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Screening of citrus cultivars against root-knot nematode (*Tylenchulus semipenetrans*)

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Abstract

Citrus is a highly remunerative horticultural crop in Pakistan. Due to many biotic factors, its yield in Pakistan is low as compared to other citrus-producing countries. Among the biotic factors, citrus nematode (*Tylenchulus semipenetrans*) is considered a vital yield-limiting factor. The present study was planned to investigate resistance or susceptibility levels of different citrus cultivar to *T. semipenetrans*. To examine nematode infestation with the roots of citrus cultivars, a screening nursery was established at the College of Agriculture (CoA), University of Sargodha, Sargodha during 2016-17. The results revealed that nematode infestation was significantly different ($P<0.05$) in roots and soil samples collected fromdifferent citrus cultivars. Among 10 citrus cultivars, four cultivars *i.e.*, Musambi, Succari, Feutrell's early, and Kinnow proved to be susceptible tothe nematode. Rough lemon was moderately susceptible. Sweet lime proved to be moderately resistant while four cultivars including grapefruit, Rubby blood, Eustis lime, and Taroccowere resistant to *T. semipenetrans*.

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Introduction

Citrus is a highly remunerative horticultural crop and has vital significance in world trade. Citrus is grown in the Mediterranean region, and southern and northern hemisphere, contributing 20%, 35% and 20%, respectively (FAO, 2018). The successful cultivation of citrus depends on the good climate, fertile soil, good quality irrigation water, and effective internal drainage (Nemec, 1986). Citrus tree decline (CTD) is the subject of substantial research all around the world. It is also known as decay, frenching, neglectosis, chlorosis and amachamients. CTD is also referred to as die-back due to no sharp difference between the symptoms of die-back and CTD. However, the term die-back represents a deadly condition that leads towards the quick death of the citrus tree from apex to bottom involving many biotic factors, whereas the CTD denotes a gradual fall in the productivity of orchards due to both biotic and abiotic factors (Ghosh, 1985).Among the biotic factors which are involved in CTD, citrus nematode (*Tylenchulus semipenetrans*) is considered as the most vital yield-limiting factor. Depending on the infection level, this nematode can reduce the yield of citrus groves up to 30%. Mature citrus trees having the ability to tolerate large populations of these nematodes show decline symptoms quite late; however, on the other hand, young trees poorly grow if planted in nematode-contaminated soils (Duncan and Cohn, 1990). The symptoms caused by this nematode are determined by the general look of the orchard and trees show slow growth, yellowing, stunting, and reduced foliage and fruit size. These symptoms are similar to other symptoms produced by some other factors and could only be differentiated by doing sampling and then extraction of nematodes from these soil and root samples. This nematode attacks the root system of the citrus tree and impairs its ability to absorb nutrients and water which are required for the normal growth. Damage caused by this nematode starts increasing when other root-detrimental factors includingwater stress, fungal infections, or reduced growth during the first phase of development, are associated (Verdejo-Lucas and MsKenry, 2004).Regarding underground symptoms, nematode infected feeder roots are littledense than non-infected and give a dirty lookdue to clinging of soil particles,which are adhered to roots because of the gelatinous matrix dropped by nematode female on the surface of the root.As slightly infected roots do not exhibit prominent symptoms; therefore, nematode infestations in nursery remainun-noticed (Duncan and Cohn, 1990). Nematode population assessments are necessary for making any management decision against these nematodes. This helps to relate densities and kinds of nematodes to the expected yield performance of citrus grove. In this way, the nematode data obtained also assists to check the potential of various control approaches. As the spatial distribution of *T. semipenetrans* nematodes population is aggregated, this necessitates the composite sample collection from different cores of soil (Abd-Elgawad, 1992).

Citrus nematode attack becomes more serious when citrus orchards have problems with some other biotic and abiotic factors. Sometimes, the citrus nematodeis not the only limiting factor in orchards having nematode infection, instead of other biotic and abiotic factors collectively hamper the availability of inputs.If the yield of citrus grovedoes not improve after the control of nematode, this means that citrus nematode is not the only limiting factor (Thomason andCaswell, 1987). There are some other possible yields restricting factors that include citrus greening disease and soil stresses.Still, different approaches are applied to manage this nematode (Thomason and Caswell, 1987).

