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Occurrence and Population Status of Citrus Nematode on Lemon in Udaipur, Rajasthan

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ABSTRACT: *Citrus limon* (L.) is a most important fruit crop of India belongs to the Family-Rutaceae. Citrus is the third largest fruit crop which is grown commercially inlarge areas of Rajasthan, Maharashtra, Andhra Pradesh, Karnataka, West Bengal and Assam in arid & semi-arid regions to humid tropical regions of India. Citrus is affected by number of insect pests and pathogens including plant parasitic nematodes. Among nematode, *Tylenchulus semipenetrans* is most economically damaging the all citrus crops. Therefore, to ascertain the prevalence and population analysis of the citrus nematode, *Tylenchulus semipenetrans*, a survey was conducted in the Udaipur district of Rajasthan from July to December 2022. A total of 350 soil and root samples were collected from lemon orchards and farmer's field from Udaipur district. In surveyed areas, out of twenty six localities, incidence of citrus nematode, *T. semipenetrans* was recorded from 25 localities (RCA farm, Kaya, Ramarate, Ramabaori, Kherwarakhalsa, Vallabhnagar, Kewdakhurd, Sakariya, Nandeshma, Paaner, Gogunda, Bansiwara, Rohimala, Biroti, Khakhar, Kolar, Bhakoomba, Phalasiya, Kheroda, Bagar, Amarpura, Gumanpura, Jawanjika Kheda, Gadariyawas, Seriya). Observation *viz.*, number of females/5 g roots, number of egg masses/5 g roots and nematode population/200cc soil were recorded. At the time of survey, GPS coordinates (longitude and latitude) were also taken to determinate the geographic location on maps of different localities where samples were collected. Occurrence of *T. semipenetrans* was obtained 71.92% with an average population of 86.40 females/5 g roots, 53.27 egg masses/5 g roots and 588.46 juveniles/ 200 cc soil. Extreme infestation of citrus nematode was marked from Gogunda followed by RCA farm and Gumanpura with nematode population 1355.00, 1050.00 and 965.00 per 200 cc soil, respectively. However, only one locality (Parda) where it was not seen.

Keywords: Survey, Lemon, Citrus nematode, *Tylenchulus semipenetrans*.

INTRODUCTION

Citrus limon (L.) is a tree with evergreen leaves and yellow edible fruits belong to the Family-Rutaceae. It is also known as lemon (English), Le citron (French), Zitrone (German), limón (Spanish) and níngméng (Chinese).

Citrus is the third largest fruit crop which is grown commercially in large areas of Rajasthan, Maharashtra, Andhra Pradesh, Karnataka, West Bengal, Sikkim, Punjab and Assam in arid & semi-arid regions to humid tropical regions of India. Presently, area under citrus is 1.09 mha with a production of 14.50 million tonnes and average productivity of 11.80 tonnes/ha in India (Agristat, 2020). The most important commercial citrus species in India are the mandarin orange (*C. reticulata*), sweet orange (*C. sinensis*), acid lime (*C. aurantifolia*) & lemon (*C. limon*) sharing production 28.36, 44.29 and 27.34 percent, respectively. In Rajasthan, lime & lemon occupy an area of 2.69 thousand ha with 14.47 thousand MT production and productivity of 5.66 tonnes/ha (Horticulture Statistics at a Glance, 2018). Nutritional facts of citrus per 100 g is Calories 60, Fat N/A, Sodium N/A, Fiber 3 g, Sugar 12 g, Protein 1g, Vitamin A 14 mcg and Vitamin C 70 mcg. In *C. limon* seed oil, the main ingredients are fatty acids, such as arachidonic acid, behenic acid and linoleic acid and also tocopherols and carotenoids (Malacrida *et al*., 2012; Mucci *et al*., 2013). The main components of the *C. limon* essential oil are monoterpenoids. In addition to terpenoids, the essential oil also contains linear furanocoumarins (psoralens) and polymethoxylated flavones (Russo *et al*., 2015; Kaskoos, 2019; González-Molina *et al*., 2010). Analysis of macro elements in *C. limon* fruit showed the presence in pulp and peel of calcium, magnesium, phosphorus, potassium and sodium (Czech *et al*., 2020).

