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A SURVEY OF OKRA (*ABELMOSCHUS ESCULENTUS*) IN THE PUNJAB PROVINCE OF PAKISTAN FOR THE DETERMINATION OF PREVALENCE, INCIDENCE AND SEVERITY OF ROOT-KNOT DISEASE CAUSED BY *MELOIDOGYNE* SPP.

MUHAMMAD ARSHAD HUSSAIN[1], TARIQ MUKHTAR[2,*], MUHAMMAD ZAMEER KAYANI[3], MUHAMMAD NAVEED ASLAM[4] AND M. IRFAN UL-HAQUE[2]

[1]*Plant Pathology Section, Regional Agricultural Research Institute, Bahawalpur, Pakistan.*
[2]*Department of Plant Pathology, Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi, Pakistan.*
[3]*Green Belt Project, Department of Agriculture, Rawalpindi, Pakistan.*
[4]*University College of Agriculture and Environmental Sciences, The Islamia University, Bahawalpur, Pakistan.*
[*]*Corresponding author e-mail: drtmukhtar@uaar.edu.pk; Tel.: +92 51 9290239*

Abstract

Okra (*Abelmoschus esculentus* (L.) Moench) is one of the important vegetables of the tropical and subtropical areas. In Pakistan okra is cultivated on thousands of hectares but the yield obtained is relatively lower, for which there are many constraints including prevalence of diseases caused by different pathogens. Among various pathogens responsible for the low yield, the root-knot disease incited by *Meloidogyne* spp., is of considerable economic importance and cause annual losses in tropics to an extent of 22 per cent. As there is meager information regarding the association of root-knot nematodes with okra in the country, the present studies were conducted in 17 districts of the Punjab province of the country to determine the occurrence, prevalence and intensity of root-knot nematodes in okra cultivations in the vegetable growing areas of Punjab. The survey revealed that root-knot nematodes prevailed in 85.28% of okra fields with an average incidence of 38.89%. Hundred per cent prevalence was recorded in Multan, Okara, Dera Ghazi Khan, Bahawalnagar, Vehari, Rahim Yar Khan and Rawalpindi districts and a minimum prevalence of 22.4% was found in Lodhran district. The incidence was above 60% in Bahawalnagar, Rahim Yar Khan, Dera Ghazi Khan and Vehari and was only 4.44% in Lodhran. The severity of infection of the nematodes was highest in Bahawalnagar and Vehari, while it was the lowest in Lodhran. Of the four most common root-knot species, *M. incognita* constituted 74.74%, *M. javanica* 24.02%, *M. arenaria* 1.57% and *M. hapla* 0.78%.

Introduction

Among the nematode pathogens, root-knot nematodes (*Meloidogyne* spp.) pose a serious threat to crops throughout the world and are considered to be the most destructive and cause huge losses. Almost all the vegetables in tropical and warm areas of the temperate regions are severely attacked by this nematode (Sikora & Fernández, 2005). Okra (*Abelmoschus esculentus* (L.) Moench.) is considered to be one of the world's oldest crops and is cultivated in almost all the Inter-tropical and Mediterranean regions for its young fruits. The vegetable is an important source of vitamins and essential mineral salts including calcium, which lacks in the diet of poor people of most of the developing countries of the world. This vegetable, vernacularly known as "Bhindi" is one of the most important summer vegetable crops of Pakistan. In the Punjab province of Pakistan, okra is sown from mid February to mid May. The total area under okra cultivation in Pakistan is about 2.21×10^5 hectares with production of 2.86×10^6 tons of green pods (Anon., 2006). The yield obtained in Pakistan is relatively lower, for which there are many constraints including prevalence of diseases caused by different pathogens (Ahmad *et al*., 2012; Arain *et al*., 2012). Among various pathogens responsible for the low yield, the root-knot nematodes are of considerable economic importance (Hussain *et al*., 2011a; Kayani *et al*., 2012a) and cause annual losses in tropics to an extent of 22% (Sasser, 1979). In India the losses have been estimated up to 99% (Bhatti & Jain, 1977). In Pakistan, damages to plants by nematodes are more serious and complex than in the developed countries due to many reasons. The country is situated in the tropical region where environmental conditions are conducive throughout the year for infection, development and reproduction of these nematodes. In the arid zone of the country, the sandy soils favour the activities of the nematodes. In irrigated areas perennial crops or susceptible crops grown in the same field year after year allow the nematodes to reproduce profusely and consequently cause severe infections and damage. In addition, these parasites also interact with other disease causing organisms to produce disease complexes (Begum *et al*., 2012) and break down resistance against other pathogens and reduce plant tolerance to environmental stress (Taylor, 1979). Plant parasitic nematodes, in Pakistan, have received little attention and only a few surveys have been made in the past (Brown, 1962; Kafi, 1963; Ahmad & Khan, 1973; Saeed & Ashrafi, 1973; Khan *et al*., 2005). As there is meager information regarding the association of root-knot nematodes with okra in the country, therefore, the present studies were conducted with the objective to determine and document the occurrence, prevalence and intensity of root-knot nematodes in okra cultivations in the vegetable growing areas of Punjab.

