Plant Parasitic Nematodes Associated with Pomegranate (*Punica granatum*) and Grapes (*Vitus vinifera*) in District Swabi

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ABSTRACT

Various pomegranate and grapes orchards were surveyed during the year 2022 at various localities District Swabi, the study aimed the incidence as well as identification of different genera of plant parasitic nematode (PPN). The nematodes are simple, colorless roundworms, and their bodies are un-segmented and tapering towards the head and tail, but some plant parasitic species have different body shapes, like pear, kidney, or lemon. Nematodes are small, and throughout the world, including Pakistan, important pests of crops and plants are plant parasitic nematodes. The living plant cells and tissues are fed by PPN. For investigation of PPN soil samples were collected from various localities. PPN was extracted through Baermann funnel technique from the rhizoshpere, and counted via genera. After fixation of nematodes on slides species were identified through morphological identification keys. There were many species recorded which belong to seven different genera. *Helicotylenchus, Tylenchorhynchus, Meloidogyne, Longidorus, Trichodorus, Xiphinema,* and *Pratylenchus* were found in pomegranate. Among these genera, *Meloidogyne* species were recorded in large population followed by *Helicotylenchus* in all collected samples of pomegranate. While *Helicotylenchus, Filenchus, Tylenchorhynchus, Xiphinema, Tylenchus,* and *Pratylenchus* were found in all collect samples from grapes orchards. Among all genera species of *Filenchus* were recorded in large numbers and the second highest species of *Tylenchus* genera were recorded. The rate of incidence and inhabitants are highly diverse among all surveyed localities which simply reveals the fact that studied sites are extremely infected by diverse species of PPN which eventually damage the cultivation of grapes and pomegranates.

Key words: ***Longidorus, Filenchus, Xiphinema, Helicotylenchus, Pratylenchus, Meloidogyne.***

INTRODUCTION

The survey was conducted in Swabi, a 1,543-square-kilometer district in Khyber Pakhtun Khuwa province, between the Indus and Kabul rivers, with a moderate, temperate climate with four seasons, 639 mm of precipitation, hot summers, and an average winter temperature of 22.2 °C.

The Greek word "nematode" originates from a combination of two words: "toid," which means form, and "nema," which means thread. These are small, warm-shaped organisms that range in length from 0.2 to 2.5 mm. All nematodes are organized in the phylum Nematoda. This phylum is a member of the super phylum Ecdysozoa, which comprises all exuviated creatures, and it evolved in aquatic habitat during the early Cambrian explosion (Aguinaldo *et al*., 1997). While most nematodes are uncomplicated, colorless roundworms with unpartitioned bodies that taper toward the head and tail, certain kinds of parasitic species have distinct body forms, such as pear, kidney, or lemon.

Nematodes are the largest class of organisms with multiple cells on Earth, according to the number of individual cells. Even though there are currently around 4,100 identified species of PPN, more are continually being identified, and when cropping habits change, some species that were formerly regarded as benign or non-harmful are now considered pests. Nevertheless, only a small number of highly specialized subgroups that either directly injure their hosts or serve as widespread carriers can be isolated from the economically applicable PPN. For every square meter of moderately fertile soil that is 30 cm beneath the surface, there are about 50 million nematodes. Among all nematode species, half are marine, 25% are free-living, soil-dwelling

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nematodes, 5% are animal and human parasites, and 0% are parasites of plants. Developed countries still lose 5–10% of their crop productivity because of parasites, even with advanced technology (Indoulia *et al*., 2021).

PPN is considered to be one of the plants' hidden enemies. They are rarely considered by farmers and remain unnoticed because there is a lack of knowledge about their presence and the damage they cause to plants. They seriously damage plants and trees, and this has an immense impact on plant health and crop yields. Many variables affect the existence of plant parasitic nematodes and the way they affect plants. State that a few of these variables are critical to the nematode infection, according to Kleynhans *et al*. (1996).

