



Official publication of Pakistan Phytopathological Society
Pakistan Journal of Phytopathology

ISSN: 1019-763X (Print), 2305-0284 (Online)

<http://www.pakps.com>



PLANT PARASITIC NEMATODE OF GENERA *APHELENCHUS* AND *APHELENCHOIDES* (NEMATODA: APHELENCHOIDEA) FROM DISTRICT LAKKI MARWAT, KHYBER PAKHTUNKHWA, PAKISTAN

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ABSTRACT

The research work is based on the study regarding nematodes belonging to the order of Aphelenchida. Research work was carried out in four sites of District Lakki Marwat, Khyber Pakhtunkhwa Pakistan, where no such research was conducted about nematode fauna presence in the past. For the said purpose, numbers of soil samples from different rhizosphere were collected from various sites in the year 2019. Upon completion of all pre-requisite, the related correspondence and data of the surveyed sites with their results including, occurrence percentage, Relative Frequency, Simpson Diversity Index and Dendrogram of Cluster Analysis were ensured. However, detailed analysis revealed two types of genera i.e. *Aphelenchus* (49%) while *Aphelenchoides* (51%). Resultantly, two species of *Aphelenchus* namely *Aphelenchus avenae* and *Aphelenchus isomerus*; while four species of *Aphelenchoides* namely *Aphelenchoides bicaudatus*, *Aphelenchoides richardsoni*, *Aphelenchoides unisexus* and *Aphelenchoides macrospica* were surfaced. The *Aphelenchus avenae* was found most frequently abundance species (40%) from all sites and followed by *Aphelenchoides richardsoni* (20%). The highest abundance occurrence site of both genera was village Aba Khel (50% each) and the lowest one village Aghzar Khel *Aphelenchus* (22.7%) and *Aphelenchoides* (18.1%). All these species were previously reported for Pakistani nematode fauna except *Aphelenchoides macrospica* which was surfaced for the first time from Pakistan. It is also pertinent to mentioned here that remaining species were surfaced from new host and location from Pakistan.

Keywords: *Aphelenchus*, *Aphelenchoides*, District Lakki Marwat, New record, New host and locality.

INTRODUCTION

Lakki Marwat is one of the Southern Districts of Khyber Pakhtunkhwa Province, Pakistan and located at 32°17'-32°53' N Latitude and 70°23' - 71°16' E Longitude. Total area covered of 3,164 Square kilometers (ESA, 2012) and having population of 876,182 as per last census conducted in year 2017. On the North side covered by District Bannu and District Karak while on the East side bounded by District Mianwali, Punjab. Towards South East of the District Dera Ismail Khan while southwest covered by the District Tank. On the western side joined by the tribal areas of District Tank including some areas

of South Waziristan Agency. Region of district Lakki Marwat has all the characteristics of a desert due to its sand dunes, scorching heat and dry weather with hot summers and moderately cool winters. However, rainfall is very rare and intermittent and generally arises in July and August. The component elements of the area's soils are extremely and deeply drained, calcareous and uneven surfaced and likewise loamy sand. The fine textured soils are sandy clay and clay loam of the area and contained variety of rocks of Bhattani and Waziristan ranges. Climate of the area is arid to semi arid and sub-tropical continental. Variation in temperature scale commonly noted during the summer season from 27-42°C and in winter season from 4-20°C (Haq, 2009). Geography of the region is a mixture of hills and sandy plains. Maximum boundaries of the district contained on hills including East, Southeast, Southwest as well as Northwest. General dimension and elevation

Submitted: August 20, 2020

Revised: October 08, 2020

Accepted for Publication: October 21, 2020

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of these hills ranging from 500 to 1000 meters and most of the district region is almost shown 200 to 300 meters above level of sea.

Most of region consists on arid plains and required a lot number of sources which is appropriate and commonly considered fruitful for the development of agriculture field. However, the area unfortunately facing acute shortage in constraints i.e. shortage of water and non-existence of irrigation system due to which the land is extremely weak for agriculture purpose (ESA, 2012). Lakki Marwat area is not rich with any sources to highlight but entirely depends upon rain water. The two prominent crops are wheat and gram and mostly the drought has severely affected both crops in the sandy areas of district. These crops have been grown on vast tract of land in the barani (rain fed) belt. The crops especially wheat and gram have been affected due to low perspiration in winter season, which is a major cause of concern for farmers. Other than aforementioned; some other crops are also growing up including maize, rice, Bajra, Jowar (Sorghum), Sugarcane, Gaura, Barley, Rape and Mustard, Masoor, Dates, Orange, Mango, Guava, Watermelon, Melon, Pomegranates, Banana, Grapes, Mulberry and Ber.

