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Meloidogyne spp. Root-knot Nematode Distribution and Population Status in Autumn Pulses in Rajasthan's Zone IIIA

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

A survey was conducted to determine the distribution and occurrence of root-knot nematode, *Meloidogyne* spp. and other plant parasitic nematodes associated with Autumn pulses in Zone IIIA of Rajasthan. A total of 387 soil and root samples were collected from 25 different localities of zone IIIA in Rajasthan. Among them *Meloidogyne* spp. was found highest in 354 samples with 91.47%

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absolute frequency, 40.16 absolute density and 3.84 prominence value. *Helicotylench*us spp., *Pratylenchus* spp., *Hoplolaimus* spp. *etc*., were also reported in surveyed localities. During the survey mung bean plants were found diseased with Cercospora leaf spot and anthracnose.

Keywords: Root-knot nematode; Meloidogyne spp.; plant parasitic nematodes; cropping system.

1. INTRODUCTION

The pulses form an integral part of the cropping system of farmers all over the country because these crops fit well in the crop rotation and occupy an important position in the human diet, due to being a good source of vegetable protein (17-43%) and a supplement to a cereal based diet. India is one of the largest producers and consumers of pulses in the world. Pulses account for around 20 percent of the area under foodgrain and contribute around 7-10 percent of the total foodgrain production in the country. Pigeon pea (tur or arhar), chickpea (gram), mung bean, urd (black gram), masur (lentil), pea and various types of beans are major pulses grown in India. Mung bean is an excellent source of low cost and high-quality protein" [1]. The nutritional value in mung bean per 100 gram is protein (2.04 g), fat (0.18 g), carbohydrate (5.9 g), dietary fiber (1.8 g), sugar (4.13 g), calcium (13 mg), iron (0.92 mg) and phosphorus (54 mg) [2]. It is a source of high quality protein for human consumption and serves fodder or green manure. All the pulses have the ability to fix nitrogen by association with rhizobium at 58-109 kg/ha [3]. The crop is believed to be a native of India and Central Asia, but is now widely grown in Southeast Asia, Africa, South America and Australia. In India, it is grown in almost all parts of the country, such as, Madhya Pradesh, Rajasthan, Maharashtra, Karnataka, Uttar Pradesh, Andhra Pradesh, Gujarat, Tamil Nadu, Jharkhand, Odisha, Chhattisgarh, Telangana, Bihar and West Bengal.

Various factors have been recognized for the low yield of pulses, including are its poor quality of seed, incidence of disease and pests including nematode and adverse climatic conditions. Among all the stages of the mung bean crop, right from germination to maturity, is attacked by a large spectrum of diseases caused by fungi, bacteria, viruses, and nematodes.

In India, root-knot nematode was first reported by Barber [4] on tea roots from the Devala territory of Kerala state. In India- the root-knot disease caused by *M. incognita* and *M. javanica* on mung beans was first reported by Singh [5]. In Rajasthan, Arya [6] reported root-knot nematode on tomato from Jodhpur.

The nematode problem may be highly destructive to the mung bean crop and is characterized by arial disease symptoms on the infected plant with suppressed growth and yield. The extent of damage caused by root-knot nematode invasion varies with the initial nematode density present in the soil, host, cultural conditions and weather parameters like temperature, moisture *etc. Meloidogyne javanica* may complete its biological cycle in about 30-34 days when soil temperatures range from 25–30°C [7]. Location and region having differences in soil conditions and environmental variations may influence the infectivity and losses caused by root-knot nematodes in pulse crops, mainly in mung bean.

2. MATERIALS AND METHODS

2.1 Survey

A systematic survey was carried out to determine the percent occurrence and distribution of root-knot nematodes, *Meloidogyne* spp. and other plant parasitic nematodes in Autumn pulses from 25 localities in zone IIIA of Rajasthan. A total of 390 soil and root samples were collected from different pulse fields. Samples were collected randomly from fields using khurpi. Collected soil and root samples were processed in the and counted for the presence of a nematode population in 200 cc soil and 5 g roots. Calculate the occurrence, absolute frequency, absolute density, and prominence value with the help of the formula.

2.2 Soil Sampling

Soil samples were collected randomly from the rhizosphere of the plants with the help of khurpi from 4-5 places in each at a depth of 15-20 cm, homogenized, filled in a polythene bag, labeled, and tagged with supporting information, *viz.* date of sampling, locality, host plant. These samples were then tied with the rubber band brought to the laboratory and stored in a refrigerator at about 100 C. The samples were processed within a week.

