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OCCURRENCE OF PLANT PARASITIC NEMATODES FROM DIFFERENT FRUIT GROWING AREAS OF BAJAUR, KHYBER PAKHTUNKHWA, PAKISTAN

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ABSTRACT

Agriculture is the most important sector of Pakistan. Effective improvement in agricultural productivity depends on appropriate crop protection from pests and diseases. Among these, the nematode problem in agricultural productivity is of significant importance. Plant-parasitic nematodes are one of the limiting factors in agricultural production. The economic importance of nematodes is well recognized all over the world as most agricultural crops are damaged by their continuous feeding on roots, buds, stems, crowns, leaves, and even seeds, resulting in low yield and poor quality fruits. The present study was conducted in 12 localities of district Bajaur, Khyber Pakhtunkhwa, Pakistan. A total of 200 samples of soil and roots of different crops and vegetables, grown in adjoining areas were collected and 22 nematode species were identified belonging to 5 orders, 11 families, and 12 genera, in which 18% were soil nematodes whereas 81% were plant-parasitic nematodes.

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INTRODUCTION

Agricultural production is the pillar of Pakistan's economy. Economy of Pakistan depends on the development of agriculture (Ali et al., 2013). This represents 31% of overall national products and benefits 70% of the population, directly or indirectly (Anonymous, 2019). The production of agricultural crops is significantly affected by many pests and various diseases, and the issue of nematodes poses a serious risk. In Pakistan it is more serious than that of other underdeveloped countries (Rehman et al., 2015; Sanaullah and Urooba, 2019). The very same situation exists in district Bajaur, because the climate is suitable and the land is very fertile and most of the land is under agriculture. Nematodes also cause damages to agriculture of Bajaur and reduce crop production, because nematodes affect farming. District Bajaur is a newly merged district of Khyber

Pakhtunkhwa, Pakistan. District Bajaur has two main sub-divisions Khar which consists of tehsils of Khar, Salarzai, Utmankhel, and nawagai comprises tehsils of Nawagai, Chamarkand, Mamund, and Barang. In Bajaur, the cultivated area under irrigation covers approximately 2739.22 hectares and over 74426 hectares is barani (un-irrigated) and around 20003.99 hectares are uncultivated. Of the total cultivation fields, three fourths depends upon precipitation and managed by farmers. There is a potential for developing agriculture in the Bajaur region, but most of the agriculture depends on groundwater. The main agricultural areas in the district Bajaur receive about 800 mm of average rainfall per annum.

In Bajaur there are two distinct seasons Rabi and Kharif. Main crops and vegetables are grown in the region are Maize, Rice (paddy crop), wheat, tomato, okra, squash, shimla rapeseed and mustard, eggplant, tenda and

fodder i.e. jowar, bajra etc. (Anonymous, 2019). Several surveys have been carried out in Pakistan to investigate the genera of nematodes associated with several important crops and the damage caused by nematodes on these crops. Plant parasitic nematodes cause a loss of US\$ 77 billion all over the world (Sasser and Freckman, 1987). Parasitic nematodes are widespread in nature and have almost all important crops associated with agriculture and pose major food safety constraints. At least one species of nematode is damaging to all plants. Nematode-borne diseases have multiple and varied financial consequences resultantly the quality and quantity of crop yields are reduced (Bernard et al., 2017). As no information is available on the presence of plant parasitic nematodes in Bajaur, therefore, the present study was carried out to determine the occurrence of nematodes associated with fruit trees in the area.

MATERIAL AND METHODS

Study area

The current study was carried out in thirteen different locations of district Bajaur KPK Pakistan. District Bajaur laying between 34.3669° to 34.4669° North, and 71.2099° to 71.3999°E and at 800 to 2400 meter elevation and covers an area of 1290 sq km. Four zones were selected on the bases of mountainous areas, plane areas, availability of water and soil fertility: Zone I (Mountaneous area of Khazan Baig Banda to Tarano and Batwar, Zone II (Pashat and Haji Lawing, and Raghagan), Zone III (Qazafi, Alizo and Khar) and Zone IV (Inayat Qilla and Mamundo Arang). Soil samples were collected from four different zones of the region. For authentic survey data and observation were recorded.

Storage of soil samples

The soil samples were immediately brought to laboratory in cool boxes or any other insulated containers, preferably among ice packs. The samples were processed or stored at 10 to 20°C in the chamber to prevent from hot sunlight and excessive heat, because nematodes may affect or kill from heat.

Extraction of nematodes or sieving of soil sample

The soil samples were firstly placed in a plastic tub. The roots were then separated from the soil samples carefully for studying of root knot nematodes (Cobb, 1918). The rest of the samples were sieved for the extraction of nematodes. For sieving process decanting and sieving and Baermann funnel method was used (Baermann, 1917). The active nematodes were isolated

from the soil sample very carefully by Baermann funnel method (Baermann, 1917). The deposit of 350 sieve mesh was obtained by Cobb's sieving and decanting method in a beaker. The 350 suspension was transferred gently into the funnel; a plastic basket was placed on the funnel which was covered with tissue paper. The funnel was partly filled with tap water. After 48 hours, 100 ml suspension was drawn in a clean beaker and studied under binocular.

Killing of nematodes

For concentration of the nematodes, the suspension was left for 2 hours to settle down. Decanted the supernatant water with the help of a dropper and reduced the water from 100 ml to 10 ml. Nematodes were heat killed by keeping the nematodes suspension on a microwave having 65-75°C temperature until the nematodes assume the heat death character. Nematodes assumed curved, straight or spiral shaped depending upon genera. Nematodes were also killed with hot water added to the cavity block of nematodes suspension or in an oven of 45°C temperature.