Lemon fruit stands out as having well-known nutritional properties, its valuable biological activities are underestimated in modern phytotherapy and cosmetology (Goetz, 2014). It is extensively used in the food, pharmacy and cosmetics industries etc. (Abad-García *et al.*, 2012; García-Salas *et al.*, 2013). Lemon

juice has traditionally been used as a remedy for scurvy disease of human system.

An alarming situation in citrus cultivation has developed due to dwindling of the area under citrus as it is of late threatened with the serious disease commonly known as "Citrus decline". It is well known that citrus decline is a complex disease. Citrus crop is attacked by number of insect pests (Citrus aphid, Lemon butterfly, Citrus psylla, mealy bug, leaf minor and fruit sucking moth) and pathogens (Citruscanker, scab, citrus tristeza disease, greening, anthracnose and sooty mould) including nematodes. Among nematodes, *Tylenchulus semipenetrans* Cobb, 1913 is one of the most destructive nematode species associated with citrus (Fig. 1) and is known to occur in all the citrus growing areas of the world (Thorne, 1961; Nasir *et al*., 2021). The citrus nematode was first discovered in California by Cobb (1913) and placed it in a new genus and named this species *Tylenchulus semipenetrans* on the basis of position in the root. This nematode is present in about 92 per cent of Southern California citrus orchard and is believed to be one of the important causal factors of the citrus replant problem (Thomas, 1913; Baines and Clark 1952; Abd-Elgawad and Mahfouz 2020). A significant yield drop of 20-30% is caused by the citrus nematode (Philis, 1989; Kumar *et al*., 2020). In India, the first report on the presence of *T. semipenetrans* was made by Siddiqi (1961). It is now known to be widely distributed in the country including Punjab, Delhi, Rajasthan, Uttar Pradesh, Maharashtra, Assam, Orissa and Kerala and causing great damage directly or indirectly in citrus (Swarup *et al*., 1964; Chona *et al*., 1965). It can be manage by eco-friendly management options may be employed in place of pesticides against citrus nematode, *T. semipenetrans* on lemon to promote organic and economical cultivation of lemon and INM (Mukesh Jaiman *et al*., 2024).

MATERIAL AND METHOD

The agro-climatic condition of Rajasthan is suitable for the growing Lemon. The citrus nematode, *Tylenchulus semipenetrans* has been found more frequently associated with lemon (*Citrus limon*) and which results for severe economic losses. A survey was carried out in Udaipur district to find out occurrence and population status of citrus nematode on lemon. Soil and root samples were collected from surveyed areas and brought to the laboratory for processing. Two hundred centimetre cubic soil were processed for estimation of nematode juveniles by Cobb's sieving and decantation method followed by Baermann's funnel technique. Roots were be stained using 0.1 % acid fuchsine lacto phenol solution, rinsed in water to remove excess amount of stain and keep in clear lacto phenol at least for 24 hours before examination (Mc Beth *et al*., 1941) and different observations were taken *viz*., number of females/5 g roots and number of egg masses/5 g roots and nematode population/200 cc soil etc. At the time of survey, GPS coordinates (longitude and latitude) were also taken to determinate the geographic location on maps of different localities where samples were collected (Table 1).

A. Occurrence of citrus nematode:

The per cent occurrence of *T. semipenetrans* was calculated by under given formula

$$\% \text{ Occurrence} = \frac{\text{Number of samples}}{\text{Total number of samples collected}} \times 100$$

