SELINUS UNIVERSITY
OF SCIENCES AND LITERATURE

ASSESSMENT OF PLANT PARASITIC NEMATODES AND THEIR MANAGEMENT IN AGRICULUTURAL CROPS IN NEPAL

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A DISSERTATION

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CERTIFICATE

We, the undersigned, members of the Advisory of **Mr. Suraj Baidya**, a candidate for the degree of Doctor of Philosophy in Agriculture of Nematology, with major in Plant Nematology under the Department of Nematology Program at Selinus University, Faculty of Life & Science, agree that the dissertation entitled **"Assessment of Plant parasitic Nematodes and their management in Agricultural crops in Nepal"** has been submitted for the requirement for the degree of Doctor of Philosophy (PhD) in Agriculture of Nematology.

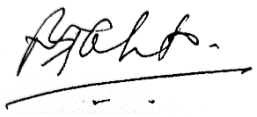

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TABLE OF CONTENTS

TABLE OF CONTENTS

TABLE OF CONTENTS

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF FIGURES

ABBREVIATIONS /ACRONYMS

$	**Dollar**
&	**And**
%	**Percentage**
@	**at the rate of**
°C	**Centigrade**
µm	**Micro metre**
AD	**Anno Domini**
AMF	**Arbuscular micorrhizal fungus**
BCA	**Biological control agent**
cfu	**Colony forming unit**
cm	**Centi meter**
CV	**Coefficient of Variance**
DAP	**Di-ammonium Phosphate**
DBCP	**1, 2- dibromo -3-Chloropropane**
DMRT	**Duncan's Multiple Range Test**
Dr	**Doctor**
DS	**Disease severity**
EC	**Emulsifiable Concentrate**
EDB	**Ethyl di-bromide**
etc	**et cetera**
g	**gram**
G	**Granule**
GI	**Galling index**
hrs	**Hours**
HYB	**Hybrid**
i.e.	**Example**
J2/s	**Juvenile/s**
JA	**Jasmonic acid**

kg	**Kilo gram**
l	**Litre**
LSD	**Least significant difference**
mins	**Minutes**
ml	**Milli litre**
mm	**Milli meter**
MOP	**Murate of Potash**
NaCl	**Sodium Chloride**
NaOCL	**Sodium hypochlorite**
NARC	**Nepal Agricultural Research Council**
NPPRC	**National Plant Pathology Research Centre**
PDA	**Potato dextrose agar**
Pf	**Final population**
pH	**Potential of hydrogen**
PhD	**Doctor of Philosophy**
Pi	**Initial population**
PPD	**Plant Pathology Division**
PPN	**Plant Parasitic Nematode**
Psi	**Pound force per square inch**
Prof	**Professor**
Pvt Ltd	**Private Limited Company**
RCBD	**Randomized complete block design**
Rf	**Reproductive factor**
RKNs	**Root Knot Nematodes**
SA	**Salicyclic acid**
SEM	**Standard error of mean**
spp	**Species**
Th	***Trichoderma harzianum***
TV	***Trichoderma viride***
USA	**United States of America**
WP	**Wettable Powder**

ABSTRACT

Field studies were conducted to monitor the distribution and occurrence of different plant parasitic nematode on 15 different crops. During the study, in total of 339 soil samples (211 from vegetable crops and 128 from cereal crops) were collected fron different districts.

Two different experiments laid on efficacy of different species of *Trichoderma* for the management of root knot nematodes (*Meloidogyne* spp) in tomato (HYB. Shrijana) were carried out in screen house conditions. In first set of pot experiment, there were six different commercial products of *Trichoderma* spp were tested against *Meloidogyne* spp including one control. The pots were artificially inoculated with 5000 eggs of root knot nematode (RKN) per pot (@ 5 eggs/g of soil) after a week of transplanting. Commercial product of three different *Trichoderma harzianum* (*Trichoderma. harzainum*-Nemastin, *Trichoderma harzainum* (T22) - Root shield plus and *Trichoderma asperellum*), and three commercial product of *Tricoderma viride* (*T. viride* – Ashtha TV, *T. viride*- Biocare-F and *T. viride*- Prarambha) were multiplied in PDA plates. The spore suspension collected in a beaker was adjusted to 1×10^6 spores/ml after counting spores using haemocytometer.

Another experiment was also conducted to test the efficacy level of locally isolated *Trichoderma* spp from different geographical regions of Nepal. The pot experiment was designed in RCBD with three replication having eleven treatments including control. In this study, 1000 J2s were applied to each pot having 1 kg soil for each treatment. The spore suspensions of *Trichoderma* collected in a beaker were adjusted to 1 X 10^7 CFU/ml after counting spores using haemocytometer.

Out of 211 samples from vegetable crops, 137 samples, twelve different genera of plant parasitic nematodes were identified. They are *Meloidogyne, Rotylenchus, Tylenchus, Hoplolaimus, Belonolaimus, Criconemoides, Helicotylenchus, Tylenchorhynchus, Pratylenchus, Hirschmanniella, Aphelenchoides* and *Longidorus.* Among them, the most commonly detected genera were *Helicotylenchus* (28%) followed by *Tylenchorhynchus* (19%), *Meloidogyne* (10%) and *Pratylenchus* (10%). They found either singly or in combination with two or more than two genera.

Out of 128 samples from cereal crops, 90 samples (78%) had plant parasitic nematodes and rest 22% samples had only saprophytic nematodes. A total of seven different genera (*Meloidogyne, Hirschmanniella, Criconemoides, Helicotylenchus, Aphelenchoides, Hemicriconemoides* and *Tylenchorhynchus*) were recorded from rice fields. The genera *Helicotylenchus, Tylenchorhynchus, Pratylenchus, Meloidogyne* and *Longidorus* are commonly recorded in maize fields. Seven different nematodes genera namely, *Helicotylenchus, Hirschmanniella, Tylenchorhynchus, Pratylenchus, Aphelenchoides, Longidorus* and *Meloidogyne* were detected from wheat fields.

The treatments of commercial *Trichoderma* varied significantly in root galing index and number of galls per root system, but not in fresh root weight. The lowest galling index on 0-10 scale was observed in *T. harzianum* (Nemastin) (1.6), followed by *T. harzianum*-T22 (Root Shield plus) (2.37) and *T. viride* (Ashtha TV) (3.43), which however, was not significantly different to one another. Considerably, lowest numbers of galls (9.13) was also observed in *T. harzianum*-Nemastin than all other treatments.

Significantly highest suppressive effect on the nematode population was achieved by *T. harzianum* (Nemastin) (*Rf* 0.45), followed by *T. harzianum* (T22) (*Rf* 0.61), *T. viride,* (Ashtha TV) (*Rf* 1.43) and *T. viride,* (Prarambha Trichoderma) (*Rf* 1.51). *T. asperellum* (*Rf* 2.10) and *T. viride,* (Prarambha Trichoderma) (*Rf* 1.51*)* did not show significant difference from each other.

The effect of application of locally isolated *Trichoderma* spp on *Meloidogyne incognita*, the result showed that the lowest *GI* was observed in T2 (*Trichoderma lixii* - T 209) follwed by T7 (*Trichoderma virens* - T 444) and T6 (*Trichoderma afroharzianum* – T 448). In term of reducing effects of RKNs on tomato seedling, the most effiecient ones were obtained from T 209 (74.79%) followed by T 448 (70.93%) and T 444 (62.12%). The lowest reproduction factor in T 209 (*Rf* 0.59) followed by T 444 (*Rf* 0.62) and T 448 (*Rf* 0.86) respectively. When the application was compared the strain of *Trichoderma* sp. collected from Sarlahi district of Medhesh Province (T 209) was found the most effective to reduce the population of root knot nematodes followed by the strains of Kanchanpur district of Karnali Province (T 444) and Chitwan district of Bagmati Province (T 448) respectively.

CHAPTER 1 - INTRODUCTION

Plant-parasitic nematodes are microscopic, pseudocoelomate, unsegmented worms, commonly described as filliform or thread – like multicellular invertebrates, a characteristic reflected by greek origin of the taxon name *nema* (= thread) and its nominative plural *nemata* (Bird and Bird, 1991). Plant parasitic nematodes live in host tissues to feed upon to grow and reproduce, and some of them can also feed on other microorganisms. They inhabit all parts of plant, including developing flowers, buds, leaves, stems, and roots. Every cultivated plant species has at least one nematode reported to parasitize it (Luc *et al.*, 2005). Different nematodes and sometimes numerous species can survive in the same feeding site. When there are two or more nematode species/genera present, their co-existence in the same location is possible, but never long-term stable and they tend to push out one another. The strength of competing is not exactly the same between the competitors and those who have more strength; they can survive and less competitive ones may either vanish, even extinct, or try to find another feeding site (Schoener, 1983). When the nematodes are present on a host in large population, they cause yield reduction. The overall average annual yield loss of the world's major crops due to damage by plant parasitic nematodes have been estimated at 12.3% (Sasser and Freckman, 1987). The average loss for the life-sustaining crops is estimated at 10.7%; for the economical important crops the average loss is estimated at 14.0%. Comparing losses of the life-sustaining crops in developing countries with comparable crop losses in developed countries, it found 12.6% and 7.0%, respectively (Sasser and Freckman, 1987). Nematodes can cause both direct and indirect losses. Direct losses are caused by both quantitative and qualitative yield reduction. Indirect losses are due to control measures and to the quarantine status of some nematodes. Some nematodes reduce a plant's

ability to resist fungal infection, thus compounding the damage; others transmit pathogenic viruses among plants. Sudden outbreak of the nematode problem has noticed to occur some times due to the favorable condition for nematodes in the certain area of the country.

About 95% of plant parasite live in the soil and feed in or on roots. Most agricultural sites are infested with at least one species of plant parasitic nematodes (Luc *et al.,* 2005). They feed with the help of special apparatus called stylet. According to their feeding strategy, plant-parasitic nematodes are broadly divided into three major groups: sedentry endoparasites, migratory endoparasites and ectoparasites. Among different plant parasitic nematodes root-knot nematode *Meloidogyne* spp are considered as the most important genus in worldwide basis in terms of number of species parasitized and extent of crop damage and loss.

In Nepal, more than 50 different plant parasitic nematodes have been reported in different cultivated crops. The first report on plant parasitic nematode (PPN) from Nepal was reported eleven different nematodes. They were *Aphelenchoides besseyi, Criconemoides* sp, *Ditylenchus dipasci, Hemicycliopora gracilis, Heterodera cruciferae, Longidorus elongates, Tylenchulus semipenetrans, Xiphinema index* and *Xiphinema diversicaudatum* (Bhatta, 1967).

Similarly, the genera *Aphelenchoides, Helicotylenchus, Heterodera*, *Criconemoide, Ditylenchus, Meloidogyne, Longidorus, Radopholus* and *Xiphenema* were reported from rice field across the rice growing area of the country (Amatya and Shrestha, 1969; and Sharma *et al.*, 2002). Root-knot nematode (*Meloidogyne graminicola*) is commonly found in rice field. In addition, others cereal nematodes such as rice root nematode (*Hirschmanniella* spp), foliar nematodes (*Aphelenchoides* sp and *Ditylenchus* sp) have also been reported from rice fields of Chitwan valley (Pokharel, 2001). The genera, *Helicotylenchus* and *Hirschmenniella* were detected from Kathmandu valley and its adjoining districts (PPD, 2013). Eight different parasitic nematodes

were detected from six districts in which the population of *Helicotylenchus* and *Meloidogyne* were found moderate level to cause yield loss in rice fields. (Baidya *et al.,* 2015). Some other genera such as *Aphelenchoides, Cactodera, Ditylenchus, Heterodera, Xiphinema, Radopholus* and *Longidorus* were also reported from different part of the rice growing area of the country (Baidya, 2013). In presence of large population, they cause significant yield reduction. The infestation may be visible in the field where the nematodes cause a stunted and yellowish plant growth. Sometimes, lower nematode number may occur without obvious symptoms of attack and so the effects may easily be overlooked (Baidya, 2013).

A survey report from Kaski district showed that root knot nematodes can be caused in an average 30 percent yield reduction in plastic house tomato cultivation. The damage may be visible in the field where the nematodes cause stunted and yellowish in patches plants growth. Lower nematode number may occur without obvious symptoms of nematode attack and so the effects of nematodes may easily be overlooked (Baidya *et al.,* 2017a).

Furthermore, plant parasitic nematodes are the most devastating and widespread parasites of the tomato (Sasser and Freckman, 1987). Among the plant parasitic nematodes, root-knot nematodes *(Meloidogyne* spp) are the most commonly prevalent parasite in vegetable crops in the country (Baidya *et al,* 2017b). *Meloidogyne incognita*, *M. javanica*, *M. hapla* and *M. arenaria* are the main four species of great significance for the reason of their wide spread distribution (Castagnone-Sereno, 2002). They cause dramatic yield losses in different agricultural crops worldwide that result to reduction in growth, quality and yield along with reduced resistance of the host against biotic and abiotic stresses. Second stage juvenile is the infective stage that enters host roots with the help of the stylet and becomes sedentary getting into the vascular cylinder where it makes giant cell as a specialized nutrient source, induced by the secretion of effector

protein (Subedi *et al.,* 2020). The disease is characterized by swelling/knots and galls all over the root system and affected plants may be killed in case of severe infestation (Sikora and Fernandez, 2005).

Once high population of *Meloidogyne* have developed in a field, it is difficult to suppress and maintain population at sufficiently low level. It has been reported that 26.5 to 73.3% reduction in yield in tomato due to root-knot nematodes causing about $125 billion annual losses world widely (Ansari and Asif, 2016). Nematode not only limits the crop yield but also makes the host favorable for other fungal and bacterial infections (Ashraf and Khan, 2010).

Nematodes can be managed by applying nematicides, practicing crop rotation, proper cultivar selection, and organic amendments or fallowing. The most reliable practices are preventive, including sanitation and choice of plant varieties. Beside this, Chemical control of plant parasitic nematode by the involvement of synthetic nematicides is one of the most potent and effective methods for nematode management. However, apart from its very high cost, chemical measures disturb the ecological equilibrium of environment. Recently, biological control through several antagonistic microbes such as *Trichoderma harzianum*, *Paecilomyces lilacinus*, and *Pseudomonas fluorescens* has gained special significance (Sharon *et al.,* 2001; Holajjer *et al.,* 2018). Organic soil amendments such as application of compost, poultry manures etc work to control plant parasitic nematode via different mechanism through stimulation of the activities of microorganisms that are antagonistic to nematodes, improvements of soil structure and fertility, alteration of the level of plant resistance, release of nemato-toxic compounds), parasites (fungi and bacteria) and other nematode antagonistic, biological control agents (Akhtar and Malik, 2000). *Meloidogyne*, one of the most harmful polyphagous endoparasites is the emerging threat that damages plants by devitalizing root tips and causing the formation of swellings of the roots

which limits the supply of water and nutrients that ultimately reduces the yield (Agrios, 2005). In this context, it is very important to assess the control measures of root-knot nematode disease. Considering these facts, research work will be the crucial step for the effective management practices of *Meloidogyne* and it is expected that this research in turn would increase production and productivity of tomato.

Nematicides either fumigants, which are usually liquids and enter the soil water solution from a gas phase or non-fumigant, granular or liquid compounds which are water-soluble are also used to control root knot nematodes. Commonly used nematicides such as non fumigants Carbofuran, Fostiazate (Taba *et al.,* 2006), and fumigants Methyl bromide, Chloropicrin (Luc *et al.,* 2005), etc have also been found effective in control of the root knot nematode.

Plant parasitic nematodes have long been known to cause extensive crop losses through reduced yields, shortened productive life, or lowered value of produce. Plant parasitic nematodes are cosmopolitan. The plants infected with root-knot nematodes have an unthrifty appearance and often show symptoms of yellowing, rotting, wilting and premature shedding of the foliage with severe stunting that result in huge losses to the infected crops (Saifullah *et al.,* 1990). It destroys crops and causes economic losses equal to those of' any other plant parasitic species. Plant-parasitic nematodes cause an estimated crop yield loss of 14.6% in tropical and sub-tropical climates and losses of 8.8% in developing countries (Juan *et al.,* 2013).

The occurrence and distribution of common plant parasitic nematodes in different vegetable crops is a continuous effort to be given for monitoring and identification plant parasitic nematodes which also help to find out the real situation of nematode variability and their population in the field condition.

Generally, chemical control is more widely used than resistant varieties in many developed countries. However, increasing concern of the environmental risks posed by nematicides and development of number of viable alternatives, the concept of chemical control is not only inefficient and uneconomic, but also biologically unsound and unacceptable to the community. Plant resistance and tolerance to parasitic nematodes have been increased in importance in the past decades with the cancellation of the permits for the use of DBCP (1, 2-dibromo-3-chloropropane) and EDB (ethyl dibromide) fumigant nematicides (Horst, 1983; Dubois *et al.*, 1990; Boerma and Hussay, 1992).

The suitability of a host for plant-parasitic nematodes is expressed as the ability of the nematode to multiply on the host. Continuous mono-cropping of highly susceptible host plants like tomato, brinjal, cucumber etc. for several years will increase the population of nematodes pest and cause severe damage of crops (Bridge, 1987). However, use of resistant/ tolerance cultivars or not host crops with susceptible crops reduced damaged caused by nematodes.

Host plant resistance has become an important strategy in the management of plant parasitic nematodes. Resistant crops provide an effective and economical method for managing nematodes in both high and low value cropping systems. In annual cropping systems, they can reduce nematode population to levels that wihout damaging to subsequent crops. Resistant cultivars can also produce higher yield of many crops and appear to hold the solutions to most nematode problems, particularly with the recent increase in the gene transfer (Luc *et al.*, 1990). In less developed countries and in low-cash crop systems plant resistance is probably the only viable long term solution to nematode problems.

1.1. OBJECTIVES

1.1.1. General objective

- To access the occurrence and distribution of common plant parasitic nematodes in different crops and identify non-chemical means (biological control agents) to manage root knot nematode.

1.1.2. Specific objectives

- To determine the presence of various plant parasitic nematodes in commonly cultivated crops in the country.
- Estimation and distribution of population density of different nematodes species.
- Efficacy testing of different isolates of *Trichoderma* spp against *Meloidogyne* spp on tomato plant.

1.2. SCOPE OF THE STUDY

The information collected from the study help know the status of existing plant parasitic nematodes in crop cultivated fields and also update the national pest list regarding plant parasitic nematodes presence in the country. It provides the information about the major problematic plant parasitic nematodes in respective crops and also prioritizes for their management activities.

Determining the presence of various nematode species and their relationships to plants will lay the ground work for future control of plant parasitic nematodes through proper use of bio-control agents like *Trichoderma* spp. Such information also may be useful in reducing minor crop damage previously attributed to other causes. The research findings are useful to farmers, researchers, non-government and international agencies and the students working in the field of plant pathological research.

CHAPTER 2 - LITERATURE REVIEW

In this chapter, an attempt has been made to review the work carried out on the investigation of plant parasitic nematodes from different crops and vegetables. An effort had been made to review the literatures pertaining to the present study on the management of root knot nematode through bio-control agents like *Trichoderma* spp.

2.1. General description of Plant Parasitic Nematodes

Plant-parasitic nematodes are microscopic (usually less than 1 mm long) and are armed with a spear-like device known as stylet that they use for feeding. This apparatus is inserted into the plant's cell and is used to withdraw the cell contents. These are elongated, cylindrical worm, tapering more of less at the head and tail ends and encased in a very tough and impermeable transparent of semi-transparent cuticle. They are generally vermiform, however in certain cases of nematodes where degree of parasitism has advanced, the males remain vermiform while the females are spherical, kidney shaped, saccate etc. Plant parasitic nematodes constitute one of the most important groups of organism inhabiting the soil in and around the roots of the plants. The first known report of the observation of plant parasitic nematode was made by John T. Needham in 1743, when he observed thousand of nematodes within the wheat gall (*Anguina tritici*). In 1855, a second plant parasitic nematode was recorded by M.J. Berkeley. He observed that galls produced on green house grown cucumber contained nematodes; the *Meloidogyne* species. Of the hundreds of different kinds of nematodes that infect plants, only a dozen or so species are known to be economically serious root-feeding pathogens in the tropics and subtropics (Schmitt and Sipes, 2000). There are fewer kinds of plant-parasitic nematodes that cause significant

damage by feeding on foliage, but as with the root feeding nematodes, new species are being discovered that cause significant foliar damage. If the numbers of harmful nematodes are large, plant growth is adversely affected. The primary groups of nematodes recognized as problems in the tropics and subtropics are the root-knot, cyst, burrowing, lesion, foliar, and reniform nematodes. Although these are associated with severe crop losses, information on actual yield loss is generally lacking. Other nematodes, such as the spiral, pin, and lance nematodes, may occur in abundance but usually do not cause sufficient damage to warrant concern (Schmitt and Sipes, 2000).

2.2. Prevalence of plant parasitic nematode in Nepal

The first report on plant parasitic nematode (PPN) from Nepal was reported in 1967. On the report, eleven different species of PPN was reported first time from Nepal. They are *Aphelenchoides besseyi*, *Criconema* spp, *Ditylenchus dipsaci*, *Hemicycliopora gracilis*, *Heterodera cruciferae*, *Longidorus elongatus*, *Tylenchulus semipenetrans*, *Xiphinema index* and *Xiphinema diversicaudatum* (Bhatta, 1967). Similarly, another extensive survey reported in addition 23 genera of PPN from different vegetable fields. Among them, the most commonly occurring genera are *Helicotylenchus*, *Criconemoides*, *Pratylenchus*, *Xiphinema*, *Heterodera*, *Longidorus*, *Meloidogyne*, *Anguina* and *Tylenchorhynchus*. The commonly recorded plant parasitic nematodes from the field of vegetables growing areas are *Aphelenchoides besseyi* in potato and chilly; *Aphelenchoides* in cauliflower, potato, and pea; *Criconemoides* in tomato, potato, radish, cauliflower, chilly, brinjal; *Helicotylenchus* in potato, tomato, brinjal, chilly, radish, mustard, cabbage, cauliflower; *Heterodera* in brinjal, radish, tomato, and cauliflower; *Longidorus* in chilly, cabbage, and potato; *Meloidogyne* in tomato, okra, brinjal and chilly,

cabbage, chinease cabbage, broccoli, radish, ginger, coriander, turmeric, pea, bean, black gram and lentil; *Pratylenchus* in potato, tomato, cauliflower, chilly, carrot, and pea; *Paratrichodorus* and *Paratylenchus* in potato; *Radopholus* in pea, black gram, *Rotylenchulus* in cauliflower, *Rotylenchus* in tomato, *Trichodorus* in tomato, *Tylenchus* in chilly; and *Xiphinema* in brinjal, chilly, mustard, potato, tomato etc (Amatya and Shrestha, 1969). Three more species, *Aphelenchoides avenae*, *Helicotylenchus dihystera* and *Meloidogyne javanica* were reported by Sam Page from Rothamsted Experimental Station, United Kingdom during 1980 (Mathur *et al.*, 1992). Likewise, one more species, *Meloidogyne incognita* was reported from potato field (Hooger, 1981).