Fixing of nematodes

For fixing of nematodes, TAF (Triethanolamine 40% 2 ml, formaldehyde 37% formalin 7 ml, and Distilled water 91 ml) was added to the cavity block of nematodes and kept for 24 hours, following the method described by Courtney et al. (1955). The TAF is used as preservatives for external and internal body parts of nematodes. After qualitative and quantitative analyses of nematodes, process of killing, fixing, and slow dehydration were done for permanent slide mounts (Hooper et al., 2005). Identification of nematodes was made through measurements given by De Man (1880) formula with an ocular micrometer under a compound microscope and identification was based on the systematics given by Siddiqi (2000). Illustrations were prepared with the help of a drawing tube attached to the compound microscope and photographed of nematodes were made with a Nikon DS, Film camera, attached to the same Nikon Eclipse E400 microscope.

RESULTS

Occurrence of nematodes species at different localities of Bajaur

The result showed the occurrence percentage of nematode taxa encountered in the order viz. Tylenchida (45%) Rhabditida (25.4%), Dorylaimida (15.4%), Aphelenchida (10.5%), Mononchida (3.5%) and Plectida (2.2%) (Figure 1 and Table 1). About 200

soil and root samples were captured from 12 different areas of district Bajaur. A total of 22 nematode species were recovered from above mentioned localities, in

which 79% were plant parasitic nematodes whereas 21% nematodes were identified as saprophytic nematodes (Figure 1).

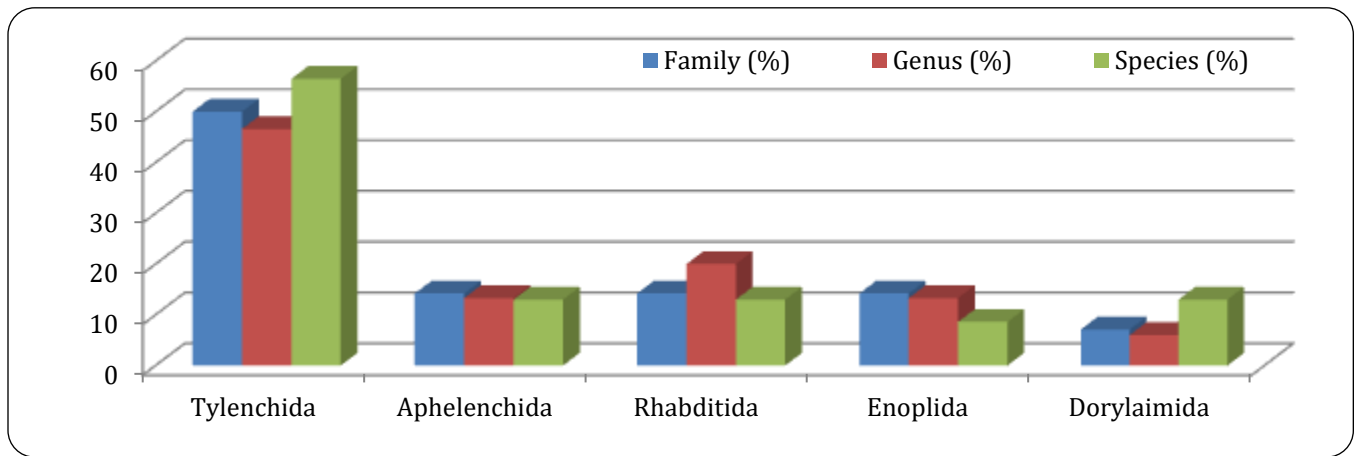


Figure 1. Overall distribution of nematode taxa in different orders.

Table 1. Nematode fauna (%) composition encountered during survey.

S. No.	Order	Family (%)	Genus (%)	Species (%)
1.	Tylenchida	50.0	46.6	56.5
2.	Aphelenchida	14.2	13.3	13.0
3.	Rhabditida	14.2	20.0	13.0
4.	Enoplida	14.2	13.3	8.6
5.	Dorylaimida	7.1	6.0	13.0

From Alizo eleven nematode species were found out of 22 nematode species (Figure 2). Out of these, 18% were soil nematodes whereas 81% were plant parasitic nematodes. From Arang, 12 species were detected out of total 22 species (Figure 3). From the soil samples of this area, 33% free living nematodes and 66% plant parasitic nematodes were found. The third locality was Batwar,

from where 25% soil and 75% plant parasitic nematodes were encountered (Figure 4). From Gambat (Figure 5) and Haji Lawang (Figure 6), the biodiversity of plant and soil nematodes were the same as in Batwar. Out of 13 nematode species, 38% nematodes were found as free-living soil nematodes while 61% nematodes were plant parasitic from Inayat Qilla (Figure 7).

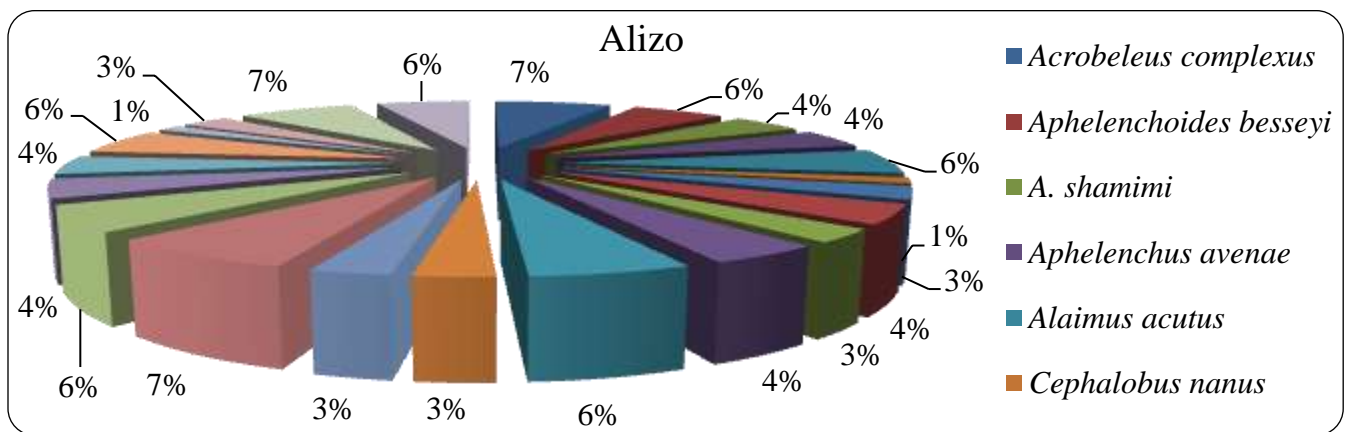


Figure 2. Occurrence (%) of nematode species in Alizo.