The planet earth is engrossed by immense diversity of life and existence of nematodes is the second most species rich phylum next to Arthropoda (Keshari *et al.*, 2019). Nematodes are parasites and microscopic round worms and it may be source of threat to food insecurity in numbers of countries. Nevertheless, Pakistan face the issues of damaging of plants by nematodes due to its geographical location particularly in the sultry areas in which the climate is considered utmost appropriate for reproduction including activities of nematodes all over the year (Maqbool, 1988). The Order Aphelenchida is one of the abstemiously limitless nematodes order which is consists on numbers of ecological sets including fungal feeding, obligate insects' parasites, predator while some of them belongs to phytoparasite related to the genera *Bursaphelenchus* Fuchs, 1937 and *Aphelenchoides* Fischer, 1894 (Nickle, 1970), but large number of species of *Aphelenchoides* are fungal feeder or predators (Kanzaki and Giblin-Davis, 2012). Generally, the *Aphelenchus* are highly active nematode, predominantly mycetophagous in habit, cosmopolitan in soil, plant debris free-living and association with lichens, on tree bark as well as in the tunnels of wood-boring beetles (Hunt, 1993).

Aphelenchus belong to be genera *Aphelenchus* and *Aphelenchoides* are the utmost and widely spread and being primarily mycetophagous almost certainly represent the nearby to the ancestral forms (Hunt, 1993). Although, most *Aphelenchoides* are entirely mycetophagous, while few species under the sobriquet 'bud and leaf nematodes', parasitize higher plants. However, *Aphelenchoides* may be retrieved from mosses, soil, decaying organic materials, mushrooms while from plant tissues (Khusainov, 2013). Sanchez-Monge *et al.*, 2015 search out plant parasitic nematodes with presented 13 plant parasites considered most important all over the world.

Currently, nineteen species of genus *Aphelenchoides* Fischer, 1894 and four species of genus *Aphelenchus* Bastian, and 1865 has been reported from Pakistan (Shahina *et al.*, 2019). The objective of this plan was to study the Aphelenchid nematodes by conducted nematological survey from different areas of District Lakki Marwat, Khyber Pakhtunkhwa (KPK), and Pakistan.

MATERIALS AND METHODS

Area of Exploration: Nematological survey was undertaken in the months of October and November 2019 in various sites including villages Aba Khel, Aghzar Khel, Sarai Naurang and Tajori of the District Lakki Marwat, Khyber Pakhtunkhwa, Pakistan. Total 61 soil samples of different plants were retrieved and subsequently these samples were safely brought to laboratory of National Nematological Research Centre, University of Karachi, Pakistan. The aim of said survey was to establish the biodiversity of Aphelenchid nematodes.

Collection and Processing of Soil Sample: The soil samples (500 gm) each from different infected plant host were collected and packed in clean plastic bags, tied up and labeled appropriately containing relevant information i.e. host, locality, time and date of collection and shifted to the laboratory of National Nematological Research Centre, University of Karachi, Pakistan and accordingly stored at 5-10°C. The nematodes from soil was extracted by means of Cobb's sieving and decanting method (Cobb, 1918) and further purified by Baermann's funnel technique (Baermann, 1917). The extracted nematodes were quantified in a chamber within 5 ml suspension through counter under the binocular microscope and the same procedure was

repeated 3 times and the average reading result found the number of nematodes as per unit of soil sample. Retrieved nematodes were killed instantaneously by pouring a hot water (80-90°C) in a glass cavity block and immediately preserve in TAF (Tri-ethanol Amine Formalin) solution for 24 hours (Courtney, Polley and Miller, 1955). After that fixed nematodes were sanitized thrice with distilled water. Specimens were kept for 5-6 days in an incubator at 55°C in 2 ml of 1.25 % glycerin for slow dehydration (Seinhorst, 1959). Permanent mounting was done by transferring of nematodes to a

clean microscopic glass slide having pure drop of glycerin and sealed with paraffin wax by gently heating the slide. Later on, measurements were undertaken by de Man's, 1884 formula through compound microscope Nikon Eclipse E-400.