In exploratory survey from Indian scientist, a new cyst nematode species, *Cactodera johanseni* also found on radish in a village Nagarkot in Bhaktapur district (Sharma e*t al.*, 2004). In 2009, a survey was carried out in the field of vegetable growing area of Chitwan, Kaski, and Kathmandu valley and identified the nematode. The commonly recorded nematode were *Meloidogyne* spp in tomato, broad bean, cowpea, radish, coriander, potato, pumpkin, chilly, brinjal, onion, and bitter gourd; *Helicotylenchus* spp, in radish, tomato, potato, broad leaf mustard; *Pratylenchus* spp in tomato, carrot, potato, *Tylenchorhynchus* spp in chilly, coriander; *Ditylenchus* spp in onion; *Rotylenchulus* spp in cowpea, cauliflower; *Paratrichodorus* in potato; *Hoploliamus* spp in cow pea; broad bean; *Trichodorus* sp in tomato; *Criconemoides* spp in brinjal and chilly; *Longidorous* spp in potato, chilly, cauliflower and *Xiphinema* spp in potato, tomato, chilly and brinjal (PPD, 2009). In fruit crops, the semi-endoparasitic nematode, *Tylenchulus semipenetrans* has been encountered in the citrus growing areas at Dhankuta and Pokhara valley (Manandhar and Amatya, 1985). In addition, nine plant parasitic nematode genera, namely *Pratylenchus, Meloidogyne, Tylenchorhynchus, Hoplolaimus, Helicotylenchus, Rotylenchulus, Xiphinema,*

Trichodorus and *Longidorus* have recorded in the soil samples collected from the field of papaya plants at Chitwan district. *Pratylenchus, Meloidogyne, Xiphinema* and *Tylenchorynchus* are more favored by papaya as compared to other genera. Similarly, *Rotylenchus, Helicotylenchus* and *Meloidogyne* in banana, litchi, papaya and pineapple, *Hemicriconemoides, Hemicycliophora* and *Tylenchorhynchus* in litchi, papaya and pine apple, *Aphelenchus* in pineapple and papaya and *Hoplolaimus* in banana and pineapple have also been recorded. These results indicated that *Rotylenchus, Helicotylenchus* and *Meloidogyne* are common nematodes associated with fruits trees in chitwan valley. In 1983, Khan has identified thirteen genera of plant parasitic nematodes including one new genus, *Psylenchus* associated with pineapple in Chitwan (Khan, 1983). All together 25 genera of plant parasitc nematodes were recorded in various crops fields in different time interval in the country (Table 1).

Table 1. List of Plant Parasitic Nematodes with their feeding habit and sites recorded in Nepal

S.N.	**Genus**	**Common Name**	**Feeding Habit**	**Feeding site**
1	*Anguina*	Seed gall nematode	Migratory endo-parasite	Seed, stem, leaf
2	*Criconemoides*	Ring nematode	Sendentary endoparasite	Root
3	*Ditylenchus*	Stem and Bulb nematode	Migratory endoparasite	Stem, leaf, bulb, tuber
4	*Helicotylenchus*	Spiral nematode	Migratory, ecto, endo parasite	Root
5	*Hirschmanniella*	Root rot nematode	Migratory endoparasite	Root
6	*Hoplolaimus*	Lance nematode	Migratory ectoparasite	Root
7	*Meloidogyne*	Root- Knot nematode	Sendentary endoparasite	Root
8	*Pratylenchus*	Lesion nematode	Migrat ecto Endoparasite	Root
9	*Radopholus*	Burrowing nematode	Migratory endoparasitic	Root, tuber
10	*Rotylenchulus*	Reniform nematode	Sedentary semi-endoparasitic	Root
11	*Tylenchulus*	Citrus root nematode	Sedentary semi-endoparasite	Root
12	*Xiphinema*	Dragger nematode	Migratory endoparasite	Root
13	*Belonolaimus*	Sting nematode	Sedentary ectoparasite	Root
14	*Aphelenchoides*	Foliar nematode	Ecto to endoparasitic	leaf and foliar part
15	*Tylenchorhynchus*	Stunt nematode	Migratary ectoparasitic	Root
16	*Paratylenchus*	Pin nematode	Migratary ectoparasitic	Root
17	*Trichodorus*	Stuby root nematode	Migratary ectoparasitic	Root
18	*Hemicycliophora*	Sheath nematode	Sedentary ectoparasitic	Root
19	*Rotylenchus*	spiral nematode	Sedentary ectoparasitic	Root
20	*Paratrichodorus*	Stuby root nematode	Migratory ectoparasitic	Root
21	*Scutellonema*	Dry rot nematode	Sedentary ectoparasitic	Root
22	*Longidorus*	Needle nematode	Migratory endoparasite	Root
23	*Heterodera*	Cyst nematode	Sendentary endoparasite	Root
24	*Cactodera*	Cyst nematode	Sendentary endoparasite	Root
25	*Tylenchus*	Filiform nematode	Ecto parasite	root

2.3. Different Features of Plant Parasitic Nematodes

About 95% of plant-parasitic nematodes live in the soil and feed in or on roots. Some invade leaf and stem tissues (Bird and Bird, 1991; Luc *et al.,* 2005). Plant-parasitic nematodes feed by inserting a needle like structure, the stylet, into plant cells. They have a great variety of feeding habits. Some species feed only on the outermost plant tissue, others penetrate to deeper tissues,

and still others induce their hosts to produce special nutrient sources upon which the parasites subsist. On the basis of their feeding habit plant parasitic nematodes are broadly classified into ecto and endo parasites. Some common genera (*Paratylenchus, Trichodorus, Tylenchorhynchus, Belonolaimus, Criconemoides, Helicotylenchus, Hemicycliophora, Hoplolaimus, Longidorus, Rotylenchus, Sclutellonema* and *Xiphinema*) are ectoparasites that can penetrate root tissues only by means of their stylets and not with their head; other genera are either semi-endoparasite or endoparasite. The semi-endoparasites genera (*Rotylenchulus, Tylenchulus*) penetrate the root by their anterior body end, the posterior end remaining outside the root surface and becoming highly swollen. Due to this phenomenon, they loose their locomotion and become sedentary. Endoparasitic nematodes are either sedentary or migratory. Some endoparasitic nematodes, such as root-knot nematodes (*Meloidogyne*) and cyst nematodes (*Heterodera, Globodera, Cactodera*), can develop their feeding site inside the root and become sedentary whereas other endoparasitic nematodes migrate in the root and form root lesions by burrowing the tissue and again invading other healthy root tissue. The genera *Pratylenchus*, *Radopholus* and *Hirschmanniella* belong to migratory endoparasites.

Most of the ectoparasitic nematodes have a rather long and powerful stylet. The stylet is used to pierce the epidermal cells of the root and the underlying layers of the cortex to suck out the cell contents. Sometimes, the nematodes bury its head and even its entire body into the host root. Some of them interact with soil inhabiting fungi. The genera *Paratylenchus, Trichodorus*, and *Tylenchorhynchus* feed on surface tissues and other genera feed on subsurface tissues: *Belonolaimus, Criconemoides, Helicotylenchus, Hemicycliophora, Hoplolaimus, Longidorus, Rotylenchus, Scutellonema* and *Xiphinema*.

Migratory endoparasites aggregate at the root surface and attack root hair cells with stylet thrusts. The cells are punctured several times. When the stylet enters the cell, salivation commences. Shortly after salivation the nematode starts feeding (pumping of medium bulb) and repeated stylet thrusts occur. They enter also the root tissues of the cortex. The nematode moves parallel to the central stele and feeds periodically. During migration the nematode punctures the cell wall. The cell wall is cut and the nematode moves to the next cell. All juvenile and adult stages may enter a root, but they can wander out, live for some time in the soil, and seek a new root.

Semi-endoparasitic nematodes penetrate the root by their anterior body end, the posterior end remaining outside on the root surface and becoming highly swollen. Due to this phenomenon, the nematode loses its ability for active movement. It becomes a sedentary ectoparasite or a semi-endoparasite. The genera *Rotylenchulus* and *Tylenchulus* are belong to the semi-endoparasitic nematodes.

Foliar nematodes may be endoparasitic or ectoparasitic. They are not attracted by host plants and their orientation is not affected by gravity or light. The nematode enters leaves through stomata when the surface is covered with a thin film of water or by penetrating the epidermis of the under surface. The nematode feeds on and destroys mesophyll cells. Movement within leaves seems to be delimited by leaf veins. Sometimes, they lives ectoparasitically within the folded crowns and runner or flower buds, so they are also called leaf and bud nematode. The nematode under genus *Aphelenchoides* sp is an example of foliar nematode and they can go overwinter in dormant buds and growing point. They exhibit considerable crytobiotic ability. It can survive in dry state for few years, mostly at the later stage of juveniles and adults.

Stem nematode, *Ditylenchus* is a migratory endoparasite and its all stages of development are capable of infecting plants. The fourth stage juvenile is the most important infective stage due to its outstanding ability to withstand desiccation and to undergo anabiosis. The nematode feeds on parenchymatous tissue are stems, but it is also found in the foliage, in inflorescences, buds, rhizomes, stolons and bulbs. They enter plant tissue through the stomata or penetrate directly at the base of stems and leaf axils. They cause the breakdown of the middle lamellae of cell walls. Feeding often causes swelling and distortions and mating and egg deposition and their further development take place within plant tissue. In drying plant tissue, the nematodes tend to aggregate and go to anhydrobiosis and survive for a long time.

2.4. Feeding and plant cell responses to nematode species

Various genera of plant parasitic nematodes have evolved intimate relationships with their hosts by the development of specialized feeding sites within plant tissues. This relation has led in most cases to a loss of nematode mobility, the adult females becoming sedentary endoparasites. The nematodes therefore become dependent on the feeding cells for nutrients and must regulate their demands so that the host cells remain alive.

Endoparasitic nematodes modify cells around their feeding sites. Two forms can be distinguished by those in which neighbouring cells fuse as a result of cell wall degradation and breakdown (syncytia), and those where wall degradation and cell fusion are not evident (giant cell). Both types have the same function providing nutrients for the developing nematodes. The giant cell induces by root knot nematodes divide without forming new cell walls. It is most likely formed through repeated endomitosis without cytokineses. At the beginning of the giant cell formation the cells are predominatly occupied by the cell vacuole, the nuclei are in the

peripheral cytoplasm. As cytoplasmic contents increase, the cells expand laterally and wall ingrowths develop on the wall of cells adjacent to vascular elements. As the food demand of the nematode increases with increasing egg laying, the giant cell cytoplasm shows signs of intense metabolic activity.

Syncytia induced by cyst forming nematodes originate in the pericycle, endodermis or adjacent cortex. The juvenile inserts its stylet into the initial cell of the syncytium and around this, a plug develops. The wall near the stylet doesnot dissolve but becomes thickened as the syncytium develops. Developing syncytia prevent the formation of new xylem elements. Wall fragments within syncytia, and walls at the cortical limit are thickened (polysaccharides). The cytoplasm of cells within a syncytium is typical of cells with high metabolic acivity, such as in root meristems. The density increases with many ribosomes, mitochondria, golgi bodies.

The permanent feeding site of adult *Rotylenchus* is usually initiated in an endodermal cell. The initial cell usually located near a protoxylem pole, expands to 4-5 times its normal size. The nucleus enlarges, cytoplasmic organelles increase and lysis of cell walls occur centripetally to the pericycle and producing a syncytium.

The nurse cells associated with *Tylenchulus* are situated in the cortex which is modified cells by nematodes around the head of the nematode. The cells contain an enlarged nucleus and are filled with cytoplasm. The head of the nematode is located in an empty cell in the middle of the feeding site.

Ectoparasite nematode like *Trichodorus* has feed on epidermal cells. They use their onchiostylet which is closed at the tip. The feeding of this nematode can typically be divided into five distinct phases that can also be recognized with other plant parasitic nematodes; they are exploration of the cell wall, wall perforation by stylet thrusting, salivation, ingestion of nutrient and withdrawal

of the stylet from the cell and movement away from the feeding site. Exploration of the cell wall is done by lip-rubbing without stylet movement. After selection of the feeding site, rods present in the pharyngeal lumen, are drawn forward and the onchiostyle is rapidly thrust at the wall in an attempt to perforate it. Repeated thrusts at the same spot perforate the cell wall. A tube formed along the rods of the protracted pharyngeal lumen is anchored in the perforation hole. Simialrly, genus *Longidorus* is equipped with a long, needle like stylet which enables them to feed on cells far below the epidermis. Feeding of *Xiphinema* species invariably causes the root tips to swell and to become terminal galls when feeding is continued for several days. They feed on a column of cells, feeding for a while on a cell at the surface and then moving progressively to cells below. This feeding at one site varies from a few minutes to several hours. Likewise, the stylet of *Hemicycliophora* penetrates to a depth of a few cells. The salivation period takes about two hours. Upon selecting a feeding site, the nematode becomes firmly attached to the surface by means of an adhesive polysaccharide plug formed between the stylet and an epidermal cell. Ingestion (pumping) takes several days. Contents from cells bordering the cell occupied by the stylet are removed (Figure 1).

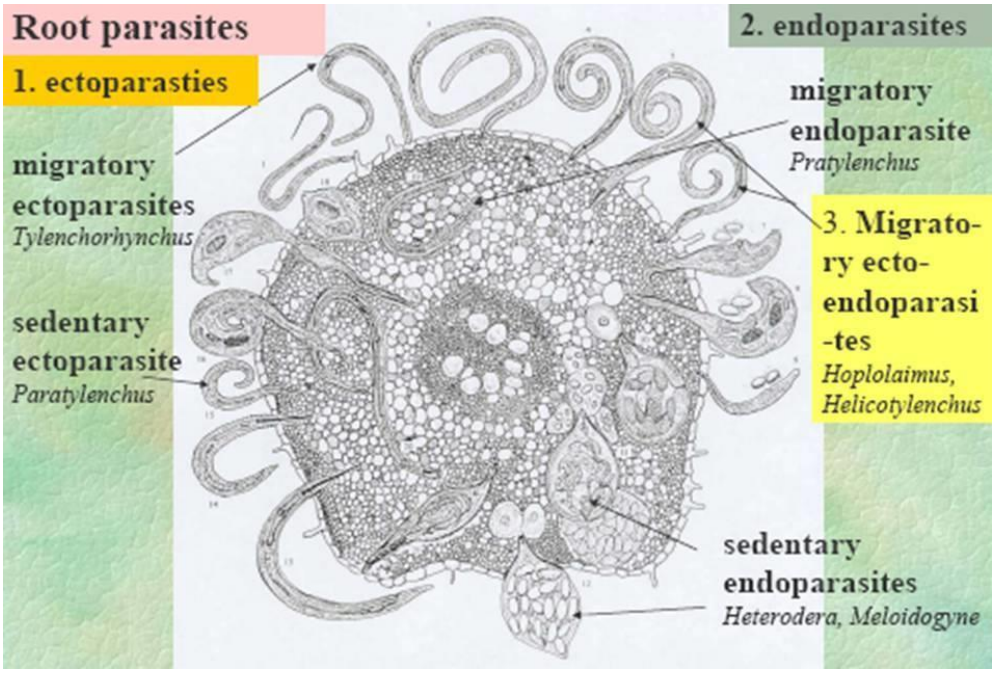


Figure 1. Diagramatic presentation of various types of nematodes feeding in root system
(*Source: Post Graduate International Nematology Course, PINC, 2005*)

2.5. Management of plant parasitic nematodes

2.5.1. Management of plant parasitic nematode through Soil amendment

Management of the plant parasitic nematodes has become an essential regarding the substantial loss in the crop yield and quality. Various strategies have been employed by researchers as well as farmers for the control the plant parasitic nematodes around the world. The root knot nematodes can be effectively minimized their population by the application of soil organic amendments such as oil cakes, saw dust, urea and bagasse (Sikora *et al.*, 1979). Barbarola (1982) achieved good control of root knot nematode with cow dung and poultry manure. Amending soil with animal manure and agricultural byproducts has reduced *Meloidogyne* spp number on variety of crops. The organic amendment tends to alter the host parasite relationship in favour of the crop and also increases plant vigor, enabling plant to withstand nematode attack (Singh *et al.*,

1986). Furthermore, organic amendments are also used against nematodes and act in one of two ways: those that contain high concentrations of nitrogen may release ammonical nitrogen in sufficient concentrations to kill the nematode and decomposing organic materials may also enhance the activity of biological control agents such as parasitic fungi and predatory nematodes. Because of environmental health hazards, application of synthetic nematicides (Velum Prime, Fosthiazate) is the last resort in integrated disease management in situations only where nematode population is potentially sufficient to cause economic damage to the next crop. However, they are the most rapid means to control root-knot nematode infection (Hay *et al.*, 2014).

2.5.2. Management of plant parasitic nematode through bio-control agent

Biological control is considered to encompass control that results from the action of soil microorganisms and the soil microfauna and is mediated through mechanisms such as parasitism, predation, competition and antibiosis (Stirling, 1991). When one living organism is used to inhibit the activity of a living plant pathogen, it is said to be a biological control agent (BCA). Biological control agents of plant diseases are most often referred to as antagonists. Many fungal species are known to infect nematodes but only a few are considered suitable as biological control agents (Siddiqui and Mahmood, 1996).The development of biological control agents is also considered an effective alternative for nematode control on vegetables (Kerry, 1987; Sikora, 1992).

Nematodes have also been successfully controlled by the bio-control agent (Kerry, 1984; Morgan-Jones *et al*., 1984; Khan and Hussain, 1988). *Pasteuria penetrans* is an obligatory parasite of nematode including *Meloidogyne* (Birchfield and Antonpoulos, 1976). Similarly, root

knot nematode can also be controlled by *Pochonia chlamydosporia* (Van damme *et al.,* 2005). *T. harzianum*, which is known to be effective against fungal diseases, also has capacity to minimize the population of root knot nematode in soil condition (Baidya *et al.,* 2008). Control of root knot nematode with an Indian strain of *T. harzianum* was enhanced by adding the antagonist to the soil amended with neem cake (Rao *et al.,* 1997). Both *T. harzianum* and *T. lignorum* increased plant growth and reduced *M. javanica* galling in tomato and aubergine in soil treated with the fungi 18 days prior to planting in greenhouse tests (Sharon *et al.,* 2001).

Soil application with conidal suspension of *T. harzianum* significantly reduced nematode population densities and root knot development in okra and mungbean. *T. harzianum* also elevated plant height and fresh shoot weight of both okra and mungbean (Siddiqui *et al.,* 2001). A greenhouse experiment was conducted to evaluate the effects of different inoculum densities of two Saudi isolates of *Trichoderma harzianum* and *T. viride* against *Meloidogyne javanica* on tomato. Four densities (10^4, 10^6, 10^8 and 10^{10} spores/g of soil) of each fungus were used. The result indicated that all four inoculum densities of the two *Trichoderma* species suppressed the nematode reproduction and root galling; and increased the growth of tomato (Ahmad and Tariq Javeed, 2016). Based on the results by Mayer *et al.*, 2001, the biological control agents, *Trichoderma virens* and *Burkholderia cepacia* were applied alone or in combination agasint *M. incognita* in soil one week after nematode inoculation were significantly effective in improving plant growth and reducing number of galls and juveniles penetration per root system. *T. virens* was more suppressive than *B. cepacia*. Dos Santos *et al* (1992) reported *T. harzianum* as an effective egg parasite of *M. incognita*. *T. harzianum* was able to grow on the egg surface and penetrated the egg shell (Saifullah and Thomas, 1996). Saifullah (1996) has used *T. harzianum* against potato cyst nematode (*Globodera rostochiensis)* with excellent results. The protective

application of *T. harzianum* and *Moringa oleifera* leave showed lowest number of juveniles of *Meloidogyne javanica* on eggplant as compared to other treatment (Murslain *et al.,* 2014). In vitro studies demonstrated that all tested isolates of *Trichoderma harzianum* and *T. viride* for the biological Control of *Meloidogyne incognita* on tomato were effective in causing second-stage juvenile (J2) mortality compared with the control (Dababat and Sikora, 2007). Nematode populations were significantly reduced in the *Trichoderma harzianum* T22 treated soils on cowpea varieties. One month after treatment soil nematode population reduced from well over 200 to as low as between 6-9 in 200 ml soil and the untreated soil almost doubled the initial population (Izuogu *et al.,* 2014).

Like *Trichoderma* spp, other biological agent such as found that *Bacillus subtilis* was effective in controlling *M. incognita* on cotton and sugar beet, *M. arenaria* on peanut and *Rotylenchulus reniformis* on cotton (Sikora, 1988). Strains of *Pseudomonas chitinolytica* also were shown to reduce *M. javanica* on tomato as reported by Spiegel *et al* (1991).

The population of *M. hapla* was reduced more effective at higher rates of chlamydospores. *P. chlamydosporia* colonized eggs of *M. hapla* and reduced population growth at the lower levels of nematode infection but failed to reduce population growth at 8 eggs per cm^3. This resulted in higher growth reduction of lettuce plants (Vianene and Abawi, 2000).

When tomato, cucumber and clover were treated with rhizobacteria as soil drenches or root dip treatments they suppressed significantly the penetration of nematodes in the roots and reduced the root galling of the root-knot nematode, *M. incognita* under greenhouse conditions as reported by Zavaleta-Meija and Van Gundy (1982) and Becker *et al* (1988). The same results were obtained by Sikora (1988) on cotton, tomato, peanut and sugar beet when treated with *Bacillus subtilis* to control *M. incognita, M. arenaria* and *Rotylenchulus reniformis* under greenhouse

conditions. He revealed that *B. subtilis* reduced *M. incognita* 43% to 66% on cotton when applied as a powder and 38% to 62% when applied as a liquid seed dressing.

Pasteuria spp which is bacterial parasites of various plant-parasitic nematodes occurring naturally in Florida, may be promising biological control agents. In an experiment on tobacco showed that *M. incognita* and *M. javanica* were suppressed exclusively by *P. penetrans*. Tobacco plants treated with *P. penetrans* had fewer galls, egg masses, and eggs than plants that either did not receive *P. penetrans* (Krueger and McSorley, 2008).

Treatment with filtrate of *P. luminescens* and combined effect between *Verticillium chlamydosporium* and *P. luminescencs* (Bacterial and fungal bio-agents) showed the highest effect on the numbers of egg-masses and females of *M. incognita* infecting cucumber (Zakaria *et al.,* 2013). *Pasteuria penetans* is a common parasite of *Meloidogyne* and is often found attached to juveniles. The spore forms of *P. penetrans* can resist both draught and exposure to non-fumigant nematicides (Mankau and Prasad. 1972). The fungal bio-agents like *Aspergillus fumigates, A. terrus, P. lilacinus* and *Trichoderma viride* improved plant growth parameters and reduction in nematode multiplication in Balsam (Goswami *et al.,* 2005).

2.5.3. Management of plant parasitic nematode through chemical

Nematicides either fumigants, which are usually liquids and enter the soil water solution from a gas phase or non-fumigant, granular or liquid compounds which are water-soluble are also used to control root knot nematodes. Commonly used nematicides such as non fumigants Carbofuran, Fostiazate (Taba *et al.,* 2006), and fumigants Methyl bromide, Chloropicrin (Luc *et al.,* 2005), etc have also been found effective in control of the root knot nematode.

