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Root-knot Nematodes (*Meloidogyne* spp.) Infecting Peach (*Prunus persica* L.) in the Pothwar Region of Pakistan

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ABSTRACT

The aim of the present study was to determine the prevalence and severity of root-knot nematodes in peach orchards in the Pothwar Region of Pakistan. Thirty-seven peach orchards were surveyed in the region and root and soil samples were collected from 10 randomly selected plants in each orchard for analysis. In the region, an overall incidence rate of 19.8% and a prevalence rate of 65.7% of root-knot nematodes were observed. The district Attock exhibited the highest prevalence rate of 71.43% and incidence rate of 34.29%. Conversely, the district Islamabad displayed the lowest prevalence rate of 50.5% and incidence rate of 10%. The surveys encountered five distinct peach varieties, and Early Grand had the highest prevalence (85.71%) of root-knot nematodes while Local Aroo showed the lowest (50%). Florida Gold and Aroo-5 were not infested with root-knot nematodes. In the region, peach trees were found to be infected by two types of root-knot nematodes, namely, *Meloidogyne incognita* and *M. javanica*., which was more prevalent than *M. incognita*, and it dominated all districts, except for Attock, where *M. incognita* was dominant. *M. incognita* was not present in Islamabad district. The occurrence of *M. incognita* and *M. javanica* as single populations were observed in 26.09 and 43.48% of orchards, respectively, while mixed populations were found in 30.43% of peach orchards. The overall galling index of the root-knot nematodes was 1.33, with the highest index in Attock and the lowest in Islamabad districts. Among the different peach cultivars, the Early Grand cultivar exhibited the maximum galling index, whereas no galling index was observed in the Florida Gold and Aroo-5 cultivars. It is recommended that stringent control strategies should be adopted to prevent the spread of nematodes to new plantations and to eradicate them from established orchards.

Keywords: Early grand cultivar, Galling index, Incidence rate, Phytopathogenic nematodes, Prevalence rate.

INTRODUCTION

Peach (*Prunus persica* (L.) Batsch) occupies a central position among stone fruit, which is grown widely throughout the world (Eldem *et al*., 2012). Peach is the second largest stone fruit after apricots among stone fruits of Pakistan. In Pakistan, peaches were fundamentally developed in Khyber Pukhtunkhwa, Balochistan and some low chill areas of Pothwar Zone of Punjab. It has been cultivated on an area of 9,800 hectares with a total production of 75,400 metric tons (FAO, 2021). The lucrative production of peaches and nectarines has been threatened for many years by an assortment of biotic factors including diseases like peach leaf curl, Peach Tree Short Life (PTSL) and nematodes. Phytopathogenic nematodes viz. root-knot nematodes, cyst nematode, root lesion nematodes etc. have economic significance in agriculture and are directly and/or indirectly associated with crop damages leading to yield losses in various vegetables, fruits and crops (Bogner *et al*., 2017; Asghar *et al*., 2020; Tariq-Khan *et al*., 2017, 2020; Ahmed *et al*., 2021; Ullah and Khanum, 2022). These nematodes have been reported

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to incur about $173 billion yield losses annually on different agricultural crops. The genus *Meloidogyne* exhibits the highest level of destructiveness and holds the top position among phytopathogenic nematodes (Termorshuizen *et al*., 2011; Kim *et al*., 2016; Gamalero and Glick, 2020; Shahid *et al*., 2022, 2023).

Root-knot nematodes (*Meloidogyne* spp.) have been found seriously infecting peaches and have become a severe issue for the majority of peach growers and nurserymen in many regions having tropical and Mediterranean climates (Lamberti, 1979). Root-knot nematodes have been found prevalent in temperate, tropical, and equatorial regions of the world (Moens *et al*., 2009; Nyczepir, 2011; Kayani and Mukhtar, 2018; Mukhtar and Kayani, 2019, 2020). Root-knot nematodes cause reduction in fruit production of many economically important species of *Prunus* including *Prunus persica*. Among different species of root-knot nematodes, *Meloidogyne incognita* and *M. javanica* are the most common in peach and plum orchards (Nyczepir and Becker, 1998). The occurrence of *M. incognita* and *M. javanica* was recorded in 95% and 5% of sampled peach orchards, respectively, in South Carolina (Nyczepir *et al*., 1997).

