Open Access
Full Length Article

ORIGINAL ARTICLE

Plant Parasitic Nematodes Associated with Maple (Acer Caesium) and Poplar *(Populous Nigra)* in District Swabi

Bilal Muhammad*, Mian Sayed Khan, Saira
Department of Zoology, University of Swabi, Anbar, Khyber Pakhtunkhwa, 23561, Pakistan

ABSTRACT

Background: Parasitic nematodes are very destructive to crops, ornamental plants and woody plants as well which cause most severe diseases in plants and feed on the roots of these plants.

Objective: Main aim of this study is to identify different species of nematodes associated with Maple (Acer caesium) and Poplar (*populous nigra*) trees at district Swabi, Khyber Pakhtunkhwa, Pakistan.

Methodology: Some Maple and Poplar growing areas of District Swabi were surveyed during the year 2019, for the occurrence and incidence of parasitic nematodes. Soil samples collected from different localities were analyzed for the nematodes. Plant-parasitic nematodes were extracted from soil by *Cobb* methods, and identified the species after fixation.

Result: Maximum analyzed simples were infected and among all localities seven different species of nematodes has been recorded. Maximum infection was recorded in Zaida, Manki and Anbar. Following nematodes were found named *Paratylenchus* Larvae, *Aphelenchus avenae*, *helicotylenchus dihystera*, *Tylenchus, Trichodorous* and *Meloidogyne incognita.* Ratio of occurrence among all different varieties of nematodes *Meloidogyne incognita* species shows most abundance in two localities Lahor and Zaida.

Conclusion: The results clearly indicate the fact that studied area is mostly infected with different types of parasitic nematodes species which highly affect the Maple (*Acer caesium*) and Poplar (*populous nigra*) plants.

Keywords	*Address of Correspondence	Article info.
Nematodes, plant parasitic nematodes, maple, poplar, infection, Meloidogyne incognita, Paratylenchus, Tylenchus.	*bilaluoswabi2021@gmail.com*	*Received: October 26, 2021* *Accepted: December 20, 2022*

Cite this article: *Muhammad B, Khan MS, Saira. Plant Parasitic Nematodes associated with Maple (Acer caesium) and Poplar (populous nigra) in district Swabi. 2022; 13(2):183-192.*

INTRODUCTION

Nematodes commonly known as round worms the word Nematoda is a Greek word “Nemos,” means “thread”. Nematodes are worm-like, generally minute, colorless and unsegmented animals. Nematodes are aquatic animals. Plant parasitic nematodes colonize in the rhizosphere. And accomplished in the rhizosphere and exert beneficial effects on the plants[1].The most numerous, multicellular animals are nematodes on earth. The size of nematodes is 0.2mm-6mm dwelling in many hosts like plants and animals[2]. Mostly plant and animals are infected at least single species of parasitic nematode. Nematodes make a wild range of relationship with other species. At large numbers of nematodes species are parasitic in nature which defined on host plant and animals[3].

Mostly nematodes are live in deep water soil. Nematodes are found almost every imaginable habitat including in the rhizosphere of plant, salt water, and fresh water as well as with other organism as a parasite. But mostly nematodes are abounded in the upper layer of soil where plant roots, other organic matters are abounded. According to survey there are 1–10 million nematodes/m are present in soil[4,5]. Nematodes are classified on the base of their feeding

habits and structure of their mouth parts. We enlist the feeding habits of some most common nematodes groups which are fungal-feeder, predators, omnivores, bacterial-feeder, and plant parasites. The Predators nematodes feed on protozoa and some other soil nematodes as well. Omnivore feeding habits depends on food availability and environmental conditions; for example during lack of their primary food source omnivores act as predators and also feed on bacteria and fungi. A small amount of soil contains numerous numbers of nematodes. Phylum Nematoda consists of 20,000 described species. Members of phylum nematoda have existed from one billion year which gives them the credibility of the ancient organism on Earth[6].The phylum nematode diverged into two classes Chromadorea and Enoplea so long ago (figure.**1**).

According to this classification ecologist easily understand the position of nematodes in soil food web chain.

Plant parasitic nematodes almost feed on all parts of Plants like seeds, roots, stem, flower, and leaves. Parasitic nematodes use specialize organ called stylet through which they puncture the plant cells during feeding and suck the nutrients from plant and having special esophageal glands[7].The 1st plant parasitic nematode was reported by Needham (1743) for the 1st time he observed the symptom of galling in wheat plant[8] .One of the important species in agriculture of parasitic nematodes are identified by Berkeley, he first time observed that in roots of cucumber are root-knot nematodes[1]. Plant parasitic nematodes are different from each other in life style. Some nematodes are take nutrition externally known as ectoparasitic nematodes. Ectoparasitic nematodes feed from the outside the host plant. While other endoparasitic nematodes enter to the host root and take nutrition from it. *Xiphinema* (California dagger nematode) is the example of ectoparasitic nematodes it cause viral infection in Grapevine and cause on large scale of economics loses world widely[9].

