

**PREVALENCE OF PHYTONEMATODES ASSOCIATED WITH
CITRUS FAMILY (Orange, Lime and Lemon) OF MARPAK,
DHADING DISTRICT, NEPAL**



Entry : 60

M.Sc. Zoo Dept. Parasitology

Signature Sophiya Pandey

Date: २०८०/०१/२१

२०२३/०५/०४

Sophiya Pandey

T.U. Registration No: 5-2-0037-1797-2014

T.U. Examination Roll No: 852/076

Batch: 2076

A Thesis submitted

**In the partial fulfillment of the requirements for the award of degree of Master of
Science in**

Zoology with special paper Parasitology

Submitted to

Central Department of Zoology

Institute of Science and Zoology

Tribhuvan University

Kirtipur, Kathmandu

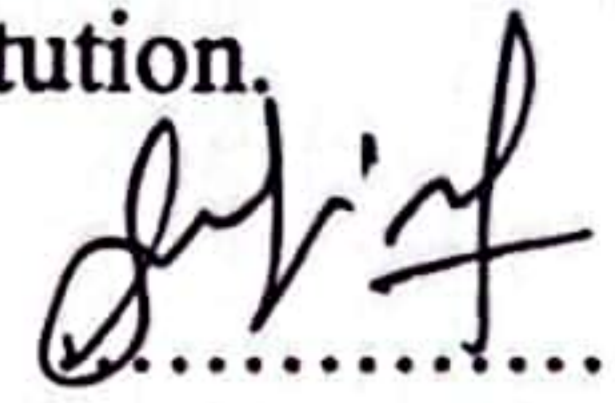
Nepal

May, 2023

DECLARATION

I hereby declare that the work presented in this thesis has been done by myself and has not been submitted elsewhere for the award of any degree. All the sources of information have been specifically acknowledged by reference to the author/s or institution.

Date: 2020/01/21



Sophiya Pandey



TRIBHUVAN UNIVERSITY

01-4331896

CENTRAL DEPARTMENT OF ZOOLOGY

Kirtipur, Kathmandu, Nepal.

Ref.No.:



RECOMMENDATION

This is to recommend that the thesis entitles “PREVALENCE OF PHYTONEMATODES ASSOCIATED WITH CITRUS FAMILY OF MARPAK, DHADING DISTRICT, NEPAL” has been carried out by Ms. Sophiya Pandey for the partial fulfillment of Master’s degree of Science in Zoology with special paper Parasitology. This is her original work and has been carried out under my supervision. To the best of my knowledge, this thesis work has not been submitted for any other degree in any institutions.

Date: २०८०/०१/२१

.....
Mr. Janak Raj Subedi
Lecturer

Central Department of Zoology
Tribhuvan University
Kirtipur, Kathmandu, Nepal



Ref.No.:

TRIBHUVAN UNIVERSITY

01-4331896

CENTRAL DEPARTMENT OF ZOOLOGY

Kirtipur, Kathmandu, Nepal.



LETTER OF APPROVAL

On the recommendation of supervisor “Mr. Janak Raj Subedi” this thesis submitted by Ms. Sophiya Pandey entitled “PREVALENCE OF PHYTONEMATODES ASSOCIATED WITH CITRUS FAMILY OF MARPAK, DHADING DISTRICT, NEPAL” is approved for the examination and submitted to the Tribhuvan University in the partial fulfillment of the requirements for Master’s Degree of Science in Zoology with special paper Zoology.

Date: 2080/01/21

.....
Prof. Dr. Kumar Sapkota
Head of Department
Central Department of Zoology
Tribhuvan University
Kirtipur, Kathmandu, Nepal



Ref.No.:

TRIBHUVAN UNIVERSITY

01-4331896

CENTRAL DEPARTMENT OF ZOOLOGY

Kirtipur, Kathmandu, Nepal.



CERTIFICATE OF ACCEPTANCE

This thesis work submitted by Ms. **Sophiya Pandey** entitled” **PREVALENCE OF PHYTONEMATODES ASSOCIATED WITH CITRUS FAMILY OF MARPAK, DHADING DISTRICT, NEPAL.**” has been accepted as a partial fulfilment for the requirements of Master’s Degree of Science in Zoology with special paper Parasitology.

EVALUATION COMMITTEE

.....
Supervisor
Janak Raj Subedi
Lecturer
Central Department of Zoology
Tribhuvan University
Kirtipur, Kathmandu

.....
Head of Department
Prof. Dr. Kumar Sapkota
Central Department of Zoology
Tribhuvan University
Kirtipur, Kathmandu

.....
External Examiner

.....
Internal Examiner

Date: 2080/03/19.....

ACKNOWLEDGEMENTS

I would like to start by expressing my deep appreciation and sincere thanks to my supervisor, **Mr. Janak Raj Subedi**, for his valuable guidance, encouragement, and friendly supervision in helping me prepare this thesis on the topic of **“PREVALENCE OF PHYTONEMATODES ASSOCIATED WITH CITRUS FAMILY OF MARPAK, DHADING DISTRICT, NEPAL”**

I am also thankful to the Professor and Head of the Department Dr. Kumar Sapkota for his supporting role throughout the preparation of this thesis work. Additionally, I wish to convey my profound gratitude and heartfelt appreciation to Professor Dr. Arvind Kumar Keshari for their unwavering support and invaluable guidance throughout the completion of this thesis. I deeply appreciate his assistance, especially given the circumstances. It is important to note that I am also grateful to all the non-teaching staff members of this department for their technical assistance and for helping to improve the quality of my work. However, I would like to express my appreciation for the trust placed in me by the citrus farm owners, Mr. Yam Bahadur Adhikari and Mr. Sabin Adhikari, who allowed me to collect samples for this study.

Lastly, I would like to express my sincere gratitude to my friends and family members for their unwavering support and assistance throughout the completion of this study. Their help has been invaluable and greatly appreciated.

Thank you all.....

TABLE OF CONTENTS

Declaration	i
Recommendation	ii
Letter of Approval	iii
Certificate of Acceptance	iv
Acknowledgements	v
List of Tables	viii
List of Figures	viii
List of Photographs	viii
List of Maps	viii
List of Abbreviations	ix
Abstract	x
1. INTRODUCTION	1-5
1.1 General Background	1
1.2 Introduction to citrus	2
1.3 Disease in citrus	3
1.4 Nematodes in citrus	4
1.5 Citrus in Nepal	4
1.6 Research Objectives	4
1.7 Rationale of Study	5
1.8 Limitations of the study	5
2. LITERATURE REVIEW	6-9
3. MATERIALS AND METHODS	10-17
3.1 Study area	10
3.2 Materials used	11
3.2.1 Materials for field	11
3.2.2 Materials for laboratory	11
3.2.3 Chemicals used	11
3.3 Study Design	12
3.3.1 Selection of study site	12
3.3.2 Study period	12
3.4 Methods	13-16
3.4.1 Collection of soil samples	13
3.4.2 Preparation of chemicals	13
3.4.3 Processing of soil samples	14
3.4.4 killing and fixation of nematodes	15
3.4.5 Mounting of nematodes	15
3.4.6 Observation and morphometric analysis for species identification.	15
3.4.7 pH analysis	16
3.5 Statistical analysis	16

4. RESULTS	17-22
4.1 Occurrence of phytonematodes associated with citrus	17-19
4.2 Generic composition and Ordinal diversity of phytonematodes in soil	20
4.3 Trophic diversity of plant nematodes	21
4.4 Soil color and pH analysis of soil samples of citrus farm.	22
5. DISCUSSION	23-25
6. CONCLUSION	26
7. RECOMMENDATIONS	27
8. REFERENCES	28-36
9. PHOTO PLATES	37-39

LIST OF TABLES

Table 1- Frequency of plant nematodes associated with citrus	17
Table 2: Relative and absolute frequency table of citrus nematode.	17
Table 3: Frequency table displaying the occurrence of phytonematodes categorized by different species of citrus.	18
Table 4: Trophic diversity of plant nematode with its order and family	21
Table 5: Table displaying the average pH values of soil samples taken from citrus farm	22

LIST OF FIGURES

Figure 1: Flow Chart of Research Design	12
Figure 2: Bar diagram showing comparative AF of Phytonematodes in Orange, Lime, and Lemon	19
Figure 3: Bar diagram showing comparative RF of Phytonematodes in Orange, Lime, and Lemon	19
Figure 4 Pie chart showing genera composition of phytonematodes	20
Figure 5: Pie chart showing ordinal diversity of encountered phytonematodes	20
Figure 6: Bar diagram showing frequency of trophic diversity of phytonematodes	21

LIST OF PHOTOGRAPHS

Photograph 1: Collecting soil samples	13
Photograph 2: Baermann funnel set up for extraction of nematodes.	14
Photograph 3: slide preparation of nematodes	15
Photograph 4: Observation of nematodes in compound microscope	16
Photograph 5: Mobile photography of phytonematodes from collected samples	37-39

LIST OF MAPS

Map 1: Map of study area	10
--------------------------	----

LIST OF ABBREVIATIONS

PPN: Plant Parasitic Nematode
RKN: Root-Knot Nematode
FLN: Free-Living Nematode
FAO: Food and Agriculture Organization
FAOSTAT: Food and Agriculture Organization Statistics
MoAD: Ministry of Agricultural Development
RF: Relative Frequency
AF: Absolute Frequency
HLB: Huanglongbing
ACP: Asian Citrus Psyllid
MoAC: Ministry of Agriculture and Cooperatives
MoALD: Ministry of Agriculture and Livestock Development
Mt: Million tons
VDC: Village Development committee
pH: Potential of Hydrogen

ABSTRACT

Parasitic feeding has been a successful strategy for organisms since the beginning of life, with about half of all parasite species infecting plants, including those in the citrus family. In the Marpak community of Dhading district, a study was conducted to investigate the prevalence of nematodes in citrus plants. Between November and February, 120 soil samples were collected and analyzed using the Baermann method to isolate and extract nematodes. The study revealed the presence of both parasitic and free-living nematodes, with 9 different genera from 3 orders identified. *Tylenchulus semipenetrans* had the highest prevalence rate at 51.6%, followed by *Mononchus* spp. at 42.5%, *Xiphinema* spp. and *Iotonchus* spp. at 29.1%, *Mesodorylaimus* spp. at 24.1%, *Meloidogyne* spp. (Juvenile) at 30%, *Rhabditis* spp. at 48.3%, *Dorylaimus* spp. at 35%, and *Discolaimus* spp. at 7.5%. Of the genera encountered, *Tylenchulus semipenetrans* was found to be the most common, while *Discolaimus* spp. was the least common. The 9 identified genera of soil nematodes, which were classified into 3 orders, included 5 genera (44%) categorized as Plant Parasitic Nematodes (PPN), and 4 genera (44%) classified as predatory Nematodes and 11% is classified as free living nematodes (FLN). In terms of taxonomic classification, the order Dorylaimida had the highest representation, accounting for 67% of the total nematode genera identified. The order Tylenchida accounted for 22%, while the order Rhabditida accounted for 11%. Similarly, the soil pH samples collected from the citrus farms were found to be slightly acidic, with an average pH value of 6.8 for orange farms and 6.6 for lemon and lime farms. The study did not observe any significant differences ($p>0.05$) in the prevalence of nematodes from the citrus farms with respect to soil pH value or color. This suggests that continued cultivation in the same manner, with the presence of suitable hosts, could potentially lead to an increase in the number of plant parasitic nematodes in the future.

1. INTRODUCTION

1.1 General background

Parasitic feeding in the living organism has been very successful from the very beginning of origin of the life where about 25000 nematodes, 5000 cestodes and 18000 trematodes has been recorded in all kinds of habitats in different species. About half of the species of parasites have been infecting the plants where the citrus family can't be left untouched.

The main source of income for the majority of people in Nepal is agriculture, which is agriculturally diverse and provides both direct and indirect livelihood opportunities (Kesari *et al.*, 2019). Singh and colleagues (2015) suggest that in a sustainable production system, the cropping sequence has a crucial role in controlling phytonematode problems in economically significant crops. Highly diverse in nature and distribution, phytonematodes pose a major threat to valuable agricultural crops and are considered one of the most significant pests. Considering the impact of phytonematodes on fruits production, crop protection measures are essential in the context of modern agriculture and sustainable development. Therefore, addressing the issue of phytonematodes is a crucial step in achieving sustainable fruits production.

Plant-parasitic nematodes are responsible for causing substantial economic losses to commercial crops worldwide. The use of chemical nematicides poses environmental and health risks, highlighting the need for environmentally friendly and safe management methods (Hanada *et al.*, 2022). Nematodes are the most abundant multicellular organisms on Earth, and they can be found living in various environments such as soil, water, and even oceans. Plant-parasitic nematodes are small, eel-like roundworms with stylets that reside in soil and can cause damage to plants by feeding on their roots or tissues. Plant-parasitic nematodes can feed on plant roots either internally or externally, while some nematodes feed on leaves instead (Bahadur & Bahadur, 2021). An infestation of nematodes in the soil can lead to secondary infections with bacterial and fungal diseases, as well as the transmission of viruses that infect plants, all of which can have a negative impact on crop production.