DATA ANALYSIS

The aim of area explored in District Lakki Marwat was to ascertain the existence and percentage of Aphelenchid nematodes. The occurrence percentage and relative frequency of nematodes was assessed as follows:

$$\text{Occurrence (\%)} = \frac{\text{Total number of positive samples}}{\text{Total number of Samples}} \times 100$$

$$\text{Relative Frequency (\%)} = \frac{\text{Frequency of Genus/ Species in a sample}}{\text{Sum of frequencies of all genus/ species present}} \times 100$$

Statistically, it was also analyzed by dendrogram of cluster analysis and Simpson diversity index (Simpson, 1949). Diversity index was determined by using the formula as follows:

$$D = 1 - \frac{\sum n(n - 1)}{N(N - 1)}$$

D=Diversity, N=Total number of individuals (Genus), n=Number of each individual (Particular Genus).

RESULTS

During the study of nematodes belonging to the order of *Aphelenchida*, it was revealed that various locations of district had large number of plant-parasitic nematodes genera including *Ditylenchus*, *Tylenchorhynchus*, *Pratylenchus*, *Hoplolaimus*, *Helicotylenchus*, root knot nematodes and other free-living soil nematodes. However,

special focus was given only on plant parasitic *Aphelenchus* and *Aphelenchoides* due scope of work.

Occurrence percentage: 30 soil samples out of 61 were found positive and detailed analysis of overall samples revealed two types of genera i.e *Aphelenchus* (49%) as well as *Aphelenchoides* (51%). In term of overall occurrence percentage of both genera reported from each site along with Global Positioning System (GPS) was illustrated (Table 1) and earmarked the highest abundance occurrence of both genera in Aba Khel (50% each) and the lowest occurrence in the village Aghzar Khel *Aphelenchus* (22.7%) and *Aphelenchoides* (18.1%). Furthermore, occurrence of genus was further probed to ascertain the fact and figure of each genus according to locations where surfaced through applied Relative Frequency (RF).

Table 1. Site Wise Occurrence % of each Genus *Aphelenchus* and *Aphelenchoides*

Sites	Latitude	Longitude	NS	NPS	Genus	SCG	Occurrence	RF
Aba Khel	32°34'09"N	70°49'39"E	04	03	<i>Aphelenchus</i>	02	50%	11.7
					<i>Aphelenchoides</i>	02	50%	10.5
Aghzar Khel	32°23'37"N	70°44'16"E	22	07	<i>Aphelenchus</i>	05	22.7%	29.4
					<i>Aphelenchoides</i>	04	18.1%	21.0
Sarai Naurang	32°49'43"N	70°46'33"E	29	16	<i>Aphelenchus</i>	08	27.5%	47.0
					<i>Aphelenchoides</i>	11	37.9%	57.8
Tajori	32°37'51"N	70°34'59"E	6	04	<i>Aphelenchus</i>	02	33.3%	11.7
					<i>Aphelenchoides</i>	02	33.3%	10.5

NS=Number of Samples, NPS=Number of Positive Samples, SCG=Samples Containing Genus, RF=Relative Frequency

Dendrogram: The dendrogram of cluster analysis among the various sites of surveyed areas was applied and accordingly presenting (Figure 1). These locations were divided into two main clades. Sarai Naurang and Tajori has been placed in clade I; while, Aba Khel and Aghzar Khel formed clade II. In the

same context, cluster analysis of six nematodes species including *Aphelenchus avenae* and *Aphelenchoides richardsoni* formed clade I; while, *Aphelenchus isomerus*, *Aphelenchoides bicaudatus*, *Aphelenchoides macrospica* and *Aphelenchoides unisexu* form clade II (Figure 2).