Endoparasitic nematodes are further divided into two groups.

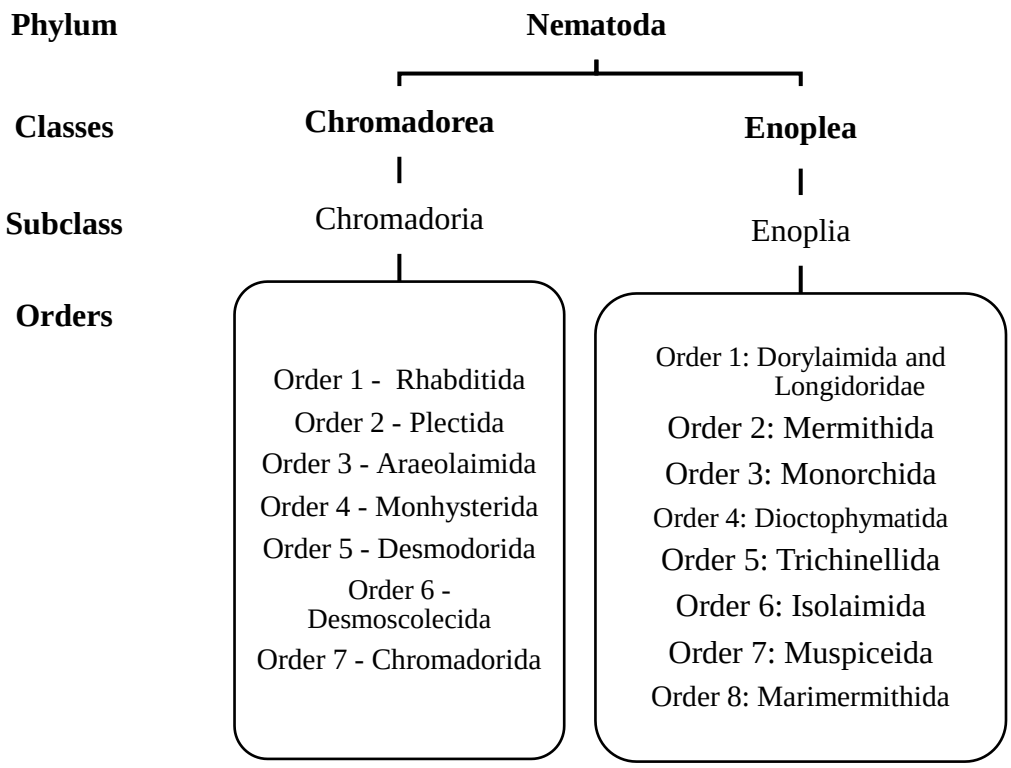

Figure 1. Classification of nematodes[5]

Migratory Nematodes.

These types of nematodes feed briefly on a particular site and then move to another site e.g. *Xiphinema, Longidorus* and *Trichodorus.*

Sedentary Nematodes.

These typed of nematodes are feed on one site of the host cell for long time and remain attached with it e.g. *Pratylenchus* and *Hirschmanniella*[10].

Root-knot Nematodes.

Root-knot nematodes contain over one hundred species which are most threaten species to the agriculture field including *Meloidogyne javanica, Meloidogyne arenaria*, *Meloidogyne incognita* and *Meloidogyne hapla*. *Meloidogyn spp*. globally distributed, having a large number of hosts to be attach[11].

Cyst Nematodes.

Second in list is cyst nematodes (*Heterodera and Globodera spp.*) are ranked to economic and agricultural more damaging nematodes. Cyst nematodes enter to the host plant root tips and secret the esophageal gland from the stylet. Esophageal gland promotes degradation of protoplast and cell wall fusion of other adjacent cells and form syncytium[12].

Lesion Nematodes.

Lesion nematodes *(Pratylenchus spp.)* are ranked on 3rd position in the list of more damaging nematodes to agriculture. Lesion nematodes get nutrition from vascular tissues for which they damage cortex of roots and then enter to vascular tissues, as a result plants show some above ground symptoms of chlorosis, necrosis on root and reduction of leaf size and number[13].

Burrowing Nematode.

Burrowing nematode (*Radopholus similis*) is migratory plant parasitic nematodes. Burrowing nematodes are listed quarantine plant pest in worldwide[14].