When nematodes colonize the roots of plants, they can disrupt the intake of water and nutrients and prevent the movement of minerals, which can lead to poor plant growth. This can be reflected in the shoot-to-root (S/R) ratios, with affected plants often showing a decrease in shoot growth compared to root growth (Gullino *et al.*, 2019). Analyzing the community of phytonematodes present in a particular region is an essential criterion for evaluating their pathogenic potential and identifying hotspots where nematode attacks are prevalent (Sahu *et al.*, 2011).

1.2 Introduction of Citrus

Citrus, a genus of plant which belongs to Rutaceae family. This family consists of about 160 genera and over 2,000 species of plants. Within the family Rutaceae, the genera Citrus- oranges (*Citrus sinensis*), mandarins (*Citrus reticulata*), pomelos (*Citrus maxima*), grapefruit (*Citrus paradisi*), lemon (*Citrus limon*), lime (*Citrus aurantifolia*) and citrons

(*Citrus medica*)- *Fortunella* (kumquats, *Fortunella Japonica* and *Poncirus* (trifoliolate oranges, *Poncirus trifoliata*) contain the principal commercial species (Swingle and Reese, 1967). Citrus plants may be tropical or subtropical trees or shrubs with glossy oval-shaped leaves and some species have thorns. They have thick large and fleshy fruits with thick skins. All the members of citrus are fruit bearing which contain juice filled vesicles known as hesperidium. Citrus spp. is naturally deep-rooted plants (Ford, 1954) and optimum growth requires deep, well drained soils. Citrus may have originated from the history of the cultivated species, and it is cultivated from 15°N to 35°S between sea level and 100m (Okwulehie, 1998). Oranges (53%), mandarins and tangerines (21%), lemons and limes (11%), grapefruit and pomelos (6%) are the citrus crops with the highest production tonnage (Cimen and Yesiloglu, 2016).

Citrus plants have greater value of medical importance. Citrus fruits have multiple nutrients like potassium, folate calcium, thiamine, niacin, vitamin B₆, phosphorus, magnesium, copper, riboflavin, and pantothenic acid. (Nekrich, 2020). Consumption of citrus plays an important role in vascular protection, reduces inflammation, improves gastrointestinal function, and plays an important role against diabetes, cancer, and neurological diseases. (Nekrich, 2020). Giacosa *et al.* (2013) reported that citrus fruits are the most commonly consumed fruits and have been linked to a reduced risk of degenerative diseases, including cancer. In addition to vitamin C, citrus fruits are also rich in flavonoids, which are a group of antioxidants that help protect the body against damage from free radicals. Flavonoids have been linked to a reduced risk of several chronic diseases, including heart disease and cancer.

While citrus is a valuable commodity, its yield has been significantly reduced as a result of parasitic infections, such as Root-knot nematodes (These microscopic roundworms infect the roots of citrus trees and cause the formation of small, knotty galls. This can lead to stunted growth, reduced fruit yield, and even death of the tree), Citrus nematode (This is another type of nematode that infects the roots of citrus trees and causes similar symptoms to root-knot nematodes), Citrus bud mite (This tiny mite feeds on the buds of citrus trees, causing them to become distorted and malformed. This can lead to reduced fruit yield and poor fruit quality), Citrus leafminer (This small moth larvae burrow into the leaves of citrus trees, causing them to become distorted and curled. This can lead to reduced photosynthesis and weakened trees), Citrus psyllid (This pest feeds on the leaves and shoots of citrus trees and can transmit a bacterium that causes a serious disease called citrus greening. Infected trees may exhibit stunted growth, yellowing leaves, and reduced fruit yield).

To control these endoparasites, it is important to regularly inspect citrus trees for signs of infestation and take appropriate measures such as using nematode-resistant rootstocks, applying nematode-controlling chemicals, and using insecticides or beneficial insects to control pests such as citrus psyllids or leafminer. Additionally, maintaining healthy trees through proper fertilization and watering practices can help prevent infestations.

1.3 Disease in citrus

Citrus plants are an economically significant crop that are vulnerable to soil-borne pathogens and pests, which can cause damage or destruction to newly formed roots of the plant. According to (Timmer *et al.*, 2000), citrus can be affected by 16 different fungal diseases, 42 viruses or virus-like organisms, 7 bacterial diseases, and some nematodes that cause diseases.

Although numerous diseases are known to affect citrus crops in Nepal, the major diseases that limit their yield include citrus greening (HLB), diseases caused by *Phytophthora* such as citrus canker, citrus tristeza, twig blight, and powdery mildew, as reported by MoAC (2011). Adhikari (2016/17) identified anthracnose, sooty mold, pink diseases, gummosis, and scab diseases as other factors contributing to the decline in citrus production and quality. Crown gall disease has been recognized as plant disease of worldwide importance to many plant species (Burr and Otten, 1999). Citrus greening is a disease caused by vector pathogen. It is of two forms namely: African and Asian citrus greening. Another common disease found in citrus is citrus huanglongbing. Farmers in southern China first observed Citrus huanglongbing (yellow shoot disease) as a problem of unknown origin in the late nineteenth century (Zhao, 1981). Asian citrus psyllid (ACP) is also the most important pest of citrus worldwide. (Boina& Bloomquist, 2014). The high prevalence of diseases not only leads to severe citrus decline syndrome in trees, but also reduces the quality of fruits in regions where citrus is grown.

1.4 Nematodes in citrus plants

The first association between nematode pests and citrus was observed by Neal (1889), and numerous nematode species have since been associated with citrus roots and their surrounding rhizosphere (Cohn, 1972): Duncan, 1999: Nehru *et al.*, 2005: Siddiqui: 2005: Park *et al.*, 2009: Pathak and Chandra, 2010; Bakr *et al.*, 2011; Zalpuri *et al.*, 2013; Ibrahim *et al.*, 2016). Yeates *et al.*, (1993) classified nematodes into various categories based on their feeding habits, which include plant feeders, bacterial feeders, fungal feeders, algae feeders, animal predators, and omnivores. All the types of nematodes occur in soil.

Like other plants, citrus is also affected by nematodes According to (O'Bannon and Esser, 1985), citrus nematodes do not cause the death of the citrus trees they infest. Nematodes mostly make their habitat around the roots of plants and highly affect the production of crops. The most common parasitic citrus nematodes are under the order of Tylenchida, Dorylaimida and Aphelenchida. Some nematodes like *Rhabditis* spp., *Mesorhabditis* spp., *Cephalous* spp. and *Eucephatobus* spp. etc. are the free-living nematodes which are common in plants and benefit plants by providing nutrients through decaying organic matter (Yeates *et al.*, 1993). The detection of *T. semipenetrans* on citrus roots in California dates back to 1912, and it was named and described in the following two years by (Cobb, 1913, 1914). With the notable exception of *T. semipenetrans*, most nematode species capable of damaging mature citrus tend to be regional or local problems, due either to edaphic conditions or to the natural distribution of a particular nematode (Duncan, 1999; Etebu and Nwauzoma, 2014). Despite not being fatal to citrus trees, when present in high numbers, citrus nematodes can cause a gradual decline in the health of

infected trees to varying degrees (Kwaye *et al.*, 2008). Infected citrus trees suffer from less yielding of fruits. As conditions become less suitable for tree growth, the effects of citrus nematode parasitism are more apparent (Van Gundy *et al.*, 1964; Heald and O'Bannon, 1987).

1.5 Citrus in Nepal

In Nepal, Citrus has been grown in 62 districts. According to (Adhikari, 2013/14), the main producers of citrus are Dhankuta, Terathum, Sindhuli, Ramechhap, Dhading, Kavre, Gorkha, Lamjung, Tanahun, Kaski, Syangja, Myagdi, Palpa, Salyan, Dailekh, Baitadi, and Dadeldhura. Citrus fruit cultivation thrives in the mid-hill regions of Nepal, which have an altitude range of 800 m to 2100 m from the east to west of the country, thanks to their favorable climatic conditions (Lama, 1988). It wasn't until 1970 that Nepal began commercial cultivation of citrus. (NCRP, 2010). According to MoALD (2017), citrus cultivation in Nepal spans an area of 46,328 hectares, out of which only 26,759 hectares are productive. The total production of citrus in Nepal is 239,773 metric tons, with a productivity rate of 8.96 metric tons per hectare. According to Kaini (2019), despite having more favorable climate and soil conditions for citrus cultivation compared to other South Asian countries, Nepal's citrus productivity has been on the decline. Subedi *et al.* (2008) attributed the low and stagnant productivity, as well as the decline, of citrus in Nepal to a range of biotic and abiotic factors.

According to Adhikari *et al.*, (2019), the decline of citrus in Nepal can be attributed to several factors, such as unsuitable soils, drought, inadequate nutritional management, use of low-quality planting materials, improper orchard management, and the occurrence of various diseases and insects in the field. Chinese citrus fly, citrus greening, canker, and root rot are among the major insect pests and diseases that contribute to the decline of citrus in Nepal. There is a high susceptibility of citrus to a wide range of diseases caused by various pathogens.

1.6 Objectives

1.6.1 General objective

- To identify the prevalence of phytonematodes associated with citrus family of Marpak community of Dhading.

1.6.2 Specific objectives

- To determine frequency of plant parasitic nematodes around rhizosphere of citrus.
- To determine trophic diversity of different plant nematodes.

1.7 Rationale of study

Among the six constituents of the nutrients where vitamin C is crucial part of which citrus family is the great source and is under constant effect of the nematodes. The world produces more citrus than any other kind of fruits, an average of 87 million tons (Mt) per year in recent years. (Anonymous, 2001) where Nepal can't be left different. Many farmers are dependent over production of citrus fruits as the source of income in Nepal and farms has been greatly affected by Nematodes. So, recognizing the problem of nematodes in citrus family in time and working for the prevention of serious infection of Nematodes preventing huge loss due to this, it could be great boon for the improvement of economic status of the farmer as well as country too. Despite the great source of income for farmers, there is huge loss due to parasitic infection (nematodes) in cultivation of citrus fruits. Neither governmental nor nongovernmental organizations have shown interest in the identification and prevention of parasitic infection (nematodes) on the citrus family yet. Thus, this research may help and provide guidelines for further research and help to control the parasitic nematodes of citrus which will increase the yielding of citrus fruits.

1.8 Limitations of the study

- The study was limited to sampling certain regions, soil types, or plant species, therefore the findings may not accurately reflect the overall prevalence of Phyto nematodes in other locations.
- The precision of the outcomes could rely on the techniques utilized for detecting and recognizing phytonematodes.
- The occurrence of phytonematodes can fluctuate based on the season, and the samples were collected during the winter season. (November to January)
- The incidence of phytonematodes can be impacted by additional soil elements like moisture, temperature, and nutrient levels, but this research is constrained to these variables.
- The study may show a correlation between the prevalence of phytonematodes and certain factors, but it may not establish a causal relationship. This means that other factors may be influencing the prevalence of phytonematodes that were not accounted for in the study.

2. LITERATURE REVIEW

Nepal is buffer state between two highly developed economic power centers of the world where economy of Nepal is totally based upon agriculture. 25, 22,520 hectars of total land mass are suitable for agriculture. Rice, maize, and wheat are dominant grown crops and sugarcane, tobacco, jute is mostly grown cash crops along with citrus fruits like tangerine, orange, lemon also have higher possibility to be grown as major cash crops which can be cultivated in sub-tropical climatic zone of Nepal, but they are not free from parasitic infections which is mainly due to nematodes. There have been numerous studies conducted globally on phytonematodes linked with citrus, but only a limited amount of research has been carried out in Nepal on this topic.

In Global Context,

In a survey of the irrigation districts of Merbein and Robinvale, settlements on plants like grape vines and citrus, (Sauer, 1962) identified several genera of plant parasitic nematodes, including *Meloidogyne*, *Tylenchulus*, *Pratylenchus*, *Paratylenchus*, and *Criconeoides*. Edward and Rai (1970) observed *Boleodorus citrin* sp., *Scutellonema sherin* sp., *Helicotylenchus dihystra*, *Xiphinema basiri*, and *Tylenchulus semipenetrans* in the soil around the roots of hill orange (*Citrus reticulata*) in Kalimpong, Sikkim. (Abivardi *et al.*, 1970) conducted a survey on nematodes in citrus orchards in southern Iran and identified 8 species of plant parasitic nematodes. Of these, only *Tylenchulus semipenetrans* was widely distributed and occurred in high populations, making it economically important. *Helicotylenchus nannus* and *Diptherophora communis* were occasionally found in high numbers. Philis and Siddiqi (1976) conducted a survey in Cyprus and identified 58 species of nematodes, including *Tylenchulus semipenetrans* Cobb and *Anguina tritici* Steinbuch in citrus farm.