2.3 Processing of Samples

Initially, 200 cc of soil from each sample was refined by using Cobb's decanting and sieving technique [8], followed by Baermann's funnel technique [9]. After 24-48 hours, suspension was drawn in a beaker from the funnel and kept for some hours to allow the nematode to settle down at the bottom. The supernatant liquid was gently removed to get a concentrated nematode population. The suspensions were made up to 100 ml, bubbled and only 5 ml was drawn with the help of a pipette and poured in a counting disc. An average of three times was taken and then multiples to total suspension counts exercise was taken. The root-knot nematode and other plant parasitic juveniles were counted under a stereoscopic binocular microscope.

2.4 Estimation of Root Population

Infested roots were washed thoroughly and stained with 0.1 percent acid fuchsin lactophenol at 80 ^{o}C for 2-3 minutes [10]. After a gentle wash in tap water, roots were kept in clear lactophenol for at least 24 hours, and then they were examined under a stereoscopic binocular microscope. After staining, the roots were teased out, and the number of root-knot nematodes and other plant parasitic nematodes was recorded under a stereoscopic binocular microscope.

Percent Occurrence of Plant Parasitic Nematodes: The frequency of occurrence percentage of the plant parasitic nematode infection in each locality was calculated by the following formula:

(a) $$\text{Absolute frequency} = \frac{\text{Number of samples containing a species}}{\text{Total no. of samples collected}} \times 100$$

(b) $$\text{Absolute frequency} = \frac{\text{Number of samples containing a species}}{\text{Total no. of samples collected}} \times 100$$

(c) $$\text{Prominence value} = \frac{\text{Absolute density} \times \sqrt{\text{Absolute frequency}}}{100}$$

Occurrence of percentage disease index (PDI): During August to September of the Autumn season 2022-23, surveys on Cercospora leaf spot disease and anthracnose of mung bean were carried out in Zone IIIA of Rajasthan. Five plots were taken to indicate Cercospora leaf spot affected plants. In each plot, two spots of one-meter square quadrant were taken at random and each spot having diseased as well as healthy plants was recorded. Observations will be recorded according to the 12grade scale. The percent disease index will be determined according to Horsfall and Barratt [11].

$$\text{Percent Disease Index (PDI)} = \frac{\text{Sum of all disease rating}}{\text{Total number of rating x Maximum disease grade}} \times 100$$

List 1. Occurrence of percentage disease index

Scale	Frequency
1	0%
2	0-3%
3	3-6%
4	6-12%
5	12-25%
6	25-50%
7	50-75%
8	75-87%
9	87-94%
10	94-97%
11	97-100%
12	100%

3. RESULTS AND DISCUSSION

3.1 Occurrence of Nematode Associated with Autumn pulses in Zone IIIA of Rajasthan

A study was carried out to determine the distribution and occurrence of root-knot nematodes, *Meloidogyne* spp., and other plant parasitic nematodes associated with Autumn pulses in Zone IIIA of Rajasthan. A total of 387 soil and root samples were collected from 25 different localities of zone IIIA of Rajasthan in Autumn season 2022-23 from August to September months. The samples were processed by using Cobb's decanting and sieving technique, followed by Baermann's funnel assembly. The extracted nematodes were examined under a stereoscopic binocular microscope. Observations on the number of root-knot nematodes, *Meloidogyne* spp., and other phytonematodes in 200 cc soil were taken and described in Table 1.

Data presented in Table 1 showed that out of 387 samples, *Meloidogyne* spp. was found highest in 354 samples with 91.47% absolute frequency, 40.16 absolute density and 3.84 prominence value.

The results recorded during the survey showed that pulses is susceptible to the attack of root-knot nematodes. The results showed that the 100% infection of root-knot nematode was recorded in 10 localities, *i.e.*, Sanjhariya, Fatehpura, Begus, Nimeda, Chomu, Akhaipura, Lalsot, Dungarpur, Mandawar and Jobner.