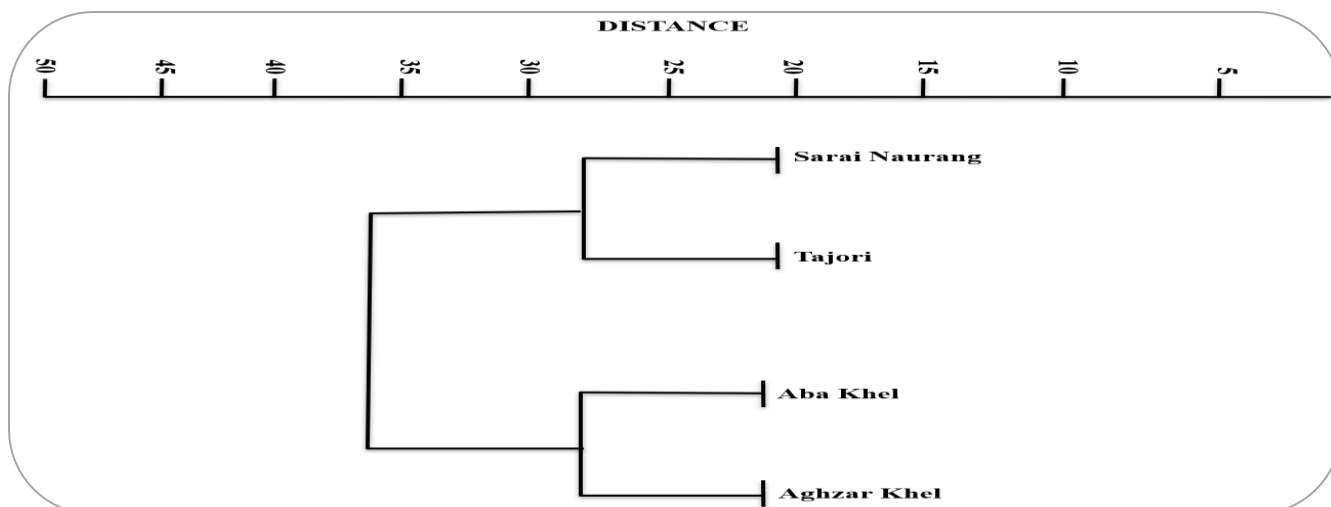


Figure 1. Dendrogram resulting from the average linkage of 04 sites of District Lakki Marwat

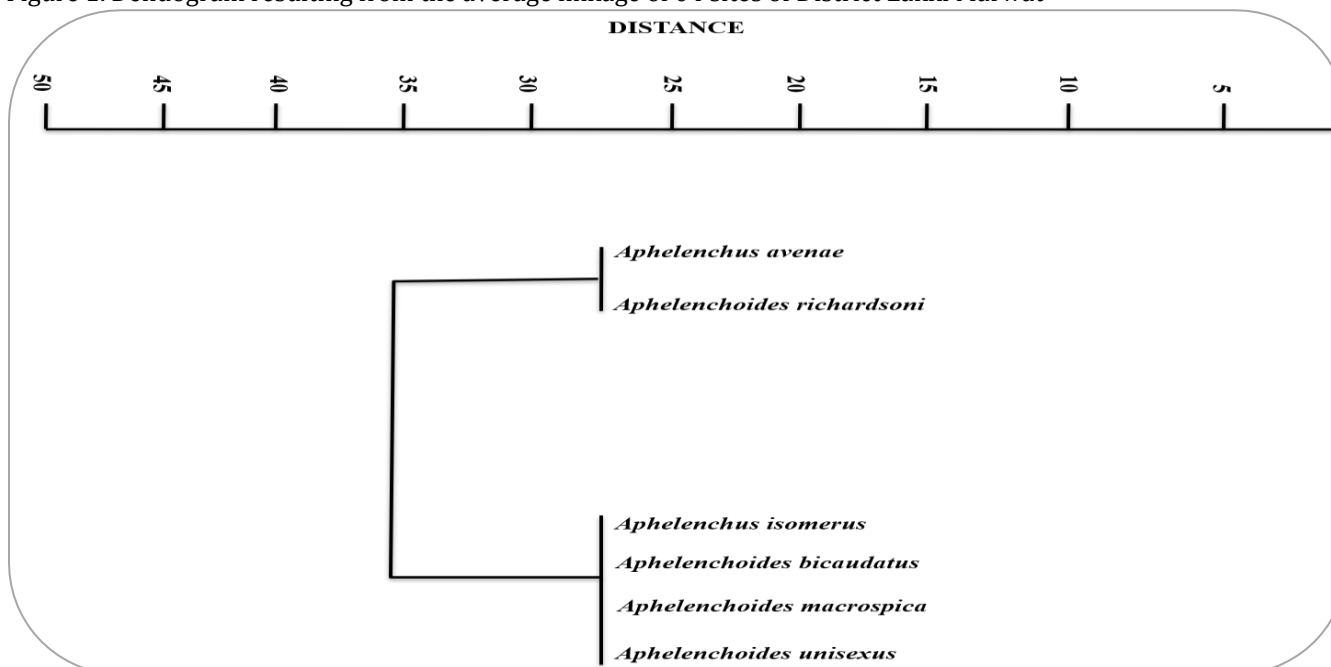


Figure 2. Dendrogram resulting of cluster analysis of 06 species

Simpson Diversity Index: In the same context, diversity index was ensured in true letter and spirit on all four surveyed sites and resultantly all areas had approximately similar diversity i.e. 0.50 as analyzed (Table 2). It has been assessed that environment of district Lakki Marwat is not quite stressful or more ecological niches. However, food webs in the areas are relatively simple and do not contain any complexity of food. Absolutely, any change in the environment is more likely to be damaging the ecosystem.

