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ACCEPTED ARTICLE

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

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Running title: Root-knot Nematodes Infecting Peach in 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*. *M. javanica* 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 the Islamabad district. The occurrence of *M. incognita* and *M. javanica* as single populations was 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. 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.

Key words: *Meloidogyne* spp., incidence; prevalence; galling index; *Prunus persicae*.

37 INTRODUCTION

38 Peach (*Prunus persica* (L.) Batsch) occupies a central position among stone fruit which is
39 grown widely throughout the world (Eldem *et al.*, 2012). Peach is the second largest stone fruit
40 after apricots among stone fruits of Pakistan. In Pakistan, peaches were fundamentally
41 developed in Khyber Pukhtunkhwa, Balochistan and some low chill areas of Pothwar zone of
42 Punjab. It has been cultivated on an area of 9,800 hectares with a total production of 75,400
43 metric tons (FAO, 2021). The lucrative production of peaches and nectarines has been
44 threatened for many years by an assortment of biotic factors including diseases like peach leaf
45 curl, peach tree short life (PTSL) and nematodes. Phytopathogenic nematodes viz. root-knot
46 nematodes, cyst nematode, root lesion nematodes etc. have economic significance in agriculture
47 and are directly and/or indirectly associated with crop damages leading to yield losses in various
48 vegetables, fruits and crops (Bogner *et al.*, 2017; Asghar *et al.*, 2020; Tariq-Khan *et al.*, 2017,
49 2020; Ahmed *et al.*, 2021; Ullah and Khanum, 2022). These nematodes have been reported to
50 incur about \$173 billion yield losses annually on different agricultural crops. The genus
51 *Meloidogyne* exhibits the highest level of destructiveness and holds the top position among
52 phytopathogenic nematodes (Termorshuizen *et al.*, 2011; Kim *et al.*, 2016; Gamalero and Glick,
53 2020; Shahid *et al.*, 2022, 2023).

54 Root-knot nematodes (*Meloidogyne* spp.) have been found seriously infecting peaches and
55 have become a severe issue for majority of peach growers and nurserymen in many regions
56 having tropical and Mediterranean climates (Lamberti, 1979). Root-knot nematodes have been
57 found prevalent in temperate, tropical, and equatorial regions of the world (Moens *et al.*, 2009;
58 Nyczepir, 2011; Kayani and Mukhtar, 2018; Mukhtar and Kayani, 2019, 2020). Root-knot
59 nematodes cause reduction in fruit production of many economically important species of
60 *Prunus* including *Prunus persica*. Among different species of root-knot nematodes,
61 *Meloidogyne incognita* and *M. javanica* are the most common in peach and plum orchards
62 (Nyczepir and Becker, 1998). The occurrence of *M. incognita* and *M. javanica* was recorded in
63 95 and 5% of sampled peach orchards respectively in South Carolina (Nyczepir *et al.*, 1997).

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

71 In addition to their direct effects, root-knot nematodes can also interact with pathogenic fungi
72 and bacteria, forming disease complexes that exert additional detrimental effects on plant health
73 (Aslam and Mukhtar, 2023a, b). Peach trees infected with PTSL or with unthrifty growth have
74 been found cohabiting with *Meloidogyne* spp. and *Criconemella xenoplax* (Raski) Luc and
75 Raski. The infestation of peach trees with both *C. xenoplax* and *M. javanica* have also been
76 associated with sparse root system, premature leaf drop and peach shoot die-back (Hugo and
77 Meyer, 1995). More than 50 percent peach orchards in the southeastern United States with a
78 history of PTSL showed the presence of root-knot nematodes and *C. xenoplax* (Nyczepir *et al.*,
79 1985). Similarly, a synergistic interaction between both the nematodes resulting in an increased
80 reduction in growth of peach has also been reported (Nyczepir *et al.*, 1993).

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

91

92 MATERIALS AND METHODS

93 Sample collection

94 A total of 37 peach orchards in the Pothwar region of Pakistan were surveyed for the
95 determination of incidence, prevalence, and infestation of root-knot nematodes (Figure 1). For
96 this purpose, 10 plants were randomly selected from each orchard. From each 10 randomly
97 selected plants, root and soil samples (250 g) were collected from the four sides of the tree, 2.5
98 feet away from the main trunk using an auger. The four collected cores were mixed together to
99 create a composite sample of one kilogram. The samples were immediately brought to the Plant
100 Nematology Laboratory for further studies. The roots were separated and washed free of
101 adhering soil particles.

