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Distribution of citrus nematode, *Tylenchulus semipenetrans* on lemon (*Citrus Limon* L.) in citrus orchard

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Abstract
Soil and root samples were collected to find out the horizontal and vertical distribution of citrus nematode, *Tylenchulus semipenetrans* in Lemon (*Citrus Limon* L.). Horizontal distribution of citrus nematode in *Citrus limon* L. exhibited an inverse relationship with distance where the average population of female / 5 g roots (113.10, 87.54 and 28.64); egg masses / 5 g roots (78.10, 56.38 and 16.74) declined as the distance from tree trunk was increased from 2 ft. to 4 ft. and 6 ft., respectively. While in case of eggs and larvae / egg mass, no significant change was seen. In vertical distribution of citrus nematode, nematode population declined sharply when increased the depth. Maximum density of citrus nematode was observed at a depth of 1 ft. followed by 1.5 ft. and 2 ft. Nematode population decreases in horizontal and vertical distribution may be due to lesser feeder roots in roots system.

Keywords: *Tylenchulus semipenetrans*, lemon, horizontal and vertical distribution

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), uhacw (Hindi), 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. The most important commercial citrus species in India are the mandarin orange (*C. reticulata*), sweet orange (*C. sinensis*) and acid lime/lemon 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). 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) [17, 12, 10]. 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) [7]. Other uses for lemon juice, known from traditional medicine, include treatment of high blood pressure, the common cold, and irregular menstruation. Beside from being rich in vitamin C, it also assists in warding off infections.

A threatening situation in citrus cultivation has developed due to dwindling of the area under citrus as it is of late concerned 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 (Citrus canker, scab, citrus tristeza disease, greening, anthracnose and sooty mould) including nematodes. Among the plant pathogens, nematodes cause significant damage to citrus crop either independently or in combination with other pathogens, thereby reducing the vigour and life span, resulting in uneconomic returns. Plant-parasitic nematode *viz.*, *Tylenchulus semipenetrans*, *Helicotylenchus*, *Hoplolaimus*, *Longidorus*, *Pratylenchus*, *Psilenchus*, *Radopholus*, *Trichodorus*, *Tylenchorhynchus*, *Xiphinema* and *Criconemoides* spp., while the saprophytic nematode genera were *Araeolaimid* sp., *Cephalobid* sp., *Diplogastrid* sp., *Mononchid* sp., *Plectus* sp. and *Rhabditid* sp., have been found associated with citrus crop (Reddy and Singh, 1979; Khanzada *et al.*, 2008) [16, 13].

Among nematodes, *Tylenchulus semipenetrans* Cobb, 1913 [19] is one of the most destructive nematode species associated with citrus and is known to occur in all the citrus growing areas of the world (Thorne, 1961; Nasir *et al.*, 2021) [22, 15]. The citrus nematode was first discovered in California by Cobb (1913) [19] 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; Mahfouz, 2020) [21, 2, 14]. 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) [20, 3].

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.

Soil samples collected from rhizosphere of lemon plants from the orchard. Soil samples were collected at a depth of about 30-60 cm using soil auger. Samples were collected from three different points in a triangular fashion. Each soil sample collected approx. 200 cc soil as well as also collect infected roots 5-10 g with each sample. These samples scooped up with the help of khurpi/spade and filled into poly bag and tightly tied with rubber bands and tagged with label card indicating on it the name of the host, locality, and date of collection etc. For estimation of nematode population in soil, the soil samples collected from the 8 years old trees from citrus orchard to the laboratory. Two hundred cubic soil was processed using Cobb's sieving and decanting technique (Cobb, 1918) [6] followed by Baermann's funnel assembly (Christie and Perry, 1951) [4]. After 24 hours the suspension was drawn in a beaker from the funnel and kept for some time to allow the nematode to settle down. The volume of suspension was made to 100 ml and then suspension was drawn with the help of a pipette and poured over a counting dish for counting. Population count was done under a stereoscopic binocular microscope and determine accordingly. Root samples were collected from each tree, labelled properly and brought to the laboratory. Roots were gently washed in running tap water to remove and clean adhering soil particles. These roots stained by using acid fuchsine and lacto phenol. Roots were completely observed under stereoscopic binocular microscope for counting of females, egg masses and eggs and larvae of nematode. Eggs and larvae could be separated from egg mass and squeezed gently and count eggs and larvae by using telecounter.

In horizontal distribution, ten plants selected from the lemon orchard. After selecting the lemon plants, marked them for further observations. After marking these plants, took the root samples from the 2, 4 & 6 feet distance from the plants at a depth of 1 ft. Observations were taken from horizontal distribution 2, 4 & 6 ft. While in vertical distribution, we took the root samples from these marked plants at 1, 1.5 & 2 ft. of depth from 4 ft. distance from the rhizosphere of plants. Observations were similar in both horizontal and vertical distribution i.e. Number of females per 5 g roots, number of egg masses per 5 g roots, number of eggs & larvae per egg mass. These observations were analysed by correlation and regression with slope estimation of graph.