The characteristic underground symptoms caused by root-knot nematodes are the formation of galls on roots and the stunting of aboveground parts of 1 to 2 years old peach trees. Defoliation at early stages, unthrifty tree growth, and reduction in biomass and fruit yield are among the other aboveground symptoms. The nematodes cause occasional death of infected trees (Nyczepir *et al*., 1993; Nyczepir and Thomas, 2009). Under drought conditions, the observable signs of the nematode on the foliage become more noticeable, particularly in sandy soil.

In addition to their direct effects, root-knot nematodes can also interact with pathogenic fungi and bacteria, forming disease complexes that exert additional detrimental effects on plant health (Aslam and Mukhtar, 2023a, b). Peach trees infected with PTSL or with unthrifty growth have been found cohabiting with *Meloidogyne* spp. and *Criconemella xenoplax* (Raski) Luc and Raski. The infestation of peach trees with both *C. xenoplax* and *M. javanica* have also been associated with sparse root system, premature leaf drop and peach shoot die-back (Hugo and Meyer, 1995). More than 50 percent peach orchards in the southeastern United States with a history of PTSL showed the presence of root-knot nematodes and *C. xenoplax* (Nyczepir *et al*., 1985). Similarly, a synergistic interaction between both nematodes resulting in an increased reduction in growth of peach has also been reported (Nyczepir *et al*., 1993).

Production of peach in Pakistan is not as much as many advanced countries. There are many limiting factors for this low production, but peach leaf curl and some insect pests are the major concerns of the farmers and are managed accordingly. Despite the implementation of various management strategies in peach orchards, the condition of the orchards is poor. As there is no data on the presence of nematodes, especially root-knot nematodes, in peach orchards, the current study aimed to scientifically investigate and assess the occurrence, spatial distribution, and intensity of root-knot nematodes within the peach orchards located in the Pothwar Region of the Punjab Province in Pakistan. The findings of this study will aid in the development of effective control strategies to manage root-knot nematodes in the region, ultimately promoting the sustainable production of peaches.

MATERIALS AND METHODS

Sample Collection

A total of 37 peach orchards in the Pothwar Region of Pakistan were surveyed for the determination of incidence, prevalence, and infestation of root-knot nematodes (Figure 1). For this purpose, 10

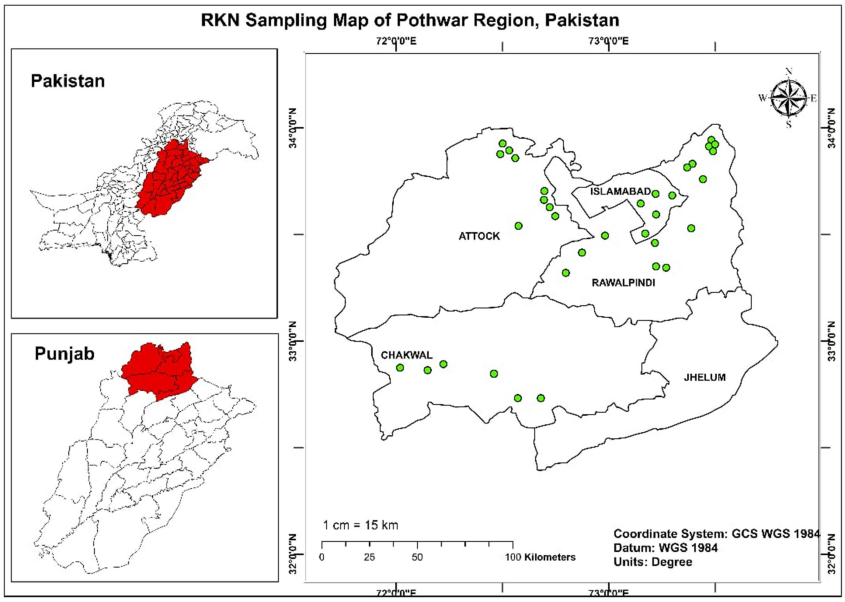

Figure 1. Map showing the locations of peach orchards for sampling root-knot nematodes.

plants were randomly selected from each orchard and root and soil samples (250 g) were collected from the four sides of the trees, 2.5 feet away from the main trunk, using an auger. The four collected cores were mixed together to create a composite sample of one kilogram. The samples were immediately brought to the Plant Nematology Laboratory for further studies. The roots were separated and washed free of adhering soil particles.