Root-knot nematodes parasitize a wide range of crops, often impacting both the quantity and quality of plant causing substantial yield losses. Use of nematicides is one of the most reliable means of managing root-knot nematodes. Chemical control of root knot nematodes have primarily been achieved through nematicides which can be non-fumigants or fumigants (Widmer and Abawi, 2000).

Non fumigants namely, Nemacur, Aldicarp, Oxamyl and Carbofuran are applied during planting and are systemic affecting the nematodes behavior. Fumigants are volatile liquids that are fast acting and dissolve in the soil killing the nematodes and their eggs. Examples of fumigants are 1, 3 dichloropropene methyl bromide, ethylene, Metham sodium and Dazomet (Njoroge, 2014). Although chemical nematicides have been widely used in commercial agriculture to control nematodes, they are both highly toxic and very expensive leading to banning of some fumigants like methyl bromide. Chemical agents like carbofuran are efficient in controlling nematodes (Adegbite and Agbaje, 2007). They had confirmed the suppressive effect of Furadan (Carbofuran) application on root knot nematode *M. incognita* race 2 multiplications on yam hybrids. Bhardwaj *et al* (1985) evaluated the nematicide, Furadan (3 G) to control the root knot nematodes *Meloidogyne* spp infesting okra and egg plant in Chitwan, Nepal.

Ethylene bromide (15%) @ 20 gallons per acre gave the best control of *Meloidogyne incognita* with the highest yield of tobacco in Nathalis, Victoria (Meagher *et al.,* 1966). Pretreatment of seedlings with Marshal 250 EC-chemical agents, whose active ingredients is Carbosulfan, was the most effective in reducing both the number of galls and juvenile (J2) population in experiments conducted by Njoroge, 2014.

2.5.4. Management of plant parasitic nematode through cultural practices

The combined use of rotation, resistant cultivars, fallowing, deep tillage, soil solarization, trap crops (marigold), flooding and other cultural practices minimize nematode damage and over time, reduce the nematodes to low population levels (Meadows *et al*., 2018). Also, phytochemicals provide another environment-friendly, safe and reliable option to suppress plant pathogenic nematodes (Mukhtar *et al*., 2004; Khan *et al*., 2019).

The degree of root knot damage to processing tomato crops in Philippines was less severe in rotations of rice and tomato than in rotations without rice (Sikora, 1989). Similarly, another report showed that root knot juveniles are killed after exposure to anaerobic condition after few days of flooding (Padgham *et al.*, 2003). Neem based oil cakes and related products have been studied intensely in India for control of root knot nematodes. They have been used alone (Singh and Sitaramaiah, 1967) and combined with biocontrol agents *Trichoderma harzianum* (Naik *et al*., 1998). Solarization applied in the summer in Morocco before the next tomato in plastic greenhouses led to a 90% reduction in *M*. *javanica* densities when compared with the controls (Eddaoudi and Ammati, 1995).

Plantation of trap crop for a short duration of time to ensure good nematode penetration and then the developing sedentary juveniles in the root tissue are killed by root removal from soil or by destruction of the root by physical means or herbicide. Good hosts like raddish, lettuce were used as trap crops for root knot nematodes for 35 days in soil could be killed large numember of RKN juveniles in Cuba (Plotter and Olthof, 1993). Similarly, non host crops as cover crops are also reduced RKN population in soil. Planting of velvet bean (*Mucuna pruriens*), horse bean (*Canavalia ensiformis*) and joinvetch (*Aeschynomene americana*) were used to control nematodes population in the Southern USA (McSorley *et al.*, 1994).

Aother crops have nemacdial properties which as consider the antagonistic crops are also used to control the nematode problem (Pandey *et al.,* 2003). Marigold, sunnhemp, castorbean, partridge pea, asparagus and sesame have been studied extensively for nematode control activity. They were used in rotations to control *M. incognita* on tomato. Among which marigold had the greatest negative effect (Swamy *et al.,* 1995). Tagetes (Merigold) known to produce tertheinyl and derivatives of bithienyl that are toxic to root knot nematodes (Zavaleta-Mejia and Guandy, 1982). Ploeg (1999) demonstrated that *Tagetes patula*, *T. erecta, T. signata* and *Tegates* hybrid reduced galling in a subsequent susceptible tomato crop compared with tomato tomato rotation.

One of the most effective and innovative techniques developed for root knot nematode control is the grafting of commercially valuable crop cultivars on the nematode and other disease resistant rootstocks. When the shoot of tomato were grafted on to the rootstocks of *Solanum torvum*, *S. sysimbriifolium* and *S. aethiopicum* and compared with plants maintained on their own roots, the Solanum and tomato rootstocks all reduced plant susceptibility to *Meloidogyne*, with *S. torvum* the best combination for both control and yield (Mian *et al.,* 1995). Whereas, application of grafrted plant with wild brinjal (*S. sysimbriifolium*) with susceptible tomato cultivar Thim-16 planted in root knot nematode infected fields at Hemja, Kaski district increased an average of 37% of tomato yield as compared to narmal tomato plants. The survival duration of plant life was also increased more than two months than the normal plants (Baidya *et al.,* 2017b).

2.6. Abiotic influences on Plant Parasitic Nematodes

Soil inhabiting nematodes appear well adapted to live in the soil pores. Due to small sizes and their type of locomotion, nematodes can easily move through most of the soil cavity. The hydrophilic cuticle ensures that any water in contact with the nematode is distributed in a film

over its body. Extreme moisture conditions in a soil do not favor nematodes. Flooding may kill nematodes and is used as a control method. Drying out of the soil also reduces nematodes and can be used in controlling the nematodes. Soil moisture affects survival of nematodes. In saturated soils, the air spaces are reduced and so lack of aeration probably reduces nematode activity. *Meloidogyne* eggs are well protected in their gelatinous matrix. Increasing the humidity will increase their survival even in the protecting matrix.

Nematodes activity increases with aeration. Oxygen concentration influences the emergence of juveniles from cysts or eggs, the higher the concentration, the higher the emergence. Carbon dioxide has a negative influence on growth of nematode; it may be stimulatory at low concentration.

Different nematodes activities (hatching, movement, penetration etc) may have different temperature requirements. Temperature relations may vary with the host plant. Depending on their age, individuals may have different requirements even between populations of the same species differences in requirements may exist. Most plant parasitic nematodes become inactive at temperature between 5-15 °C, their optimum ranges is about 15-30 °C, and the high temperature range for inactivity is 30-40 °C. Outside this range, temperatures are often lethal for nematode development. Nematode development is frequently related to so-called day temperature and the lower temperature limit (8 °C).

Due to differences in salt concentrations in the nematode body and in the soil solution, water tends to move through cuticle to restore osmotic equilibrium. This may result in shrunken body or juviniles inside eggs. The survivability of nematodes is high in low salinity in soil containing host plants.

Soil texture is strongly related with other physical parameters such as particle size, pore space, percentage of organic matter. The size of particles or crumbs affects nematode movement directly in that it controls the pore size. Small pores prevent nematode passage, if the pores are too large leverage becomes a problem. It was demonstrated that the maximum nematode mobility is obtained when the nematode length is three times the diameter of soil particles. If the water film in which the nematode lives is too great, there is slippage and if the film is too thin, there is too much friction and mobility is inhibited.

2.7. Biotic influences on plant parasitic nematodes

It has been demonstrated that plant parasitic nematodes distribution is often correlated with root distribution. The plant parasitic nematodes are attracted to the roots. This implies that there is some nematode movement in the rhizosphere, but it also implies that not all movement is random. The area of the root tip is very attractive since it is a region of high metabolic activity from where many substances diffuse. Host plants govern nematode distribution both horizentally and vertically. Besides, the host plant many other biological factors influence plant parasitic nematodes. Some of them are thought to be potential biological agents. Some bacteria like *Pasturia penetrans* parasitic to phytopathogenic nematodes. More is known about nematodes trapping and parasitic fungi. Some of them are egg parasites; others penetrate nematode bodies after their spores germinated on the nematode's cuticles. Biological agents control nematodes through two main kinds of mechanisms of action, i.e., those that include the production of secondary metabolites (antibiosis), lytic enzymes, parasitism and space, nutrient and other resource competition by bio agents (Poveda *et al.,* 2020). *Meloidogyne* species have been successfully suppressed by using several biological control agents (Akram *et al*., 2020). Root-

knot nematodes problem can also be decreased by the application various bio agents like *Pochonia chalamydosporia* (Van damme *et al.,* 2005), *Trichoderma,* ambuscular micorrhizal fungi (Schoutenden *et al.,* 2015), *Paecilomyces lilacinus* and a group of microorganism like Rhizobacteria (Luc *et al.,* 2005).

Trichoderma, arbuscular micorrhizal fungi (AMF), *Paecilomyces lilacinus, Glomus mossae* and a group of microorganism like Rhizobacteria (Luc *et al.,* 2005). The biological control agents like *Trichoderma harzianum, T. virens, and Catenaria anguillulae* have showed promising results against the nematode (Pathak and Kumar, 2003). The research by Sharon *et al* (2007) showed that *T. asperellum* parasitizes on egg masses, their derived eggs and the second stage juveniles (J2s). When the egg masses are destroyed, the number of the infective juveniles is reduced as well as the overall number of nematodes. Thus, this explains the reason for the reduction in the number of J2 nematodes that were observed from the soils treated with *T. asperellum* compared to the soil in the control.

CHAPTER 3 - MATERIALS AND METHODS

3.1. Identification of Plant Parasitic Nematodes in various crops

3.1.1. Soil Sample collection

Field survey was carried out in different districts of the country to find out prevalence of plant parasitic nematodes in soil under cultivation of major crops. Soil samples were collected according to season of the crops. In total of 339 soil samples were collected from 15 different crops including cereal (rice, wheat and maize) and vegetables (cruciferous and solonaceus crops). Out of 339 samples, 211 samples were collected from vegetables and 128 samples from cereal crops fields. In each site, around 200 g soil was taken from 6 spots each, from surface to a depth of 15-20 cm, close to the plant roots, with help of soil auger. The soil samples of 6 spots were mixed well and taken again 200 g soil as a final composite sample. Sampling was done in a zigzag pattern in each field. Immediately after collection, soil samples were kept inside plastic bags, mouth closed with rubber bands tightly to prevent moisture loss and labeled with sample number, location, name of crop and date of sampling. These samples were brought to laboratory of National Plant Pathology Research Centre, NARC, Khumaltar and stored in a cool place until use.

3.1.2. Selection of crops

The samples were collected from 15 different crops (Rice, wheat, maize, tomato, potato, lady finger, chilly, capsicum, broad leaf mustard, radish, carrot, cress, brocauli, cabagge and

cauliflower) from 10 districts (Kathmandu, Dhading, Kavrepalanchok, Lalitpur, Chitwan, Ramechhap, Bhaktapur, Sindhuli, Nuwakot and Dolkha) during 2020-2022 AD.

3.1.3. Extraction of nematodes from soil

Juveniles and adult nematodes were extracted from soil following the method described by Hooper *et al.* (2005). Each sample was crumbled finely with hand. Tissue paper was spread inside plastic sieve placed on extraction plates (plastic plate, extraction tray, plastic tray). The soil samples were spread over the tissue paper as shown in Figure 2. Clean tap water was carefully added from one side of the extraction tray until the soil layer was wet totally. The extraction tray and the sieves were labeled to identify the soil samples. The extraction sets were left as such for 24 hours and motile juveniles were moved from soil to the extraction tray.

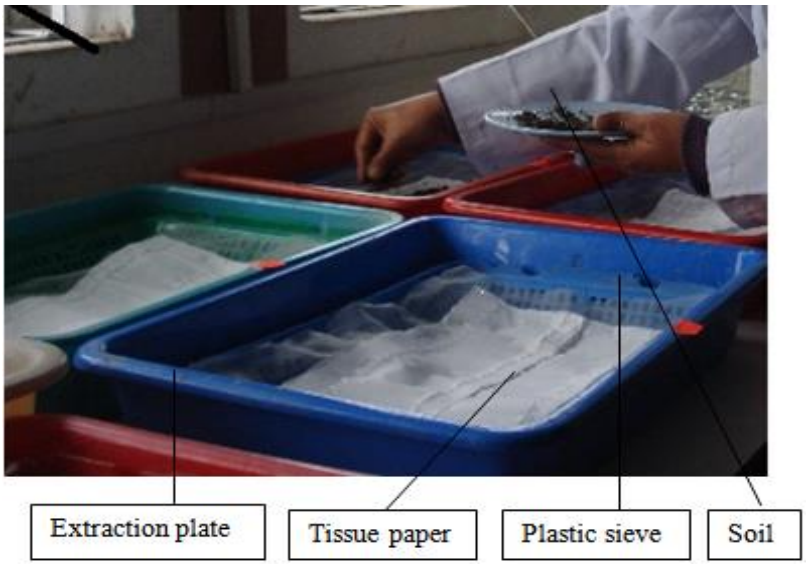


Figure 2. Preparation of sieve and spreading of soil for extraction of nematodes

After 24 hours, the plastic sieves with soil were lifted up to drain the water into the extraction tray, and the soil was removed. The water with nematodes from the extraction tray was poured

into labeled beaker. Wash bottle was used to rinse the tray and the water was also added into the beaker. The volume of water with nematode juveniles was 300 ml. It was taken as a stock sample or suspension. The suspension was left as such for an hour to settle down the nematodes. Ten milliliter suspension from the stock sample was pipetted out after stirring well and kept into empty petri-plates for isolation of juveniles. Around 10 ml solution from the stock sample was pipetted out mixing uniformly and kept in the petri plates for extraction. Manual fishing was done using nematode fishing needle and collected them in the cavity blocks. The nematodes numbers and genera/species were diagnosed under compound stereo-microscope and compound microscope.

3.1.4. Identification of nematodes

The juveniles and adult plant parasitic nematodes were observed and identified under stereoscope and compound microscope (40 to 100 × magnification). The plant parasitc nematodes and saprophytic nematodes were diagnosed with the help of stereoscope and all the plant parasitic nematode were picked up and kept into cavity block with fishing needle (Figure 3). The genera of plant parasitic nematodes were identified under compound microscope with their external and internal morphological features on the microscope in 40-100 × magnification based on photographs and description (Luc *et al.,* 1990).

Figure 3. Identification of nematodes under microscope

3.1.5. Occurrence of plant parasitic nematodes in soil

Total number of each type of plant parasitic nematodes present in 10 ml of suspension was counted, and from this number, total number of nematodes present in 300 ml stock suspension (i.e. 100 g soil) was calculated. Number of juveniles/adults of each genus present in total number of soil samples of each crop was added and also mean number/sample calculated, by dividing total number of each genus by the total number of samples diagnosed for each crop. Finally, total numbers of all genera with respect to crops were also computed.

3.2. Efficacy testing of different species of *Trichoderma* for the management of root knot nematode (*Meloidogyne* spp) in tomato

3.2.1. Collection and multiplication of *Meloidogyne* spp

Juveniles and eggs of *Meloidogyne* spp were collected from soil and galls of tomato plants from infested fields. The galled roots and soil samples were multiplied in sterilized soil containing pots with plainting tomato plants in screen house for multiplication of the nematodes at National Plant Pathology Research Centre (NPPRC), NARC, Khumaltar (Figure 4). Three weeks old, healthy, three tomato seedlings were transplanted in each pot. The plants were grown under screen house and nematodes were allowed to multiply into the roots of tomato until their use.

Figure 4. Multiplication of *Meloidogyne* spp in roots of tomato inside screen house

3.2.2. Inoculum preparation of root-knot nematodes

The gall infected tomato roots were uprooted and cut into 1-2 cm pieces. These roots were kept in 250 ml conical flask containing 200 ml of 0.5% sodium hypochlorite (NaOCl) solution then shaken vigorously for 4 mins to extract nematode eggs by sieving method as mentioned by

Barker *et al*. (1985). For collection of juveniles, the egg suspension were poured on filter paper and incubated at 28±2 ºC to obtain freshly hatched juveniles (J2). Juveniles were collected within 2-4 days after incubation (Figure 5).

A

B

C

D

Figure 5. Daigramatic illustration of RKN eggs extraction from tomato roots; Cutting of gall infected root in 1-2 cm (A), Well shaking of roots in NaOCl solution (0.5%) for 4 mins , Seiving of solution in 30 µm mesh sieve for extraction of eggs (C) and collection of eggs from sieve (D)

3.2.3. Nursery bed preparation

Seeds of Shrijana hybrid variety of tomato were sown in plastic trays filled with cocopeat. The trays were covered with a polythene sheet till germination starts. After 6 days, the trays with germinated seeds were placed individually on the raised beds inside the shade net. Regular watering was provided to proper growth of plants.

3.2.4. Pot filling with soil for experiment

A 100 kg of soil was mixed with 1 kg of urea, 500 g of DAP, 300 g of MOP and 2 kg of well decomosed poultry manures to prepare the potting mixture and then was sterilized in an oven at a temperature of 120 °C for 30 mins. Each plastic pot was filled with 1 kg of mixture. Three weeks old seedling was transplanted to a pot and watered as per required (Figure 6).

Figure 6: Transplantation of tomato seedlings at screenhouse

3.2.5. Screen house experiment

With an objective to find out the efficacy of management strategies for root knot nematode, two different screen house experiments were conducted to explore the possibilities of employing *Trichoderma* spp against root knot nematodes (*Meloidogyne* spp). First experiment was established with seven different treatments including one control in three replications including one control in RCBD. Tomato (HYB. Shrijana) seedlings were transplanted in a pot of diameter

12.5 cm. Each pot was filled with sterilized soil and compost at ratio of 4:1. The texture of soil was 69.3% sand, 22% silt and 8% clay i.e., sandy loam soil. In screenhouse, the temperature was recorded with an average of not less than 24 ºC during cropping period. The soil moisture adjusted to 40-50% of the field capacity. Five pots were used for each treatment. Pots of each treatment were adjusted in 10 cm distance and each block of replication was arranged in 50 cm distances as shown in figure 7.

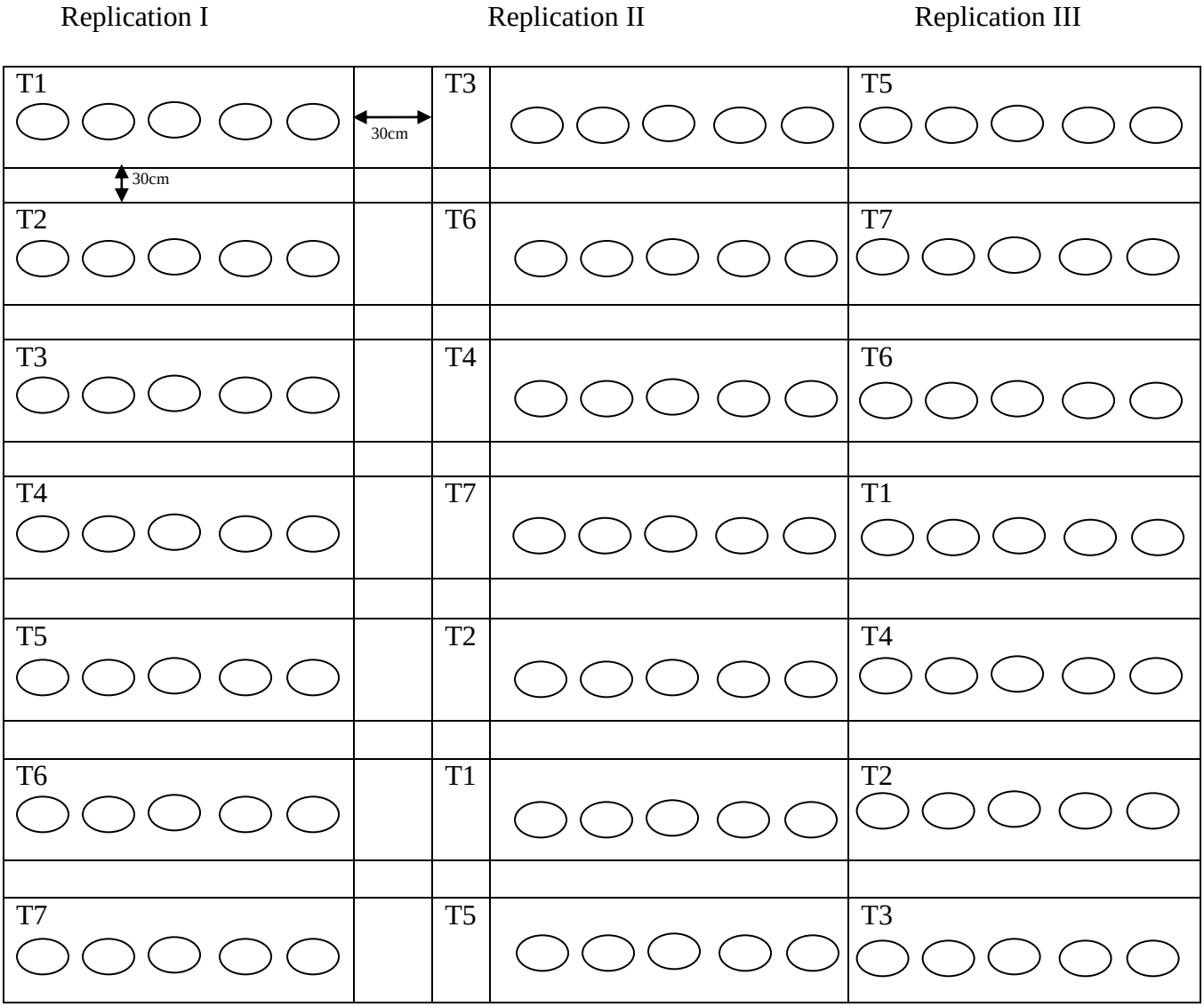


Figure 7. Sktch diagram of pots arrangement in RCBD in screen house, Khumaltar

3.2.6. Cultural practices

The plants were grown at 24-28 °C inside the screen house. Hand weeding and irrigation was done as per required. Staking of plants was done to each plant with the help of bamboo sticks. Other foliar pests like powdery mildew and aphid were managed with sparying Karathane 1.5 ml/l and Cypermethrin 2 ml/l in 10 days interval for 2-3 times respectively.

3.2.7. Inoculation of eggs inoculum of root knot nematode

The pots were inoculated with 5000 eggs of root knot nematode (RKN) per pot (@ 5 eggs/g of soil) after a week of the transplantation. The inoculum density was maintained at 500 eggs per ml. Shallow holes were made close to each plant and nematode eggs were inoculated using a glass pipette.Three equidistant shallow holes (3 cm) surrounding the root zone of the plant were made and nematode eggs were inoculated uniformly using a pipette as shown in figure 8. Immediately after inoculation the holes were covered with soil.