Table 1. Percent occurrence of root-knot nematode and other phyto-nematodes associated with mung bean in Zone IIIA of Rajasthan

No.	Nematode spp.	Total Samples Collected	Samples Containing nematode spp.	Absolute frequency	No. of individuals of a spp.	Absolute Density	Prominence Value
1.	*Meloidogyne* spp.	387	354	91.47	29145	40.16	3.84
2.	*Helicotylenchus* spp.		197	50.90	495	1.22	0.08
3.	*Pratylenchus* spp.		178	45.99	934	2.55	0.17
4.	*Hoplolaimus* spp.		163	42.11	254	0.76	0.04

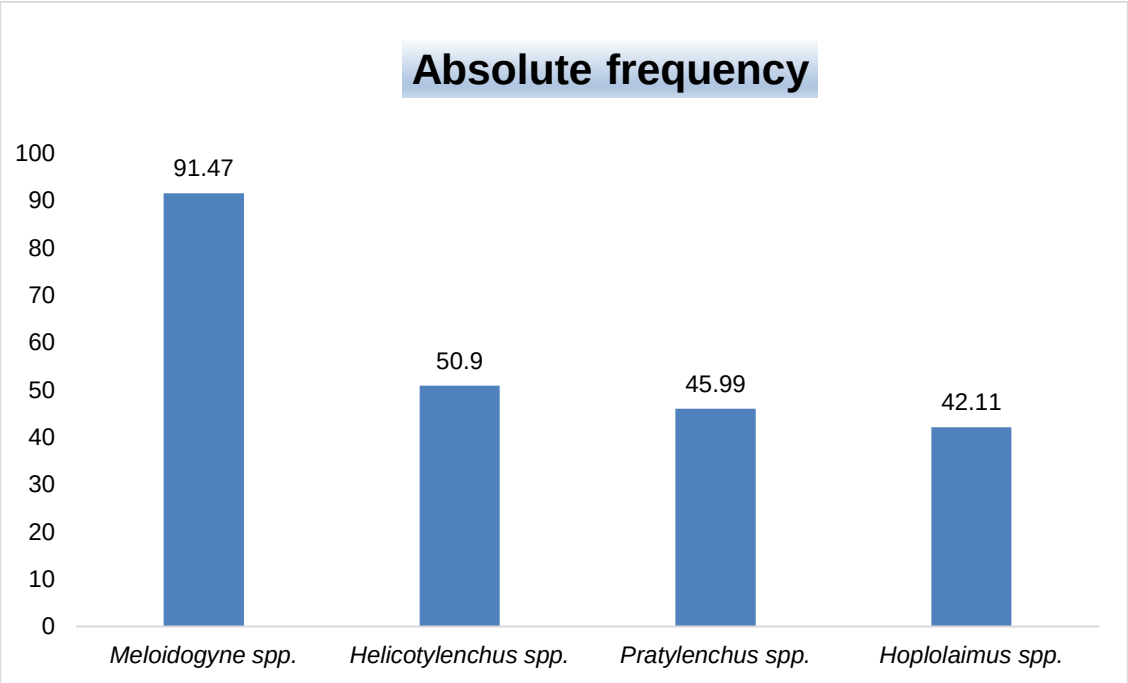

Fig. 1. Absolute frequency of root-knot nematodes and other plant parasite nematodes

Table 2. Distribution and occurrence of root-knot nematode, *Meloidogyne javanica* and other plant parasitic nematodes Zone IIIA of Rajasthan