Maple and Poplar trees:

Maple (*Acer caesium*) and poplar (*Populus nigra*) trees are mostly planted or self-grown trees in Pakistan. The plantations of *Populus nigra* are the 1st choice of farmer because of his rapid growth and domesticated importance. The woods of *Populus nigra* are commonly used for the manufacturing of matchsticks. The Poplar trees are disseminated throughout Pakistan**.** There are 20 species of Poplar tree found in Pakistan but mostly Poplar (*Populus nigra*) specie is found in district Swabi which are cultivated or self-growing in this area. Maple (*Acer caesium*) is slow growing plant approximately 0.2 to 0.5 cm growth/year. 20 to 25m tall and the shape of leaves are simple having 3 to 5 lobes and 8 to 20 cm wide. It can reproduce from both sources seed and from vegetative means. It distributed throughout Pakistan, Nepal and India. In Pakistan mostly found in Swat, Muree Hills, Chitral, Dir, Swabi and Azad Kashmir.The wood of *Acer caesium* use in bobbins, carving, Furniture and ornamental[15].

LITERATURE REVIEW

According to Malakhov (1994) the parasitic nematodes having negatively impact on agriculture field, human health and other many wildlife animals. There are parasitic nematodes present everywhere having special host to be affected[16]. Gregory and Egnin, (2017) were conducted that from his research that parasitic nematodes cause lot of destruction in agriculture field in past. Cyst nematodes, lesion nematodes, and Root-knot nematodes listed on top of the scientifically most economically important species[17].

Another study was conducted by Gnamkoulamba *et al.,* (2018) which proposed that rice is the second most important cereal in Togo. Among parasites which affect rice production, nematodes as parasites are considered to be contributing too much regarding detrimental effect over all as about 71% nematodes parasitic genera like *Hirschmanniella spp., Xiphinema spp., Meloidogyne spp., Helicotylenchus spp., Tylenchid spp.,* and *Pratylenchus spp.,* were been collected during study which established the evidence that nematodes could be important constraints for rice production. Study of nematodes declared that the species of Root-knot nematode is commonly most pathogen to tomato crop including Ethiopia which is seen as been spread worldwide attacking a vast variety of crop plants and destroying economy of different countries[18].

Bridge *et al.,* (2005) concluded from his survey that Rice production is affected by over 100 species of nematodes. *Meloidogyne spp.* is on the top of this list that is been distributed through world, but the *Hirschmanniella oryzae* is most effective regarding rice production which also

known as rice root nematode which has been reported in following Asian countries such as Pakistan, India, Nepal, Bangladesh, Thailand, Vietnam, Sri Lanka, China, Japan, and Korea[19].

Al-mohithef *et al.,* (2018) conducted a survey on some genera of parasitic nematodes associated with vegetable crops in Riyadh, (Saudi Arabia). Survey showed that among all the identified genera *M. javanica* is more common among all vegetables crops. From Tennessee nurseries in 1981, 27 red maple, 48 dog woods, and 17 peach were sampled for nematodes identification 57 species of nematodes in 24 genera were found. The most detected species of nematodes were *Xiphinema americanum* and *Paratylenchus projectus*, which were found 78% and 88% respectively found in sites. And others species of nematodes were found in 10% in sites or above than 10%. But the effect of *Paratylenchus projectus,* were more than others is mean *Xiphinema americanum* and *Paratylenchus projectus,* have been large effect on maple, dogwood, and peach plants in Tennessee[20]. Hussein *et al.* (2016) surveyed the Montpellier maple from the different regions Boyer-Ahmad, Sepidar and Kohgiluyeh of Iran. He studied the rhizosphere of Montpellier maple during his study he found a new species which belong to genus glenchus. The new species have some different characters from other species of genus glenchus like they have long stylet approximately 7-8 µm in length[21].

The first report on nematodes associated with poplar tree was published from Pakistan in 1993 by Dr. Aly Khan and his colleagues who mentioned in their book "Nematodes associated with some trees of Khyber Pakhtunkhwa" that they collected 120 soil samples from the rhizosphere of *populous nigra*. They collected those samples from six different locations of district Mardan. Ten different species and nine genera were isolated from the rhizosphere of *populous nigra. Tylenchorhus annulatus* showed large population with 180-205 nematodes per 100 ml of soil residue, *Helicotylenchus dihystera* 95-130 nematodes per 100 ml of soil residue and *Hoplolaimus seinhorst* and *Pratylenchus penetrans* showed the lowest percentage of occurrence with 2-8 nematodes per 100 ml of soil residue[22].

MATERIALS AND METHOD

Study Area:

The area of interest for research on plant parasitic Nematodes on Maple Tree and Poplar (*Populus nigra*) was District Swabi. Swabi lies between Indus River and Kabul River 100 kilometer from country capital Islamabad and 100 kilometer from provincial capital Peshawar. District Swabi is situated at 34° 7', 48° N and 72° 28', 11" E of KPK, Pakistan. There are five tehsil in district Swabi. Lahor is a tehsil of Swabi which is the largest tehsil in district Swabi which consist of 10 villages (figure. **2**).