Figure 8. Innoculation of nematodes in pot inside screen house at Khumaltar, NARC

Disease Severity (DS %) was calculated by using the equation given by Galanihe *et al*. (2004) as:

Disease Severity (DS %) = $\frac{\sum(S \times n)}{N \times Z} \times 100\ \%$

where, S= severity score (Table 2), n = number of affected plants having same score, N = Total number of plants observed, and Z = Maximum scale number (10)

3.2.8. Counting of nematodes (eggs/juveniles)

Tomato plants were uprooted and washed to remove adhering soil and eggs were extracted using NaOCl by sieving method (Barker *et al.*, 1985) and counted under stereomicroscope. Similarly, the number of juveniles from soil sample by modified Baermann tray method (Figure 9).

The soil from rhizosphere of the plants of each respective pot was mixed properly and 100 g of soil was placed uniformly over the paper tissue, which was superimposed on a stainless steel screen. It was then placed in the plastic tray and water was added just to cover the soil and incubated at 21-24 °C for 24 hrs. The juveniles were collected from the trays. Fishing of juveniles was carried out and collected in cavity block and then counted the juveniles of *Meloidogyne* based on their morphology (Figure 10).

Figure 9: Juveniles extraction through modified tray method

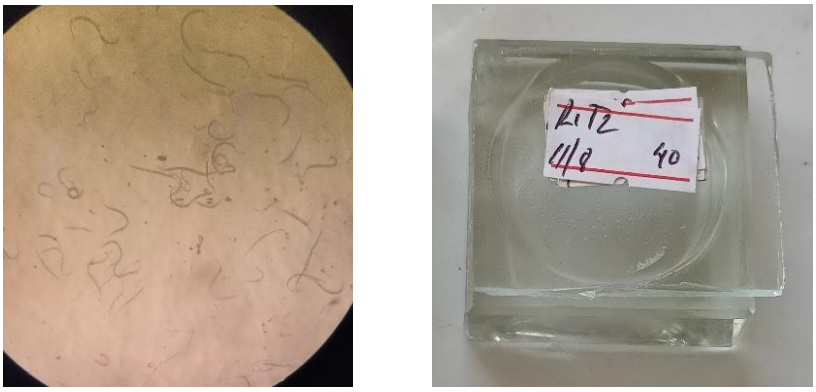


Figure 10. Fishing of juveniles and collected in cavity block for counting the Juveniles

- % reduction of nematode population (eggs and juveniles)

It was calculated as given by Raddy *et al.* (2013):

$$\% \text{ of reduction} = \frac{Control - treatment}{control} \times 100$$

- Nematode reproduction factor (*Rf*)

$Rf = \frac{Pf}{Pi}$ where *Pi* = nematode initial inoculum, *Pf* = nematode final population

The smaller the reproduction factor the greater the efficacy of the nematicide (Raddy *et al.*, 2013).

Table 2. Estimation of galling index with explanation of rating

Galling index (0-10)	Root system galled (%)	Explanation of rating
0	0	Complete and healthy root system, no infection
1	10	Very few small galls can only be detected upon close examination
2	20	Small galls/ knot only but clearly visible main root clean
3	30	Some larger knot visible, main root clean
4	40	Larger knot predominate but main root clean
5	50	50% of root infested. Knotting on some main roots. Reduced root system
6	60	Knotting on main roots
7	70	Majority of main roots knotted
8	80	All main roots, including tap root, knotted. Few clean root visible
9	90	All roots severely knotted. Plant usually dying
10	100	All root severely knotted. No root system. Plant usually dead.

Source: John Bridge & S.L.J. Page.1980. Estimation of root-knot nematode infestation levels on roots using a rating chart. *Tropical Pest management* 26: 296-298

3.2.9. Preparation of *Trichoderma* spp

Commercial product of three different *Trichoderma harzianum* (*T. harzainum*-**Nemastin**, *T. harzainum* (T 22) - **Root shield plus** and *T. asperellum*), and three commercial product of *T. viride* (*T. veride* – **Ashtha TV,** *T. viride*- **Biocare-F** and *T. viride*- **Prarambha**) (Table 3) were multiplied in PDA plates. The plates were incubated at 25 ^{0}C for two weeks. As the *Trichoderma* colony grew on PDA, it formed concentric rings and changed colour from white to green as it matured and sporulated. The plates were then used for conidia extraction. The mycelia and conidia developed on PDA were carefully scraped with a slide, and suspended in 500 ml distilled

water for each species. Spores were separated from mycelia by sieving through a muslin cloth. The spore suspensions collected in a beaker were adjusted to cfu 1×10^6 spores/ml after counting spores using a haemocytometer. One week after transplanting of seedlings, 3 cm deep three holes were made with a stick around each plant at rhizosphere and 5 ml spore suspension was poured/hole (15 ml/plant). The same concentration of *Trichoderma* spores solution was prepared and again inoculated in the pot after one month from the first inoculation (Figure 11).

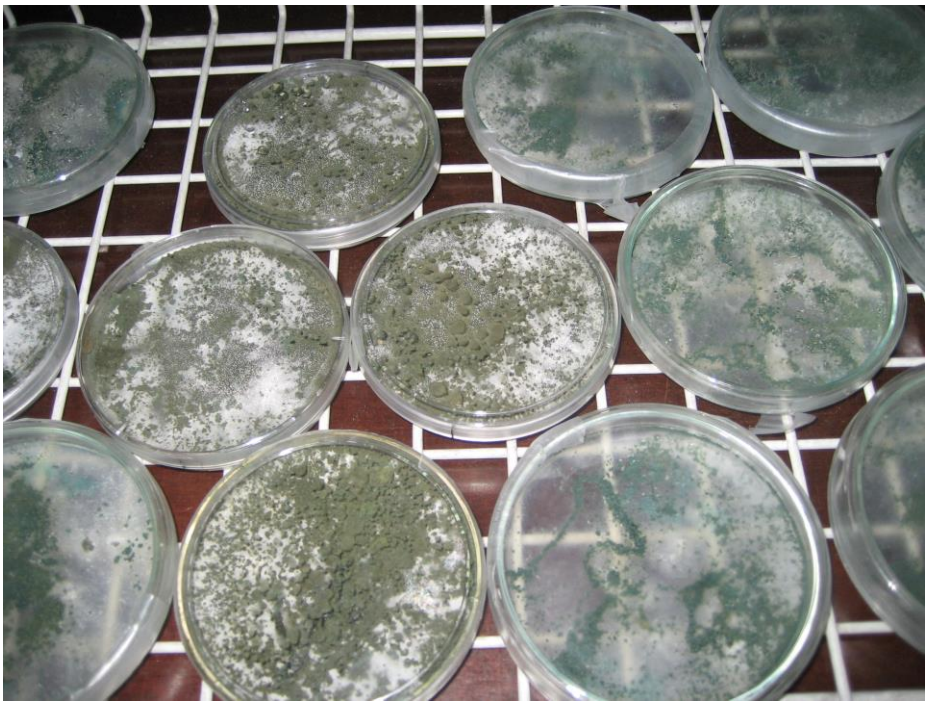


Figure 11. Multiplication of different *Trichoderma* spp on PDA media

Table 3. List of different *Trichodema* spp with their concentration for inoculation

S.N.	Treatments	Symbol	Inoculation ml /pot	Source
1.	*Trichoderma harzianum* (Nemastin)	T_1	30 ml	Kan Biosys Pvt. Ltd, Maharashtra, India
2.	*Trichoderma harzianum* (T22) (Rootshield Plus)	T_2	30 ml	AP Biochemical, Gujarat, India
3.	*Trichoderma asperellum*	T_4	30 ml	DORA Agri Tech, Suzhou, China
4.	*Trichoderma viride* (Ashtha TV)	T_3	30 ml	Lila Agrotech Pvt. Ltd. Kolkata, West Bangal, India
5.	*Trichoderma viride* (BIOCARE-F) 1.25% W.P.	T_5	30 ml	Harsidhi Biotech Pvt. Ltd. Bihar, Uttar Pradesh, India
6.	*Trichoderma viride* (Prarambha *Trichioderma*)	T_6	30 ml	Prarambha Biotech, Pvt. Ltd. Kalanki, Kathmandu, Nepal
7.	Control	T_7	(water)	

3.2.10. Calculation of final population of Nematode

Final population of *Meloidogyne* was estimated from soil and root systems of each pot. At 65 days from the date of inoculation of eggs, all plants were cut and the root system collected from the soil and weighed separately (Figure 12). Root stubbles and fine roots were separated by sieving the soil. A 100 g of soil sub sample was taken from well-mixed soil sample of each pot to extract the 2^{nd} stage juveniles by modified tray method. Similarly, eggs and juveniles extracted from whole root system. The extracted solution was homogenized and 0.5 ml sample was taken with the help of a pipette and added 1 ml of tap water in a counting disc. The aliquots were counted till the CV below 15% and the final number of eggs and juveniles estimated from the total suspension. The reproductive factor (*Rf*) was calculated by dividing the final population (*Pf*) to initial population (*Pi*). Root galling was indexed from 0-10 scale as described by Bridge

& Page (1980). Gelatinous matrix of root galls stained by washing roots thoroughly without soil or other debris and soaked in the stain (Phloxine B solution of 0.015% = 15 g per liter of water) for 15 minutes, then the roots rinsed in the beaker with water and dried in blotting paper to make egg masses of root knot nematodes more visible for counting as well as easy for grading root gall severity.

Figure 12. Collection of root for galling index (*GI*) estimation

3.2.11. Statistical Analysis

The collected data were entered, tabulated and processed in Microsoft Excel version 12. The recorded data on different parameters were analyzed by using R Stat and the means between treatments were separated using Duncan's multiple range test (DMRT) at 5% level of significance.

3.3. Efficacy Tesing of locally isolated *Trichoderma* spp against Root knot nematode (*Meloidogyne incognita*)

3.3.1. Collection and multiplication of *Trichoderma* spp

Ten different locally isolated *Trichoderma* spp were collected from different parts of the country. The isolates were represented plain to hill of eastern to western parts of the counry. Among ten isolates, each four isolates were collected from hill and plain regions and two isolated represented from high hill. Similarly, each three isolates represented to eastern and central parts of the country whereas, one from western and far-western region. Rest two isolates of *Trichoderma* were from mid-western part of the country (Table 4).

For rapid growth and uniform availability of nutrients, one pure culture disc of 0.7 mm of each isolate was placed at two edges of each PDA plate. The petridishes were incubated at 25 ^{0}C. As the *Trichoderma* colony grew on PDA, it formed concentric rings and changed colour from white to green as it matured and sporulated. The fungi sporulated within seven days forming compact tufts of white and green shades. These plates were used for conidia extraction.

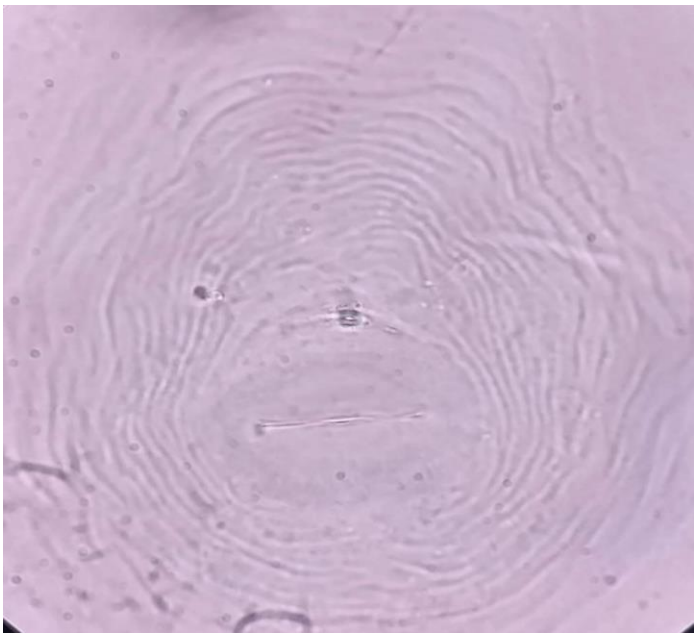
Table 4. List of *Trichoderma* isolates collected from different parts of the country

S.N	*Trichoderma* isolates	Species Identification	District/ Province	Location	Region
1	*Trichoderma* sp (T 517)	*Trichoderma harzianum*	Kathmandu/ Bagmati	Central	Hill
2	*Trichoderma* sp (T 209)	*Trichoderma lixii*	Sarlahi/ Madhesh	East	Plain
3	*Trichoderma* sp (T 354)	*Trichoderma afroharzianm*	Sunsari/ Koshi	East	Plain
4	*Trichoderma* sp (T 460)	*Trichoderma afroharzianum*	Lalitpur/ Bagmati	Central	Hill
5	*Trichoderma* sp (T 362)	*Trichoderma* sp	Pyuthan/ Lumbhini	Mid-West	Hill
6	*Trichoderma* sp (T 448)	*Trichoderma afroharzianum*	Chitwan/ Bagmati	Central	Plain
7	*Trichoderma* sp (T 444)	*Trichoderma virens*	Terhathum/ Koshi	East	High hill
8	*Trichoderma* sp (T 282)	*Trichoderma* sp	Kanchanpur/ Sudurpashchim	Far-West	Plain
9	*Trichoderma* sp (T 486)	*Trichoderma* sp	Lamjung/ Gandaki	West	Hill
10	*Trichoderma* sp (T 260)	*Trichoderma afroharzianum*	Jumla/ Karnali	Mid-West	High hill

3.3.2. Identification of nematode

The adult females were taken out from the root galls by teasing with the needle and preserved in 0.5% NaCl solution. The female was transferred to a small drop of 45% lactic acid in a plastic petri dish. A female body was then pushed out of a drop in a small isthmus of lactic acid solution, so that it was held in place by surface tension. The end of razor blade was embedded into the plastic plate and cut off the posterior end of nematode. The body tissue from posterior region was gently removed with dissecting needle. Then, the cuticle was trimmed into a square with the perenial pattern in the center. It was then transferred to a microscope slide and covered by a cover slip which was observed under microscope. Thus, species of *Meloidogyne* was

identified as *M. incognita* based on the perineal pattern, observed under compound microscope as described by Eisenback *et al*. (1981) as shown in figure 13.



Common feature in perineal pattern of *M incognita*
Oval to rounded, striae usually wavy, high squared dorsal arch, lateral field absent or weakly demarcated by forked striae. Often there are striae that bend toward vulva.

Figure 13. Perineal Pattern of *Meloidogyne incognita* of root knot nematode

3.3.3. Collection of Juveniles

Eggs of root knot nematode were extracted from the infected root of tomato plant grown in screen house with a 0.5% solution of NaOCl solution. After eggs were released, they were collected using a modified technique described by McClure *et al*., 1973. The collected eggs were rinsed with tap water through a 125 µm sieve and collected in a 30 µm water sieve and transferred into distilled water to form egg suspension. The egg suspension was poured into a blotting paper and incubated at 25±2 °C. Emerged second stage juveniles (J2s) of nematodes were collected after after 48 hours to five days after hatching all eggs and stored them in refrigerator at 5 °C.

3.3.4. Screen house experiment set up

To determine the effects of some locally isolated *Trichoderma* spp from different parts of the country agasint *Meloidogyne incognita* in tomato, pot experiment was conducted in screen house at National Plant Pathology Reseasrch Centre (NPPRC). This experiment was laid in RCBD with three replication having eleven treatments including control (Figure 14 & 15). In this study 1000 J2s were applied to each pot having 1 kg soil for each treatment. Seven days after inoculation of juveniles the solution of different *Trichoderma* species were applied 30 ml at 1×10^7 cfu. Afer a month, second application of *Trichoderma* spp was also done after first application of *Trichoderma* in same concentration. After 65 days, efficiency of the *Trichederma* spp on the root knot nematodes was evaluated with respect to gall formation ratio on root system and calculation of J2s in the soil and root surface. At the end of the experiment, all plants were carefully removed from pots and their root parts were cut and collected separately. The roots were carefully washed with tap water in order to be freed from soil. Meanwhile, the washed water was collected and counted the juveniles under stereomicroscope. Side by side, galling index was also recorded from the root system.

Figure 14. Pot experiment set up inside screenhouse at NPPRC, NARC, Khumaltar

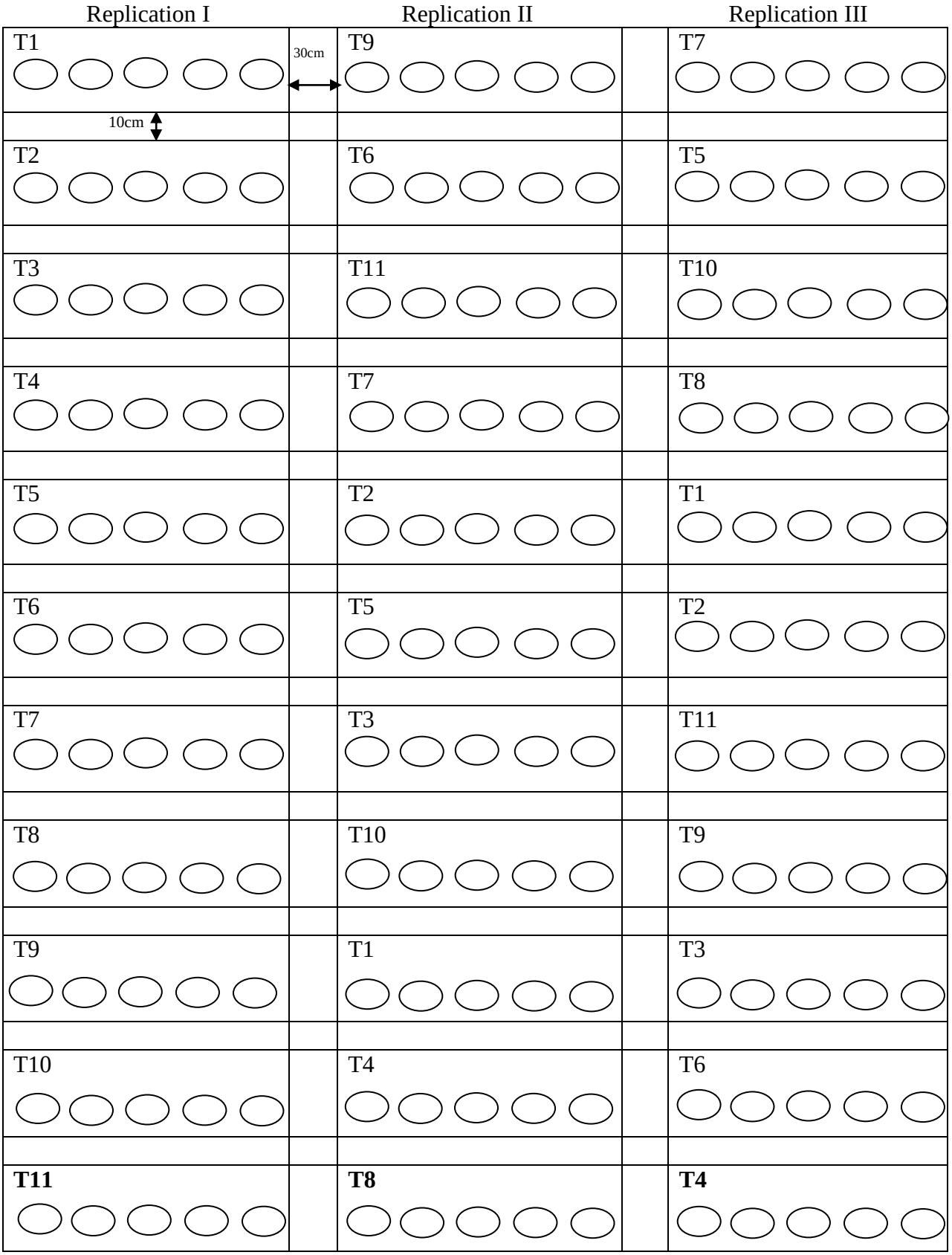


Figure 15. Sketch diagram of lay out of pots arrangement inside screen house, Khumaltar

3.3.5. Inoculation of plants with *Meloidogyne incognita*

One week after transplanting of tomato seedlings, 3 cm deep three holes were made close to each plant with a glass rod and the plants were inoculated with 20 ml inoculum/plant using a plastic syringe. The inoculum contained 50 juveniles per ml of solution. Thus, each plant was inculated with 1000 juveniles of *Meloidogyne incognita*. After inoculation, the holes were covered with the surrounding soil.

3.3.6. Application of different *Trichoderma* spp

The mycelia and conidia of *Trichoderma* grown on PDA were carefully scraped with a slide, and suspended in 100 ml distilled water. Spores were separated from mycelia by sieving through a muslin cloth. The spore suspensions collected in a beaker were adjusted to cfu 1×10^7 spores/ml after counting spores using haemocytometer. Immediately after transplanting of seedlings, 3 cm deep three holes were made with a glass rod around each plant at rhizosphere and 3 ml spore suspension was poured/hole (9 ml/plant). The same concentration of *Trichoderma* spores solution was prepared and again inoculated in the pot after one month from the first inoculation.

3.3.7. Determine Nematode population

To determine the changes of J2s populations, the reproduction ratio was determined with Equation developed by Ferris and Noling, 1987.

Reproduction factor (*Rf*) = Final population (*Pf*) / Initial population (*Pi*)

It is accepted that when reproduction factor ($Rf = Pf/Pi$) is ≥ 1, nematodes can grow and develop a population, but when the ratio is ≤ 1, nematodes neither grow nor develop a population in the

agro-ecosystem. In this condition, the nemtodes can not inpact in the yield of plants/crops under threshold level of nematodes population.

3.3.8. Filling of pots with soil

Sandy loam soil collected from the field at NARC, Khumaltar, was cleaned by removing plant debris and pebbles and autoclaved for 30 min at 15 psi and 120 ^{0}C. The cooled soil was mixed well with properly decomposed compost in the ration of 4:1, i.e. 800 g soil with 200 g compost. One hundred and sixty five plastic pots of 12.5 cm diameter and 18.0 cm height were filled with 1 kg soil/pot upto 1 inch below the mouth of pots. The pots were kept in the screen house.

3.3.9. Seedling raising and transplanting

Tomato (*Lycopersicon esculentum*) seeds of hybrid "Shrijana" were sown in plastic trays (40 cm × 18 cm × 10 cm) filled with field soil, continuously in lines 5 cm apart and placed in a screen house. Immediately after sowing, mulching with a thin layer of straw and light irrigation was given. Three weeks old seedlings were transplanted as one plant per pot. Watering was done immediately after transplanting, then daily for a week and two times at weekly intervals afterwards.

3.3.10. Disease assessment

Sixty five days after transplanting, all tomato plants were carefully uprooted, roots were cleaned with tap water and assessed for root gall intensity produced by *Meloidogyne* spp. The galling index (root gall index) was determined of each root system based on a 0-10 scale and its explanation given by Bridge & Page (1980) as described in table 2 and figure 16. Mean values of

root gall system were computed. All lateral roots were separated from tap root by cutting carefully with scissors. Galls on each lateral root and tap root of each plant of each treatment were counted with naked eyes (Figure 17). After galling index was calculated the roots were used for extraction of eggs and juveniles.