Locality	Nematode Genera	No. of Samples	Samples Con. Nema. Spp.	Absolute Freq.	No. of Ind. Spp./200 CC soil	no. of ind. Spp./5g roots	Absolute den.	PV
Sanjhariya	*Meloidogyne*	21	21	100	867	432.00	30.17	3.02
	Helicotylenchus	21	6	28.57	35	432.00	0.83	0.04
	Pratylenchus	21	9	42.86	27	19.00	1.07	0.07
	Hoplolaimus	21	7	33.33	17	0.00	0.40	0.02
Fatehpura	*Meloidogyne*	16	16	100.00	811	447.00	25.34	2.53
	Helicotylenchus	16	12	75.00	45	0.00	1.41	0.12
	Pratylenchus	16	9	56.25	28	22.00	0.88	0.06
	Hoplolaimus	16	10	62.50	23	0.00	0.72	0.06
Begus	*Meloidogyne*	20	20	100.00	922	576.00	23.05	2.30
	Helicotylenchus	20	10	50.00	26	0.00	0.65	0.04
	Pratylenchus	20	8	40.00	18	14.00	0.45	0.03
	Hoplolaimus	20	11	55.00	40	0.00	1.00	0.07
Bobas	*Meloidogyne*	18	17	94.44	890	512.00	24.72	2.40
	Helicotylenchus	18	8	44.44	16	0.00	0.44	0.03
	Pratylenchus	18	10	55.56	24	17.00	0.67	0.05
	Hoplolaimus	18	6	33.33	14	0.00	0.39	0.02
Bassi Jhajhra	*Meloidogyne*	12	10	83.33	790	358.00	32.92	3.00
	Helicotylenchus	12	4	33.33	25	0.00	1.04	0.06
	Pratylenchus	12	7	58.33	30	16.00	1.25	0.09
	Hoplolaimus	12	5	41.67	11	0.00	0.46	0.03
Keshyawala	*Meloidogyne*	15	14	93.33	815	350.00	27.17	2.62
	Helicotylenchus	15	9	60.00	13	0.00	0.43	0.03
	Pratylenchus	15	7	46.67	23	26.00	0.77	0.05
	Hoplolaimus	15	6	40.00	9	0.00	0.30	0.02
Nimeda	*Meloidogyne*	14	14	100.00	788	425.00	28.14	2.81
	Helicotylenchus	14	8	57.14	11	0.00	0.39	0.03
	Pratylenchus	14	6	42.86	15	32.00	0.54	0.03
	Hoplolaimus	14	4	28.57	6	0.00	0.21	0.01
Mundiya Ramsar	*Meloidogyne*	16	15	93.75	857	466.00	26.78	2.59
	Helicotylenchus	16	7	43.75	14	0.00	0.44	0.03
	Pratylenchus	16	5	31.25	21	14.00	0.66	0.04
	Hoplolaimus	16	9	56.25	11	0.00	0.34	0.02
Phagi	*Meloidogyne*	22	20	90.91	812	410.00	18.45	1.76
	Helicotylenchus	22	10	45.45	15	0.00	0.34	0.02

Locality	Nematode Genera	No. of Samples	Samples Con. Nema. Spp.	Absolute Freq.	No. of Ind. Spp./200 CC soil	no. of ind. Spp./5g roots	Absolute den.	PV
	Pratylenchus	22	5	22.73	3	9.00	0.07	0.00
	Hoplolaimus	22	4	18.18	7	0.00	0.16	0.00
Doobli	*Meloidogyne*	13	12	92.31	618	345.00	23.77	2.28
	Helicotylenchus	13	5	38.46	14	0.00	0.54	0.03
	Pratylenchus	13	8	61.54	6	18.00	0.23	0.01
	Hoplolaimus	13	4	30.77	2	0.00	0.08	0.00
Chomu	*Meloidogyne*	18	18	100.00	847	457.00	23.53	2.35
	Helicotylenchus	18	11	61.11	8	0.00	0.22	0.01
	Pratylenchus	18	13	72.22	14	12.00	0.39	0.03
	Hoplolaimus	18	9	50.00	6	0.00	0.17	0.01
Bassi	*Meloidogyne*	19	18	94.74	884	568.00	23.26	2.26
	Helicotylenchus	19	11	57.89	12	0.00	0.32	0.02
	Pratylenchus	19	9	47.37	27	39.00	0.71	0.04
	Hoplolaimus	19	4	21.05	13	0.00	0.34	0.01
Siya ka Was	*Meloidogyne*	15	14	93.33	687	356.00	22.90	2.21
	Helicotylenchus	15	8	53.33	22	0.00	0.73	0.05
	Pratylenchus	15	12	80.00	16	38.00	0.53	0.04
	Hoplolaimus	15	10	66.67	9	0.00	0.30	0.02
Akhaipura	*Meloidogyne*	16	16	100.00	786	467.00	24.56	2.45
	Helicotylenchus	16	10	62.50	26	0.00	0.81	0.06
	Pratylenchus	16	5	31.25	15	28.00	0.47	0.02
	Hoplolaimus	16	9	56.25	5	0.00	0.16	0.01
Charanwas	*Meloidogyne*	14	12	85.71	766	348.00	27.36	2.53
	Helicotylenchus	14	7	50.00	21	0.00	0.75	0.05
	Pratylenchus	14	9	64.29	22	32.00	0.79	0.06
	Hoplolaimus	14	6	42.86	5	0.00	0.18	0.01
Malpura	*Meloidogyne*	10	4	40.00	486	208.00	24.30	1.53
	Helicotylenchus	10	3	30.00	21	0.00	1.05	0.05
	Pratylenchus	10	2	20.00	5	14.00	0.25	0.01
	Hoplolaimus	10	1	10.00	2	0.00	0.10	0.00
Diggi	*Meloidogyne*	10	3	30.00	360	150.00	18.00	0.98
	Helicotylenchus	10	2	20.00	11	0.00	0.55	0.02
	Pratylenchus	10	2	20.00	8	20.00	0.40	0.02
	Hoplolaimus	10	1	10.00	5	0.00	0.25	0.01
Avikanagar	*Meloidogyne*	14	9	64.29	634	389.00	22.64	1.81
	Helicotylenchus	14	7	50.00	16	0.00	0.57	0.04