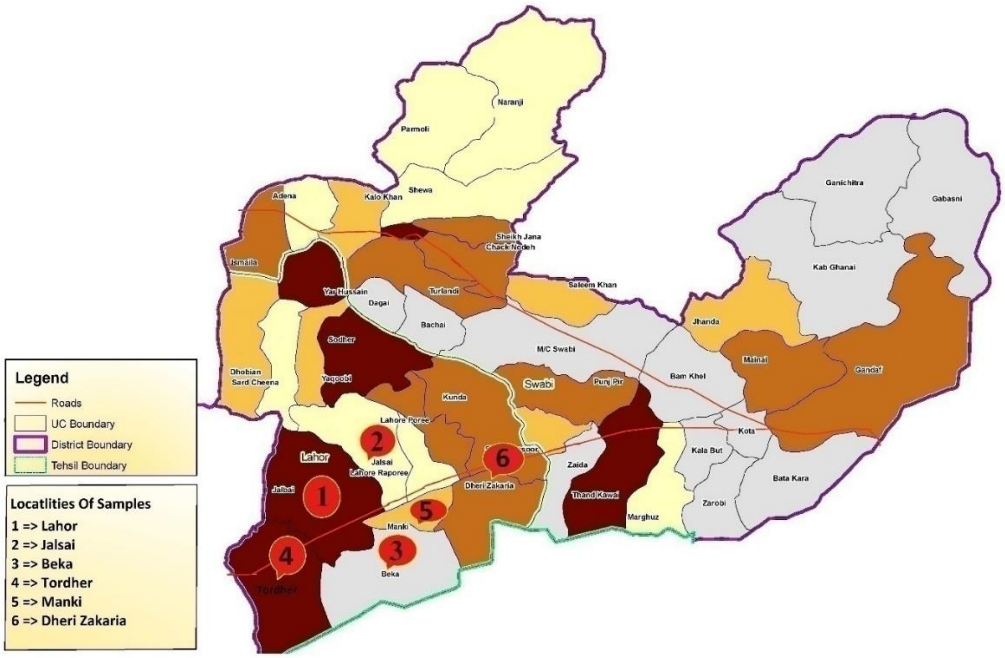

Figure 2. Map of district Swabi showing different villages (Online, Accessed December, 2019)

From which we have collected the samples of Maple and Poplar trees initially. The climate of district Swabi is subtropical warm humid in summers and mild in winters. Average Temperature is 22.2 C and annually average Precipitation is 639 mm of district Swabi. Average rainfall in November is 12 mm therefore this month is a driest month of year. While the precipitation in August (wettest month of year) is 137 mm. Hottest month is June average temperature of this month 41.2 ° C and the coldest month of year is January average temperature of this month is 10.2 °C. The climate of Tehsil Lahor is suitable for cultivation of Maple and Poplar trees. Generally the cultivation starts between mid-February and mid-March. Poplar Tree is one of the poplar wood producing trees. Poplar tree can grow above 85 feet in height and 36 inches diameter in 5 to 6 years. Mostly Maple and Poplar trees can survey in any environment Hot and cool both condition can't effect the cultivation of Maple and Poplar.

Collection of samples:

In this study the soil were taken for samples collection and some roots with it from approximately 1 to 2 feet deep from the base (rhizosphere) of Maple and Poplar trees and dump it in plastic bag. At least 2 samples were collected from each locality (Maple and Poplar) from one area, and brought these collections to the lab and analyzed them for the nematodes.

Extraction of Nematodes:

In this study Cobbs sieving method (1918)[23] were used and Baermann funnel technique (Schindler, 1961)[24] for the extracting of nematodes from the collected soil samples. In lab mixed the soil with water in tub and stirred it. Then that liquid passed from 100µ mesh sieves in tub. Discord the residue which present in 100 meshes and passed the liquid which passed previously from 100µ mesh sieves now passed them through 200µ mesh sieves, and then passed the liquid from 300µ mesh sieves discord the liquid after passing it from 300µ mesh sieves. Washed the residue of 200µ and 300µ mesh sieves and kept it in beaker. Take the Baermann Funnel, Rubber tube, and circular wire mesh and filter paper. Attached the rubber to the mouth of Baermann Funnel and clamped it shut. Put the wire mesh on Baermann Funnel and placed the filter paper on them. And then added the residue on filter paper mixed with water. Filled the Baermann Funnel with water and left it for 24 hours that all nematodes sinked down in bottom. After 24 hours took 50 ml water from the rubber tube the nematodes were sinked there.

Isolation of Nematodes:

First take the 50 ml of water from bearrman funnel in beaker then poured some of water from the respective beaker in petry dish to observe through stereoscopic microscope in order to find out nematodes which were picked up with the help of dropper after seeing in the respective arrangement and placed them into the cavity block.

