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To study the distribution and occurrence of root knot nematode & other phyto-nematodes associated with okra in and around Jaipur district

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Abstract
Survey of okra crop in and around Jaipur district of major okra growing areas like Dindhol, Baseri, Bagru kalan, Dudu, Bagus, Sitapura, Vinovapuri, Bassi, Madau revealed the occurrence of root knot nematode (*Meloidogyne* spp.) in rhizosphere of the okra crop. In Analysis of soil and root sample from the rhizosphere of okra plant, the highest population is found to be of *Meloidogyne* spp. (*Meloidogyne incognita* and *Meloidogyne javanica* both) with highest Absolute frequency, Absolute density and per-cent Prominence value; 98.13 %, 12.86% and 3.82% respectively.

Keywords: Root nematode, phyti-nematodes, associated with okra

Introduction
Okra (*Abelmoschus esculentus (L.) Moench*) is a commercial vegetable crop which is ordinarily known as 'Bhindi'. It is one of the most important summer and rainy season vegetable crops and is cultivated in various states of India. Okra is a nutritive vegetable crop which plays a very significant role in meeting the demand of vegetable scanty market. Pal *et al.* (1952) [11] and Rashwan (2011) [12] found that okra is a good source of calcium, vitamin, potassium, carbohydrate minerals and fibers. It is also considered to be the best source of iodine and contains generous dosage of vitamin c (30 mg/100 g), calcium (90 mg/100g) and iron (1.5 mg/100g) and include contains of crude fiber 6.60 to 10 %, protein 14.40 to 18.60% and ash 8.20 to 9.15 % of the total weight and is also rich source of vitamin B_6 and folic acid, in dry seed of okra contain about 20-30 % crude protein. (Anonymous 2018) [9]. Root-knot nematodes (*Meloidogyne* spp.) are one of the most economically damaging genera of plant-parasitic nematodes on horticultural and field crops. Root-knot nematodes are distributed all over the world, and are obligatory parasites of the roots of thousands of plant species. *Meloidogyne javanica* is considered as an agricultural pest, as it is extremely abundant and damaging (Alford 2012) [10]. Nematode abundance and composition are significantly influenced by soil properties, rainfall and temperature. The geographic distribution of *M. incognita* is largely dependent on environmental factors such as temperature and moisture.

Material methods
To find out the status of the nematode, a systematic survey was undertaken on various annual vegetable crops in and around Jaipur district. Other experiments were conducted at Rajasthan Agricultural Research Institute, Durgapura, Jaipur during 2019-20. An extensive and intensive survey was conducted for the presence of *Meloidogyne* spp infesting annual vegetables from different localities in and around Jaipur district *i.e.* Dindhol, Baseri, Bagru kalan, Dudu, Bagus, Sitapura, Vinovapuri, Bassi and Madau villages.

Soil sampling
An average 200cc soil sample were collected from various annual vegetables crop *viz.*, chilli, cucumber, okra, tomato, brinjal, carrot, pea, spinach, radish, bottle gourd. The samples were collected randomly from the rhizosphere of the plants with the help of khurpi from 4-5 places in each field of okra at the depth of 15-20cm, homogenized, filled in a polythene bag, labeled, tied, brought to the laboratory and stored in a refrigerator at about 10^0c temperature.

A composite sample of size 200 cc along with 5g roots should be obtained from one acre field.

Processing of sample

To isolate the nematodes from soil, each soil sample was processed by Modified Cobb's sieving and decanting technique. A 200cc soil sample was put in a bowl half filled with water and mixed thoroughly to make it homogenous and left for 1/2 minutes only to allow heavy particle to settle down at the bottom. The mixture was sieved into another bowl through a coarse sieve (16 mesh) to remove the debris and other undesirable particles. The suspension in the second bowl was then poured through sieves in a sequence, with 60 mesh first followed by 100, 200 and 400 mesh sieve. The water flowing through 400 mesh sieve was allowed to run down and residue on the sieve was collected in a beaker. The process was repeated twice/thrice for complete recovery of nematodes.

Nematode extraction

The nematode suspension obtained by sieving was poured on wire gauge containing double layer tissue paper placed on Baermann funnel holding sufficient water in contact with the bottom of the gauge. After 24-48 hrs. Suspension passing through tissue paper, containing nematodes at the bottom of Baermann funnel tube was collected in a beaker for further studies.