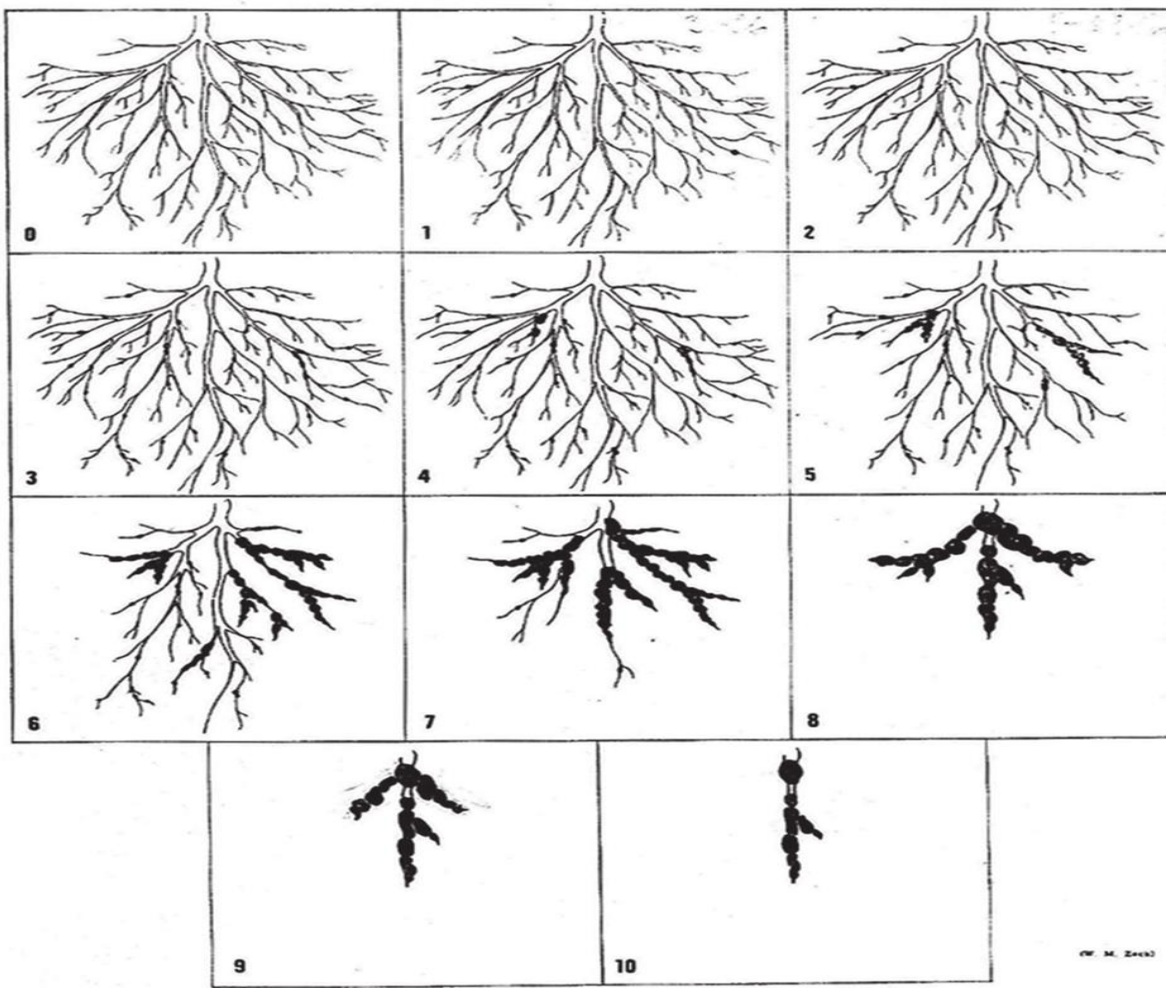


Figure 16. Diagramatic preseantation of different level of severity of RKN in root system (Source: Luc *et al*., 2005)

Figure 17. Tomato root system without gall (a) and with gall (b) of RKN

3.3.11. Extraction of juveniles of *Meloidogyne* spp from soil and eggs

The process of juveniles (J2s) extraction from soil and eggs was same as above mentioned in 5.1.3. " Extraction of nematodes from soil" and collection of juveniles respectively.

5.3.12. Statistical analysis

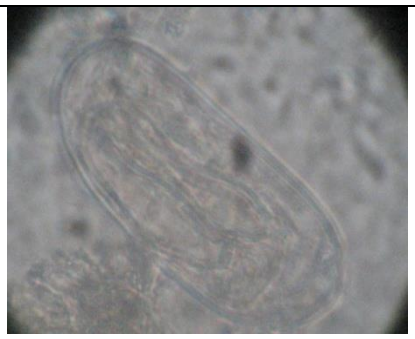
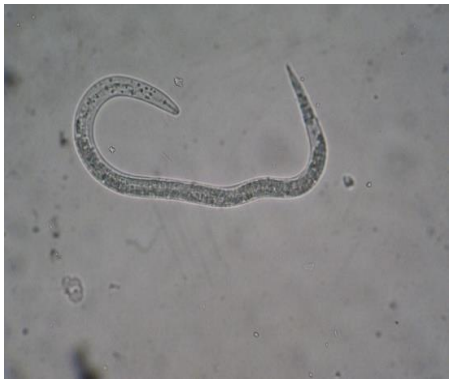
The collected data were compiled and entered as per Ms-excel program. Analysis of variance for all parameters was carried out as per the procedures given in M STAT. Duncan's Multiple Range Test (DMRT) was done for mean separation from the reference of Gomez and Gomez (1984). Graphs and tables were constructed by using the MS-excel computer software program.

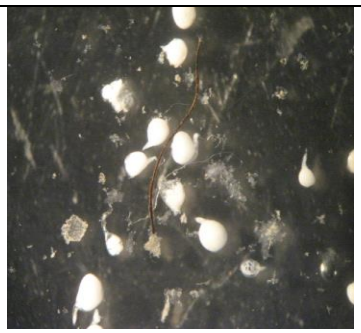
CHAPTER 4 - RESULT

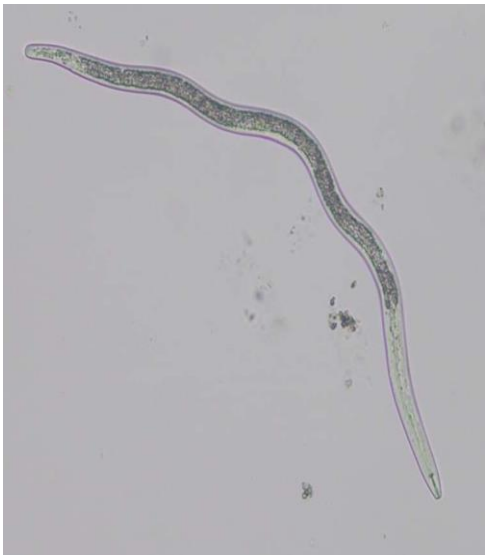
4.1. Identification of plant parasitic nematodes

The juveniles of nematodes were observed and identified under stereomicroscope and compound microscope (at 40 and 100 × magnification). The genera of plant parasitic nematodes were identified based on photographs and description (Luc *et al.*, 1990). Photographs and morphological features (external and internal body parts) of plant parasitic nematodes used for identification were as mentioned in table 5.

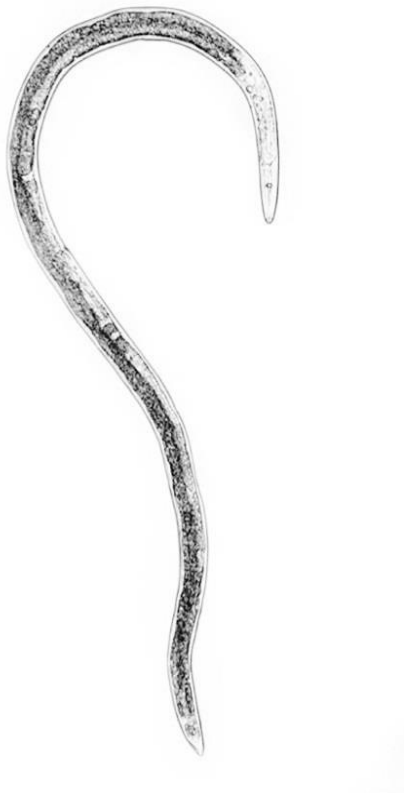
Table 5. Common feature of some genera of Plant parasitic nematodes

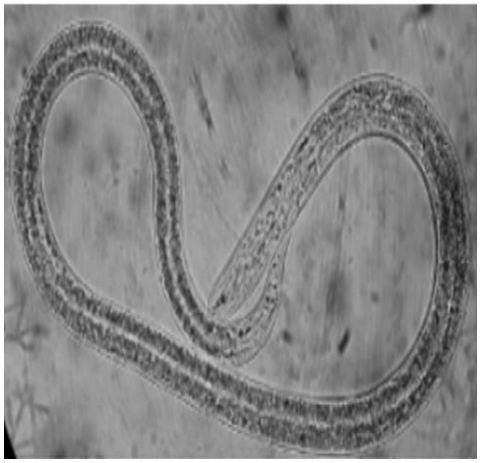
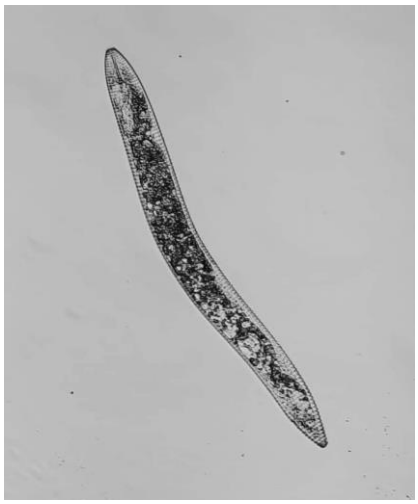
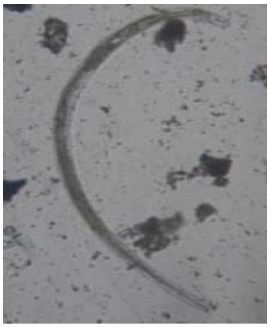
S. N.	Nematode genera with key features	Microscopic view
1.	***Meloidogyne*** **(Sedentary endoparasite)** - Female globose with 0.3-0.7 mm diameter - Cuticle whitish, thin and annulated - Sexually dimorphic - Female adult is pear shaped, pearly white, with a selender neck - Two long convoluted ovaries - Female embedded in root tissue - Body size = 0.5 – 0.7 mm - Unique fingerprint-like perineal pattern - Vulva subterminal near anus - Male very long and thin, head cap indistinct, weakly sclerotized - Juvenile has moderately sclerotized head - Body of juvenile is slender and vermiform around 450µm size. - Excretory pore located anterior to medium bulb valve plate - Strong stylet with well-developed knobs - Esophagus overlaps intestine ventrally,	 **Egg**  **Juvenile**

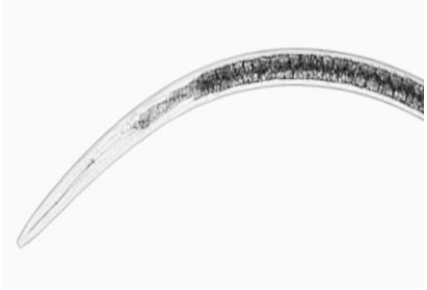
	- Adult male has one testis, spicules open a short distance from the tail tip - Short, rounded tail, - Bursa absent - Juvenile has short and thin body - Stylet short and weak with indistinct knobs - Tail conical with hyline portion starting near the tail tip.	 **Adult**
2.	***Helicotylenchus* (Migratory ecto-, endoparasite)** - Body size 0.4-1.2 mm - Moderately long body, cylindrical - Spiral shaped when cold and relaxed - Head region conical, strong short to moderate long well develop stylet - Cup shaped knob of stylet - Three to four times width of lip - Valva near mid body - Tail usually offset, often with a small projection - Male tail short, bursa reaching at tail tip - Female tail usually dorsally convex-conoid or hemispheral. - Pharyngeal glands longest ventrally and laterally - Vulva position at 60-70% of body. - Both genital tracts usually fully develop	
3.	***Tylenchorhynchus* (Ectoparasite)** - Body size 0.6 – 1.0 mm - Round head continue with body - Scleritized head - Backward slopping basal knob. - Offset gland position with intestine - Vulva position at 50% of body. - Spermatheca round shape - Lateral field with two, three or four lines - Two genetal tract equally distributed - Sub-cylindrical with round tip tail. - Male tail elongated, conical pointed, bursa extending to tail tip. - Male spicule straight or slightly curved ventrally. - Short, moderately strong stylet, cephalic framework weak, Spicule slightly curved	

4.	***Hemicriconemoides* (Ectoparasite)** - Body short and stout around 0.5 mm body size. - Body annule = Few annules and very closely adpressed with double cuticles. - Deep annulations, edges smooth - Stylet knob with anteriorly directed process. - Tail short, coniod. - Stylet long, thick, anchor shaped knobs - Body tapering at short dinstance in both ends - Vulva locates at posteriorly with plain vulva lips. - Female has one ovary - Labial is heavely sclerotized. - Osophagous degenerated in male. - Procorpus fused with metacarpus - Strong sexual dimorphism - Juveniles resembling female but posterior margin of body annules ornamented with scales or short denticles.	
5	***Hoplolaimus* (Semi-endoparasite)** - Body size = 1-2 mm length - Labial region high, offset - Basal lip annule may divide into small squares - Bodies slightly curve ventrally. - Anteriorly flatten head - Massive scleriotized lip - Well develop basal knob with anterior tooth like projection - Oesophagus gland dorsally overlapping gland lobe containing either three or six nuclei. - Phasmids enlarged to form scutella, one between anus and vulva and other anterior to vulva. - Didelphic genitical organs. - Arcurate tail and bursa ended at tail tip. - Vulva position at 60% of body. - Very long and thick robust stylet with anchor shaped knobs - Short and bluntly rounded tail in females and juveniles - Pointed tail in male with small bursae. - Bursa extends to tail tip, Well develop spicule	

6	***Pratylenchus* (Migratory endoparasite)** - Short, narrow body with less than 1mm body length - No sexual dimorphim - Hemispherical lip with low flattens appear black cap under stereomicroscope. - Sclerotized cephalic region. - Broad, flattened anterior end - Short, strong stylet with large anteriorly concave knobs - Vulva position at posterior region or near tail. - Metacarpus or medium bulb well develop or prominent. - Esophagus overlaps intestine ventrally - One ovary or genitical tract anteriorly. - Spermatheca oval shape. - Tail cylindrical with narrowly rounded. - Some species have truneate terminus tail or annulate tail. - Male tail short and dorsally convex-conoid - Male has bursa ended upto tail tip.	 
7	***Belonolaimus* (Semi-endoparasite)** - Long and slender body with more than 2mm long. - Lip region offset with four lobes. - Moderately active, Rounded head region, distinctly set off from body. - Stylet long (more than 100µm) and thin with small rounded knobs. - Oesophagus gland ventrally overlapped. - Vulva position at near mid body at 50% of body. - Medium bulb well develops with prominent. - Amphididelphic genetal tract. - Tail tip cylindrical and round terminus.	

8	***Aphelenchoides* (Ecto-, endoparasite)** - Slender and moderately long with 1- 1.2 mm long. - No sclerotized or weak head region. - Round cephalic region with slightly offset. - Short, weak stylet with very small knobs. - Well developed ovoid or spherical mediam bulb with central vulve plates. - Esophagus overlaps intestine dorsally - Oesophageal bulb well developed, spherical to rounded-rectangular in shape and more or less filling the body diameter - Vulva position posteriorly at 60-75% of body. - One ovary at anteriorly genetical tract. - Tail medium coniod with terminal micron may or may not present. - Specule thorn shaped developed and no bursa. - Male tail medium conoid with curved ventrally like walking stick.	
9	***Rotylenchus* (Semi-enctoparsite)** - Body size around 0.3 to 0.6 mm long. - Body spiral to C- shaped - Lip not offset but slightly depression with body - Labial region hemispherical with four annuli at mid of the body - Week scleritized head region - Stylet robust with large, rounded and sometimes intended knob - Basal knob in 90°C position with stylet - Oesophagus gland with well develop median bulb overlapping intestine dorsally - Vulva situates at 55-60% of the body length from anterior length - Reproductive tract didelphic- amphidelphic - Spermatheca indistinct to rounded, not filled - Phamid pore anterior to annus - Tail very short with commonly truncated tip - Tail length about equal to annual diameter of body	

10.	***Hirschmanniella* (Migratory endoparasite)** - Body size long around 1 – 4 mm long length. - Stright body sometimes ventrally arcuate. - Hemispherical head and central part flat. - Strong and dark stylet with round basal knob. - Basal knobs large, rounded and close to shaft. - Head high with not offset. - Oesophagus overlaps intestine ventrally. - Tail tip mucronate and pointed. - Vulva median of the body. - Equal size two genetical tracts at both sides. - Specule slender and arcuate. - Male bursa not reaching at tail tip. - Tail elongate and coniod. - Terminal mucron may or may not present.	
11.	***Criconemoide*s (Ectoparasitic)** - Short body size with 0.2 – 1.0 mm. - Straight or slightly curve body shape. - Head rounded anterior end. - Cuticle provides with 40-200 prominent, restrose annule which become smooth or finely create posterior position. - Strong stylet, basal knob with a forwarded directed process (anchor shaped). - Well develp median bulb which fused procorpus. - Gland has small basal knob with offset. - Vulva at posterior position at 90% of the body. - One genetical tract anteriorly position. - Cephalic region rounded or conoid, lateral field with three to four incisures. Bursa distinct, subterminal in male	
12.	***Longidorus* (Semi ecto parasite)** - Body size with 11 mm long. - Cuticle is smooth and lateral fields are absent. - Anterior part has amphid pouch like shape. - Odontostyle/odontophore junction not forked. - Odontophore lacks flages. - Guide ring in anterior half of odontostyle. - Oesophagus consists of a narrow anterior section	 Juvenile

	and posterior cylindroids expansion which is both muscular and glandular. - Mostly monodelphic female reproductive organ posteriorly (Opisthodelphic) - Well develop male spicule with lateral guiding pieces - No gubernaculums or bursa in male organ - Present series of sensory supplements in ventral region at anterior of cloaca. - Somatic cuticular pores are present along the body - No excretory pore, phasmids, deirids and cephalids absent	 Anterior part of nemadote (*Longidorus* sp)  Posterior part of nematode (*Longidorus* sp)
13.	***Tylenchus*** **(Ecto parasite)** - Body size 0.5 to 1.0 mm long - No scleritized head - Lip region rounded and elevatd with annulation - Transverse striations with longitudinal ridge cuticle - Cephalic region continuous. - Conus half or more than stylet length - Median bulb with valvular apparatus - Posterior sloping basal knob with spear shape - Oesophagous glands offset - Monodelphic, anterior ovary outstretched and posterior uterine branch rudimentary - Vulva at posterior position at 70% of the body - Spermathecae round to oval - Spicule arcuate, small and selender - Cardia present - Bursa adenal, simple or lobed - Phasmid not visible - Tail of both sexes similar, elongate conoid to filliform	

4.2. Plant Parasitic Nematodes Recorded in Vegetable Crops

Out of 211 samples, 137 samples had presence of plant parasitic nematodes i.e., 35 percent of total samples and rest 65 percent had only saprophytes nematodes (Figure 18). From 137 samples, twelve different genera of plant parasitic nematodes were identified. They are *Meloidogyne, Rotylenchus, Tylenchus, Hoplolaimus, Belonolaimus, Criconemoides, Helicotylenchus, Tylenchorhynchus, Pratylenchus, Hirschmanniella, Aphelenchoides* and *Longidorus.* Among them, the most commonly detected genera were *Helicotylenchus* (28%) followed by *Tylenchorhynchus* (19%), *Meloidogyne* (10%) and *Pratylenchus* (10%) as shown in figure 19. They found either singly or in combination with two to more than two genera. Around 57 percent sample had presence of single genus. Similarly, 31% sample had two different types of nematode (Figure 20). Ten different species of nematodes in solanaceous and nine in cruciferous crops were recorded. The population of six species was at high level in cruciferous crops whereas four species in solanaceous crops at moderate to high level of population distributed. Population of both ecto-parasite (*Helicotylenchus, Tylenchorhynchus*, and *Belonolaimus*) and endo-parasites (*Meloidogyne, Pratylenchus* and *Hoplolaimus*) were recorded at moderate to high level to cause yield loss in crops (Table 6).

In solanaceous crops, ten different nematodes *Tylenchorhynchus* sp, *T. vulgaris, T. acutus*; *Helicotylenchus* sp, *H. indicus*; *Pratylenchus* sp, *P. coffeae, P. penetrans*; *Tylenchus* sp; *Meloidogyne* sp, *M. javanica*, *M. incognita, Criconemoides ornatus; Aphelenchoides* sp; *Belonolaimus* sp; *Hoplolaimus* sp and *Rotylenchus* sp were recorded. Among these nematodes, *T. vulgaris, M. javanica*, *Helicotylenchus* sp and *Hoplolaimus* sp were recorded moderate to high population density to cause the yield loss in solanaceous crops. Similarly, in crucifers, *Helicotylenchus* sp, *H. dihystera*; *Belonolaimus* sp, *B. longicaudatus*; *Longidorus* sp, *L.*

elongatus; *Tylenchorhynchus brassicae, T. acutus*; *Pratylenchus brachyurus, P. penetrans, P. crenatus*; *Hoplolaimus indicus*; *Criconemoides oranatus*; *Meloidogyne* sp, *M. incognita*; and *Hirschmanniella* sp. Among these nine different types of nematodes, *Tylenchorhynchus, Pratylenchus* sp, *Meloidogyne* sp, *Belonolaimus* sp, *Hoplolaimus* sp, and *Helicotylenchus* sp were found medium to high population density in cruciferous crops (Table 7).