Materials and methods

To determine the infestation of nematodes with the roots of different citrus cultivars, a screening nursery was established at the College of Agriculture (CoA), University of Sargodha, Sargodha (UOS) during 2016-17. Before the plantation of the nursery, the nursery area was deeply ploughed and covered with a double polythene sheet for seven days to sterilize the soil of the nursery. After that, formalin was added with irrigation water to ensure complete sterilization of nursery soil (Irshad *et al.,* 2012). Later, normal agronomic practices were performed to prepare the nursery for the plantation of citrus cultivars. Ten mostly grown citrus cultivars, having the age of six months, were collected from Citrus Research Institute (CRI) Sargodha and sown in RCBD with three replications in prepared sterilized soil of nursery.

The names of cultivars with their botanical names are mentioned in Table 1. The second stage (J2) freshly hatched juveniles of*T. semipenetrans* @ 2000were inoculated around plants. Irrigation was applied daily to provide the moist conditions to nursery (Aatif *et al.*, 2018). The nursery was maintained for two years*i.e.* 2017 and 2018, and data was recorded.

Nematodes extraction from root and soil

After two months of inoculation, inoculated plants were uprooted for root and soil samples.Whitehead and hemming tray method were used for the isolation of nematodes (Van-Bezooijen, 2006). Nematodes were counted using an inverted microscope. Roots of each inoculated plant were thoroughly rinsed with tap water and after rinsing stained with lactophenol (Saeed *et al.,* 2019). To remove the extrastain, roots were rinsed with glyceroland distilled water solution (50:50). Data were recorded by counting thenumber of females in 1gm of roots and J_2 in 100g of soil. Resistance and susceptibility of cultivars were measured using the following rating scale (Javed *et al.,* 2008).

Results and discussion

Nematode infestation was significantly different ($P<0.05$) in roots and soil samples collected from different citrus cultivars. Among 10 citrus cultivars, four cultivars i.e., Musambi, Succari, Feutrell's early, and Kinnow proved to be susceptible to nematode population1030, 1121, 1560 and 1174 J_2/100 cm^3 of soil, and 548, 414, 577 and 561 females/g of roots, respectively. Rough lemon was moderately susceptible with 1088 J2/ 100 cm^3 of soil and 168 females/g of roots. Sweet lime proved to be moderately resistant with 289 J2/100 cm^3 of soil and 113 females/g of roots. Four citrus varieties *i.e.,* grapefruit, Rubby blood, Eustis lime, and Tarocco were resistant with 163, 254, 203 and 117 J2/100 cm^3 of soil, and 57, 61, 67 and 55 females/g of roots, respectively (Table 3).*T. semipenetrans* nematodes are a serious problem to many fruit crops such as citrus, mango, tomato, melon, guava, banana, grapes, apple, apricot, and almond (Iqbal *et al.*, 2005; Freitas *et al.,* 2017; Ali *et al.*. 2018).

Table 1. Names of cultivars with their botanical names.

Sr. No.	Common Name of Cultivars	Botanical Name
1.	Musambi	*Citrus sinensis* Osbeck.
2.	Succari	*Citrus sinensis* Osbeck.
3.	Tarocco	*Citrus sinensis* Osbeck.
4.	Grapefruit	*Citrus paradise* Mcaf.
5.	Sweet Lime	*Citrus limettoides*
6.	Feutrell's Early	*Citrus reticulata* Blanco.
7.	Rubby Blood	*Citrus sinensis* Osbeck.
8.	Eustis Lime	*Citrus aurantifolia* Swing.
9.	Rough Lemon (JattiKhatti)	*Citrus jambhiri* Lush.
10.	Kinnow	*Citrus reticulata* Blanco.

The present study revealed that these nematodes are associated withmost of the citrus cultivars of Punjab.This is in agreement with the findings of Atif *et al.* (2018).They evaluate different citrus cultivars against citrus nematodes and found that Musambi, Feutrell's early, Succari and Kinnow were susceptible to these nematodes. Iqbal *et al.* (2005) found that rough lemon is among susceptible rootstock against citrus nematodes. Citrus nematodes significantly affect root, shoot growth, and yield of rough lemon

(Irshad *et al*., 2012).Four citrus varieties including grapefruit, Rubby blood, Eustis lime, and Tracco were found resistant during this study.The same has been reported by Khanzada *et al*. (2008). In their study, grapefruit, lemon, and sweet lime were resistant against citrus nematodes in Punjab. The nematode juveniles per gram of soil and the number of females per gram of root varied with different varieties.

