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ASSOCIATION OF ROOT-KNOT NEMATODES (*MELOIDOGYNE* SPP.) WITH CUCUMBER IN THE POTHOWAR REGION OF THE PUNJAB PROVINCE OF PAKISTAN

Muhammad Zameer Kayani[1]*, Tariq Mukhtar[2] and Muhammad Arshad Hussain[3]

[1]*Green Belt Project, Department of Agriculture, Rawalpindi, Pakistan.*
[2]*Department of Plant Pathology, PMAS-Arid Agriculture University, Rawalpindi, Pakistan.*
[3]*Plant Pathology Section, Regional Agricultural Research Institute, Bahawalpur, Pakistan.*
Corresponding author E-mail: kianizmr@gmail.com

ABSTRACT

The root knot nematodes (*Meloidogyne* spp.) are one of the most harmful and most ubiquitous, there is no data about their association with cucumber in the Pothowar region of the Punjab province. Therefore, a survey was conducted to assess the incidence and intensity of root-knot nematodes on cucumber in 4 districts of the Pothowar region. The study revealed variation in incidence and severity of root-knot nematodes in the four districts. The incidence of root knot nematodes was the highest in district Rawalpindi (21.86%) followed by 13.89% in Attock. Of the four districts, minimum incidence of 10.97% was recorded from district Jhelum. The maximum mean severity measured in terms of galling index was found in district Rawalpindi (3.79) while the minimum was observed in district Jhelum (1.86). The mean severity of root-knot nematodes in districts of Attock and Chakwal were 2.66 and 2.19, respectively. Of all the associated species of root-knot nematodes, *M. incognita* constituted 78.53%, *M. javanica* 19.03%, *M. arenaria* 1.82 % and *M. hapla* 0.62%. All the four main *Meloidogyne* species were found in all the districts of Pothowar region except district Chakwal where *M. hapla* was not found. *M. incognita* was predominantly found in the all the four districts.

Key words: *Meloidogyne incognita*, *M. javanica*, *M. arenaria*, *M. hapla*, incidence, severity.

INTRODUCTION

Pakistan lies between 24-36° North and 61-76° East, with a subtropical climate in the warm temperate zone. This type of climate is suitable for the activity and reproduction of root-knot nematodes throughout the year. Similarly, sandy and warm soils, found in arid zones, are favourable for nematode development and infestation. Moreover, in the irrigated areas, monocropping causes considerable increase in the nematode population by constantly providing the same host. In many areas of the Pothowar region of Punjab province, 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 nematodes are of considerable economic importance and cause annual losses in tropics to an extent of 22 per cent (Sasser, 1979). To date more than 100 species of *Meloidogyne* have been reported from different parts of the world (Karssen & Moens, 2006). Among these four viz. *M. incognita*, *M. javanica*, *M. arenaria* and *M. hapla* are economically important (Eisenback & Triantaphyllou, 1991) and responsible for 95% of the infestations in cultivated lands (Sasser & Carter, 1982). These occur in the following order; *M. incognita*, 47%; *M. javanica*, 40%; *M. arenaria*, 7% and *M. hapla*, 6% (Sasser, 1980). The approximate distribution of *Meloidogyne* spp. in the soils of Pakistan is; *M. incognita*, 52%; *M. javanica*, 31%; *M. arenaria*, 8%; *M. hapla*, 7% and other species are about 2% (Maqbool, 1987).

Root-knot nematodes are becoming one of the most serious calamities for the successful cultivation of cucumber in the Pothowar region of the country. The estimated losses in cucumber have been reported up to 33 % (Sasser, 1979). The infestation of root-knot nematodes on cucumber in Pakistan has been found up to 52 % (Khan *et al*, 2005). Increased information on incidence and severity of root-knot nematodes in cucumber production areas is important for sustainable production of cucumber. Plant parasitic nematodes, in Pakistan, have received little attention and only a few surveys have been made in the past (Brown, 1962; Kafi, 1963; Saeed & Ashrafi, 1973; Ahmad & Khan, 1973; Khan *et al.,* 2005). There is limited information regarding the association of root knot nematodes with cucumber in the country. Therefore, the objective of present studies was to conduct a survey to determine and document the incidence and intensity of root-knot nematodes on cucumber cultivations in the vegetable growing areas of Pothowar region of the Punjab province of the country.