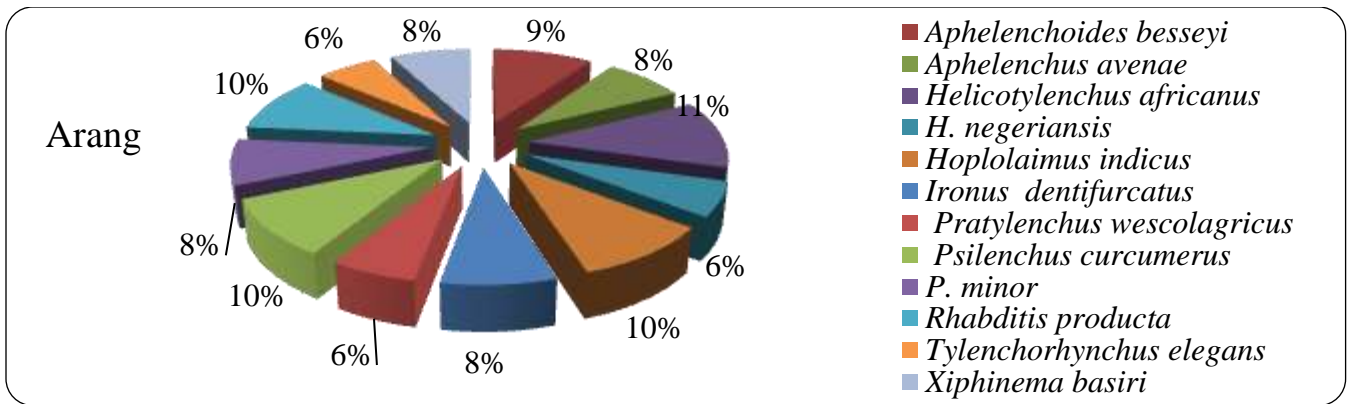


Figure 3. Occurrence (%) of nematode species in Arang.

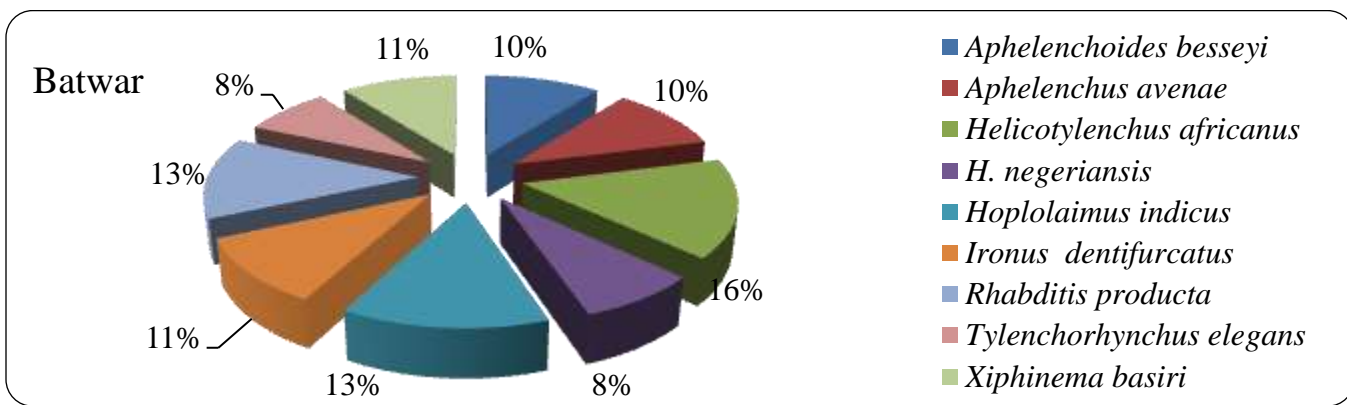


Figure 4. Occurrence (%) of nematode species in Batwar.

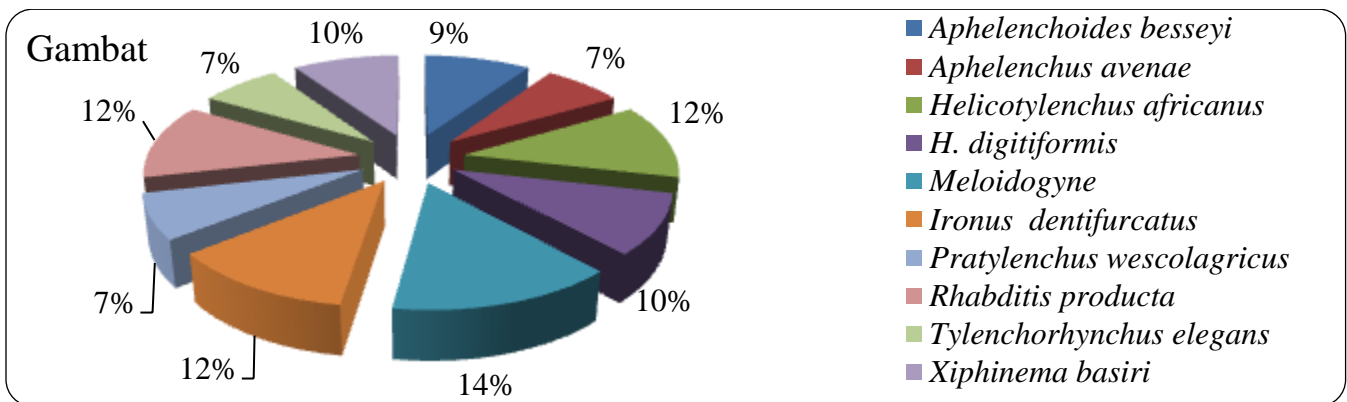


Figure 5. Occurrence (%) of nematode species in Gambat.