Killing of Nematodes:

Then after isolation kept the cavity block on heater at very low temperature because high temperature is destructive to the cell morphology of the respective entity (nematode) for the purpose of killing respective nematode through proper scientific protocol to observe accurately. Following heat killing then water was removed from the cavity block.

Dehydration:

After removing water from cavity block TAF were added to cavity block and kept it for 48 hours in room temperature to get it dehydrated. After removing water from cavity block TAF were added to cavity block and kept it for 48 hours in room temperature to get it dehydrated.

Staining:

After 48 hours TAF were removed from cavity block while glycerin were added instead to cavity block for staining and kept it in oven for 72 hours.

Preparation of slides:

After the staining the next step was to prepare permanent slides. This started with cleaning of the slide and cover slip with xylene with the help of tissue paper gently so to avoid rupturing and scratches of slides. Then Glycerin was dropped over the central part of cleaned slide and then through eyelash brush nematodes picked up from cavity block and placed into the glycerin drop on respective slide. Maximum number of nematodes which are allowed to be placed on a single slide range 7-9 for certain and accurate result which was kept in mind during the process for having maximum accuracy, after keeping nematodes over slide four pieces of paraffin wax were placed on four spots around the glycerin drop so that to fully surround the drop

along with our study object over which cover slip was kept to cover the object then heated it up by keeping on hot plate in order to melt paraffin wax which covered all the surface of slide and hold the slip tightly to prevent object movement during observation, after fully melting of wax over slide, slide was removed from hot plate and kept at room temperature till getting it fully dried. After this entire described process finally slid were ready to be observed for nematode identification.

RESULTS

Soil samples from six localities were collected and analyze for the identification of parasitic species of nematodes associated with Poplar (*Populus nigra*) and Maple (*Acer caesium*).

The results of study shows that samples were found infected with nematodes showing high infection. Maximum samples of Poplar (*Populus nigra*) tree were found infected. Table. **1** is shown that four different species of parasitic nematodes *Aphelenchus avenae* and *Tylenchus* larvae were identified in Anbar and one species of *Paratylenchus* Larvae was identified in Manki and one species of parasitic nematodes *helicotylenchus dihystera* was found in Tordher district Swabi.

Table 1. Plant Parasitic Nematodes in Poplar tree *(Populus nigra)*.

S. No.	Tree Name	Number of Soil Samples	Location	Nematodes
1	Poplar	2	Manki	*Paratylenchus* Larvae
2	Poplar	2	Anbar	*Aphelenchus avenae*
3	Poplar	2	Tordher	*Helicotlenchus dihytera*
4	Poplar	2	Anbar	*Tylenchus* larvae
5	Poplar	2	Lahor	Free Living
6	Poplar	2	Beka	Free living

