



Root-Knot Nematodes Infecting Okra in Major Vegetable Growing Districts of Punjab, Pakistan

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ABSTRACT

The lucrative production of okra throughout the globe is threatened by root-knot nematodes (*Meloidogyne* spp.). As very little information is available on the distribution of root-knot nematodes in okra plantations, therefore, in the present studies surveys were conducted to record incidence, prevalence, and severity of root-knot nematode species associated with okra in the major vegetable growing districts of the Punjab province of Pakistan. The overall incidence of 39%, prevalence of 85% and severity of 4.1 in terms of the galling index was recorded throughout the province. Differences in the incidence of root-knot nematodes were recorded from all the districts ranging from 11% to 70%. The incidence was found to be the maximum (70%) in districts of Bahawalnagar and Rahim Yar Khan followed by Dera Ghazi Khan and Vehari showing incidences of 66 and 63%, respectively. On the other hand, the incidence was found to be the minimum (4%) in Lodhran followed by Jhang and Rajan Pur districts showing incidences of 11 and 13%, respectively. The rest of the districts showed incidences ranging from 13 to 63%. Variations were also recorded in the prevalence of root-knot nematodes in the seventeen districts of the Punjab province. The prevalence was found to be 100% in seven districts while the remaining districts showed prevalence below 100% and the minimum was found in district Lodhran. As regards the severity of root-knot nematodes, it was found to be the maximum (6.2) in Bahawalnagar followed by Vehari and Rahim Yar Khan with severities of 6.0 and 5.8, respectively. On the contrary, the severity was the minimum (0.6) in district Lodhran. Of all the associated species of root-knot nematodes, *M. incognita* constituted 73%, *M. javanica* 24%, *M. arenaria* 2% and *M. hapla* 1%. The study will aid in developing control strategies for the management of these nematodes accordingly.

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Authors' Contribution

MAH and TM designed the study, conducted the surveys, executed experimental work, analyzed the data and prepared the manuscript. TM supervised the work.

Key words

Incidence, Prevalence, *Meloidogyne* spp., Root-knot nematodes.

INTRODUCTION

The lucrative production of vegetables throughout the globe is threatened by large number of biotic factors including plant-parasitic nematodes (Hussain *et al.*, 2016; Kayani *et al.*, 2017, 2018; Khan *et al.*, 2017; Mukhtar *et al.*, 2017a, b, 2018; Tariq-Khan *et al.*, 2017). Different nematode species attack vegetables and among these, nematodes of the genus *Meloidogyne*, commonly known as root-knot nematodes have been found seriously affecting okra all over the world (Hussain *et al.*, 2016a, b). These nematodes have great destructive potential and can incur enormous yield losses (Mukhtar, 2018).

Okra, *Abelmoschus esculentus* (L.) Moench is regarded as one of the most important and oldest crops of the world. It is cultivated in all the regions having Mediterranean, tropical and subtropical climates. The vegetable is rich in vital mineral salts like calcium and vitamins. In native language, it is called "Bhindi" and

enjoys a significant position among the summer vegetables in the country. It is normally cultivated during February to May in the Punjab province of the country. It is cultivated on an area of about 1.55×10^4 hectares with an annual production of 1.19×10^5 tons. The obtained yield of okra in the country is comparatively low as compared to high yielding countries due to legions of abiotic and biotic limitations including insect pests (Javed *et al.*, 2017a, b; Iftikhar *et al.*, 2018; Kassi *et al.*, 2018a, 2019a, b; Nabeel *et al.*, 2018) and diseases (Ashfaq *et al.*, 2017; Aslam *et al.*, 2017a, b, 2018; Fateh *et al.*, 2017).

Among biotic factors which account for the reduction in crop production, root-knot nematodes are regarded as serious pests and are of substantial economic significance. These nematodes have been reported to incur yearly losses to the tune of 22% in tropical environments (Sasser, 1979). Bhatti and Jain (1977) reported yield losses to the extent of 99% in India. Losses in Pakistan due to nematodes to crops have been found more serious and complex as compared to the developed countries owing to numerous causes. Firstly, the country is positioned in the tropical region where the environmental conditions are encouraging for infectivity, growth, and reproduction of these nematodes all the year

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round. Secondly, the arid zone of the country being sandy in nature is favorable for the activities of these nematodes. Lastly, the cultivation of perennial crops or susceptible crops year after year in the same piece of land in the irrigated plains permits rapid multiplication of nematodes which results in severe infections and damage. On the other hand, entomopathogenic nematodes can reduce the incidence and severity of root-knot nematodes (Rahoo *et al.*, 2017, 2018a, b, 2019a, b). Root-knot nematodes have also been found associated with fungal and bacterial pathogens resulting in disease complexes and aggravate the severity of the latter (Kayani and Mukhtar, 2018).