Stirling (1976) surveyed vineyards in all five of South Australia's grape growing districts. Root-knot nematodes (*Meloidogyne* spp.) occurred in four districts and were present in almost all vineyards with sandy soil in the Riverland, Barossa Valley, and Central districts. Four species (*M. arenaria*, *M. hapla*, *M. incognita* and *M. javanica*) were identified. The Riverland vineyards had a widespread infestation of the citrus nematode (*Tylenchulus semipenetrans*), while isolated occurrences of infestation were discovered in other districts. Other plant parasitic nematode genera found during the survey were *Helicotylenchus*, *Macroposthonia*, *Paratrichodorus*, *Paratylenchus*, *Pratylenchus*, *Tylenchorhynchus* and *Xiphinema*. Abu-Gharbieh (1975), Abu-Gharbieh and Hammou (1972), AbuGharbieh *et al.* (1978), Anon (1970, 1971) and Qasem (1970) reported the presence of *Meloidogyne* spp. Also, both *Anguina tritici* (Steinbuch) Chitw and *Merlinius rugosus* (Siddiqi) were reported to occur in the country by (Qasem, 1970) and (Hashim, 1979) respectively.

Z. Hashim (1979) collected samples from Jordan Valley, Southern Ghors, WadiShu'aib, Jubeiha, Sweileh, Salt, Jerash and Irbid which revealed nematode species such as *Helicotylenchus Steiner*, *Meloidogyne Goeldi*, *Pratylenchus Filipjev*, *Tylenchorhynchus Cobb*, *Tylenchulus Cobb*, *Xiphinema Cobb*, and *T. semipenetrans* Cobb.

Kaplan (1985) identified *Radopholus citrophilus*, *Tylenchulus semipenetrans*, *Pratylenchus brachyurus*, and *P. coffeae* as the most serious nematodes affecting citrus

crops in Florida, USA. During a survey of ten citrus orchard areas in Libya, (Siddiqui et al., 1987) discovered the presence of 26 genera of parasitic nematodes, among which *Tylenchulus semipenetrans* was identified. Meanwhile, (Edwards, 1989; Sasser, 1989) reported *Xiphinema* species from important crops globally like grapevines and citrus, while *Xiphinema americanum* has impacted 37% of vineyards. The trial sites were found to have plant parasitic nematodes, including root-knot nematodes (*Meloidogyne javanica* (Treub) Chitwood) and citrus nematodes (*Tylenchulus semipenetrans* Cobb). In a survey conducted on citrus groves, (Walter and Kalplan, 1990) encountered species like *Radopholus citrophilus* and *Rhabditis* spp. by using Sieving and Baermann funnel method. In another study, (Elekcioglu et al., 1994) found several nematode species associated with 12 different crop plants in the East Mediterranean region of Turkey, including *Tylenchulus*, *Meloidogyne*, *Pratylenchus*, *Helicotylenchus*, and *Xiphinema* species. Among these, *T. semipenetrans* Cobb was the most prevalent nematode species found and was associated with Citrus spp.

In a survey of plant parasitic nematodes in the East Mediterranean region of Turkey, (Elekcioglu, 1995) identified 16 nematodes associated with citrus, but only four species, namely *Helicotylenchus pseudorobustus*, *Rotylenchulus macroratus*, *Tylenchorhynchus goffarti*, and *Tylenchulus semipenetrans*, were found to be capable of infecting citrus. In a plant nematode survey conducted by (Bridge et al., 1996) in Belize, Central America, a range of crops including citrus, other fruit plant and vegetable crops were sampled. A total of 47 species of plant-parasitic nematodes were identified. Among them, The most commonly found nematodes included *Tylenchulus semipenetrans* on citrus, as well as some spiral nematodes such as *Helicotylenchus multicinctus*, *H. mucronatus*, and *Meloidogyne* spp. According to (Handoo, 1998) the most devastating nematode species associated with citrus is the citrus nematode (*Tylenchulus semipenetrans*), which is found in all citrus producing regions of the world and another is burrowing nematode (*Radopholus similis*).

A study conducted in Punjab revealed the presence of 85 plant parasitic nematodes belonging to 38 genera in vegetables. These included *Meloidogyne incognita*, *M. arenaria*, *Heterodera avenae*, *Aphelenchoides composticola*, *Ditylenchus myceliophagus*, *Rotylenchulus reniformis*, *Pratylenchus coffeae*, and *Tylenchulus semipenetrans*. (Mahajan and Kaur, 1991) and (Mahajan and Chhabra, 2009). Roca et al. (1992) found *X. belmontense* and *X. abarticum* in apples and peaches in Portugal.

Feil et al., (1997) studied the influence of seasonal and site-specific factors on the population of *Xiphinema* index in two vineyards in California. Crozzoli et al., (1998) carried out nematode survey in the main citrus growing areas of Venezuela. Thirty four species were identified: *Aorolaimus holdemani*, *A. macbethi*, *Criconema demani*, *Gracilacus aculenta*, *Helicotylenchus crenacauda*, *Helicotylenchus dihystra*, *H. erythinae*, *H. multicinctus*, *H. multicinctus*, *Hemicriconemoides communis*, *H. mangiferae*, *Hoploaimusseinhorsti*, *Meloidogyne exigua*, *M. incognita*, *Mesocriconem aonoense*, *M. ornatum*, *M. sphaerocephalum*, *Monotrichodoros monohystera*, *Paratrichodoros minor*, *Paratylenchus elachystus*, *P. minutus*, *Pratylenchus sbrachyurus*, *P. zaeae*, *Rotylenchulus reniformis*, *Rotylenchus caudaphasmidius*, *Scutellonema brachyurum*, *Tylenchorhynchus*

annulatus, *T. capitatus*, *Tylenchulus semipenetrans*, *Xiphinema brasiliense*, *X. brevicolle*, *X. krugi*, *X. peruvianum*, *X. simillimum* and *X. vulgare*.

Clark (1960) described *Mononchus* from New Zealand, while Mulvey (1961) described it from Canada, and Ahmad et al. (2005) described it from Singapore. Coetzee (1965) described various new and previously known species of *Mononchus* from South Africa and Malaysia, as noted by (Loof, 2006). Additionally, Khan and Araki (2002) described several species of *Mononchus* found in Japan. Jairajpuri (1971) reported numerous genera of *Mononchus* from India, while Badri et al. (1978) described *Iotonchus* and new species of *Cobbonchus* sp. from India. Zaki and Mantoo (2003) reported the occurrence of one predaceous species of *Mononchus* sp. in fruit trees in the Kashmir valley.

Bakr et al., (2011); Divsalar et al., (2011) identified plant parasitic nematodes in citrus orchards of Egypt and Guilan and Mazandaran provinces respectively using morphometrical characteristics and species were identified like *Aphelenchoides asterocaudatus*, *A. bicaudatus*, *A. sacchari*, *Aphelenchus avenae*, *Basiria graminophila*, *Boleodo rusthylactus*, *Criconemoides xenoplax*, *C. curvatum*, *Ditylenchus myceliophagus*, *Filenchu sfacultativus*, *F. vulgaris*, *Helicotylenchus digonicus*, *H. dihystra*, *H. exallus*, *H. pseudorobustus*, *H. vulgaris*, *Nagelus obscurus*, *Ogmacivellae*, *Paratylenchu snanus*, *Pratylenchus loosi*, *P. neglectus*, *P. jaehni*, *P. zea*, *Psilenchushilarulus*, *Seinura lii*, *S. prospera* and *Tylenchulus semipenetrans*. Zalpuri and colleagues (2013) conducted a survey on citrus crops in India and found that *Xiphinema* and *Pratylenchus* species had the highest prominence value among nematode species, followed by *Hoplolaimus*, *Tylenchulus semipenetrans*, and *Meloidogyne javanica*. Ibrahim and colleagues (2015) collected soil samples from various crops in Jordan and found that nectarine and plum orchards had the highest nematode infestation (100%), followed by citrus (97.6%), apple (88.7%), pear and quince (85.7%), cherry (81.4%), almond (66.6%), and apricot (66.6%). The nematodes detected in the samples included *Meloidogyne* spp., *Tylenchulus*, *Xiphinema*, *Rotylenchus*, *Pratylenchus*, and *Longidorus*, with citrus trees mostly affected by *Tylenchulus* and *Radopholus* spp.

Hussain et al. (2016) reported nematodes like *Helicotylenchus digonicus*, *H. indicus*, *H. pseudorobustus*, *Hoplolaimus indicus*, *Meloidogyne incognita*, *M. javanica*, *Pratylenchus holdemani*, *P. penetrans*, *P. scribneri*, *P. thornei*, *Rotylenchus dalhousiensis*, *Scutylenchus rugosus*, *Tylenchorhynchus annulatus*, *T. curvus*, *T. trilineatus*, *Tylenchus filiformis*, *Xiphinema americanum*, *X. basiri*, *X. index* and *X. rivesi* from the rhizosphere soil of Pomegranate, Grapes, Peach, Plums, Tobacco, Apple and Apricot at three localities of Pishin district, Balochistan province. In their study, (Eisvand and colleagues, 2019) conducted a survey on the plant-parasitic nematode diversity present in citrus orchards in Khuzestan province, identifying six nematode species including *Helicotylenchus abunaamai*, *H. crenacauda*, *Pratylenchus allius*, *P. musii*, *Psilenchushilarulus*, and *Tylenchulus semipenetrans*.

In addition, Kaur et al. (2010) reported that *Longidorus siddiqii* had a distribution rate of 13.8% in banana crops. A review by Sikandar et al. (2020) also noted *M. incognita* as a major issue for agricultural crops worldwide, requiring careful management in the near future. Pascual et al. (2014), Gnamkoulamba et al. (2018), War et al. (2019), and Nyaku

et al. (2021) also found *M. graminicola* and *H. oryzae* to be the most frequent pests of rice plants, but Mussarat et al. (2016) observed *Hirshmanniella* spp. and *Helicotylenchus* spp. as the most frequent genus in Pakistan. Talavera et al. (2019) identified *Meloidogyne* spp., particularly *M. hapla*, as the major pest of strawberries, which was later confirmed by a review by Mahfouz and Abd-Elgawad (2019). Singh et al. (2019) reported *Meloidogyne* spp. as the major pest of pomegranate, while *Helicotylenchus* spp. was the most frequent genus for pineapple in the Philippines (Benzonan et al. 2021). Citrus crops, on the other hand, were found to be most affected by *Tylenchulus semipenetrans* and *Helicotylenchus* spp. according to Zoubi et al. (2022).

In National context,

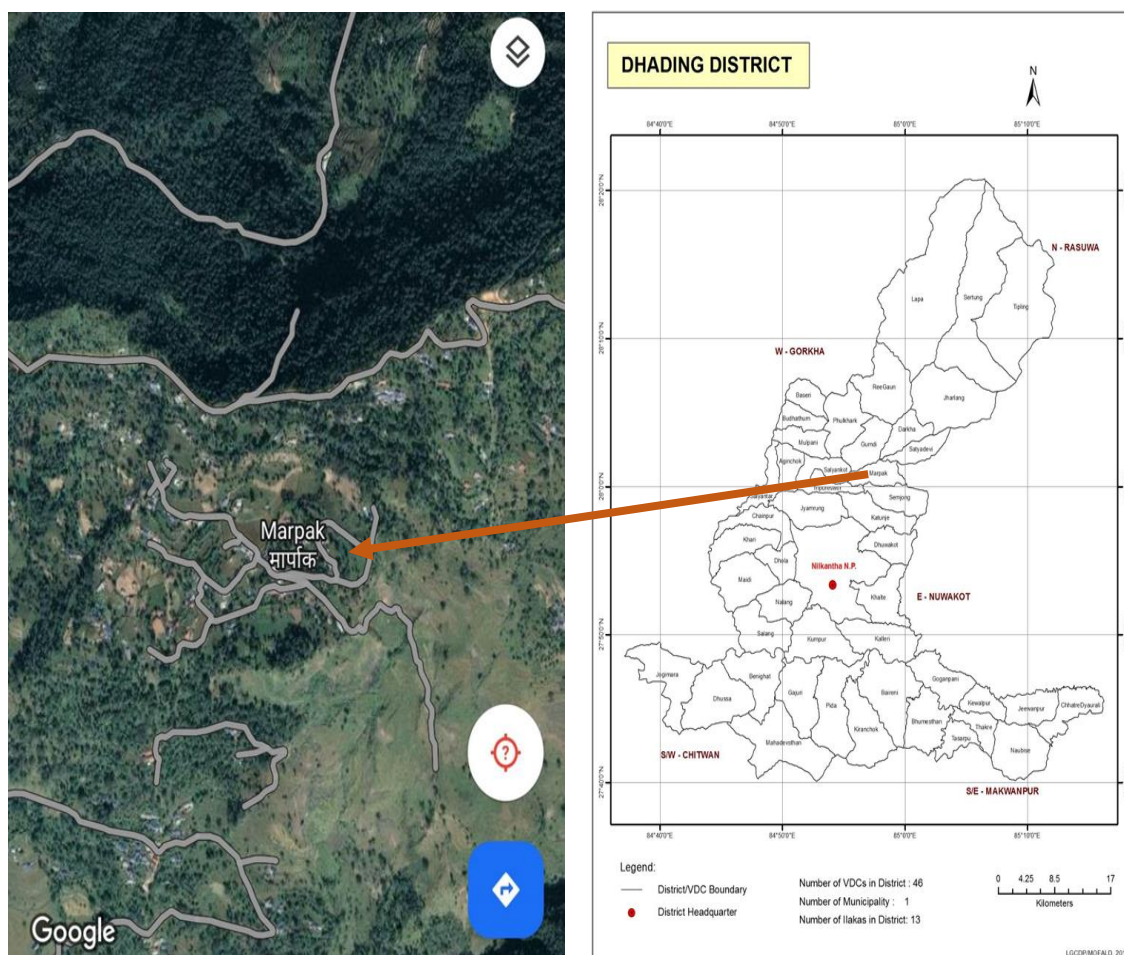
In Nepal, the agricultural sector is a major contributor to the country's economy, and phytonematodes have been identified as a major constraint to crop production. Despite their significant impact, there is a limited amount of research on the diversity, distribution, and management of phytonematodes in Nepal. Keshari et al. (2018) reported *Mononchus aquaticus* from major vegetable crops in the Bhaktapur and Kavre districts of Nepal. Chettri and Subedi (2019) reported *Mononchus* from kiwi plants, while Shrestha (2015) found it in pear plants in Nepal. Various types of plant parasitic nematodes, including root knot nematode (*Meloidogyne* sp.), *Hirshmanniella oryzae*, *Aphelenchoides besseyi*, *Ditylenchus angustus*, *Tylenchorhynchus* sp., *Hoplolaimus* sp., and *Helicotylenchus* sp., are widespread in rice crops in Nepal. Additionally, (Upreti, 2000) found that *Meloidogyne ramnicola* is more prevalent in light soil compared to heavy soil. Nematode species such as *Tylenchorhynchus*, *Hemicycliophora*, *Pratylenchus*, *Helicotylenchus*, *Hoplolaimus*, *Rotylenchus*, *Cricinemoides*, and *Xiphinema* are associated with rice, while *Anguina*, *Aphelenchus*, *Pratylenchus*, *Heterodera*, *Xiphinema*, and *Hoplolaimus* are associated with wheat (Upreti, 2000).

