ONLINE ISSN: 3007-3197 | PRINT ISSN: 3007-3189

http://amresearchreview.com/index.php/Journal/about

Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 3, Issue 4 (2025)

Investigates The Incidence And Population Densities Of Plant Parasitic Nematodes In Citrus Orchards Across Three Localities

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ABSTRACT

This study investigates the incidence and population densities of plant parasitic nematodes in citrus orchards across three localities: Palai Katlang, Rabbot Dir L, and Dargai Jabbon. The highest disease incidence (70%) was observed in Palai Katlang, followed by Rabbot Dir L (50%) and Dargai Jabbon (40%). Palai Katlang also exhibited the highest nematode population density, particularly in samples S8 (90%) and S9 (85%). Soil analyses revealed that all three locations had similar silt-loam textures, with Palai Katlang having the highest pH (8.94) and electrical conductivity (228 µS). The presence of Tylenchulus semipenetrans and Radopholus similis, the main culprits of citrus nematode diseases, was confirmed, with severe root stunting and dieback observed in Palai Katlang. The study underscores the role of soil conditions, such as pH and texture, in influencing nematode survival and density. Recommendations for controlling nematode populations emphasize Integrated Disease Management (IDM) practices, including regular soil sampling and exploration of resistant citrus varieties. Further research is needed to develop effective control strategies to minimize the impact of nematodes on citrus crops.