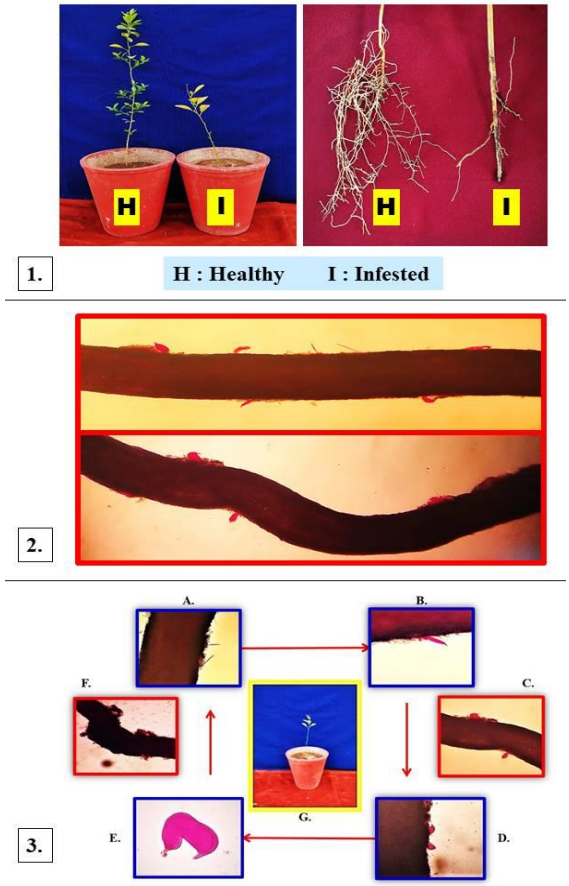

Fig. 1: (1) Healthy and Citrus nematode infested lemon; (2) Severe infection of *Tylenchulus semipenetrans* on lemon roots (3) Life-Cycle of Citrus nematode, *Tylenchulus semipenetrans* on Lemon. (3A: Establishment of feeding site by second stage juveniles (J2) of *Tylenchulus semipenetrans*; 3B: Third stage juvenile; 3C: Pre mature female and fourth stage juvenile of citrus nematode; 3D&E: Mature female buried their mouth in roots and separate embedded female from infected root with damaged mouth parts due to make a permanent feeding site; 3F: Soil dirt particles clinging the roots due to gelatinous matrix which is secreted by female of *T. semipenetrans*)

Table 1: Name of localities and GPS coordinates of surveyed citrus growing areas in and around Udaipur, Rajasthan.

Sr. No.	Name of Locality		Locality No.	Latitude	Longitude
	Tehsil	Locality			
1.	Girwa	RCA farm	L1	24.574929	73.706398
		Kaya	L2	24.441485	73.667985
		Ramarate	L3	24.409972	73.625660
2.	Rishabdev	Ramabaori	L4	24.078730	73.691182
3.	Kherwara	Kherwarakhalsa	L5	24.005565	73.597942
4.	Vallabhnagar	Vallabhnagar	L6	24.672116	74.004934
5.	Jaisamand	Kewdakhurd	L7	24.417359	73.768012
6.	Sayra	Sakariya	L8	24.985987	73.414292
		Nandeshma	L9	24.830324	73.465970
7.	Gogunda	Paaner	L10	24.860778	73.576067
		Gogunda	L11	24.757379	73.525819
8.	Jhadol	Bansiwara	L12	24.395659	73.324585
		Rohimala	L13	24.395635	73.324586
		Biroti	L14	24.377439	73.306608
		Khakhar	L15	24.384121	73.435809
		Kolar	L16	24.121020	73.861014
		Parda	L17	24.394702	73.340874
		Bhakoomba	L18	24.352995	73.310005
		Phalasiya	L19	24.236542	73.413527
9.	Bhinder	Kheroda	L20	24.570318	74.056119
		Bagar	L21	24.558071	74.043474
		Amarpura	L22	24.575773	74.104083
		Gumanpura	L23	24.685941	74.011471
10.	Mavli	Jawanjika Kheda	L24	24.780504	73.968271
		Gadariyawas	L25	24.798189	73.971926
11.	Salumber	Seriya	L26	24.138949	74.052785