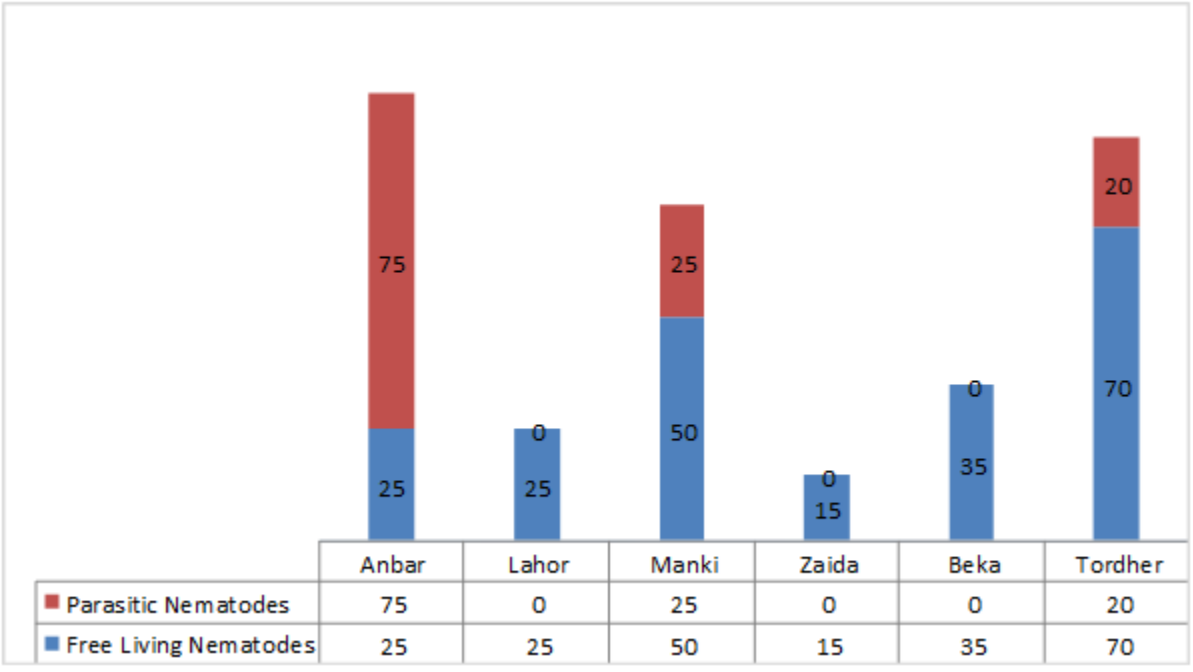

Figure 3. Frequency of free living and parasitic nematodes in Populus nigra.

Figure **3** shows that the most infected area is Anbar which shows 75% infestation followed by Manki with 25% of infestation rate.

Table. **2** is shown that three species of parasitic nematodes identified in which *Trichodorous* larvae determine in Zaida and two species of *Meloidogyne incognita* were identified in Lahor and Zaida.

The most abounded species of nematodes *Paratylenchus*, *Aphelenchus avenae*, *helicotylenchus dihystera*, *Tylenchus, Trichodorous* and *Meloidogyne incognita* were mostly distributed among the different area of district Swabi. The most infected area is Zaida with 70% of infestation rate followed by Lahor which having 30% of infestation rate which has been shown in table. **3** and figure **4**.

In below figure. **4** we determine that Zaida is the most infected site with 70% infestation rate.

Table 2. Plant Parasitic Nematodes in Maple tree (Acer caesium).

S. No.	Tree Name	Number of soil samples	Location	Nematodes
1	Maple	2	Lahor	*Meloidogyne incognita*
2	Maple	2	Zaida	*Meloidogyne incognita*
3	Maple	2	Zaida	*Trichodorous* larvae
4	Maple	2	Tordhair	Free living
5	Maple	2	Manki	Free living
6	Maple	2	Anbar	Free living

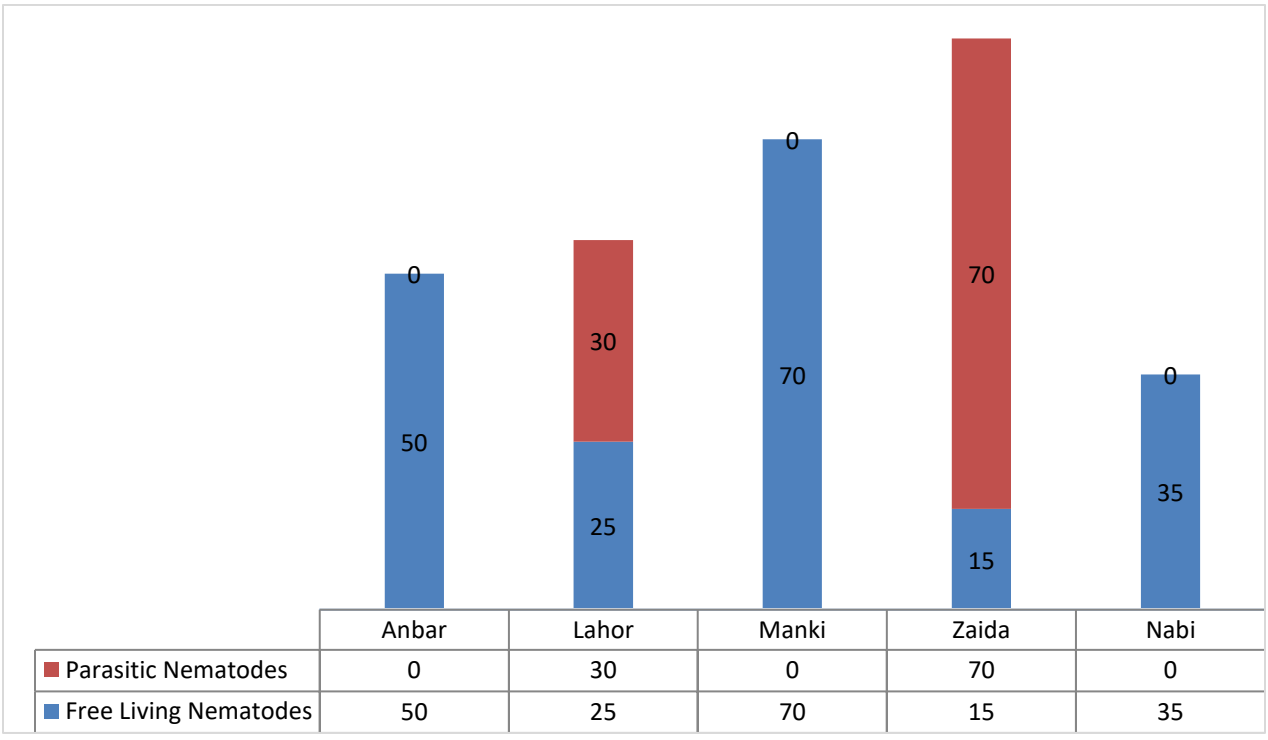

Figure 4. Frequency of free living and parasitic nematodes in *Acer caesium*.