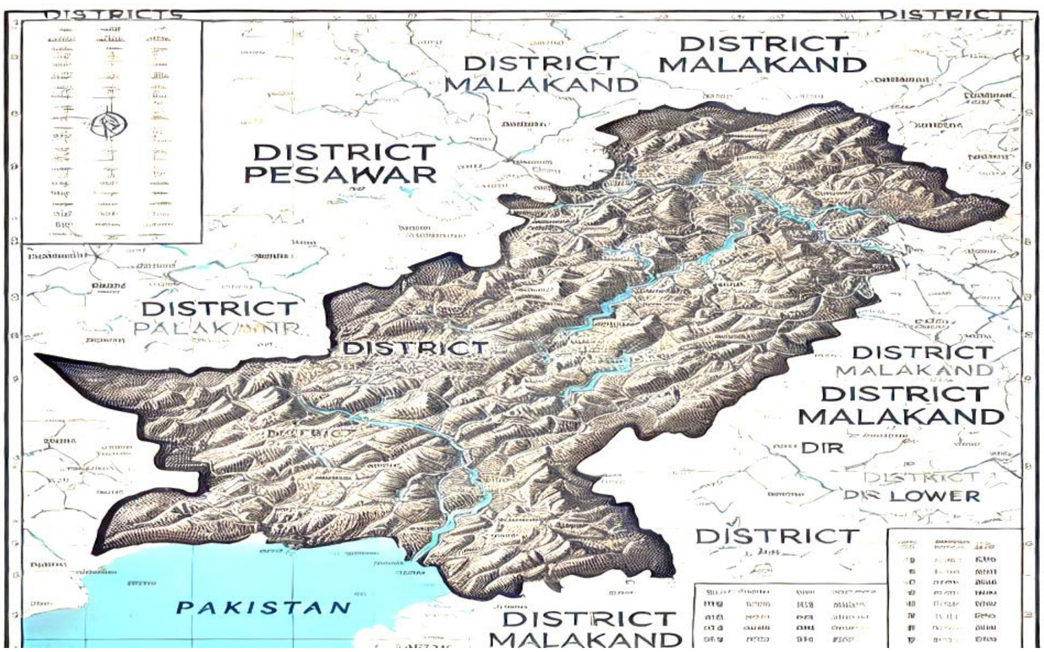
Introduction

Citrus is one of the most common genera of the flowering plants and belongs to (family *Rutaceae*). The various species of Citrus are all believed to be native to the subtropical and tropical regions of Asia and the Malay Archipelago, and to have spread from there to other sections of the world. Citrus has been cultivated through the ages, and in some pretty remote places (Perez and Marvin, 2015). Citrus is a rich source of sugar, vitamin C, minerals like magnesium, calcium and organic acid (Duncan and Cohn, 2005). In terms of international trade, citrus is the highest value fruit. It is grown in more than 125 countries in a belt within 35^{o}latitude south or north of the equator. Around 70% of the world's total citrus production is grown in the Northern Hemisphereand the United States. Brazil is also one of the biggest citrus producing countries. Citrus support world's biggest agriculture industries (Duncan and Cohn, 2005). Pakistan is the sixth biggest producer of oranges and kinnow mandarin and one of the chief citrus producing countries in the world (MINFAL, 2005). Dubai, Saudi Arabia, Kuwait, Qatar, Oman, Netherland, Indonesia, Singapore, Malaysia and UK are the major Pakistani markets of Kinnow. About ninetyfive percent of citrus kinnow in the world is provided by Pakistan. In Pakistan citrus is produced on an area of 193,985 hectares with an annual production of 2,001,685 tonnes. In Khyber Pakhtunkhwa citrus fruits were grown on an area of 2,970 hectares with total production of 24,355 tonnes. Similarly, the land under cultivation of citrus in Sindh, Balochistan and Punjab are 5,140, 1,395 and 183,296 hectares at the production rate of 31,394, 6,920 and 1,930,082 tonnes, respectively (Anonymous, 2013). A wide range of plant parasitic nematode has been associated with citrus rhizosphere but only a few species cause damage to trees (Verdejo-Lucas and McKenry, 2004). These include migratory endoparasites (lesion and burrowing nematodes), sedentary endoparasites (citrus root knot nematodes) and several other ectoparasites that cause serious damages to citrus. The most common nematodes affecting citrus trees are citrus nematodes, *Tylenchus semipenetrans* (Cobb, 1913). The nematode causes "Slow Decline" of citrus all around the world and limits citrus fruits production under a wide range of environmental conditions (Duncan, 2005). All varieties of citrus are attacked but rootstocks like trifoliate orange (*Poncirus trifoliata*) are highly resistant to citrus nematode attack. Others such as Troyer and Carrizo citrange are moderately tolerant and some like sweet orange are highly susceptible (Halbert,2012). Citrus nematodes are ectoparasitic and feed on root tissues from outside the plant. With their hindquarters buried in the soil, the larvae pierce the roots to feed, usually just behind the growing tips. Feeding on the roots does not kill the citrus tree. However, the root's capacity to carry water and nutrients is impeded and yields are noticeably reduced early in the life of the tree. Nematode damage of the roots also promotes entry of secondary diseases (Godwin *et. al.*, 2007). Citrus nematodes are most active in the warmer months from September to April. It is difficult to diagnose these hidden nematodes of citrus Spreading decline is another important disease of citrus caused by *Radopholu ssimilis* (Cobb, 1893) Thorne, 1949. This nematode caused severe losses in citrus in central Florida (Duncan, 2005). Symptoms in the citrus included severe necrosis and root destruction (Machon and Brdige, 1996). Other species of nematodes i.e *Pratylenchus coffeae* (Zimmermann, 1898), *Xiphenema* spp., *Paratrichodorus* spp. (Topham PB. 1985), *Belonolaimus longicaudatus* (Mulrooney RP, 2010) and *Helicotylenchus* spp., (Whittaker LM. 1984) have been reported in citrus orchards. In Pakistan, information on plant parasitic nematodes attacking citrus orchards is very limited. No reliable data on incidence, occurrence, frequency distribution and losses incurred by parasitic nematodes infesting citrus orchards is available in the Khyber Pakhtunkhwa province. The present study was undertaken to estimate the incidence and to identify major parasitic nematodes of citrus in selected localities. Keeping in view, the importance of plant parasitic nematodes, a survey was conducted in citrus growing orchards at Dargai jabbon, Palai katlang and Rabbot Dir Lower to achieve the following objectives.

MATERIALS AND METHODS

Survey and collection of samples.

