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Research Article

Phytonematode Problems associated with Some Economically Important Plants in Pishin District, Balochistan, Pakistan

Faisal Hussain[1]*, Saadullah Khan Laghari[2], Diam Ali Darban[3], Farzana[1], and Muhammad Akbar[4]

[1]Department of Botany, Federal Urdu University of Arts, Science & Technology, Gulshan-e-Iqbal Campus, Karachi, Pakistan
[2]Department of Botany, University of Balochistan, Quetta, Pakistan
[3]Department of Agriculture, School of Agriculture Policy and Development, The University of Reading, Reading, UK
[4]Department of Environmental Sciences, Karakoram International University, Gilgit-Baltistan, Pakistan

Abstract: Present study was conducted for nematodes communities associated with Pomegranate (*Punica granatum* L.), Grapes (*Vitis vinifera* L.), Peach (*Prunus persica* L.), Plums (*Prunus domestica* L.), Tobacco (*Nicotiana tabacum* L.), Apple (*Malus pumila* Mill.) and Apricot (*Prunus armeniaca* L.) at three localities of Pishin district, Balochistan province. In all investigated plant species twenty nematode species were recorded *viz., Helicotylenchus digonicus, H. indicus, H. pseudorobustus, Hoplolaimus indicus, Meloidogyne incognita, M. javanica, Pratylenchus holdemani, P. penetrans, P. scribneri, P. thornei, Rotylenchus dalhousiensis, Scutylenchus rugosus, Tylenchorhynchus annulatus, T. curvus, T. trilineatus, Tylenchus filiformis, Xiphinema americanum, X. basiri, X. index* and *X. rivesi* from the rhizosphere soil of above mentioned plant species. Among all species, *Meloidogyne incognita* and *Xiphinema rivesi* were dominant. The nematodes problem is rapidly increasing in Pakistan and widely damaging the host range by several species of phytonematodes. This investigation was designed to check the association, soil analysis, nematodes population density, incidence (%) and prevalence of nematodes associated with some crop and orchard plants of Pishin district, Balochistan.

Keywords: Phytonematodes, population density

1. INTRODUCTION

Plant-parasitic nematodes are widespread and are among the most damaging pests of different crops, causing not only severe crop losses of commercial fruits that are meant for export but also seriously limit the production and viability of many other crops. Phytonematodes vary in their feeding behavior; each species causing somewhat diverse type of damage to the crops. Pomegranate (*Punica granatum* L.), grapes (*Vitis vinifera* L.), peach (*Prunus persica* (L.)), plums (*Prunus domestica* L.), tobacco (*Nicotiana tabacum* L.), apple (*Malus pumila* Mill.) and apricot (*Prunus armeniaca* L.) are important fruits and economic crops of Pakistan particularly of Pishin district, Balochistan. Balochistan along with its huge natural resources is one of the rich agricultural zones with great variety of dry and fresh fruits. The province produces 60% percent of peach, pomegranate and apricot, 90 percent of grapes, almond and cherry, 34 percent of apple and 70 percent of date of the total fruit production of Pakistan [1]. The study area (Pishin district) in Balochistan province lies between 30o 04' to 31o 17' north latitudes and 66o 13' to 67o 50' east longitudes. It falls in the tropical agro-ecological zone of bearing a total potential agricultural area

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*Corresponding author: Email: Faisal Hussain: faisal.botanist2011@gamil.com

of 185,280 hectares [2], which is approximately 23.5% of the total geographical area of the Pishin district. Main crops of the district are wheat, barley, cumin, tobacco, apple, pomegranate, grapes, peach, plums and apricot. These plants are affected by many pest diseases but the infection of nematodes in Pakistan, particularly in Pishin district is more serious compared to other developing countries. There is paucity of investigation regarding the host range of nematodes and their damage to commercial fruit species [3].