The soil-borne diseases of plants that attack plant roots and reduce the yield and quality of agricultural crops are one of the greatest issues facing global agriculture (Aslam *et al*., 2017a, b and Katan, 2017). Worms and harmful mold destroyed many crops in Pakistan (Zarins and Shahina, 2010). Vegetables are becoming less prevalent and of lesser quality because of their receptivity to various root and soil diseases (Chehri *et al*., 2010). One of the most prevalent plant diseases is Fusarium, and most of its varieties are known to be significant root infection sources. It is the root cause of significant losses in grain crop yields globally and has been reported in Sindh and Balochistan (Savary *et al*., 2012). Nematodes destroy plants whole, from the root through the tip. Thousands of parasitic insects feed on the subterranean parts of trees. More diseases of plants have been able to infect the roots as a result of the nematode damage to the tree's root system, damaging the plant (Jibril *et al*., 2016).

Plant parasitic nematodes (PPN) are believed to be the largest crop-related problem around the world. Annually, PPN damages fruits and vegetables worth more than one hundred billion dollars (Muhammad *et al*., 2024). Therefore the main objective of this study was to investigate the occurrence and detection of various types of plant parasitic nematodes (PPN).

MATERIALS AND METHODS

Samples Collection

During the months of April–August 2021, a total of 60 samples of soil from the rhizosphere of pomegranate and grapes were randomly collected. 8 pomegranate and 6 grape orchards from different localities like Manki, Lahor, Anbar, Kunda, Shahmansoor, and Panjpir from each locality (4 quadrats) were taken in District Swabi, Pakistan. The site was selected on the basis of Tehsil Lahor (Figure 1). By using a soil hoe or trowel, at a depth of 25–30 cm, samples were taken. The size of the sample was 50–100 g/locality. The samples were placed and stored in plastic bags at 25–30 °C before being taken to the lab.

Figure 1. Map of Tehsil Lahor District Swabi (Online, Accessed December, 2021).

Sieving of sample

Initially, the soil was mixed in water, and then the mixture was sieved through 100µm, 200µm, and 300µm following the Cobb sieving method (1918).

Extraction of Nematodes

After sieving, the extraction of nematodes from residue was done through a dropper using the Baermann funnel technique (Schindler, 1961).

Fixing of Nematodes and preparation of slides

Nematodes were heat killed by an electric heater (avoid high temperature because it destroyed the morphology of nematodes) and preserved in TAF. Isolate the preserved nematodes and immerse them in 1.25 g of glycerin in a microwave oven at 54 °C (for 72 hours) for staining. Then the Muhammad *et al*. (2022) method was followed for slide preparation.

Identification of nematodes

Morphological identification of nematode genera was done through formulas and keys by Siddiqi (1986).

RESULTS AND DISCUSION

The study's findings showed that a number of PPNs were connected to pomegranates *(Punica granatum)*, with the most frequent PPNs in Manki comprising *Meloidogyne* (80%) and *Xiphinema* (19%), followed by *Tylenchorhynchus* (12%), as seen in Tables (1 and 2). In Shahmansor and Punjpir, *Helicotylenchus* was the most commonly recorded genera, whereas *Meloidogyne* was more abundant in Lahore and Anbar (Figure 2).

Table 1. (F.O. %) shows frequency of occurrence, and (P.D.) shows population density while (-) show no occurrence of PPN nematode genera associated with Pomegranate (*Punica granatum*) in district Swabi

Host Plant	Locations	Identified Genera	P.D.	F.O. (%)
Pomegranate (*Punica granatum*)	Manki	*Helicotylenchus*	73	7.8 %
		Tylenchorhynchus	123	12 %
		Meloidogyne	680	80 %
		Longidorus	9	1 %
		Trichodorus	40	4 %
		Xiphinema	115	19 %
		Pratylenchus	-	-
Pomegranate (*Punica granatum*)	Lahor	*Helicotylenchus*	105	10 %
		Tylenchorhynchus	37	3.9 %
		Meloidogyne	170	17 %
		Longidorus	-	-
		Trichodorus	4	0.4 %
		Xiphinema	-	-
		Pratylenchus	17	1.7 %
Pomegranate (*Punica granatum*)	Anbar	*Helicotylenchus*	275	61 %
		Tylenchorhynchus	34	3.3 %
		Meloidogyne	312	67 %
		Longidorus	-	-
		Trichodorus	1	0.01 %
		Xiphinema	13	1 %
		Pratylenchus	20	2 %
Pomegranate (*Punica granatum*)	Kunda	*Helicotylenchus*	80	8 %
		Tylenchorhynchus	47	4%
		Meloidogyne	91	9%
		Longidorus	3	0.03 %
		Trichodorus	12	1 %
		Xiphinema	7	0.7 %
		Pratylenchus	21	2%
Pomegranate (*Punica granatum*)	Shahmansor	*Helicotylenchus*	232	58 %
		Tylenchorhynchus	27	2%
		Meloidogyne	167	11%
		Longidorus	-	
		Trichodorus	-	
		Xiphinema	-	
		Pratylenchus	53	5%
Pomegranate (*Punica granatum*)	Punjpir	*Helicotylenchus*	87	9%
		Tylenchorhynchus	15	2%
		Meloidogyne	32	3%
		Longidorus	5	0.5%
		Trichodorus	-	
		Xiphinema	2	0.2%
		Pratylenchus	7	1%