In addition, *Helicotylenchus* sp., *Pratylenchus* sp., *Heterodera* sp., *Meloidogyne* sp., *Anguina* sp., and *Tylenchorhynchus* sp. have been reported from vegetables (Amatya and Shrestha, 1969). Nematode species such as *Tylenchorhynchus mashhoodi*, *Hoplolaimus indicus*, *Helicotylenchus incisus*, *Microposthonia paraxestis*, and *Hemicriconemoides cocophilus* have been recorded as plant parasitic nematodes in vegetables in the hilly district of Kathmandu (Keshari and Gupta, 2016). Sharma et al. (2001) also reported a new species of *Cactodera johanseni* from radish in Bhaktapur. Furthermore, papaya and maize crops have been found to be affected by nematode species such as *Pratylenchus* sp., *Meloidogyne* sp., *Tylenchorhynchus* sp., *Hoplolaimus* sp., *Helicotylenchus* sp., *Rotylenchus* sp., *Xiphinema* sp., *Trichodorus* sp., *Hirshmanniella* sp., and *Longidorus* sp. (Pokharel et al., 1994; Sharma-Poudyal, 2004). Altogether, there have been reports of 32 species of plant parasitic nematodes in Nepal (Gupta, 1997). According to Amatya and Shrestha (1996), *Longidorus* sp. has been found in various crops including cauliflower, chili, wheat, paddy, cabbage, and tomato in Nepal. Additionally, Shrestha (2015) reported finding *Longidorus* sp. in summer and autumn samples of pear trees in Nepal. Dangal et al., (2009) discovering its wide distribution in rice nurseries in Chitwan.

3. MATERIALS AND METHODS

3.1 Study Area

This study was carried out at Marpak village development committee of Dhading district. Marpak is a village development committee (VDC) positioned in the central development region of Dhading district in the Bagmati province of Nepal. It is extended over 28°0'33"N latitude and 84°59'1"E longitude. The climate type of this region is subtropical highland climate or temperate oceanic climate with dry winters. It is in the main hilly region at an altitude of 669.54m. In the study area, citrus plants are cultivated immense area. As we know, nematodes are distributed widely in the soil which may parasitize the citrus species. So, this study is carried out for the prevalence of soil nematodes in citrus.



Map 1: Map of study area

3.2. Materials used:

3.2.1 Materials for field

- i. Digging rod funnel
- ii. Supporting nets
- iii Tissue paper
- iv. Measuring tape
- v. Polythene bags

3.2.2 Materials for laboratory

- | | | |
|-------------------------|----------------------|--------------|
| i. Binocular microscope | x. Tissue paper | xix. Clamp |
| ii. Compound microscope | xi. Beaker | xx. pH meter |
| iii Ocular microscope | xii. dropper | |
| iv. Polythene bags | xiii. Petridisk | |
| v. Marker | xiv. Bucket | |
| vi. Slide | xv. Dessicator | |
| vii. Cover slip | xvi. Baermann Funnel | |
| viii Gloves | xvii. Stand | |
| ix. weighing balance | xviii. Rubber tube | |

3.2.3 Chemicals used

- i. Formalin
- ii. Triethanolamide
- iii. Distilled water
- iv. Ethanol
- v. Glycerin
- vi. Calcium chloride

3.3 Study Design

The study was designed to assess the soil nematodes of citrus family of Marpak community of Dhading district. The study design includes:

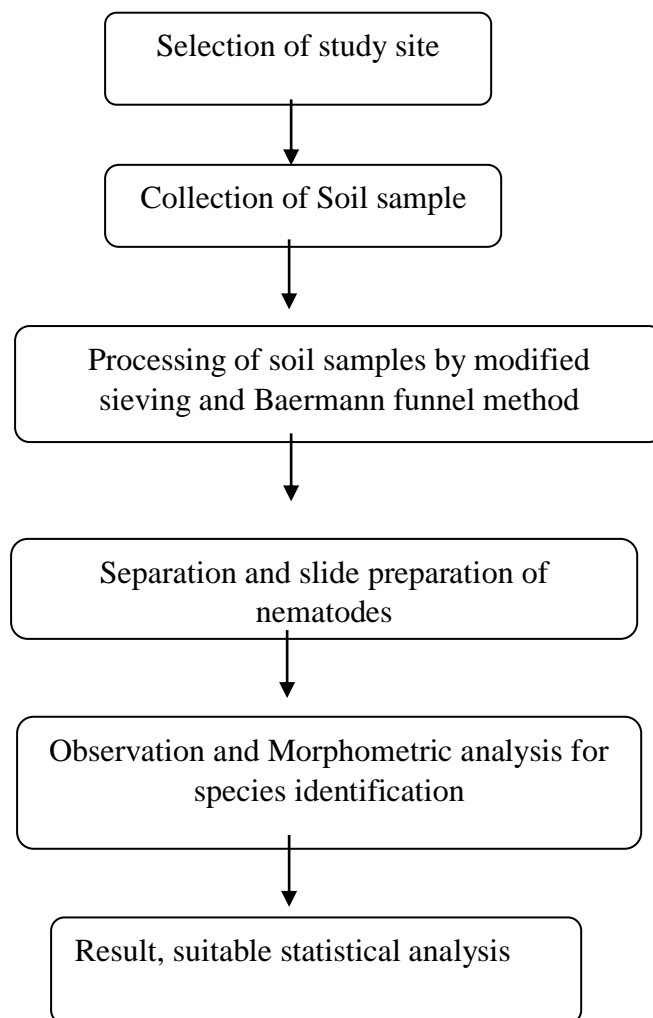


Fig 1: Flow Chart of Research Design

3.3.1 Selection of study site

The study was carried out in Marpak community of Dhading district positioned in the central development region, Bagmati province of Nepal.

3.3.2 Study Period

The study was carried out from November 2022 to February 2023.

3.4 Methods

3.4.1 Collection of soil samples



Photograph 1: Collecting soil samples

Nematodes that parasitize plants are typically located near the roots, with only a small number present in the top 5cm of soil. A total of 120 soil samples of citrus plants were taken. (viz; 40- lime, 40- oranges and 40 lemons). Soil samples are usually obtained from a depth of 15-20cm and then placed in polythene bags while being kept moist. To preserve the samples, they should be kept cool at a temperature of approximately 10-16°C. This is the process used for collecting soil samples that will be examined (Chhetri and Subedi 2019).

3.4.2 Preparation of the chemicals

The laboratory processing of collected samples requires two specific solutions: TAF solution and Glycerol Ethanol solution. TAF solution is used for fixing the nematodes, while Glycerol Ethanol solution is utilized for processing the soil samples.

TAF solution is prepared by mixing the chemicals in given proportions. Viz.

- a. Formalin (40% Formaldehyde) – 7ml
- b. Triethanolamide – 2ml
- c. Distilled water – 91ml

Although TAF is commonly used as a fixative, it is not suitable for long-term preservation. Nematodes fixed in TAF will retain their life-like appearance for several hours, but to achieve optimal results, they should be kept in the fixative for a minimum of 48 hours or longer. During this time, the process of tissue and collagen hardening occurs, which improves the preservation of the specimens. When fixed in TAF and mounted in glycerol, the specimens remain in good condition.

Glycerol ethanol is used for processing nematodes. This solution is prepared by mixing given chemicals in the given proportion.

- i. Ethanol – 5ml
- ii. Glycerol (30%) – 95ml

3.4.3 Processing of soil samples.



Photograph 2: Baermann funnel set up for extraction of nematodes.

The collected sample of soil is further processed by the modified sieving and decantation technique followed by the Baermann funnel technique (Baermann 1917) for the extraction of soil nematodes. Initially in this method, about 500gm of soil sample is taken and that is filled with water. Then the heterogeneous mixture of mud and water is stirred gently with hands to break down the lumps. Stone and debris are removed from the mixture. The muddy water is stirred and poured through sieve of mesh size of 500 μm , 300 μm and 150 μm respectively. The residue is disposed of, and the filtrate is poured into another bucket through sieve of mesh size of 75 μm . The suspension is washed by gentle flow of water and the filtrate is disposed of. Again, the residue present in the sieve is collected in the clean water in a beaker. This residue is poured over the tissue mounted on the course of supporting net and placed in Baermann funnel. The stem of funnel is connected with rubber tube and its tip is closed by the clip. The funnel is filled with water to the level just touching the lower surface of tissue paper on supporting net for avoiding the bubbles. Then the apparatus is left for 24 hours. From the tip of rubber tube in a cavity block, a small quantity of suspension was collected, and the nematodes move toward the bottom by penetrating towards the tissue paper so that they could be collected from the few drops of water at the rubber tube in the Petri disk.

3.4.4 Killing and fixation of nematodes.

After collection, the nematodes are allowed to settle undisturbed for several minutes. Excess water is then removed using a pipette. The nematodes are subsequently transferred to a cavity block containing TAF solution, which serves as an effective fixative. By preventing damage to the epidermal layer of the nematodes, TAF solution preserves their life-like appearance for several hours (Seinhorst, 1959).

3.4.5 Mounting of nematodes.

To mount the nematodes, they are transferred from the cavity block to a drop of anhydrous glycerin on a slide using the wax ring technique (Seinhorst, 1959). Water-colored nail polish was applied around the coverslip to provide additional support.

3.4.6 Observation and Morphometric analysis for species identification.

Finally, the slides should be observed using low and high-power magnification, such as 4X, 10X, and 40X for the nosepiece and 10X for the eyepiece (Shrestha and Bam 2015). The structures and parts of the nematode's body should be measured in detail using an ocular micrometer to aid in species identification. Identification of various nematodes was done under compound microscope on the basis of morphological characters with the help of key book of Mai and Lyon, 1975.



Photograph 3: slide preparation of nematodes



Photograph 4: Observation of nematodes in compound microscope

3.4.7 pH analysis

To determine the pH of the soil samples, a pH meter was utilized following calibration. Each soil sample, weighing 20gm, was dissolved in 50ml of distilled water by stirring with a glass rod and left undisturbed for some time. The calibrated pH meter was then immersed into the solution, and the constant reading on the device was recorded, as per the methodology outlined by Ndava et al. (2018).

3.5 Statistical analysis

The R-software was utilized to conduct a Chi-square test and determine significant differences among variables. Results were considered significant at a level of $p < 0.05$, following the approach outlined by Wu and Shi (2011). Norton's formulae (1978) were applied to compute the parameters for the community analysis of different nematode genera.

Frequency (N): Frequency of nematode genus (i.e., the number of samples in which the genus was present).

$$\text{Absolute frequency} = \frac{\text{No. of samples containing a species}}{\text{No of samples collected}} \times 100$$

$$\text{Relative frequency} = \frac{\text{Frequency of a species}}{\text{Sum of frequencies of all species}} \times 100$$

4. RESULTS

Various diseases, including plant parasitic nematodes, have been reported to infect citrus plants. In order to examine these nematodes, soil samples from the rhizosphere of the citrus were screened. The analysis identified the presence of nine distinct nematode species. Based on their morphological characteristics, the isolated nematodes were classified into three groups: plant parasitic, predator, and free-living.

