

**PREVALENCE OF NEMATODES IN RHIZOSPHERE OF TOMATO
(*Solanum lycopersicum* LINNAEUS, 1753) PLANTS IN TWO
DIFFERENT HIGH TUNNEL FARMS IN DAHACHOWK,
KATHMANDU, NEPAL**



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Central Department of Zoology

Institute of Science and Technology

Tribhuvan University

Kirtipur, Kathmandu, Nepal

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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 sources of information have been specifically acknowledged by reference to author/s or institution.

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RECOMMENDATION

This is to recommend that the thesis entitle “Prevalence of Nematodes in Rhizosphere of Tomato (*Solanum lycopersicum* Linnaeus, 1753) Plants in Two Different Tunnel Farms in Dahachowk, Kathmandu, Nepal” has been carried out by Ms. Deepa Acharya 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 has not been submitted for any other degree in any institution/s.

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On the recommendation of supervisor **Mr. Janak Raj Subedi**, this thesis submitted by **Ms. Deepa Acharya** entitled "**Prevalence of Nematodes in Rhizosphere of Tomato (*Solanum lycopersicum* Linnaeus, 1753) Plants in Two Different Tunnel Farms in Dahachowk, Kathmandu, Nepal**" is approved for the examination and submitted to Tribhuvan University in the partial fulfillment of the requirements for Master's Degree of Science in Zoology with special paper Parasitology.

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CERTIFICATE OF ACCEPTANCE

This thesis submitted by Ms. Deepa Acharya entitled "Prevalence of Nematodes in Rhizosphere of Tomato (*Solanum lycopersicum* Linnaeus, 1753) Plants in Two Different Tunnel Farms in Dahachowk, Kathmandu, Nepal" has been accepted as the partial fulfillment for the requirements of Master's Degree of Science in Zoology with major Parasitology.

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LIST OF ABBREVIATIONS

PPN: Plant Parasitic Nematode

RKN: Root-Knot Nematode

RLN: Root Lesion Nematode

FLN: Free-Living Nematode

FAO: Food and Agriculture Organization

FAOSTAT: Food and Agriculture Organization Statistics

NARC: National Agricultural Research Council

MoAD: Ministry of Agricultural Development

ICAR: International Centre for Agricultural Research

RARS: Regional Agriculture Research Station

GI: Galvanized Iron

ToMV: Tomato Mosaic Virus

CMV: Cucumber Mosaic Virus

TYLCV: Tomato Yellow Leaf Curl Virus

RCBD: Randomized Complete Block Design

HRD: Horticulture Research Division

RA: Relative Abundance

ABSTRACT

The present study was conducted to determine nematode prevalence in tomato plants in two monoculture tunnel farms in Dahachowk. A total of 40 soil samples were collected from November-January at the time of harvest and subjected to Baermann set up for nematode isolation and extraction. The study reported occurrence of both parasitic and free living nematodes. 9 nematode genera belonging to 3 orders were identified. Among them 56% were parasitic and 44% were free living. Among parasitic nematodes, 22.9% *Meloidogyne* juvenile, 20.84% *Tylenchorhynchus*, 12.99% *Helicotylenchus*, 11.88% *Hoplolaimus* and 0.5% *Discocriconemella* were encountered while among free living, 27.19% *Rhabditis*, 1.91% *Dorylaimus*, 1.4% *Eudorylaimus* and 0.35% *Filenchus* were reported. In general, *Meloidogyne* juvenile and *Rhabditis* was the most frequent parasitic and free living genus with 22.96% and 27.19% composition while *Discocriconemella* and *Filenchus* were the least frequent parasitic and free living genus with 0.5% and 0.3% composition in both tunnels. Also soil samples from both tunnels were found to be acidic with average pH of 4.6 and 4.1 while average moisture measured were 93.22% and 77.42% for both tunnels. No significant differences ($p > 0.05$) were observed in nematode distribution from both tunnels in relation to depth, pH and moisture contents. However, findings suggested mixed nematode diversity that tunnel 2 to having more nematode prevalence than tunnel 1 showing phyto-pathogenic tendencies for hybrid mono-culture practice. Therefore, regular researches on particular criterion can unfold hidden secrets on nematode prevalence for the region.

1. INTRODUCTION

1.1 Background of the study

Tomato (*S. lycopersicum*) is also known as poor man's apple in Nepal and belongs to Solanaceae family. The family comprises some of the most economically important flowering plants like potato, tomato, eggplant and peppers, constituting the major horticultural production in the world (Morris and Taylor 2016) and are frequently grown in poly-house. It is the third most important vegetable in terms of area and production in Nepal (MoAD 2015/16). Tomato is herbaceous, warm-season sprawling plant growing to one-three meters high with weak woody stem and yellow flowers and variable sized fruit known as an excellent source of vitamin minerals and organic acids often used as raw vegetable, salad, pickle, syrup, ketchup etc. (Maharjan 2019).

World contributed average production of 37.46 tons/h (Ghimire et al. 2018). China is the largest producer of tomatoes in the world (FAOSTAT, 2019). Recently tunnel farming is adapted for their off-seasonal cultivation where more than 90 countries have opted for cultivation of vegetables, flowers, and fruits contributing the maximum share (Dutta et al. 2021). In Nepal, tomato has been one of the high yielding crops with 400,674 ton productions and average productivity of 19t/ha (Gurung et al. 2020). Changes in consumption pattern and escalating market demand resulted in increased production (Maharatha et al. 2019). Thus, tomato cultivation is known to have higher prospects with better management and channelization (Rajkarnikar and Shrestha 2021).

1.2 Overall Nematode Burden

Nematodes are found everywhere, in all habitats, particularly abundant in marine, freshwater and soil habitat but often over-looked because of their microscopic size, colorless appearance, remains hidden in soil and water, parasitize hosts and causes few direct impacts on human and other animals. Some scientists' estimated over one million nematodes kind present on Earth. Of all, about 50% are small living in marine, 25% in soil or fresh water and feeds on bacteria, fungi and spros while only 10% are parasites of plants (McSorley 2019). Nematodes may be useful indicator of soil quality because of their tremendous diversity and their participation in different levels of soil food web (Blair et al. 1997). Soil has been

excellent habitat for nematodes and has potential to cause great economic damages to cultivated crops imposing severe global threats in cultivation however not all nematodes are plant parasites but are beneficial in decomposition of organic matters, often referred as free-living nematodes (Abd-Elgawad and Askary 2015). Soil nematodes are a group of diverse functional and taxonomic type known to have evolved ability to detect and respond to soil chemical complexity cues for successful foraging (Rasmann et al. 2012). Plant Parasitic Nematode (PPN) are small worm-like transparent, bilaterally symmetrical, multi-cellular, pseudo-coelomate, free-living or parasitic or symbiotic micro-organisms that are predatory, aquatic or terrestrial, ecto-parasite or endo-parasite or semi-endoparasite (e.g. *Tylenchulus semipenetrans*) or sedentary in nature (Shah and Mahamood 2017; Mandal et al. 2021) reported to cause loss of billions of dollars annually in crops worldwide (Tileubayeva et al. 2021). PPNs are called plant parasites because they receive nutrients directly from plants as they bear “stylet” that helps them to pierce cell wall and feed upon the cell contents (Bernard et al. 2017; Tileubayeva et al. 2021).

Over the course of time, PPNs have been major serious pathogens known to attack several vegetable crops in both open-field and greenhouses world-wide (Al-Yahya et al. 2018) impacting on both quality and quantity of crop yield and hence known to be important yet dangerous and notorious pests of all sorts of crops worldwide (Krif et al. 2020). Nearly 4100 reported PPN species are known as serious constraint for global food security having caused projected 12.3% yield loss annually to global food production, let alone 10 billion USD losses annually in the US (Hassan et al. 2013). Several PPNs genera have been reported to be associated as greatest threat to crops worldwide, including *Meloidogyne*, *Scutellonema*, *Trichodorus*, *Pratylenchus*, *Tylenchorhynchus*, *Helicotylenchus*, *Criconemoides*, *Tylenchus*, *Tylenchulus*, *Xiphinema*, *Rhabditida*, *Paratylenchus*, *Aphelenchus*, *Heterodera*, *Rotylenchus*, *Criconemella*, *Belonolaimus*, *Hoplolaimus*, *Longidorus* and *Hemicycliophora* (Khan et al. 2019; Krif et al. 2020; Nyaku et al. 2021; Tileubayeva et al. 2021; Ramadhani et al. 2022) of which *Meloidogyne* spp. supposedly has more than 90 described species (Almohitthef et al. 2018; Pehlivan et al. 2020; Tileubayeva et al. 2021) with more than 3000 host species (Mokbel 2014). Root Knot Nematode (RKN, *Meloidogyne* spp.), Root Lesion Nematode (RLN, *Pratylenchus* spp.) and Cyst nematode (*Heterodera* spp.) has global distribution and

implicates significant economic loss (Abd-Elgawad and Askary 2015). RKNs single has been the most devastating pests for almost every horticultural and ornamental plant in most part of the world (Tileubayeva et al. 2021). These nematodes can influence the forage and inter-cropped agricultural crops from the establishment up to harvest stages (Avelino et al. 2019). In addition to host plants, several other factors like environment, temperature, rainfall, soil substrate, texture, moisture, pH and nutrient content have known to be widely affected by their prevalence and diversity (Fleming et al. 2016). Changes in soil conditions have however have been reported to have direct and indirect effects on soil nematodes and community structure that plays an important role in indicating changes in soil environment and their stability (Zheng et al. 2012).

Free-living Nematodes (FLN) are also microscopic worms found in all sorts of soil but reported to cause no harm to plants but are portrayed as the beneficial organism to crops (O'Callaghan et al. 2018) and also very vital and beneficial in decomposition of organic materials and recycling organic soil nutrients making them accessible back to the plant roots and enhancing the plant productivity (Zheng et al. 2012; McSorley 2019) and therefore regarded as the most beneficial secondary consumer in an ecosystem (Mulder et al. 2005). Herbivore FLN feed on plant roots by injecting stylet into root tissues making them susceptible to stress, lesions, access to other pests like bacteria or fungi to causing significant crop damage by transmitting virus which has been reported in potatoes known to transmit Tomato blacking virus and Tobacco rattle virus (O'Callaghan et al. 2018). Different genus and species of nematodes have economic importance as they are known as the important biological pests of crops mostly with mixed occurrence with variable host range from weeds to vegetable crops usually vegetable and field crops as the prime host (Noling 2016).