MATERIALS AND METHODS

Incidence and severity of root-knot nematodes

A survey of cucumber fields located in different randomly selected localities of the four districts (Rawalpindi, Jhelum, Chakwal and Attock) of Pothowar region of the Punjab province of Pakistan was conducted for the determination of incidence and severity of root-knot nematodes. Six localities were randomly selected from each tehsil and from each locality; three fields of cucumber were randomly selected**.** From each cucumber field, 20 plants were selected after each 10 steps following zigzag pattern. The selected plants were carefully uprooted up to 15-30 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 incidences of root-knot nematodes of individual cucumber fields were determined as followed.

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

The root systems of individual plants were rated following the galling index developed by Bridge and Page (1980) for the determination of 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 Department, Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi for identification of root-knot nematode species.

Identification of *Meloidogyne* Species

Root-knot nematodes (*Meloidogyne* species) were identified on the basis of female perineal patterns described by Taylor & Netscher (1974). Mature females of root-knot nematodes were dissected out from the infected cucumber 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 percent 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 *Meloidogoyne* species was identified. In this way perineal patterns of 40 females were prepared from each infected cucumber field and the distribution of each *Meloidogyne* species in each tehsil was calculated.

RESULTS

Incidence of Root-Knot Nematodes

The root knot nematodes (*Meloidogyne* spp.) were recorded with varying degrees in cucumber fields of districts of Rawalpindi, Attock, Jhelum and Chakwal. The highest disease incidence of 21.86% was recorded in Rawalpindi district followed by 13.89% in Attock. Of the four districts, minimum incidence of 10.97% was recorded from district Jhelum as shown in Table 1.

Within the districts, the variations in incidence were also found. In District Rawalpindi, maximum incidence (31.11 %) was recorded in Tehsil Taxila while minimum (6.94 %) was observed in Tehsil Kotlisattian. In District Attock, maximum incidence was recorded in Tehsil Pindi Gheb and minimum in Tehsil Fateh Jang. The mean incidence in other tehsils ranged between 8.06 to 17.50 %. Similarly, in district Jhelum, the incidence of root-knot nematodes ranged between 7.78 to 14.44 %; Tehsil Pind Daden Khan showing the maximum incidence of 14.44 per cent. In district Chakwal, the maximum incidence of 15.56% was found in Tehsil Choa Syedan Shah and minimum of 10 and 10.28% were in Kallar Kahar and Talagang as shown in Table 2.

It is evident from the dendrogram in Figure-I that on the basis of incidence, two main clusters were formed. Cluster-1 comprised five tehsils viz. Taxila, Murree, Kahuta, Kallar Syedan and Gujjar Khan with mean incidence above 20%. All the tehsils in this group fall in District Rawalpindi. The rest of the tehsils form cluster-2 with mean incidence below 18%. The cluster-2 is subdivided into two clusters. In cluster 2A, tehsils showing mean incidence from 6.94 to 11.11% are included, while in cluster 2B, tehsils of Pind Daden Khan, Choa Syedan Shah, Attock, Hazro, Pindi Gheb and Rawalpindi are included with mean root-knot incidence ranging from14.44 to 17.78%.

Severity of Root-Knot Nematodes

The maximum mean severity as measured in terms of galling index suggested by Bridge and Page (1980) was found in district Rawalpindi (3.79) while the minimum was observed in district Jhelum (1.86). The mean severity of root-knot in districts of Attock and Chakwal were 2.66 and 2.19, respectively (Table 1).

Differences in root-knot severity were also observed among tehsils of each district. In district Rawalpindi, mean severity of 5.39 was observed in tehsil Taxila while it was the minimum in Tehsil Kotlisattian. The rest of tehsils

showed severity above 3. In District Attock, tehsil Attock and Pindi Gheb showed severity above 3 while a minimum severity of 1.56 was recorded in tehsil Fateh Jang. The mean intensities of root-knot nematodes were found to be lower in tehsils of Jhelum and Chakwal and ranged from 1.22 to 2.72 (Table 2).

The dendrogram in Fig. 2 constructed on the basis of severity of root-knot nematodes formed two main clusters at 2.5 linkage distance. There are three tehsils in Cluster-1 viz. Taxila, Murree and Kallar Syedan with mean severities of 5.39, 4.33 and 4.83, respectively. All the tehsils in this group fall under District Rawalpindi. The second cluster comprised the rest of the tehsils with mean severity ranging from 1.22 to 3.33. The second cluster is divided into two sub-clusters. In cluster 2A, tehsils of Sohawa, Kallar Kahar, Talagang, Jhelum, Fateh Jang and Kotlisattian showing mean severity of root-knot nematode from 1.22 to 1.83 are included, while the tehsils included in cluster 2B ranged in severity from 2.17 to 3.33.