Table 3. Categorization of identified species on the bases of their abundance.

Nematodes	Host	Localities	Abundance
Paratylenchus Larvae	Poplar	Manki	65-70
Aphelenchus avenae	Poplar	Anbar	20-30
Helicotlenchus dihytera	Poplar	Tordher	30-35
Tylenchus larvae	Poplar	Anbar	70-80
Meloidogyne incognita	Maple	Lahor	43-60
Meloidogyne incognita	Maple	Zaida	75-80
Trichodorous larvae	Maple	Zaida	45-50

DISCUSSION

Present survey confirms the occurrence of parasitic nematodes in Maple and Poplar trees in district Swabi and we also aim that from discussion our study clear this fact that Meloidogyne *spp.* and *Pratylenchus spp.* is not host specific and cause destruction only in rice and vegetables but also destructive for poplar and maple tree also it prove that the determine species are not host specific they can cause infestation in any maple and poplar too. The damaging of ornamental, woody, crops due to the effect of parasitic nematodes is a universal problem including Pakistan. Malakhov (1994) stated that every parasitic nematodes have specific host to affect these creatures are very disaster for crop, animal health and livestock[16].

According to the survey of Aly in 1993 of *populous nigra* of district Mardan, ten different species and nine genera were isolated from the rhizosphere of *populous nigra*. *Tylenchorhus annulatus* showed large population with 180-205 nematodes species per 100 ml of soil residue, *Helicotylenchus dihystera showed* 95-130 nematodes. Species per 100 ml of soil residue, and *Hoplolaimus seinhorsti* and *Pratylenchus penetrans* showed the lowest percentage of occurrence with 2-8 nematode per 100 ml of soil residue[22]. While during our survey four different species of parasitic nematodes *Aphelenchus avenae, Tylenchus* larvae, *Paratylenchus* Larvae, and *Helicotlenchus dihytera* were recorded in the samples of Popalr trees, and two different species of parasitic nematodes *Trichodorous* larvae and *Meloidogyne incognita* larvae were recorded on samples of Maple trees.

Al-mohith *et al*., (2018) survey in Riyadh region of Saudi Arabia for the parasitic nematodes was associated with vegetable crops. The results of this survey showed that the most threaten root nematodes species to crops is *M. javanica.* Which mostly affect the vegetable crops in Riyadh region. From Tennessee nurseries in 1981, 27 red maple, 48 dogwoods, and 17 were samples for nematodes identification 57 species of nematodes in 24 genera were found. The most detected species of nematodes were *Xiphinema americanum* and *Paratylenchus projectus*, which were found 78 % and 88 % respectively found in sites, and other species of nematodes were found in 10 % in sites or above than 10 %. But the result showed that the *Paratylenchus projectus* effect were more than *Xiphinema* americanum on maple, dogwood, and peach plants in Tennessee[20].

Bridge *et al.,* (2005) concluded from his survey that over 100 species of parasitic nematodes affect production of rice world widely, *Meloidogyne spp* is the most widely distributed specie throughout the world, while *Hirschmanniella oryzae* which is mostly associated with ricehas been reported in Asian countries such as Pakistan, India, Nepal, Bangladesh, Thailand, Vietnam, Sri Lanka, China, Japan and Korea[19].

CONCLUSION

The results of this study show that samples were found infected with nematodes showing high infection. The most abounded species of nematodes *Paratylenchus*, *Aphelenchus avenae*, *Helicotylenchus dihystera*, *Tylenchus, Trichodorous* and *Meliodogyne incognita* were mostly distributed among the all Maple and poplar cultivated area of district Swabi. There were two different species of parasitic nematodes *Trichodorous* larvae and *Meloidogyne incognita* larvae were identified in Zaida and

one species of parasitic nematodes *Meloidogyne incognita* larvae were identified in Lahor district Swabi, and four different species of parasitic nematodes associated with *Populus nigra* were found. *Aphelenchus avenae* and *Tylenchus* larvae were identified in Anbar and one species of *Paratylenchus* Larvae was identified in Manki and one species of parasitic nematodes *Helicotlenchus dihytera* were found in Tordher district Swabi.

From above results and discussion we conclude that, the various villages of district Swabi are suffering from various parasitic nematode infestation which are ultimately become the cause for many diseases in Maple and Poplar trees.