From Khar, out of 11 nematode species, 45% nematodes were found as free-living soil nematodes while 54% were plant parasitic nematodes (Figure 8). In Khazan Baig Banda, the soil analysis revealed 38% soil nematodes while 61% nematodes were plant parasitic (Figure 9). From Mamundo (Figure 10) both nematode fauna were equally distributed which was 50%. Whereas in Pashat, 43% soil nematodes were

found while 57% plant parasitic nematodes were encountered (Figure 11). In the locality of Qazafi, free-living soil nematodes were found 38% while plant parasitic nematodes were 61% (Figure 12). From Tarano, 42% soil nematodes whereas 57% plant parasitic nematodes were found (Figure 13). The nematodes and host plants recorded in these areas are given in Tables 2-4.

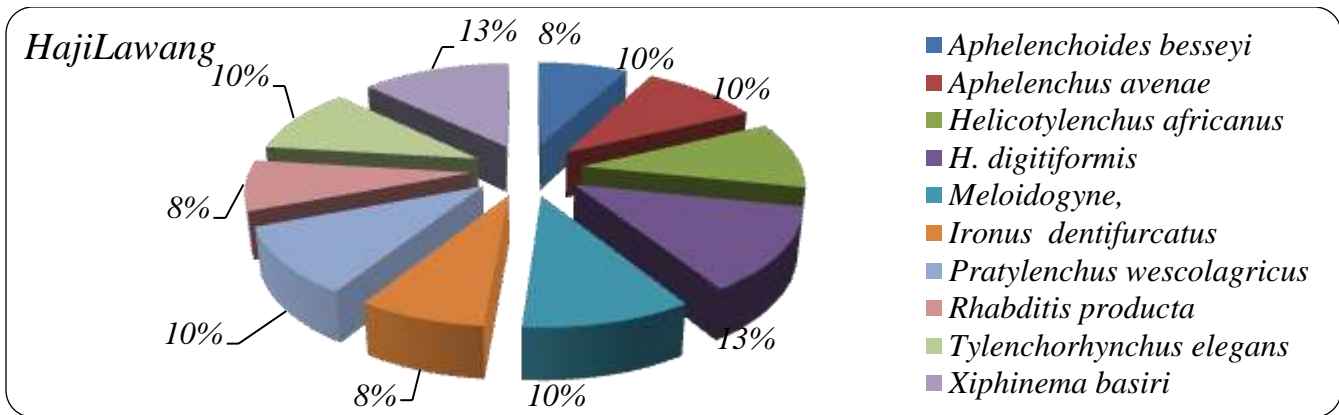


Figure 6. Occurrence (%) of nematode species in Haji Lawang.

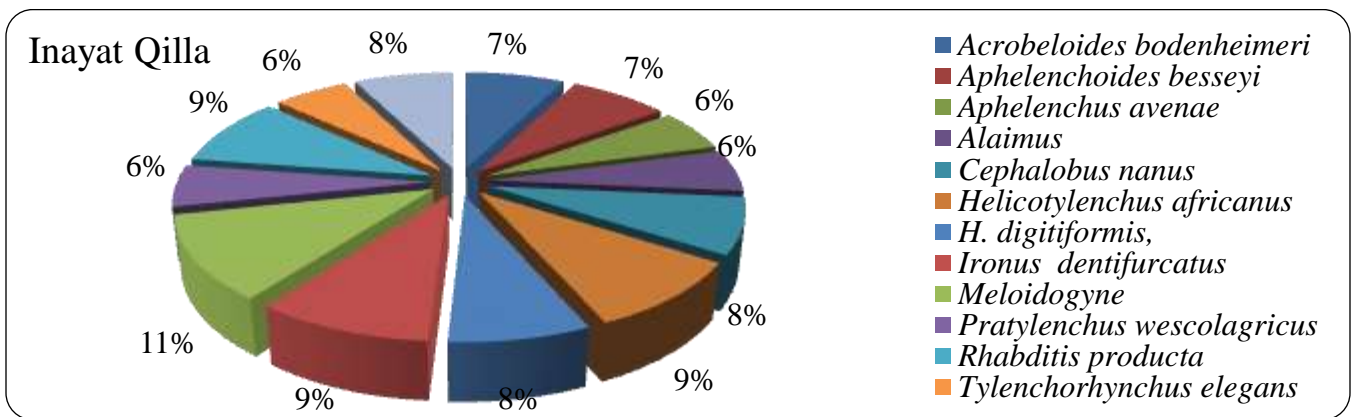


Figure 7. Occurrence (%) of nematode species at Inayat Qilla.

