1

INTRODUCTION

About 95% of plant parasites live in the soil and feed in or on roots. They are microscopic, pseudocoelomate, unsegmented worms commonly described as filliform or thread like (Bird and Bird 1991*)*. The birth of nematology can be dated on 1743 with the observation of Needham of the wheat seed gall nematode or "ear cockle eelworm" (Luc *et al.,* 1990). Since then the role of nematode in crop losses have been closely monitored. The overall annual yield loss of the world's major crops due to damage by plant-parasitic nematode is at 20% with average loss of 14% for economic important corps (Sasser and Freck man, 1987). Crop production problem induce by nematodes therefore generally occur as a result of root dysfunction, reducing rooting volume and foreging and utilization of efficiency of water and nutrients. 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 is considered as the most important genus in worldwide basis in terms of number of species parasitized and extent of crop damage and loss.

Nepal is the home place of natural beauty with traces of artifacts. Agriculture is the source of livelihood for the majority of population in Nepal. Diverse climatic condition favours the cultivation of different kinds of fruits and vegetables in Nepal. Among various crop-cultivations, off-season tomato cultivation is one of the important income-generating source for small farmers of the hills (Budhathoki *et al.,* 2004). Tomato is one of the most popular and widely grown vegetables in the world remaining second in importance to potato in many countries. The fruits are eaten raw or cooked. Large quantities of tomato are used to produce soup, juice, ketchup, paste and powder. Tomato is also popular because it supplies vit-c and adds variety of colours and flavours to foods. Cultivation of this crop is getting popular day by day for quick and high income generation. It is reported that income of Rs. 65,000 to 100000 per ropani of plastic house is obtained by farmers at Kathmandu, Pokhara, Lamjung, Lalitpur, Bhaktapur, Palpa, Parbat etc. *(*Budhathoki *et. al*., 2004). Tomato growers are very discouraged and depressed due to heavy reduction in production and productivity by root-knot nematodes (DADO Kaski and Lamjung, 2006).

The infestation level of root-knot nematode alone has been expected up to 82.5 percent on tomato and okra (Saxena, 1986*)*. Bhatti and Jain (1977) observed losses worth of 91, 46 and 27 percent in okra, tomato and brinjal, respectively due to root-knot nematode. Krishnappu *et al*; (1992) found 15 to 60% yield loss in tomato.

Generally, chemical control is more widely used than resistant varieties. 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 (Webster, 1987). 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. Nematode multiplication is a standard measurement of resistance which, in nematology, is defined as the ability of a host plant to prevent multiplication of the nematode (Cook and Evans, 1987; Trudgill, 1991). Host plant resistance has become an important strategy in the management of plant parasitic nematodes. Although considerable genetic resources of resistance are available in crop plants of their wild relatives, relatively few crops have resistant cultivars for commercial use (Roberts, 1992).

Resistant cultivars can 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). Resistant cultivars have several advantages over other methods of reducing nematode populations: (i) their use requires little or no technology and is cost effective (ii) they allow rotation to be shortened and best use to be made of the land (iii) they do not leave toxic residues (Trudgill, 1991).

To this point research priority discussions (Bird, 1980) have identified plant resistance consistently over chemical, biological, cultural and regulatory control components as the highest research priority for pest management. Resistant crops provide an effective and economical method for managing nematodes in both high and low value cropping systems. Resistant crops in annual cropping systems can reduce nematode population to levels that are non damaging to subsequent crops. In less developed countries and in low-

cash crop systems plant resistance is probably the only viable long term solution to nematode problems.

Screening experiments for resistance can be established in the field, micro plots, green-house, shade-house, growth chamber and *in vitro* (Starr, 1990). The cultural conditions can greatly influence the growth of plants, the survival, development and reproduction of migrating plant parasitic nematodes, it is necessary to conduct screening experiments under carefully defined conditions (Peng and Moens 2002). A growth chamber can effectively avoid the variation in the environment conditions between different seasons (Peng, 2001). Laboratory-based systems use plants in various types of container, growing in sand (Foot, 1977) or other media. Peng and Moens (2002) recommended that for the screening of rose cultivars, the screening conditions are best in a growth chamber and by using smaller pots (50 ml).