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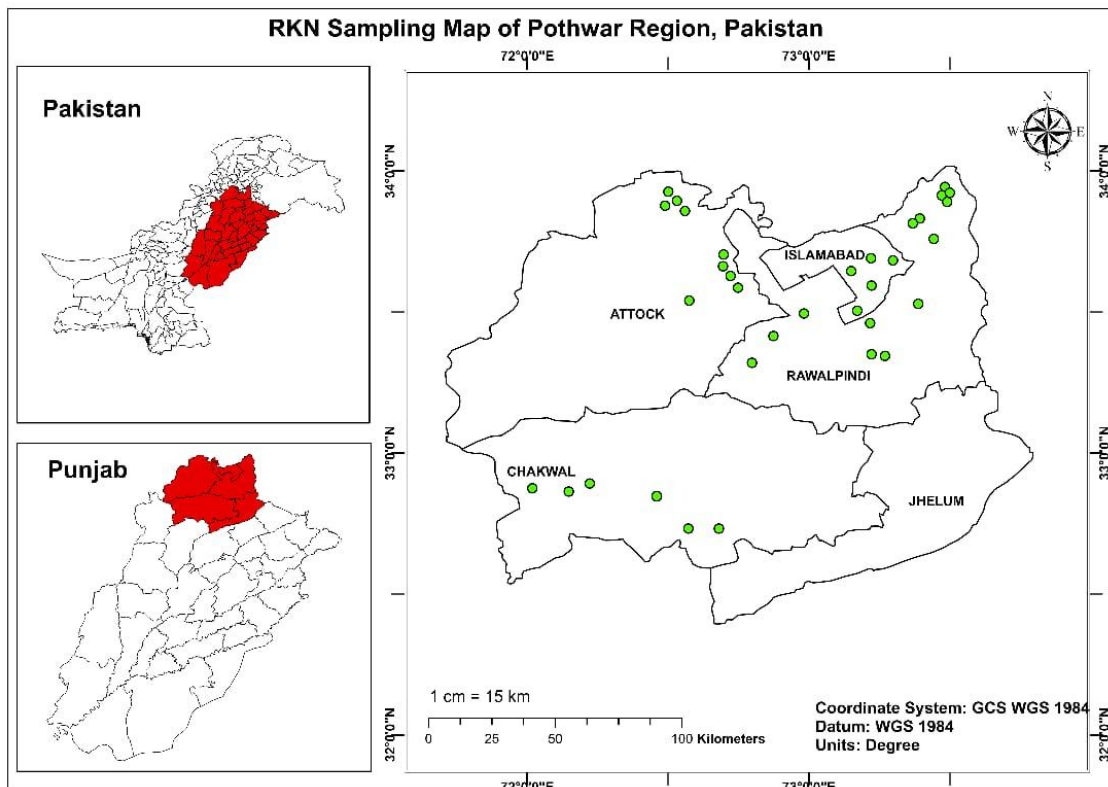
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106 **Determination of Incidence and prevalence of root-knot nematodes**

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

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

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117 **Determination of galling index**

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

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122 **Identification of *Meloidogyne* species**

123 **A composite sample was created for the detection of *Meloidogyne* species by combining and**
124 **thoroughly mixing the infected root samples from all the peach orchards in each district.**
125 **Perineal patterns of females were made for the identification of root-knot nematodes as outlined**
126 **by Taylor and Netscher (1974) and observed under a stereomicroscope at 4x. *Meloidogyne***
127 **species were identified by comparing the perineal patterns with the standard diagrams**

128 (Eisenback et al., 1981). Similarly, perineal patterns of 20 randomly selected females from each
129 infected composite root sample from each peach orchard were made and the prevalence of each
130 *Meloidogyne* species was determined. The occurrences of sole and mixed *Meloidogyne*
131 populations were also found out in the four districts on each variety in the Pothwar region.