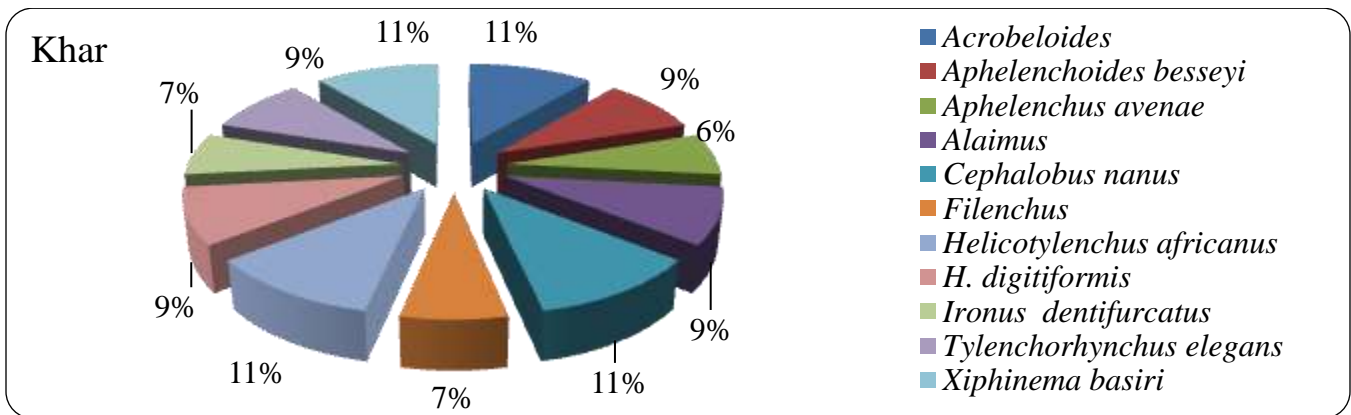


Figure 8. Occurrence (%) of nematode species in Khar.

The current studies were based on the survey conducted and assessment of the frequency of occurrence of economically important nematode species associated with different fruits and crop. The maximum nematodes were found in the order Tylenchida (45%) followed by the order Rhabditida (25.4%), Dorylaimida (15.4%), Aphelenchida (10.5%), Mononchida (3.5%) and Plectida (2.2%). The

results have shown the prevalence of 22 nematode species belonging to 12 genera, 11 families and 5 orders viz. Aphelenchida, Dorylaimida, Enoplida, Rhabditida and Tylenchida. Plant Parasitic nematodes of ten genera were encountered viz. *Aphelenchus*, *Aphelenchoides*, *Filenchus*, *Helicotylenchus*, *Meloidogyne*, *Pratylenchus*, *Psilenchus*, *Sinura*, *Tylenchorhynchus*, *Xiphinema*.

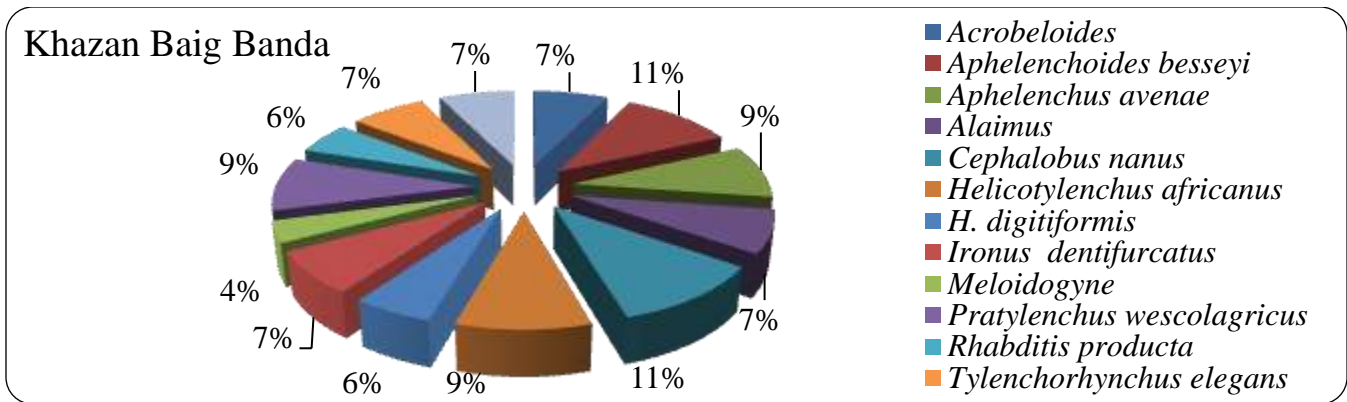


Figure 9. Occurrence (%) of nematode species in Khazan Baig Banda.

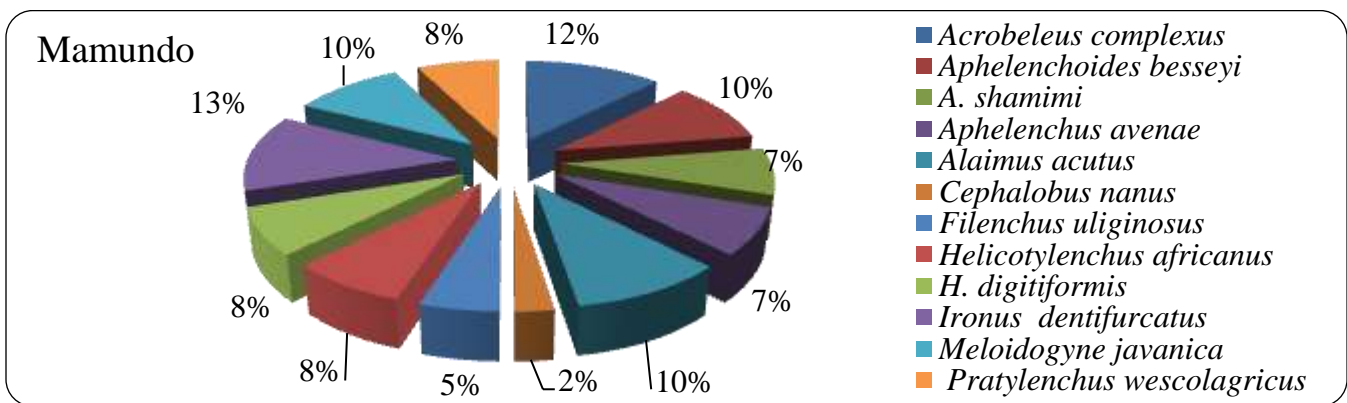


Figure 10. Occurrence (%) of nematode species at Mamundo.