Table I. District wise incidence and severity of root-knot nematodes in Pothowar region.

District	Mean incidence	Mean intensity
Rawalpindi	21.83 ± 1.51	3.79 ± 0.25
Attock	13.89 ± 1.38	2.66 ± 0.26
Jhelum	10.97 ± 1.49	1.86 ± 0.25
Chakwal	12.22 ± 1.39	2.19 ± 0.24
Overall	**15.66 ± 0.78**	**2.80 ± 0.13**

Table 2. Tehsil wise incidence and severity of root-knot nematodes in Pothowar region.

District	Tehsil	Mean incidence	Mean intensity
Rawalpindi	Rawalpindi	17.78 ± 3.16	3.33 ± 0.60
	Gujjar khan	21.67 ± 3.98	3.61 ± 0.76
	Kallar Syedan	23.33 ± 3.45	4.83 ± 0.62
	Taxila	31.11 ± 4.34	5.39 ± 0.64
	Kahuta	24.17 ± 4.51	3.44 ± 0.67
	Murree	26.11 ± 3.85	4.33 ± 0.50
	Kotlisattian	6.94 ± 1.62	1.53 ± 0.39
	Average	**21.83 ± 1.51**	**3.79 ± 0.25**
Attock	Hasan Abdal	12.50 ± 3.06	2.61 ± 0.60
	Hazro	15.83 ± 4.70	2.78 ± 0.75
	Attock	15.83 ± 3.48	3.44 ± 0.72
	Fateh Jang	8.06 ± 2.72	1.56 ± 0.50
	Jand	13.61 ± 3.52	2.56 ± 0.71
	Pindi Gheb	17.50 ± 2.40	3.00 ± 0.38
	Average	**13.89** ± 1.38	**2.66** ± 0.26
Jhelum	Jhelum	10.56 ± 2.86	1.78 ± 0.48
	Pind Daden Khan	14.44 ± 3.68	2.33 ± 0.60
	Sohawa	7.78 ± 2.15	1.22 ± 0.32
	Dina	11.11 ± 3.04	2.17 ± 0.57
	Average	**10.97** ± 1.49	**1.86** ± **0.25**
Chakwal	Chakwal	13.06 ± 2.69	2.50 ± 0.47
	Choa Syedan Shah	15.56 ± 3.02	2.72 ± 0.51
	Talagang	10.28 ± 2.73	1.83 ± 0.47
	Kallar Kahar	10.00 ± 2.74	1.72 ± 0.47
	Average	**12.22** ± 1.39	**2.19** ± 0.24
Overall Mean		**15.66** ± 0.78	**2.80** ± 0.13

Occurrence of Root-Knot Nematodes Species

The different species of *Meloidogyne* associated with cucumber in different districts are given in Table 3. Of all the associated species of root-knot nematodes, *M. incognita* constituted 78.53%, *M. javanica* 19.03%, *M. arenaria* 1.82 % and *M. hapla* 0.62%.

All the four main *Meloidogyne* species were found in all the districts of Pothowar region except district Chakwal where *M. hapla* was not found. *M. incognita* was predominantly found in all the four districts. The individual percentage of each *Meloidogyne* species in each district is given in Table 3.

In district Rawalpindi, the percentage of *M. incognita* was the highest in tehsil Kahuta and Taxila; being 90.00 and 88.33% respectively and was the lowest (47.5%) in tehsil Gujjar Khan. As regards *M. javanica*, it was found in highest degree in tehsil Gujjar Khan and was the lowest in tehsil Kahuta. *M. hapla* was recorded from only Murree and Kotlisattian by 9.33 and 3.33% respectively. On the other hand *M. arenaria* was recorded from Rawalpindi and Gujjar Khan tehsils as shown in Table IV. In other districts, *M. incognita* and *M. javanica* were recorded with varying degrees; *M. incognita* being predominant in all the tehsils. *M. hapla* was recorded from tehsil Attock only and not from tehsils of Jhelum and Chakwal districts. On the other hand *M. arenaria* was recorded from only Attock and Chakwal tehsils. In district Jhelum, *M. arenaria* was found in all the tehsils except tehsil Dina as shown in Table 4.