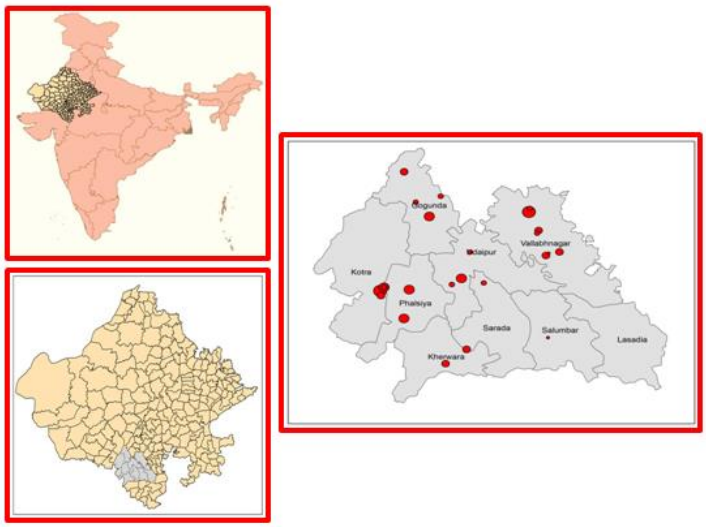

Fig. 2. GPS locations of surveyed citrus growing areas in and around Udaipur to find out population status of Citrus nematode on lemon.

RESULT

To ascertain the prevalence and population analysis of the citrus nematode, *Tylenchulus semipenetrans*, a survey was conducted in the Udaipur district of Rajasthan from July to December 2022. A total of three hundred fifty soil and roots samples were obtained from citrus groves. Soil and roots samples collected, brought to the laboratory and stored them in the refrigerator till processing. After processing the samples using Cobb's sieving and decanting method followed by Baermann's funnel assembly, the samples were checked for citrus nematode presence using a stereo binocular microscope. Results expressed that the occurrence of *T. semipenetrans* was observed 71.92 per cent from Udaipur district with an average population of 86.40 females per 5 g of roots, 53.27 egg masses per 5 g of roots and588.46 Nematode per 200 cc soil. Observations on females per 5 g of roots, egg masses per 5 g of roots and nematode population per 200 cc

soil were recorded and presented in Table 2 with occurrence per cent and illustrated through Fig. 2 and 3.

1. Girwa. In the Girwa tehsil, three localities were surveyed, which were RCA farm, Kaya and Ramarate. Number of soil and root samples collected from these localities was 20, 10 and 20, respectively. Among them *Tylenchulus semipenetrans* was recorded at 100%, 80% and 60% occurrence, respectively. Nematode population *viz.*, number of females per 5 g roots was 110.25, 96.20 and 56.00 and number of egg masses per 5 g roots was 76.15, 65.90 and 32.00 and number of nematodes per 200 cc soil was 1050.00, 355.00 and 475.00, respectively from each locality. Highest number of females, egg masses per 5 g roots and nematodes population per 200 cc soil were found in RCA farm within Girwa tehsil with a number of 110.25, 76.15 and 1050.00 with 100% frequency of occurrence.

2. Rishabdev. Ramabaori, a village in the Rishabdev tehsil, was the localities that underwent survey. Ten soil and root samples were taken from the area. *Tylenchulus semipenetrans* was found in 80% of them. In number, 100.20 females and 72.70 egg masses were found in 5 g of roots. While, the nematode population per 200 cc soil were 400.00.

3. Kherwara. Kherwarakhalsa, a locality in the Kherwara tehsil, was the one that underwent a survey. A total of ten samples of the soil and roots were taken. A 60% occurrence of the citrus nematode was noted among them. There were 69.00 females and 38.20 egg masses per 5 g of roots and the number of nematode in 200 cc soil were 425.00.

4. Vallabhnagar. In the tehsil of Vallabhnagar samples were collected from nearby areas. Ten soil and root samples were collected from the locality. Among them citrus nematode (*Tylenchulus semipenetrans*) was recorded at 70% occurrence. Number of females per 5 g roots was 80.10 and egg masses per 5 g of roots were 50.50 and nematodes per 200 cc soil was 670.00.

5. Jaisamand. Kewdakhurd, a hamlet in the Jaisamand tehsil, was surveyed. Ten samples of the soil and root system were taken. 70 percent of them were found infested with the citrus nematode *Tylenchulus semipenetrans*. The ratio of females to 5 g of roots was 76.35. The egg masses per 5 g of roots were 42.40, while the nematode population per 200 cc of soil was 455.00.

6. Sayra. Sakariya and Nandeshma, two settlements in the Sayra tehsil, were surveyed. A total of 20 and 10 samples of soil and roots were taken from each location, respectively. *Tylenchulus semipenetrans* was found with the occurrence of 80% and 90% of them, respectively. There were 102.00 and 114.30 females per 5 g of roots, respectively and the egg masses per 5 g of roots were 68.00 and 73.10, respectively, while the larval densities per 200 cc of soil were 405.00 and 545.00 from each site. Nandeshma had the highest female populations, egg masses per 5 g roots, and nematode population per 200 cc soil.