1.1 Significance of the study

While viewing the above mentioned importance of the resistant cultivars the present study has been under-taken with the attachment on the regular project of Plant Pathology Division under NARC.

1.2 Limitations of the study

- The study was carried out in screen house condition instead of regularly infested field. So field data was not included.
- In this study only 14 different tomato cultivars were used for screening against root-knot nematode, however some commonly, cultivable cultivars were excluded in the experiment.
- The experiment was conducted for 45 days, hence no yield data was recorded. In addition to this physical parameters of plants such as plant height, branching, data of flowering and fruiting etc had not been included.
- The innoculum was collected from Hemja, Pokhara but species of *Meloidogyne* was not identified during experiment. It might be mix-population of different species of genus *Meloidogyne*.

2

A BRIEF INTRODUCTION OF *MELOIDOGYNE*

2.1 Taxonomic position of *Meloidogyne* spp

Nematodes are classified within the phylum Nematoda with two classes: Enoplea and Chromadorea (De Ley and Blaxter, 2000). The order Domylaimida within the Enoplea and Order Rhabditida within Chromadorea contain all plant-parasitic nematodes. The genus *Meloidogyne* under Meloidogynidae family belongs to order Rhabditida and commonly known as root-knot nematode. *Meloidogyne* was first observed by Berkeley in 1855.

2.2 The taxonomical position of *Meloidogyne* species is as follow

Phylum: Nematode

Class: Chromadorea

Order: Tylenchida

Suborder: Tylenchina

Superfamily: Tylenchoida

Family: Meloidogynidae

Subfamily: Meloidogyninae

Genus: *Meloidogyne*

Common name: Root knot nematode

2.3 Measurements:

Female: - length =0.44-1.30mm, width=0.35-0.700mm, stylet=10-24µm usually 14-15µm, Dorsal oesophgeal gland orifice (DEGO)=2-10 µm (Eisenback and Traintophyllou, 1991).

Male: - Length =1000-1500μm i.e. 1-1.5mm, stylet=13-30μm av1.8-24μm, spicule length =19-40μm, DEGO =2-13μm posterior to stylet knob base (Eisenback and Traintophyllou, 1991).

2nd stage juvenile: - length=280-500μm, stylet=10μm (13-30μm),DEGO distance= 2-8μm, tail length=15-100μm (Eisenback and Triantophyllou, 1991).

Egg: - Length=80μm and width=35μm (Orion *et al*., 1994).

Female

Mature females are swollen to pear shaped or nearly spherical shape except for an elongate anterior end. Its body remains soft, pearl white in colour and does not form a cyst, the neck protrudes anteriorly and the excretory pore is anterior to the median bulb often near stylet base, the vulva and anus are terminal, flush with or slightly raised from the body counter, the cuticle of the terminal region forms a characteristic pattern; the perennial pattern. The female stylet is shorter with a small basal bulb. The stylet is moved by protactor muscles and functions like a hypodermic needle. The paired gonads have a extensive convoluted ovaries that fill most of the swollen body cavity. There are six large unicellular rectal glands in the posterior body, which produce a gelatinous matrix to form an egg sac in which the eggs are deposited (Eisenback and Triantophyllou, 1991; Kleynhans, 1991).

Male

They are vermiform. Their lip region has a distinct head cap, which includes a labial disc surrounded by lateral and medial lips. The oesophagus has normally developed procorpus, metacarpus with a valve, narrow isthmus and a ventrally overlapping glandular basal bulb. Its stylet is strongly developed with a large basal knob. Spicules and gubernaculums are nearly terminal and the blunt rounded tail, which has no bursa. The tail is short and hemispherical. Body usually twisted through 180 along its length on heat relaxation (Luc *et al*., 2005). One gonad is present in normal males, whereas sex-reversed male has two gonads. Most of the gonads consist of long vas deferens packed with developing sperm (Eisenback and Triantophyllou, 1991).

2nd stage juvenile

It is the infective stage and often found free living in the soil. The stylet is slender and bear rounded basal knobs. The median oesophageal glands are extensive, overlapping the intestine for several body widths mainly ventrally. The tail is canoid often ending in a narrow rounded terminus. Stylet and head skeleton are weakly sclerotized. The position of the excretory pore is variable (Eisenback and Triantophyllou, 1991).