Locality	Nematode Genera	No. of Samples	Samples Con. Nema. Spp.	Absolute Freq.	No. of Ind. Spp./200 CC soil	no. of ind. Spp./5g roots	Absolute den.	PV
	Pratylenchus	14	6	42.86	14	26.00	0.50	0.03
	Hoplolaimus	14	5	35.71	2	0.00	0.07	0.00
Lalsot	*Meloidogyne*	16	16	100.00	753	345.00	23.53	2.35
	Helicotylenchus	16	8	50.00	14	0.00	0.44	0.03
	Pratylenchus	16	6	37.50	11	5.00	0.34	0.02
	Hoplolaimus	16	9	56.25	9	0.00	0.28	0.02
Ramgarh – Pacchwara	*Meloidogyne*	12	11	91.67	588	298.00	24.50	2.34
	Helicotylenchus	12	9	75.00	13	0.00	0.54	0.05
	Pratylenchus	12	7	58.33	9	4.00	0.38	0.03
	Hoplolaimus	12	8	66.67	3	0.00	0.13	0.01
Baswa	*Meloidogyne*	14	13	92.86	754	467.00	26.93	2.59
	Helicotylenchus	14	8	57.14	30	0.00	1.07	0.08
	Pratylenchus	14	6	42.86	5	24.00	0.18	0.01
	Hoplolaimus	14	8	57.14	11	0.00	0.39	0.03
Dungarpur	*Meloidogyne*	18	18	100.00	879	516.00	24.42	2.44
	Helicotylenchus	18	6	33.33	17	0.00	0.47	0.03
	Pratylenchus	18	10	55.56	25	13.00	0.69	0.05
	Hoplolaimus	18	13	72.22	13	0.00	0.36	0.03
Mandawar	*Meloidogyne*	11	11	100.00	689	300.00	31.32	3.13
	Helicotylenchus	11	7	63.64	23	0.00	1.05	0.08
	Pratylenchus	11	4	36.36	19	30.00	0.86	0.05
	Hoplolaimus	11	2	18.18	3	0.00	0.14	0.00
Jobner	*Meloidogyne*	16	16	100.00	789	466.00	24.66	2.46
	Helicotylenchus	16	11	68.75	21	0.00	0.66	0.05
	Pratylenchus	16	7	43.75	13	23.00	0.41	0.03
	Hoplolaimus	16	3	18.75	15	0.00	0.47	0.02
Durgapura	*Meloidogyne*	17	16	94.12	851	578.00	25.03	2.43
	Helicotylenchus	17	10	58.82	26	0.00	0.76	0.06
	Pratylenchus	17	6	35.29	18	32.00	0.53	0.03
	Hoplolaimus	17	9	52.94	10	0.00	0.29	0.02

Table 3. Per cent occurrence of cercospora leaf spot and anthracnose disease associated with mung bean in Zone IIIA of Rajasthan

S.N.	District	Village	Cercospora leaf spot PDI (%)	Anthracnose PDI (%)
1.	Jaipur	Sanjhariya	10.02	8.26
2.	Jaipur	Fatehpura	16.50	9.02
3.	Jaipur	Begus	10.09	6.32
4.	Jaipur	Bobas	11.26	10.62
5.	Jaipur	Bassi Jhajhra	9.36	10.32
6.	Jaipur	Keshyawalya	7.10	6.01
7.	Jaipur	Nimeda	22.23	17.52
8.	Jaipur	Mundiya Ramsar	19.56	12.32
9.	Jaipur	Phagi	29.33	15.11
10.	Jaipur	Doobli	1.02	0.00
11.	Jaipur	Chomu	0.00	0.00
12.	Jaipur	Bassi	10.32	10.22
13.	Jaipur	Siya Ka Was	21.65	11.32
14.	Jaipur	Akhaipura	0.00	0.00
15.	Jaipur	Charanwas	10.03	9.01
16.	Tonk	Malpura	21.02	30.32
17.	Tonk	Diggi	37.71	29.66
18.	Tonk	Avikanagar	25.31	23.07
19.	Dausa	Lalsot	19.35	11.74
20.	Dausa	Ramgarh Pacchwara	5.02	6.1
21.	Dausa	Baswa	8.36	9.15
22.	Dausa	Dungarpur	10.02	2.81
23.	Dausa	Mandawar	12.31	10.37
24.	Jaipur	Jobner	5.01	8.06
25.	Jaipur	Durgapura	18.6	17.68
Average			13.64	11.00