Table 2. Simpson diversity index of nematode genus of four sites

Sites	Simpson diversity index
Aba Khel	0.5
Aghzar Khel	0.5
Sarai Naurang	0.5
Tajori	0.5

Composition of *Aphelenchus* and *Aphelenchoides* species: During the study two species of *Aphelenchus* i.e. *Aphelenchus avenae* (Bastian, 1865), *Aphelenchus isomerus* Anderson and Hooper, 1980 (Ebsary, 1991) and four

species of *Aphelenchoides* comprising *Aphelenchoides bicaudatus* (Imamura, 1931) Filipjev and Schuurmans Stekhoven, 1941, *Aphelenchoides richardsoni* (Grewal *et al.*, 1992), *Aphelenchoides unisexus* (Jain and Singh, 1984) and *Aphelenchoides macrospica* (Golhasan *et al.*, 2017) were identified. It is pertinent to mention herewith that the genus *Seinura* could not be ascertained from district Lakki Marwat. The *Aphelenchus avenae* was found in highest frequency (40%) from all areas, the second

highest *Aphelenchoides richardsoni* (20%) and followed by the lowest all remaining species (10%) and the same also evaluated *vide* (Figure 3). All these species previously encountered from different areas of Pakistan; while current survey result showing presence of species (Table 3) and elaborates their area wise presence (Figure 4). Notably, *Aphelenchoides macrospica* was surfaced for the first time in Pakistan during the research work (Salma *et al.* 2020).

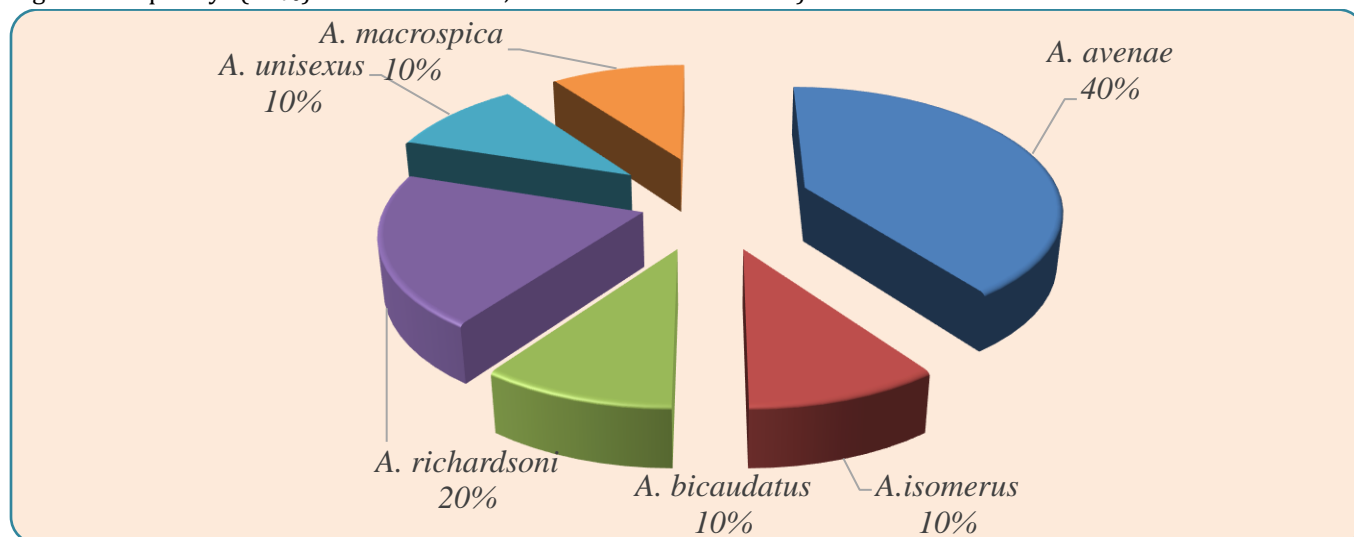


Figure 3. Occurrence percentage of species

Table 3. Species encountered during current survey according to new hosts and localities