4.1 Occurrence of plant nematodes associated with citrus

From soil samples taken from a citrus farm, A total of 9 genera of plant nematodes has been encountered. The most commonly found species is *Tylenchulus semipenetrans* with frequency 62 followed by *Rhabditis* spp. of frequency 58 whereas *Discolaimus* spp. has least frequency of 9.

Table 1- Frequency of plant nematodes associated with citrus

Phytonematodes	Citrus			Total frequency
	Orange	Lime	Lemon	
<i>Tylenchulus semipenetrans</i> (Juvenile)	21	17	24	62
<i>Mononchus</i> spp.	15	19	17	51
<i>Xiphinema</i> spp.	14	9	12	35
<i>Mesodorylaimus</i> spp.	9	6	14	29
<i>Iotonchus</i> spp.	16	11	8	35
<i>Meloidogyne</i> (Juvenile) spp.	18	8	10	36
<i>Rhabditis</i> spp.	11	20	27	58
<i>Dorylaimus</i> spp	12	9	21	42
<i>Discolaimus</i> spp.	3	2	4	9

Table 2: Relative and absolute frequency table of citrus nematode.

Out of the nine plant nematodes that were identified, *Tylenchulus semipenetrans* has the highest absolute frequency of 51.6% and relative frequency of 17.3%, while *Rhabditis* spp. followed closely behind with an absolute frequency of 48.3% and relative frequency of 16.2%.

Phytonematodes	Frequency	Absolute frequency (%)	Relative frequency (%)
<i>Tylenchulus semipenetrans</i>	62	51.6	17.3
<i>Mononchus</i> spp.	51	42.5	14.2
<i>Xiphinema</i> spp.	35	29.1	9.8
<i>Mesodorylaimus</i> spp	29	24.1	8.1
<i>Iotonchus</i> spp.	35	29.1	9.8
<i>Meloidogyne</i> (Juvenile) spp	36	30	10.08
<i>Rhabditis</i> spp.	58	48.3	16.2
<i>Dorylaimus</i> spp.	42	35	11.7
<i>Discolaimus</i> spp.	9	7.5	2.5

In the soil of the citrus farm, *Tylenchulus semipenetrans* is the most frequent genus followed by *Rhabditis* spp. On the other hand, *Discolaimus* spp is the least frequent genus.

Table 3: Frequency table displaying the occurrence of phytonematodes categorized by different species of citrus.

Phytonematodes	Orange (N=40)	AF (%)	RF (%)	Lime (N=40)	AF (%)	RF (%)	Lemon (N=40)	AF (%)	RF (%)
<i>Tylenchulus semipenetrans</i>	21	52.5	17.64	17	42.5	16.8	24	60	17.5
<i>Mononchus</i> spp.	15	7.5	12.60	19	37.5	18.8	17	42.5	12.4
<i>Xiphinema</i> spp.	14	35	11.76	9	22.5	8.9	12	30	8.7
<i>Mesodorylaimus</i> spp.	9	22.5	7.56	6	15	5.9	14	35	10.2
<i>Iotonchus</i> spp.	16	40	13.4	11	27.5	10.8	8	20	5.8
<i>Meloidogyne</i> (Juvenile) spp.	18	45	15.1	8	20	7.9	10	25	7.2
<i>Rhabditis</i> spp.	11	27.5	9.2	20	50	19.8	27	67.5	19.7
<i>Dorylaimus</i> spp.	12	30	10.08	9	22.5	8.9	21	52.5	15.3
<i>Discolaimus</i> spp.	3	7.5	2.5	2	5	1.9	4	10	2.9

A total of 120 soil samples were taken to detect phytonematodes in citrus plants, specifically in orange (40), lemon (40), and lime (40). Out of the three citrus species, the frequency of phytonematodes in lime was lower i.e., 101 compared to orange (119) and lemon (137).

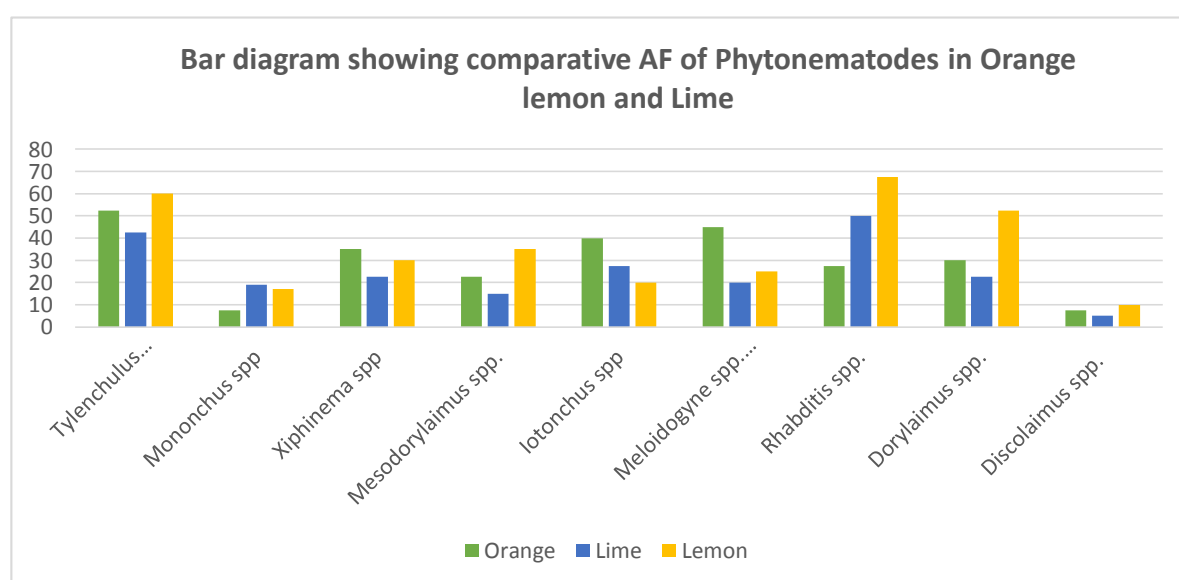


Figure 2: Comparative AF of Phyto nematodes in Orange, Lime, and Lemon

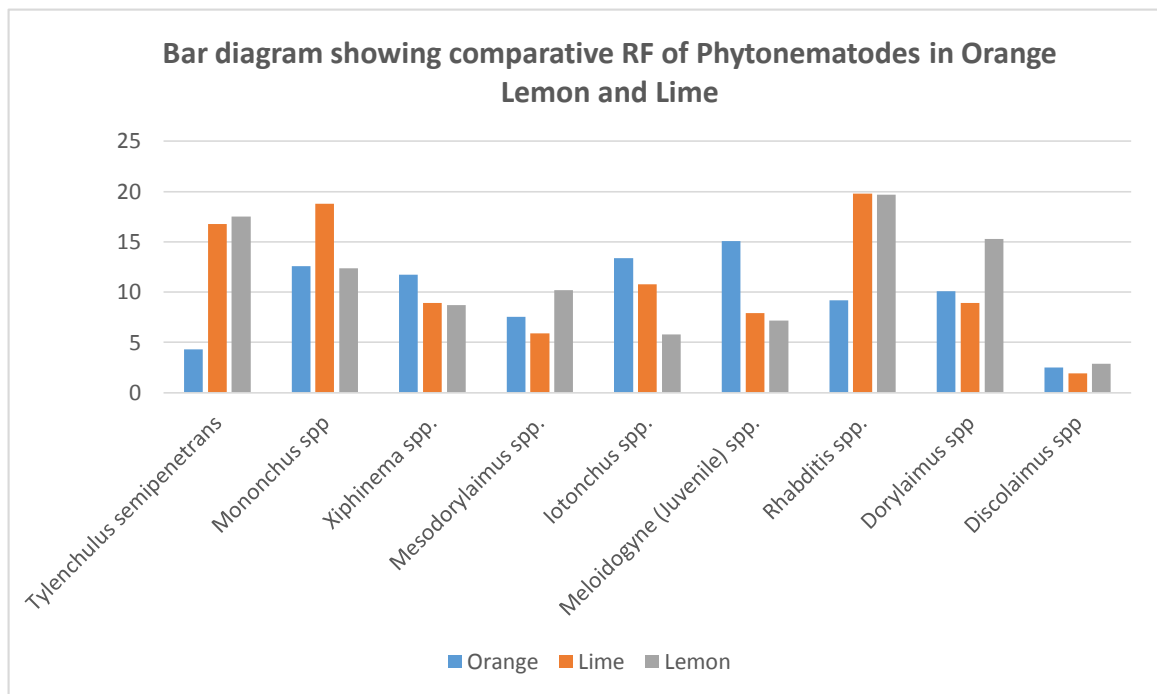


Figure 3: Comparative RF of Phyto nematodes in Orange, Lime, and Lemon

4.2 Generic Composition and Ordinal Diversity of Nematode in Soil

Out of the 9 identified genera of soil nematodes that are classified into 3 distinct orders, 4 of them (44%) were categorized as Plant Parasitic Nematodes (PPN) and the remaining 4 genera (44%) were classified as Predatory Nematodes and only one (11%) was classified as free-living nematodes (FLN) as shown in figure 4.

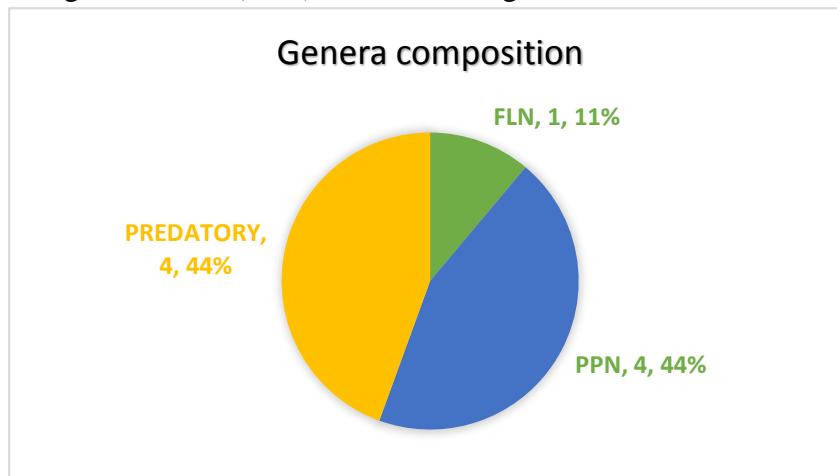


Figure 4: Pie chart showing genera composition of phytonematodes

When it comes to their taxonomic classification, the order Dorylaimida had the highest representation among the identified genera and orders, with 6 genera, accounting for 67% of the total. This was followed by the order Tylenchida, which had 2 genera and accounted for 22%, and the order Rhabditida, which had only one genus and accounted for 11%.

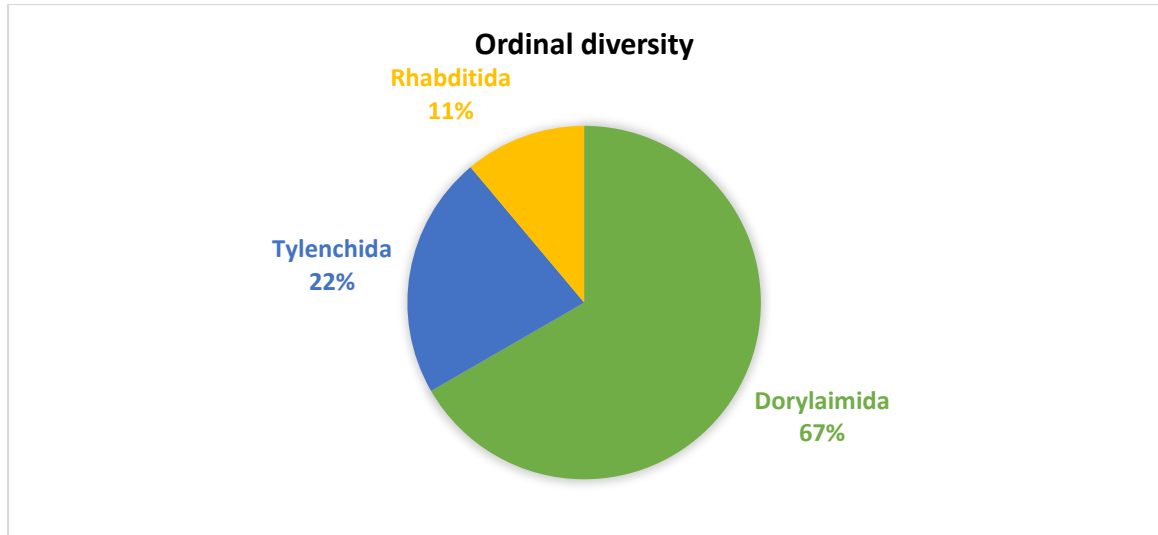


Figure 5: Pie chart showing ordinal diversity of encountered phytonematodes

4.3 Trophic diversity of plant nematodes

Table 4: Trophic diversity of plant nematode with its order and family

Phytonematodes	Order	Family	Trophic Diversity	Frequency
<i>Tylenchulus semipenetrans</i>	Tylenchida	Tylenchulidae	Parasitic	68
<i>Mononchus</i> spp.	Dorylaimida	Mononchidae	Free living and predatory	77
<i>Xiphinema</i> spp.	Dorylaimida	Longidoridae	Parasitic	42
<i>Mesodorylaimus</i> spp.	Dorylaimida	Dorylaimidae	Bacterivores	29
<i>Iotonchus</i> spp.	Dorylaimida	Iotonchusidae	Predatory	56
<i>Meloidogyne</i> spp.	Tylenchida	Heteroderidae	Parasitic	40
<i>Rhabditis</i> spp.	Rhabditida	Rhabditidae	Predatory	67
<i>Dorylaimus</i> spp.	Dorylaimida	Dorylaimidae	Parasitic, Predatory and fungivores	46
<i>Discolaimus</i> spp.	Dorylaimida	Dorylaimidae	Predatory	16