A comprehensive survey of citrus orchards in selected localities at district. and Peshawar was carried out. Three sites i.e Palai Babozai , Dargai jabbon and Rabbot Dir Lower were selected for collecting roots and soil samples. Samples were collected from citrus orchard following atri-anglular design (Zarina *et al*., 2015). From every site ten trees were selected. Citrus orchards from selected localities., . (Palai katlang) and Dargai jabbon Farm were surveyed. Soil sample (each 10 g) was collected with help of soil augur up to a depth of approximately 15-20cm (Southey, 1986). Roots samples (each 50 g) were also collected from the same citrus orchards from where soil cores were collected. Ten soil and root samples were collected from each orchard. Samples were randomly taken with help of trowel and augur. Debris were removed from the upper 15 cm soil and samples were taken from 25-30 cm soil depth in a rhizosphere of the diseased and healthy trees at a distance of 60 to 90 cm away from the tree trunk. A composite of 100g soil with roots were taken in polythene bags, properly labelled and brought to the laboratory of Plant Pathology for further study.



Extraction I: Whitehead and Hemming Tray method (Soil)

Juveniles and adults were extracted from the soil by Whitehead and Hemming method (Whitehead and Hemming, 1965). Fifty grams (50 g) of powder soil was placed over a tissue paper in a sieve. The sieve was placed in steel tray. About two hundred ml water to the tray from the corner was added until the soil was properly moistened. The sample was left for 24 hours at room temperature. Nematodes extracted from soil sample were counted counted under stereomicroscope at 60X magnification and their population density of nematodes was recorded (Southey, 1986).

Extraction II: Root Incubation method (Roots)

Citrus roots were washed carefully with tap water to remove soil particles and then air dried. Feeder roots were soaked in a beaker having 180-200ml of distilled water for about 24-48 hours. Roots were washed and cut into 1.0 cm pieces. Fifty grams (50 g) roots were placed in petri plates containing distilled water. Plates were incubated for 24 hours at a room temperature and nematodes that crawled out of roots were observed under Stereo-microscope and their density was calculated at 60 X magnification (Southey, 1986).

Microscopy:

Major nematodes extracted from citrus roots and rhizosphere were identified at 40 X magnification using key of(Cohn E 1969). Male and female nematodes were identified based on their morphological characteristics. Parasitic nematode genera associated with citrus roots were identified and morphological features were observed and compared with the key of (Albert et al., 2010).

Soil Analysis

The percentage of sand, silt and clay in the inorganic fraction of soil was measured by Hydrometer Method (Bouyoucos, 1962). Fifty (50g) of soil, clear from debris and gravel was taken in special dispersion cup and 10 ml of dispersing solution was added to it. The cup was then filled with water and placed on a stirrer for 5 minutes. The treated soil was transferred to 1000 ml glass cylinder and filled with distilled water up to 1000 ml mark. Hydrometer and thermometer were placed in it. After 40 seconds the first hydrometer value and temperature were

recorded. Then the suspension was re-shaked and left over for 2 hours. After 2 hours the 2nd hydrometer value and temperature were recorded.0.3 was added to the readings of the samples for every 1 °C above 20 °Cand subtract 0.3 for every 1 °C below 20 °F. (Bouyoucos,1962).The Silt, Sand and Clay percentage was calculated as;

$$\text{Silt} + \text{Clay} = \frac{40 \sec reading}{\text{weight of sample}} X\,100$$

$$\text{Clay} = \frac{Corrected\ value\ at\ 2\ hrs}{\text{weight of sample}} X\,100$$

Sand = 100- Silt + Clay

Soil electrical conductivity (EC) and pH

Measurements of electrical conductivity (EC) and pH were determined on a saturation extract of soil or supernatant liquid of1:2 soils, water suspension. A15g of soil sample was shaken with 30ml of distilled water in 250 ml conical flasks for 10 minutes. After that the suspension was passed through filter paper to remove the soil particles. The EC was then measured by the electrical conductivity meter and pH by pH-meter (Hendershot *et al*., 1993).