Estimation of nematode population

The volume of nematode suspension was adjusted to 100 ml. The numbers of *Meloidogyne* species were counted per 5ml of suspension with the help of a counting dish, and an average of two counts was taken as the number of nematodes per ml. The volume of the suspension was multiplied with average count of nematodes per ml to arrive at the total no. of nematode population per 200cc of soil.

Staining and counting of nematodes in roots

For counting the population of the nematodes, the roots were carefully uprooted, washed with water to remove soil and stained in boiling 0.05% acid fuchsin lactophenol solution (Franklin and goodey, 1949) [8] for two minutes. After cooling the stained roots were washed gently in plain water and then kept in plain lactophenol solution till they were teased and examined under stereoscopic microscope to count the number of nematode *viz*., no. of females, eggs and egg masses/5 g roots and calculated/plant root system.

Killing and fixing of nematodes

Killing and fixing of nematodes was achieved by using hot fixatives F.A. 4:1; F.A. 4:10, in quick method of lactophenol. T.A.F. was also used which was found to be better as nematodes were killed and fixed together in this process.

Mounting of nematodes

Killed and fixed nematodes were transferred into a cavity block containing Seinhorst solution-1 (96% ethanol-20 parts, glycerol-1-part, distilled water-79 parts). This cavity block was kept into an airtight desiccator having 96% ethanol for 24hrs at room temperature. Later on, the nematodes were transferred to another cavity block with Seinhorst solution-2 (96% ethanol-95 parts, glycerol-5 parts) and kept in another desiccator containing calcium chloride for 24 hrs to remove the water contents. Then the nematodes were mounted in anhydrous glycerol (Seinhorst, 1959) [6].

Nematode diversity

To study the nematode community structure with reference to Absolute frequency of *Meloidogyne* spp. per soil sample, Absolute density of *Meloidogyne* spp. and the prominence value, formulae proposed by Norton, (1978) [7] have also been thoroughly applied

$$\text{Absolute frequency of } \textit{Meloidogyne} \text{ spp. per soil sample} = \frac{\text{Number of samples containing a species}}{\text{Total no. of samples collected}} \text{X} 100$$

$$\text{Absolute density of } \textit{M}\text{eloidogyne spp.} = \frac{\text{No. of individuals of a species in a sample}}{\text{volume of sample}} \text{X} 100$$

$$\text{Prominence value of } \textit{Meloidogyne} \text{ spp.} = \frac{\text{Absolute density X } \sqrt{\text{Absolute frequency}}}{100}$$

Experiments result

Survey of okra growing areas to know association of various plant parasitic nematodes

A survey was carried out on the occurrence of root knot nematode and other Phyto nematodes associated with okra in and around Jaipur district. Soil samples were collected from nine localities *viz*; Dindhol, Baseri, Bagru kalan, Dudu, Bagus, Sitapura, Vinovapuri, Bassi, Madau (Table 1). In these nine localities of Jaipur district, soil samples were collected from ten different annual vegetables like: chilli, cucumber, okra, tomato, brinjal, carrot, pea, spinach, radish, bottle gourd *etc*. (Table-2). The soil and root samples collected from all the different localities were brought to the laboratory and were analyzed for the presence of root knot nematode.

Total 107 soil and root samples were collected from okra growing areas of Jaipur district. In this survey, four species of plant parasitic nematodes were found which belong to genera *Meloidogyne, Helicotylenchus, Pratylenchus* and *Tylenchorhynchus etc.* (Table-3).

Results of community analysis showed that out of four spp. of nematodes, *Meloidogyne* spp. (*M. incognita* and *M. javanica*) was found to be the most frequently occurring plant parasitic nematode. In the nine localities, per-cent occurrence of *Meloidogyne* spp. was 98.13 % followed by *Pratylenchus* spp. 65.42 %, *Helicotylenchus* spp. 61.68 % and *Tylenchorhynchus spp*. 52.33 %. Absolute density of *Meloidogyne* spp. was 12.86 %, followed by *Helicotylenchus* spp. 0.77 %, *Pratylenchus* spp. 0.67 % and *Tylenchorhynchus* spp. 0.67 %. Per-cent prominence value of *Meloidogyne* spp. is 3.82 %, followed by *Helicotylenchus* spp. 0.18 %, *Pratylenchus* spp. 0.16 % and *Tylenchorhynchus* spp. 0.14 %. (Table 4).