Nematodes	Hosts	Locations	First Description
<i>Aphelenchus avenae</i> (Bastian, 1865)	<i>Acacia nilotica</i> (Kikar)	Tajori, Aghzar Khel	First time, it was surfaced from the lower sheaths of oats leaves from stubble field, Broadmoor, Berks, England
	<i>Ziziphus mauritiana</i> (Ber)	Tajori	
	<i>Bougianvillea spectabilis</i> (Paper flower)	Sarai Naurang	
	<i>Punica granatum</i> (Pomegranate)	Aba Khel	
<i>Aphelenchus isomerus</i> Anderson and Hooper, 1980 (Ebsary, 1991)	<i>Citrus aurantium</i> (Bitter orange)	Sarai Naurang	In the beginning, it was reported from Cuba, associated with potatoes
<i>Aphelenchoides bicaudatus</i> (Imamura, 1931) Filipjev and Schuurmans Stekhoven, 1941	<i>Ziziphus mauritiana</i> (Ber)	Sarai Naurang	The said nematode was initially retrieved from paddy field, Japan
<i>Aphelenchoides macrospica</i> * (Golhasan <i>et al.</i> , 2017)	<i>Bougianvillea spectabilis</i> (Paper flower)	Sarai Naurang	Earlier it was associated with the rhizosphere of rose plants in Hasanlou old hill, Northwestern Iran
<i>Aphelenchoides richardsoni</i> (Grewal, Siddiqi and Atkey, 1992)	<i>Citrus aurantium</i> (Bitter orange)	Sarai	Previously, the same was founded from rhizosphere of mushroom, UK
	<i>Bougianvillea spectabilis</i> (Paper flower)	Sarai Naurang	
<i>Aphelenchoides unisexus</i> (Jain and Singh, 1984)	<i>Ziziphus mauritiana</i> (Ber)	Sarai Naurang	At the earliest, it was obtained from soil around the roots of <i>Chrysanthemum</i> (<i>Chrysanthemum</i> spp) from Lucknow, India

Note: * New record

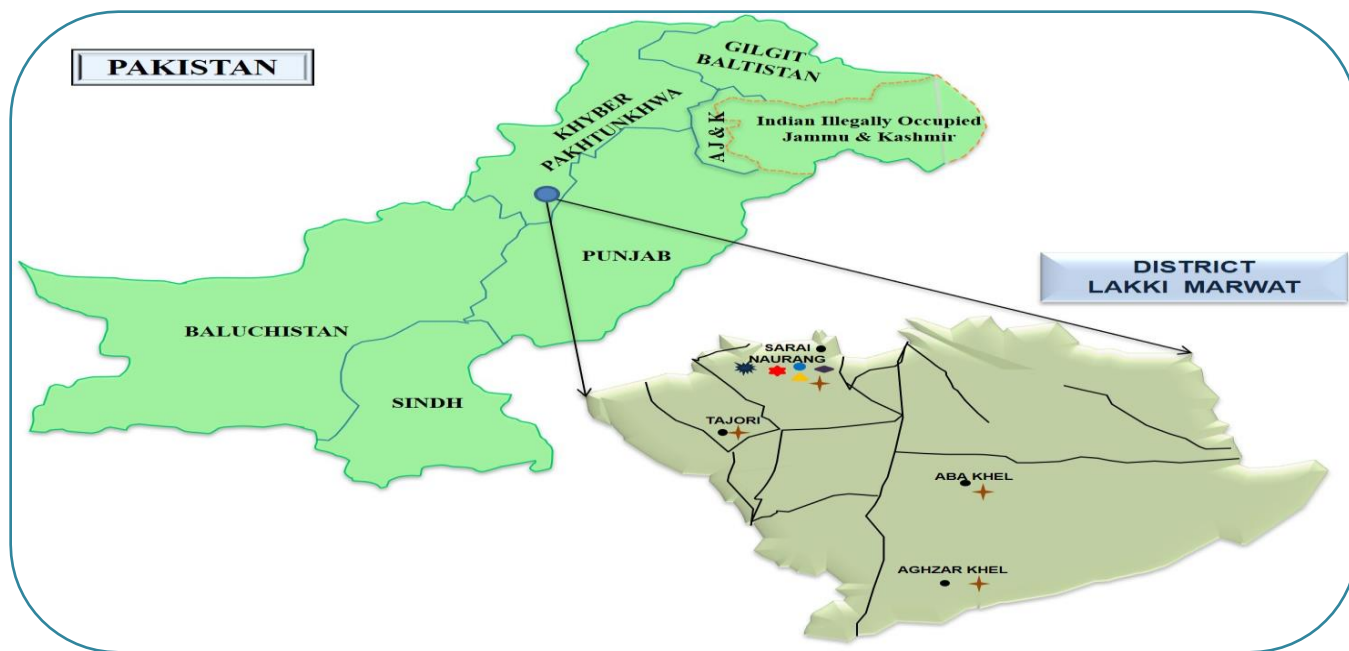








Figure 4. Species found from different locations of District Lakki Marwat, KPK, Pakistan