All the methodology was similar in aspect of extraction of nematode from soil and plant roots as per requirement. Observations of nematode parameters from 4 ft. distance horizontally and 1 ft. depth vertically was similar in both horizontal and vertical distribution.

Result

Estimation of horizontal and vertical population of citrus nematode, *Tylenchulus semipenetrans* periodically in the citrus orchard situated at RCA Horticulture Farm, MPUAT. This experiment was carried out to know the population dynamics of citrus nematode, *Tylenchulus semipenetrans* at different horizontal distance and different vertical depth in citrus orchard at RCA farm. Observations on nematode parameters (number of egg masses per 5 g roots, number of females per 5 g roots and number of eggs & larvae per egg mass) were recorded, analyzed and presented in Table- 4 & 5 and illustrated through Figure 2, 3, 4 & 5.

A. Horizontal distribution

A. Females per 5 g roots

At the time of sampling, females per 5 g of roots were recorded at the distance of 2 ft., 4 ft. and 6 ft. from the tree trunk, as 113.10, 87.54 and 28.64, respectively. It was recorded maximum at a distance of 2 ft. (128.00) in plant no. 4&7, 4ft. (106.80) and 6ft. (35.60) both from plant no.4 from the stem of plants. Regression was analyzed on the nematode parameters and results were recorded for R-Square 0.95, p-value 0.14 (p>0.05) and regression coefficient -0.045. Regression equation for number of females/5 g roots was calculated Y = 7.441-0.045x. Correlation analysis was performed on the given set of data and the correlation factor was found to be -0.97. Slope value was obtained -21.12 when distance was presented at X-axis and number of females at Y-axis (Fig. 2).

B. Egg masses per 5 g roots

Egg masses per 5 g of roots were recorded at a distance of 2 ft., 4 ft. and 6 ft. from the stem of plants. It was observed 78.10, 56.38 and 16.74, respectively (Table-4). It was recorded maximum at a distance of 2 ft. (98.60), 4 ft. (78.00) in plant no.10 and 6 ft. (26.80) plant tag no 4, from the stem of plants. Regression analysis was estimated on the parameters and results were obtained for R-Square 0.97, p-value 0.106 (p>0.05) and regression coefficient -0.063. Regression equation for number of females/5 g roots was obtained -52.12 when depth was presented at X-axis and number of egg masses at Y-axis.

Table 4: Horizontal distribution of citrus nematode, *Tylenchulus semipenetrans* on Lemon.

Plant number	No. of females / 5g roots			No. of egg masses / 5g roots			No. of eggs& larvae / egg mass		
	2 FT.	4 FT.	6 FT.	2 FT.	4 FT.	6 FT.	2 FT.	4 FT.	6 FT.
1.	110.00	86.00	30.40	78.60	58.40	18.40	48.20	47.60	46.20
2.	106.60	77.20	27.00	69.80	55.60	15.20	46.80	45.40	44.80
3.	109.20	90.40	33.40	91.20	66.60	18.40	47.40	46.60	48.00
4.	128.00	106.80	35.60	88.40	65.20	26.80	50.20	51.40	49.20
5.	88.80	68.00	29.20	54.60	30.00	18.80	50.00	52.60	49.40
6.	116.00	85.60	32.40	70.00	46.00	20.20	51.20	47.80	50.80
7.	128.00	102.20	28.80	85.00	60.00	16.60	46.80	45.20	47.60
8.	103.40	70.00	18.20	55.00	42.00	11.00	44.40	46.00	45.40
9.	115.60	90.60	26.00	89.80	62.00	10.00	49.20	47.20	48.60
10.	125.40	98.60	25.40	98.60	78.00	12.00	51.80	49.40	51.20
Total	1131.00	875.40	286.40	781.00	563.80	167.40	486.00	479.20	481.20
Mean	113.10	87.54	28.64	78.10	56.38	16.74	48.60	47.92	48.12
S.Em±	3.393			3.868			0.735		
CD at 5%	10.008			11.409			NS		