RESULTS

The incidence of citrus nematodes across three localities, namely Palai Katlang, Rabbot Dir L, and Dargai Jabbon, was observed in various percentages, with Palai Katlang showing the highest disease incidence at 70%, followed by Rabbot Dir L at 50%, and Dargai Jabbon at 40%. The population density of citrus nematodes, measured in different samples, was highest in Palai Katlang, with S8 showing a 90% density, followed by S9 at 85% and S6 at 80%. In contrast, the nematode density in Rabbot Dir L was highest in sample S5, with a 55% population density, and in Dargai Jabbon, the highest density was recorded in sample S9 at 39%, followed by S10 (33%) and S1 (30%). When combining the mean population densities, Palai Katlang recorded the highest mean population density of 599, followed by Rabbot Dir L at 460, and Dargai Jabbon at 243. In terms of nematode density in citrus roots, the highest densities were recorded in S1 of Palai Katlang and Dargai Jabbon. The soil properties, including pH, electrical conductivity (EC), and texture, were also analyzed. Palai Katlang had a soil pH of 8.94 with an EC of 228 µS and a silt-loam texture. Rabbot Dir L had the highest soil pH at 8.98, with an EC of 220 µS and a similar silt-loam texture. Dargai Jabbon exhibited the lowest soil pH at 8.77 with an EC of 206 µS and also had a silt-loam texture. The soil samples from all three locations showed a similar silt-loam texture with varying percentages of silt, sand, and clay, contributing to the overall soil structure. These findings indicate that the highest nematode population densities were observed in silt-loam textured soils, especially in Palai Katlang, which also exhibited the highest disease incidence.

