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INCIDENCE AND DISTRIBUTION OF NEMATODES IN CITRUS ORCHARDS OF PUNJAB CAUSING DECLINE

Shahid Ahmed Khanzada[1]*, Anjum Munir[1], Khurshid Burney[1], Shahid Hameed[1] and Hafeez ur Rehman[2]
[1]Crop Diseases Research Program (CDRP), Institute of Plant and Environmental Protection (IPEP)
[2]Horticulture Research Program (HRP), Institute of Field and Horticultural Crops (IFHC),
National Agricultural Research Center, Islamabad
*Email: sbashirk@yahoo.com

Abstract

Citrus nematode *Tylenchulus semipenetrans*, one of the major pest of citrus crop and responsible to cause slow decline in citrus nurseries and orchards. Surveys were conducted to access the incidence and distribution of citrus nematode in different major citrus growing areas of Punjab including Sargodha, Faisalabad, Sahiwal, Jhang, Toba Tek Singh and Pattoki. Soil and root samples were collected from different citrus varieties comprising Feutrell's Early, grapefruit, Kinnow, lemon, malta, mitha, musambi and orange. Among all the areas the incidence of *T.semipenetrans* in all citrus varieties varies from maximum as 56% Feutrell's Early followed by 40% Lemon and Orange, 35% Kinnow and Musambi. The minimum incidence was observed 20% in Grapefruit. Apart from *T.semipenetrans*, following genera of plant parasitic nematodes were also recorded i.e. *Belonolaimus sp*, *Helicotylenchus sp, Hoplolaimus sp, Longidorus sp, Paratylenchus sp, Psilcuclus sp, Radopholus sp, Trichodorus sp*, *Tylenchorhynchus sp*, *Xiphinema sp, and Zygothlenchulus* spp. While the saprophytic nematodes genera were *Alamid sp, Araeolamid sp, Cephalobid sp, Diplogastid sp, Monhysterid sp, Mononchid sp, Plectus, Rhabditid* in citrus verities of all given districts.

Key words: Citrus nematodes, *Tylenchulus semipenetrans*, incidence and decline.

INTRODUCTIOPN

The *Citrus*, family Rutaceae commonly edible fruits in this group are sweet oranges (*Citrus sinensis* Osbeck), mandarins (*C. reticulata* Blanco), grapefruit (*C. paradisi* Macf.), lemon (*C. limonia* L), lime (*C. aurantifolia* Swingle), pummelo (*C. grandis* Osbeck), Sour orange (*C. aurantium* L), Wild orange (*C. macroptera* Montrouzier), Citron (*C. medica* L.), Calamondin (*C. mitis* Blanco), Kaffir lime (*C. hystrix* DC) and *Fortunella* spp. (Mukhopadhyay, 2004).

It is the world's recognized agricultural commercial fruit crop that maintains a prominent position in the fruit industry. World trade in citrus is second only to bananas and more than double the volume of apples. It is highly nutritive and refreshing being a source of vitamins A and C, organic acids, amino acids, sugars and carotenoids and it is grown in more than 125 countries in a belt within 35 latitude north or south of the equator (Duncan and Cohn 1990).

Pakistan is one of the top ten citrus producing countries of the world and sixth largest producer of Kinnow mandarin and oranges in the world. In Punjab province it is mostly grown in the plain areas of Sargodha, Bhalwal, Sahiwal, Faisalabad, Lahore districts (GOP, 2004). It is the largest group of fruits produced over an area of 195 thousand hectares with an annual production of 1670 thousand tonnes out of which 206 thousand tonnes were exported earning 4202 million rupees of foreign exchange in Pakistan (MINFAL, 2005).

The province of Punjab is leading in fruit production as it contributes 65% of the total fruit production in Pakistan, and 85% of the citrus with major concentration of Kinnow mandarin, 10% of Mausambi, 4% of Feutrell's Early and 1% of Red Blood while root stock of rough lemon *Citrus jambhiri* is extensively used.

In Pakistan, the nematode problem is more serious and complex than in the developed countries. The reasons for this are many and varied. Firstly, the country lies in the tropical and sub-tropical regions where climate suitable for the activity and reproduction of the nematode throughout the year. Secondly, soils are favorable for nematode infestation, especially in irrigated areas. Perennial and other crops grown in the same field year after year are often heavily attacked by nematodes. Numerous nematode species are associated with the citrus rhizosphere, however, relatively few have been shown to be of economic importance (Cohn, 1972).

Citrus is attacked by a number of insect pests and diseases causing heavy losses both in quality and quantity. Among the nematodes, citrus nematode, *Tylenchulus semipenetrans,* (Cobb) has been recognized as one of the greatest threats throughout the world. It occurs in all citrus producing regions of the world and limits production of citrus fruits under a wide range of environmental conditions (Cobb, 1914).