Little attention has been paid to plant-parasitic nematodes in Pakistan and a small number of surveys were conducted in the past to record association and infestation of these nematodes with vegetables and other crops (Khan *et al.*, 2005). To develop suitable and sustainable approaches to manage and control root-knot nematodes, a better comprehension of nematode distribution in a territory and association of root-knot nematodes with the host is essential. As very little information is available about the distribution of root-knot nematodes in okra plantations, therefore, in the present studies surveys were conducted to record incidence, prevalence, and severity of root-knot nematode species associated with okra in the major vegetable growing districts of the Punjab province of the country. The study will aid in developing control strategies for the management of these nematodes accordingly.

MATERIALS AND METHODS

Distribution of root-knot nematodes

The distribution and severity of root-knot nematodes in okra plantations were determined in randomly selected sites of major vegetable producing districts of Punjab. For this purpose, fields were selected at random from each randomly selected locality and from each field; twenty five plants were marked after every ten steps in a zigzag pattern. The marked plants were excavated up to a soil depth of 20 cm using a spade. The adhering soil particles were gently removed from the root system and roots were examined for the presence or absence of galls.

The incidence of root-knot nematodes in each okra field was calculated as followed:

$$\text{Incidence (\%)} = \frac{\text{Total No. of infected plants}}{\text{Total No. of observed plants}} \times 100$$

Similarly, the prevalence of root-knot nematodes in each district was determined as followed:

$$\text{Prevalence (\%)} = \frac{\text{No. of fields infected with RKN}}{\text{Total No. of fields surveyed}} \times 100$$

Severity of root-knot nematodes

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 severity of root-knot nematodes of the root system of each randomly selected plant was assessed by following the galling index (Table I) developed by Bridge and Page (1980).

Table I.- Description of root-knot galling index.

Galling index	Description
0	No knots/galls
1	Few small knots difficult to find
2	Small knots only but visible, main root clean
3	Some larger knots, main root clean
4	Larger knots predominance but main root clean
5	50% roots infested, knotting on some main roots, reduced root system
6	Knotting on main roots
7	Majority of main roots are knotted
8	All main roots including tap root, knotted, few clean roots visible
9	All roots severely knotted, plant usually dying
10	All roots severely knotted, no root system, plant usually dead

Identification of Meloidogyne species

Identification of root-knot nematodes (*Meloidogyne* species) was done on the basis of female perineal patterns (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 a 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 coverslip was applied and sealed with nail polish, and was observed under microscope. The perineal pattern was compared with standard diagrams and *Meloidogyne* species was identified. In this way, perineal patterns of forty females were made from each infected okra field and the distribution of each *Meloidogyne* species in each district was calculated.

RESULTS

The overall incidence, prevalence, and severity of root-knot nematodes in Punjab have been shown in Figure 1.

Incidence

Differences in the incidence of root-knot nematodes were found from all the districts ranging from 11% to 70% (Fig. 2). The incidence was found to be the maximum (70%) in districts of Bahawalnagar and Rahim Yar Khan followed by Dera Ghazi Khan and Vehari showing incidences of 66 and 63%, respectively. On the other hand, the incidence was found to be the minimum (4%) in Lodhran followed by Jhang and Rajan Pur districts showing incidences of 11 and 13%, respectively. The rest of the districts showed incidences ranging from 13 to 63% (Fig. 2).

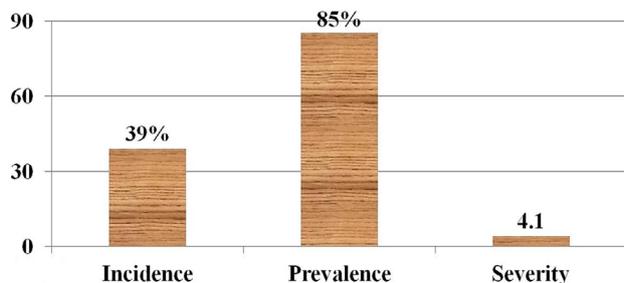


Fig. 1. Overall incidence, prevalence and severity of root-knot nematodes on okra in Punjab.

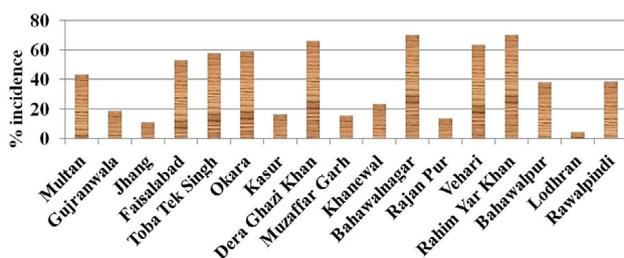


Fig. 2. Incidence of root-knot nematodes on okra in seventeen districts of Punjab.