Determination of Incidence and Prevalence of Root-Knot Nematodes

In order to determine the incidence of root-knot nematodes, the root samples of each plant from each orchard were checked for the presence of galls produced by root-knot nematodes, and the incidence of that orchard was calculated as described by Mukhtar *et al*. (2013a). Similarly, incidence of each orchard and each district was calculated. Prevalence of each district was computed by dividing infected orchards by the total orchards. Similarly, the occurrence and frequency of root-knot nematodes were assessed on individual peach varieties.

Determination of Galling Index

The galling index on root system of each infected plant from each orchard was determined (Bridge and Page, 1980). Similarly, galling index from each district and on each variety was determined.

Identification of *Meloidogyne* Species

A composite sample was created for the detection of *Meloidogyne* species by combining and thoroughly mixing the infected root samples from all the peach orchards in each district. Perineal patterns of females were made for the identification of root-knot nematodes as outlined by Taylor and Netscher (1974) and observed under a stereomicroscope at 4×. *Meloidogyne* species were identified by comparing the perineal patterns with the standard diagrams (Eisenback *et al*., 1981). Similarly, perineal patterns of 20 randomly selected females from each infected composite root sample

from each peach orchard were made and the prevalence of each *Meloidogyne* species was determined. The occurrences of sole and mixed *Meloidogyne* populations were also found out in the four districts on each variety in the Pothwar region.

RESULTS

Incidence and Prevalence of Root-Knot Nematodes in Peach Orchards

In the Pothwar region of Pakistan, the occurrence of root-knot nematodes on peach trees was documented to have an overall incidence of 19.8% and a prevalence of 65.7% (Figure 2). When examining specific districts, district Attock exhibited the highest prevalence (71.43%) and incidence (34.29%), while district Islamabad had the lowest prevalence (50.5%) and incidence (10%) (Figure 3). The survey identified five different peach varieties in the region. The variety Early Grand displayed the highest prevalence of root-knot nematodes (85.71%), whereas Local Aroo had the lowest prevalence (50.0%). No infestation of root-knot nematodes was observed on Florida Gold and Aroo-5 varieties. Conversely, Florida King had the highest incidence of root-knot nematodes, while Local Aroo had the lowest incidence of 15% (Figure 4). Table 1 provides information on the occurrence and distribution of root-knot nematodes across the four districts of the Pothwar Region, categorized by different varieties.

Occurrence of Root-Knot Nematode Species

In the investigated region, peach trees were infected by two distinct species of root-knot nematodes, namely, *Meloidogyne incognita* and *M. javanica*. Through observations, it was determined that *M. javanica* exhibited a higher prevalence compared to *M. incognita*, as illustrated in Figure 5. Across all districts, *M. javanica* was the dominant species, except in Attock

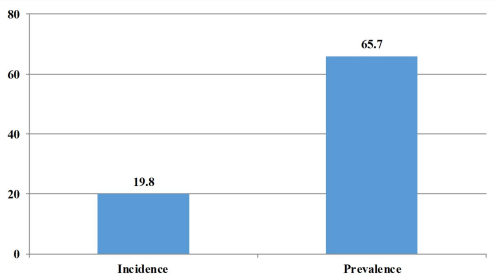

Figure 2. The overall incidence and prevalence of root-knot nematodes in the Pothwar Region.

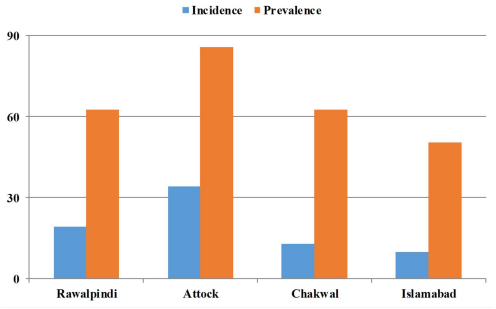

Figure 3. District wise incidence and prevalence of root-knot nematodes in Pothwar Region.