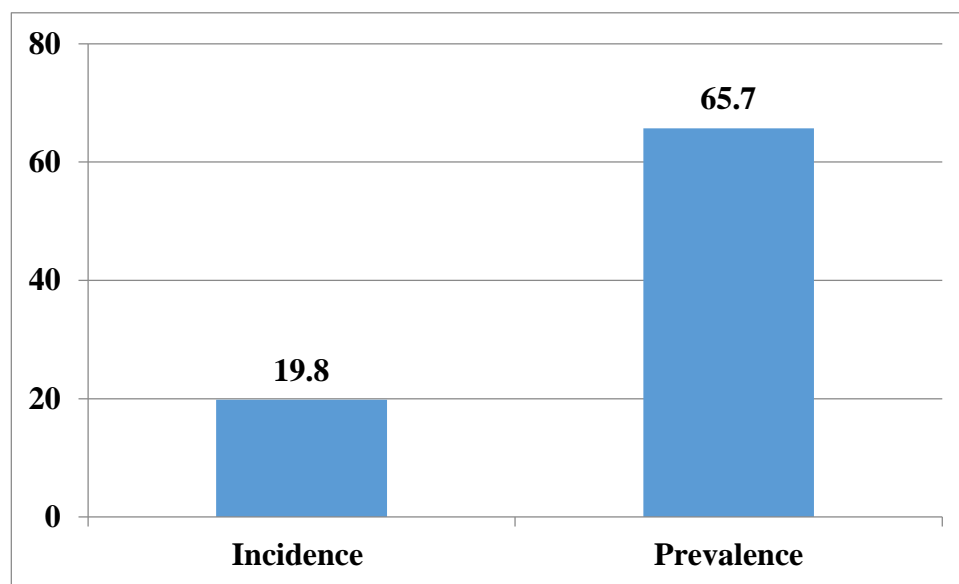
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133 RESULTS

134 Incidence and prevalence of root-knot nematodes in peach orchards

135 In the Pothwar region of Pakistan, the occurrence of root-knot nematodes on peach trees was
136 documented to have an overall incidence of 19.8% and a prevalence of 65.7% (Figure 2). When
137 examining specific districts, district Attock exhibited the highest prevalence (71.43%) and
138 incidence (34.29%), while district Islamabad had the lowest prevalence (50.5%) and incidence
139 (10%) (Figure 3). The survey identified five different peach varieties in the region. The variety
140 Early Grand displayed the highest prevalence of root-knot nematodes (85.71%), whereas Local
141 Aroo had the lowest prevalence (50.0%). No infestation of root-knot nematodes was observed
142 on Florida Gold and Aroo-5 varieties. Conversely, Florida King had the highest incidence of
143 root-knot nematodes, while Local Aroo had the lowest incidence of 15% (Figure 4). Table 1
144 provides information on the occurrence and distribution of root-knot nematodes across the four
145 districts of the Pothwar region, categorized by different varieties.

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

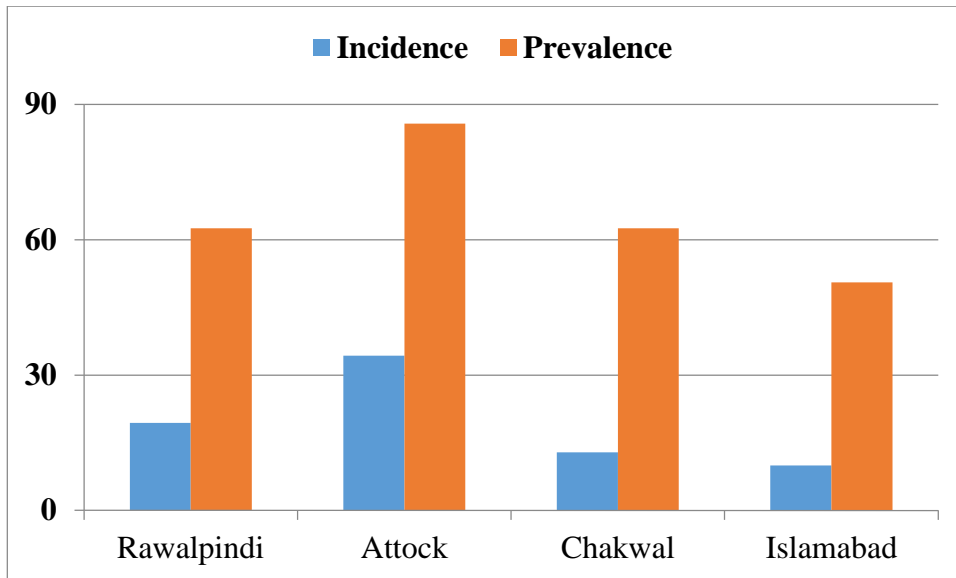


Figure 3. District wise incidence and prevalence of root-knot nematodes in Pothwar Region.