Prevalence

Variations were also recorded in the prevalence of root-knot nematodes in the seventeen districts of the Punjab province. The prevalence was found to be 100% in seven districts while the remaining districts showed prevalence below 100% and the minimum was found in district Lodhran as shown in Figure 3.

Severity

As regards the severity of root-knot nematodes,

it was found to be the maximum (6.2) in Bahawalnagar followed by Vehari and Rahim Yar Khan with severities of 6.0 and 5.8, respectively. On the contrary, the severity was the minimum (0.6) in district Lodhran. The severity in terms of the galling index in each district has been given in Figure 4.

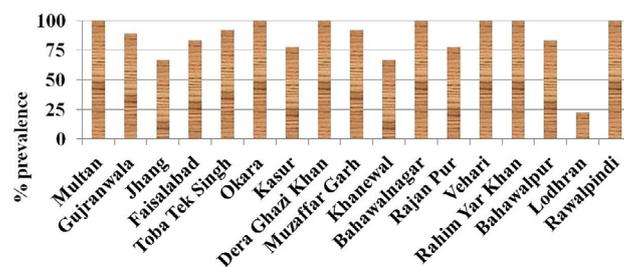


Fig. 3. Prevalence of root-knot nematodes on okra in seventeen districts of Punjab.

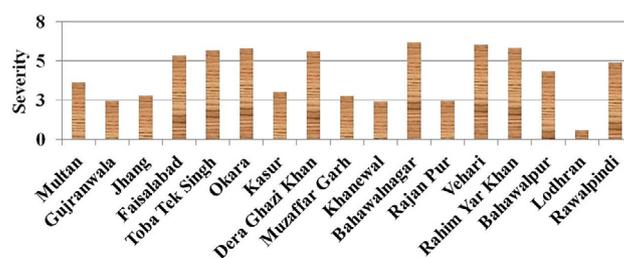


Fig. 4. Severity of root-knot nematodes on okra in seventeen districts of Punjab.

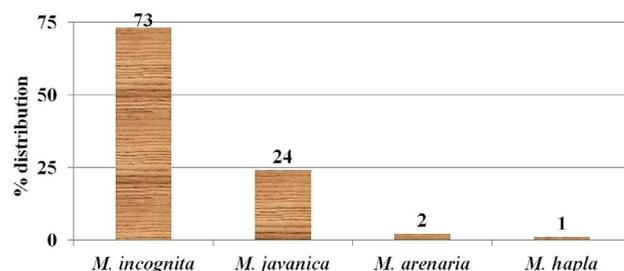


Fig. 5. Overall distribution of *Meloidogyne* species associated with okra in Punjab.

Distribution

The association of *Meloidogyne* species with okra in the surveyed districts also showed variations in distribution. Of all the associated species of root-knot nematodes, *M. incognita* constituted 73%, *M. javanica* 24%, *M. arenaria* 2% and *M. hapla* 1% (Fig. 5). *M. incognita* and *M. javanica* were reported from all the districts while *M. arenaria* and *M. hapla* were recorded from district Rawalpindi only. Of all the root-knot nematode species, *M. incognita* was found to be the most predominant (Fig. 5).