Incidence of citrus nematodes

	nce (%)						. Jabbon)			on Density					

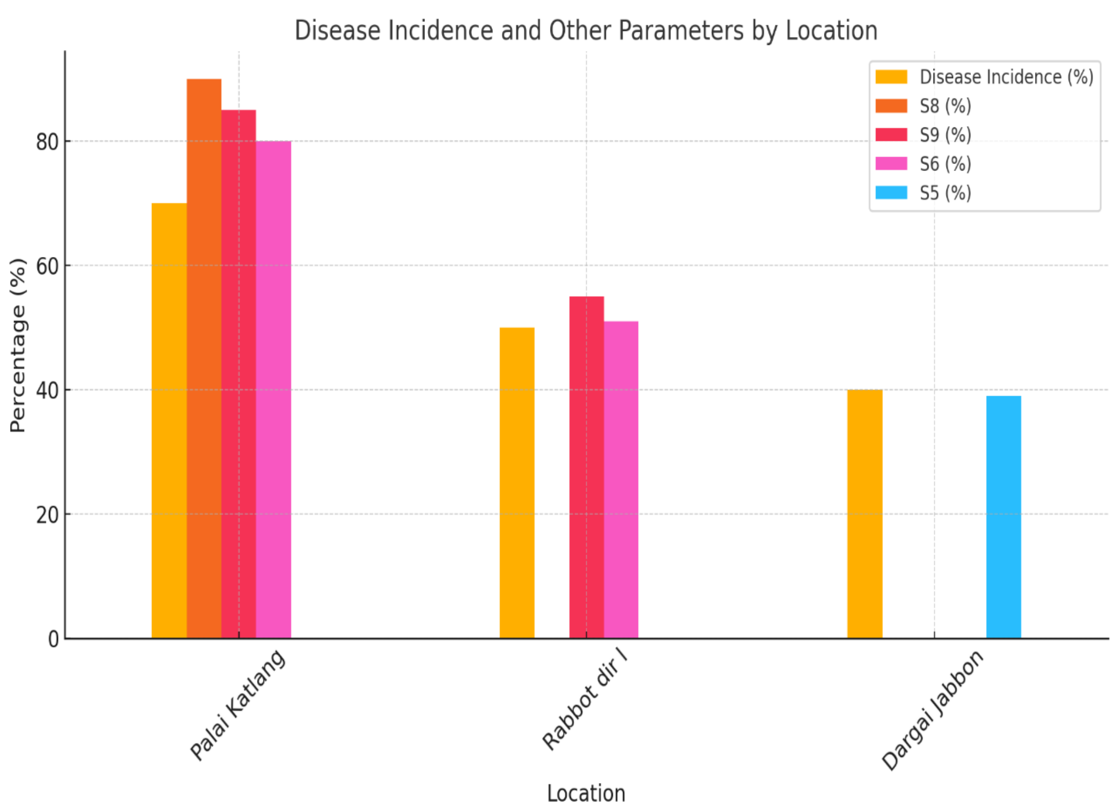


Table pH, electrical conductivity (EC) and soil texture values of soil samples collected from citrus orchards at three different selected localities.

.NO	Locality	pH	EC	Percent	Percent	Percent	Soil Texture

		(μS)		Silt	Sand	Clay	
1	Palai Katlang	8.94	207	62.2	42.0	4.0	Silt loam
2	Rabbot dir L	8.98	228	68.0	33.7	4.2	Silt loam
3	Dargai Jabbon	8.77	206	58.0	38.0	3.4	Silt loam

Symptoms and morphology of plant parasitic nematodes in citrus

Two major genera of plant parasitic nematodes were isolated from citrus rhizosphere and roots. Infected citrus trees showed typical decline symptoms caused by citrus nematodes, *Tylenchulus semipenetrans* (Figure4.2.1). Infested roots showed severe stunting and decay. Severely infected trees showed yellowing and thinned canopy, exposure of bare crown limbs, reduced leaf and fruit size.*Tylenchulus semipenetrans* exhibited sexual dimorphisim. Male and female showed differences in their morphology. Fig 4.2.2 showed typical male nematode of *Tylenchulus semipenetrans* isolated from citrus roots. The nematode showed a translucent, white slendered vermiform shape under 40 X magnification (Fig 4.2.2 a). The nematode possessed a delicate stylet with small knobs. A single elongated testis and dark paired spicules were observed in adult males. Figure 4.2.2 (b)showed structure of a typical sedentary female of *T. semipenetrans.* Posterior portion of a mature female was swollen with elongated neck whereas its head was embedded into root tissues as shown in figure. Burrowing nematode *Radopholus similis* exhibits marked sexual dimorphism. Male nematodes possess a raised lip region and a reduced feeding apparatus (stylet and esophagus) because they are not infective. The tail of male burrowing nematode has a distinctive bursa extending at least two-thirds of the tail length that it uses to clasp the female body during mating (Fig 4.2.3 a) Females do not have a raised lip region, but do have a heavily sclerotized and thickened framework. The female stylet is robust with three distinct knobs. The vulva, the opening of the reproductive system, is located slightly below mid body (Fig 4.2.3 b) Infected roots of citrus revealed another genus of plant parasitic nematode i.e spiral nematode, *Helicotylenchus* spp. The nematode showed typical spiral structure as shown in the figure (4.2.4). Figure 4.2.6 showed the female of spiral nematode. The nematode is curved into spiral shaped when relaxed or dead. The vulva of the female is located around 2/3 of the nematode's body length from the anterior terminusas shown in figure (4.2.6). The tail of nematode is asymetrical. Male is straight as shown in the figure (). The nematode produced dark necrotic lesions on roots and caused roots dieback.