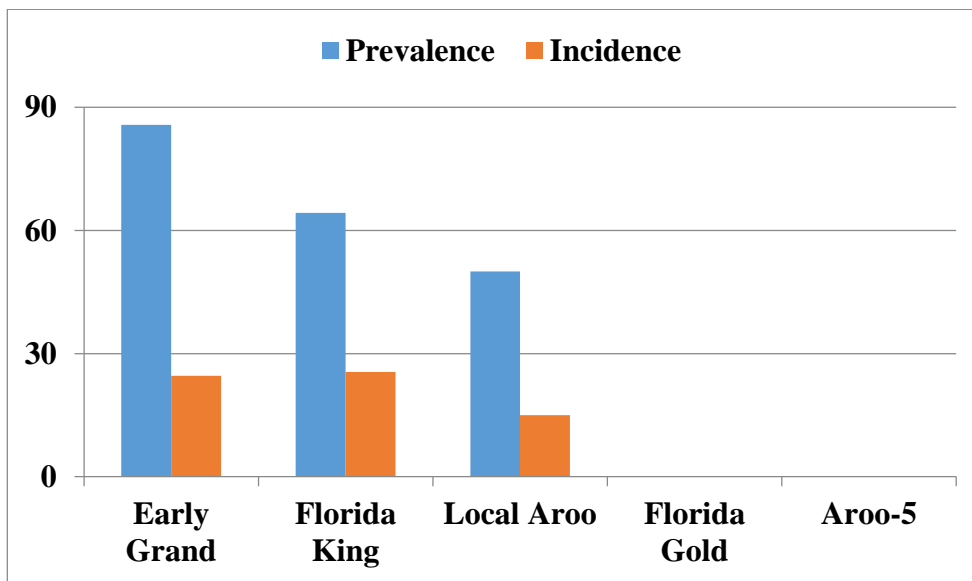


Figure 4. Variety wise incidence and prevalence of root-knot nematodes in the Pothwar Region.

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161 **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

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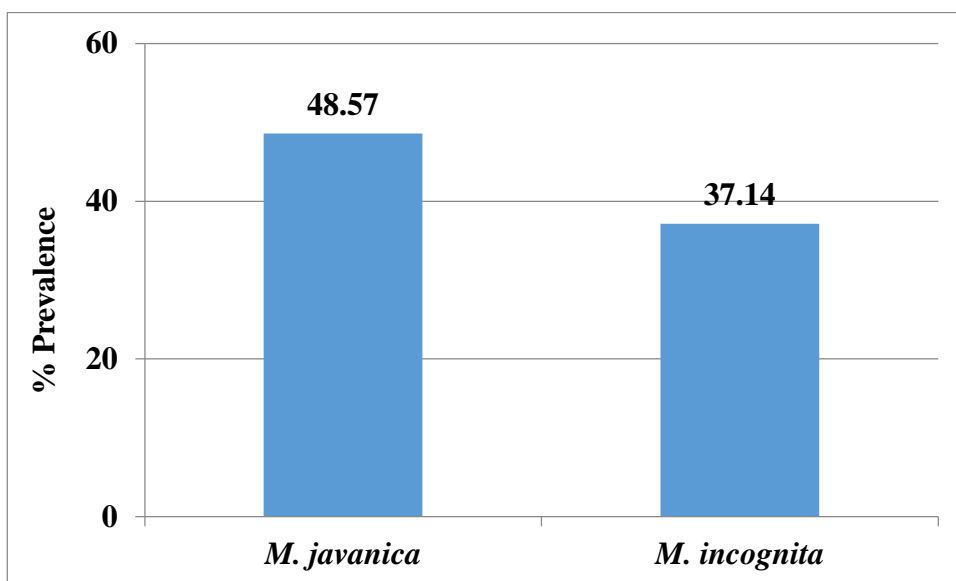
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167 **Occurrence of root-knot nematode species**