Most nematodes in soil are not plant parasites instead help in disease control and nutrient cycle. Bacterivore feed on bacteria, Fungivore feed on fungi by consuming their sap while predatory nematodes eat all types of smaller nematodes and protozoa or attach to themselves to cuticle of larger nematodes scraping away until prey's internal body parts can be extracted (Ingham 2021). *C. elegans*, a Bacterivore model nematode has distinction of being first multi-cellular organism to have its entire genome sequenced and annotated and serves as

basis for several molecular researches, *Aphelenchus*, a fungivore nematode, is commonly found in soil and moss, *Mononchus*, a predatory nematode, usually preys upon other nematodes and soil-dwelling creatures using its large tooth (Iqbal and Jones 2017).

1.3 Tunnel House in Nepal

Tunnel house is an infrastructure based technology that facilitates crop production for an extended period and is comparatively new to Nepal than other developed countries (K.C et al. 2021). In Nepal, tunnel house was first introduced in 1996 by Regional Agriculture research Station- Lumle (Kafle and Shrestha 2017; K.C et al. 2021). The technique is simple and low cost and offers protection to crops in all seasons, yields maximum, maintains land fertility, controls temperature and humidity, protects from wilds and insects and ensures water conservation (FAO 2013). The tunnel structure generally consists of bamboo or galvanized pipe (GI) framework, covered with transparent silpaulin plastic, usually 45-90 gsm equipped with drip irrigation (KC et al. 2021). Crops like cucumber, melons, capsicum, tomato, pepper, bitter gourd, eggplant are highly valued and show significant increase in yield (Budhathoki 2006). Several districts have adopted tunnel farming practice since it is promising technique and requires smaller land areas showing bumping yield and earning annually (Lamichane et al. 2018).

Hybrid varieties are preferably grown in tunnel as it allows farmers to production expansion period and hereby increases the productivity (Simkhada and Pokharel 2012). “**Srijana**” is a Nepali hybrid variety developed by NARC (National Agriculture Research Council), widely cultivated in tunnels for its adaptability including off-season production, superior taste and tolerance to bacterial wilt disease and hence is getting popular amongst farmers (Chapagain et al. 2011; Thapa Magar et al. 2016) and also the popular variety registered by National Seed Board with effort of NARC in 2010 (MoAD 2013; NARC 2014b). This variety has been highly recommended for tunnel farming as it is identified as the potential crop for agricultural benefits (Pokharel and Thakur 2012; Thapa Magar et al. 2016).

However, tomato production is most likely hampered by biotic stress like RKN posing major threat in production (Gowda et al. 2020). Moreover, crop damages from nematodes are not readily apparent in most of the cases as it often remains hidden or over-shadowed by many other factors limiting the plant growth (Schmitt and Sipes 2000).

Following the same, *Meloidogyne* spp. are microscopic, sedentary, obligatory and microscopic endo-parasitic soil inhabiting roundworm that are most susceptible to tomato plants whose first occurrence in Nepal was reported by Amatya and Shrestha (1996) on tomato, okra, egg-plant and chilly from Illam, Pokhara and Yugapuri, characterized by presence of characteristic root galls/swelling usually found in clusters. Above ground symptoms includes stunted plant growth, chlorosis and wilting of plant (Schwarz and Gorny 2020). Nematodes of genus *Meloidogyne* are considered dangerous parasitic pathogens of cultivated and wild plants reducing 60-80% yields (Saidova et al. 2020). Other nematodes like *Rotylenchulus reniformis* (Usman and Siddiqui 2012) *Pratylenchus* spp., *Helicotylenchus* spp. and *Hoplolaimus* spp. have also been reported to attack tomato plant extensively (Kavitha and Das 2016). Moreover, nematodes are familiar with all soil types and environment worldwide (Mandal et al. 2021).

Continuous and Multi-cropping have been the most common and traditional agricultural practice in most of parts of world including Nepal and China. Continuous cropping for a long term has led to significant declination in soil pH, organic matter contents, enzymatic activities and soil properties (Xiong et al. 2015). Many crops grown together under the same roof sharing the same soil and resources by small farm holders' having limited resources have adapted to integrated continuous multi-cropping system so as to overcome poverty, longing for better earnings and livelihood with the aim to improve nutrition and poverty in developing world (Francis and Porter 2017).



Photograph 1: Framework of Tunnel for tomato cultivation in Chandragiri Municipality.

1.4 Rationale of the Study

Tomato has been extensively grown in tunnels along with several other companion crops spinach, beans etc. in Thankot continuously. Hybrid variety of tomato, Srijana is grown by almost every tunnel farmers throughout the year. Due to lack of local information on prevalence of soil nematodes, the main focus of this study will be to emphasize nematode prevalence in the rhizosphere soil collected within various depths of tomato plants. Also this study will account for evaluation of parasitic and free-living nematodes in the two tunnel houses. This study is expected to light on impacts of nematodes on tomato cultivation.

1.5 Research Objectives

→ General Objective:

- ✖ To explore nematode prevalence in rhizosphere of tomato plants during early winter.

→ Specific Objectives

- ✖ To analyze both free living and parasitic nematodes present in the soil.
- ✖ To determine the nematode prevalence with relation to their depth of collection.

1.6 Limitations of the Study

The study however had few limitations. They were:

- ❖ The study period was pretty much limited.
- ❖ Only rhizosphere soil samples were collected for the study excluding root samples.
- ❖ Only two tunnels were selected for the study purpose under the given time frame.

2. LITERATURE REVIEW

There is large scale diversity in soil nematodes and thereby are the most numerous soil animals. Because of their microscopic size and complex extraction from soils, they are seldom studied and taxonomic studies tended towards significant plant parasites (Huang and Cares 2006; Myint et al. 2017). Like several other pathogens, plant nematodes particularly have been focused recently. Because plant parasites are obligatory and soil inhabiting parasites, their documentation on pathogenicity experimentally involves long term challenges (Barker 2003). These PPNs are among important and abundant animals in all parts of world and widely known to attack all types of plants including vegetables, fruits, ornamental and medicinal plants and causes farmers and nurserymen severe loss and have ability to survive in any environment and often known as invisible enemies of crops (Neher 2010; Coyne et al. 2018; Ndava et al. 2018). It is also very important to know that RKNs have heterogeneous spatial distribution (Duncan and Philips 2009). Therefore, RKNs have been tagged as the most devastating PPN in the world (Sikandar et al. 2020; Tileubayeva et al. 2021).

Bernard et al. (2017) addressed the significant impacts of PPNs on every agricultural crops and global food security. Phyto-parasitic nematodes ranks as Root-knot Nematodes (*Meloidogyne* spp.); Cyst nematodes (*Heterodera* and *Globodera*) and lesion nematodes (*Pratylenchus* spp.) as top of list of the most economically and scientifically important species due to their intricate relationship with host plants, wide host range and level of damage caused by infections. Also chemical pesticides focused as alternative nematode control method showing identification and implementation of host resistance. Jones et al. (2013) too listed root knot, cyst and root lesion nematodes as the top three nematodes of scientific and economic importance. Another nematological survey by Fahad and Al-Yahya (2018) mentioned RKNs, cereal cyst nematode and citrus nematode as the most dangerous nematode pests to agricultural crops in Saudi Arabia. Similarly, Pehlivan et al. (2015) in their journal identified *Globodera rostochiensis* and *Meloidogyne chitwood*, *M. hapla*, *M. incognita* and *M. javanica* in potato in Turkey by morphological and molecular methods respectively.

Bhardwaj and Hogger (1984) reported three species of RKN (*Meloidogyne* spp.) identified to be *M. incognita*, *M. javanica* and *M. arenaria* associated with 26 commercially important crops in Chitwan district of Nepal. Later, Sharma and his associates (2007) in Indian Agriculture Research institute observed high intensity of RKN (*M. incognita*) infestation in tomato and cucumber despite management practices under poly-house cultivation. Likewise, Singh et al. (2019) documented *M. incognita*, *R. reniformis*, *H. indicus*, *T. indicus*, *T. brassicae*, *Pratylenchus* spp., *Helicotylenchus* spp., *Xiphinema* spp. and *Longidorus* spp. from nineteen vegetable crops reported to cause 30% yield loss. Furthermore, the root knot and cyst nematodes have been economically important pests in numerous crops (Mandal et al. 2021). Supporting the fact, Rusinque et al. (2021) also reported *Meloidogyne* as the most widely distributed pests known to cause economic damages in larger number of greenhouse and field plants worldwide in their extensive research survey in *Meloidogyne* spp. in an island molecularly identified as *M. incognita*, *M. arenaria* and *M. javanica* and *M. incognita* was the most common one found to be the most prevalence. Singh and Kumar (2013) also revealed highest number of RKN, *Meloidogyne incognita* from soil sample of sponge gourd and tomato along with other ecto-parasites like *Tylenchorhynchus* spp., *Pratylenchus* spp. and *Hoplaimus* spp. and semi-endoparasitic nematode like *Rotylenchulus reniformis* on cucurbits and capsicum in Delhi, India. Another survey by Anwaret al. (2013) on four different crops under tunnel revealed nine species of plant parasitic nematodes, *M. incognita* and *M. arenaria* as the most common in all crops. Similarly, a survey by Singh and Khanna (2015) also documented alarming prevalence of *M. incognita* from India under protected cultivation. Almothithet et al. (2020) and Tileubayeva et al. (2021) investigated PPNs that affect greenhouse vegetables in Rostov and Saudi Arabia and documented *Meloidogyne* as the most observed genus in all sampled crops followed by *Helicotylenchus*, *Pratylenchus* and *Globodera* in each sampled crops.

In a purposive experiment on ‘Srijana’ variety by Shrestha et al. (2018), eight treatments were laid in RCBD and replicated thrice and concluded that treatment with ½ RDC with 12.5 ton/ha compost performed better over other treatments so can be recommended to for commercial tomato producers but for farmers adopting organic farming with 15ton/ha compost with fermented CU was the best for plastic tunnel tomato farming as well as improves soil fertility. Later, Maharjan (2019) further stated necessity of off season tomato cultivation in Nepal and

use of ‘Srijana’, a hybrid for their best agricultural and economical properties. Another experiment carried out by Gurung et al. (2020) to evaluate growth and yield performance of hybrid tomato lines at RARS laid in RCBD with 7 hybrid tomato lines as treatments and 3 replications confirmed ‘Srijana’ as the superior variety in terms of yield attributing characters like marketable fruit per plant, total fruit yield and for several qualitative parameters like TSS and hence was recommended in Bara District.