CONFLICTS OF INTEREST

No conflict of interest.

FUNDING SOURCE

None.

ACKNOWLEDGEMENTS

We are thank full to University of Swabi for providing research facility.

LIST OF ABBREVIATIONS

Spp	Specie
KPK	Khyber Pakhtoon Khawa
TAF	Tri ethylamine formaldehyde
N	North
E	East

REFERENCES

1. Berkeley M. The Botany of the Antarctic Voyage II. Florae Novae-Zealandiae, 1855: p. 172-210.
2. Mark B, and Dee RD.*The worm in the world and the world in the worm.* BMC biology, 2012. 10(1): p. 57.
3. Jones JT, Annelies H, Etienne GJD, Hari SG,Johannes H, et al.Top 10 plant-parasitic nematodes in molecular plant pathology. Molecular plant pathology, 2013. 14(9): p. 946-961.
4. DavisEL, MacGuidwinAE.*Lesion nematode disease.* Plant Health Instr, 2000.
5. Poinar G. The natural history of nematodes., 1983.
6. Wang MS, Limin Li, *Rapid screening assay methods and devices*. 1999, Google Patents.
7. MaierC, Bilas F, Weinbauer MG, Watremez P, Peck MA, et al.*Respiration of Mediterranean cold-water corals is not affected by ocean acidification as projected for the end of the century.*Biogeoscience, 2013.
8. Needham F, John T, Robert, Henry. New microscopical discoveries: London: Smithsonian Libraries; 1745.
9. Villate L,Fievet V, Hanse B, Delemarre F, Plantard O, et al. *Spatial distribution of the dagger nematode Xiphinema index and its associated Grapevine fanleaf virus in French vineyard.* Phytopathology, 2008. 98(8): p. 942-948.
10. WilliamsonVM, HusseyRS.*Nematode pathogenesis and resistance in plants.* The Plant Cell, 1996. 8(10): p. 1735.
11. Poulin R,aseeb SR.*Evolution of parasitism along convergent lines: from ecology to genomics.* Parasitology, 2015. 142(S1): p. S6-S15.
12. GheysenG, Fenoll C.*Gene expression in nematode feeding sites.* Annual review of phytopathology, 2002. 40(1): p. 191-219.
13. Jones M, Nyarko J.*Molecular biology of root lesion nematodes (Pratylenchus spp.) and their interaction with host plants.* Annals of applied biology, 2014. 164(2): p. 163-181.
14. SasserJ, Freckman D.*A world perspective on nematology; the role of the society. Pp: 7-14.* Vistas on nematology. Society of Nematologists, Hyatsville, MD, 1987.
15. MahmoodA, Caccamo DV,Tomecek FJ, Malik GM.*Atypical and malignant meningiomas: a clinicopathological review.* Neurosurgery, 1993. 33(6): p. 955-963.
16. MalakhovV.*Classification of the Pseudocoelomates.* Nematodes, structure, development, classification and phylogeny. Smithsonian Institute Press, Washington, 1994: p. 175-201.
17. Gregory B, Egnin , Bonsi C. The impact of plant-parasitic nematodes on agriculture and methods of control. Nematology-concepts, diagnosis and control. 2017; 10(1): 121-151.
18. GnamkoulambaA, Tounou AK, Tchabi A, Kolombia AG, Agboka K, et al.*Occurrence, abundance and distribution of plant-parasitic nematodes associated with rice (Oryza spp.) in different rice agroecosystems in Togo.* International Journal of Biological and Chemical Sciences, 2018. 12(2): p. 618-635.
19. Bridge J, Plowright RA, Peng D.*Nematode parasites of rice.* Plant parasitic nematodes in subtropical and tropical agriculture, 2005. 2: p. 87-130.
20. Almohithef, A.H, Al-YahyaF.A, Al-HazmiA.S, Dawabah A.A.M, Lafi H.A, Prevalence of plant-parasitic nematodes associated with certain greenhousevegetable crops in Riyadh region, Saudi Arabia. J. Saudi Soc. Agric. Sci.2018. 19 (1),22–25.

21. HusseinM,Abdollahi M,Karegar A.*Description of Aglenchus Microstylus n. sp.(Nematoda, Tylenchidae) from Iran with a modified key to the species of the genus.* Nematropica, 2016. 46(1): p. 38-44.
22. Khan, A. and Bilqees, F.M. Basiria bajorensis n. sp. (Nematoda: Tylenchida) from Pakistan. Proceedings of Parasitology,1993.15: 14-16.
23. Cobb N. Estimating the nematode population of the soil. Agric. Tech. Circ. U.S. Dept. Agriculture. 1918; 48 p.
24. Schindler A. F, A simple substitute for a Baermann funnel. Pl. Dis. Replr; 1961 p 747-748.