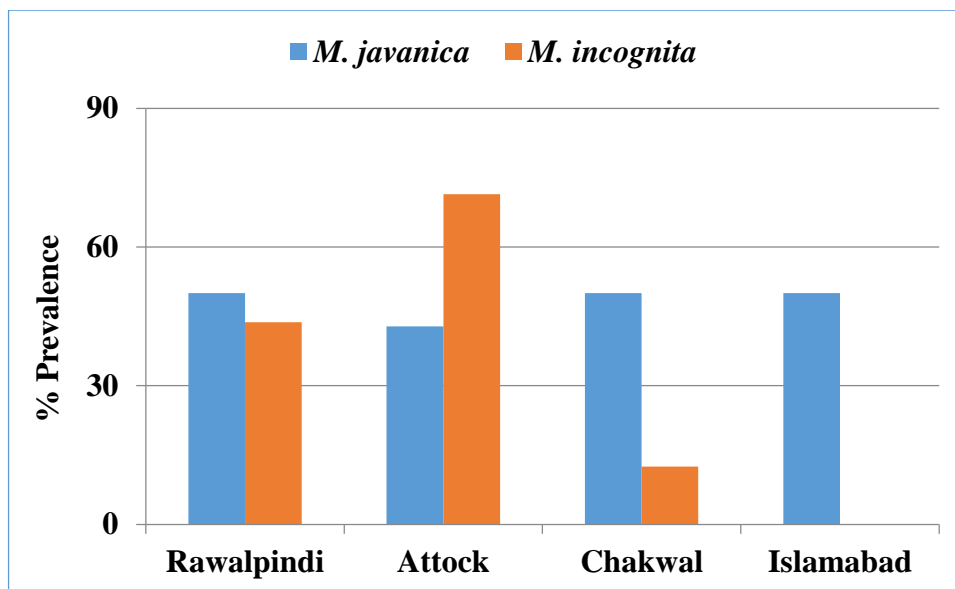
168 In the investigated region, peach trees were found to be infected by two distinct species of
169 root-knot nematodes, namely *Meloidogyne incognita* and *M. javanica*. Through observations,
170 it was determined that *M. javanica* exhibited a higher prevalence compared to *M. incognita*, as
171 illustrated in Figure 5. Across all districts, *M. javanica* was the dominant species, except in
172 Attock where *M. incognita* was found to be the prevailing nematode. Notably, no instances of
173 *M. incognita* infestation were recorded in the Islamabad district (Figure 6). *M. javanica* as a
174 sole population was the most prevalent nematode species in the peach orchards of the Pothwar
175 region with a prevalence of 43.48%, followed by *M. incognita* with a prevalence of 26.09%.
176 On the other hand, the combined prevalence of both species was 30.43% (Figure 7). The district
177 wise and variety wise occurrence of root-knot nematode species in infected peach orchards as
178 sole and mixed populations are given in Tables 2 and 3.

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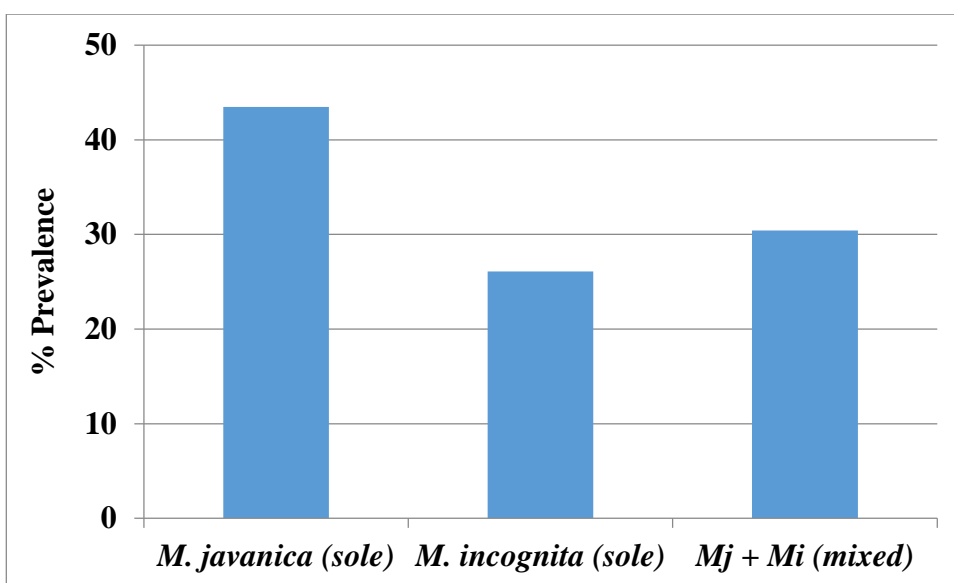
180 **Figure 5.** Overall prevalence of root-knot species in the Pothwar Region.
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Figure 6. District wise prevalence of root-knot species in the Pothwar Region.