LEGEND	
	<i>Aphelenchoides bicaudatus</i>
	<i>Aphelenchoides macrospica</i>
	<i>Aphelenchoides richardsoni</i>
	<i>Aphelenchoides unisexus</i>
	<i>Aphelenchus avenae</i>
	<i>Aphelenchus isomerus</i>

DISCUSSION

As per previous held record, earlier many species of *Aphelenchus* of the family Aphelenchidae (Fuchs, 1937) Steiner, 1949 and *Aphelenchoides* of the family Aphelenchoididae (Skarbilovich, 1947) Paramonov, 1953 were surfaced from case to case research work and accordingly published in several papers from time to time with respect to numerous hosts, locations and time period from Pakistan. Till to date most of the abundance occurrence species belonging to the family *Aphelenchidae* from Pakistan is *Aphelenchus avenae* (Bastian, 1865). The said species has already reported by Anwar and Sarwar, 1981; Gul and Saifullah, 1991 from sample of Citrus spp. collected from Sargodha,

Faisalabad, Sahiwal, Multan, Kalam, Mardan, Peshawar, Mansehra and Hazara. As well as by Khan *et al.*, 2002 found around the root of Citrus trees and to evaluate its control by applying indigenous nematicides. Khan, 1991; Maqbool, 1992 from sample of lime collected from district Thatta. Khan and Bilqees, 1985 and Zahid, 2014 found in sample of date palm collected from district Thatta and district Khairpur respectively. Anwar *et al.*, 1986; Maqbool, 1992; Gul and Khan, 2005 found in samples of sugarcane collected from Sargodha, Lahore, Faisalabad, Sahiwal and Charsadda. Gul and Saifullah, 1991; Maqbool, 1988; Saeed *et al.*, 1986; Maqbool, 1986; Maqbool and Zaki, 1992; Maqbool, 1992; Aatika, 2015 from tobacco at Swat, Kalam, Mardan, Mansehra, Abbotabad, Charsada, Hazara, Rawalpindi, Islamabad, Gujrat, Lahore, Sheikhpura and Punjab. Gul and Saifullah, 1991; Qasim and Ahmed, 1989; Maqbool, 1988; Maqbool, 1986; Maqbool and Zaki, 1992; Maqbool, 1992; Khan and Hussain, 2004; Bhutta, 2008; Aatika, 2015 from potato samples collected from various places including Hunza, Kalam, Peshawar, Kaghan Valley, Bannu, D I Khan, Mansehra, Abbotabad, Hazara, Gujrat, Gujranwala, Faisalabad, Therparker, Hyderabad, Thatta, Karachi, Northern areas and Punjab. Solangi, 1981 found *Aphelenchus* and *Aphelenchoides* population in different region of district Hyderabad on the basis of symptoms appearance. Gul and Saifullah, 1991; Maqbool, 1988; Maqbool, 1992; Aatika, 2015 from maize sample collected from Kalam, Peshawar, Mansehra, Abbotabad,