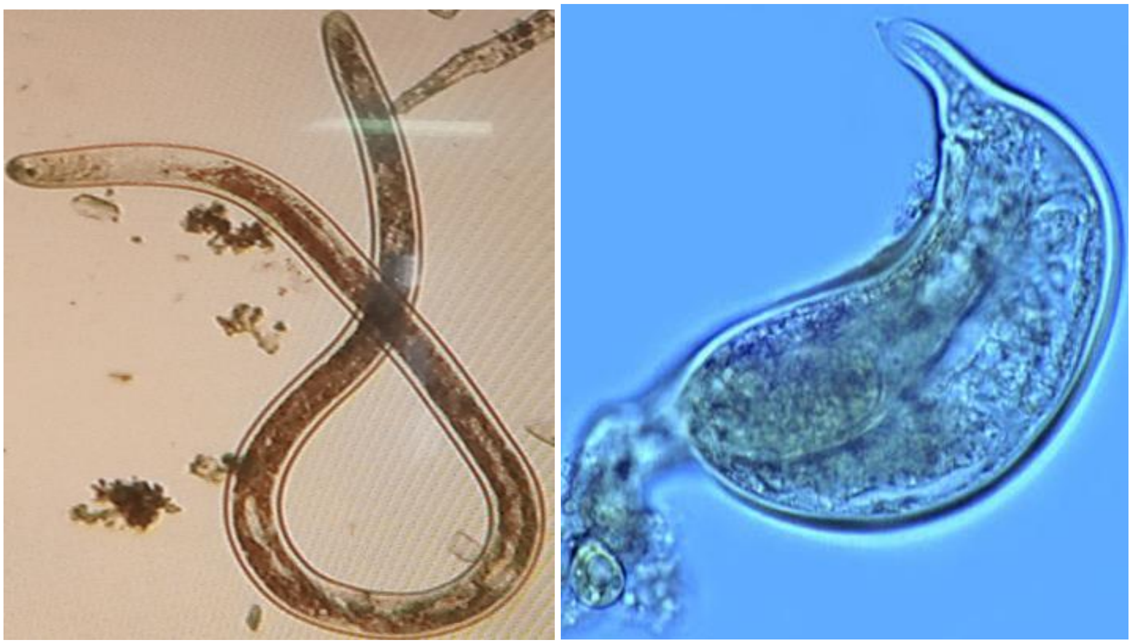


Fig Adult male (a) and female (b) of *Tylenchulus semipenetrans* isolated from citrus roots.

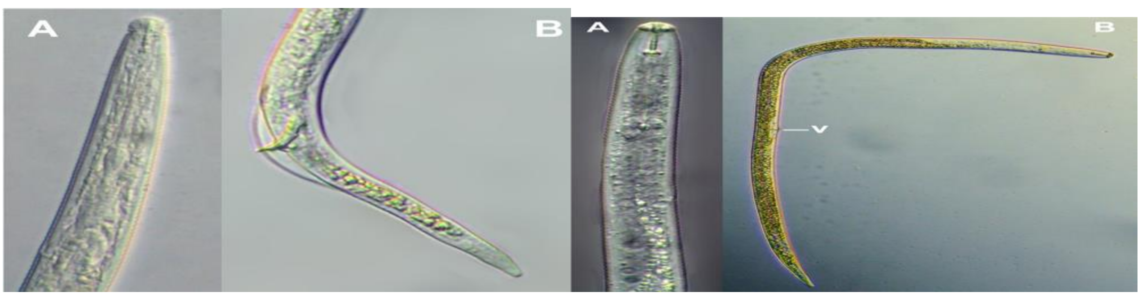


Fig 4.2.3 *Radopholus similis* male head (A) and tail (B) Left. Female of *R. similis* (right) with head region (A) and full body with vulva [v] near midbody (B). (Internet Picture: Photograph by Nicholas Sekora)

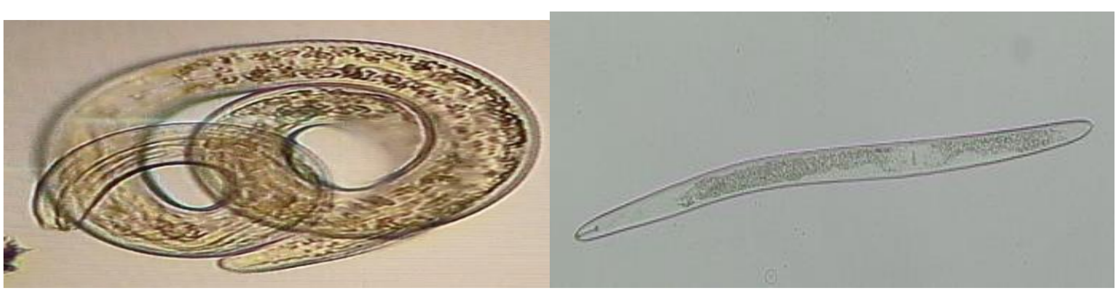


Fig Adult female of *Helicotylenchus spp* (A) isolated from citrus. The body of nematode is curved into spiral shaped when nematode is dead or relaxed. B) Male spiral nematode (*Helicotylenchus* spp.)