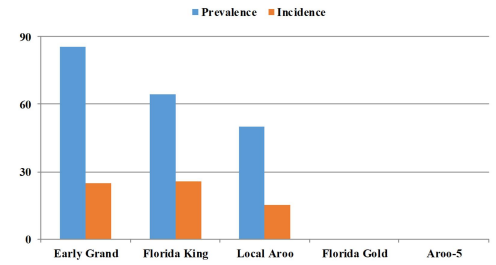

Figure 4. Variety wise incidence and prevalence of root-knot nematodes in the Pothwar Region.

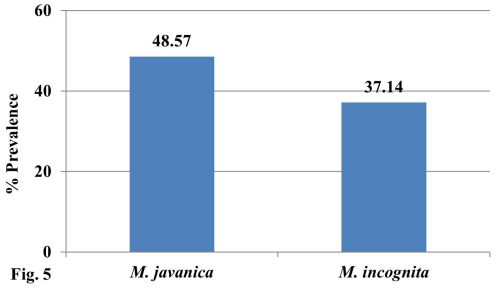

Figure 5. Overall prevalence of root-knot species in Pothwar Region.

Table 1. Variety wise incidence (%) and prevalence (%) of root-knot nematodes in the four districts of the Pothwar Region.

Variety	Rawalpindi		Attock		Chakwal		Islamabad	
	Incidence (%)	Prevalence (%)	Incidence (%)	Prevalence (%)	Incidence (%)	Prevalence (%)	Incidence (%)	Prevalence (%)
Early Grand	28.33	83.33	23.33	66.67	21.25	100.00	20.0	100.00
Florida King	20.00	75.00	42.50	100.00	4.5	25.00	10.0	50.00
Local Aroo	15.00	50.00	0.00	0.00	0.00	0.00	0.00	0.00
Florida Gold	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Aroo-5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

where *M. incognita* was the prevailing nematode. Notably, no instances of *M. incognita* infestation were recorded in the Islamabad District (Figure 6). *M. javanica*, as a sole population, was the most prevalent nematode species in the peach orchards of Pothwar Region with a prevalence of 43.48%, followed by *M. incognita* with a prevalence of 26.09%, while the combined prevalence of both species was 30.43% (Figure 7). The district wise and variety wise occurrence of root-knot nematode species in infected peach orchards as sole and mixed populations are given in Tables 2 and 3.

Quantification of Root-Knot Nematode-Induced Galling on Peach Roots

The comprehensive galling index of root-knot nematodes in the studied area was recorded as 1.33. District Attock exhibited the highest galling index, whereas the lowest was observed in Islamabad District (Figure 8). In a similar vein, Early Grand exhibited the highest galling index of 1.46, whereas Local Aroo displayed the lowest index of 1.08. Notably, Florida Gold and Aroo-5 cultivars remained free from root-knot nematode infestation, resulting in a galling index of zero (Figure 9). Table 4 presents the galling index of root-knot nematodes categorized by variety across the four districts of the Pothwar Region.

DISCUSSION

In the present study, differences were observed in the incidence, prevalence, severity, and species of root-knot nematodes in the surveyed peach orchards in all the districts of Pothwar Region of the Punjab Province of Pakistan. The variations observed in the distribution of root-knot nematodes across the studied areas can be attributed to a range of biotic and abiotic factors. These differences may arise from variations in environmental and edaphic conditions found throughout the districts of the region. Many earlier studies have confirmed that fluctuating agro-climatic factors and environments in the Pothwar Region, like cropping sequences, soil pH, moisture level, soil temperature, soil texture

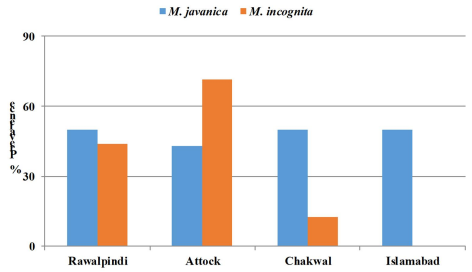

Figure 6. District wise prevalence of root-knot species in Pothwar Region.