3.2 Occurrence of Disease Associated with Mung Bean Other Than Nematode Disease in zone IIIA of Rajasthan

Total 25 mung bean-growing villages surveyed zone IIIA of Rajasthan to record the occurrence of different diseases during the Autumn season of 2022. The villages were surveyed from the flowering and pod-forming stages of the mung bean crop. The data indicated in Table 3 revealed that the Cercospora leaf spot intensity ranged from 0.00 to 37.71 percent with an average of 13.64 percent during August to September 2022. While anthracnose intensity ranged from 0.00 to 30.02 percent with an average of 11.00 percent. The maximum Cercospora leaf spot intensity was observed at Diggi (37.71%), followed by Phagi (29.33%), Avikanagar (25.31%), Siya ka Was (21.65%), and Malpura (21.02%). Whereas, maximum anthracnose intensity in mung bean was found in Malpura (30.32%). followed by Diggi (29.66%) and Avikanagar (23.07%).

The results of the present investigation were similar to those of Mishra and Chakrabarti [12], who reported the association of root-knot nematodes, cyst nematodes, lesion nematodes, reniform nematodes, and various ectoparasitic nematode groups with all the pulse crops in pulse-producing areas of India. Olowe [13] surveyed cowpea areas in Nigeria and showed that *Meloidogyne incognita* (51.8%) was the most prevalent nematode, followed by *Meloidogyne javanica* (44.1%) and *Meloidogyne arenaria* (4.1%), respectively. Amponsah et al. [14] investigated the distribution of plant parasitic nematodes associated with peanut, cowpea and soybean in northern Ghana. Ten genera of plant parasitic nematodes were encountered in the three sites and host crops. These were *Helicotylenchus*, *Heterodera*, *Hoplolaimus*, *Longidorus*, *Meloidogyne*, *Pratylenchus*, *Rotylenchus*, *Scutellonema*, *Tylenchorhynchus* and *Xiphinema*. Soil and root samples of cowpea growing areas from Churu, Jhunjhunu, Udaipur, Rajsamand and Chittorgarh districts of Rajasthan showed high prominence values of *Meloidogyne* and *Rotylenchulus reniformis* and *Tylenchorhynchus* spp. [15]. Whereas, thirteen nematode genera (*Hoplolaimus, Tylenchorhynchus, Helicotylenchus, Tylenchus, Heterodera cajani, Pratylenchus, Basiria, Aphelenchus, Meloidogyne, Filenchus, Boleodorus, Rotylenchulus* and *Scutellonema*)

are associated with pigeon pea from the Bundelkhand region of Uttar Pradesh (India) [16]. However, infection of root-knot nematodes in tomatoes showed in all surveyed localities in and around the Sanganer Tehsils, Jaipur (Rajasthan) [17]. Manandhar et al. [18] collected 211 soil samples in different crop fields; among them, 137 samples were diagnosed for different plant parasitic nematodes (*Meloidogyne*, *Helicotylenchus*, *Pratylenchus*, *Tylenchus*, *Tylenchorhynchus*, *Hoplolaimus*, *Belonolaimus*, *Criconemoides*, *Aphelenchoides, Hirschmanniella*, *Longidorus* and *Rotylenchulus*). In the present study also, root-knot nematode (*Meloidogyne* spp.) was found to be the prominent economically important plant parasitic nematode genera above the economic threshold level affecting mung bean, around zone IIIA of Rajasthan. This might be due to favorable environmental conditions of this area, availability of light soil, host suitability, sowing time of crop, *etc*.

4. CONCLUSION

In the present study, root-knot nematode (*Meloidogyne javanica*) was found to be the only prominent economically important plant parasitic nematode genera and polyphagous nature. A survey showed that pulses is susceptible to the attack of root-knot nematode. The root-knot nematodes, *Meloidogyne* spp., is a major key pest in Rajasthan. Root-knot nematode *Meloidogyne javanica* is a major nematode pest in a Autumn pulse in zone IIIA of Rajasthan. During the survey, mung bean plants were also diseased with Cercospora leaf spot and anthracnose.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

I hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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