Data are the average of five replications

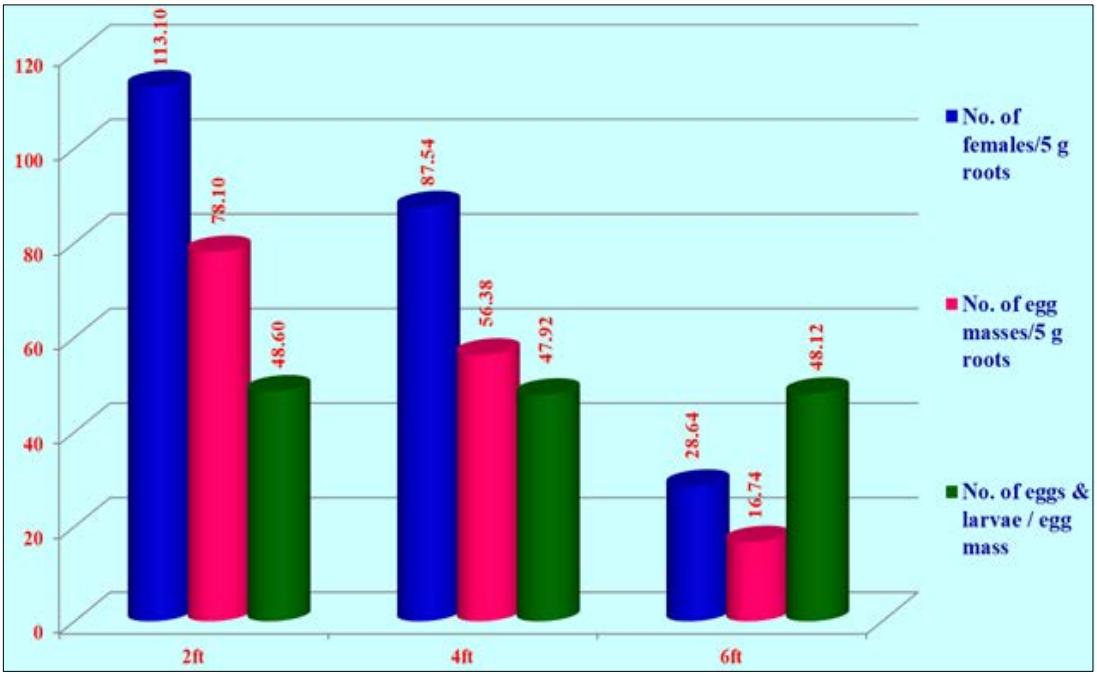

Fig 1: Horizontal distribution of citrus nematode, *Tylenchulus semipenetrans* on Lemon (*Citrus limon* L.).

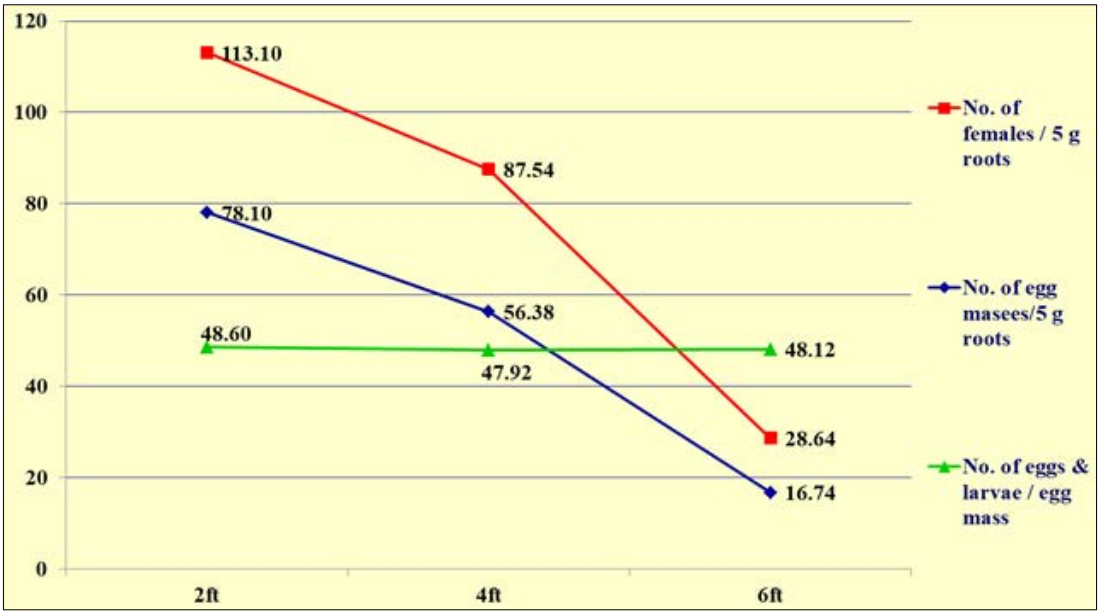

Fig 2: Line Diagram showing horizontal distribution of citrus nematode, *Tylenchulus semipenetrans* on Lemon.