Root knot nematode, *Meloidogyne* spp. (mix population of *M. incognita* and *M. javanica*) was found most predominant

species in different localities of Jaipur district. In Dindhol, *Meloidogyne* spp. population was found to be 218 followed by *Helicotylenchus*, *Pratylenchus* and *Tylenchorhynchus* to be 9, 10, and 10. In Baseri village, *Meloidogyne* spp. population was found to be 309 followed by *Helicotylenchus*, *Pratylenchus* and *Tylenchorhynchus* which was 15, 15, and 12. In Bagru kalan village, *Meloidogyne* spp. population is found to be 305 followed by *Helicotylenchus*, *Pratylenchus* and *Tylenchorhynchus* is 13, 13, and 22. In Dudu village, *Meloidogyne* spp. population is found to be 318 followed by *Helicotylenchus*, *Pratylenchus* and *Tylenchorhynchus* is 10, 12, and 17. In Bagus village, *Meloidogyne* spp. population is found to be 360 followed by *Helicotylenchus*, *Pratylenchus* and *Tylenchorhynchus* is 14, 21, and 19. In Sitapura village, *Meloidogyne* spp. population is found to be 355 followed by *Helicotylenchus*, *Pratylenchus* and *Tylenchorhynchus* is 21, 25 and 18. In Vinovapuri village, *Meloidogyne* spp. population is found to be 370 followed by *Helicotylenchus*, *Pratylenchus* and *Tylenchorhynchus* is 23, 24 and 9. In Bassi village, *Meloidogyne* spp. population was found to be 340 followed by *Helicotylenchus*, *Pratylenchus* and *Tylenchorhynchus* is 25, 11 and 16. In Madau village, *Meloidogyne* spp. population was found to be 300 followed by *Helicotylenchus*, *Pratylenchus* and *Tylenchorhynchu-s* is 40, 16 and 26. (Table 5).

Table 1: List of localities from where soil samples were collected.

S. No.	Locality No.	Name of Localities
1.	L 1	Dindhol
2.	L 2	Baseri
3.	L 3	Bagru kalan
4.	L 4	Dudu
5.	L 5	Bagus
6.	L 6	Sitapura
7.	L 7	Vinovapuri
8.	L 8	Bassi
9.	L 9	Madau

Table 2: List of annual vegetables and the locality from which soil samples were collected.

S. No.	Name of annual vegetables	Botanical Names	Locality
1.	Chilli	*Capsicum annum*	L_1, L_2, L_3, L_4, L_5, L_7, L_8,
2.	Cucumber	*Cucumis sativus*	L_1, L_2, L_3, L_5, L_6, L_8, L_9
3.	Okra	*Abelmoschus esculentus*	L_1, L_2, L_4, L_5, L_6, L_7, L_8, L_9,
4.	Tomato	*Solanum lycopersicum*	L_1, L_2, L_3, L_4, L_5, L_6, L_8
5.	Brinjal	*Solanum melongena*	L_1, L_3, L_5, L_6, L_7, L_8
6.	Carrot	*Daucus carota*	L_1, L_2, L_6, L_7
7.	Pea	*Pisum sativum*	L_4, L_6, L_9
8.	Spinach	*Spinacia oleracea*	L_4, L_6, L_7, L_8
9.	Radish	*Raphanus sativus*	L_6, L_7, L_8
10.	Bottle gourd	*Lagenaria siceraria*	L_3, L_4, L_7, L_9

Table 3: Frequency of occurrence of plant parasitic nematodes in various localities in and around Jaipur

Locality No.	Order of prevalence in descending order
L 1	*Meloidogyne* spp., *Pratylenchus* spp., *Tylenchorhynchus* spp., *Helicotylenchus* spp.,
L 2	*Meloidogyne* spp., *Helicotylenchus* spp., *Pratylenchus* spp., *Tylenchorhynchus* spp.
L 3	*Meloidogyne* spp., *Tylenchorhynchus* spp., *Helicotylenchus* spp., *Pratylenchus* spp.
L 4	*Meloidogyne* spp., *Tylenchorhynchus* spp., *Pratylenchus* spp., *Helicotylenchus* spp.
L 5	*Meloidogyne* spp., *Pratylenchus* spp., *Tylenchorhynchus* spp., *Helicotylenchus* spp.
L 6	*Meloidogyne* spp., *Pratylenchus* spp., *Helicotylenchus* spp., *Tylenchorhynchus* spp.
L 7	*Meloidogyne* spp., *Pratylenchus* spp., *Helicotylenchus* spp., *Tylenchorhynchus* spp.
L 8	*Meloidogyne* spp., *Helicotylenchus* spp., *Tylenchorhynchus* spp., *Pratylenchus* spp.
L 9	*Meloidogyne* spp., *Helicotylenchus* spp., *Tylenchorhynchus* spp., *Pratylenchus* spp.