7. Gogunda. In the Gogunda tehsil, two localities were surveyed, which were Paaner and Gogunda. Ten soil and root samples were collected from each locality. Out of them citrus nematode (*Tylenchulus semipenetrans*) was recorded at 90% and 80% occurrence, respectively. Number of females per 5 g roots was 98.60 and 146.70, respectively. Number of egg masses per 5 g roots was 54.90 and 82.40 and nematode population per 200 cc soil was 730.00 and 1355.00 from each locality. Highest females, egg masses per 5 g roots and nematode population per 200 cc soil were found in Gogunda.

8. Jhadol. Eight localities were surveyed in Jhadol tehsil, which were Bansiwara, Rohimala, Biroti, Khakhar, Kolar, Parda, Bhakoomba, and Phalasiya. Numbers of soil and root samples collected from each locality were 20, 10, 20, 10, 10, 10, 10 and 10, respectively. Out of them citrus nematode was recorded at 80%, 70%, 60%, 70%, 50%, 0%, 80% and 70% occurrence, respectively. Number of females / 5g roots was 79.20, 78.80, 98.50, 99.30, 56.40, 0, 110.00 and 67.60, respectively. Egg masses/5g of roots were 41.65, 39.30, 68.80, 65.30, 28.60, 0, 72.00 and 34.20, respectively and nematode population/200 cc soil were 450.00, 375.00, 800.00, 530.00, 295.00, 0, 860.00 and 645.00 from each locality. Highest females, egg masses/ 5g roots and nematode population/ 200 cc soil were found in Bhakoomba.

9. Bhinder. In the tehsil of Bhinder four localities were surveyed, which were Kheroda, Bagar, Amarpura and Gumanpura. Number of soil and root samples collected was 20, 10, 10 and 10 from each locality. Out of them 85%, 80%, 60% and 80% samples were infested with the citrus nematode (*Tylenchulus semipenetrans*), respectively. Number of females (108.60, 78.30, 95.00 and 116.50) and egg masses (70.35, 44.70, 60.00 and 78.00) per 5 g of roots were, respectively and nematode population per 200 cc soil (925.00, 510.00, 745.00 and 965.00) was recorded from each locality. Highest females, egg masses per 5 g roots and nematode population per 200 cc soil were found in Gumanpura.

10. Mavli. Jawanjika Kheda and Gadariya was were the two locations in the Mavli tehsil that were surveyed. 20 and 10 samples of soil and roots, respectively, were taken from each location. Citrus nematode was found in 65% and 100% of them, respectively. There were 85.20 and 86.70 females per 5 g of roots, respectively. The egg masses per 5 g of roots from each site were 62.30 and 48.20, respectively, while the nematode population per 200 cc soil was 955.00 and 280.00. Jawanjika Kheda had the highest number of females, egg masses per 5 g roots, and nematode population per cc soil.

11. Salumber. In the tehsil of Salumber, one locality was surveyed, which was Seriya. Number of soil and root samples collected was twenty from the locality. Out of them citrus nematode (*Tylenchulus semipenetrans*) was recorded at 60% occurrence. Number of females (36.60) and egg masses (15.40) per 5 g of roots were recorded and nematode population per 200 cc soil were 100.00.

Table 2: Frequency of occurrence of citrus nematode, *Tylenchulus semipenetrans* and population status of citrus nematode, *Tylenchulus semipenetrans* on lemon in and around Udaipur, Rajasthan.