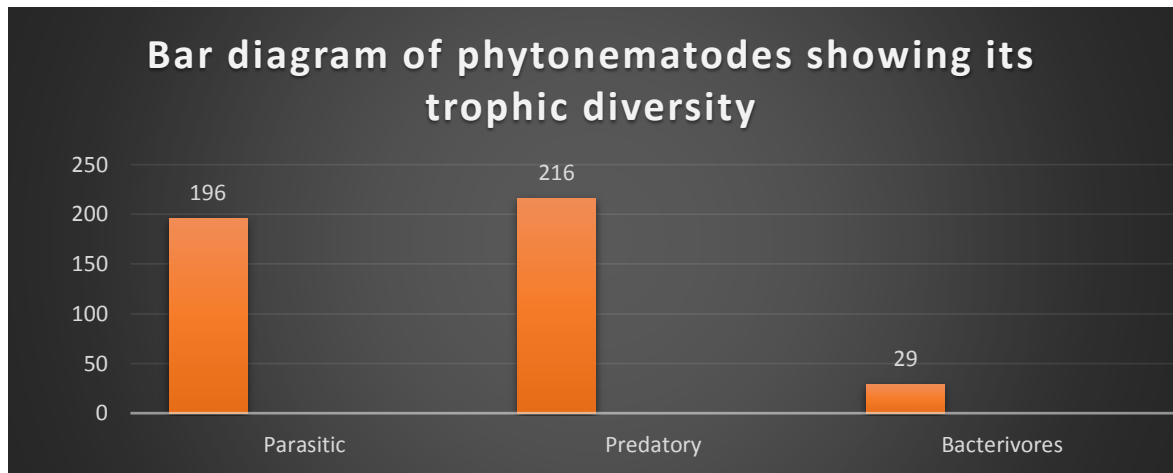


Figure 6: Bar diagram showing frequency of trophic diversity of Phyto nematodes

4.4 Soil color and pH analysis of soil samples of citrus farm

The soil in the citrus farm appeared reddish in color, which is an indication of the presence of ferric oxide. The red soil is characterized by a deficiency of nitrogen, humus, phosphoric acid, magnesium, and lime. However, it is rich in potassium and typically has a pH level that ranges from neutral to acidic (Dwevedi and Kayastha, 2017). In a similar manner, the pH values of soil samples from three different species of citrus farms were measured using a calibrated pH meter, and the average reading was noted and recorded.

Table 5: Table displaying the average pH values of soil samples taken from citrus farms.

Citrus species	Average pH value of soil samples
Orange	6.8
Lime	6.6
Lemon	6.6

The table above indicates that the pH levels of soil samples collected from citrus farms were slightly acidic. The average pH value for orange farms was found to be 6.8, while that of lime and lemon farm was 6.6. The pH levels of the soil samples collected did not appear to have any significant impact ($p > 0.05$) on the prevalence of nematodes among the various species of citrus, as indicated by the readings.

Based on the analysis of soil parameters such as pH and color, it has been demonstrated that lemon farms tend to have a higher frequency of nematodes compared to lime and orange farms. Although no significant differences ($p > 0.05$) were found between the pH and color of soil between citrus farms, the analysis did suggest that soil from lemon farms was more conducive to nematode habitat. This indicates a potential for an increase in the number of plant parasitic nematodes with continued cultivation in the same manner and with the presence of suitable hosts in the future. However, existing free-living nematodes may also assist in maintaining and regulating soil health.

5. DISCUSSION

Soil nematodes are a significant and widespread type of soil organism. However, their abundance, concentration, and diversity differ according to ecological and soil conditions, even though they can be found in different environments. (Boag and Yeates, 1998; Khatoon *et al.*, 2001). Soil mineral levels, soil type, pore space, and root secretions are additional factors that impact the overall presence and concentration of nematodes. Within this group, the plant-parasitic nematodes (PPN) can cause financial harm to agricultural crops, whereas the free-living group can serve as an indicator of soil quality and health (Ferris *et al.*, 2001).

Nematodes are a crucial component of soil and are found everywhere, making them a ubiquitous presence. They are considered to be one of the most significant elements of soil, coexisting in various ecosystems and exhibiting a wide range of diversity (Lazarova *et al.*, 2021). Nematodes play a direct role in nutrient cycling and contribute indirectly to soil decomposition, according to (Khatoon *et al.*, 2001). Nematodes, particularly the Bacterivores and Fungivores, aid in the decomposition of organic matter by accelerating the process of decomposition. This contributes to the recycling of nutrients and their return to the soil, which is beneficial for plant roots (Mussarat *et al.*, 2016). On the other hand, plant-parasitic nematodes (PPNs) are known for their ability to cause economic damage and yield loss (Keshari *et al.*, 2016).

During the study in the citrus farm of Marpak community in Dhading district, nine genera of Phytonematodes were identified, belonging to three different orders. The dominant group among these were the plant-parasitic nematodes (PPNs), with the free-living nematodes (FLNs) being less prevalent. The results of the study indicated that a higher percentage of nematodes identified belonged to the order Dorylaimida. This phenomenon has been noted in the majority of research studies conducted on various crop types worldwide. A similar finding was reported in the Ghizer district of Pakistan, where several fruit plants were found to have a higher population of phytonematodes belonging to the Dorylaimida order, which is consistent with the results of the current study (Erum *et al.*, 2021).

A study conducted in 2007 by Sen, D., Chatterjee, A., and Manna, B. investigated the levels of dorylaimids, tylenchids, and mononchids in a guava orchard located in Shalipur (West) in the Baruipur Subdivision of the South 24-Parganas district in West Bengal, India. The findings of this research were comparable to those of the current study. RKNs have been observed to affect a variety of crops, including ornamental grass in Korea (Mwamula and Lee 2021), horticultural crops in Portugal (Rusique *et al.*, 2022), banana farms in Egypt (El-Sagheer, 2020), strawberry farms in Spain (Talavera *et al.*, 2019), and citrus farms in Egypt (R.A. Bakr *et al.*, 2011). The prevailing assumption is that the presence of a host plant is the primary factor that determines the nematode diversity affecting crops (Ali *et al.*, 2018; Sabeh *et al.*, 2018; Tileubayeva *et al.*, 2021). In line with this observation, the current study focused on citrus plants and demonstrated, as also shown by Keshari and Mallikarjun 2022 and Shokoohi and Ducan 2018, that citrus plants, being the most cultivated plants, are hosts to the most common genera of RKNs – *Meloidogyne* spp. However, the present study only found the juvenile forms of

Meloidogyne, with a high incidence, which could be attributed to the earlier collection of soil samples and the soil being relatively young for cultivation.

High prevalence of the citrus nematode, *Tylenchulus semipenetrans*, in citrus farms has been reported in previous studies by Shokoohi and Ducan (2018), Keshari and Mallikarjun (2022), Ayazpour *et al.*, (2010), Kumar and Arthurs (2021), and Ducan (2005), which is consistent with the findings of the present study. However, In the present study, it was observed that only juvenile forms of *Tylenchulus semipenetrans* were present in the soil samples. This could be due to the fact that the soil was relatively young for cultivation, and the soil samples were collected at an early stage.

Ardakani *et al.*, (2014) conducted a study in Iran and found that the most suitable pH for nematode activity was around seven. An increase or decrease in soil pH resulted in a decrease in nematode population. Similarly, Zoubi *et al.*, (2022) and Herrera *et al.*, (2019) reported that the distribution of plant-parasitic nematodes was correlated with soil physicochemical properties such as soil texture and pH levels. Porazinsha *et al.*, (1998) also found that soil pH and texture significantly correlated with nematode community indices and the presence of nematode genera. However, in the present study, no relation was found between soil pH and the presence of plant-parasitic nematodes, which may be due to other factors such as temperature, moisture, and nutrient availability. Moreover, the relationship between soil pH and plant-parasitic nematodes may vary depending on the type of nematode and the availability of suitable hosts in the future with continued cultivation.

The results of the present study show that the most frequent and dominant trophic group was Predatory, followed by Parasitic, while Bacterivore was the least. This is different from the findings of other researchers such as (Mokbel, 2014), (Keshari *et al.*, 2016), and (Sikandar *et al.*, 2021), where PPNs were dominant over other groups. This difference could be due to the soil being continuously mono cropped for almost three years and the physicochemical properties of the soil. On the other hand, (Bulluck III *et al.*, 2002), (Keshari *et al.*, 2016), (Wachira *et al.*, 2009), and (Chhetri and Subedi, 2019) reported that bacterivores were more abundant than other trophic groups, which could be attributed to the use of cow manure as an organic amendment in agricultural soil and the use of organic manures and fertilizers. In addition, (Ansari and Ahmad, 2000), (Moreno and Ferris, 2007), and (Wardle and Yeates, 1993) reported more predatory nematodes than other trophic groups, similar to the findings of the present study. This could be due to the excretory products of nematodes, other organisms grazing on microbes, more microbial biomass, and the ability of predatory nematodes to switch between different food sources as needed, allowing them to persist even in variable environments.

A survey was carried out by (Bakr *et al.*, 2011) in Egypt to investigate the occurrence of citrus and other vegetable crops. The study revealed that *Meloidogyne* spp. and *T. semipenetrans* had occurrence rates of 96.26% and 85.18%, respectively, in the surveyed fields. The prevalence of *T. semipenetrans* in the present study is similar to that reported in previous studies, including those conducted by (Abivardi *et al.*, 1970) in Iran, (Edward and Rai, 1970) in Sikkim, (Philis and Siddiqi, 1976) in Cyprus, (Siddiqi *et al.*, 1987) in

Libya, (Edwards, 1989) in Northeast Victoria, (Walter and Kalplan, 1990) in Florida, (Elekcloglu *et al.*, 1995) in Turkey, (Bridge *et al.*, 1996) in Central America, (Handoo, 1998), (Divsalar *et al.*, 2011) in Gulan east and Mazadaran west, (Zalpuri *et al.*, 2013) in India, and (Ibrahim *et al.*, 2015) and (Hussain *et al.*, 2016) in Pakistan, where the highest prevalence of citrus nematode was reported.

In an earlier study, (Zaki and Mantoo, 2003) reported the existence of a predatory *Mononchus* species in fruit trees in Kashmir valley. Similarly, Keshari *et al.*, (2018) found *Mononchus aquaticus* in significant vegetable crops in Bhaktapur and Kavre districts of Nepal. Additionally, Chettri and Subedi (2019) identified *Mononchus* spp. in kiwi plants and pears (Shrestha, 2015), likewise (Baqri, 1991) also encountered *Mononchus* spp in citrus from Sikkim, India. The present study also discovered a predatory species of *Mononchus* spp. with a higher frequency of occurrence, at 42.5% AF and 14.2% RF, which is consistent with the findings of the previous studies. The current investigation found the most predatory nematode such as *Discolaimus* spp., which is consistent with the findings of previous studies by (Pervez ,2004), (Sauer and Annells, 1985), and (Walter and Kalplan, 1990). The reason for this is that they consume other organisms that reside in the soil, which have the potential to cause harm to citrus trees such as *Tylenchulus semipenetrans*, *Xiphinema* spp., *Meloidogyne* spp., and *Dorylaimus* spp.

The dissimilarities observed between the results of this study and those of previous research may be specifically caused by several factors, including the soil being relatively new and young and not having a long history of cultivation, the lack of accurate information about the field's history, the utilization of organic manures and fertilizers, the traditional practice of mono-cropping with only seasonal companion crops, and other factors that may have influenced the soil's quality and nutrient availability.

6. CONCLUSION

The study focused on the presence of plant parasitic, free-living and predatory nematodes in the rhizosphere of citrus plants such as orange, lemon, and lime in the Marpark community of Dhading district. A total of 9 genera of nematodes, belonging to 3 orders and 7 families, were identified. These included both parasitic, free-living and predatory species. They are: *Tylenchulus semipenetrans*, *Mononchus* spp., *Xiphinema* spp., *Mesodorylaimus* spp., *Iotonchus* spp., *Meloidogyne* (Juvenile) spp., *Rhabditis* spp., *Dorylaimus* spp., and *Discolaimus* spp. Among the encountered genera, *Tylenchulus semipenetrans* has highest prevalence whereas *Discolaimus* spp. is least prevalent.