Table 4.4: Survey of okra growing areas of Jaipur district to know association of various nematodes

S. No.	Localities	Nematode population			
		Nematode spp.	Total no. of sample collected	Sample containing nematode	Average Population per sample
1	L_1	*Meloidogyne* spp.	14	14	218
		Helicotylenchus spp.		9	9
		Pratylenchus spp.		9	10
		Tylenchorhynchus spp.		7	10
2	L_2	*Meloidogyne* spp.	12	12	309
		Helicotylenchus spp.		8	15
		Pratylenchus spp.		9	15
		Tylenchorhynchus spp.		5	12
3	L_3	*Meloidogyne* spp.	16	15	305
		Helicotylenchus spp.		10	13

		Pratylenchus spp.		8	13
		Tylenchorhynchus spp.		8	22
4	L_4	*Meloidogyne* spp.	15	15	318
		Helicotylenchus spp.		10	10
		Pratylenchus spp.		10	12
		Tylenchorhynchus spp.		9	17
5	L_5	*Meloidogyne* spp.	9	9	360
		Helicotylenchus spp.		6	14
		Pratylenchus spp.		6	21
		Tylenchorhynchus spp.		5	19
6	L_6	*Meloidogyne* spp.	10	10	355
		Helicotylenchus spp.		6	21
		Pratylenchus spp.		7	25
		Tylenchorhynchus spp.		5	18
7	L_7	*Meloidogyne* spp.	13	12	370
		Helicotylenchus spp.		8	23
		Pratylenchus spp.		10	24
		Tylenchorhynchus spp.		8	9
8	L_8	*Meloidogyne* spp.	8	8	340
		Helicotylenchus spp.		5	25
		Pratylenchus spp.		5	11
		Tylenchorhynchus spp.		5	16
9	L_9	*Meloidogyne* spp.	10	10	300
		Helicotylenchus spp.		4	40
		Pratylenchus spp.		6	16
		Tylenchorhynchus spp.		4	26

Table 5: Community analysis of nematodes (200 cc soil + 5gm roots)

Nematode spp.	Absolute frequency	Absolute density (soil + 5 gm root)	Percent prominence Value (soil + 5 gm root)
Meloidogyne spp.	98.13	12.86	3.82
Helicotylenchus spp.	61.68	0.77	0.18
Pratylenchus spp.	65.42	0.67	0.16
Tylenchorynchus spp.	52.33	0.67	0.14

Discussion

Result of this survey confirmed that okra is very susceptible to root-knot nematode infection. Wherever the okra crop, grown in Jaipur, root-knot nematode infects the crop. Similar results were also reported by Esfahani (2009) [2] that the *Meloidogyne incognita* and *M. javanica* both species infect tomato in different areas. The species were either found singly or in mixed populations. Out of the two, *M. javanica* was more frequent. It was found in 7 localities out of 8, either, singly or in concomitantly with *M. incognita*. Similarly, Singh *et al.* (2012) [3] found that among the different plant parasitic nematodes, *Meloidogyne* spp. was more frequently encountered in most localities of Jammu and Samba districts. Similarly, Gautam *et al*. (2014) [1] found that the *Meloidogyne* species are economically important pathogens, especially infesting vegetable crops. In the tropics and subtropics, *Meloidogyne incognita* cause an estimated yield loss of 5-43% in vegetable crops. According to, Ralmi *et al.* (2016) [4], the presence of RKN in the crops becomes one of the major problems nowadays because they cause great agriculture loss. Among the many genera of nematodes having some economic impact, *Meloidogyne* spp. is responsible for a large part of the annual 100-billion-dollar losses attributed to nematode damage. According to Hussain *et al.* (2012) [5] the four most common root-knot species, *M. incognita* constituted 74.74%, *M. javanica* 24.02%, *M. arenaria* 1.57% and *M. hapla* 0.78%.

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