Locality No.	No. of Sample collected	No. of sample containing *T. semipenetrans*	%Frequency of Occurrence	Mean population of nematodes			Range of nematodes density		
				No. of Females/5g roots	No. of Egg masses/5g roots	No. of Nematodes /200cc soil	Females /5g roots	Egg masses/5g roots	Nematodes/200cc soil
L1	20	20	100	110.25	76.15	1050.00	21-168	13-117	335-1785
L2	10	8	80	96.20	65.90	355.00	0-184	0-125	0-640
L3	20	12	60	56.00	32.00	475.00	0-108	0-65	0-925
L4	10	8	80	100.20	72.70	400.00	0-169	0-109	0-875
L5	10	6	60	69.00	38.20	425.00	0-131	0-88	0-1010
L6	10	7	70	80.10	50.50	670.00	0-144	0-92	0-1380
L7	20	14	70	76.35	42.40	455.00	0-160	0-87	0-905
L8	20	16	80	102.00	68.00	405.00	0-183	0-100	0-700
L9	10	9	90	114.30	73.10	545.00	0-146	0-89	0-940
L10	10	9	90	98.60	54.90	730.00	0-129	0-75	0-1305
L11	10	8	80	146.70	82.40	1355.00	0-188	0-126	0-1995
L12	20	16	80	79.20	41.65	450.00	0-153	0-69	0-850
L13	10	7	70	78.80	39.30	375.00	0-136	0-84	0-755
L14	20	12	60	98.50	68.80	800.00	0-174	0-109	0-1330
L15	10	7	70	99.30	65.30	530.00	0-185	0-111	0-1035
L16	10	5	50	56.40	28.60	295.00	0-126	0-74	0-710
L17	10	0	0	0	0	0	0	0	0
L18	10	8	80	110.00	72.00	860.00	0-159	0-97	0-1575
L19	10	7	70	67.60	34.20	645.00	0-99	0-62	0-1130
L20	20	17	85	108.60	70.35	925.00	0-129	0-102	0-1950
L21	10	8	80	78.30	44.70	510.00	0-121	0-73	0-800
L22	10	6	60	95.00	60.00	745.00	0-158	0-102	0-1370
L23	10	8	80	116.50	78.00	965.00	0-183	0-134	0-2055
L24	20	13	65	85.20	62.30	955.00	0-178	0-101	0-1210
L25	10	10	100	86.70	48.20	280.00	19-118	5-86	25-435
L26	20	12	60	36.60	15.40	100.00	0-64	0-38	0-220
Total	**350.00**	**253.00**	**71.92**	**2246.40**	**1385.05**	**15300.00**	-	-	-
Mean	-	-	-	**86.40**	**53.27**	**588.46**	-	-	-

DISCUSSION

Citrus nematode, *Tylenchulus semipenetrans* was first reported from California citrus orchards in United States of America by Cobb (1913). Later in 1961 its occurrence was reported in India in Aligarh, Uttar Pradesh. However, in India very little work has been done under survey. To fill this gap, survey was undertaken during the July, 2022 to December, 2022 in and around Udaipur district of Rajasthan. During survey, three hundred and fifty soil and root samples were collected from Udaipur (RCA farm, Kaya, Ramarate, Ramabaori, Kherwarakhalsa, Vallabhnagar, Kewdakhurd, Sakariya, Nandeshma, Paaner, Gogunda, Bansiwara, Rohimala, Biroti, Khakhar, Kolar, Parda, Bhakoomba, Phalasiya, Kheroda, Bagar, Amarpura, Gumanpura, JawanjikaKheda, Gadariyawas, Seriya) district of Rajasthan. Results expressed occurrence of *T. semipenetrans* 71.92 per cent from surveyed area of Udaipur district. Highest occurrence was observed in RCA & Gadariya was (100%) followed by Nandeshma & Paaner (90%) and Kheroda (85%). Maximum population of females per 5 g roots were recorded from Gogunda (146.70) followed by Gumanpura (116.50) and Nandeshma (114.30). In case of egg masses per 5 g roots, highest egg masses were found from Gogunda (82.40) while 78.00 & 76.15 egg masses per 5 g roots were observed from Gumanpura and RCA, respectively. Further, Nematode per 200 cc soil were found 1355.00, 1050.00 & 965.00 in descending order from Gogunda, RCA farm and Gumanpura, respectively.