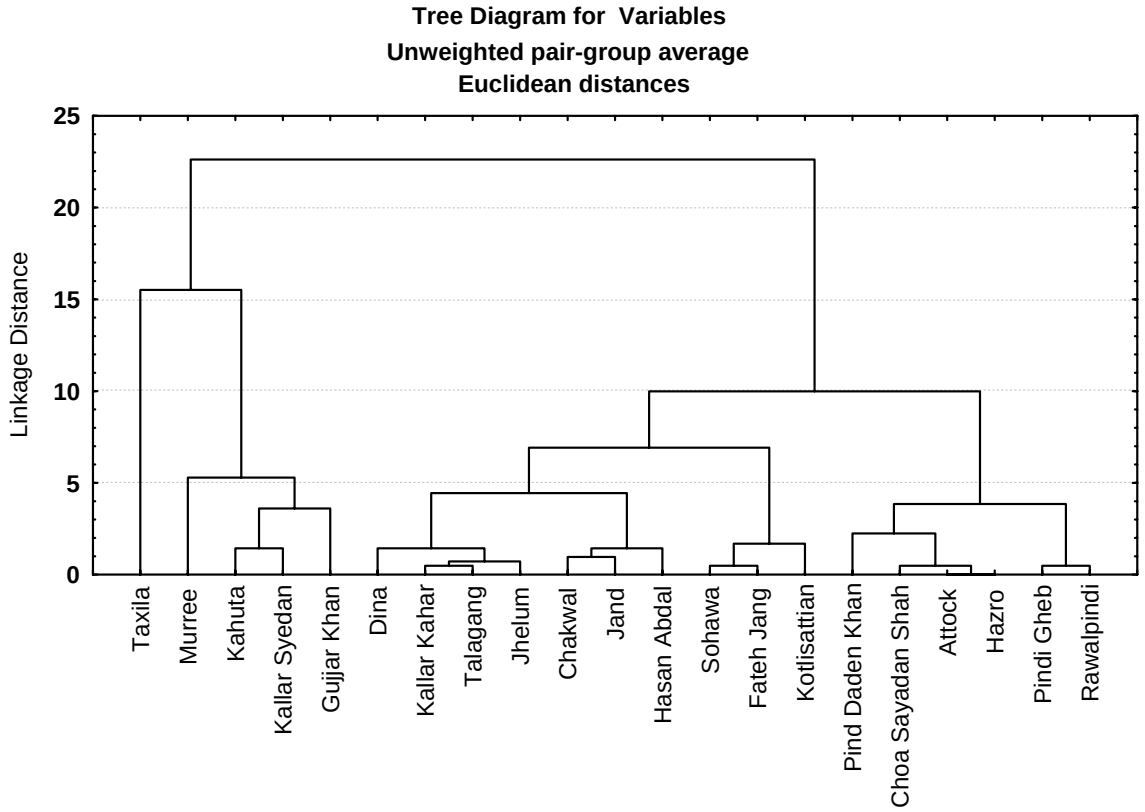

Fig. I. Dendogram showing clusters on the basis of incidence of root-knot nematodes in 21 tehsils of four districts of Pothowar region of the Punjab Province.

Table 3. District wise distribution of *Meloidogyne* species in Pothowar region.

District	**Percentage of *Meloidogyne* species**			
	M. incognita	***M. javanica***	***M. hapla***	***M. arenaria***
Rawalpindi	74.17	21.69	1.81	2.33
Attock	80.74	17.38	0.56	1.32
Jhelum	84.93	12.25	0.11	2.71
Chakwal	74.27	24.79	0.00	0.94
Over all	**78.53**	**19.03**	**0.62**	**1.82**