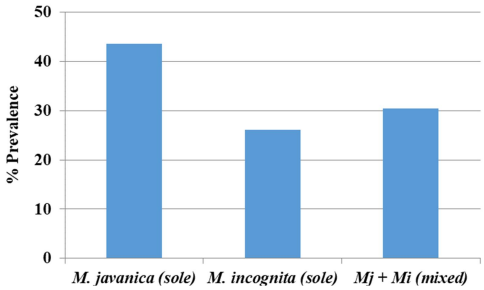

Figure 7. Prevalence of *Meloidogyne* spp. as sole and mixed populations in Pothwar Region.

Table 2. District wise occurrence (%) of root-knot nematode species as sole and mixed populations in the Pothwar Region.

District	*M. incognita*	*M. javanica*	*M. incognita* + *M. javanica*
Rawalpindi	20.00	30.00	50.00
Attock	50.00	16.67	33.33
Chakwal	20.00	80.00	0.00
Islamabad	0.00	100.00	0.00

Table 3. Variety wise occurrence of root-knot nematode species as sole and mixed populations in the four districts of the Pothwar Region.

Variety	Root-knot species	% Occurrence of root-knot species			
		Rawalpindi	Attock	Chakwal	Islamabad
Early Grand	*M. incognita*	20.00	16.67	20.00	00.00
	M. javanica	10.00	16.67	60.00	50.00
	Mi + Mj	20.00	00.00	00.00	00.00
Florida King	*M. incognita*	0.00	33.33	00.00	00.00
	M. javanica	10.00	00.00	20.00	50.00
	Mi + Mj	20.00	33.33	00.00	00.00
Local Aroo	*M. incognita*	0.00	00.00	00.00	00.00
	M. javanica	10.00	00.00	00.00	00.00
	Mi + Mj	10.00	00.00	00.00	00.00

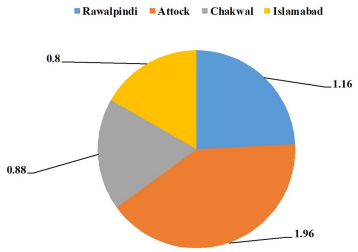

Figure 8. District wise galling index of *Meloidogyne* species in the Pothwar Region.

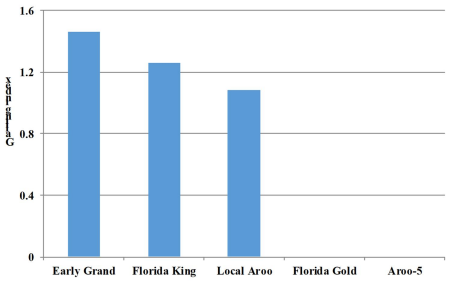

Figure 9. Variety wise galling index of *Meloidogyne* species in Pothwar Region.

Table 4. Variety wise galling index of *Meloidogyne* species across the four districts in Pothwar Region**.**

Variety	Rawalpindi	Attock	Chakwal	Islamabad
Early Grand	1.47	1.40	1.45	1.70
Florida King	1.38	2.38	0.30	0.75
Local Aroo	1.10	0.00	0.00	0.00
Florida Gold	0.00	0.00	0.00	0.00
Aro-5	0.00	0.00	0.00	0.00

and structure greatly affect incidence, prevalence, severity, and distribution of root-knot nematodes (Van Gundy, 1985; Kayani *et al*., 2013).

Attock District exhibited the highest occurrence, prevalence, and intensity of root-knot nematodes, whereas the lowest levels were observed in Islamabad. The high incidence, prevalence, and severity of root-knot nematodes can be attributed to several factors. One significant factor is that a large number of farmers in the area have planted uncertified peach plantlets that were raised and developed in soils infested with root-