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

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

District	<i>M. incognita</i>	<i>M. javanica</i>	<i>M. incognita + M. javanica</i>
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

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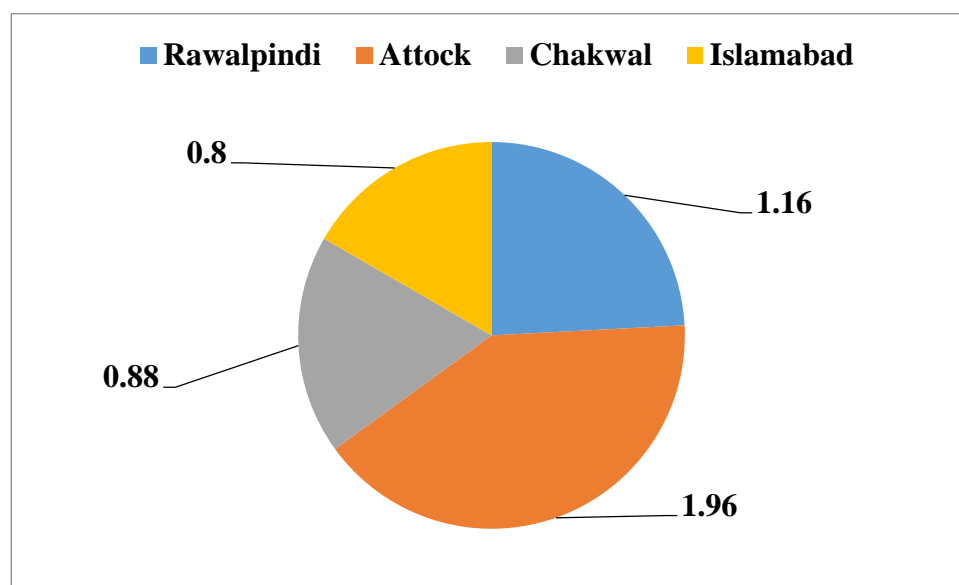
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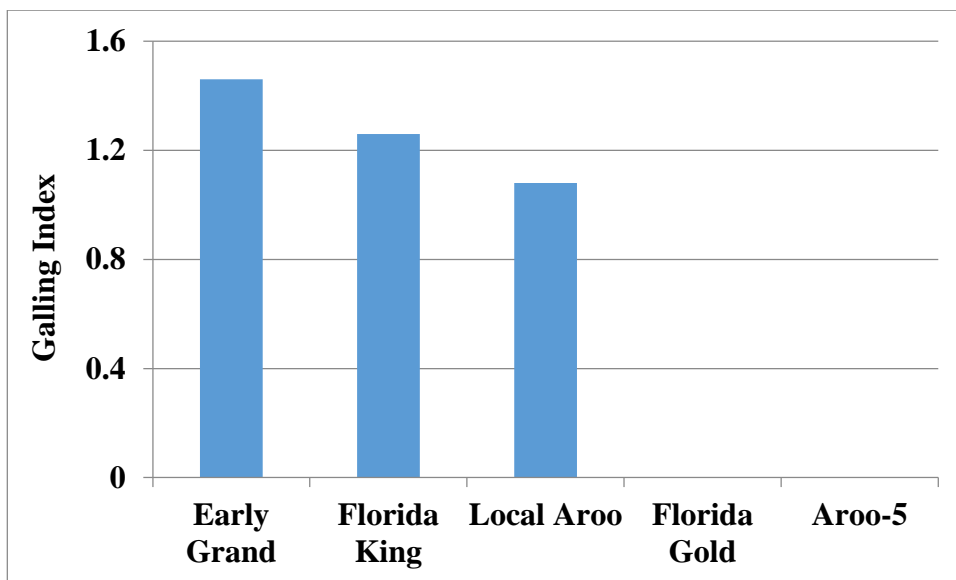
196 **Table 3.** Variety wise occurrence of root-knot nematode species as sole and mixed
 197 populations in the four districts of the Pothwar Region.