Discussion

Plant parasitic nematodes are widely distributed pests and are abundant in agriculture soils. In the present study we detected and identified two major genera of plant parasitic nematodes i-e *Helicotylenchuus spp* (spiral nematode) and *Tylenchulus semipenetrans.* These plant parasitic nematodes were found associated with citrus in three different localities i.e (Dargai jabbon, Palai katlang and Rabbot Dir Lower). We found the highest incidence of citrus nematodes in Palai katlang (district .) and minimum in the Dargai jabbon soils. The population densities of citrus nematodes were the greatest in palai katlang locality. Higher nematodes density in roots produced severe stunting, dieback symptoms on roots particularly in roots samples that were collected from Palai katlang. Higher disease incidence and densities of nematodes in palai katlang locality could be considered due to favourable soil conditions, temperature and cultivation of susceptible citrus rootstocks. Numerous citrus varieties are cultivated in Pakistan. These include rough lemon, sour oranges, kinnow, sweet oranges, mandarins and wild oranges. The nematode population depends on the type of host infested. *T. semipenetrans* cause slow declines in all citrus nurseries and orchards but its incidence varies in all citrus varieties. We reported higher incidence of citrus nematodes in sweet oranges. Similar results were reported by other researchers who observed maximum incidence of citrus nematodes in Feutrells variety, lemon and orange and minimum incidence in grapefruit (Khanzada *et al*., 2007).Some researchers documented that rough lemon and sour oranges contain maximum number of female citrus nematodes as compared to feutrells and sweet orange (Ahmad *et al*., 2007). Soil texture also affect nematode population densities, reproduction factor and movement of nematodes. As a generalization, sandy soils are nematode-loving soils. *T. semipenetrans* and *Helicotylenchus* spp., do not persist well in dry soil and population densities on drought-stressed trees decline rapidly (Tsai and Van Gundy, 1988). However, infection and population growth are rapid in localized areas of drought in the rhizosphere if other portions of the root system have adequate water. However, nematodes exist in soils of all textures some even being favoured by a more fine textured.Soil pH is a useful measurement because it is a predictor of various chemical activities within the soil. As such, it is also a useful tool in making management decisions in controlling different soil borne plant pathogens including plant parasitic nematodes. Soil pH appears crucial to nematode survival. Acid or alkaline soils tend not to favours nematodes. In conclusion, control of plant-parasitic nematodes typically requires an integrated approach. However, there are often no or very few chemical options. In field crops, due to the cost of nematicides, their use is not economically justified. Therefore, cultural tactics should often be the first line of defence for management of plant-parasitic nematodes. The importance of maintaining proper fertility and optimizing soil pH has been emphasized here to reduce the impact of nematodes.

Conclusions:

Nematodes are widely distributed in citrus growing areas of Districts Peshawar and .. Three types of nematodes genera viz., citrus nematode, *Tylenchulus semipenetrans* and *radoopholus similis Helicotylenchus* spp (spiral nematode) and were detected and identified. The highest percentage disease incidence (70%) was recorded in Palai katlang and minimum disease incidence (40%) was recorded in Dargai jabbon. The highest Population density of nematodes occurred in palai katlang citrus orchards.

Recommendations:

Integrated disease management (IDM) practices are recommended to the growers in order to achieve better yield and good quality of citrus. Sampling at least once or twice a year is recommended to observe nematodes density and devise control strategies.Further study is required to investigate and explore resistant varieties and other control measures to reduce the population densities of these nematodes in agricultural soils.

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