Egg and egg sac

The egg of *Meloidogyne* has an oblong shape, with a surface of two distinctive topographical structures under scanning electron microscope (SEM). The eggs are laid in gelatinous matrix (GM) in a single celled stage and undergo development to first stage juveniles and hatches into second stage juvenile. The eggs and the GM form the egg mass, which is generally found at the interface between the gall surface and the soil. The GM is produced by six rectal glands, during egg laying which are arranged radially around the female anal opening. The density of the layered material in the GM appeared to change with age with a diameter of 0.5μ in a newly formed egg mass and of 2μm in mature egg mass. The GM contains cellulytic and pectolytic enzymes and was suspected to protect the nematode against soil borne microorganisms (Orion *et al*., 1994).

2.4 Host range of *Meloidogyne* spp

Root knot nematodes occur throughout most of the world, infect all major crop plants and cause substantial reduction in crop yield and quality. The genus *Meloidogyne* with more than 80 species shows a wide range of host specificity except few species that are species specific. Some common hosts of *Meloidogyne* reported from different parts of Nepal are mentioned as below: -

Vegetables

Brinjal (*Solanum melongena*), Potato (*Solanum tuberosum*), Tomato (*Lycopersicon esculatum*), Broccoli (*Brassica oleracea var. ilalica*), Radish (*Raphanus sativa*), cabbage (*Brassica olreacea* var. *capitata*), Chinese cabbage (*Brassica chinensis*), Cauliflower *(Brassica olracia var. botrytis*), and okra *(Abelmoschus culenestus (*Bhardwaj, 1982, Rana *et al., 1992).*

Legumes

Broad bean (*Vicia faba*), Pigeon pea *(Cajanu cajan*), Pea (*Pisum sativum),* Gram *(Cicer arentinum*), and Lentil *(Lens esculenta*) (Bhardwaj, 1982, Rana *et al.,* 1992).

Spices and narcotics

Chilly (*Capsicum annum),* Ginger (*Zingiber officinale*), Turmuric (*Curcuma longa*), Anise *(Pimpinella anisum*), and Coriander (*Coriandrum sativa)*(Bhardwaj, 1982).

Fruits and other crops

Banana *(Musa spp*), Rape (*Brassica campestris var.tori*), papaya (*Carica papaya*), and Jute (*Corchorus spp*) (Pokharel, 1993).

Weeds

Common vetch (*Vicia sativa*), Vetch (*V. hirsute*), black night shade (*Solanum nigrum*), Datiyun (*Achyranthes aspera*), Lunde kada *(Amaranthus spinosus),* Krishnanil (*Anagallis arvensis*), Bhang (*Cannabis sativa*), Taprejhar (*Cassia tora*), Bethe (*Chenopodium aibum*), Jaluka/ wild taro (*colocasia esculenta*), Banpat (*Corchorus aestuans*), Chitre banso (*Digitaria ciliaris*), Bhadaure banso (*Echinochloa colona*), Mulapate (*Emilia sonchifolia*), Dudhe (*Euphorbi*a *heterophylla*), and Gandhejhar (*Ageratum houstonianum*) (Rana *et al.,* 1993).

2.5 General symptom and Feeding behavior of *Meloidogyne* spp:

Root-knot nematodes affect plant growth adversely causing morphological and physiological changes in the roots, expressed as deformation and sometimes reduction of the root mass and formation of galls and giant cells in the root and other below ground parts. The damage to plants by *Meloidogyne* is due largely to the disruptions of vascular tissues and extensive hypertrophy and hyperplasia of root cells. The infected plants show unthriftiness, general wilt and poor growth with increasing population of the nematode (Swarup and Sharma, 1965). The damage is aggravated by the parasites interaction with other microorganisms such as fungi and bacteria as it induces the plant to become susceptible to normally non-pathogenic or weakly parasitic organisms (Kleynhan, 1991).

The juveniles of 2nd stage of *Meloidogyne* is the only stage that can infect a new plant, they perceive stimuli and are attracted by plants. Co_2 is considered as being most important root excretion for attracting the 2nd stage juvenile which accumulate at the

region of cell elongation just behind the root cap and are also attracted to apical meristems, points where lateral roots emerge, penetration of juveniles involves mechanical action by thrusting of the stylet. Cellulytic and pectolytic enzymes may also be involved. Following penetration, especially with multiple infections on the same root, the root tip may enlarge and root growth often stopped for a short period. The juveniles then migrate intracellularly in the cortex to the region of cell elongation. This causes cells to separate along the middle lamella.