Eggs & Larvae per Egg mass

Eggs & larvae per egg mass were recorded at a depth of 1 ft., 1.5 ft. and 2 ft. from the soil surface as 47.92, 47.22 and 47.74, respectively. It was recorded maximum at a depth of 1 ft. (52.60) in plant no. 5, 1.5 ft. (49.60) in plant no. 6 and 2 ft. (50.20) in plant no. 1. Regression analysis was estimated on the parameter and results were obtained for R-Square 0.06, p-value 0.841 (p>0.05) & regression coefficient -0.340. Regression equation for number of females/5 g roots was calculated Y = 17.72-0.340x. Correlation analysis was performed on the given set of data and the correlation factor was found to be -0.92. Slope value was obtained -0.18 when depth was presented at X- axis and number of eggs & larvae per egg mass at Y-axis (fig.5).

Discussion

Citrus nematode population was found to show a substantial decline as the distance from the plant was increased. Sample was taken at 1 ft. depth at a distance of 2, 4 & 6 ft. from the tree trunk which exhibited female per 5 g roots 113.10, 87.54 & 28.64, respectively. The number of egg masses per 5 g roots at 2 ft., 4 ft. and 6 ft. were recorded as 78.10, 56.38 and 16.74, respectively. The number of eggs and larvae per egg mass at 2 ft., 4 ft. and 6 ft. were recorded as 48.60, 47.92 and 48.12, respectively. Number of females and egg masses decreases when move outward from tree trunk. But in case of eggs & larvae per egg mass, no significant change were seen. Thus we can conclude that an inverse relationship is shared between distance and nematode population parameters.

Regression analysis were calculated for the nematode parameter and the following results showed that the R-Square value is close to 1 that show these parameters are related closely and the p-value is greater than 0.05 (p>0.05) that means the horizontal distance did not affected the nematode population but the negative regression coefficient and negative correlation coefficient as well as the negative slope value showed the inversely relation between distance and nematode population. That means when moving outward from tree trunk, the population of nematode is also decreased due to the lesser feeder roots. But the R-square value (R^2) and "r"-value is not close to one in case of eggs & larvae per egg mass that showed the number of eggs & larvae per egg mass was not much affected when the distance increased and it also showed by the slope value -0.12 is very closely to zero that mean not significant change in number when increase the distance.

Similar results were also observed by Duncan (1989) [9] who reported a decline in citrus nematode population when the distance was increased from the plant. He also reported the lack of feeder root in the distant roots of the plant which were the primary point of infection for citrus nematode. Baghel and Bhatti (1982) [1] observed that as the distance of sampling was increased from 30 cm to 90 cm, the population of the nematode showed a decline upon moving far from the tree trunk.

Vertical distribution of citrus nematode population was found to decline as the depth of sampling was increased. Greatest densities of citrus nematode occurred at the depth of 1 ft. following which it declined at 1.5 ft. and least concentration of citrus nematodes occurred at 2 ft. Sample taken at the depth of 1 ft., 1.5 ft. and 2 ft. at the plant which showed the number of females per 5 g roots 87.54, 46.64 and 11.28, respectively. The number of egg masses per 5 g of roots at 1 ft., 1.5 ft. and 2 ft. were recorded as 56.38, 27.98 and 4.14, respectively. Number of eggs & larvae per egg mass at 1 ft., 1.5 ft. and 2 ft. were recorded as 47.92, 47.22 and 42.90, respectively. These results showed that female's count and egg masses decrease when depth increase that means the number of citrus nematode population inversely proportional to the depth. In case of eggs and larvae per egg mass is similar or non-significant variation when increasing depth. The correlation value -0.25 is also nearly to zero that show trifling relation between eggs & larvae per egg mass and depth. While in case of both female and egg masses values of correlation coefficient (r) are -0.99. That means both are negatively correlated with the depth. When regression analysis performed that also showed the R-squares value is near to 1 that means the results are close to regression line. The p-value is less than (< 0.05) that showed the nematode population is affected by depth and nematode population exist a relationship and the negative regression coefficient and the negative slope value showed the inversely relation between nematode population and depth.

Similar observations recorded by Davis (1984) [8] who found that the highest concentrations of citrus nematode occurred at the 0 to 15 cm of soil depth. Citrus nematode population showed a sharp decline when compared at 45 cm when compared to 30 cm depth as inferred by Saeed, *et al.* (2019) [18].

These finding showed that the distribution of citrus nematode was directly correlated with the number of feeder roots present in soil. When distance from the plant was increased either horizontally or vertically, the number of feeder roots decreased and consequently, the concentration of citrus nematodes population decreased.

Conclusion

Soil and root samples were collected to find out horizontal and vertical distribution of citrus nematode on lemon. Results revealed that citrus nematode population declined progressively when samples collected from 2ft. to 6ft. distance from tree trunk in horizontal distribution. In vertical distribution nematodes population sharply decreased progressively when sampling from 1 ft. to 2 ft. depth. Highest density of T. Semipenetrans was observed at a distance of 2 ft. horizontally and 1 ft. vertically. Minimum population was noticed at 6 ft. distance horizontally and at 2 ft. depth vertically.

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