The "citrus nematode", *T. semipenetrans* Cobb, causal agent of slow decline of citrus, is a small microscopic worm, which feeds within the roots of citrus. Slow decline is a universal problem and it was reported in Pakistan in an orange orchard at Lyallpur as early as 1962 (Brown, 1962). It limits production of citrus fruits under a wide range of environmental and edaphic condition. Root feeding by the citrus nematode results in the breakdown of root cells which are then frequently invaded by secondary organism which eventually kill the root. The death of many roots over a period of years causes reductions in tree growth and size and a gradual dieback of the tree canopy, increased leaf drop during periods of environmental stress and a reduction in uniform fruit size and yield. High populations of *T. semipenetrans* can cause rapid deterioration of the root system during the early stages of tree development.

A survey of 1977 in Vidarbha region, Nagpur India reported that 20% of mandarin orchards showed conspicuous sign of decline and the diseases incidence progressed with the highest incidence of 28% (Diware and Kolte, 1990).

As citrus orchards are declining in citrus growing areas of Punjab therefore present survey was conducted to ascertain the occurrence of citrus nematode in the citrus growing districst. The current studies have been carried out to find out the population of nematodes causing the severe losses in citrus orchards of Punjab.

MATERIALS AND METHOD

Extensive surveys were conducted in the citrus growing areas of Punjab i.e. Sargodha, Bhalwal, Faisalabad, Sahiwal, Jhang, Toba Take Singh and Patoki in 2005 and 2006 to determine the incidence and distribution of citrus nematode population in relation to citrus variety. Soil and roots samples from the rhizosphare of citrus were collected from 20 different citrus orchards of 3-40 years old at different localities in each area. Soil and feeder roots were taken at the depth of 4-6 inches under the canopy of each tree with the help of shovel. Samples were taken from citrus orchards of each district by having composite samples that included 50 sub-samples (cores) collected in X shape sampling procedure. From each orchard randomly 10 trees were selected and form each tree 5 sub samples were taken from the different surrounding sites of the trunk and a composite sample of 1000 g was taken in the polyethylene bag. The samples were properly labeled

and brought in to Plant Nematology Laboratory CDRP, NARC, Islamabad for isolation and identification of plant parasitic nematode.

The nematode population extracted from the soil samples by tray modification method of Baermann funnel and the Flegg sieving technique (Flegg and Hooper, 1970). Specimens were later killed by heat and then fixed in TAF for microscopic examination (Courtney et al., 1955). Number of nematodes per ml suspension was determined and the nematodes identified to generic level. Nematode population was counted in a 10 ml suspension in a Doncaster counting dish and a mean of 3 counts was taken in each case. Citrus roots were washed free from soil and cut into 5-10 cm long and immersed in water for migratory endoparasitic nematode under 20-25C for 4-7 days by root incubation technique (Southey, 1986). The roots were stained in lacto-glycerol for the presence of females of *T. semipenetrans* (Bridge et al., 1982).

RESULTS AND DISCUSSION

The present research work was conducted in the citrus growing areas of Punjab province of Pakistan, i.e. Sargodha, Faisalabad, Sahiwal, Jhang, Toba Tek Singh and Okara (Pattoki) to estimate the incidence and distribution of citrus nematode, *Tylenchulus semipenetrans*, its population in relation to citrus variety as shown in (Table 1). In total 215 samples were selected for different varieties of citrus from given districts of Punjab. The highest number of samples was collected from Sargodha district while least number of samples was taken from Jhang district (Table 1). The occurrence of *T.semipenetrans* in Jhang, Okara (Pattoki), Toba Tek Singh, Sargodha, Faisalabad, Sahiwal and were calculated 100%, 43%, 42%, 40%, 32%, and 29% respectively. The highest infestation percentage of *T.semipenetrans* was found in Jhang while least was found in Sahiwal (Table 1).

Incidence of *Tylenchulus semipenetrans* among Citrus Varieties

In over all given districts of Punjab, the incidence of *T. semipenetrans* in all citrus varieties i.e Feutrell's Early, Kinnow , Orange, Lemon, Musambi, Malta and Grapefruit variation of *T. semipenetrans* were calculated 56%, ,40%, 40%, 36%, 35%, 20% and 18%, respectively (Table 2).

Isolation of the Population of Other Genera Related to Citrus Fruit Varieties

During soil analysis apart from *T.semipenetrans*, following genera of nematodes (parasitic & saprophytic) (Table 3) were also recorded i.e. *Belonolaimus sp*, *Helicotylenchus sp,Hoplolaimus sp, Tylenchulus semipenetrans sp, Longidorus sp, Paratylenchus sp, Psilenchus sp, Radopholus sp, Trichodorus sp*, *Tylenchorhynchus sp*, *Xiphinema sp, Zygotylenchulus sp* and saprophytic nematodes are *Alamid sp, Araeolamid sp, Cephalobid sp, Diplogastid sp, Monhysterid sp, Mononchid sp, Plectus, Rhabditid* in citrus varities of all given districts of Punjab.