Four of the nine genera of soil nematodes identified, which make up 44% of the total, were determined to be Plant Parasitic Nematodes (PPN). The other four genera, comprising 44% of the total, were classified as Predatory Nematodes and only one genus is free-living nematodes (FLN). A study discovered a varied population of plant nematodes residing in the rhizosphere, consisting of a combination of distinct groups. The nematodes identified were classified as either Parasitic, Predatory, or Bacterivores. *Tylenchulus semipenetrans*, which is the most commonly found nematode, is a parasitic species, while *Discolaimus* spp., the least frequently observed nematode, is a predatory species.

7. RECOMMENDATIONS

Absolutely, addressing the significant impact of nematodes requires the formulation of some recommendations.

- To improve the analysis of nematode prevalence, it is necessary to place greater emphasis on various soil and environmental factors.
- This research is limited to identifying the group of phytonematodes known as genera, so further research should be carried out for the identification of Phyto nematodes on species level.
- Due to the potential negative impact on citrus plant production, a comprehensive investigation of parasitic nematodes across various citrus crops is necessary.
- Performing regular laboratory tests on soil, roots, and plant tissues can aid in accurately diagnosing and identifying the prevailing nematodes. Additionally, these tests can be used to compare and validate findings with other research studies and field observations.

8. REFERENCES

- Edward, J. C. and Rai, B. B. 1970. Plant parasitic nematodes association with hill orange (*Citrus reticulata Blanco*) in Sikkim. Allahabad Farmer, **44**(4):251-254.
- Abivardi, C., Izadpanah, K., Saffarian, A. and Sharafeh, M. 1970. Plant-parasitic nematodes associated with citrus decline in southern Iran. Plant Disease Reporter, **54**(4):339-342.
- Elekcioglu, L.H., Lung, G., Ohnesorge, B. and Uygun, N. 1994. Plant parasitic nematodes in the east Mediterranean region of Turkey. Nematologia Mediterranea, **22**(1):59-63.
- Edwards, M. 1989. Resistance and tolerance of grapevine rootstocks to plant parasitic nematodes in vineyards in north-east Victoria. Australian Journal of Experimental Agriculture, **29**(1): 129 – 131.
- Divsalar, N., Jamali, S., Pedramfar, H. and Taheri, H. 2011. Identification of plant parasitic nematodes of citrus orchards in Guilan east and Mazandaran west province. Journal of plant protection (agricultural science and technology), **25**(2): 168-177.
- Stirling, G.R. 1976. Distribution of plant parasitic nematodes in South Australian vineyards. Australian Journal of Experimental Agriculture and Animal Husbandry, **16**(81): 588 – 591
- Hashim, Z. 1979. A preliminary report on the plant-parasitic nematodes in Jordan. Faculty of Agriculture - University of Jordan - Amman – Jordan, Semaloi. medii. **7**: 177-186.
- Elekcioglu, I. 1955. Plant parasitic nematodes associated with citrus in the East Mediterranean region of Turkey. Journal of Turkish Phytopathology (Turkey), **24**(1): 29-37.
- Bisheya, F., Faroogi, N., Rashid, A. and Siddiqui, Z.A. 1987. A survey of plant parasitic nematodes associated with citrus in Libya and trials on chemical control. Indian Journal of Nematology, **17**(1): 76-80.
- Sauer, M.R. 1962. Distribution of plant parasitic nematodes in irrigated vineyards at Merbein and Robinvale. Australian Journal of Experimental Agriculture and Animal Husbandry, **2**(4): 8 – 11
- Ibrahim, S. K., Ibrahim, A., Christian, Naser., Badran, A. and Ibrahim, L. 2016. Plant-parasitic nematodes on stone fruits and citrus in Lebanon. Lebanese Science Journal, **17**(1): 9-24.
- Zalpuri, L., J.S. Tara, and Singh, V.K. 2013. Prevalence of Plant Parasitic Nematodes (Citrus Species) in Various Villages of Jammu Region. International Journal of Scientific and Research Publications, **3**(6): ISSN 2250-3153.

- Bakr, R.A., Mahdy, M.E. and Mousa, E.M. 2011. A survey of root-knot and citrus nematodes in some new reclaimed lands in Egypt. *Pak. J. Nematol.*, **29** (2): 165-170
- Kaplan, D. T. 1985. Plant-parasitic nematodes affecting citrus. *Fruit Varieties Journal* **39**(3): 26-30.
- Philis, J. and Siddiqui, M.R. 1976. A list of plant parasitic nematodes in Cyprus. Agricultural Research Institute, Nicosia, Cyprus, and Commonwealth Institute of Helminthology, St. Albans, U.K., *Nematol. medit.* **4**(2): 171-174.
- J. Bridge, J., Hunt, D.J. and Hunt, P. 1996. Plant-Parasitic Nematodes of Crops in Belize. *Nematropica* **26**: 111-119.
- Walter, D.E. and Kalplan, D.T. 1990. Antagonists of Plant-parasitic Nematodes in Florida Citrus. *Journal of Nematology*, **22**(4): 567-573.
- Crozzoli, R., Greco, N., Lamberti, F. and Rivas, D. 1998. Plant parasitic nematodes associated with citrus in Venezuela. *Nematol. Medit.* **26**: 31-58.
- Burr, T.H., and Otten, L. 1999. Crown Gall of Grape: Biology and Disease Management. *Annual review of Phytopathology*. **37**:1, 53-80.
- Zhao, X. Y. 1981. Citrus yellow shoot disease (Huanglongbing) in China. *Proc. of the International Society of Citriculture*. **1**(28):466-469.
- Boina, D.R., and Bloomquist, J.R. 2014. Chemical control of the Asian citrus psyllid and of Huanglongbing disease in citrus. *Pest Manag Sci*, **71**: 808–823.
- O'Bannon, J. H., and R. P. Esser. 1985. "Citrus declines caused by nematodes in Florida. II. Physiological races." *Nematology Circular* 116.
- Kwaye, R. G., Mashela, P. W., Shimelis, H., and Mapope, N. 2008. Determination of *Tylenchulus semipenetrans* biotype in Zebediela and Champagne, Republic of South Africa. *Plant Dis.* **92**:639-641.
- Parvez, I., Mukhtar, T., and Fiaz, M. 2003. Occurrence of citrus nematode (*Tylenchus semipenetrans*) in Sargodha district. *Journal of Research (Science)*, Bahauddin Zakariya University, Multan, Pakistan, **14**(1): 91-95
- Chona B. L., Sethi, L.C., Swarup, G. and Gill, J. S. 1965. Citrus Nematode (*Tylenchulus Semipenetrans* Cobb, 1913) Associated with Die-Back Disease of Citrus Trees in India. *Indian Journal of Horticulture*, **22**(3):370-373.
- Pathak, R. K. and Chandra, J. 2010. A note on the occurrence of plant parasitic nematodes on citrus fruit trees in Doon Valley. *Plant Archives*, **10**(1): 425-428.

Nehru, R.K., Bhagat, K.C. and Koul, V. 2005. Nematode fauna associated with citrus rhizosphere in Jammu and Kashmir. *Annals of Plant Protection Sciences*, **13**(2): 524-525.

Yeates, G. W., Bongers, T., De Goede, R. G., Freckman, D. W., & Georgieva, S. S. 1993. Feeding habits in soil nematode families and genera—an outline for soil ecologists. *Journal of nematology*, **25**(3): 315.

Gundy, V. and Seymour, D. 1965. Factors in Survival of Nematodes. *Annual Review of Phytopathology*, **3**(1): 43-68.

Etebu, E. and Nwauzoma, A.B. 2014. A review on sweet orange (*Citrus Sinensis* Osbeck): health, diseases, and management. *American Journal of Research Communication*, **2**(2): 33-70.

Anonymous (2002) “Pakistan Statistical Yearbook”, Federal Bureau of Statistics, Statistical Division, Government of Pakistan.

Desar, A. 2019. Distribution of soil nematodes associated with Grapevine plant in Central Horticultural center (CHC). M.Sc. Thesis. Central Department of Zoology, Tribhuvan University, Kathmandu, Nepal.

Cobb, N.A. 1913. New nematode genera found inhabiting freshwater and brackish soils. *Journal of Washington Academy of Sciences*, **3**: 432-444.

Ezeibekwe, I. O. 2021. Prevalence of citrus disease on four citrus varieties. *International Journal of Enology and Viticulture*, **8**(1): 001- 005.

Cimen, B., & Yesiloglu, T. 2016. Rootstock breeding for abiotic stress tolerance in citrus. In *Abiotic and Biotic Stress in Plants-Recent Advances and Future Perspectives*. IntechOpen.

Bahadur, A., & Bahadur, A. (2021). Nematodes Diseases of Fruits and Vegetables Crops in India. *Nematodes - Recent Advances, Management and New Perspectives*. <https://doi.org/10.5772/INTECHOPEN.98850>

Hanada, K., Hu, Y., Pan, F., Chen, J., & Abd-Elgawad, M. M. M. (2022). Exploiting Plant–Phytonematode Interactions to Upgrade Safe and Effective Nematode Control. *Life* 2022, Vol. **12**, Page 1916, 12(11), 1916. <https://doi.org/10.3390/LIFE12111916>.

Khatoon, M., Sharma, S., and Saxena, S.K. (2001). Community analysis of predatory nematodes of Rohilkhand division. *UP. Current Nematology*, **12**, 11- 14.

Neher, D.A. (2001). Role of nematodes in soil health and their use as indicators, *Journal of Nematology*, **33**: 161- 168.

Norton, D.C. (1978). Ecology of plant parasitic nematodes. New York: John Willey and Sons. pp268.

Southey JF. Laboratory methods for working with plant and soil nematodes. HMSO, 1986.

Ferris, H., Bongers, T. and Goede, R.H.M. de (2001). A framework for soil food web diagnostics: extension of the nematode faunal analysis concept. Applied Soil Ecology, **18**, 13-29.

FAO, Food and Agriculture Organization of the United Nations. The importance of soil organic matter. Viale delle Terme di Caracalla, 00100 Rome, Italy, 2005, 64.

Čerevková, A. Diversity and Distribution of Nematode Communities in Grassland in Relation to Its Establishment Age and Utilization. Nova Science Publishers, Inc., 2011, 1-15.

Boag, B., and Yeates, G.W. (1998). Soil nematode biodiversity in terrestrial ecosystem. Biodiversity and Conservation, **7**, 617-630.

Zaki, F.A. and Mantoo, M.A. 2003. Plant parasitic nematodes associated with temperature fruits in Kashmir valley. Pest management and Economic Zoology, **1**:97-101.

Whitehead, A.G. 1998. Semiendoparasitic nematodes of root (*Helicotylenchus*, *Rotylenchulus* and *Tylenchulus*) pp 90-137 in plant nematode control. CAB International Wallingford, UK.

Yeates, G.W. and Coleman, D.C. 1982. Nematodes and decomposition. Pages 55-80 in D.

W. Freckman, editor. Nematodes in soil ecosystems. University of Texas Press, Austin, Texas, USA.

Yeates, G.W., Mercer, C.F. and Newton, P.C.D 2008. Population of *Longidorus elongatus* (Nematoda: Longidoridae) in New Zealand soils under grazed pasture. New Zealand Journal of Zoology, **35**: 287-296.

Barker, K.R. 2003. Perspectives on plant and soil nematology. Annu. Rev. Phytopathol **41**: 1-25. <https://doi.org/10.1146/annurev.phyto.41.052002.095647>.

Bhardwaj, L.N. and Hogger, C.H. 1984. Root-knot nematodes of Chitwan district of Nepal. Institute of Agriculture and Animal Science, Rampur, Chitwan, Nepal. Nematologia Mediterranea **12**: 155-158.

Chhetri, B. and Subedi, J.R. 2019. Nematodes associated with Kiwi plants in Central Horticulture Centre, Kirtipur, Kathmandu, Nepal. International Journal of Applied Agricultural Sciences **5** (3): 71-74. <https://doi.org/10.11648/j.ijaas.20190503.12>.