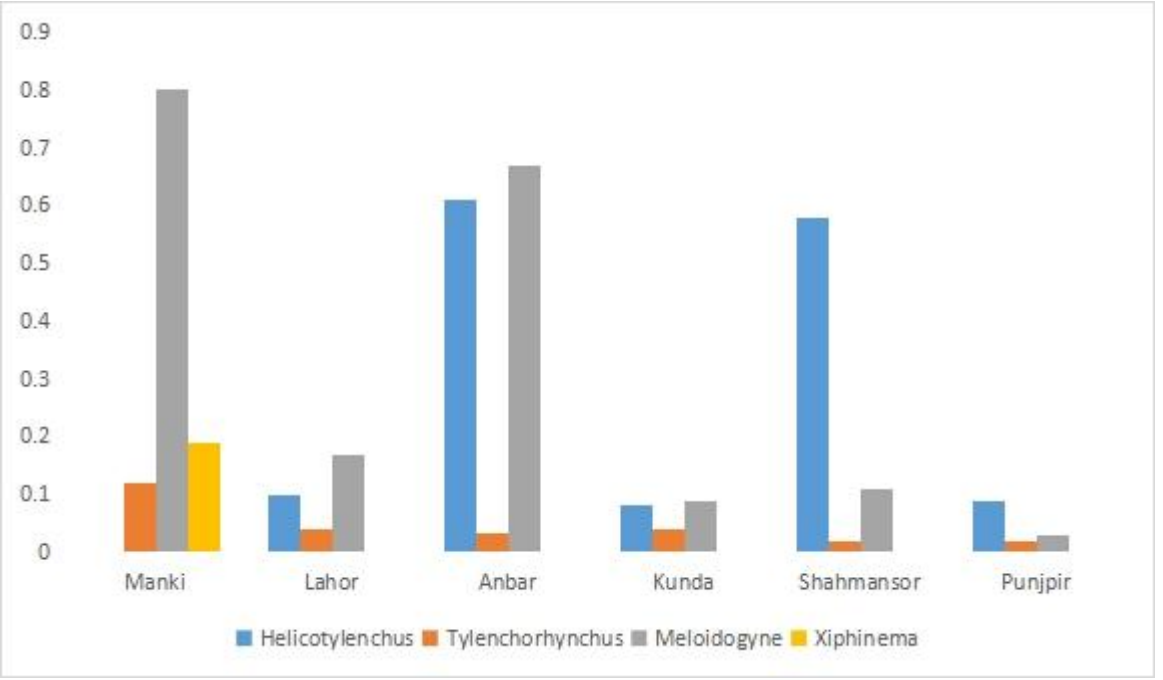

Figure 2. Shows the large abundance of recorded genera in each localities

Table (2) describes the different PPN species identified from the grape (Vitus *vinifera*) samples. The *Filenchus* species were the most commonly recorded, with a substantial number of records, followed by the *Tylenchus* species, as indicated in Figure (3). *Tylenchus* and *Filenchus* genera have a higher density of populations in Lahor. In Punjpir, *Meloidogyne* dominated, but in Shahmansor and Kunda, *Helicotylenchus* dominated.