Table 2. Nematodes and host plants recorded in Alizo, Arang, Batwar, Gambat, Inayat Qilla and Khazan Baig Banda.

Locality	Host Plant	Nematodes
Alizo	<i>Prunus persica</i>	<i>Aphelenchoides</i> , <i>Cephalobes</i> <i>Aphelenchus</i> , <i>Aphelenchoides</i>
	<i>Zea mayz</i>	<i>Helicotylenchus</i> , <i>Mononchus</i> <i>Tylenchorhynchus</i>
	<i>Capsicum annum</i>	<i>Aphelenchus</i> , <i>Aphelenchoides</i> <i>Mononchus</i> , <i>Pratylenchus</i>
	<i>Abelmoschus esculentus</i>	<i>Aphelenchus</i> , <i>Aphelenchoides</i> <i>Helicotylenchus</i> , <i>Pratylenchus</i>
	<i>Phaseolus vulgaris</i>	<i>Aphelenchoides</i> , <i>Aphelenchus</i> <i>Pratylenchus</i>
Arang	<i>Solanum lycopersicum</i>	<i>Aphelenchus</i> <i>Aphelenchoides</i> <i>Pratylenchus</i> <i>Tylenchorhynchus</i>
	<i>Prunus percica</i>	<i>Aphelenchoides</i> <i>Aphelenchus</i> <i>Helicotylenchus</i> <i>Mononchus</i>
	<i>Triticum aestivum</i>	<i>Aphelenchus</i> <i>Cephalobus</i> <i>Tylenchorhynchus</i> , <i>Xiphenima</i>
Batwar	<i>Prunus armeniaca</i>	<i>Aphelenchus</i> <i>Helicotylenchus</i>
	<i>Diospyrus kaki</i>	<i>Tylenchorhynchus</i>
	<i>Juglans spp</i>	<i>Aphelenchus</i> , <i>Aphelenchoid</i> <i>Aphelenchus</i> , <i>Aphelenchoides</i>
Gambat	<i>Coriandrum sativam</i>	<i>Pratylenchus</i> , <i>Tylenchorhynchus</i>
	<i>Citrus sinensis</i>	<i>Rhabditis</i> <i>Aphelenchus</i> , <i>Rhabditis</i>

	<i>Capsicum frutescens</i>	<i>Aphelenchus</i> , <i>Helicotylenchus</i> <i>Tylenchorhynchus</i>
	<i>Eriobotrya</i> spp	<i>Aphelenchus</i> , <i>Helicotylenchus</i> , <i>Meloidogyne</i> , <i>Xiphenima</i>
	<i>Cucurbiata</i> spp	<i>Aphelenchus</i> <i>Aphelenchoides</i> , <i>Pratylenchus</i> , <i>Tylenchorhynchus</i> ,
Inayat Qilla	<i>Solanum melongena</i>	<i>Aphelenchus</i> , <i>Mononchus</i> , <i>Pratylenchus</i> , <i>Tylenchid</i>
	<i>Capsicum annum</i>	<i>Acrobeloides</i> , <i>Alaimus acutus</i>
	<i>Capsicum frutescens</i>	<i>Cephalobids</i> , <i>Ironus</i>
Khazan Banda	Baig <i>Diospyros kaki</i>	<i>Helicotylenchus africanus</i> <i>Tylenchorhynchus</i> <i>Acrobeloides besseyi</i> <i>Alaimus</i> , <i>Cephalobids</i> , <i>Ironus</i> ,

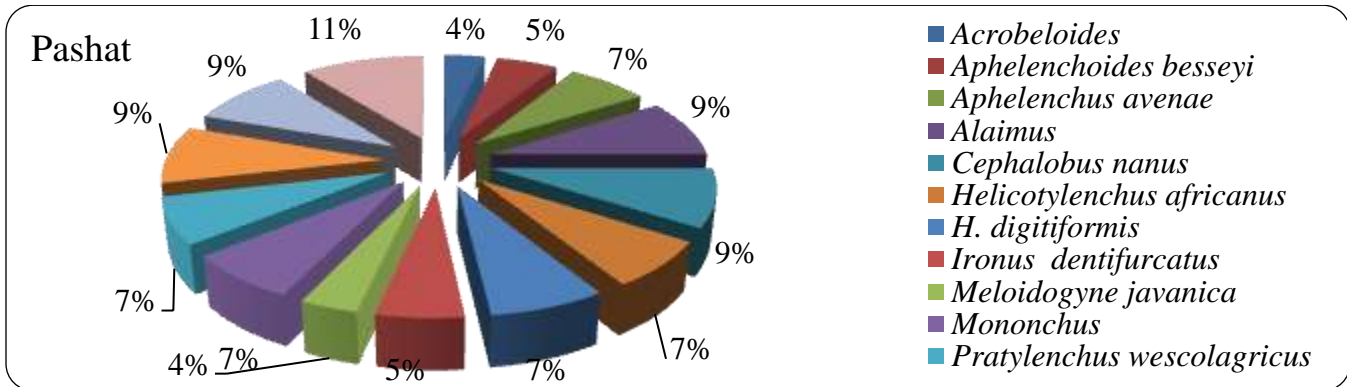


Figure 11. Occurrence (%) of nematode species in Pashat.