Pomegranate (*Punica granatum* L.) is an important fruit crop of tropical and subtropical regions of Pakistan [4]. Most of the pomegranate orchards were found infested with root-knot nematodes [5, 6]. Sudheer et al. [7] reported that most of the nematode infested plants showed yellowing of foliage and stunted growth and these plants produced less or undersized fruits or did not bear any fruits. Khan and Shaukat [8] recorded twelve genera from the rhizosphere of pomegranate and the most dominant species was *Meloidogyne incognita* from Khuzdar and Kalat districts of Balochistan province. Grape (*Vitis vinifera* L.) which is an economically important crop has been facing many soil-borne pathogens and pests that damage or completely destroy the new roots of plants. Aballay et al. [9] and Baginsky et al. [10] established that several species of plant-parasitic nematodes (PPNs) have been reported to cause economic damage to grapevines and the most frequent species were *Xiphinema index, Meloidogyne ethiopica, Mesocriconema xenoplax*, and *Tylenchulus semipenetrans.* Peach (*Prunus persica* L.) is an important economic fruit plant of Balochistan, with an area under cultivation of 60.22 ha and production of 30.31 tons [11] also faces different diseases that cause reduction in its annual productivity. Di Vito et al. [12] noticed that the peach trees are subjected to various diseases throughout their lives and phytonematode infections in these trees are common. Several species of phytonematodes have been reported to damage the peach crops. Malo [13] reported that root-knot nematodes attack peach trees and weaken them to such an extent that they are easily affected and often killed by other pathogens or physiological conditions. Chitwood et al. [14] pointed out that *Xiphinema americanum* was predominant form in the vicinity of peach trees. Philis [15] found that *Meloidogyne* spp., attack young roots of peach which leads to stunting of trees and shortens lifespan. Apple (*Malus pumila* Mill.) and apricot (*Prunus armeniaca* L.) are also affected by many pests and diseases worldwide, but nematodes are considered important pests of apple and apricot as noticed by Aballay and Erikson [16], Islam et al. [17], Karanastasi et al. [18] and Khan et al. [19].

The current study focuses on the phytonematodes associated with fruit trees in Pakistan and that widely damaged by several species of phytonematodes. The investigation is designed to check the association, population density, incidence (%) and prevalence of nematodes associated with some important plants were studied in the fields of Pishin district, Balochistan.

2. MATERIALS AND METHODS

2.1 Collection and Isolation of Phytnematodes

Soil samples from three localities namely Killi Karbala, Huramzai and Barshore of Pishin district Balochistan, Pakistan during December, 2013 were collected for the estimation of infestation, population and prevalence of phytonematodes on different fruit trees including pomegranate (*Punica granatum* L.), grapes (*Vitis vinifera* L.), peach (*Prunus persica* (L.)), plums (*Prunus domestica* L.), tobacco (*Nicotiana tabacum* L.), apple (*Malus pumila* Mill.) and apricot (*Prunus armeniaca* L.). Samples were collected from the soil close to tree trunk at a depth of 20-30 cm depth. Approximately 2 kg of soil adhering the roots was procured. All Nematodes were isolated from 200 cm^3 soil by using method of Cobb's Decanting Sieving method followed by a modified Baermann's technique as described by Southey [20].

2.2 Soil Analysis

A total of 70 soil samples from three designated localities (Killi Karbala, Huramzai and Barshore) in the vicinity of fruit trees and crops were collected to analyze soil types, soil pH and water holding capacity of nematodes. Soil texture was determined manually [21]. The pH of soil was measured in soil paste (1:5 soil/dist. water) while water holding capacity was determined in accordance with the procedure of Shaukat et al. [22]. The soil was saturated in tin cans (with a hole in the bottom) and

weighed. The soil was then oven-dried (100 °C for 24 h) and the maximum water holding capacity was calculated as loss in weight (saturated soil – oven dried soil) and expressed as a percentage of oven-dry soil [22, 6].

2.3 Population Density, Prevalence and Incidence Percentage

Nematode densities in rhizosphere soil were determined by extracting nematodes from a 200 cm^3 by using the centrifugal-flotation method [23]. The frequency of occurrence (prevalence) and incidence of the disease in each locality was calculated by the following formula:

$$\text{Prevalence (\%)} = \frac{\text{Number of fields with nematode infections}}{\text{Total number of fields surveyed}} \times 100$$

$$\text{Incidence (\%)} = \frac{\text{Number of plants galled}}{\text{Total number of plants sampled}} \times 100$$

2.4 Identification of Nematdes

The nematodes and their different morphological stages and characters were identified with the help of Hartman and Sasser [24], Siddiqi [25], Ahmad [26] and Handoo [27]. Nematodes specimens were killed by hot-water bath and fixed in 3% formaldehyde. For identification of the ectoparasitic nematode species, nematode specimens were processed by Seinhorst's glycerol-ethanol method Seinhorst [28] and stained by NaOCl-Acid Fuchsin method Byrd et al. [29]. The root-knot nematode species were identified with the help of perineal pattern as described by Taylor and Netscher [30]. The nematodes were viewed through an electronic and stereomicroscope.