In several locations in Balochistan, Pakistan, Khan *et al*. (2017) reported species from the PPN, including *Meloidogyne, Pratylenchus, Xiphinema, Merilinius Helicotylenchus, Helicotylenchus,* and *Aphelenchus.*

Khan *et al.* conducted a survey in 2014 in Balochistan, Pakistan, where sixty-six grapevine samples were collected from two districts. It included the PPN species *Aphelenchus, Helicotylenchus, Hoplolaimus, Meloidogyne,* and *Xiphinema.*

Many studies and investigations have been conducted on pomegranate plantations around the globe. Pomegranates have been identified as having *Melodogyne javanica, M. incognita X. index, Xiphinema basiri, Helicotylenchus multicinctus, H.indicus, Ditylenchus spp., Pratylenchus coffeae, Rotylenculus reniformis, Longidorus iranicus, Aglenchus spp., Basiroides obliquus, Basiria graminophila, Tylenchorhynchus Tylenchulus semipenetrans,* and *Ditylenchus sp., brassicae*. The orders Rhabditida, Dorylaimida, Aphelenchida, Chromadorida, Triplonhida, and Mononchida, included the species that were identified. In Turkey, the most common species found in pomegranates were *Boleodorus thylactus, Helicotylenchus digonicus* and *Pratylenchoides alkani* (Bajestani & Dolatabadi 2018 and Ilangovan & Poornima, 2017).

Plant parasitic nematode genera *(Pratylenchus, Trichodorus, Rotylenchulus, Longidorus, Tylenchorhynchus, Helicotyleuchus, Xiphinema Tylenchulus,* and *Meloidogyne*). According to 94.5 of all soil samples examined, the most frequent genus of root-knot nematode in grape assays was *Meloidogyne.* According to soil samples taken from 10.25 grape plants, *Tylenchorhynchus,* the stunt nematode, was the second most prevalent genus.
Xiphinema and *Rotylenchulus* were another two genera that were commonly found; they were found in 5.75 to 10.25% of the grape samples.

The present survey identified three new species: *Tylenchulus semipenetrans, Rotylenchulus reniformis,* and *Meloidogyne incognita*. *Meloidogyne* was the most common nematode genera, appearing in 35.5% of the soil samples examined, according to the frequency of nematode genera discovered in soil samples taken during the present assessment of pomegranate orchards. In 13.3% of the pomegranate soil samples examined, *Tylenchorhynchus* was the second most common genus.

Table 2. (F.O.%) shows the frequency of occurrence, and (P.D.) shows population density while (-) shows no occurrence of PPN nematode genera associated with Grapes (*Vitus. vinifera*) in district Swabi

Host Plant	Locations	Identified Species	P.D.	F.O. (%)
Grapes (*Vitus. vinifera*)	Manki	*Helicotylenchus*	227	45%
		Tylenchorhynchus	81	16%
		Meloidogyne	163	32%
		Filenchus	407	81%
		Tylenchus	343	68%
		Xiphinema	-	-
		Pratylenchus	-	-
Grapes (*Vitus. vinifera*)	Lahor	*Helicotylenchus*	116	23%
		Tylenchorhynchus	13	2.6%
		Meloidogyne	139	27.8%
		Filenchus	219	43.8%
		Tylenchus	311	62%
		Xiphinema	3	0.6%
		Pratylenchus	7	1.4%
Grapes (*Vitus. vinifera*)	Anbar	*Helicotylenchus*	18	3.6%
		Tylenchorhynchus	31	6%
		Meloidogyne	65	13%
		Filenchus	34	6.8%
		Tylenchus	17	3.4%
		Xiphinema	11	2%
		Pratylenchus	2	0.4%
Grapes (*Vitus. vinifera*)	Kunda	*Helicotylenchus*	4	0.8%
		Tylenchorhynchus	9	1.8%
		Meloidogyne	192	38.4%
		Filenchus	86	17%
		Tylenchus	41	8%
		Xiphinema	-	-
		Pratylenchus	-	-
Grapes (*Vitus. vinifera*)	Shahmansor	*Helicotylenchus*	67	13.4%
		Tylenchorhynchus	13	2.6%
		Meloidogyne	37	7.4%
		Filenchus	7	1.4%
		Tylenchus	-	-
		Xiphinema	7	1.4%
		Pratylenchus	2	0.4%
Grapes (*Vitus. vinifera*)	Punjpir	*Helicotylenchus*	113	22.6%
		Tylenchorhynchus	21	4%
		Meloidogyne	78	15.6%
		Filenchus	24	4.8%
		Tylenchus	19	3.8%
		Xiphinema	13	2.6%
		Pratylenchus	1	0.2%