Materials and Methods

Prevalence, incidence and severity of root-knot nematodes: A survey of okra fields located in different randomly selected localities of the major vegetable growing districts of the Punjab province of Pakistan was conducted during the year 2009-10 for the determination of prevalence, incidence and severity of root-knot nematodes. Form each locality, three fields of okra were randomly selected**.** From each field, 25 plants were selected after each 10 steps following zigzag pattern**.** The

selected plants were carefully uprooted up to 15-20 cm soil depth with the help of trowel. The soil adhering to the root system was gently removed and roots were observed for root-knot nematodes infection (presence or absence of galls).

The root systems of individual plants were rated for the determination of severity of root-knot nematodes following the galling index developed by Bridge and Page (1980). The infected roots along with soil were put into polythene bags, labeled properly and brought to the laboratory of Plant Pathology Section, Regional Agricultural Research Institute, Bahawalpur for identification of root-knot nematode species.

The incidence of root-knot nematodes of individual okra fields was determined as follows:

$$\text{Incidence (\%)} = \frac{\text{Total number of infected plants}}{\text{Total number of observed plants}} \times 100$$

The prevalence of root-knot nematodes in each district was calculated as follows:

$$\text{Prevalence (\%)} = \frac{\text{Number of fields infected with root-knot nematodes}}{\text{Total number of fields surveyed}} \times 100$$

Identification of *Meloidogyne* species: Root-knot nematodes (*Meloidogyne* species) were identified on the basis of female perineal patterns described by Taylor and Netschler (1974). Mature females of root-knot nematodes were dissected out from the infected okra roots and placed in watch glass containing distilled water. The live mature females were then picked up with fine bristle and were placed in plastic Petri dish containing 45% lactic acid and were left for two hours. The posterior end then was cut off with a fine needle and the body tissues were removed by lightly brushing the inner surface of the cuticle with a flexible bristle. When all the tissues were removed, the cuticle was transferred to a drop of glycerin where it was carefully trimmed, the piece of cuticle containing vulval portion with the typical perineal pattern was then transferred to a drop of glycerin on a micro slide, a cover slip was applied and sealed with nail polish, and was observed under microscope. The perineal pattern was compared with standard diagrams and *Meloidogyne* species were identified. In this way perineal patterns of 40 females were prepared from each infected okra field and the distribution of each *Meloidogyne* species in each district was calculated.

Results

Prevalence of root-knot nematodes: The prevalence of root-knot nematodes in different districts is given in Table 1. It is evident from the table that maximum prevalence (100%) was recorded in districts of Multan, Okara, Dera Ghazi Khan, Bahawalnagar, Rahim Yar Khan, Vehari and Rawalpindi, while the minimum (22.4%) was found in district Lodhran. The prevalence in Gujranwala, Jhang, Faisalabad, Toba Tek Singh, Kasur, Muzaffar Garh and Bahawalpur ranged from 66.62 to 91.65%.

Table 1. Prevalence, incidence and galling index (severity) of root-knot nematode (*Meloidogyne* species) on okra in different districts of Punjab.