An article published by FAO in the year 2013 under FAO Strategic Objectives 5, also stated necessities and positive outcomes of agricultural practice of growing crops under tunnel farms for providing finances for household women and ensures food supply to local farmers at household level. Later, following the cases, Rayemajhy et al. (2020) also mentioned the risen use greenhouse/tunnels for growing vegetable crops in recent decades with documentations on various types of greenhouse technologies adopted in Nepal depending upon latitude of site of construction, light, temperature, relative humidity, soil quality and crop requirements. Study showed use of: Low Plastic Tunnels; Bamboo greenhouse tunnels; Shade Net House; Insect-proof net house; fan-and-cooling system greenhouse and Greenhouses with several formulated government policies and programs for promotion of greenhouse technology/protected cultivation in Nepal. The following year, K.C. et al. (2021) stated the increased practice of tunnel farms in many parts of Nepal as a tool to enhance livelihood by production of agricultural crops among smallholder farmers and tackle climatic variations hereby aid in better livelihood.

Alvani et al. (2016) recorded four new plant parasitic nematodes in Barberry (*Berberis vulgaris*) identified to be *Merlinus acuminatus*, *Geocenamus dobroticus*, *Paratylenchus vandenbrandei* and *Criconemoides morgensis* from Iran. Along with this, Keshari and Gupta (2016) reported five species of PPNs for the first time in Nepal and identified to be *Hoplolaimus indicus*, *Tylenchorhynchus mashoodi*, *Helicotylenchus incisus*, *Microposthonia paraxestis* and *Hemicriconemoides cocophilus* from agricultural crops in three hilly districts of Nepal. Another research by Fleming et al. (2016) in grassland and cereals in Northern Ireland reported *Meloidogyne* spp., *Heterodera* spp. and *Pratylenchus* spp. above the economic threshold in both sites. Variations in findings where *Rhabditis* as the most abundant genus among FLNs and *Helicotylenchus* among PPNs but *Meloidogyne* as the most dominant genus

in terms of frequency and density have been documented by Keshari et al. (2018) from soil samples sampled among vegetable crops from Nepal. However, Askary (2017) reported *Meloidogyne*, *Heterodera*, *Pratylenchus*, *Rotylenchulus*, *Tylenchorhynchus* and *Helicotylenchus* spp in pulses like chick pea, pigeon pea, common bean, mug bean, urd bean and lentils.

In the first extensive survey by Ibrahim and Al-Yahya (2002), twenty phyto-parasitic nematode genera from ornamental plants were reported of which, *Helicotylenchus*, *Tylenchorhynchus* and *Meloidogyne* were the most prevalent one in general with new host records for RKN in Saudi Arabia. Another very first Indonesian research by Kurniawati and Kumala (2021) reported six genera of phytonematodes identified as *Meloidogyne*, *Helicotylenchus*, *Hoplolaimus*, *Criconemoides*, *Rotylenchus* and *Xiphinema* from Guava root samples. Similarly, Luquini and Amorim (2019) reported frequent occurrence of *Helicotylenchus multicinctus* (87%), *Rotylenchus reniformis* (63%), *M. incognita* (41.5%), *M. arenaria* (37%), *M. javanica* (18%), *Pratylenchus* spp. (3%) from banana production area in Brazil. Another statistical trial by Chormule et al. (2017), the maximum incidence of *M. incognita* was observed in December and minimum in May showing highly significant negative correlation between number of root-knot nematodes, no. of root galls and egg masses with air and soil temperatures. Mahalik and Sahoo (2017) revealed presence of various PPNs in Okra in which *Meloidogyne incognita* was found to be more prominent followed by *Rotylenchulus reniformis*, *Tylenchorhynchus mashoodi*, *Helicotylenchus dihystra*, *Hoplolaimus indicus*, *Hirschmaniella mucorata* with the lowest prominence in *Criconemella ornate* in PPNs Dorylaimids with the highest frequency of occurrence and highest density and prominence value followed by *Rhabditis* and Monochids in FLNs.

Rotylenchulus reniformis and *Meloidogyne incognita* along with *Helicotylenchus dihystra* had been frequently encountered species among vegetable crops like tomato and Okra in India (Mukhopadhyaya and Roy 2006; Srivastava et al. 2012). Similarly, Adamou et al. (2013) observed the presence of 10 genera of PPNs in the soil and root analysis in three important vegetable producing areas in Nigeria and showed their susceptibility to *M. javanica* representing that RKNs are present in all three tree species and are destructive to vegetable crops as they have potential to attack more than 200 plants species and colonize in many

habitats. Later, Baidya et al. (2017) in their joint research observed two species: *M. incognita* and *M. javanica* in a single plant and concluded grafting technique from rootstock of *S. sisymbriifolium* as an alternative way especially beneficial to the tunnel farm practices to reducing nematode population over commercially available chemical nematicides. Later, the thesis work by Ngeno (2019) reported 24 genera of nematodes from tomato including 14 plant parasitic nematodes, 5 bacterivores 3 fungivores and 2 predator species identified to be *Meloidogyne*, *Alaimus*, *Aporcelaimus* and *Monunclus* the prevalent one from respective trophic group. Another nematological survey by Juma (2021) in five different areas of Kiambu and Embu counties recorded RKN as the most abundant and common genera followed by *Pratylenchus* and *Helicotylenchus* among the samples of tree tomato. Upon broadening the horizon on plant diseases in tunnels, Manandhar et al. (2020) presented 37% (53) fungal infections from eleven districts and identified to be root/collar rot, leaf spot, early leaf blight, late blight and powdery mildew; 20% (28) bacterial infections from five districts and identified as bacterial wilt, pith necrosis and bacterial stem rot; 37% (54) viral infections from ten districts and identified as ToMV, CMV and TYLCV and 6% (8) nematode infections from six districts identified as RKN caused by *Meloidogyne* spp. to making plants susceptible to bacterial and fungal infections herby affecting agronomy among farmers.

Osei et al. (2012) and Lutuf et al. (2018) both reported eight associated species in tomatoes from Ghana, *M. incognita* being most abundant and predominant pest in all sampled areas. A review article by Sikandar et al. (2020) also mentioned *M. incognita* as the most notorious and serious issue among agricultural crops in all parts of the world and requires careful analysis for management in near future. Several other studies have also mentioned incidence of *Meloidogyne graminicola* in rice crop. Dangal et al. (2009) discovered wide distribution of *M. graminicola* intherice nurseries in Chitwan. In addition, Analysis by Pascual et al. (2014); Gnamkoulamba et al. (2018); War et al. (2019) and Nyaku et al. (2021) discovered *Meloidogyne graminicola* and *Hirshmanniella oryzae* as the most abundant and frequent pests of rice plants but slightly differed with findings of Mussarat et al. (2016) who observed *Hirshmanniella* spp. *Helicotylenchus* spp. as the frequent genus in Pakistan. Likewise, Talavera et al. (2019) documented *Meloidogyne* spp. especially *M. hapla* as the major pest of strawberry. Later, a review by Mahfouz and Abd-Elgawad (2019) also confirmed *Meloidogyne* spp. as major pest of strawberry. Same was the finding with Pomegranate as reported by Singh

et al. (2019). However, *Helicotylenchus* spp. was the frequent genus for Pineapple in the Phillipines (Benzonan et al. 2021). A different finding has been observed among citrus. *Tylenchulus semipenetrans* and *Helicotylenchus* spp. has been reported as the most predominant one (Zoubi et al. 2022).

Tauheed et al. (2020) updated information about biology, epidemiology, symptomatology and stated RKNs as the major threat to plants worldwide and addressed recent management strategies practiced for nematode management with urge for several novel approaches purely effective for control of RKN population.

3. MATERIALS AND METHODS

3.1 Study Area

Study area was Dahachowk, a beautiful town in western Kathmandu, Bagmati province. It is a part of Chandragiri municipality. It is situated nearby to Thankot and north of Matatirtha. The burial ground on hill top of Kaji Kalu Pande is a popular hiking spot that lies in western outskirts of Kathmandu from where Gorkha can be seen.

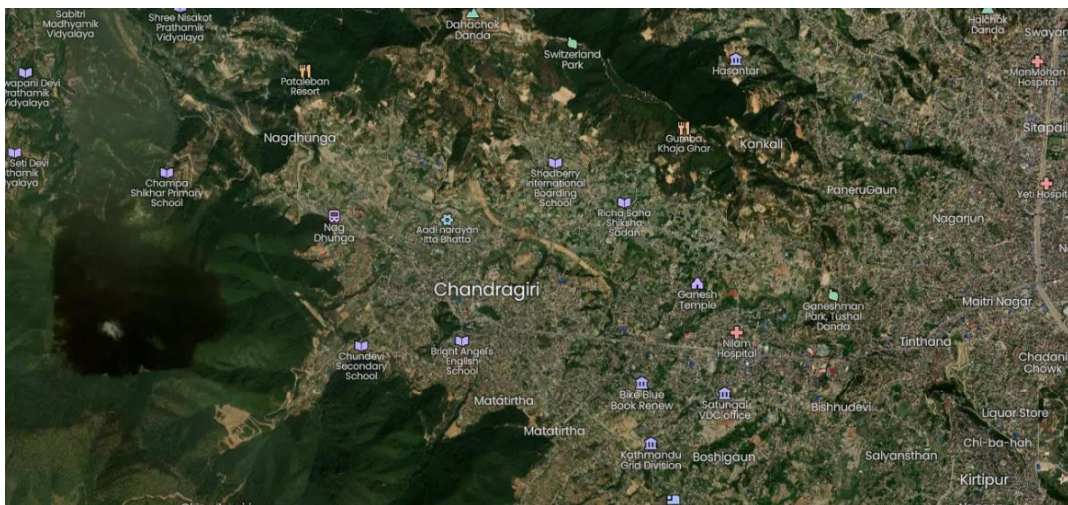
Latitude: 27.6928° or 27°41'34" north

Longitude: 85.2248° or 85°13'29" east

Elevation: 1451 meters (4760 feet).