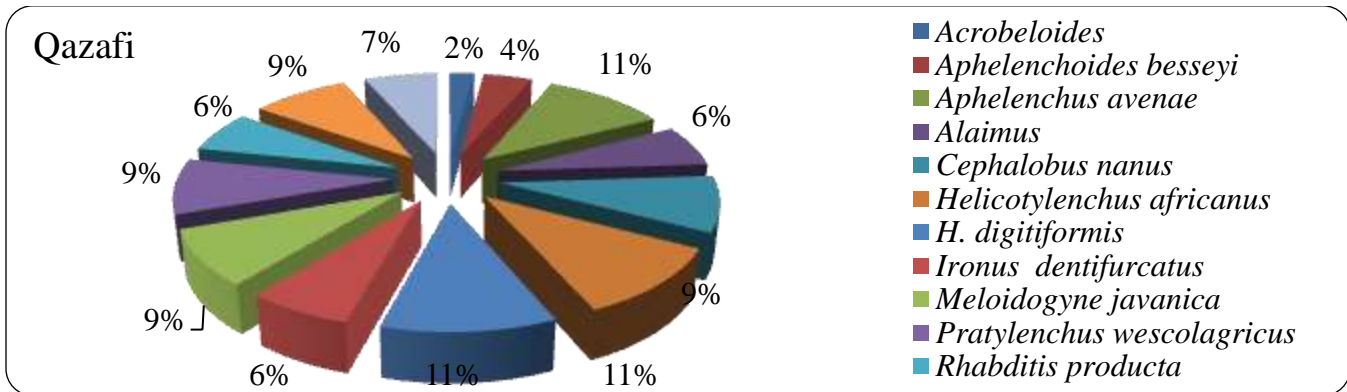


Figure 12. Occurrence (%) of nematode species in Qazafi.

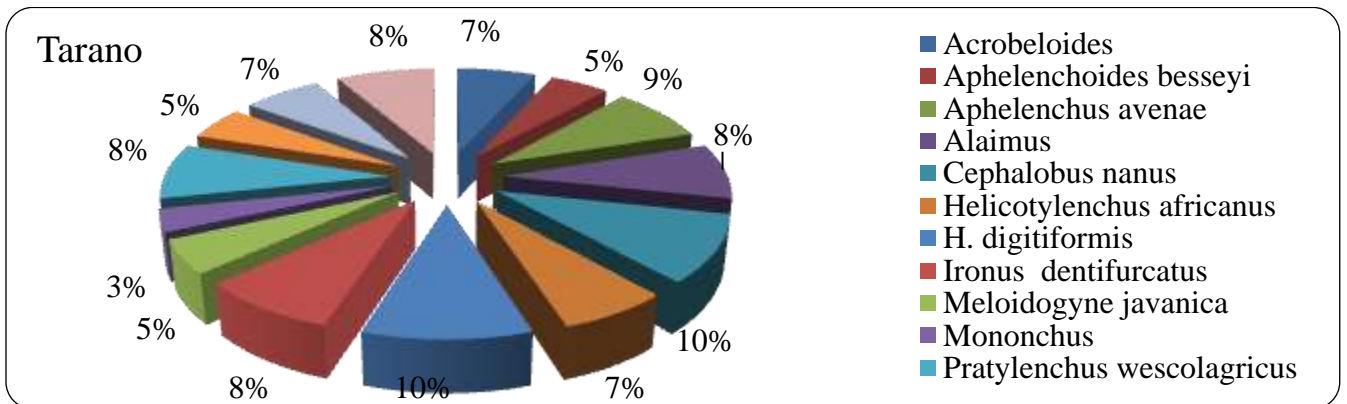


Figure 13. Occurrence (%) of nematode species in Tarano.

Table 3. Nematodes and host plants recorded in Khazan Baig Banda, Khar, Mamundo and Qazafi.

Locality	Host Plant	Nematodes
Khazan Baig Banda	<i>Phaseolus vulgaris</i>	<i>Acrobeloides, Alaimus, Cephalobids, Ironus</i>
	<i>Solanum melongena</i>	<i>Meloidogyne</i>
	<i>Allium sativum</i>	<i>Aphelenchus, Helicotylenchus, Tylenchorhynchus</i>
	<i>Solanum lycopersicum</i>	<i>Acrobeloides, Alaimus Cephalobus, Tylenchorhynchus</i>
Khar	<i>Zea mays</i>	<i>Aphelenchoides, Filenchus Cephalobus, Rharbditis, Tylenchorhynchus,</i>
	<i>Capsicum frutescense</i>	<i>Acrobeloides, Cephalobids, Ironus</i>
	<i>Oriza sativa</i>	<i>Acrobeloides, Cephalobids, Ironus</i>
	<i>Triticum aestivum</i>	<i>Acrobeles, Aphelenchus, Aphelenchoides, Cephalobus, Tylenchorhynchus</i>
	<i>Solanum melongena</i>	<i>Aphelenchus, Aphelenchoid</i>
Mamundo	<i>Cucurbita</i>	<i>Acrobeloides, Aphelenchus, Alaimus, Cephalobids, Dorylims, Filenchus, Ironus, Psilenchus</i>
	<i>Cucurbita</i>	<i>Aphelenchus, Discolaimus, Mononchus, Tylenchorhynchus</i>
	<i>Solanum melongena</i>	<i>Aphelenchus, Discolaimus, Mononchus, Tylenchorhynchus</i>
	<i>Cucurbita</i>	<i>Acrobeloides, Aphelenchus, Alaimus, Cephalobids, Dorylims, Filenchus, Ironus, Psilenchus,</i>
	<i>Cucurbita</i>	<i>Aphelenchus, Discolaimus, Mononchus, Tylenchorhynchus</i>
Qazafi	<i>Triticum aestivum</i>	<i>Aphelenchus, Mononchus, Pratylenchus,</i>
	<i>Prunus percica</i>	<i>Cephalobus, Rharbditis</i>
	<i>Capsicum annum</i>	<i>Aphelenchoides, Aphelenchus,</i>
	<i>Capsicum annum</i>	<i>Cephalobus, Meloidogyne, Mononchus,</i>
	<i>Citrus spp</i>	<i>Acrobeloides, Alaimus, Cephalobids, Ironus, Tylenchorhynchus</i>
	<i>Brassica rape</i>	<i>Mononchus, Aphelenchus, Pratylenchus,</i>

Table 4. Nematodes and host plants recorded in Pashat and Tarano.