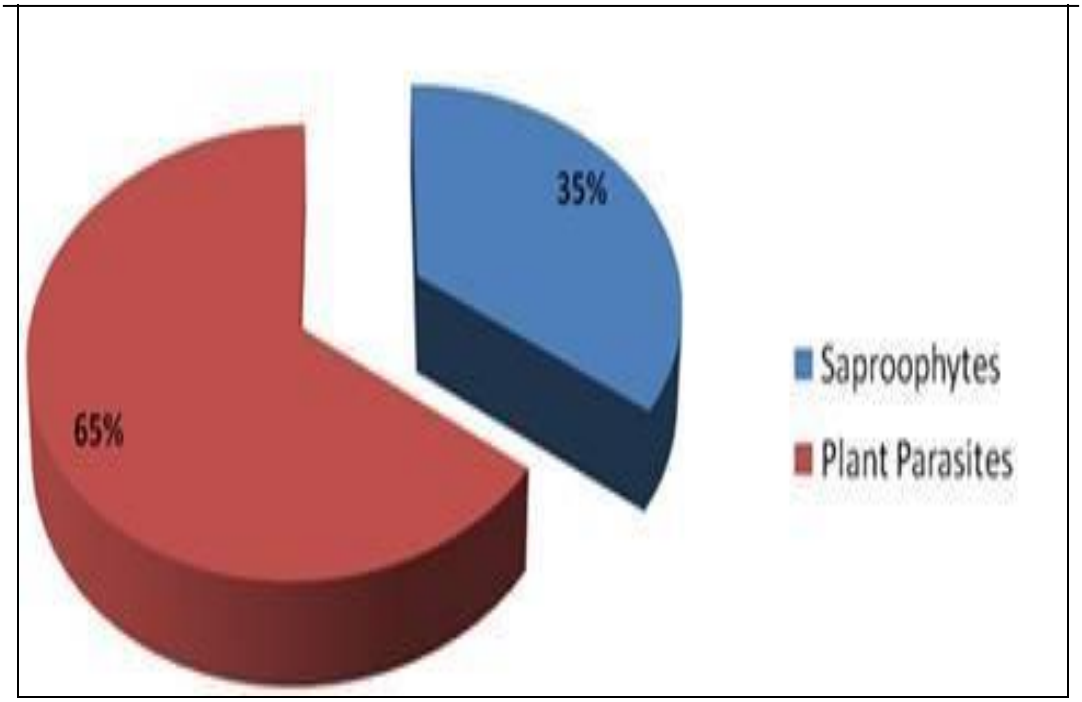


Figure 18. Distribution of plant parasitic nematodes in the samples

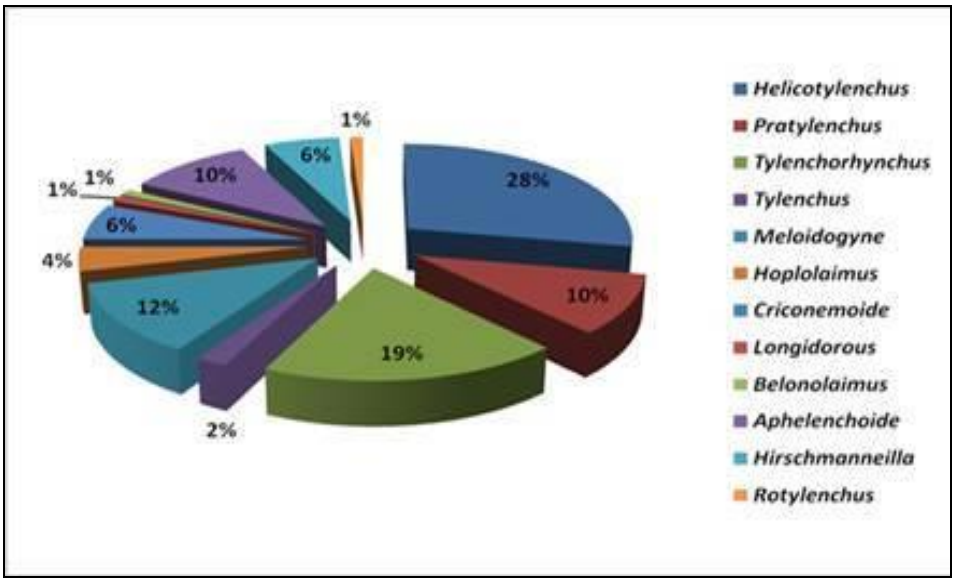


Figure 19. Frequency patterns of nematode genera in vegetable fields

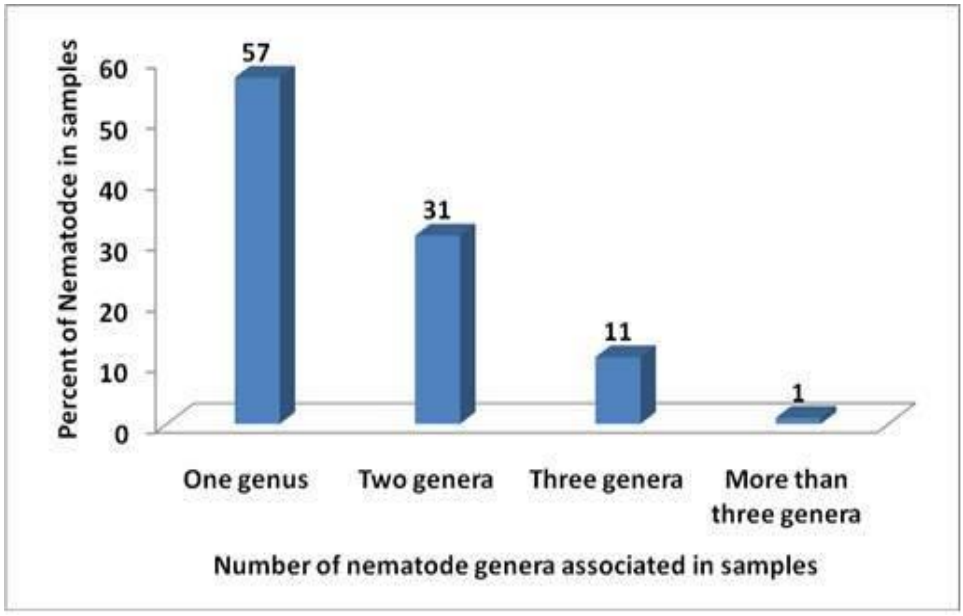


Figure 20. Existing patterns of nematode genera in vegetables

Table 6. Distribution and population density of plant parasitic nematodes in different vegetables of cruciferous crops

S.N.	Crops	Nematodes species	No. of samples	Population density / g soil	Level of threshold
1.	Cress	*Longidorus* sp	4	1	Low
		Tylenchorhynchus sp		1	Low
		Hoplolaimus sp		1	Low
2	Cauliflower	*Hoplolaimus* sp	26	4	High
		Belonolaimus longicaudatus		2	Moderate
		Meloidogyne sp		1	Low
		Tylenchorhynchus sp		2	Moderate
		Hirschmanniella sp		1	Low
		Helicotylenchus sp, *H. dihystera, H. indicus*		5	High
		Criconemoides sp, *C. annulatus*		1	Low
3	Cabbage	*Pratylenchus* sp, *P. penetrans*	4	1	Low
		Helicotylenchus indicus		2	Moderate
4	Broad leaf mustard	*Helicotylenchus* sp, *H. dihystera*	31	5	High
		Belonolaimus sp		1	Low
		Longidorus sp, *L. elongates*		1	Low
		Tylenchorhynchus brassicae, T. acutus		5	High
		Pratylenchus brachyurus, P. penetrans, P. crenatus		3	High
		Hoplolaimus indicus		1	Low
		Criconemoides oranatus		1	Low
		Meloidogyne sp		2	Moderate
5	Carrot	*Pratylenchus* sp	1	2	Moderate
6	Brocauli	*Tylenchorhynchus* sp	2	1	Low
7	Radish	*Tylenchorhynchus* sp, *T. vulgaris*	6	2	Moderate
		Longidorus elongates		1	Low
		Helicotylenchus dihystera, H. indicus		1	Low
		Belonolaimus sp		1	Low
		Meloidogyne sp, *M. incognita*		1	Low
		Hoplolaimus sp		1	Low

Table 7. Distribution and population density of plant parasitic nematodes in different vegetables of solanaceous crops

S.N.	Crops	Nematodes species	No. of samples	Population density/g soil	Level of threshold
1.	Tomato	*Helicotylenchus* sp, *H. vulgaris, H. indicus*	28	2	Moderate
		Meloidogyne incognita, M. javanica		3	High
		Tylenchorhynchus sp, *T. acutus*		1	Low
		Hoplolaimus sp		2	Moderate
		Rotylenchus sp		1	Low
		Criconemoides sp		1	Low
2.	Potato	*Tylenchorhynchus* sp	12	2	Moderate
		Helicotylenchus sp		4	High
		Pratylenchus sp, *P. coffeae, P. penetrans*		1	Low
		Tylenchus sp		1	Low
		Meloidogyne sp		1	Low
		Criconemoides ornatus		1	Low
3.	Chilly	*Meloidogyne javanica*	18	2	Moderate
		Tylenchorhynchus sp		2	Moderate
		Helicotylenchus sp		1	Low
		Pratylenchus sp, *P. coffeae*		1	Low
		Belonolaimus sp		1	Low
		Hoplolaimus sp		1	Low
4.	Lady finger	*Meloidogyne* sp	2	1	Low
		Helicotylenchus sp		1	Low
5.	Capsicum	*Aphelenchoides* sp	3	1	Low
		Tylenchorhynchus vulgaris		2	Moderate

4.3. Plant Parasitic Nemaodes recorded in Cereal Crops

In total of 128 samples in which 37 from rice, 64 from wheat and 27 from maize fields were collected. Out of 128 samples, 90 samples, (78%) had presence plant parasitic nematodes with saprophytic nematodes and rest 22% samples had only saprophytic nematodes (Figure 21). In

cereal crops, eleven different plant parasitic nematode genera (*Hirschmanniella, Meloidogyne, Criconemoides, Tylenchorhynchus, Aphelenchoides, Helicotylenchus, Hemicriconemoides, Longidorus, Pratylenchus, Tylenchus* and *Hoplolaimus*) were recorded.

In which, seven different genera namely, *Meloidogyne, Hirschmanniella, Criconemoides, Helicotylenchus, Aphelenchoides, Hemicriconemoides* and *Tylenchorhynchus* were recorded from the rice fields of thirteen districts. Among them, *Meloidogyne, Hirschmanniella* and *Helicotylenchus* were most dominant than other nematodes (Figure 27). The population density of genus *Hirschmanniella* was comparatively higher than other genera in most the district except in Dhading district whereas the population of *Meloidogyne* sp. was high in Dhading district. The population of *Hirschmanniella oryzae* was recorded highest number than other nematodes that was more than five nematodes per gram of soil in different districts. Whereas the population of *Helicotylenchus, Tylenchorhynchus* and *Hemicriconemoide* were under threshold level and others genera were recorded moderate to high level to impact on the yield of the rice crop (Table 8).

Similarly, seven different genera of plant parasitic nematodes (PPNs) were identified in wheat fields. The genera namely, *Helicotylenchus, Tylenchorhynchus, Pratylenchus, Hirschmanniella, Aphelenchoides* and *Longidorus* were identified from the samples (Figure 22). In wheat field, more than 75% of nematodes were observed the plant parasitic nematodes and rest was saprophytic nematodes. The plant parasitc nematode was dominant in the wheat fields (Figure 23).

Among the PPNs, nearly half samples had presence of genus *Helicotylenchus* followed by *Tylenchorhynchus* and *Pratylenchus*. Both parasitic and saprophytic nematodes were detected in vegetative and reproductive stages of crop. Two third samples had ecto and semi-parasitic

nematodes and rest were migratory and sedimentary endoparasitic nematodes (Table 8). A total of 60 percent samples detected a single type of nematode and rest has either in combination of one or more than one genus/species (Figure 24). Population of plant parasitic nematodes was higher in vegetative stage than in reproductive stage in wheat fields. The trend of plant parasitic nematodes are decreasing pattern with plant stage or maturity of plant.

In rice field, more than 50 percent samples had single genus and 28 percent had two different genera (Figure 25). The population of nematode was varying with the stage of crop. More saprophyte nematodes were detected in rice field as compare to parasitic nematodes. The population of saprophytic nematode was higher in both vegetative and reproductive stage of crops (Figure 26).

In maize field, more plant parasitic nematodes were recorded than the saprophytic nematodes. The trend of population of plant parasitc nematodes was higher in reproductive stage (Figure 28). There were also recorded seven genera from different locations of eight districts. They are *Meloidogyne, Tylenchorhynchus, Longidorus, Pratylenchus, Helicotylenchus, Tylenchus* and *Hoplolaimus* (Figure 29). The distribution pattern of plant parasitic nematodes was similar to rice crops. Around 50 percent samples had only one genus whereas 17% samples had four different genera was existed (Figure 30). The genus *Pratylenchus* was recorded moderate to high population in Dolkha, Kathmandu, Kavre, whereas in Dhading, Lalitpur and Bhaktapur the population was under threshold level. The genus *Helicotylenchus* was predominant nematodes reported many locations with moderate to high level of population in maize fields (Table 8). The population of *Meloidogyne, Tylenchorhynchus* and *Tylenchus* were also under threshold level while the population of *Hoplolaimus* was low to moderate at different location (Table 8).

Table 8. Distribution and population density of plant parasitic nematodes in different cereal crops

S.N.	Crops	Nematodes species	No. of samples	Population density/g soil	Level of threshold
		Hirschmanniella spp, *H. oryzae*,	4	5	High
		Meloidogyne sp, *M. graminicola*	3	4	High
1	Rice	*Criconemoides annulata, C. oryzae*	1	3	High
		Tylenchorhynchus annulata	1	1	Low
		Aphelenchoides besseyi	1	2	Moderate
		Helicotylenchus indicus	4	1	Low
		Hemicriconemoides sp	1	1	Low
		Helicotylenchus sp, *H. indicus, H. dihystera*	26	2	Moderate
		Hirschmanniella sp	5	1	Low
	Wheat	*Longidorus* sp	3	1	Low
2		*Pratylenchus* sp , *P. zeae, P. brachyurus*	8	2	Moderate
		Aphelenchoides sp	3	1	
		Tylenchorhynchus sp, *T. annulatus, T. vulgaris, T. acutus*	8	2	Moderate
		Meloidogyne sp	4	2	Moderate
		Pratylenchus penetrans, P. crenatus, P. zeae	5	6	High
		Tylenchorhynchus vulgaris	3	2	Moderate
		Longidorus sp	1	2	Moderate
3	Maize	*Helicotylenchus* sp, *H. dihystera, H. indicus*	5	5	High
		Meloidogyne sp	1	1	Low
		Tylenchus sp	1	1	Low
		Hoplolaimus sp, *H. indicus*	2	2	Moderate
Total			**90**		

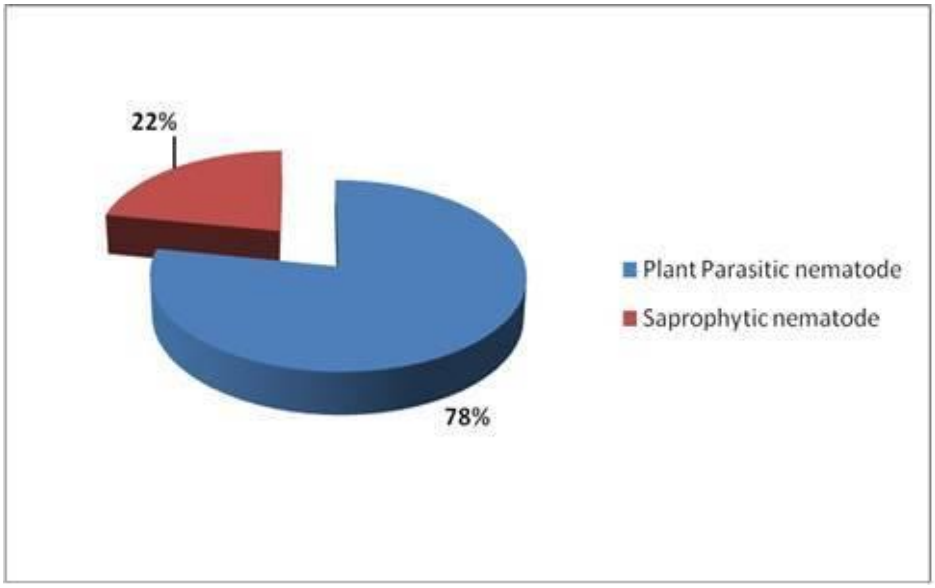


Figure 21. Ratio of plant parasitic and saprophytic nematodes presence in cereal crops