Map 1: Map of Chandragiri Municipality. *Source:*Googlemap



Map 2: Satellite map of Dahachowk. *Source:* Mapcarta.com/355675

3.2 Methods

3.2.1 Soil Sampling

40 (twenty each) soil samples were collected during early winter from two different tunnel houses at the time of harvest in the month of November – January. Samples were collected randomly in zig-zag pattern within 10-20cm depth (Brithevnan et al. 2020). The samples were collected in polyethene, labeled properly and brought to lab of Central Department of Zoology for further analysis.

3.2.2 Nematode Extraction and Identification

The laboratory procedures were done in the lab of Central Department of Zoology.



Laboratory Procedures:

1. *Processing of soil sample*: In lab, first of all, chunks of soil were gently smashed and pebbles were removed from the soil by hand (Chhetri and Subedi 2019).
2. *Nematode Isolation from soil*: thus processed soil sample was further processed by Baermann's funnel technique (Baermann 1917) for extraction of soil nematodes. In a

small coarse sieve placed on a large funnel and covered by a tissue paper, processed soil sample was added to it. The stem of funnel is connected with rubber and closed by tight clipper. Funnel is then filled with water such that water touches only up to the lower surface of tissue paper on sieve avoiding air bubbles. The set up was left undisturbed for more than 24 hours so that nematodes get to settle at the bottom of the funnel. 20cc of water suspension was obtained through bottom of funnel and analyzed further (Chhetri and Subedi 2019).



Photograph 2: Baermann set up for nematode isolation from soil

3. *Extraction of Nematode*: The water suspension from Baermann's funnel was then observed under stereoscopic binocular microscope for extraction. Motile nematodes observed were extracted using small and fine entomological pin and slides were prepared for their identification.



Photograph 3: Stereoscopic Microscope used for nematode extraction from water collected from Baermann set up.

4. *Mounting and slides Preparation:* Thus extracted nematodes were then mounted. A drop of anhydrous glycerine was placed in a clean glass slide and nematodes were transferred to it and cover slip was applied (Seinhorst 1959). For extra support, water colored nail polish was used to seal around the cover slip.



Photograph 4: Mounting and slide preparation of isolated nematodes on glass slides

5. *Observation and Nematode Identification*: Thus prepared slides were observed under microscope under different magnification i.e. 4X, 10X and 40X for nose piece and 10X for an eye-piece (Shrestha and Bam 2015). Identification of extracted nematodes were done up to the generic level based on esophageal and other anatomical structures (Liang et al. 2005) following keys to identification by Jr. Smart and Nguyen (1988), Hunt et al. (2005) and Coyne et al. (2014) and also with the help from nematode expertise. Thus identified nematodes were further allocated and analyzed into five trophic group's viz., Bacterivore, Fungivore, Omnivore, Herbivore and Predator according to Keshari et al. (2016).



Photograph 5: Nematode observation under compound binocular stereo- microscope with under-stage lighting

3.2.3 Moisture contents and pH analysis

Moisture Content: Moisture is defined as percentage of water present in the soil mass by its weight. It's usually expressed in %.

It was measured by Oven-drying method in which 100gm of soil was kept in oven with temperature between 105°C to 110°C for 36 hours and change in the soil mass was calculated using the formula (Reynold 1970).

Mathematically,

$$\text{Moisture contents} = \frac{\text{Wet Weight} - \text{Dry Weight}}{\text{Dry Weight}} * 100\%$$

pH: The pH of each soil samples was measured by using pH meter. Before measuring pH of soil, pH meter was calibrated and 20gm soil sample was weighed and dissolved in 50ml distilled water by stirring with a glass rod and let it sit for a while. Then calibrated pH meter was dipped and constant reading in the device was recorded after Ndava et al. (2018).

3.3 Data Analysis

Species Composition and Relative Abundance of extracted nematodes were calculated followed after Myint et al. (2017). Also, using R-software, significant difference among variables using Chi-square test was performed. Differences obtained at levels of $p < 0.05$ were considered to be significant (Wu and Shi 2011).

Mathematically,

$$\text{Species Composition} = \frac{\text{No. of Individuals of species} * 100}{\text{Total no. of all species}}$$

$$\text{Relative Abundance} = \frac{\text{No. of Individuals of species}}{\text{Total no. of all species}}$$

4. RESULTS

Upon numbering the identified nematode specimen, the study reports identification of a total of 993 specimens from soil samples collected from two different farms in Dahachowk.

4.1 Generic Composition and Ordinal Diversity of Nematode in Soil

Of 9 genera of soil nematodes belonging to 3 different orders identified, 5 (56%) genera belonged to Plant Parasitic Nematode (PPN) and 4 (44%) genera belonged to Free Living Nematodes (FLN) respectively (fig 1).

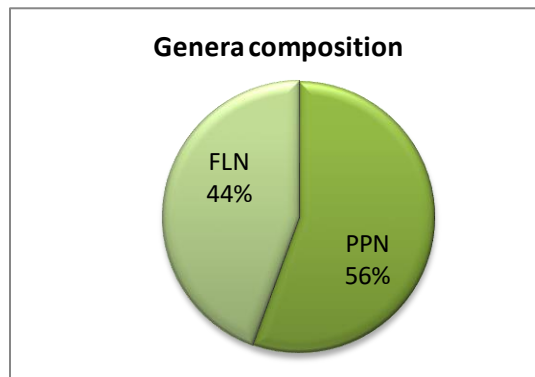


Fig 1: Generic Composition of nematode in total

In terms of their taxonomic groups, among the nine identified genera and three orders, order Tylenchida represented 67% with 6 genera followed by 22% order Dorylaimida with 2 genera and 11% Rhabditida with only one genus (fig 2).

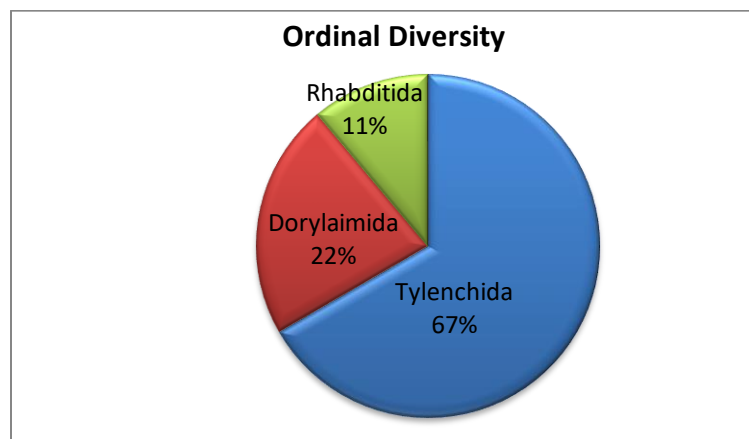


Fig 2: Ordinal diversity of nematodes in total

4.2 Occurrence of Nematodes in Tunnel 1 (T1) and Tunnel 2 (T2)

A slight variation was observed in the occurrence of nematodes in between tunnels. In T1, the number of nematodes extracted was lesser than that of T2. In both tunnels, among PPN, *Meloidogyne* juvenile was the frequent nematode followed by *Tylenchorhynchus*, *Helicotylenchus* and *Hoplolaimus*. *Discocriconemella* was only recorded in T2 and also was the least frequent nematode genus for T2 (fig 3).

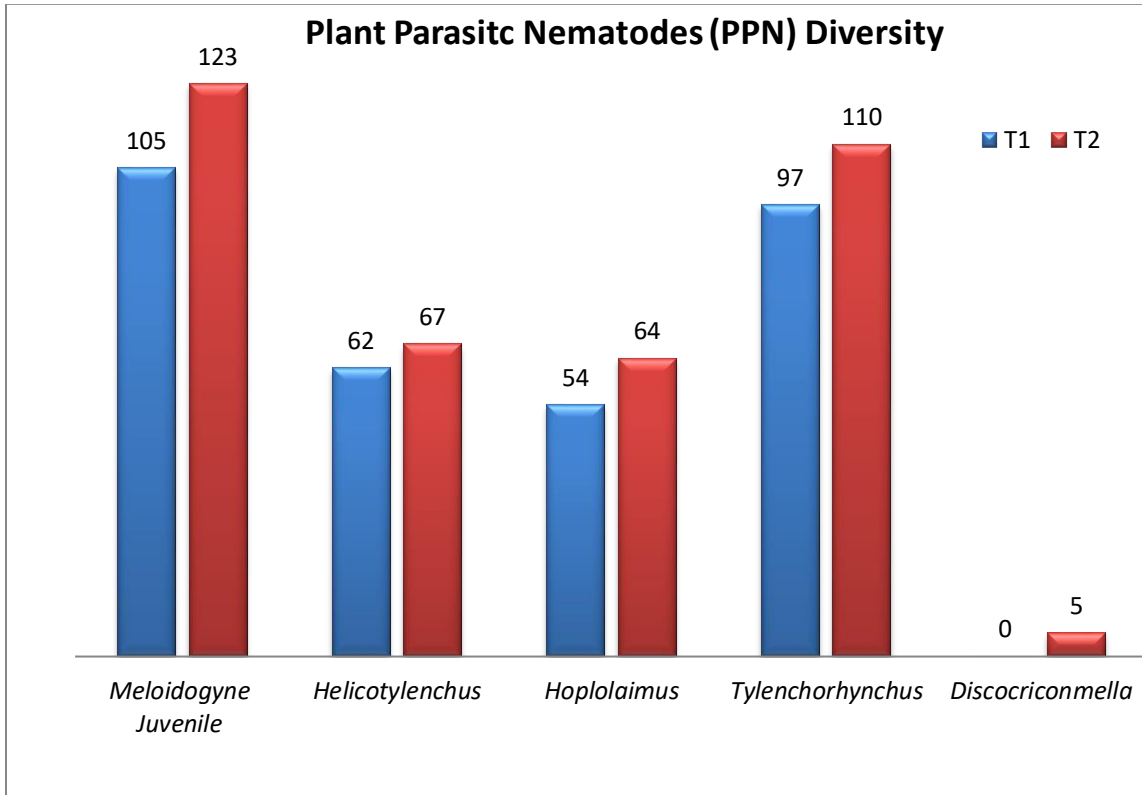


Fig 3: Plant Parasitic Nematode diversity in Tunnel 1 and Tunnel 2

In case of FLN, *Rhabditis* was the most frequent genus in both tunnels however; it was most reported and recorded with the highest number in T2. Both *Dorylaimus* and *Eudorylaimus* had slight distribution in both farms. *Filenchus* was the only detected genus in T2 making it the least frequent genus from T2 (fig 4). However no significant difference ($p > 0.05$) was obtained in distribution of PPNs and FLNs in both tunnels.