District	Disease prevalence (%)	Disease incidence (%)		Galling index	
		Range	Mean	Range	Mean
Multan	100	26.6-53.3	43.07	2-6	3.6
Gujranwala	88.86	0-33.3	18.5	2-5	2.44
Jhang	66.63	0-20	11.1	0-7	2.77
Faisalabad	83.32	0-86	52.76	0-8	5.33
Toba Tek Singh	91.65	0-86.6	57.74	0-8	5.66
Okara	100	33.3-86.6	59.23	4-7	5.77
Kasur	77.73	0-33.3	16.27	0-5	3
Dera Ghazi Khan	91.65	0-26.6	15.52	0-4	2.75
Muzaffar Garh	100	46.6-93.3	66.18	3-8	5.6
Khanewal	66.62	0-53.3	23.31	0-5	2.41
Bahawalnagar	100	53.3-80	69.98	5-7	6.16
Rajan pur	77.73	0-33.3	13.30	0-4	2.44
Vehari	100	40-86.60	63.3	4-7	6
Rahim Yar Khan	100	46.6-86.6	69.95	4-7	5.83
Bahawalpur	83.31	0-93.3	38.12	0-8	4.33
Lodhran	22.4	0-20	4.44	0-3	0.55
Rawalpindi	100	13.3-60	38.48	3-6	4.88
Average	**85.28**	**-**	**38.89**	**-**	**4.08**

Incidence of root-knot nematodes: The incidence of root-knot nematodes was recorded from all the okra growing areas. The incidence varied in all the districts and ranged from 11.1 to 69.95%. The maximum incidence (69.98%) of root-knot nematodes was found in Bahawalnagar district followed by Rahim Yar Khan (69.95%), Dera Ghazi Khan (66.18%) and Vehari (63.30%). The lowest incidence was recorded from Lodhran (4.44%) followed by Jhang (11.1%) and Rajan Pur (13.30%). The incidence ranged from 13.30 to 63.30% in Multan, Faisalabad, Toba Tek Singh, Okara, Dera Ghazi Khan, Bahawalpur, Rahim Yar Khan and Vehari districts. The mean individual incidence in each district is given in Table 1.

Severity of root-knot nematodes: The maximum severity (6.16) measured in terms of galling index suggested by Bridge & Page (1980) was found in district Bahawalnagar followed by Vehari (6.00) and Rahim Yar Khan (5.83) while the minimum was observed in district Lodhran which was only 0.55. The individual mean galling index (severity) recorded in each district is given in Table 1.

Occurrence of root-knot nematodes species: The different species of *Meloidogyne* associated with okra in different districts are given in Table 2. Of all the associated species of root-knot nematodes, *M. incognita* constituted 73.74%, *M. javanica* 24.02%, *M. arenaria* 1.57% and *M. hapla* 0.78%.

Both *M. incognita* and *M. javanica* were found in all the districts; *M. incognita* being predominantly found. *M. arenaria* and *M. hapla* were found only in Rawalpindi district. The individual percentage of each *Meloidogyne* species in each district is given in Table 2.

Maximum mean occurrence of *M. incognita* (97.5%) was observed in Toba Tek Singh and the lowest (33.3%) was recorded in Rawalpindi district. The highest occurrence of *M. javanica* (48%) was recorded in Dera Ghazi Khan and the lowest (2.5%) was found in Toba Tek Singh. The distribution of *Meloidogyne* species in each district is given in Table 2.

Table 2. Distribution of *Meloidogyne* species associated with okra in different districts of Punjab.

District	Number of *Meloidogyne* species	*Meloidogyne* spp. (percent)			
		M. incognita	*M. javanica*	*M. arenaria*	*M. hapla*
Multan	2	86.0	14.0	0	0
Gujranwala	2	86.6	13.3	0	0
Jhang	2	70.0	30.0	0	0
Faisalabad	2	70.0	30.0	0	0
Toba Tek Singh	2	97.5	2.5	0	0
Okara	2	83.3	16.6	0	0
Kasur	2	90.0	10.0	0	0
Dera Ghazi Khan	2	52.0	48.0	0	0
Muzaffar Garh	2	82.5	17.5	0	0
Khanewal	3	77.5	15.0	0	0
Bahawalnagar	2	80.0	20.0	0	0
Rajan pur	2	86.6	23.3	0	0
Vehari	2	70.0	30.0	0	0
Rahim Yar Khan	2	65.0	35.0	0	0
Bahawalpur	2	63.3	36.6	0	0
Lodhran	2	60.0	40.0	0	0
Rawalpindi	4	33.3	26.6	26.6	13.3
Average		**73.74**	**24.02**	**1.57**	**0.78**