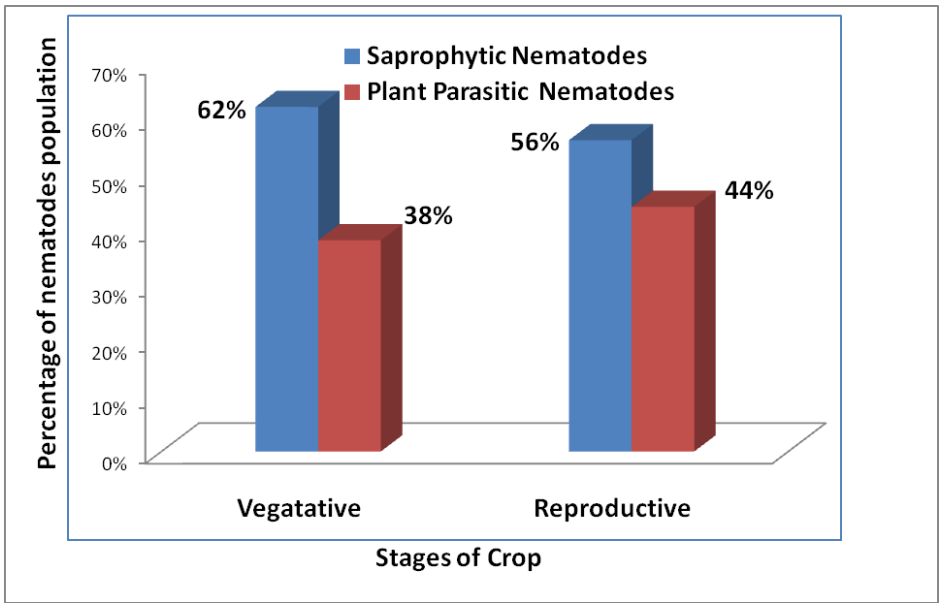


Figure 22. Population dynamic of nematodes in different stages in wheat crop

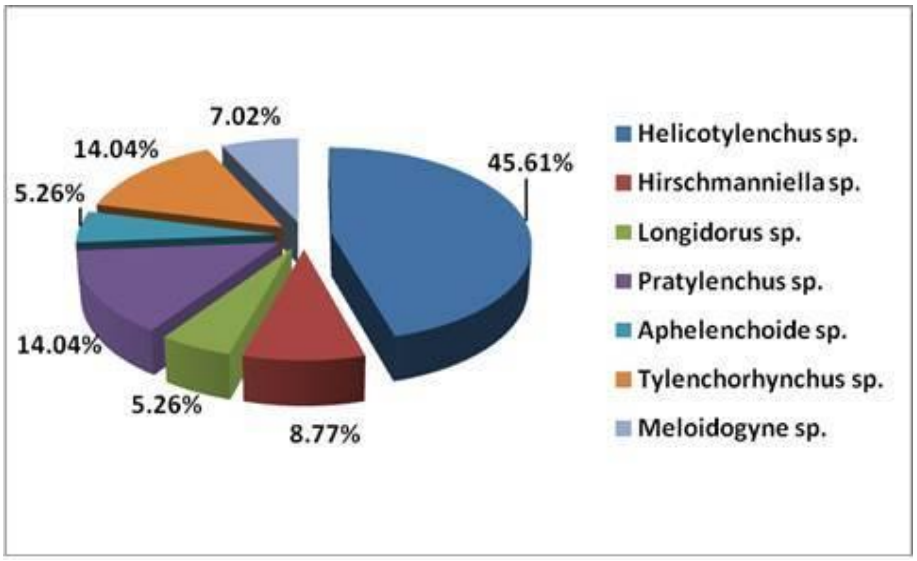


Figure 23. Distribution pattern of nematode genera in wheat fields

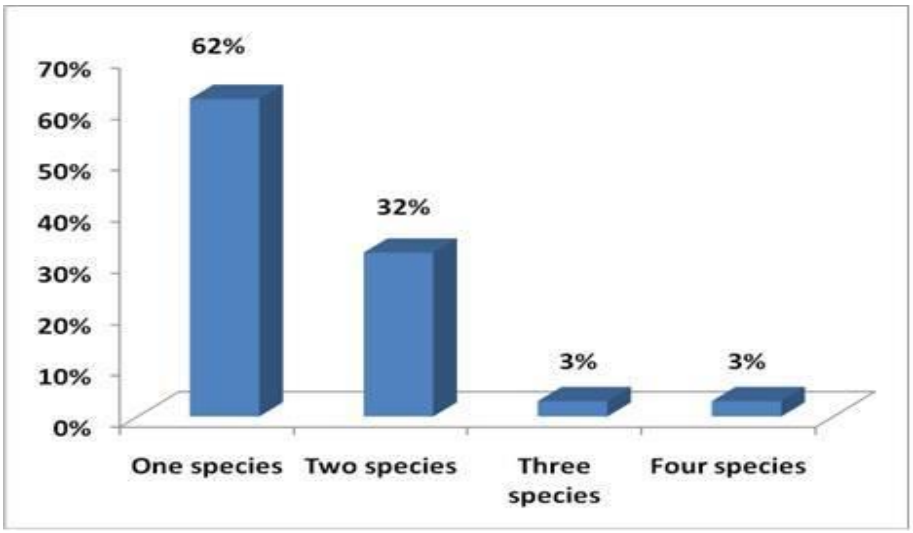


Figure 24. Existing pattern of nematode genera in wheat fields

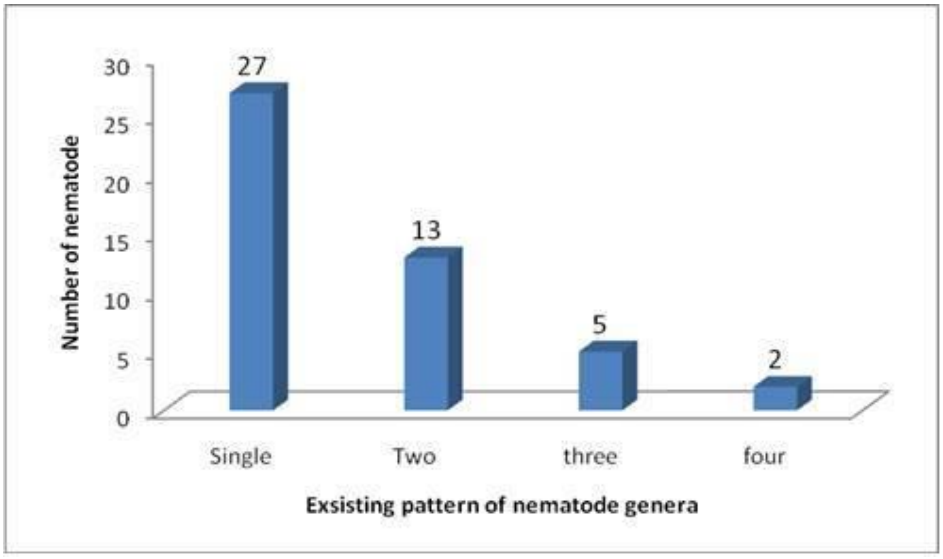


Figure 25. Existing pattern of nematode genera in rice fields

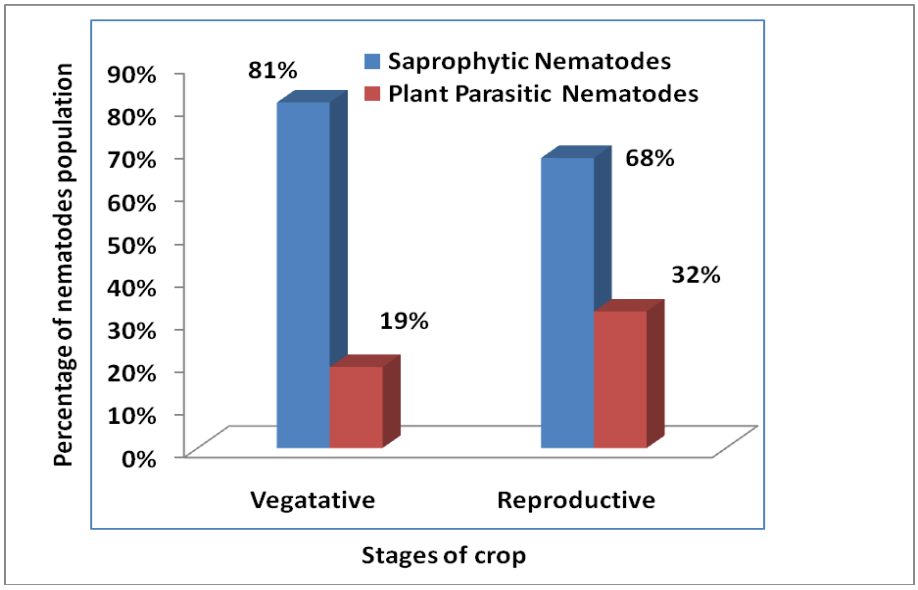


Figure 26. Population density of nematode in rice fields

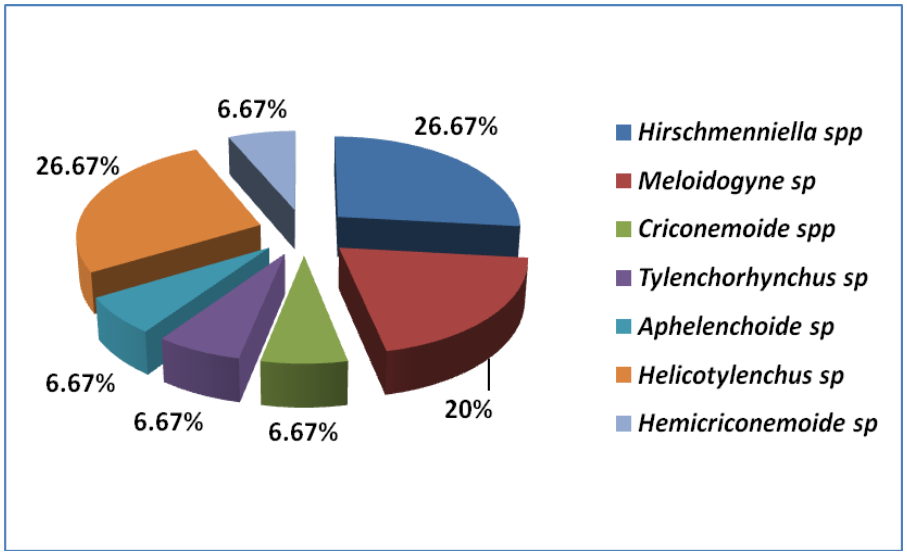


Figure 27. Distribution pattern of nematode genera in rice fields

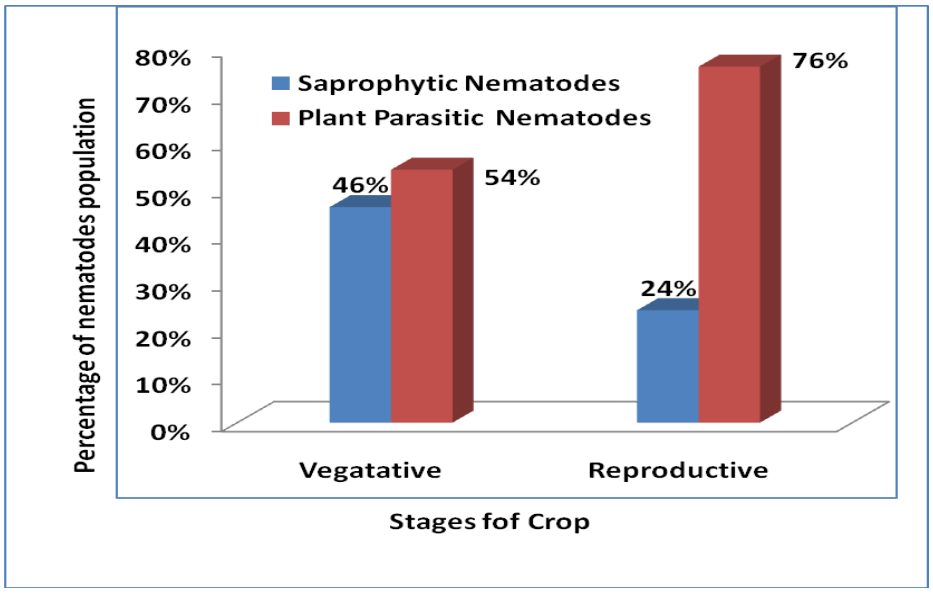


Figure 28. Ratio of plant parasitic and saprophytic nematodes presence in maize fields

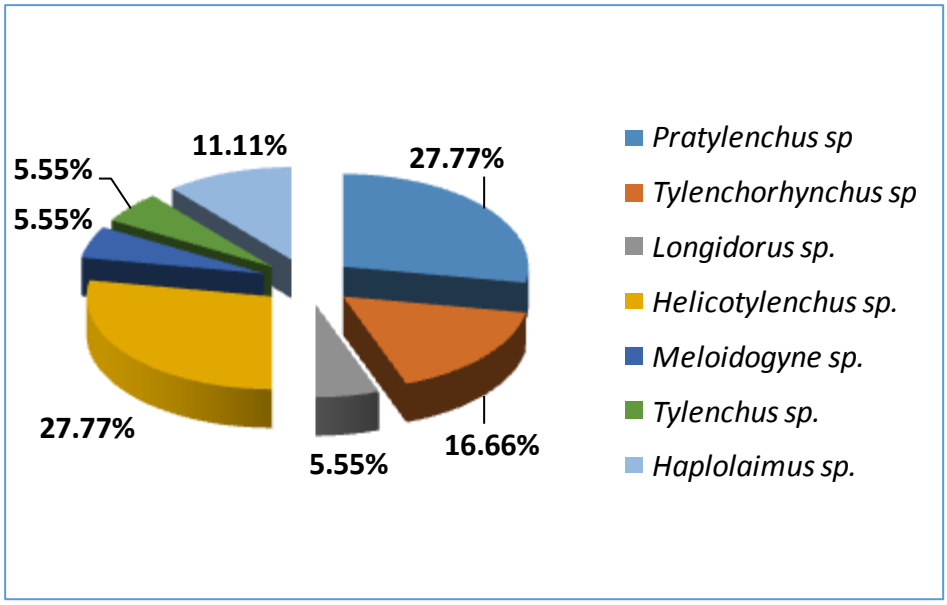


Figure 29. Distribution pattern of nematode genera in maize fields

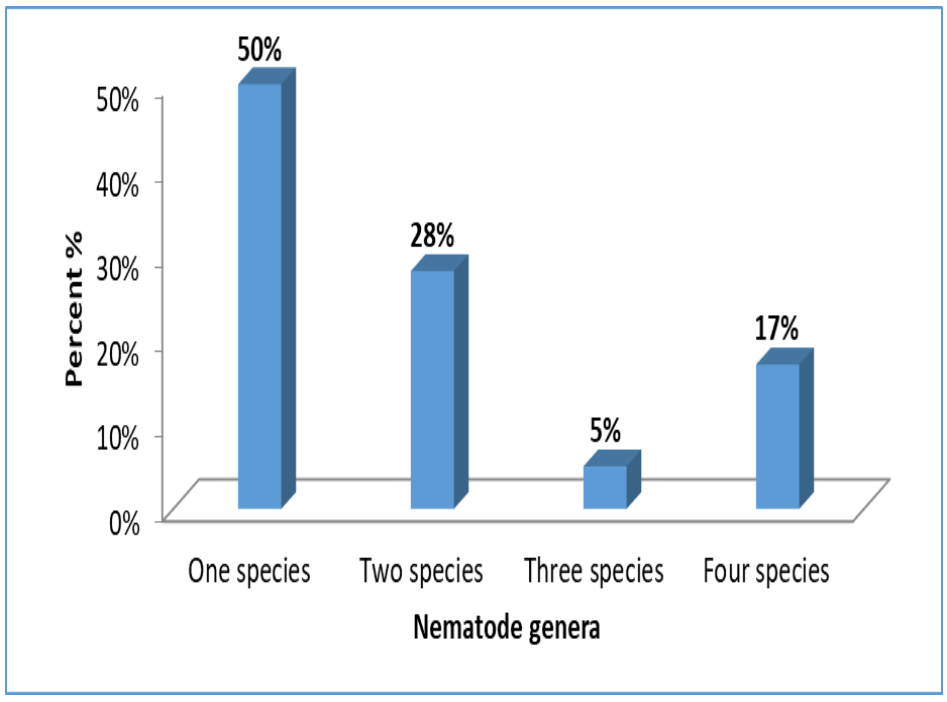


Figure 30. Existing pattern of nematode genera in maize fields

4.4. Effecacy level of commercial product of *Trichoderma* on *Meloidogyne* spp

The treatments varied significantly in root knot index and number of galls in root system, but not differ in fresh root weight per plant. The lowest gall index was observed in *T. harzianum* (Nemastin) (1.6), followed by *T. harzianum*-T22 (Root Shield Plus) (2.37) and *T. viride* (Ashtha TV) (3.43), which were, however, not significantly different to one another. Control plants showed considerably highest root gall index (6.08) which was significantly higher than others. Considerably, lowest numbers of galls (9.13) was observed in *T. harzianum* (Nemastin) than all other treatments (Table 9). The number of galls in *T. harzianum* - T22 (42.61) and *T. viride* - BIOCARE-F (71.43) inoculated plants had also significantly different with control (132.33).

There was no significant difference in fresh root weight per plant of tomato with control except *T. harzianum* (Nemastin) and *T. harzianum* (T22). However, fresh root weight appeared highest in *T. harzianum* -T22 (Root Shield Plus) had 11.3 g; followed by *T. harzianum* (Nemastin) had 8.37 g whereas in control showed the lowest weight (3.75 g).

The treatments varied considerably in number of eggs and juveniles (J2)/plant, J2/100 g soil and reproductive factor (*Rf*). Significantly highest suppressive effect on the nematode population was achieved by *T. harzianum* (Nemastin) (*Rf* 0.45), followed by *T. harzianum* (T22) (*Rf* 0.61), *T. viride*, (Ashtha TV) (*Rf* 1.43) and *T. viride*, (Prarambha *Trichoderma*) (*Rf* 1.51) (Table 10). *T. asperellum* (*Rf* 2.10) and *T. viride*, (Prarambha *Trichoderma*) (*Rf* 1.51*)* did not show any significant difference from each other. Similarly, *T. viride* (BIOCARE-F) and control had also no significantly different to each other. In the roots, population of eggs and juveniles was recorded lower number in *T. harzianum* (Nemastin) followed by *T. harzianum* (T22) and *T. viride* (Ashtha TV), whereas, in *T. viride* (BIOCARE-F), the nematode population (eggs and juveniles) did not show any different with control.

Table 9. Mean value of treatments effect on root gall index and fresh root weight of tomato in screen house at NPPRC, Khumaltar in two seasons (April-June and August-October), 2022

SN	Treatments	Root gall index (GI) (0-10 scale)#	No. of galls/root system#	Fresh root weight/plant (g)#
1	*Trichoderma harzianum* (Nemastin)	1.6cd	9.13c	8.37b
2	*Trichoderma harzianum* (T22) Root Shield Plus	2.37c	42.61bc	11.38b
3	*Trichoderma viride* (Ashtha TV)	3.43bc	88.86ab	5.51a
4	*Trichoderma asperellum*	3.91b	102.05a	4.39a
5	*Trichoderma viride* (BIOCARE-F)	3.87b	71.43b	5.07a
6	*Trichoderma viride* (Prarambha Trichoderma)	3.63b	101.60a	7.23a
7	Control	6.08a	132.33a	3.65a
	Mean	3.55	78.28	6.51
	CV%	11.18	15.97	3.75
	LSD	1.07	46.31	4.4
	(P<0.05)	*	*	*

Means followed by the same letter in a column are not significantly different by DMRT at 5% level of significance, *significant at 5% level, CV=Coefficient of variation, LSD=Least Significant difference.

Mean data transferred in root square value for analysis

Table 10. Mean value of treatments effect on reproduction of *Meloidogyne* spp on tomato in screen house at NARC, Khumaltar, during two seasons (April-June and August-Oct), 2022

SN	Treatments	*Pi* (No.)	Final population (No.)			*Pf* (No.)	*Rf*
			Eggs /root system[#]	J_2 / root system[#]	J_2 /100g soil[#]		
1	*Trichoderma harzianum* (Nemastin)	2000	812d	17bc	62d	891	0.45d
2	*T. harzianum* (T22) (Root Shield Plus)	2000	1114d	6de	97cd	1217	0.61d
3	*Trichoderma viride* (Ashtha TV)	2000	2717c	11d	137bc	2865	1.43c
4	*Trichoderma asperellum*	2000	3841b	6de	180ab	4027	2.10b
5	*T. viride* (BIOCARE-F)	2000	5242a	19b	217a	5478	2.74a
6	*T. viride* (Prarambha *Trichoderma*)	2000	3181bc	17bc	124c	3022	1.51bc
7	Control	2000	5694a	33a	201a	5928	2.96a
	Mean	2000	3186	16	145		1.227
	CV%		11.75	8.91	6.54		14.42
	LSD		671.12	5.22	43.6		0.6
	P<0.05		*	*	*		*

[#] Mean data transferred in square root value for statistical analysis

Means followed by the same letter in a column are not significantly different by DMRT at 5% level of significance, *Significant at 5% level, CV=Coefficient of variation, LSD=Least Significant difference.

Pi = Initial population of eggs of *Meloigyne* spp. inoculated into a plant
Pf = Final population of eggs and Juveniles in a plant and soil of a pot
Rf = Reproductive factor
J2 = Second stage juveniles of *Meloidogyne* spp

4.5. Interaction of gall index (*GI*) with reproductive factor (*Rf*) of *Meloidogyne* spp

There was a direct relationship between gall index and reproductive factor of *Meloidogyne* spp. Generally, as root gall index increased reproduction of nematodes also increased and vice versa. Similar trend was also reported by Baidya *et al*. (2008). Initially, there was slow or gradual increase in gall index due to suppressive effect of the treatments, and later there was abrupt rise due to sharp decline in suppression of nematode multiplication (Figure 31).

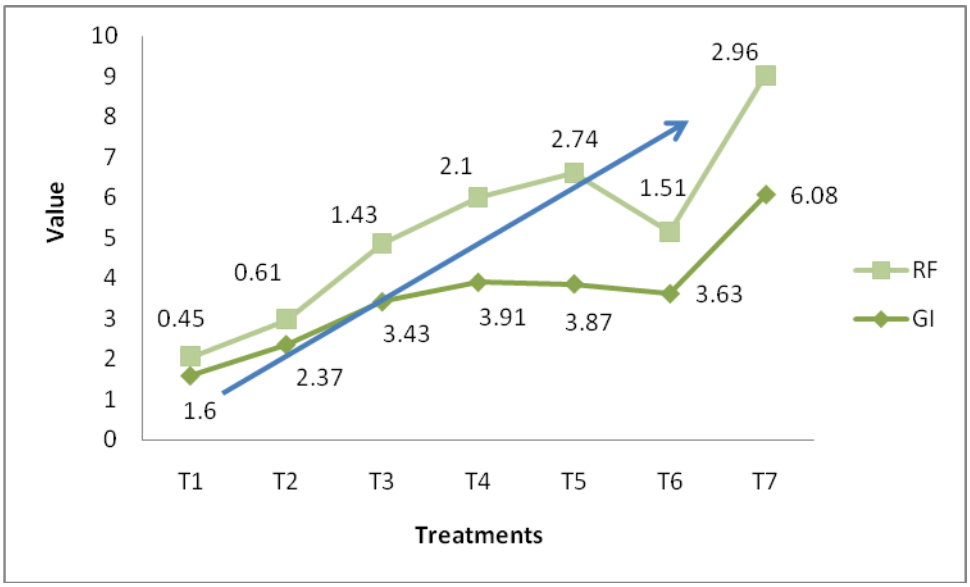


Figure 31. Interaction of gall index with reproductive factor of *Meloidogyne* spp in tomato in screen house at NARC, Khumaltar, during 2022

4.6. Effecacy level of local isolates *Trichoderma* spp on *Meloidogyne incognita*

The effect of application of *Trichoderma* spp to the tomato plant was given in Table 11. Result showed that the lowest galling index was observed in T2 (*Trichoderma lixii* - T 209) follwed by T7 (*Trichoderma virens* - T 444) and T6 (*Trichoderma afroharzianum* - T 448). In term of reducing effects of RKNs on tomato seedling, the most efficient ones were obtained from T 209 (74.79%) followed by T 444 (70.93%) and T 448 (62.12%).

4.7. Reproduction rate of root knot nematode in screen house

Effect of the application to *M. incognita* productivity was shown in table 12. In term of *Trichoderma* spp applications, the lowest reproduction ratio values in T2 (*Trichoderma lixii* - T 209) (*Rf* = 0.59) followed by T7 (*Trichoderma virens* - T 444) (*Rf* = 0.62) and T7 (*Trichoderma* - T 448) (*Rf* = 0.86) respectively. The value of galling index was directly proportionally to reproduction factors of nematodes. Number of nematodes population increased with high scale of galling index. The value of galling index was varied with the isolates of *Trichoderma*. Some isolates of *Trichoderma* such as T 209, T 444 and T 448 had low reproduction factor with lower galling index. The higher galling index showed higher reproduction factor (Figure 32).

Table 11. Effect of galling index rates (X±SEm) induced by *Meloidogyne* incognita in root system of tomato plant

S.N.	*Trichoderma* spp	Galling Index	Effect
1	**T1 (T 517)**	3.60±0.16b	-46.83
2	**T2 (T 209)**	1.70±0.26a	-74.79
3	**T3 (T 354)**	4.30±0.15bc	-30.44
4	**T4 (T 460)**	5.10±0.17cd	-18.95
5	**T5 (T 363)**	4.60±0.16cd	-35.53
6	**T6 (T 448)**	2.00±0.22a	-62.12
7	**T7 (T 444)**	1.90±0.17a	-70.93
8	**T8 (T 282)**	4.00±0.18bc	-43.66
9	**T9 (T 486)**	5.30±0.16d	-25.89
10	**T10 (T 260)**	4.90±0.22cd	-30.99
11	**T11 (Control)**	7.20±0.12e	0.00
	P≤0.05	**	
	LSD	0.98	
	CV%	8.6	

Mean within the same column with a common letter are not significantly different (p≤0.05), CV = Coefficient of vareince, Standard error of mean (SEm±)

Table 12. Effect of *Trichoderma* spp on reproduction rate of *Meloidogyne* spp on tomato

S.N.	*Trichoderma* spp	Initial Popolation (*Pi*)	Final population (*Pf*)	Reproduction factor (*Rf*)
1	**T1 (T 517)**	1000	2010	2.01b
2	**T2 (T 209)**	1000	596	0.59a
3	**T3 (T 354)**	1000	2060	2.06b
4	**T4 (T 460)**	1000	4490	4.49d
5	**T5 (T 363)**	1000	3120	3.12c
6	**T6 (T 448)**	1000	864	0.86a
7	**T7 (T 444)**	1000	620	0.62a
8	**T8 (T 282)**	1000	3180	3.18c
9	**T9 (T 486)**	1000	4800	4.8d
10	**T10 (T 260)**	1000	2280	2.28bc
11	**T11 (Control)**	1000	5120	5.12d
	P≤0.05			**
	LSD			0.87
	CV%			11.9

Mean within the same column with a common letter are not significantly different (p≤0.05), CV = Coefficient of vareince

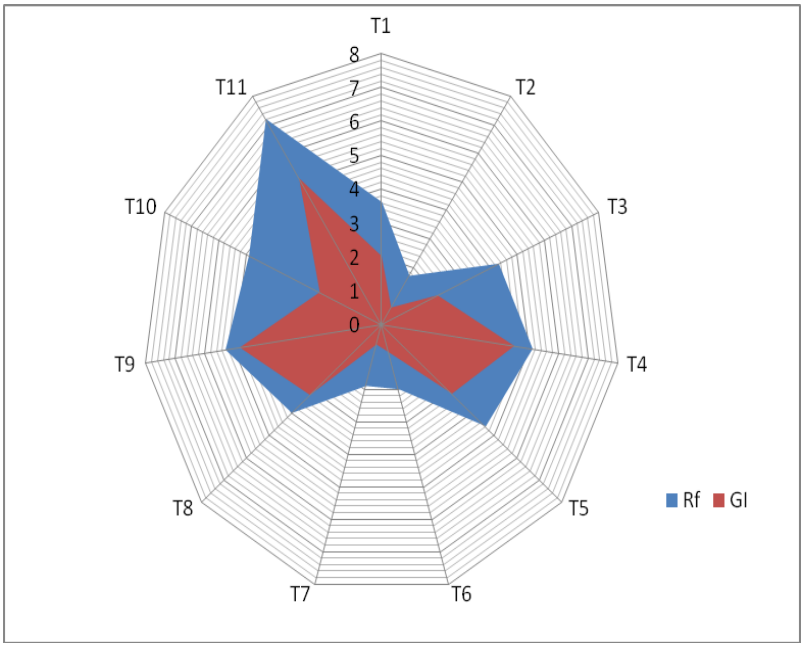


Figure 32. Interaction of gall index with reproductive factor of *Meloidogyne* spp in tomato in a screen house at NARC, Khumaltar

CHAPTER 5. DISCUSSION

5.1. Distribution of plant parasitc nematodes in cereal crops

According to their feeding strategy, plant parasitic nematodes are broadly classified into three major types; ecto-parasitic, endo-parasitic and semi-endoparasitic nematodes. These all types of nematodes were detected from the samples. The ecto-parasitic type lives outside the plant, feeding on roots with the ability to move about 3 feet to find a host, depending on the soil and species. Ecto-parasitic nematodes such as *Tylenchorhynchus, Helicotylenchus, Hoplolaimus, Longidorus, Rotylenchus,* and *Belonolaimus* etc. have long and powerful stylet with the help of that they feed in the root tissue (Luc *et al*., 2005). Endo-parasitic nematodes penetrate the root, then enter penetrates and live inside it and they are either sedentary or migratory. Some endo-parasitic nematodes such as root-knot nematode (*Meloidogyne*) can develop their feeding sites inside the root and become sedentary whereas other endo-parasitic nematodes of genera *Pratylenchus, Radopholus* and *Hirschmanniella* migrate in the roots and form root lesion by burrowing the tissue and again invading other healthy root tissue (Luc *et al*., 2005).