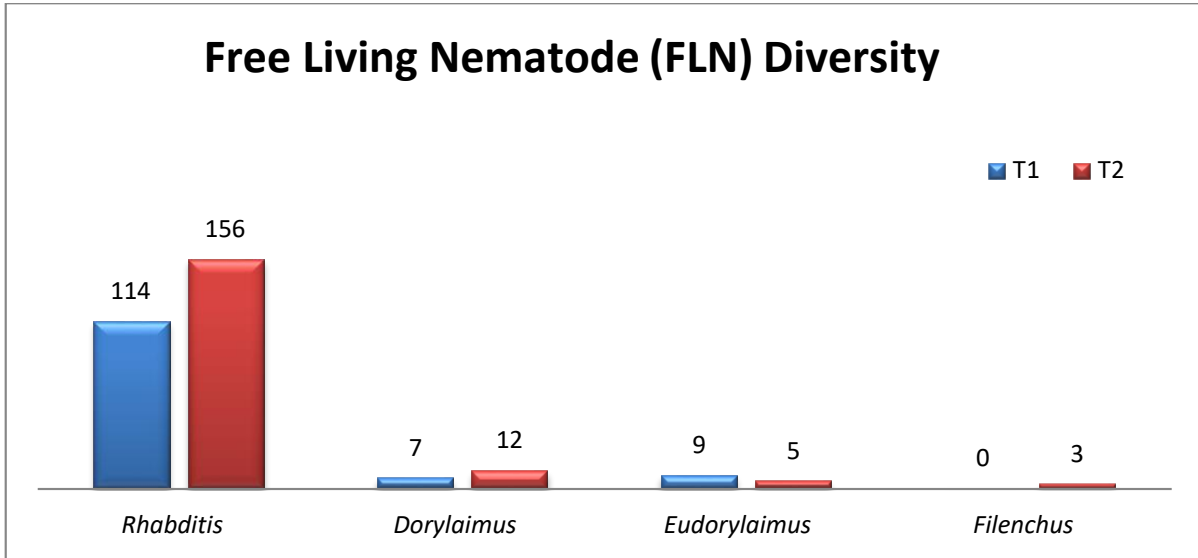


Fig: 4: Free Living Nematode Diversity in Tunnel 1 (T1) and Tunnel 2 (T2).

4.3 Trophic Diversity of Nematode Genus

Thus identified nematode genera were further allocated as per their feeding habits into different trophic groups' viz., Herbivore, Omnivore, Fungivore, Bacterivore and Predator. In overall, five genera of Herbivores, two genera of Omnivores, one genus of Bacterivore and Fungivore each was extracted. Predator were absent in both of the farms.

In the present study, slight change was witnessed among tunnels. Herbivore was the most dominant group in both tunnels but the number of genera varied. For Tunnel 1 (T1), Herbivore consist of 4 genera, Bacterivore consist of 1 genus and Omnivore consist of 2 genera respectively while for Tunnel 2 (T2), Herbivore consist of 5 genera, Omnivore consist of 2 genera and Bacterivore and Fungivore consist of 1 genus each. However, Fungivore was present only in T2 (fig5). Whatsoever, Herbivore was indeed the most dominant group for both tunnel farms.

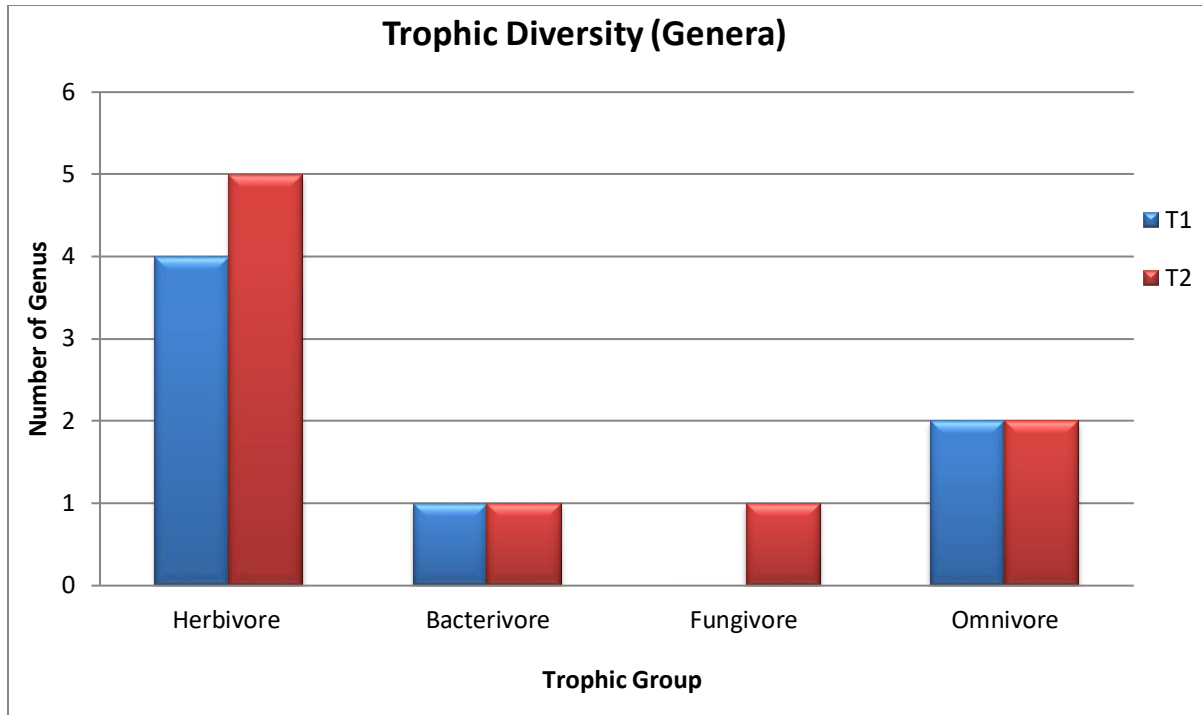


Fig 5: Trophic Diversity (Genera)

4.4 Depth-wise Representation of Nematodes

Nematodes were extracted from soil samples collected within several depths in both tunnels. During the collection, 10-20 cm was the depth for collection and hence nematodes thus extracted were further divided within the depth range of their collection. Two depth ranges, 10-14cm and 15-20 cm were considered as the basis for allocation.

Within the depth of 10-14 cm, genus *Tylenchorhynchus* (43) was encountered in the highest number followed by *Meloidogyne* juvenile (34) and *Helicotylenchus* (30) from Tunnel 1 (T1). Likewise from Tunnel 2 (T2), the highest occurrence was of *Meloidogyne* juvenile (27) followed by *Tylenchorhynchus* (22) and *Helicotylenchus* (12) respectively. The lowest occurrence was of *Filenchus* (2) only reported from T2. The genus *Discocriconemella* was absent in this depth range (fig 6). However, no significant differences between the two tunnels were observed ($p > 0.05$) in distribution of nematodes in soil collected within this depth range.

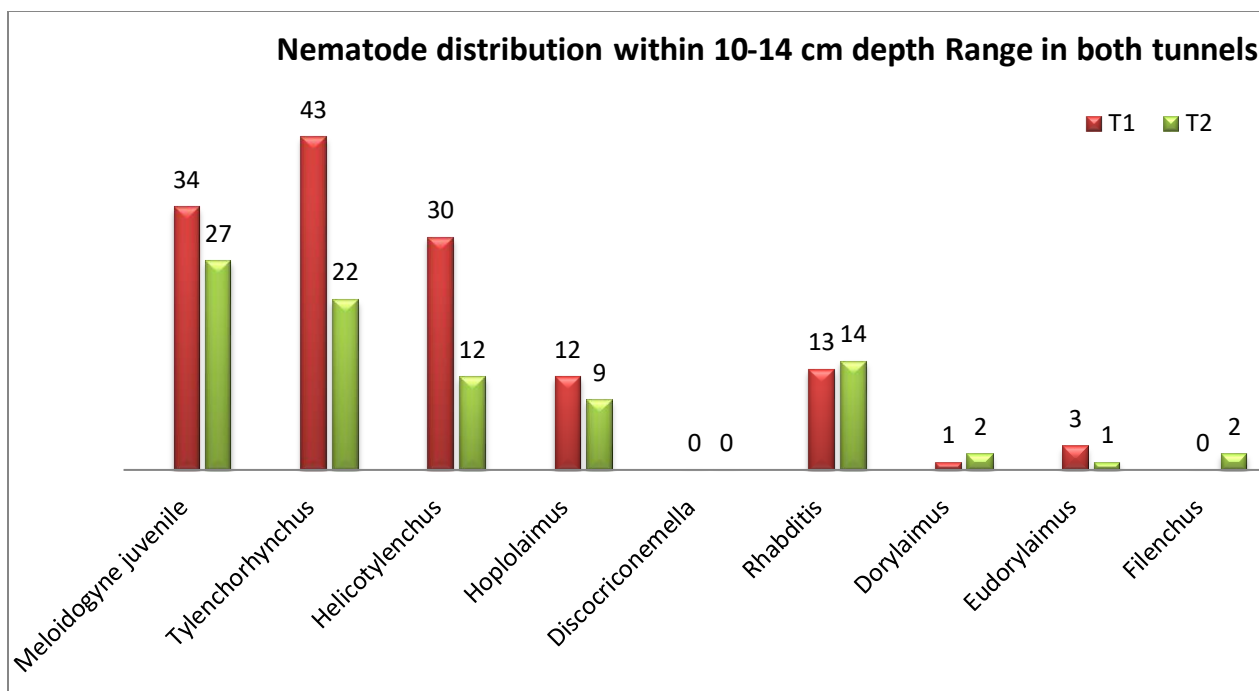


Fig 6: Nematode distribution within depth range 10-14cm in both tunnels.