Discussion

The results of the present survey showed variations in the prevalence, incidence and severity of root-knot nematodes in different districts of Punjab. Similar results were also reported by Lamberti *et al*. (1975), Bhatti and Jain (1977), Khan *et al*. (2005) and Shahid *et al*. (2007). The results of these workers confirmed the present findings regarding the prevalence of plant parasitic nematodes and occurrence of *Meloidogyne* species on vegetables. It is clear from the results of these researchers that okra crop was the most susceptible host of *Meloidogyne* spp. in vegetable growing areas. These variations in infestations are attributed to many environmental and edaphic factors, as differences in various climatic and edaphic factors of these districts have been found. There are reports which confirmed that distribution, prevalence, incidence and severity of root-knot nematodes are affected by varying agro-climatic conditions of the areas, soil type, moisture, soil pH and

particular cropping sequence (Taylor *et al.*, 1982; Sasser & Carter, 1985; Van Gundy, 1985; David, 1985).

Root-knot nematodes are also influenced by the biological, chemical and physical characteristics of the soil environment (Upadhyay *et al.*, 1972). In district Lodhran, the prevalence of root-knot nematodes is quiet low. This is because of higher amount of organic matter in the soil of the district and also due to the fact that okra was sown in the soils which were fallow for the last few years. This fact is supported by the findings of other researchers who found that fallowing increased the organic matter contents of the soils and thereby reduced the number of nematodes (Ferris & Bernard, 1971; Netscher, 1985; Aung & Prot, 1990; Floret & Serpantie, 1993).

The root-knot disease was found 100% in Multan, Okara, Bahawalnagar, Rahim Yar Khan, Vehari and Rawalpindi. This high incidence is due to intense vegetable cropping pattern and the availability of suitable host throughout the year in these districts which allowed rapid multiplication of root-knot nematodes. Earlier a number of researchers reported that abundance of root-knot nematodes is highly dependent upon the presence of the suitable host plants (Jacq & Fortuner, 1979; Yeates, 1976, 1981; Ferris *et al.*, 1985; Cuc & Prot, 1992).

The high incidence and severity of root-knot nematodes in these districts were also due to the cultivation of susceptible varieties, high temperature and comparatively less annual rainfall. These conditions favored the multiplication, development and infection of root-knot nematodes. There are reports which showed that nematode populations are influenced by soil type (Kincaid, 1946; Wallace, 1969; Prot & Van Gundy, 1981; Jain, 1992). In the present studies *M. incognita* and *M. javanica* were found in all the districts in varying proportions and *M. incognita* was predominant in all the districts. Trudgill *et al*. (2000) reported that *M. incognita* and *M. javanica* were the most widespread root-knot nematode species in all the countries. Similar results have also been reported by many workers (Khan *et al.*, 1993; Campos, 1994; Das & Das, 2000; Bhosle *et al.*, 2004; Ravichandra & Krishnappa, 2004; Rathour *et al.*, 2006). The distribution and infestation of *Meloidogyne* spp. in the soils of Pakistan was *M. incognita*, 52%, *M. javanica*, 31%, *M. arenaria*, 8%, *M. hapla*, 7% and other species about 2% (Maqbool, 1987) which further proved the present findings. *M. arenaria* and *M. hapla* were isolated only from Rawalpindi district. The climate of the district is cool, humid and mild. These results confirmed the findings of Brown (1962) that *M. arenaria* and *M. hapla* are cool, humid and hilly climate species. Gul & Saeed (1987) also reported *M. arenaria* and *M. hapla* from North West Frontier Province (NWFP) of Pakistan. It is concluded from the present studies that okra is severely attacked by root-knot nematodes and *M. incognita* is the most predominant species, which warrant that strict control measures should be adopted for its management (Hussain *et al*., 2011b; Kayani *et al*., 2012b; Mukhtar *et al*., 2013a, 2013b; Qureshi *et al*., 2012; Rahoo *et al*., 2011; Vagelas & Gowen, 2012).

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