nematode population. Potassium also enhances root development, extending the rhizosphere area for nematode invasion and feeding (Badra and Yousif, 1979). This opinion was similar to studies by Kandji *et al*. (2001); Badra and Yousif (1979); Juma *et al*. (2020).

Most of the soils in the study sites were Red Soil (RS) and Black Soil (BS). According to Talwana *et al*. (2008) above types of soil structures support the high nematode population densities. Such soils are highly porous with good aeration and favour the mobility of nematodes (Talwana *et al*., 2008; McLean and Lawerence, 2000; Norton, 1978). Since the survey was conducted during the post-monsoon season (October-December 2021) all the sites had a copious amount of moisture in the soil which favored the greater PPN populations. Wallace (1983); Jaetzold *et al*. (2006) also postulated similarly that humidity and moisture in soil received due to rainfall create favorable conditions for nematode development. Nyasani *et al*. (2008) stated that the pH 4-8 range tolerated by most PPNs and the present study also revealed that the optimum pH ranged between 7-7.3 which was found conducive for the growth and development of nematodes.

Conclusion

This study illustrated that the tomato plant is invaded by multiple PPNs at Baruasagar in Jhansi. The most invasive nematode genera are *Meloidogyne*, *Pratylenchus*, *Helicotylenchus and Rotylenchulus*. The physical and chemical characteristics of soil and cultivation methods have significantly increased the number of PPNs at tomato production sites. Therefore, additional research is required to identify the nematode species on a molecular basis so that an appropriate and effective management plan may be developed to keep the PPN population below the economic threshold and at a sustainable level (ETL). On the other, it is a need of the hour to disseminate agrarian literacy among the farmers to let them educate about hazardous pests like nematodes and the importance of organic solutions as an alternative to non-degradable chemical nematicides.

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Author's Contributions

Abha Sachan: Conducted experimental work and secured funded.

Rajesh Kumar Pandey: Designed the research question and the manuscript outline.

Shailendra Kumar: Curated and analyzed the data.

Neelam Kashyap: Coordinated the study and supervised the research team.

Manvendra Sengar: Drafted, reviewed, and edited the manuscript.

Ethics

This research project on plant parasitic nematodes was exempt from ethical approval.

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Abbreviations

BS	=	Black Soil
Cri	=	*Criconema*
Dip	=	*Diplogaster*
Dor	=	*Dorylaimus*
FLNs	=	Free Living Nematodes
Hel	=	*Helicotylenchus*
Hop	=	*Hoplolaimus*
J2	=	Larval infective stage
J4	=	Larval infective stage
K	=	Potassium
Mel	=	*Meloidogyne*
Mon	=	*Mononchus*
N	=	Nitrogen
N	=	Number of fields
OC	=	Organic Carbon
P	=	Phosphorus
PPNs	=	Plant Parasitic Nematodes
Pra	=	*Pratylenchus*
Rot	=	*Rotylenchulus*
RS	=	Red Soil
S. D.	=	Standard Deviation
Site-1	=	Harpura
Site-2	=	Basvan,
Site-3	=	Chitavar