Table 1: Species Composition and Relative Abundance for nematodes within 10-14 cm depth range

Genus	Tunnel 1(T1)	Tunnel 2 (T2)	Total	Species Composition (%)	Relative Abundance (RA)
Plant Parasitic Nematodes (PPNs)					
<i>Meloidogyne Juvenile</i>	34	27	61	27.11	0.27
<i>Tylenchorhynchus</i>	43	22	65	28.89	0.28
<i>Helicotylenchus</i>	30	12	42	18.67	0.18
<i>Hoplolaimus</i>	12	9	21	9.34	0.093
<i>Discocriconemella</i>	0	0	0	0	0
Free-Living Nematodes (FLNs)					
<i>Rhabditis</i>	13	14	27	12.0	0.12
<i>Dorylaimus</i>	1	2	3	1.34	0.013
<i>Eudorylaimus</i>	3	1	4	1.78	0.017
<i>Filenchus</i>	0	2	2	0.89	0.0089
Total	136	89	225		

The table showed that within depth of 10-14 cm, 225 nematodes were extracted in total. Of which, T1 harbored more nematodes (136) than Tunnel 2 (89). *Tylenchorhynchus* was the most abundant genus with the highest number of its occurrence (65) along with species composition and RA value of 28.89% and 0.28 followed by *Meloidogyne* juvenile and *Helicotylenchus* with 27.11% and 18.67% species composition and 0.27 and 0.18 RA values respectively. The least abundant genus with the least number of occurrences (2) with species composition and RA values was recorded in *Filenchus* with 0.89% and 0.0089. *Discocriconemella* was not reported within this depth range (table 1).

Similarly, no significant differences between the two tunnels were observed ($p > 0.05$) in distribution of nematodes in soil collected within the depth of 15-20 cm. *Rhabditis* was definitely the most occurred genus in both tunnels. In T1, *Rhabditis* (101) was the most occurred genus followed by *Meloidogyne* juvenile (71) and *Tylenchorhynchus* (54) while the least occurred genus appeared to be *Dorylaimus* (6). Likewise in T2, the highest occurrence was reported to be *Rhabditis* (142) followed by *Meloidogyne* juvenile (96) and *Tylenchorhynchus* (88). *Filenchus* (1) was the least occurred genus. *Discocriconemella* (5) was reported only within this depth range (fig 7).

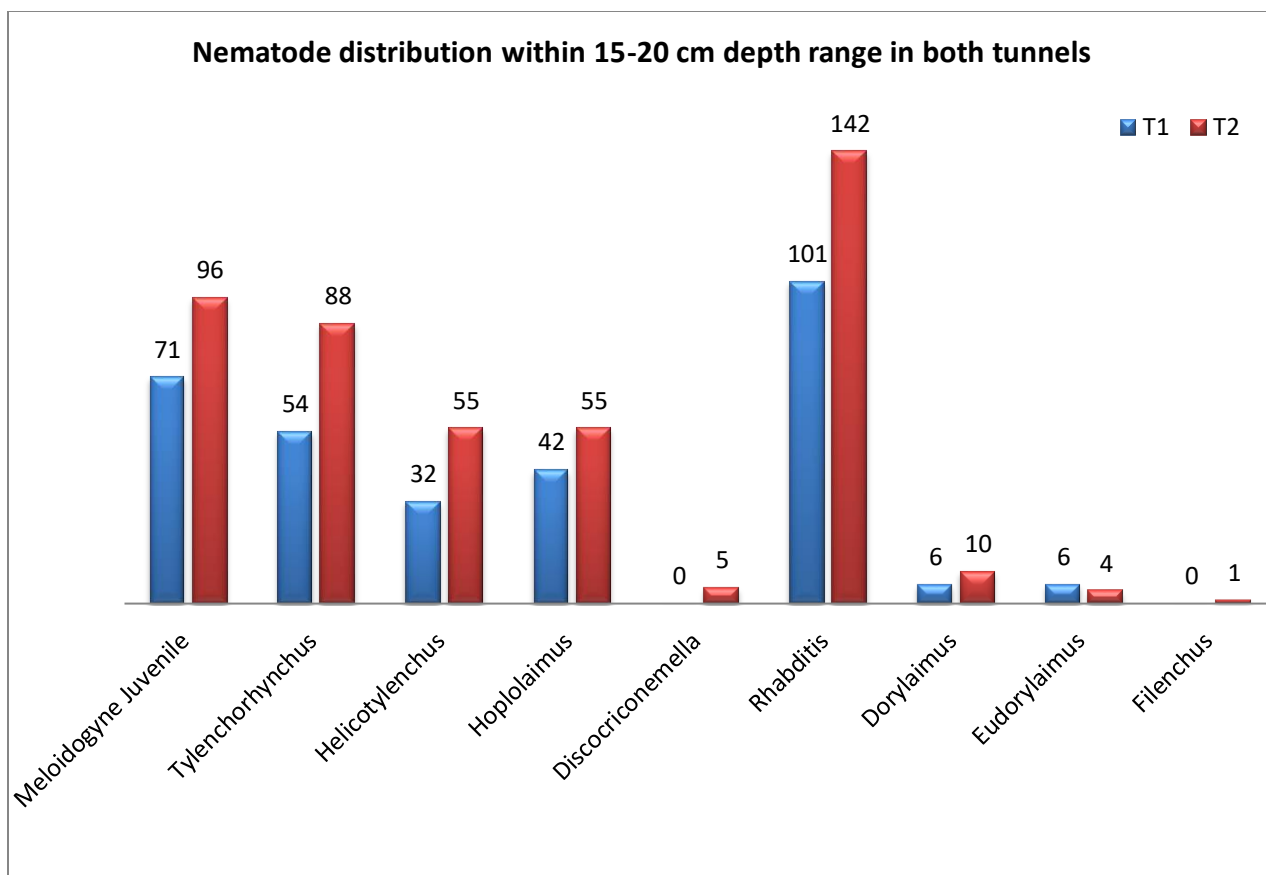


Fig 7: Nematode distribution within depth range 15-20 cm in both tunnels.

Table 2: Species composition and Relative Abundance calculation for nematodes within 15-20 cm depth range

Genus	Tunnel 1 (T1)	Tunnel 2 (T2)	Total	Species Composition (%)	Relative Abundance (RA)
Plant Parasitic Nematodes (PPNs)					
<i>Meloidogyne Juvenile</i>	71	96	167	21.74	0.21
<i>Tylenchorhynchus</i>	54	88	142	18.48	0.18
<i>Helicotylenchus</i>	32	55	87	11.32	0.11
<i>Hoplolaimus</i>	42	55	107	13.93	0.13
<i>Discocriconemella</i>	0	5	5	0.65	0.0065
Free-Living Nematodes (FLNs)					
<i>Rhabditis</i>	101	142	243	31.64	0.31
<i>Dorylaimus</i>	6	10	16	2.08	0.02

<i>Eudorylaimus</i>	6	4	10	1.30	0.013
<i>Filenchus</i>	0	1	1	0.13	0.0013
Total	312	456	768		

Moreover, within the depth range of 15-20 cm, relatively higher number of nematode genus (768) was extracted. Unlike within 10-14 cm depth range, the abundance of nematodes in T2 was found to be higher (456) than in T1 (312). *Rhabditis* was the most abundant genus with Species composition and RA of 31.64 % and 0.31 followed by *Meloidogyne* juvenile and *Tylenchorhynchus* with species composition of 21.74% and 18.48% each and RA of 0.21 and 0.18 respectively. Like within 10-14 cm depth range, *Filenchus* was the least abundant genus with species composition and RA of 0.13% and 0.0013. *Discocriconemella* was recorded within this depth range only 5 in number (table 2).

In general, *Rhabditis* was the most occurred genus from both tunnels followed by *Meloidogyne* juvenile and *Tylenchorhynchus* respectively. *Discocriconemella* and *Filenchus* were the genus only reported from T2. The result showed that T2 harbors more nematode population to that of T1 (table 3). However, no significant differences between these two tunnels were observed ($p > 0.05$) in distribution of nematodes from total soil samples collected.

Table 3: Species Composition and Relative Abundance of nematodes in both tunnel farms

Genus	Tunnel 1 (T1) (%)	Tunnel 2 (T2) (%)	Total	Species Composition (%)	Relative Abundance (RA)
Plant Parasitic Nematodes (PPNs)					
<i>Meloidogyne</i> Juvenile	105 (23)	123 (22.5)	228	22.96	0.22
<i>Tylenchorhynchus</i>	97 (22)	110 (20)	207	20.84	0.20
<i>Helicotylenchus</i>	62 (14)	67 (12)	129	12.99	0.12
<i>Hoplolaimus</i>	54 (12)	64 (12)	118	11.88	0.11
<i>Discocriconemella</i>	0	5 (0.9)	5	0.50	0.005

Free-Living Nematodes (FLNs)

<i>Dorylaimus</i>	7 (2)	12 (2)	19	1.91	0.019
<i>Eudorylaimus</i>	9 (2)	5 (0.9)	14	1.40	0.014
<i>Filenchus</i>	0	3 (0.6)	3	0.30	0.003
<i>Rhabditis</i>	114 (25)	156 (29)	270	27.19	0.27
Total	448 (45)	545 (55)	993		

4.5 Measurement of pH and Moisture content of soil

The moisture content and pH for each soil samples of both tunnels were measured. Calibrated pH meter was used to record the pH readings in each soil samples and their average reading was noted and Moisture content was measured by oven-drying method. Individual readings were noted and their average reading was recorded (table 4).

Table 4: pH and Moisture contents readings of soil samples from both tunnels

Soil Samples	pH		Moisture (%)	
	T1	T2	T1	T2
1	4.2	3.6	92.88	75.81
2	4.3	4.4	93.87	79.03
3	4.0	3.8	89.34	75.74
4	4.7	3.9	93.56	81.60
5	4.2	4.3	92.74	80.09
6	4.1	4.0	94.40	74.90
7	4.6	3.6	95.08	79.83
8	4.0	3.9	92.01	80.66
9	4.1	4.0	89.97	79.41
10	3.9	4.7	94.20	73.06
11	4.4	4.7	96.47	72.90
12	5.0	4.4	95.83	74.73
13	6.6	4.0	97.21	77.22
14	6.3	4.3	90.05	78.39

15	5.1	4.0	94.83	73.79
16	4.6	3.6	97.75	77.30
17	4.5	4.3	90.11	80.11
18	4.8	4.1	94.37	78.20
19	4.9	3.7	89.03	79.26
20	4.6	5.0	90.88	76.39
Average	4.6	4.1	93.22	77.42

Upon analyzing the average values, pH measurement showed that Tunnel 2 (T2) was more acidic (4.1) than Tunnel 1 (T1) (4.6). pH values of individual soil samples ranged from 3.9-6.6 with average of 4.6 for T1 while the range was 3.6-5.0 with average reading of 4.1 for T2. The readings however showed no significant differences ($p > 0.05$) in distribution of nematodes among both tunnels in relation to the pH of the soil samples collected.