DISCUSSION

The results show that 38% orchards were found to be infested with the citrus nematodes. The highest infestation of *T.semipenetrans* was found in Jhang and least was found in Sahiwal. Parviz et al. (2003) conducted a survey during 2002 from Sargodha and reported 54% infestations of *T. semipenetrans* while our results from Sargodha shows an infestation of 40% which closely resembles with his results. Ahmed and Khan (1999) in their survey of citrus orchards in the Punjab reported that 100% infestation of *T. semipenetrans* in citrus orchards but in our survey it was found that the infestation is 38%. They might have conducted a survey based on positive sampling while we conducted a random survey that shows this much infestation. The survey conducted during the study showed no co-relation of *T.semipenetrans* with variety or locations. However it was observed that Mitha was not infested with T. semipenetrnas. The maximum number of nematode species parasitic as well as saprophytic was found in Kinnow. The abundant occurrence of saprophytic nematode attributed to fungal and bacterial populations in those soils. We, therefore, can refer this to a complex situation that can be the cause of decline in citrus and more in Kinnow plantations. All the citrus growing areas showed infestation of *T. semipenetrans*. It was

observed that all the rootstock from which grafting being made are susceptible to *T. semipenetrans* therefore efforts should be made for resistant root stock to avoid citrus declining and better yields.

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Table 1: Detail of site sample

S. No.	Districts	Total locations (numbers)	Fruit variety	No of sample infested/Total number of sample	*T. semipenetrans* (%)
01.	Sargodha	85	Feutrell's Early, Grapefruit, Kinnow, Malta Musambi.	34/85	40
02.	Faisalabad	37	Grapefruit, Kinnow, Lemon (China & Sweet) Malta, Musambi, Sweet Orange.	12/37	32
03.	Sahiwal	51	Feutrell's Early, Grapefruit, Kinnow, Lemon Malta, Mitha, Mussambi.	15/51	29
04.	Jhang	04	Kinnow.	04/04	100
05.	Toba Tek Singh	31	Feutrell's Early, Kinnow, Mussambi, Orange	13/31	42
06.	Okara (Pattoki)	07	Grapefruit Kinnow, Lemon, Musambi	03/07	43
Total		215			

Table 2: Incidence of *Tylenchulus semipenetrasns* in different citrus growing areas

S. No.	Fruit varieties	Incidence of nematodes (number of samples/total number of samples)	*T. semipenetrans* (%)	Districts with sample infested with *T. semipenetrans* (%)					
				Sargodha	Faisalabad	Sahiwal	Jhang	T.T.Singh	Okara (Pattoki)
1.	Feutrell's Early	05/09	56%	33%	-	67%	-	-	-
2.	Grapefruit	02/11	18%	50%	-	-	-	-	100%
3.	Kinnow	58/144	40%	37%	27%	47%	100%	46%	50%
4.	Lemon	04/11	36%	-	60%	25%	-	-	-
5.	Malta	02/10	20%	50%	-	14%	-	-	-
6.	Mitha	00/02	00%	-	-	-	-	-	-
7.	Musambi	08/23	35%	80%	14%	22%	-	-	100%
8.	Orange	02/05	40%	-	100%	-	-	-	-

Table 3: Nematode species isolated from different citrus varieties.

S. No.	Fruit Variety	Nematode isolated	
		Plant parasitic	Saprophytic
1.	Feutrell's Early	*Hoplolaimus sp* *Longidorus sp* *Trichodorus sp* *Tylenchorhynchus sp* *Tylenchulus semipenetransp*	*Cephalobid sp*
2.	Grapefruit	*Trichodorus sp* *Xiphinema sp* *Tylenchulus semipenetrans*	*Cephalobid sp* *Diplogastid sp* *Monhysterodea sp* *Mononchid sp*
3.	Kinnow	*Belonolaimus sp* *Helicotylenchus sp* *Hoplolaimus sp* *Longidorus sp* *Paratylenchus sp* *Psilenchus sp* *Radopholus sp* *Trichodorus sp* *Tylenchorhynchus sp* *Tylenchulus semipenetrans* *Xiphinema sp* *Zygotyhlenchulus sp*	*Alamid sp* *Araeolamid sp* *Cephalobid sp* *Diplogastid sp* *Monhysterid sp* *Mononchid sp* *Plectus sp* *Rhabditid sp*
4.	Lemon	*Helicotylenchus sp* *Longidorus sp* *Radopholus sp* *Tylenchorhynchus sp* *Tylenchulus semipenetrans* *Xiphinema sp* *Zygotylenchulus sp*	*Araeolamid sp* *Cephalobid sp* *Diplogastid sp*
5.	Malta	*Helicotylenchus sp* *Pscylenchus sp* *Trichodorus sp* *Tylenchulus semipenetrans* *Xiphinema sp*	*Araeolamid sp* *Cephalobid sp*
6.	Mitha	*Xiphinema*	*Cephalobid*
7.	Musambi	*Hoplolaimus sp* *Helicotylenchus sp* *Longidorus sp* *Radopholus sp* *Pscylenchus sp* *Trichodorus sp* *Tylenchorhynchus sp* *Tylenchulus semipenetrans* *Xiphinema sp*	*Araeolamid sp* *Cephalobid sp* *Diplogastid sp* *Monhysterid spp* *Rhabditid sp* *Mononchid sp*
8.	Orange	*Hoplolaimus sp* *Longidorus sp* *Tylenchulus semipenetrans* *Xiphinema sp* *Zygotylenchulus sp*	*Cephalobid sp* *Diplogastid sp*