Hazara, Sargodha, Mianwali, Sibbi, Chagi, Nawab Shah, Hyderabad, Thatta and Punjab. Maqbool, 1988 and Maqbool, 1992; Aatika, 2015 from sample of wheat collected from Sailkot, Gujranwala, Sheikhpura, Bahawalpur, Rahim Yar Khan, Sukkar, Nawab Shah, Hyderabad and Punjab. Aatika, 2015 found in sample of sorghum collected from Punjab. Maqbool, 1988; Maqbool, 1992 from grapes sample retrieved from Quetta, Pishin and Chagi. Islam *et al.*, 1994; 1996 from sample of apple retrieved from Sawat. Khan and Bilqees, 1994; Khan and Shaukat, 1999; Maqbool, 1988; Maqbool, 1992 from rice sample collected from Bajore Agency, Thatta, Larkana, Nawab Shah and Sanghar. Nematode community of *Aphelenchus avenae* associated with two rice varieties namely Lateefy and Kharai Ganja were investigated in district Thatta by Khan *et al.*, 2000. Khan *et al.*, 1998 studied the effect of neem cake, neem seed coat and carbofuran on the population density of four nematodes including *Helicotylenchus indicus*, *Merlinus brevidence*, *Aphelenchus avenae* and *Meleiodogone* Sp. larvae from garlic. Khan *et al.*, 2007; Khan, 2008 from sample of papaya collected from Karachi and Hyderabad. Khan, 2008 from sample of mango collected from Karachi and Hyderabad. Brown, 1962 described *Aphelenchus* sp from sample of chilli collected from Malir, Karachi, while Khan *et al.*, 2000; Anwar *et al.*, 2013 and Zarina *et al.*, 2015; Aatika, 2015 reported from sample of chilli collected from lower Sindh, Faisalabad, Jhang, Kasur, Lahore, Nawab Shah, Sargodha, T.T. Singh, Sheikhpura, Karachi and Punjab. Nasira *et al.*, 2008 from sample of Ashok tree due to cause of great damages to the tree at Karachi. Anwar *et al.*, 2013 found from sample of tomato collected from Faisalabad, Jhang, Kasur, Lahore, Nawab Shah, Sargodha, T T Singh and Sheikhpura as well as by Samina and Erum, 2019 from Kurram Agency. Nadia, 2019 from walnut sample collected from Hazara Division. Anwar *et al.*, 2013 from cucumber sample collected from Faisalabad, Jhang, Kasur, Lahore, Nawab Shah, Sargodha, T T Singh, Sheikhpura. Anwar *et al.*, 2013 from sample of bell peppers collected from Faisalabad, Jhang, Kasur, Lahore, Nawab Shah, Sargodha, T T Singh, Sheikhpura. Israr, 2017 reported from radish, turnip and carrot samples collected from KPK and Sindh while on radish, turnip, carrot and sugar beet sample from Punjab. Tariq, 2013 reported *Aphelenchus avenae* from sample of different medicinal plants including kachnar, jungli badam, aloe, lemongrass, basil, sukhchain and castor

collected from Karachi, Thatta and Hyderabad region. Similarly, *Aphelenchus isomerus* Anderson and Hooper, 1980 (Ebsary, 1991) by Akhter and Zarina, 2014 found from chilli sample collected from Karachi. In the same way *Aphelenchoides bicaudatus* (Imamura, 1931) Filipjev and Schuurmans Stekhoven, 1941 by Maqbool, 1986; 1988; 1990; 1992; Maqbool and Zaki, 1992 from sample of rice, kidney bean, pearl millet, sugarcane and maize collected from Sukkar, Nawab Shah, Thatta, Sanghar, Larkana, Dadu, Badin and Khairpur. Shahina and Maqbool, 1992 from sample of banana collected from Sukkar, Nawab Shah and Thatta. Mussarat, 2011 reported *Aphelenchus bicaudatus* from sample of rice and soybean collected from Faisalabad, Multan, Raheem Yar Khan, Sanghar, Badin and Dir. Israr *et al.*, 2017 from sample of radish, turnip and carrot collected from Faisalabad, Tando Allahyar, Swat, Sargodha, Dadu, Peshawar, Sialkot, Badin and D. I Khan. Accordingly, in the same context, *Aphelenchoides richardsoni* Grewal *et al.*, 1992) from pumpkin and *Aphelenchoides unisexus* (Jain and Singh, 1984) from tomato sample by Samina and Erum, 2019 collected from Kurram Agency. These genera have been causing serious damages to the root hairs and epidermal tissues which resultantly minimize the capability of roots to suck water and nutrient from the nearby soil (Endo, 1975).

Notwithstanding above, the research work was carried out with an aim to get maximum information about presence of nematodes especially Order *Aphelenchida* in those areas where no research work has been carried out yet. The presence of new recorded species *Aphelenchoides macrospica* is an indicator of existence of other undiscovered species. Further research work in other parts of Pakistan will surely be helpful in identifying additional species of nematodes of *Aphelenchida*. The evidence on nematodes occurrence may be assisted the formers for planning and directing nematode management policies to handle the nematodes population below their threshold levels.

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Contribution of Authors:

Samreen Khan	:	Executed survey, collected the data and wrote the manuscript
Salma Javed	:	Supervised the research and reviewed the manuscript
Tabassum A. Khanum	:	Analyzed the data. All authors read and approved the final manuscript