knot nematodes. There is also lack of proper guidance for the farmers regarding nematode free nursery. Planting of peach orchards in infested soils is another reason for this high incidence of root-knot nematodes. Most of the peach orchards surveyed were established on soils that remained under vegetable cultivation for many years with a history of high populations of root-knot nematodes, and were not treated prior to transplantation of peach plantlets. Moreover, many peach orchards were found adjacent to vegetable fields, which had high incidences of root-knot nematodes. As the topography of Pothwar Region is undulating, receiving high rains throughout the year, so, the flooding water is the main source of their dispersal. The other reasons for the spread and development of root-knot nematodes are the incognizance of the farmers about the nematode. They are lacking knowledge or awareness regarding the mechanism of invasion and spread of the nematode. The nematode can survive on alternative plants, especially vegetables and weeds, which remain flourishing in the majority of orchards, and the inoculum continues to multiply. The farmers are mostly poor and do not apply nematicides and other pesticides for the management of nematodes or other pests. Continuous growing of vegetables in peach orchards, sandy nature of most soils, and the cultivation of suitable hosts throughout all the seasons of the year in the district favored the fast development and reproduction of root-knot nematodes. Previous findings of many scientists also confirmed that high populations of root-knot nematodes in soils were greatly influenced by, and depended upon, the cultivation of suitable crops (Cuc and Prot, 1992; Kayani *et al*., 2013). The low incidence and prevalence of root-knot nematodes in the Islamabad territory can be attributed to the establishment of orchards on uncultivated lands without a history of root-knot nematode presence. Additionally, the farmers in Islamabad are educated and demonstrate excellent orchard management practices, ensuring the health and protection of their orchards.

The current investigation revealed that Early Grand exhibited the highest occurrence and prevalence of root-knot nematodes, whereas Local Aroo demonstrated the lowest levels. There was no evidence of *Meloidogyne* spp. infestation on Florida Gold and Aroo-5. This could be due to the variations in the genetic makeup of the peach cultivars. The differences in the genetic makeup of the hosts affect different life stages of the nematode. The resistant cultivars do not allow juveniles to penetrate the roots or, if they succeed in penetrating, they are either killed ensuing penetration or unable to develop and/or reproduce, resultantly, there would be no infection of the host (Mukhtar *et al*., 2013b).

Examination of peach trees in the region unveiled the presence of two root-knot nematode species, namely, *M. incognita* and *M. javanica*. Overall, the prevalence of *M. javanica* was higher compared to *M. incognita*. *M. javanica* exhibited dominance in all districts, with the exception of Attock, where *M. incognita* prevailed. Notably, no infestation of *M. incognita* was observed in the district of Islamabad. The results are in line with the findings of Trudgill *et al*. (2000) who found that *M. incognita* and *M. javanica* were the most prevalent *Meloidogyne* species throughout the world. Many researchers have also reported similar findings in different parts of the world (Bhosle *et al*., 2004; Rathour *et al*., 2006). In Pakistani soils, the reported distribution and infestation of *M. incognita* and *M. javanica* was 52% and 31%, respectively (Maqbool, 1987), which confirm the present results.

The occurrence of *M. incognita* and *M. javanica* throughout the Pothwar area might be due to the suitable edaphic factors and environmental conditions for the nematodes, cropping pattern, and host suitability (Ploeg and Maris, 1999). In district Attock, the dominance of *M. incognita* is due to environmental conditions optimal for *M. incognita* but sub-optimal for *M. javanica*. Such factors might act in a differential

manner to enhance the penetration, infection, development, survival, and multiplication of *M. incognita* over *M. javanica* (Taylor *et al*., 1982).

In terms of exclusive populations, *M. incognita* was detected in 26.09% of peach orchards infested with root-knot nematodes, while *M. javanica* was present in 43.48% of the infested orchards. Conversely, when considering mixed populations, both species were observed in 30.43% of the affected orchards. Common occurrence of *M. javanica* alone and conjointly with *M. incognita* has also been observed in Morocco, Iran, and Spain (Sanei and Okhovvat, 2011; Hamza *et al*., 2017; Nico *et al*., 2002). The greater incidence and prevalence of *M. javanica* as a mixture of both nematodes might reveal a competitory advantage of *M. javanica* over *M. incognita* (Eisenback and Griffin, 1987).

CONCLUSIONS

The findings of the current study indicate significant occurrence of root-knot nematodes in peach orchards located in the Pothwar region of Pakistan. The frequency and distribution of these nematodes exhibited variations across different districts and cultivars. Two distinct species of root-knot nematodes, namely, *Meloidogyne incognita* and *M. javanica*, were identified as the pathogens affecting peach trees in this region, with *M. javanica* demonstrating a higher prevalence. The maximum galling index was observed in the Early Grand cultivar, while Florida Gold and Aroo-5 were not infested with root-knot nematodes.