During the diagnosis of nematodes, the genera *Helicotylenchus, Tylenchorhynchus, Pratylenchus, Meloidogyne* and *Longidorus* are commonly recorded in maize fields whereas genus *Hirschmanniella, Criconemoides, Tylenchorhynchus* and *Meloidogyne* were frequently recorded plant parasitic nematodes from rice fields. These genera have also been already reported from different locations of the country (Bhatta, 1967 and Sharma *et al*., 2002). The nematodes were also diagnosed in species level with their morphological study. Species of *Hirschmanniella oryzae,* two species of *Criconemoides* are *C. annulata* and *C. oryzae,* one species of *Aphelenchoides besseyi* and a species *Helicotylenchus indica* were commonly

recorded in rice fields. Likewise, the three species of genus *Pratylenchus* are *P. zeae, P. penetrans* and *P. crenatus*, three species of *Helicotylenchus* are *H. indicus, H. dihystera* and *H. vulgaris* and one species of *Tylenchorhychus vulgaris* and *Hoplolaimus indicus* were commonly recorded from maize fields. In maize crop, two third of toal samples had presence of moderate to high population density nematodes to cause yield loss and rest samples had the population under threshold level, whereas in rice, nearly 45% of total samples had moderate to high population of nematodes. It indicated that the population of plant parasitic nematodes has been found higher in maize field than rice field.

The distribution of nematode species showed that more than two third (73% samples), only one genus of PPNs was recorded whereas 21% had two genera of PPNs and 6% samples had three different types of genera associated. High percent of presence of single genus in the samples may be due to their competitive behavior for feeding sites and pushing one another within intra and inter species.

In rice fields, the endoparasitic nematode *Hirschmanniella* and *Meloidogyne* were not detected in same samples/fields but they were recorded with combination of ecto and semi endo-parasitic nematodes. Similarly, the foliar nematode was also recorded with soil nematodes (endo-parasite and ecto-parasite). These nematodes were either singly or combine with one or more than one nematode in a same place. The existing patterns of nematodes in samples were 57% in single genus and 28%, 11% and 4% in combination with one, two and three other genera respectively (Figure 25). Parasitic and saprophytic nematodes were detected in vegetative and reproductive stages. However, the population of both saprophytic and parasitic nematodes was higher in vegetative stage than reproductive stage of crop (Figure 26).

Population of *Meloidogyne, Helicotylenchus, Hirschmanniella* and *Hoplolaimus* were recorded more than two nematodes per gram of soil whereas other genera were observed less than two nematodes per gram of soil. Commonly, nematodes may impact on crop yield when their population recorded more than two nematodes per one gram of soil in the field condition. Lower nematode population may not show any disease symptoms caused by nematode alone and so the effects may easily be overlooked (Baidya *et al.*, 2015). All plants tolerate minor attacks by pathogens without a significant impact on vigor, but nematodes become a problem when the population level surpasses the damage threshold; at this point damage is measurable. Growing stage of plant and enough moisture level in soil may create conducive environment for nematode multiplication (Schoener, 1983). The number of saprophytic nematodes was higher in vegetative stage due to high moisture level in field condition. Likewise, vigor root system development during vegetative stage also provided surplus feeding sites for nematodes and supported to their multiplication. The population of nematodes was gradually decreased in later stage of plant (reproductive stage) due to root system damage by numerous nematodes population and toward senescence of plant itself.

More than 50% samples showed the presence of single genus and one forth percent of total samples occupied the combination of two genera. Only 15% samples contained more than two genera. Similarly, two different endo-parasitic genera were not observed in samples. It may be due to their competitive nature for feeding sties and pushing one another within intra and inter species (Duncan and Ferris, 1983).

5.2. Distribution of plant parasitic nematodes in vegetable crops

In potato field, population of *Helicotylenchus, Tylenchorhynchus, Pratylenchus* and *Tylenchus* were recorded equal or more than two nematodes per gram of soil. Similarly, in tomato, *Helicotylenchus, Meloidogyne, Hoplolaimus*, and *Tylenchorhynchus* were recorded above threshold level to reduce crop yield. Likewise, the distribution of nematodes population in the fields of cabbage, cauliflower, chilly, capsicum, broad leaf mustard, carrot and radish also had above threshold level to impact on yield.

The genera *Helicotylenchus, Tylenchorhynchus, Pratylenchus* and *Meloidogyne* were commonly recorded than the others genera (Baidya, 2013). The result showed that the presence of high population of nematodes was also one of the possible biotic factors to reduce yields in both solanaceous and cruciferous vegetables. This study indicated that the nematode might be widespread than previously known. Spiral nematode (*Helicotylenchus* spp), root knot nematode *(Meloidogyne* spp) and stung nematode (*Tylenchorhynchus* sp) were observed in all the crops, therefore, indicated their wide spread as they are the cosmopolitan pests of vegetables distributed worldwide and infesting more than 2500 kinds of host plants (Siddiqui, 1986). Estimation of vegetable crop losses in the tropics (Sasser, 1979) ranged from 24 and 38% on tomato.

More than 55 percent samples showed the presence of single genus and 33 percent samples occupied the combination of two genera and rest 12 percent contained more than two genera. Higher percentage of existence of single nematode may be due to their competition on feeding sites and pushing one another within intra and inter species. Nematodes are various and sometimes numerous species can survive in the same root zone. When there are two or more nematode species present, their co-existence in the same location is possible, but never long-term

stable and they tend to push out one another. The strength of competing is not exactly the same between the competitors and those who have more strength; they can survive and less competitive ones may either vanish, even extinct, or try to find another feeding site. The competition level may be inter specific or intra specific or both, depending on the population density with respect to the feeding area (Schoener, 1983). Competition for feeding sites is one of the factors that limit nematode establishment and reproduction in the plant roots (Duncan and Ferris, 1983). There are three possibilities of competitive interactions among plant-parasitic nematodes: Preemptive competition occurs when a unit of space is occupied by one species, thereby preventing another species from entering that space. Nematode species may inhibit other nematode species through competition for feeding sites (Duncan and Ferris, 1983). Chemical competition is another possibility that a species produces a toxin or allele-chemical that suppresses another species. Likewise, consumptive competition occurs when some essential resource, usually food, is consumed by one species, reducing or depleting the quantity available to the competing species. Inhibition of root growth and disruption of root tissues in a host plant may be indications of consumptive competition (Sikora *et al.*, 1979).

Lower nematode population may not show any symptom cause by nematode alone and so the effects may easily be overlooked, but nematode become a problem when the population level surpasses the damage threshold; at that point the damage due to the pathogen is measurable. The crops damage by nematodes invariably remains hidden by many other limiting factors operating in agriculture, especially the presence of multiple biotic and abiotic stress factors recognized as major limiting factors until all other constraints on yield increase have been removed. If the population of nematodes is increased then its causes significant yield reduction. Hence, monitoring, identification and prioritization of plant parasitic nematodes with their population

density in different crops to manage before sudden outbreak of the problem to cause significant yield loss on the favorable condition.

5.3. Effect of *Trichoderma* spp on root knot nematode

The screen house experiment results indicated that the *Trichoderma harzianum* (Nemastin) significantly reducing the gall formation on tomato roots caused by *Meloidogyne* spp among the treatments at $P<0.05$. The phytopathogenecity of *Meloidogyne* was lower in the application of *T. harzianum* (Nemastin) followed by *T. harzianum* T22 (Root Shield Plus) and *T. viride* (Ashtha TV) compared to the control. The samilar results were obtained by Sharon *et al*. (2001), who reported that *T. harzianum* reduced galling of root-knot nematode, *M. javanica,* on tomato plants. Furthermore, Dababat and Sikora (2007) used two species of *Trichoderma* (*T. viride* and *T. harzianum*) and found significant reduction in tomato root galling infested with *M. incognita*. Four inoculum densities of two Saudi isolates of *T. harzianum* and *T. viride* against *M. javanica* on tomato (10^4, 10^6, 10^8 and 10^{10} spores/g of soil) were used and results indicated that the efficacy of *T. harzianum* was better than that of *T. viride*, especially at the highest density (10^{10} spore/g soil) which resulted in better control according to Al-Hazmi and TariqJaveed, 2015. In this study also showed *T. harzianum* was found more effective against *Meloidogyne* spp than *T. viride*. Another study also supported that *Trichoderma* species applied at 10^{10} CFU supressed the nematode reproduction and root galling; and increased the growth of tomato plants. Generally, efficacy of *T. harzianum* was better than that of *T. viride* which resulted good control according to Al-Hazmi and TariqJaveed, 2015. Results have been in agreement with Pandey *et al.* (2003) who used different treatments of *Trichoderma viride* against *M. incognita* in chickpea, in which

all treatments of *T. viride* decreased galling and the final nematode population densities in both field and pot experiments.

T. harzianum significantly reduced egg number and juveniles. The production of antibiotics and extracellular lytic enzymes (Elad *et al.,* 1982) by *Trichoderma* spp are known to be involved in the antagonism. *T. harzianum* has also been found as an egg parasite of *M. incognita* racs-3 killing 53% of eggs *in-vitro* (Dos Santos *et al.,* 1992).

T. harzianum parasitizes eggs and juvenile cuticle layer by dissolving the chitin layer through enzymatic activity. They proliferate within the organism and produce toxic metabolites into the medium in which they grow (Dos Santos *et al.,* 1992; Bandyopadhyay and Cardwell, 2003). *T. harzianum* was able to grow on the egg surface and penetrated the egg shell (Saifullah and Thomas, 1996). The present study may indicate that *T. harzianum* is an egg parasite of root knot nematode. Different species of *Trichoderma* have different modes of penetration (Dumas and Boyonowski, 1992). The variation in egg infection by the *T. harzianum* isolates can be related to genetic variability among isolates yielding difference in infectivity (Naserinasab *et al.,* 2011). Thus, the enzymes produced by *Trichoderma* spp, such as chitinase, glucanases and proteases seem to play an important role in parasitism (Haran *et al.,* 1996). The research by Sharon *et al.,* 2007 showed that *T. asperellum* parasitizes on egg masses, their derived eggs and the second stage juveniles (J2s). When the egg masses are destroyed, the number of the infective juveniles is reduced as well as the overall number of nematodes. Thus, this explains the reason for the reduction in the number of J2 nematodes that were observed from the soils treated with *T. asperellum* compared to the soil in the control. Dos Santos *et al.,* 1992 reported *T. harzianum* as an effective egg parasite of *M. incognita*. *T. harzianum* was able to grow on the egg surface and penetrated the egg shell (Saifullah and Thomas, 1996).

It suggested that *T. harzianum* produced chemical compounds detrimental to nematodes in soil or that the fungus stimulated some defense mechanisms in tomato to inhibit nematode infection of the roots or to delay the development of nematodes that entered the tomato roots (Luc *et. al*., 2005). Systemic acquired resistance or induced systemic resistance may have been induced in tomato in response to *T. harzianum* infection, leading to suppression of nematode infection and development (Jindapunnapat *et al.,* 2013). Exudation of organic acids such as gluconic acid, citric acid and fumaric acid by *Trichoderma* species reduces pH of soil and finally increase solubility and absorb important micronutrients required for growth of plant such as iron, manganese, magnesium, mineral cations and phosphats Benitez *et al.*, 2004. Liu *et al* (2007) showed that *Trichoderma* species self-propagate and survive remarkably in the soil and plant rhizosphere therefore, may remain in the soil for a long period and it had very high nematicidal action to the nematodes, such as root-knot nematodes. Antagonistic fungi possessed larvicidal and oviposidal properties against root knot nematodes. It was assessed from the present investigation that the reproductive factor of *Meloidogyne* was significantly reduced by *Trichoderma harzianum* (Nemastin), followed by *T. harzianum* (T22) and *T. viride* (Ashtha TV) in comparision to other treatments and control. Differences in performance of various strains of *Trichoderma* species in this test could also possibly be attributed to differences in the secretion of biochemical substances (Murslain *et al.,* 2014). *Trichoderma* has not only been proved to parasitize nematodes also inactivate pathogen enzymes and help in tolerance to stress condition.

The experimental result indicated that the application of *Trichoderma* species also increased the root weight of tomato plants with significant differences when compared with control. Application of different species of *T. viride,* there was no significant differences among the treatments. This result agreed with the results of Dababat and Sikora (2007), which showed there

was no significant variation between weight and height of plants inoculated with different *Trichoderma* spp. Other similar findings by Ozbay and Newman (2004) showed *T. harzianum* T22 and T95 had no effect on fresh root and dry weight. Naserinasab *et al* (2011) had also reported that inoculating the tomato seedling with *T. harzianum* didn't have consistent positive effect on fresh root weight. Both *T. harzianum* and *T. lignorum* increased plant growth and reduced *M. javanica* galling in tomato and aubergine in soil treated with the fungi 18 days prior to planting in greenhouse tests (Sharon *et al.,* 2001). Similarly, another stuty on efficacy testing of *Trichoderma* showed that who reported that treatment of tomato with *T. harzianum* increased top fresh weight and dry weight *Trichoderma* isolates have also been shown to stimulate tomato growth in both sterilized and unsterilized soil (Affokpon *et al.,* 2011).

5.4. Effect of local isolates of *Trichoderma* in *Meloidogyne incognita*

Application of different strains of *Trichoderma* collected locally from different ecological zones of country showed significantely ($p \leq 0.05$) reduced formation of galls. The galling index and number of nematode population was varied with different strains of *Trichoderma* applied. The population of nematode was achieved with concomitant decrease of galling index in root system. The trend of reproduction factor (*Rf*) was lower with decreasing the galling index (*GI*) and vice-versa. In some treatments of *Trichoderma* spp, the rate of decreasing the *Rf* value was proportionately higher with respect to reduction of galling index than other treatment of *Trichoderma* spp. It might be due to the virulence capacity of different strains of *Trichoderma* against root knot nematodes. The strains of *Trichoderma* namely T 209 (*Trichoderma lixii*), T 444 (*Trichoderma virens*) and T 448 (*Trichoderma afroharzianum*) had high potential to reduce the population of root knot nematode as compare to other strains of *Trichoderma.*

When the application was compared the strains' of *Trichoderma* sp collected from Sarlahi district of Medhesh Pravince (*Trichoderma lixii* - T 209) was found the most effective to reduce the population of root knot nematodes followed by the strains of Kanchanpur district of Sudurpashchim Province (T 444) and Chitwan district of Bagmati Province (T 448), respectively. The isolates of *Trichoderma* from different parts of the country had potential to effect on the growth of root knot nematodes. The study showed that the *Trichoderma* sp from plain areas were found to reduce the population of nematodes than the isolates/strains from hill areas.These obtained values showed parallelism with RKN scale values. In a study conducted by Ahmad and Khan (2004) on tomato, by applying *Trichoderma* sp to the soil, the nematode population in plant was reduced 67-77%. Also, the gall in root system was reduced 30%. Also, Akhtar and Mahmood (1993) pointed out that pepper plants, *M. incognita* destruction were determined to be reduced when the *Trichoderma* spp added to soil. These finding showed similarity with results obtained on the study. *Trichoderma* sp is a fungus which colonizes near the plant roots and grows on roots, provide resistance to wilt and rot diseases in root system and also create physical barrier for nematodes to contact. Nemacidal activity of *Trichoderma* may be due to the eggs and juveniles being infected through the increase in chitinase and prolease activity. As chitin is a major component of egg shell of nematodes, nematophagous egg parasitic fungus can penetrate the eggs leading to the reduction in population (Wickramaarachchi and Ranaweera, 2008). Similarly, *T. harzianum* is also an effective egg parasite of *M. incognita* which is able to grow on the egg surface and penetrated the egg shell (Sikora, 2008). Babu *et al*. (1999) reported that the formation of galls on the roots and colonization of root tissue by the nematode deprives plants nutrients. The damage also occurs due to devitalization of root tips, which may stop their growth or cause excessive branching of roots.

In support of this research, Akram *et al*. (2020) recently also reported the suppression of RKNs by using such endophytic fungi *Trichoderma.* Colonization of plants by *Trichoderma* triggers host defense against PPN (Forghani and Hajihassani, 2020). A group of researchers found that root colonization by *Trichoderma* prevented nematode performance both locally and systemically at multiple stages such as invasion, gall formation and reproduction. First, *Trichoderma* primed salicyclic acid (SA)-regulated defenses, limiting nematode root invasion. It enhanced jasmonic acid (JA) regulated defenses, thereby antagonizing the deregulation of JA-dependent immunity by the nematodes, compromising galling and fecundity (Martinez-Medina *et al.*, 2017). Murslain *et al*. (2014) and Sharon *et al*. (2001) reported similar results that population of *M. incognita* was remarkably reduced by *Trichoderma* spp. Hence, *Trichoderma* spp exert its biocontrol activity against fungal phytopathogens either indirectly, by competing for nutrients and space, modifying the environmental conditions, or promoting plant growth and plant defense mechanisms and antibiosis or directly, by mechanisms such as mycoparasitism (Hafez *et al.*, 2013). Likewise, in vitro studies demonstrated that all tested isolates of *Trichoderma harzianum* and *Trichoderma viride* for the biological control of *Meloidogyne incognita* on tomato were effective in causing second-stage juveniles (J2s) mortality compared with the control (Dababat and Sikora, 2007). Similarly, Pathak & Kumar, 1996 and Affokpon *et al.,* 2011 showed that *Trichoderma asperellum T*-16 and *T. asperellum* T-12, suppressed second stage juvenile (J2) densities in roots by up to 80% and 94% respectively in tomato.

T. harzianum parasitizes eggs and juvenile's cuticle layer by dissolving the chitin layer through enzymatic activity. They proliferate within the organism and produce toxic metabolites into the medium in which they grow (Dos Santos *et al.*, 1992; Bandyopadhyay and Cardwell, 2003*). T. harzianum* was able to grow on the egg surface and penetrated the egg shell (Saifullah and

Thomas, 1996). The present study indicated that *T. harzianum* has been an egg parasite of root knot nematode. Different species of *Trichoderma* have different modes of penetration (Dumas and Boyonowski, 1992). The variation in egg infection by the *T. harzianum* isolates can be related to genetic variability among isolates yielding difference in infectivity (Naserinasab *et al.*, 2011). Thus, the enzymes produced by *Trichoderma* spp, such as chitinase, glucanases and proteases seem to play an important role in parasitism (Haran *et al.,* 1996). *Trichoderma* has not only been proved to parasitize nematodes also inactivate pathogen enzymes and help in tolerance to stress condition by enhanced root development. It participates in solubilization of organic nutrients. Thus, *Trichoderma* colonized roots require lesser supply of man made nitrogen fertilizer (Harman, 2000). In addition, once *Trichoderma* is applied in the soil there are no needs to apply repeatedly if the soil condition is menatined favourable for self multiplication of organisms in each cropping season. Hence, in favourable environment with optimum level of organic fertilizer and moisture in soil, *Trichoderma* grows well and multiply its population. *Trichoderma* treated plot showed suppression of RKN during the cropping period. Liu *et al.* (2007) showed that *Trichoderma* species self-propagate and survive remarkably in the soil and plant rhizosphere therefore, may remain in the soil for a long period and it had very high nematicidal action to the nematodes, such as root-knot nematodes.

CHAPTER 6. CONCLUSION

The genera *Helicotylenchus, Tylenchorhynchus, Pratylenchus, Hirschmanniella, Aphelenchoides, Meloidogyne* and *Longidorus* are commonly recorded plant parasitic nematodes in wheat field. Some of them (*Helicotylenchus, Tylenchorhynchus Meloidogyne* and *Pratylenchus*) were presence moderate level which could be caused crop yield loss. In high population of nematodes, they can cause both quantitative and qualitative yield loss. The genera *Helicotylenchus, Tylenchorhynchus, Pratylenchus* and *Hoplolaimus* have been commonly recorded in maize fields with moderate to high number to inpact on crop yields. Where as genus *Hirschmanniella, Criconemoides, Aphelenchoides* and *Meloidogyne* were frequently recorded plant parasitic nematodes from rice fields. Nematode itself has potential to reduce yield of crops if their population was establish two or more than two in number per gram of soil. Lower nematode population may occur with or without obvious symptom of attack and so the effects may easily be overlooked. Nematode may also one of the hidden biotic factors to reduce crop yield in the fields. Hence, management of plant parasitic nematodes has also necessary step for the increase of yield by minimizing the losses due to nematode damage in the crop.

In vegetable crops, twelve different genera of plant parasitic nematodes namely, *Meloidogyne, Rotylenchus, Tylenchus, Hoplolaimus, Belonolaimus, Criconemoides, Helicotylenchus, Tylenchorhynchus, Pratylenchus, Hirschmanniella, Aphelenchoides* and *Longidorus* were detected. Among them, the most commonly detected genera were *Helicotylenchus* followed by *Tylenchorhynchus, Meloidogyne* and *Pratylenchus*. They found either singly or in combination with two to more than two genera. More than 50% samples had presence of single genus. In solanaceous crops, the commonly recorded nematodes were *Tylenchorhynchus* spp,

Helicotylenchus spp, *Pratylenchus* spp, *Tylenchus* sp, *Meloidogyne* spp, *Criconemoides* sp, *Aphelenchoides* sp, *Belonolaimus* sp, *Hoplolaimus* sp, and *Rotylenchus* sp. Similarly, in cruciferus crops, *Helicotylenchus* spp, *Belonolaimus* sp, *Longidorus* sp, *Tylenchorhynchus* sp, *Pratylenchus* sp, *Hoplolaimus* sp, *Criconemoides* sp, *Meloidogyne* sp and *Hirschmanniella* sp were recorded. Some genera like *Helicotylenchus, Tylenchorhynchus, Pratylenchus* and *Meloidogyne* had record more than two nematodes per gram of soil that mean above threshold level to inpact on the crops yield. Lower nematode population may not show any symptom caused by nematode alone and so the effects may easily be overlooked, but nematodes become a problem when the population level surpasses the damage threshold; at that point the damage due to the pathogen is measurable and also need to manage them.

Different commercial products of *T. harzianum* appear effective agasint root knot nematode than the product of *T. viride* in terms to suppress root knot galling index and multiplication of *Meloidogyne* spp at screen house condition. In addition, the bio-control agents also reduce the number of gall formation and increase the root system. Therefore, the *T. harzianum* might be applied as an organic management tool in field to reduce the nematode population and increase yield of tomato. Also further investigation on identification of effective isolates of *Trichoderma* species has to be carried out for the management of different species of root knot nematode in vegetables.

The result of this study revealed that the local strains of *Trichoderma* sp such as *T. lixii, T. virens* and *T. afroharzianum* have potential to regulate the population of root knot nematodes by reducing egg hatching and caused juveniles mortality in screen house condition. The isolate of *Trichoderma* from plain areas Sarlahi, Kanchanpur and Chitwan districts of Madhesh, Sudurpashchim and Bagmati provinces respectively had more effective than the other isolates of

the country. However, to see their efficacy potential further research should be carried out to determine its efficacy under field conditions. Due to exorbitant cost of nemacidal chemicals and the environmental hazards they cause, it is necessary to modify the nematode management options by applying biological control agents like *Trichoderma* which is safer and cost-effective. Findings also suggested that the tested *Trichoderma* spp were effective for managing root knot nematodes. *Trichoderma* spp has potential to be a vital component of integrated management of root-knot nematodes. Several problems are associated with the use of chemicals such as their poor penetration into the nematode eggs, rapid leaching and degradation, high cost and above all, chemicals are a serious threat to the environment. Hence, biological control is an environment friendly alternative tool for the management of soil borne pathogens including nematodes.

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