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

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 199 **Quantification of root-knot nematode-induced galling on peach roots**
 200 The comprehensive galling index of root-knot nematodes in the studied area was recorded as
 201 1.33. District Attock exhibited the highest galling index, whereas the lowest galling index was
 202 observed in Islamabad district (Figure 8). In a similar vein, Early Grand exhibited the highest
 203 galling index of 1.46, whereas Local Aroo displayed the lowest index of 1.08. Notably, Florida
 204 Gold and Aroo-5 cultivars remained free from root-knot nematode infestation, resulting in a
 205 galling index of zero (Figure 9). Table 4 presents the galling index of root-knot nematodes
 206 categorized by variety across the four districts of the Pothwar region.



208 **Figure 8.** District wise galling index of *Meloidogyne* species in the Pothwar Region.
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211 **Figure 9.** Variety wise galling index of *Meloidogyne* species in the Pothwar Region.
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214 **Table 4.** Variety wise galling index of *Meloidogyne* species across the four districts in the
 215 **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
Aroo-5	0.00	0.00	0.00	0.00

216 **DISCUSSION**
 217

218 In the present study, differences were observed in the incidence, prevalence, severity, and
 219 species of root-knot nematodes in all surveyed peach orchards in all the districts of Pothwar
 220 region of the Punjab province of Pakistan. The variations observed in the distribution of root-
 221 knot nematodes across the studied areas can be attributed to a range of biotic and abiotic factors.
 222 These differences may arise from variations in environmental and edaphic conditions found
 223 throughout the districts of the region. Many earlier studies have confirmed that fluctuating
 224 agroclimatic factors and environments in the Pothwar region like cropping sequences, soil pH,
 225 moisture level, soil temperature, soil texture and structure greatly impact incidence, prevalence,
 226 severity, and distribution of root-knot nematodes (Van Gundy, 1985; Kayani et al., 2013).

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

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

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

263 In the present research, the examination of peach plants in the region unveiled the presence of
264 two root-knot nematode species, namely *M. incognita* and *M. javanica*. The prevalence of *M.*
265 *javanica* was determined to be higher overall when compared to *M. incognita*. *M. javanica*

266 exhibited dominance in all districts, with the exception of Attock, where *M. incognita* prevailed.
267 Notably, no infestation of *M. incognita* was observed in the district of Islamabad. The results
268 are in line with the findings of Trudgill et al. (2000) who found that *M. incognita* and *M.*
269 *javanica* were the most prevalent *Meloidogyne* species throughout the world. Many researchers
270 have also reported similar findings in different parts of the world (Bhosle et al., 2004; Rathour
271 et al., 2006). In Pakistani soils, the reported distribution and infestation of *M. incognita* and *M.*
272 *javanica* was 52 and 31% respectively (Maqbool, 1987) which confirm the present results.

273 The occurrence of *M. incognita* and *M. javanica* throughout the Pothwar area might be due to
274 the prevailing suitable edaphic factors and environmental conditions for the nematodes,
275 cropping pattern and host suitability (Ploeg and Maris, 1999). In district Attock, the dominance
276 of *M. incognita* is due to environmental conditions optimal for *M. incognita* but sub-optimal
277 for *M. javanica*. Such factors might act in a differential manner to enhance the penetration,
278 infection, development, survival, and multiplication of *M. incognita* over *M. javanica* (Taylor
279 et al., 1982).

280 In terms of exclusive populations, *M. incognita* was detected in 26.09% of peach orchards
281 infested with root-knot nematodes, while *M. javanica* was present in 43.48% of the infested
282 orchards. Conversely, when considering mixed populations, both species were observed in
283 30.43% of the affected orchards. Common occurrence of *M. javanica* alone and conjointly with
284 *M. incognita* has also been observed in Morocco, Iran, and Spain (Sanei and Okhovvat, 2011;
285 Hamza et al., 2017; Nico et al., 2002). The greater incidence and prevalence of *M. javanica* as
286 a mixture of both the nematodes might reveal a competitive advantage of *M. javanica* over *M.*
287 *incognita* (Eisenback and Griffin, 1987).

288

289 CONCLUSIONS AND RECOMMENDATIONS

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

297 Based on these findings, the following recommendations are made:

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

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

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

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

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

311

312 **ACKNOWLEDGEMENTS**

313 The assistance and cooperation rendered by the local farmers in the areas visited is gratefully
314 acknowledged.

315

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