Table 2. The rating scale for the evaluation of citrus cultivars against *T. semipenetrans*.

Scale	Rating	J_2/100 cm^3 of soil	Females in 1 gram of roots
1	R	<250	< 100
3	MR	250-500	100 to 200
5	MS	500-1000	200 to 300
7	S	1000-1600	300 to 500
9	HS	>1600	> 500

This variation may be due to different virulencelevels of nematode population andless resistance of different rootstocks (Duncan and Cohn, 1990). Root exudates of varieties have also marked an effect on the size of the population of nematode (Yang *et al*., 2016). There is a possibility that the cultivars found resistant during current findings might have discharged some root exudates which have restricted the population size of the nematodes.

Table 3. Nematodes infestation in soil and roots of different citrus cultivars.

Sr. #	Variety	Juveniles/ cm^3 of soil	Females/g of roots	Remarks
1.	Musambi	1037±14d	551±13a	Susceptible
2.	Succari	1121±18bc	414±9b	Susceptible
3.	Grapefruit	163±14g	57± 8d	Resistant
4.	Feutrell's Early	1560±12 a	577±8 a	Susceptible
5.	Sweet lime	289±7 e	113±6 cd	Moderately Resistant
6.	Rubby blood	254± 12 ef	61± 4 d	Resistant
7.	Eustis lime	203±11 fg	67±9 d	Resistant
8.	Rough Lemon	988± 13 cd	168± 9 c	Moderately Susceptible
9.	Kinnow	1174± 16 b	561±13 a	Susceptible
10	Tarocco	117±7 g	55±5 d	Resistant

Means sharing similar letters are not significantly different at P>0.05.

The difference in the population of females/gram of roots may also be a result of temperature and moisture levels in the vicinity of citrus plants in the nursery. High moisture does not favor the citrusnematodes in the rhizosphere asthese nematodes are highly sensitive to moisture (Van Gundy and Martin, 1964). Likewise, thecitrus nematodes population rises between 20-31°C temperatures while optimum temperature for the nematode population is 25°C.

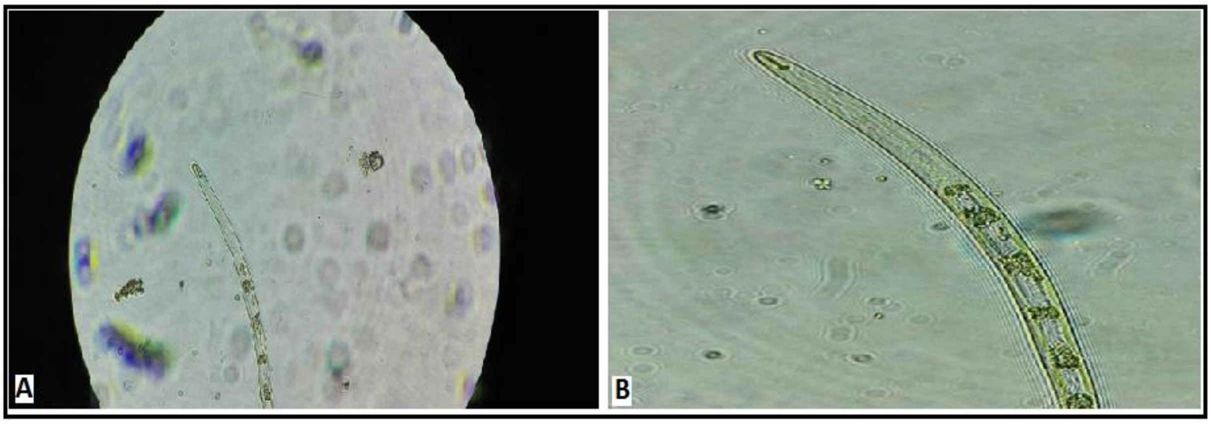

Fig. 1. Microscopic view of Citrus root-knot nematode (A, B).

The extreme temperature usually kills the nematodes. Davis (1984) investigated the population dynamics of citrus nematode in different months and found that populations were at the peak in April and dropped to the lowermost level in September.

Conclusion

The present study revealed the resistance in some cultivars of citrus against *T. semipenetrans* suggesting their further molecular characterization against citrus nematodes to develop resistant cultivars in the future. The study further recommends characterizing the exudates of resistant cultivars to investigate their role in developing resistance against citrus nematodes.

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Conflict of Interests

The author(s) declare(s) that there is no conflict of interest regarding the publication of this article.

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