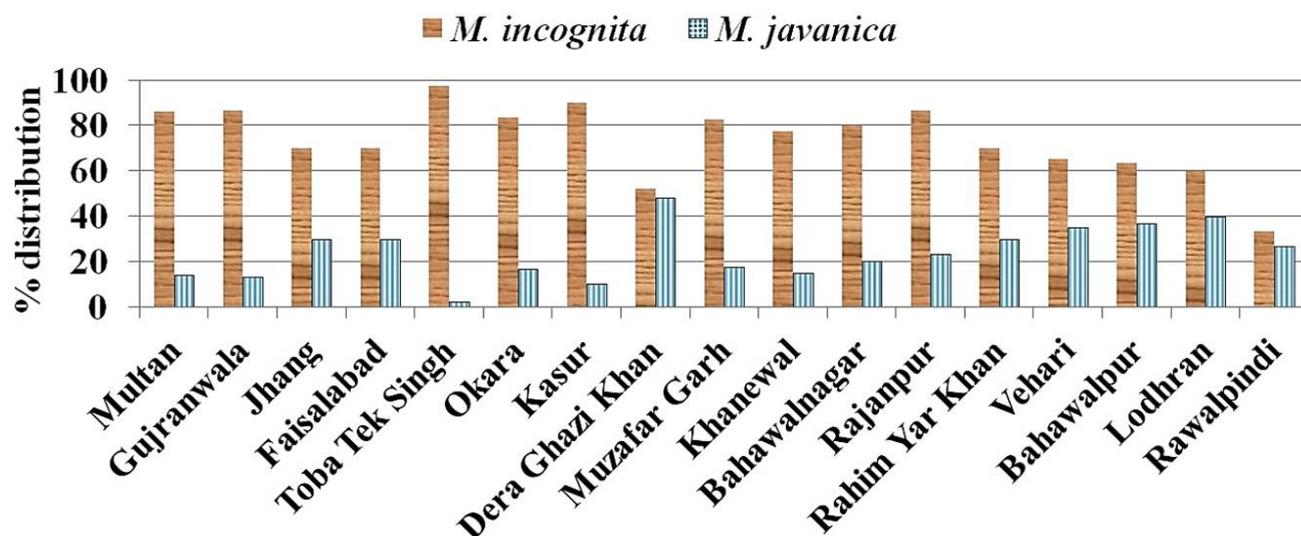


Fig. 6. Distribution of *Meloidogyne* species associated with okra in seventeen districts of Punjab.

The maximum mean incidence of *M. incognita* (98%) was recorded from Toba Tek Singh while the minimum incidence of 33% was found in Rawalpindi District. Similarly, the maximum incidence of *M. javanica* (48%) was observed in Dera Ghazi Khan and the minimum of 3% was recorded from Toba Tek Singh. The individual percentage of each *Meloidogyne* species in each district is given in Figure 6.

DISCUSSION

In the present study, differences were observed in the incidence, prevalence, and severity of root-knot nematodes in the surveyed districts of the Punjab province. Earlier surveys conducted by other researchers also showed similar results about the distribution of different plant-parasitic and root-knot nematodes associated with vegetables which corroborated the findings reported in this paper (Khan *et al.*, 2005; Shahid *et al.*, 2007). The findings of these scientists confirmed that okra was the most vulnerable to be attacked by root-knot nematodes in different vegetable growing areas. In the present study, variations were observed in the incidence, prevalence, and severity of root-knot nematodes in the surveyed districts. The variations in these parameters can be ascribed to a number of abiotic factors as differences have been observed in climatic and edaphic parameters in the surveyed districts. Incidence, prevalence, and severity of root-knot nematodes have also been reported to be influenced by changing agro-climatic conditions of the surveyed districts, soil type, moisture, soil pH and particular cropping sequence (Sasser and Carter, 1985; van Gundy, 1985).

There are reports that the distribution of root-knot nematodes is affected by the physical, chemical and biological properties of the soil environment (Upadhyay *et al.*, 1972). For example, in the present study, quite low prevalence of root-knot nematodes was recorded in case of district Lodhran. The low prevalence can be attributed to high quantities of organic matter in the soils. Another reason for this low prevalence was that okra was sown in lands which were fallow for the last few years. The findings of other researchers also confirmed this fact that fallowing resulted in an increase in the contents of organic matter and consequently caused a decline in nematode populations (Aung and Prot, 1990; Floret and Serpantie, 1993).

Hundred percent incidences of root-knot nematodes were recorded in the districts of Bahawalnagar, Multan, Okara, Rahim Yar Khan, Rawalpindi and Vehari. The possible reasons for this high incidence could be the intensive vegetable cropping patterns and the growing of susceptible vegetables on the same piece of land year after year which results in rapid multiplication of root-knot nematodes. In previous studies, many researchers have claimed that the presence of root-knot nematodes in abundant quantities mostly depends on the continuous availability of suitable hosts (Cuc and Prot, 1992). Growing of crop varieties susceptible to nematodes, relatively less annual rainfall, and high temperatures might also result in high incidence and severity of root-knot nematodes in the surveyed districts as these factors are favorable for rapid multiplication, development, and infection of root-knot nematodes. A number of researchers also reported that the type of soil also greatly influenced nematode populations in

a certain area (Wallace, 1969; Prot and van Gundy, 1981).

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 (Bhosle *et al.*, 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. In another study, Kayani *et al.* (2013) reported the prevalence of *Meloidogyne* spp. in cucumber plantations in the Pothwar region of the Punjab province of Pakistan. Of all the associated species of root-knot nematodes, *M. incognita* constituted 78%, *M. javanica* 19%, *M. arenaria* 2% and *M. hapla* 1%. *M. incognita* and *M. javanica* were recorded from all of the districts, with *M. incognita* being predominant. *M. incognita* as a pure population was recorded from 30% of the villages, while the other three species were found as mixtures. The most common mixed population was that of *M. incognita* and *M. javanica*, recorded from 70% of the villages in the region.

CONCLUSION

The results showed that root-knot nematodes are fairly distributed in okra plantations in all the districts of Punjab in varying intensities. *M. incognita* was found to be the most predominant species which warrants that strict control measures should be adopted for its management.

Statement of conflict of interest

Authors have declared no conflict of interest.

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