Also the moisture content of each soil samples collected from both tunnels was measured. It was found that moisture contents of soil samples from T1 were recorded to be way higher than T2 with the average readings of 93.22% over 77.42% respectively. The readings however showed no significance differences ($p > 0.05$) in nematodes distribution in both tunnels in relation to the moisture contents of collected soil samples.

With reference to these parameters, it's been clearly represented that T2 harbors more number of nematodes than T1. It also reflected that soil from T2 was better enough for nematode habitat and distribution, though no significant differences ($p > 0.05$) were established between both tunnels with respect to depth, pH and moisture contents, further hinting for possible increase in the number of plant parasitic nematodes with further cultivation in same pattern and with the availability of appropriate hosts in the upcoming period of time with the great assistance from existing free living nematodes aiding in nurturing and regulating the possible soil health.

5. DISCUSSION

Being an important habitant of soil, nematodes are ubiquitous in their nature and often regarded as one of the important soil components, mainly co-exist in different ecosystem varying in their diversity (Lazarova et al. 2021) directly involved in nutrient cycling and indirectly contributing to soil decomposition (Khatoun et al. 2001). Specifically, Bacterivores and Fungivores help in decomposition of organic matters by speeding up the decomposition process recycle nutrients and return to soil making accessible to plants roots (Mussarat et al. 2016) while PPNs are basically known to cause economic damages causing yield loss (Keshari et al. 2016).

In the present study, a total of 9 genera belonging to three different orders were identified from two different tunnel farms from Dahachowk in which PPNs were dominant over FLNs. The findings thus observed recorded higher percentage of nematodes belonging to order Tylenchida. The same observation has been documented in majority of researches performed in varieties of crops worldwide. In Saudi Arabia sole, several greenhouse crops have been reported to be highly infected by Tylenchids in past couple of years (Almohithet et al. 2020; Tileubayeva et al. 2021). Tylenchids have been reported from all sorts of crops: vegetables, fruit trees, horticultural and ornamental crops (Mokbel 2014). On all these, RKNs have been the major concern. Ornamental grass in Korea (Mwamula and Lee 2021), horticultural crops in Portugal (Rusique et al. 2022); Banana farms in Egypt (El-Sagheer 2020) and strawberry farms in Spain (Talavera et al. 2019) have all been affected by RKNs in the recent years. It is usually assumed that presence of host plant is the main factor for determining the nematode diversity affecting on crops (Ali et al. 2018; Sabehe et al. 2018; Tileubayeva et al. 2021). Moreover, the current study focused on tomato plant supported this observation and also with Gomes et al. (2003) and hence showed that tomato, the most cultivated and produced plant, is the host for the two most common genera- *Meloidogyne* and *Helicotylenchus* but combined occurrence of *M. incognita* and *M. javanica* had been documented in tomato in Pakistan by Zia et al. 2014 with 100% incidence. Meanwhile, in the US, *M. javanica* has been documented as the main RKN infestation known to widely affecting tomato plants grown in high tunnels (Frey et al. 2020). However, in the present study only juvenile of *Meloidogyne* was present but

was highest in occurrence, which could possibly be due to earlier collection of soil samples and soil being young for cultivation.

The results of this study showed that among PPN, *Meloidogyne* and *Helicotylenchus* as the most prevalent genera along with *Tylenchorhynchus*. This finding has been partially favored by Singh and Khanna (2015); Ibrahim et al. (2010); Ahmed et al. (2020) and Amulu et al. (2021) who in their respective study documented these PPNs as the most frequent and prevalent genera amongst several cultivated and vegetable crops like tomato. Furthermore, Lutuf et al. (2018) also states *Meloidogyne* and *Helicotylenchus* as the major pest for tomato. In addition, *Helicotylenchus* has also been documented as one of the frequent genus by Salas et al. (2021) in tomatoes and Zoubi et al. (2022) among citrus plants and the second highly prevalent genus in rice by Mussarat et al. (2016). Further supporting the finding, Nzesya et al. (2014); El-Nuby et al. (2019) and Krif et al. (2020) in their respective researches also mentioned *Meloidogyne* as the most major pest in organic farms and several vegetable crops. Furthermore, in the present study, *Meloidogyne* and *Tylenchorhynchus* have been the most dominant one and this result is fairly supported by Korayem et al. (2014) who states *Meloidogyne* and *Tylenchorhynchus* as the most predominant pests of vegetable and field crops, fruits and ornamental crops, with addition support by Nzesya et al. (2014) and Shrestha and Bam (2015). Mokbel (2014) too reported *Tylenchorhynchus* and *Helicotylenchus* as the prevalent nematodes among field crops and vegetable crops in Saudi Arabia making current findings more relevant. Moreover, various genera belonging to family Criconematidae (Ring Nematode) have been reported by several studies. This study also documents occurrence of Ring Nematode, *Discocriconemella* butas the least frequent genus contradictory to Ibrahim et al. (2010); Keshari et al. (2018); Almohithet et al. (2020); Tileubayeva et al. (2021) who reported them as the average frequent group. Souza et al. (2019) reported ring nematode, *Mesocriconema* as the second highly prevalent and aggressive genus in peach plants and Sarkar et al. (2021) reported *Criconemoides* as the genus of highest prevalence in groundnuts. The contradiction of the findings of this study to those mentioned studies could rather be due to course of evolution and acclimatization of these ring nematodes to the soil since the soil is young in cultivation of tomatoes and other companion crops. The current finding might suggest emergence and incidence of ring nematodes in the soil.

Upon diving into their trophic groups, Bacterivore was the most frequent and dominant group while Fungivore was the least. Though Bacterivore comprised single genus *Rhabditis*, its distribution and occurrence was higher for both tunnels and was the most frequent group. This finding contradicts with the findings of several other researchers like Mokbel (2014); Keshari et al. (2016); Sikandar et al. (2021) where PPNs were dominant over other groups. This could be possibly due to soil being continuously mono-cropped for almost three years and soil been supplied with organic manures and fertilizers. This assumption is further supported by Keshari et al. (2016) and Chhetri and Subedi (2019) suggesting that in agricultural soil, use of organic manures and fertilizers enables nematodes to respond quickly to increased food supply. Wachira et al. (2009) further justified increase of Bacterivores and Funivores population with use of cow manures as an organic amendment, which was the best fit for the current study. In addition, it can also be assumed that once uncultivated for relatively longer period of time made them dependent on initial community structure and hereby resulted in domination of Bacterivores population in the land but later the mono-cultivation of tomato slowly followed by other companion crops under tunnel farm for couple of years lead to the increase in assembly of plant parasitic nematodes gradually over the still-maintained population of Bacterivores. This assumption is somehow favored by Boutton and Biedermann (2009) and Lazarova et al. (2021) documenting increase in population of plant parasitic nematodes from grasslands to the cultivated lands from 10% to 40% still with more than 30% increase in Bacterivore population. The strong dominancy of Bacterivore population in the present study is strongly justified by Zheng et al. (2012) after their documentation of the higher population of Bacterivores in younger soil than in the older ones in tomato plant also a review article by Lazarova et al. (2021) quoted that “closely related nematodes phylo-type tend to co-occur more often showing that closely related lineage occupies similar niches” supports the assumption on the results of the present study.

Prior to this study, Ngele and Kalu (2015) observed highest prevalence (42%) of *Xiphinema* spp., *M. incognita* as the second most prevalent (37.5%) species among vegetable crops within depth of 10-15cm. This finding appeared to be contradictory with another work conducted by Brithevnam and his associates (2020) who documented *Meloidogyne incognita* and *Helicotylenchus dihystrera* as the most frequent (92% and 89%) species in

their study in Beetroot and also with Sikandar et al. (2021) who reported *Heterodera* (55.7%), the major host of maize; *Tylenchorhynchus* (33.33%) and *Helicotylenchus* (24.7%) as the most occurred genus from maize and other rotational plants, with the study being conducted with soil and root samples collected within the same depth range. However, the present study contradicts with the prior researches since *Tylenchorhynchus* was the most prevalent one (28.89%) in this study followed by *Meloidogyne* (27%). Interestingly, within the soil samples collected within 5-10 cm depth, similar finding was observed by Myint et al. (2017) in chilly and groundnut plant where *Meloidogyne* outnumbered the overall occurrence of nematodes with 12% occurrence in Groundnut and 22% in chilly followed by 11% occurrence of *Tylenchorhynchus* in groundnut and 14% *Helicotylenchus* in chilly respectively. These contradictory findings could possibly be due to several soil parameters, collection period and type of crops soil was collected from.

Zheng et al. (2012) and Lutuf et al. (2018) both reported high prevalence of PPNs in deeper soil, especially *Meloidogyne* and *Helicotylenchus* have been reported to be highly localized in deep soils, above 15 cm depth, in all sampled soils. This fact partially favors the present study because this study reports *Meloidogyne* juvenile and *Tylenchorhynchus* as the most prevalent genera within depth of above 15cm with further support and explanation from Zheng et al. (2012) that with the increase in depth ensure increased prevalence of phyto-parasitic nematodes for the older soils. However, Wu and Shi (2012) completely supported the present study with their observation that population of *Meloidogyne* juvenile and free living nematodes increased with increase of soil depth and years of cultivation in compliance with findings by Alabi et al. (2017) in Nigerian yam. Therefore it can be assumed that in the present study, the population of existing nematodes might increase with more cultivation. Since the soil for the present study is less than 5 years old for continuous cultivation, the findings is however supported since the nematodes showed increased trend with the increase in depth respectively. With another similar study to the present study, whatsoever carried out in Okra, *M. incognita* has been documented to be the most pre-dominant species from the soil samples surveyed within 20 cm depth (Hussain and Mukhtar 2019) and this similar finding was reported by the present in tomato plant where only juvenile form of *Meloidogyne* was detected however was known to be the dominant plant parasitic genus within depth of 20cm as in the mentioned study.

Further supported by similar research by Krif et al. (2020) to the present study, Moroccan organic tomato farms presented supporting documentation on *Meloidogyne* as the highly prevalent genus severely affecting tomato plants and several other medicinal and horticultural plants within 25 cm deep soil throughout the farmland surveyed. Anwar and McKerny (2012) reported *Meloidogyne* and *Pratylenchus* as the most common and frequent (90% and 30%) genus among all the surveyed roots and soil samples of vegetables crops within depth range of 15 to 20 cm, partially supported the present study since the study was similar to the present study, however, *Pratylenchus* was not detected in the present study but larval form of *Meloidogyne* was definitely the most frequent phyto-parasite detected. The inability to discover *Pratylenchus* in the current study was mainly due to the fact that study focused only on soil samples excluding root samples.