Locality	Host Plant	Nematodes
Pashat	<i>Triticum aestivum</i>	<i>Acrobeloides, Alaimus, Cephalobids, Ironus,</i>
	<i>Alium cepa</i>	<i>Aphelenchus, Mononchus</i>
	<i>Morus</i>	<i>Meloidogyne javanica</i>
	<i>Solanum melongena</i>	<i>Acrobeloides, Alaimus, Cephalobids, Ironus,</i>
	<i>Abelmoschus esculentus</i>	<i>Aphelenchus, Mononchus, Tylenchorhynchus, Xiphenima</i>
Tarano	<i>Juglans</i>	<i>Acrobeloides, Alaimus, Cephalobids, Xiphenima</i>
	<i>Cucurbeta</i>	<i>Aphelenchus, Discolaimus, Mononchus, Tylenchorhynchus</i>
	<i>Prunus armeniaca</i>	<i>Aphelenchus, Mononchus</i>
	<i>Zea mays</i>	<i>Acrobeloides, Aphelenchus, Alaimus, Dorylims, Ironus, Cephalobids</i>
	<i>Lagenaria siceraria</i>	<i>Aphelenchus, Sinura, Meloidogyne,</i>

DISCUSSION

The current surveys provided thorough information on the occurrence and prevalence of plant parasitic nematodes from different fruit growing areas of Bajaur, Khyber Pakhtunkhwa. In the surveys, plant parasitic nematodes of ten genera were encountered viz. *Aphelenchus*, *Aphelenchoides*, *Filenchus*, *Helicotylenchus*, *Meloidogyne*, *Pratylenchus*, *Psilenchus*, *Sinura*, *Tylenchorhynchus*, *Xiphinema*. The survey provided baseline information on the occurrence and prevalence of phytopathogenic nematodes in the area associated with fruit trees. Some of the nematodes found were of economic significance as they are considered serious pests of fruit trees.

Root-knot nematodes were recorded from the soil as well as found infecting the roots. These nematodes induce galls on the roots of trees and reduce yield and growth of plants and trees by affecting translocation of water, minerals and nutrients from the soil. Root lesion nematode, *Pratylenchus penetrans*, is a migratory endoparasitic nematode of roots and produces reddish brown lesions on the rootlets. These nematodes have also been found implicated with other soil borne pathogens and increase the severity of other diseases. There are reports that *Meloidogyne* spp. and *Aphelenchoides* have been frequently reported from strawberry in USA (Anonymous, 1991). This plant is also a host of *P. penetrans*, *Ditylenchus* spp. and *Xiphinema* spp. All these nematode pests have the potential to cause serious infections on fruit trees including strawberry.

The other identified nematodes were ectoparasites of roots. These included *Aphelenchus*, *Filenchus*, *Helicotylenchus*, *Psilenchus*, *Sinura*, *Tylenchorhynchus*, *Xiphinema*. These nematodes cause direct damage to the roots by their continuous feeding. Soil and root samples from strawberry in other parts of the world contained *Helicotylenchus* spp., *Pratylenchus* spp., *Xiphinema* spp., *Meloidogyne* spp., *Tylenchorhynchus* spp. and other nematode genera (Goocheen, 1956). These nematodes have also been found in many other commercial fruits. These findings suggest that these nematodes are indigenous and can multiply with the passage of time if the conditions were favorable.

The variations in the occurrence of different nematodes seem to be influenced by cropping patterns. It has also been found that plant parasitic nematodes in fruit trees in cultivated lands may be affected by planting cover crops, the use of alternate crop sequences and fallow

(Brodie et al., 1970a, 1970b; Brodie and Murphy, 1975; Park et al., 2005). Root-knot nematodes have been reported by many workers from fruits and vegetables from different areas of the country (Hussain et al., 2012; Hussain and Mukhtar, 2019; Kayani et al., 2013; Saeed et al., 2021; Tariq-Khan et al., 2017; Tariq-Khan et al., 2020). Similarly, citrus has been found infected with *Tylenchulus semipenetrans* and caused serious reductions in fruit production (Ahmad et al., 2004; Fateh et al., 2017; Iqbal et al., 2004b; Iqbal et al., 2004a; Iqbal et al., 2005a; Iqbal et al., 2007; Iqbal et al., 2005b; Irshad et al., 2012; Mukhtar et al., 2007; Saeed et al., 2019).

CONCLUSIONS

It is inferred from the present study that plant parasitic nematodes are fairly distributed in the fruit growing areas of Bajaur. This information on the current status of nematode prevalence will be helpful for the farmers who consider the nematode problems both currently and in future according to the characteristic of each species of the nematode. Presence of numerous economically important nematodes in fruit orchards of the area deserves stringent attention by the researchers, scientists and extension workers to persuade farmers to adopt control strategies accordingly. Further research is needed to determine the significance of these nematodes on fruits and to determine their possible interactions with other soil borne pathogens as well as with the environment. The survey provided important background information for planning and administering nematode management strategies in fruits of the area.

AUTHORS' CONTRIBUTION

SU and TAK designed and formulated the study, prepared layout of surveys, SU conducted surveys, recorded the data, took nematode samples, isolated nematodes and identified them, TAK helped in nematode identification and supervised the work, SU wrote the manuscript, and TAK proofread the manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interests.

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