Based on these findings, the following recommendations are made:

1. Farmers in Pothwar Region of Pakistan should take steps to control the root-knot nematodes in their peach orchards. This could involve using nematode-resistant peach varieties or employing other management strategies.

2. Considering the higher prevalence of *M. javanica* in the given region relative to *M. incognita*, farmers should be aware of the potential for this nematode species to infect their peach trees and take appropriate measures to manage it.

3. Farmers growing Early Grand peach cultivar should pay particular attention to nematode management as this cultivar had the highest galling index of all the cultivars surveyed.

4. Considering the resistance of Aroo-5 and Florida Gold cultivars to root-knot nematodes, it is advisable for farmers to incorporate these cultivars in their orchards as a strategic measure to mitigate the detrimental effects of nematode infestations.

5. Additional scientific investigations could be undertaken to explore alternative management strategies, thereby expanding the breadth of potential approaches.

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نماتدهای گره‌ریشه (*Meloidogyne spp*) آلوده کننده هلو (*Prunus persica* L.) در منطقه پوتوار پاکستان

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چکیده

هدف این پژوهش تعیین شیوع و شدت نماتدهای گره ریشه (root-knot) در باغ‌های هلو در منطقه پوتوار(Pothwar) پاکستان بود. ۳۷ باغ هلو در منطقه بررسی شد و در هر باغ نمونه های ریشه و خاک از ۱۰ گیاه انتخاب شده به طور تصادفی برای تجزیه جمع آوری شد. در این منطقه، نرخ بروز نماتدهای گره ریشه به طورکلی ۱۹.۸٪ و نرخ شیوع ۶۵.۷٪ مشاهده شد. منطقه Attock بالاترین میزان شیوع ۷۱.۴۳٪ و نرخ بروز ۳۴.۲۹ ٪ را نشان داد. در مقابل، ناحیه اسلام آباد کمترین میزان شیوع را با ۵۰.۵% و نرخ بروز ۱۰٪ را نشان داد. در این بررسی ها پنج رقم هلو مجزا بررسی شد و Early Grand بالاترین شیوع (۸۵.۷۱٪) نماتدهای گره‌ریشه را داشت در حالی که Local Aroo کمترین (۵۰٪) را نشان داد. گلد فلوریدا (Florida Gold) و آرو-۵ به نماتدهای گره ریشه آلوده نشدند. در منطقه، درختان هلو توسط دو نوع نماتد گره ریشه، یعنی *Meloidogyne incognita* و *M. javanica* آلوده شده بودند که نماتد اخیر شیوع بیشتری نسبت به *M. incognita* داشت، ، و بر تمام نواحی به جز اتوک (Attock) تسلط داشت. M. incognita در منطقه اسلام آباد حضور نداشت. بروز M. incognita و M. javanica به عنوان جمعیت منفرد به ترتیب در ۲۶.۰۹% و ۴۳.۴۸% از باغات مشاهده شد، در حالي که جمعیت مختلط در ۳۰.۴۳% از باغات هلو مشاهده شد.

شاخص کلی گالینگ (بروز M. incognita و M. javanica به عنوان جمعیت منفرد به ترتیب در ۲۶.۰۹% و ۴۳.۴۸% از باغها مشاهده شد، در حالي که جمعیت مختلط در ۳۰.۴۳% از باغات هلو مشاهده شد. شاخص کلی گالینگ (galling index) نماتدهای ریشه گره ۱/۳۳ بود که بیشترین شاخص در اتوک و کمترین آن در ناحیه اسلام آباد بود. در بین ارقام مختلف هلو، رقم Early Grand بیشترین شاخص گالینگ را از خود نشان داد، در حالی که در ارقام گلد فلوریدا و آرو-۵ هیچ شاخص گالینگ مشاهده نشد. توصیه می شود که برای جلوگیری از گسترش نماتدها به باغ‌های جدید و ریشه کن کردن آنها در باغ‌های مستقر، استراتژی های کنترل دقیق اتخاذ شود.