Soil moisture and pH have been detrimental factors for nematode population analysis. In the current study, the soil samples collected were measured to be acidic for both tunnels. Moreover, it was observed that the more acidic soil holds more nematode population and relatively diverse group. *Rhabditis* have been reported to be the most frequent one and its number was observed to be increased with increase in pH and moisture, since both tunnels appeared to be acidic in nature and relatively high moisture content; the observation of the highest population of *Rhabditis* was justified with that of PPNs. This finding agreed with the result observed by Rawhat and her associates (2021) in their joint studies who reported increased population of Bacterivores and PPNs in soil with higher pH and moisture contents in patched landscapes. However, different observation was reported by Ndava et al. (2018) in tomatoes grown in soil with different pH and texture conditions. Strong negative correlation ($r = - 0.935$) was observed between pH and nematode population suggesting decrease in nematode population with the increase in pH. Though only three genera were reported from field and garden where *Meloidogyne* was the most frequent (50.6%) one followed by *Helicotylenchus* (26.4%) and *Pratylenchus* (23%) respectively.

In contrast, the co-existence of many species using the same resources has been a major query for ecologists and pedologists. Hence it can be assumed that there are as many different resources as there are different species. Thus, distinct physiological and environmental requirements drive species of same functional group to play contrasting role in soil ecosystem

(Neher 2010). Nematodes do respond to different soil type and moisture contents in different ways. They are known to grow optimally over a limited temperature range. Thus, communities exhibit seasonal dynamics where dominant species differ through time followed by quality and quantity of plant litter as food resources (Ferris et al. 2004). Nematode community also tend to be changing suggesting that nematode succession on a newly cultivated cropland may unfold the unexpected succession in 5/10 years of time period (Neher 2010). Also is the fact that continuous cultivation of local cultivar favors the rapid buildup and survival of nematode population in soil (Ibrahim et al. 2000; Basyony et al. 2020; Bakr et al. 2020).

The differential findings of this study to some researches could specifically be due to soil being relatively new and young to cultivation without accurate field history, use of organic manures and fertilizer, traditional but continuous mono-cropping practice with only seasonal companion crops etc. However, other parameters like cropping practices, rainfall, temperature and physio-chemical properties of soil have been associated with diversity in nematode community over decades of period (Fleming et al. 2016) further supported by Krif et al. (2020) who stated that variability in genera could be attributed to crops grown in a particular field meaning that their distribution could be affected by parameters like environment, production systems and employed cultural practices. Also, companion crops like marigold, lettuce, mustard have been well cultivated in the tunnels here in Dahachowk. This is could be one of the reasons for controlled population of existing nematodes. Tringovska et al. (2015) also stated the positive impacts of these companion crops especially mustard and Marigold in enhancing nematode antagonistic micro-organisms for nematode suppression without affecting on tomato yield. Upon summarizing, general lack of knowledge among tomato growers about possible nematode infestation, farmers having least education level and some even illiterate and belonging to the far-western part of the country and adapted to following the traditional cultivation practices may also have contributed to the existing prevalence of nematodes in the tunnel farms. Since any communities tend to be expanding and changing with time and available resources, many soil secrets can be unfolded with more explorations in the future and hence close follow-up is necessary.

6. CONCLUSION

Prevalence of both plant parasitic and free-living nematodes in rhizosphere of hybrid tomato plants in two different tunnels in Dahachowk has been studied. Altogether 9 genera of parasitic and free living nematodes belonging to 3 orders and 7 families have been recorded and were: *Meloidogyne* juvenile, *Tylenchorhynchus*, *Helicotylenchus*, *Hoplolaimus* and *Discocriconemella* as parasitic while *Rhabditis*, *Dorylaimus*, *Eudorylaimus* and *Filenchus* as free-living nematodes.

The study showed mixed community of plant nematodes from the rhizosphere suggesting nematode diversity. Nematodes belonging to four trophic groups were observed. *Rhabditis*, a Bacterivore, was the dominant genus further confirming that free living nematodes are everywhere and can be found in all sorts of habitat but PPNs need host for their occurrence. Upon emphasizing the PPNs, juvenile form of *Meloidogyne* was frequent followed by *Tylenchorhynchus* and *Helicotylenchus*. However, more number of nematodes was observed in the soil within depth of 15-20 cm range. Acidic pH soil tends to favor more diverse nematode infestation. Diversity of nematodes thus observed in a three year soil for continuous mono-culture is really concerning especially for a resistant hybrid variety.

Most nematodes reported are known to be highly phyto-pathogenic and may present severe threat to tomato in the future with further traditional cultivation practice even the hybrid variety is cultivated. Considering the destructive nature of these nematodes, sound research and management practices have to be in place for minimization of possible crop damages in the future. The current prevalent nematodes are expected be in the course of acclimatization and might be a point of concern in the future. However, for the accurate and authentic conclusion, further more but regular researches on the particular criterion can unfold the hidden secrets of nematode prevalence and this study can be the basis for the region.

7. RECOMMENDATIONS

Given the severity of nematodes impacts, some recommendations are to be suggested:

- i. More focuses needs to be made on several soil and environmental parameters for better analysis on nematode prevalence.
- ii. Laboratory assays of soil, roots and plant tissues periodically can help for complete diagnosis and identification of prevalent nematodes and help in comparison with several existing researches and field observations.
- iii. Crop rotation is strongly recommended.
- iv. Continuous mono-culture practice is strongly not recommended.

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9. PHOTO PLATES

Photograph 6: Mobile photography of Plant Parasitic Nematodes (PPNs) collected from soil.



Discocriconemella spp. X400



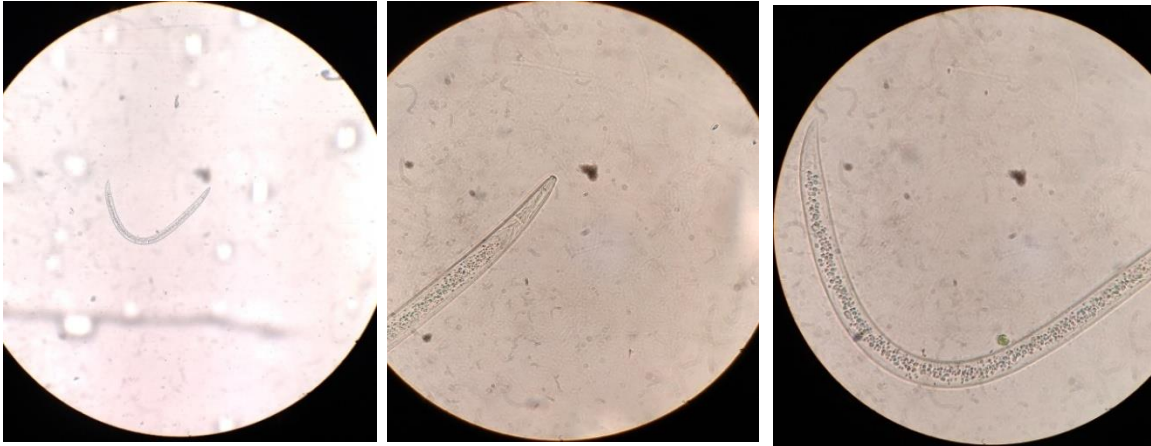
Tylenchorhynchus spp. X400



Hoplolaimus spp. X400



Helicotylenchus spp. X400

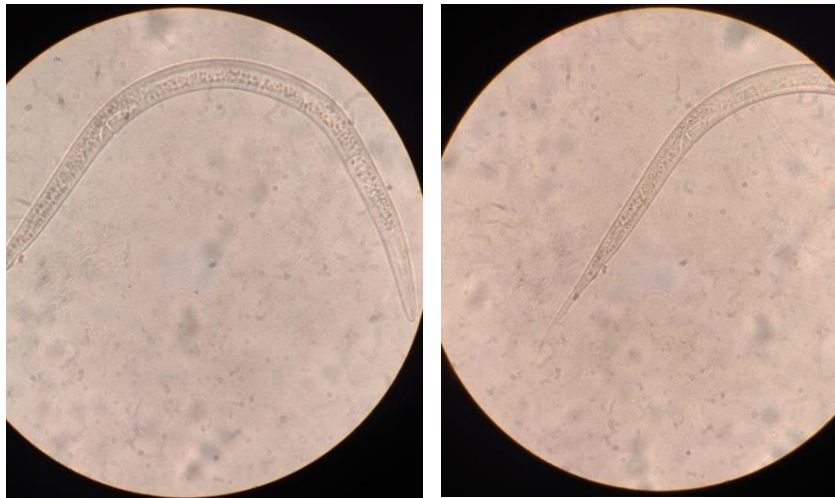


Meloidogyne juvenile X100

Anterior halfX400

Posterior halfX400

Photograph 7: Mobile photography of Free-living Nematodes (FLNs) collected from soil.



Filenchus spp. Anteriorhalf X400

Posterior halfX400



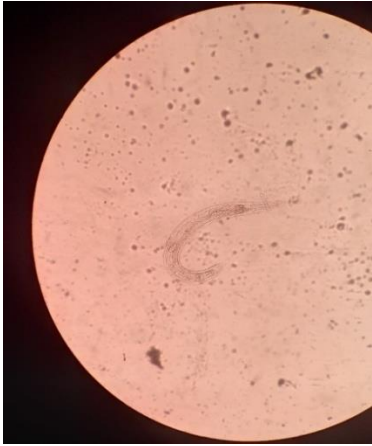
Dorylaimus spp. X100



Anterior half X400



Posterior half X400



Rhabditis spp. X100



Anterior half 400X



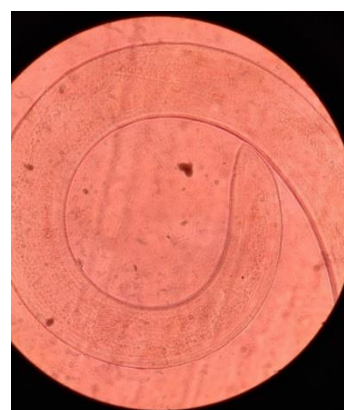
Posterior half X400



Eudorylaimus spp. X100



Anterior half X400



Posterior half X400