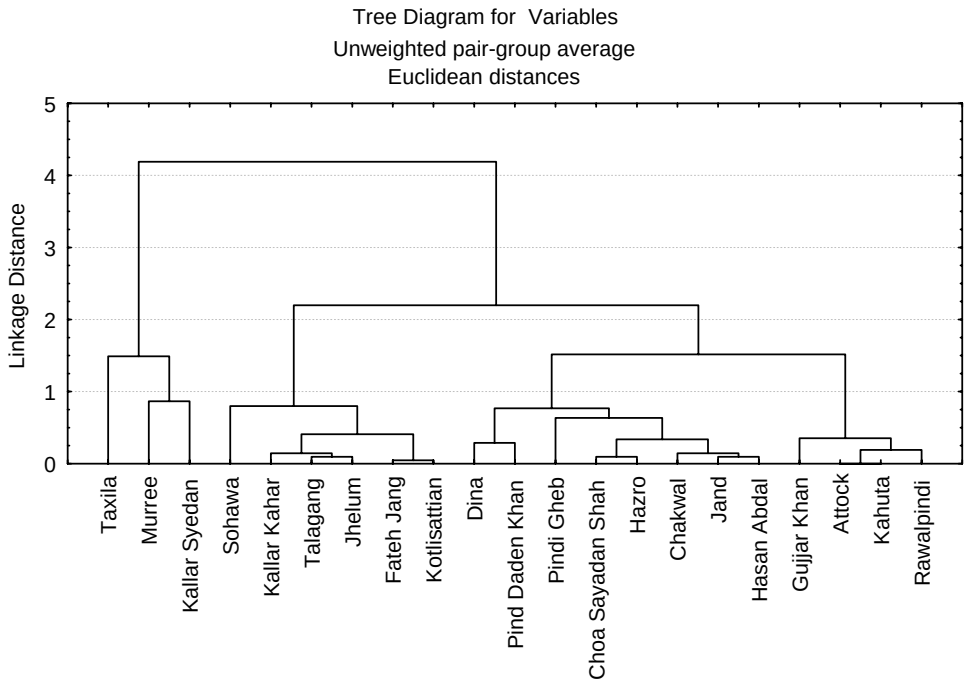

Fig. 2. Dendogram showing clusters on the basis of severity of root-knot nematodes in 21 tehsils of four districts of Pothowar region of the Punjab Province.

Table 4. Tehsil wise distribution of *Meloidogyne* species in Pothowar region.

District		**Percentage of *Meloidogyne* species**			
	Tehsil	***M. incognita***	***M. javanica***	***M. hapla***	***M. arenaria***
Rawalpindi	Rawalpindi	58.67	36.67	0.00	4.67
	Gujjar khan	47.50	40.83	0.00	11.67
	Kallar Syedan	84.00	16.00	0.00	0.00
	Taxila	88.33	11.67	0.00	0.00
	Kahuta	90.00	10.00	0.00	0.00
	Murree	67.33	23.33	9.33	0.00
	Kotlisattian	83.33	13.33	3.33	0.00
	Average	**74.17**	**21.69**	**1.81**	**2.33**
Attock	Hasan Abdal	77.92	22.08	0.00	0.00
	Hazro	86.25	13.75	0.00	0.00
	Attock	70.83	17.92	3.33	7.92
	Fateh Jang	80.56	19.44	0.00	0.00
	Jand	85.56	14.44	0.00	0.00
	Pindi Gheb	83.33	16.67	0.00	0.00
	Average	**80.74**	**17.38**	**0.56**	**1.32**
Jhelum	Jhelum	85.00	9.44	0.00	5.56
	Pind Daden Khan	88.33	8.89	0.00	2.78
	Sohawa	84.44	11.67	0.00	3.89
	Dina	86.11	13.89	0.00	0.00
	Average	**84.93**	**12.25**	**0.11**	**2.71**
Chakwal	Chakwal	82.08	14.17	0.00	3.75
	Choa Syedan Shah	70.00	30.00	0.00	0.00
	Talagang	85.56	14.44	0.00	0.00
	Kallar Kahar	59.44	40.56	0.00	0.00
	Average	**74.27**	**24.79**	**0.00**	**0.94**
Overall Mean		**78.53**	**19.03**	**0.62**	**1.82**

DISCUSSION

The results of the present survey showed variations in the incidence and severity of root-knot nematodes in different districts and tehsils of Pothowar region of the Punjab province of Pakistan. Similar results were also reported by Lamberti *et al.* (1975); Bhatti & Jain (1977); Khan *et al.* (2005); Shahid *et al*. (2007). Our results confirmed the above findings regarding the prevalence of plant parasitic nematodes and occurrence of *Meloidogyne* species on vegetables. It is clear from the results that cucumber crop is the most susceptible host of *Meloidogyne* species in vegetable growing areas. The 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).

Maximum incidence of root-knot disease was found in district Rawalpindi. This high incidence is due to intensive 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 dependable upon the presence of the suitable host plants (Jacq & Fortuner, 1979; Yeates, 1981; Ferris, 1985; Cuc & Prot, 1992).

In the present studies *M. incognita* and *M. javanica* were found in all the tehsils of all the districts in varying proportions and *M. incognita* was predominant. 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; Ravichandra & Krishnappa, 2004; 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. hapla* was only found in tehsils of Murree, Kotlisattian and Attock. The climate of these tehsils 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. *M. arenaria* was found in those tehsils where groundnut is widely cultivated. 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 cucumber 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.

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