3. RESULTS AND DISCUSSION

Balochistan province has tremendous potential for developing fruit farms, comprising of agro-ecological zones endowed with a unique environment for production of a great variety of quality fruits. Abundance, prevalence and incidence of phytonematodes associated with fruit trees were examined. A total of nine genera of nematodes were recorded from three different localities and seven crops (Table 1). *Meloidogyne incognita* was the dominant species and recorded from four crops species, while *Helicotylenchus digonicus, H. pseudorobustus, Xiphinema americanum* and *X.*

Table 1. Summary of phytonemtaode species present in different investigated plant species of Pishin district of Balochistan.

Nematode species	*Pomegranate*	*Grapes*	*Peach*	*Plums*	*Tobacco*	*Apple*	*Apricot*
Helicotylenchus digonicus	+	-	-	-	-	-	-
H. indicus	-	-	-	-	+	-	+
H. pseudorobustus	-	-	+	-	-	+	-
Hoplolaimus indicus	-	-	-	-	+	-	-
Meloidogyne incognita	+	+	+	-	-	+	-
M. javanica	-	-	-	-	+	-	-
Paratylenchus holdemani	-	-	-	-	-	-	+
P. penetrans	-	-	-	+	-	-	-
P. scribneri	-	-	-	-	-	+	-
P. thornei	-	+	-	-	-	-	-
Rotylenchus dalhousiensis	-	-	-	-	-	-	+
Scutylenchus rugosus	+	-	-	-	-	-	-
Tylenchorhynchus annulatus	-	-	+	-	-	-	-
T. curvus	-	+	-	-	-	-	-
T. trilineatus	-	-	-	-	-	-	+
Tylenchus filiformis	-	-	-	-	+	-	-
Xiphinema americanum	-	-	+	+	-	-	-
X. basiri	+	-	-	-	-	-	-
X. index	-	+	-	-	-	-	-
X. rivesi	-	+	+	-	-	+	-

(+) = Presence and (-) = Absence

Table 2. Densities of plant parasitic nematode in 200 cm^3 soil in the rhizosphere of different plants species of Pishin district of Balochistan.

Plant species	*Helicotylenchus digonicus*	*H. indicus*	*H. pseudorobustus*	*Hoplolaimus indicus*	*Meloidogyne incognita*	*M. javanica*	*Paratylenchus holdemani*	*P. penetrans*	*P. scribneri*	*P. thornei*	*Rotylenchus dalhousiensis*	*Scutylenchus rugosus*	*Tylenchorhynchus annulatus*	*T. curvus*	*T. trilineatus*	*Tylenchus filiformis*	*Xiphinema americanum*	*X. basiri*	*X. index*	*X. rivesi*
Pomegranate	52	-	-	-	127	-	-	-	-	-	-	66	-	-	-	-	-	17	-	-
Peach	-	-	26	-	190	-	-	-	-	-	-	-	22	-	-	-	28	-	-	12
Plum	-	-	-	-	-	-	-	39	-	-	-	-	-	-	-	-	33	-	-	-
Tobacco	-	70	-	63	-	210	-	-	-	-	-	-	-	-	-	39	-	-	-	-
Apple	-	-	112	-	213	-	-	-	78	-	-	-	-	-	-	-	-	-	-	27
Apricot	-	59	-	-	-	-	37	-	-	-	86	-	-	-	41	-	-	-	-	-
Grapes	-	-	-	-	179	-	-	-	-	66	-	-	-	47	-	-	-	-	35	8

rivesi were isolated from only two crops. While, all other nematodes species were observed in rare cases.

The number of second stage juveniles of *M. incognita* ranged from 127 to 213 while that of *Xiphinema basiri* 17 per 200 cm^3 soil (Table 2). The highest number of nematode species such as *Meloidogyne incognita, Xiphinema americanum, Helicotylenchus pseudorobustus, Tylenchorhynchus annulatus* and *Xiphinema rivesi* was recorded from the rhizosphere soil of peach plants. The average population of *Meloidogyne incognita, M. javanica* and *Helicotylenchus pseudorobustus* were abundant, while *Xiphinema basiri, X. index* and *Tylenchorhynchus annulatus* populations were low. *Meloidogyne incognita* was recorded from maximum number of crop plants (four), followed by *Xiphinema rivesi* which was found associated with three crop plants.