- Duncan, L.W. and Philips, M.S. 2009. Sampling root nematodes In Root-knot nematodes, Perry, R.N., Moens, M., and Starr, J.L. (eds.). Cab International, Wallingford, UK. Pp: 275-300.
- Ferris, H., Venette, R.C. and Scow, K. 2004. Soil management to enhance Bacterivore and Fungivore nematode populations and their nitrogen mineralization function. *Applied Soil Ecology* **25**: 19-35. <https://doi.org/10.1016/j.apsoil.2003.07.001>.
- Keshari, A.K. and Gupta, R. 2016. Five species of plant parasitic nematodes (Order: Tylenchidae) affecting vegetable crops of Nepal. *International Journal of Life Sciences*, **10** (1): 10-16
- Keshari, A.K., Shah, M.M. and Gupta, R. 2018. Community analysis of soil nematodes in vegetable growing areas of Dhading District, a high hill of Nepal. *Journal of Applied and Advanced Research* 3 (5): 156-160. <https://dx.doi.org/10.21839/jaar.v3i5.234>.
- Khatoon, M., Sharma, S. and Saxena, S. K. 2001. Community analysis of predatory nematodes of Rohilkhand division, UP. *Current Nematology*, **12**: 11-14.
- Mandal, H.R., Katel, S. and Shrestha, J. 2021. Plant parasitic nematodes and their management in crop production: A Review. *Journal of Agriculture and Natural Resources* 4 (2): 327-338. <https://doi.org/10.3126/janr.V4i2.33950>.
- Budhathoki, K. 2006. Market oriented, organic, and off-season vegetable production technology in Nepali. Shrimati Basanti Budhathoki, Lalitpur. Nepal.
- Amatya, P. and Shrestha, M. 1969. Preliminary survey of plant parasitic nematodes in Nepal. *Nepalese Journal of Agriculture*, **3**:17-28.
- Neher, D. A. 2010. Ecology of plant and free-living nematodes in natural and agricultural soil. *Annu. Rev. Phytopathol.* **48**: 371-94. <https://doi.org/10.1146/annurev-phyto-073009-114439>.
- Seinhorst, J.W. 1959. A rapid method for transfer of nematodes from fixative to anhydrous glycerin. *Nematologica*, **4** (1): 67-69.
- Shikandar, A., Zhang, M.Y., Wang, Y.Y., Zhu, X.F., Liu, X.Y., Fan, H.Y. et al. 2020. Review article: *Meloidogyne incognita* (Root-knot Nematode) a risk to Agriculture. *Applied Ecology and Environmental Research*, **18** (1): 1679-1690.
- Shrestha, R., and Bam, A.B. 2015. Isolation of plant parasitic nematodes in pear orchard at Chhaimale (Pharping) VDC of Kathmandu. *Nepalese Journal of Zoology* **3** (1): 76-81.
- Zoubi, B., Mokrini, F., Dababat, A.A., Amer, M., Ghoulam, C., Lahlali, R. et al. 2022. Occurrence and geographic distribution of plant parasitic nematodes associated with

Citrus in Morocco and their interactions with soil patterns. *Life* **12** (5): 637.
<https://doi.org/10.3390/life1205063>

Whitehead, A.G. 1998. Semiendoparasitic nematodes of root (*Helicotylenchus*, *Rotylenchulus* and *Tylenchulus*) pp 90-137 in plant nematode control. CAB International Wallingford, UK.

Tiefenbrunner, A. and Tiefenbrunner, W. 2004. Longidoridae (Nematoda:Dorylaimida) from the rhizosphere of wild growing grape (*Vitis venifera* ssp. *silvestris*) in the riparian woods of rivers Danube and March in Austria. *Helminthologia*, **41**: 45-53.

Sudershan, G., Singh, M. and Kaushal, K.K. 2002. *Xiphinema mali* sp. nov. (Nematoda: Dorylaimida) from Nepal with a compendium and Key to the species of group 3 Sensu Loof and Luc (1990) of the genus. *Indian Journal of Nematology*, **32**: 169-174.

Pandey, P. 2022. A Review on Major Diseases of citrus in Nepal and their management. *Journal of Tikapur Multiple Campus*, Vol.5. DOI: <https://doi.org/10.3126/jotmc.v5i1.46522>.

Almasco, A. Jr., Yabut, A. J., Zamora, M. J., Alvarez, L. V., Mapanao, C. P., Rendon, A. O., Zurbano, L. Y. and Bellere, A. D. et al. 2021. Morphological identification of plant parasitic nematodes found in roots and soil of pomelo (*Citrus maxima* (J. Burm.) Merr.). *International Journal of Agricultural Technology*, **17**(4):1273-1286.

Khan, M.R. 2015. Nematode Diseases of Crops in India. In: Awasthi, L.P. (eds) *Recent Advances in the Diagnosis and Management of Plant Diseases*. Springer, New Delhi. https://doi.org/10.1007/978-81-322-2571-3_16.

Baghel, P. P. S and Bhatti, D. S. 1981. Distribution of phytonematodes associated with citrus. *Indian Journal of Nematology*, **11**(1): 113-114.

Sharma, S. B. and Chawla, M. L. 1981. Population estimation of citrus nematode, *Tylenchulus semipenetrans* Cobb, 1913. *Indian Journal of Nematology*, **11**(1): 132-133.

Ravichandra, N.G. 2014. Phytonematodes: Threat to Horticulture. In: *Horticultural Nematology*. Springer, New Delhi. https://doi.org/10.1007/978-81-322-1841-8_2.

Khan, A., Shaukat, S.S. and Khatoon, N. 2021. Phytonematodes associated with peach (*Prunus persica* L.) seedlings in Balochistan, Pakistan. *Pakistan Journal of Nematology*, **39**(1): 93.

Abu Habib, A. H. A., Younes, H. A., Ibrahim, I. K. A., and Khalil, A.E. 2020. Plant Parasitic Nematodes Associated with Citrus Trees and Reaction of Two Citrus Cultivars to *Tylenchulus semipenetrans* in Northern Egypt. *Journal of the Advances in Agricultural Research*, **25**(2): 166-175

Mekonnen, M., Seid, A., Ayalew, A., and W/Tsadid, K. 2013. Distribution of Citrus Nematode (*Tylenchulus semipenetrans* Cobb, 1913) in Major Citrus (*Citrus sinensis* L.) Growing Areas of Ethiopia. *Journal of the Advances in Agricultural Research*, **5**(2): 166-175.

Verdejo-Lucas, S., Mc Kenry, M. V. 2004. Management of the citrus nematode, *Tylenchulus semipenetrans*. *Journal of nematology*, **36**(4): 424-432

Irshad, U., Mukhtar, T., Ashfaq, M., Kayari, M.Z., Kayani, S.B., Hanif, M. and Aslam, S. et al., 2014. Pathogenicity of citrus nematode (*Tylenchulus semipenetrans*) on citrus jambhiri. *The Journal of Animal & Plant Sciences*, **22**(4): 1014-1018.

Bam, A.B. 2005. Screening of tomato germplasm to the *Meloidogyne*: a root- knot nematode. M.Sc. Thesis. Central Department of Zoology, Tribhuvan University, Kathmandu, Nepal.

Fattah, F.A., Saleh, H.M. and Aboud, H.M. 1989. Parasitism of the Citrus Nematode, *Tylenchulus semipenetrans*, by *Pasteuria penetrans* in Iraq. *Journal of Nematology*, **21**(3): 431-433.

Kaplan, D.T., and Verdejo- Lucas, S. 2002. The citrus nematode: *Tylenchulus semipenetrans*. Plant resistance to parasitic nematodes <https://doi.org/10.1079/9780851994666.0207>.

Gurung, S., Adhikari, P., Giri, K., Gotame, T. P., & Shrestha, S.L. 2020. Growth and yield performance of hybrid tomato (*Solanum lycopersicum* L.) lines at parwanipur, Bara, Nepal. *Journal of Agriculture and Natural Resources* **3**(1): 180-189. Online Retrieved from: <https://doi.org/10.3126/janr.v3i1.27166>.

Ibrahim, A.A.M. and Al-Yahya, F.A. 2002. Phyto-parasitic nematodes associated with ornamental plants in Riyadh, Central Saudi Arabia. *Alexandria Journal of Agricultural Research* **47** (3): 157-167.

Iqbal, S., and Jones, M.G.K. 2017. Nematodes. In *Free-living Nematodes*. Encyclopedia of Applied Science (second edition), Vol.3, Pg: 113-119. Online available at: <https://doi.org/10.1016/B978-0-12-394807-6.00061-7>.

Keshari, A.K. and Gupta, R. 2016. Five species of plant parasitic nematodes (Order: Tylenchidae) affecting vegetable crops of Nepal. *International Journal of Life Sciences* **10** (1): 10-16.

Maharatha,S., Dahal, B.R., Acharya, N. and Devkota, S. 2019. Price behavior, marketing, and consumption pattern of tomato in selected region of Western Nepal. *Archives of Agriculture and Environmental Science* **4** (4): 472-477. Online available at: <https://doi.org/10.26832/24566632.2019.0404014>.

Tarjan, A. C. 1961. Two new American dagger nematodes (*Xiphinema*: Dorylaimidae) associated with citrus, with comments on the variability of *X. bakeri* Williams, 1961. Proceedings of the Helminthological Society of Washington, **31**(1): 65-76.

Siddiqi, M.R.1964. Studies on *Discolaimus* Spp. (Nematoda: Dorylaimidae) from India. Online available at: <https://doi.org/10.1111/j.1439-0469.1964.tb00720.x>.

Nurdahlia, S., Muhamat and Gaful, A. 2016. Nematoda mononchid dari kebunjerukdi kabupaten banjar, kalimantanselatan. Bioscientiae, **13**(1): 11-18

Baqri, Q. H.1991. Contribution to the fauna of Sikkim. Nematodes associated with citrus from Sikkim, India. Records of the Zoological Survey of India, **38**: 103-128.

Taylor, C.E., Brown, D.J.F. and Neilson, R. 1994. The persistence and spread of *Xiphinema diversicaudatum* in cultivated and uncultivated bio topes. Annals of Applied Biology, **124**: 467-477.

Ali, T., Mubeen, M., Jamil, Y., Ahmed, U., Khan, H.A., Iqbal, S., et al. 2021. An overview of root-knot nematode and their management. Journal of Entomology and Zoology Studies **9** (1): 35-40.

Al-Yahya, F. A. 2018. Plant parasitic nematodes: a serious threat to agricultural crops in Saudi Arabia. Journal of Experimental Biology and Agricultural Sciences **6** (3): 628-632. [https://dx.doi.org/10.18006/2018.6\(3\).628.632](https://dx.doi.org/10.18006/2018.6(3).628.632).

Smart, G.C., Jr. and Nguyen, K.B.1988. Identification of Common Nematodes in Florida. Institute of Food and Agricultural Sciences, Gainesville, Florida, 90pp.

Wu, H.Y. and Shi, L.B. 2012. Effects of continuous cropping durations on population dynamics of second stage juvenile *Meloidogyne* spp. and free-living nematodes. 51African Journal of Agricultural Research **6** (2): 307-312. Online available at: <https://doi.org/10.5897/AJAR10.402>.

Schmitt, D. P., and Siper, B. S. 2000. Plant parasitic nematodes and their management. Plant Nutrient Management in Hawaii's soil, Approaches for tropical and sub-tropical Agriculture. J.A. Silva and R. Uchida eds. College of Tropical Agriculture and Human Resources, University of Hawaii at Manoa. Pp 145-149.

Kanga, F.N., Waeyenberge, L., Hauser, S., Moens, M. 2012. Distribution of entomopathogenic nematodes in Southern Cameroon. Journal of Invertebrate Pathology, **109**(1): 41-51.

Baermann, G. 1917. Eine einsache method zur Auffindung von Ankylostomum (Nematoden) larven in Erdproben. Geneeskd Tijdschr Ned – Indie, **57**: 131-137

Pervez, R. 2004. Prevalence of plant parasitic and predatory nematodes associated with different crops of Badaun districts, (Uttar Pradesh). *Current Nematology*, **15**(11): 43-46

Sauer, M. R. and Annells, C.M. 1988. Species of *Discolaimus* (Nematoda: Dorylaimoidea) from Australia. *Nematologica*, **31**: 121-133

9. PHOTOPLATES

Photograph 5: Mobile photography of phytonematodes from collected samples.



Discolaimus spp (10X)



Anterior part (40X)



Posterior part (40X)



Xiphinema spp(10X)



Anterior part(40X)



Posterior part(40X)



Rhabditis spp (10X)



Anterior part (40X)



Posterior part (40X)



Tylenchulus semipenetrans Juvenile stage, Anterior and posterior part (10X and 40X)



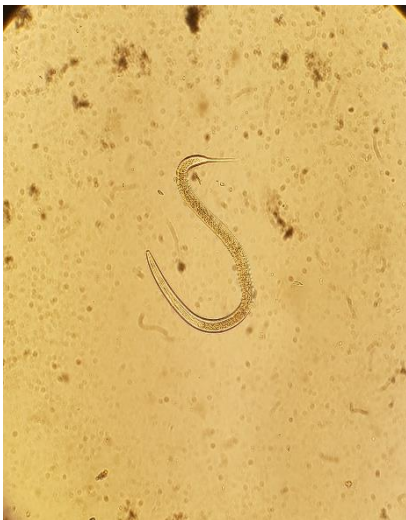
Mononchus spp., Anterior and posterior part (10X and 40X)



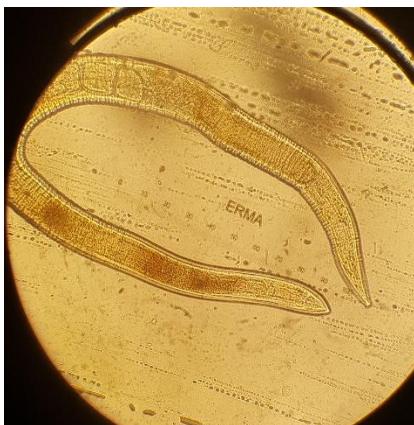
Meloidogyne spp. Juvenile stage, Anterior and posterior part (10X and 40X)



Iotonchus spp. Anterior and posterior part (10X and 40X)



Mesodorylaimus spp. Anterior and posterior part (10X and 40X)



Dorylaimus spp. Anterior and posterior part (10X and 40X)