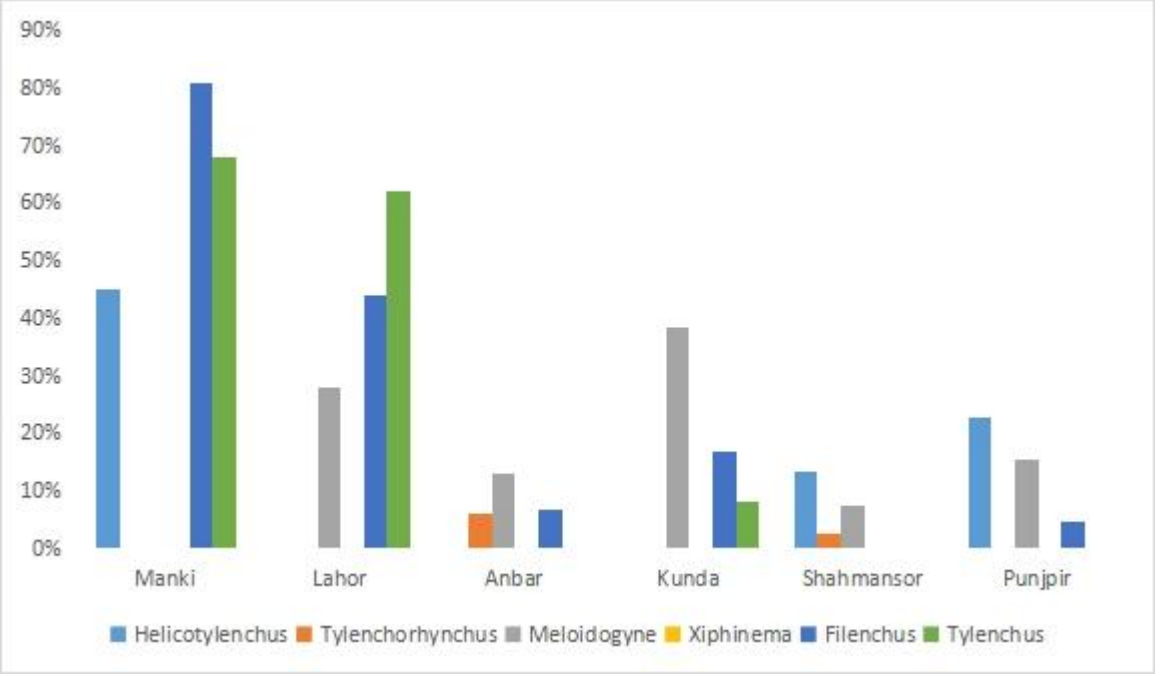

Figure 3. Shows the large abundance of recorded genera in each localities

Research indicated that the variety of plant-parasitic nematodes that infect grapes, *Meloidogyne* spp., the root-knot nematode, was more prevalent in 94.5% of the soil samples evaluated, followed by *Tylenchorhynchus* and *Xiphinema* (Mohamed *et al.*, 2017). In soil samples from four locations in the governorate of Ismailia, pomegranate fruit trees were identified to have an abundant genus of root-knot nematode, *Meloidogyne*. Under the circumstances of the North Sinai, the results concurred with those reported by Korayem *et al.* (2014). In grapes *Rotylenchulus* levels were found to be significantly lower than other species of *Meloidogyne* in El-Sharkia governorate (Adam *et al*., 2013), with mean levels ranging from 50 and 70 parasites, such as those in 250 g soil, as opposed to 429 and 20 individual nematodes in 100 g soil.

CONCLUSIONS

It is considered that many of the PPN genera that have been identified are highly infested and pose a threat to plants and crops. All of the assessed places have extremely distinct levels of occurrence and populations, which only highlights how highly infected the investigated sites are by a variety of PPN species, which ultimately affects pomegranate and grape growth. The PPN identification for agriculture in district Swabi is so necessary to reduce fruit yield loss. Managing the PPN is essential to preventing the loss of grape and pomegranate fruit yield.

Declaration

Ethical Approval and Consent to participate

Not applicable.

- **Consent for publication**

Not applicable.

- **Availability of supporting data**

Not applicable.

- **Competing interests**

The authors declare that they have no conflicts of interest.

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- **Authors' contributions**

Kubra Bibi: Conceptualization, Investigation, And Writing-original draft.

Mian Sayed Khan: Supervision.

Bilal Muhammad: Writing-review & editing.

Suleman: Review

All authors read and approved the submitted version.

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