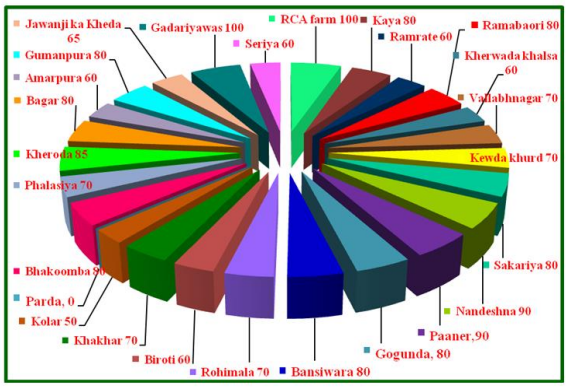

Fig. 3. Frequency of Occurrence of citrus nematode, *Tylenchulus semipenetrans* on lemon in and around Udaipur.

In Parda village, citrus nematode was not noticed. Overall highest nematode infection was found in Gogunda followed by RCA and Gumanpura. The results of present investigation are in accordance with the findings of earlier workers who reported its wide distribution and varied population from India as well as in abroad. Khanzada *et al.* (2008) recorded the incidence of *T. semipenetrans* in all citrus varieties varied from 56% for mandarin followed by 40% for lemon & orange and 35% for kinnow & musambi. Bakr *et al*. (2011) carried out a survey to know the occurrence of *T. semipenetrans* in Egypt and results revealed occurrence of *T. semipenetrans* was 85.18 %. Abu Habib*et al.* (2020) conducted a survey in Alexandria, El-Behera and Kafr El-Shiekh governorates, northern Egypt in citrus groves and reported that the citrus nematode was found to be very common frequency of occurrence (FO) = 100% in all the surveyed locations. Nasir *et al*. (2021) surveyed citrus orchards at Peshawar, Mardan, Nowshera and Malakand for citrus nematode. Maximum infestation (86.00%) was recorded at Pallai in Malakand division while minimum infestation (44.00%) was recorded in Malakandher, Peshawar. Jabbar and Abedulridah (2023) carried out a survey on *T. semipenetrans* (Cobb) and found the presence of citrus nematodes infestation in all orchards and in all regions. It was found that the severity of infection was significantly higher with older trees compared to newly established orchards with young plants. Haseeb *et al.* (2024) carried out a survey for the assessment of nematode population distribution in all 7 Tehsils of district Sargodha (Pakistan) and showed that the population was low in declining orchards as compared to the population densities found in healthy orchards.

The variation in occurrence and citrus nematode population may be due to variation in varieties, age of orchards, soil texture, soil temperature and relative humidity as well as other agro-ecological conditions during survey. Most of the citrus groves in area of Udaipur were infected with the citrus nematode, *Tylenchulus semipenetrans* with the occurrence of 71.92%. The nematode is responsible to cause slow decline with the symptoms of chlorosis and as well as dead twigs and in case of nursery stage it also reduce the root system drastically and ultimately reduced the quality & quantity of yield.

CONCLUSIONS

Tylenchulus semipenetrans is found to be predominant in lemon (*Citrus limon* L.) where verdie-back symptoms are present and is suggested that citrus nematode is one of the main factors responsible for slow decline of citrus trees in India. A survey was carried out in the month of July to December 2022 in district Udaipur of Rajasthan, to record the prevalence of citrus nematode in lemon. In surveyed areas, out of twenty six localities, incidence of citrus nematode, *T. semipenetrans* was recorded from 25 localities. Extreme infestation of citrus nematode was marked from Gogunda, RCA farm and Gumanpura with nematode population (1355.00, 1050.00 and 965.00) per 200 cc soil, respectively. However, only one locality (Parda) where it was not found. Occurrence of *T. semipenetrans* was obtained 71.92% with an average population of 86.40 females / 5 g roots, 53.27 egg masses / 5 g roots and 588.46 juveniles / 200 cc soil. This study demonstrated that the Citrus nematode is more prevalent than previously believed, with population variations varying according to the localities.The study's findings may be applied to a number of critical areas to enhance management and decrease production losses. It will also be essential to do research on the effects of nematodes on host susceptibility and the role of climate change contributes in changing the ranges of *Tylenchulus semipenetrans.* Additionally, developing rapid diagnostic tools could allow for early detection, enabling timely interventions to protect lemon crops. Together, these efforts could significantly enhance sustainable lemon production by minimizing nematode-induced losses.

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