Out of 70 soil samples from the vicinity of fruit trees and crops, 58 were infected with nematodes. The highest population and prevalence of nematodes was documented in the soil samples of Killi Karbala (26% and 66% respectively) followed by Barshore (21% and 60% respectively), while Huramzai exhibited lowest (15% and 47% respectively) as shown in Table 3.

Data presented in Table 4 indicates that *Meloidogyne incognita* was the most common nematode isolated from four plant species such as pomegranate, peach, apple and Grapes. However, *Xiphinema rivesi* was prevalent in peach, apple and grape. Maximum incidence percentage of *M. incognita* was recorded 76% from the soil samples of grape plants. However, minimum incidence percentage was 12% that was recorded from the soil of tobacco plants.

In present study, total of nine genera of nematodes were recorded from different localities of Balochistan which are highly damaging loss of fruit trees. These results are in agreement with those of Khan et al. [31] and also corroborate our study. They found eight different phytonematodes in different areas of Balochistan associated with the seedlings of peach, while most predominant nematode was *Helicotylenchus pseudorobustus* and the least frequent were *Meloidogyne javanica*

Table 3. Soil characteristics with population density and prevalence (%) of different localities in Pishin.

Locality	Soil texture	Soil pH	Max. water holding capacity (%)	Population density	Prevalence
Killi Karbala	Loamy sand	8.0	27.4	26%	66%
Huramzai	Loamy sand	8.2	26.7	15%	47%
Barshore	Sandy loam	7.6	35.6	21%	60%

Table 4. Phytonematodes incidence (%) in different plant species of Pishin district, Balochistan.

Plant species	Nematodes	Incidence (%)
Pomegranate	*Helicotylenchus digonicus*	22
	Meloidogyne incognita	42
	Scutylenchus rugosus	17
	Xiphinema basiri	32
Peach	*Helicotylenchus pseudorobustus*	57
	Meloidogyne incognita	65
	Tylenchorhynchus annulatus	33
	Xiphinema americanum	29
	X. rivesi	30
Plums	*Pratylenchus penetrans*	15
	Xiphinema americanum	31
Tobacco	*Helicotylenchus indicus*	39
	Hoplolaimus indicus	12
	Tylenchus filiformis	26
	Meloidogyne javanica	61
Apple	*Meloidogyne incognita*	56
	Helicotylenchus pseudorobustus	41
	Xiphinema rivesi	24
	Pratylenchus scribneri	19
Apricot	*Tylenchorhynchus trilineatus*	45
	Rotylenchus dalhousiensis	27
	Helicotylenchus indicus	30
	Pratylenchus holdemani	41
Grapes	*Xiphinema index*	57
	Meloidogyne incognita	76
	Tylenchorhynchus curvus	47
	Pratylenchus thornei	23
	X. rivesi	37

and *Tylenchorhynchus annulatuus*. In addition, the observations of Khan et al. [6] correspond with the findings of our study. They recorded several genera of nematodes from the rhizosphere of pomegranate and the most dominant nematode species was *Scutylenchus rugosus* followed by *Xiphinema basiri* and *Meloidogyne incognita.*

Similar findings were also reported by other researchers such as Aballay et al. [9] and Baginsky et al. [10] who showed that several species of nematodes including *Xiphinema index, Meloidogyne ethiopica, Mesocriconema xenoplax*, and *Tylenchulus semipenetrans* have been reported to cause economic damage to grapes and

completely destroying the younger roots of plants. Khan et al. [19] also concluded that *Meloidogyne incognita* and some other nematode infection cause noticeable damage to apple and apricot roots and consequently affect the fruit production.

4. CONCLUSIONS

Based on results of this study, it is concluded that soil factors are of considerable of importance to the fruit tree species and a number of nematode species are associated with the rhizosphere soil of fruit trees in Pishin district. The soils were infected by sizeable populations of phytonematode communities which can adversely affect growth and yield of some important fruit trees. Further studies on the host range, histopathology, actual damage, biological control, integrated control and strong management practices are warranted to gain better understanding of plant-nematodes associated with fruit trees of Balochistan.

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