After migrating a short distance, juveniles reside in cortical tissues in the zone of differentiation, their heads in the vascular tissue and the remainder of their bodies in the cortex parallel with the long axis of the root. Susceptible plants react to feeding by juveniles and undergo pronounced morphological and physiological changes. Giant cells, feeding sites for the nematodes are established in the phloem or adjacent parenchyma. These cells are highly specialized cellular adaptations induced and maintained by feeding juveniles. Without this host response, juveniles fail to develop. Giant cells are most likely formed through repeated endomitosis without cytokinesis (Moens, 2005).

Concurrent with the establishment of giant cells, root tissues around the nematodes undergo hyperplasia and hypertrophy causing the characteristic root gall. Galls usually develop one or two days after juvenile penetration.

2.6 Life cycle of *Meloidogyne* spp

Life cycle of *Meloidogyne* spp. completes within four weeks at 25ºc (Luc *et al*., 2005). One molt occurs in the egg, leading to hatching of the infective 2nd stage juvenile. This stage penetrates and migrates inside host tissue and starts to feed. Then their body swell, which is frequently termed as a "sausage stage", within, which three additional molts occurs. Females then continue to grow nearly spherical in form. After the last molt, however, males are seen coiled looped within the "sausage" cuticle, from which they emerge and migrate toward a female. Mating may occur but is not essential to the development since parthenogenesis occurs in this genus. Eggs from a single female numbers from a few hundred to 5000, with 300 to 500 generally considered the average. Eggs are deposited in a single celled stage and undergo development to the first and second stage juvenile prior to hatching and emergence (figure 1).

According to Bird and Wallace, *M. hapla* hatches best at 25ºc while for *M. javanica* a 30ºc optimum for hatching.

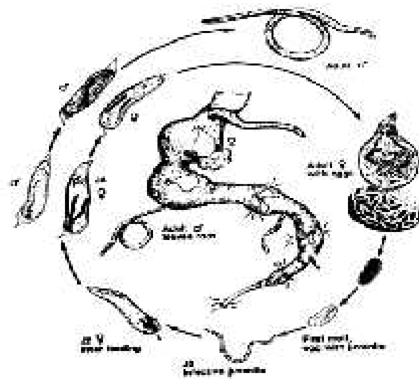

Figure 1: Diagram of the life cycle of root-knot nematode, *Meloidogyne* spp. Abbreviation: J2, second-stage juvenile; J4, fourth-stage juvenile.

2.7 Reproduction

Root knot nematodes reproduce by cross-fertilization (amphimixis), by both amphimixis and meiotic parthenogenesis (automixis) or by obligatory mitotic parthenogenesis (apomixis). Amphimictic species have a haploid chromosome number of 18, meiotic parthenogenetic species have 18 or fewer (13-17) chromosome but some are diploid, others are triploid with 50-56-chromosome number of about 45 in table 1 (Triantaphyllou, 1985) in Table 1.

Table 1. Summary of Cytogenetic information Related to Root-knot nematodes (*Meloidogyne* spp.)

Meloidogyne species	Populations studied (number)	Countries of origin (number)	Chromosome number n	Chromosome number 2n	Mode of reproduction
M. kikuyensis	1	1	7		
M. spartinae	4	1	7		
M. carolinensis	2	1	18		
M. megatyla	1	1	18		Amphimixis
M. microtyla	2	1	18-19		
M. subartica	1	1	18		
M. exigua	6	1	18		
M. graminicola	1	1	18		
M. graminis	10	5	18		Facultative
M. naasi	1	1	18		
M. ottersoni	1	1	18		meiotic
M. hapla (race A)	48	24	13-17		parthenogenesis
(Polyploid)	1	1	28		
(Polyploid)	2	2	34		
M. chitwoodi	6	3	14-18		
M. arenaria	18	13		30-38	
		34	21	40-48	
		68	32	51-56	
M. cruciani	1	1		42-44	
M. enterolobii	1	1		46	
M. hapla (race B)	6	3		30-32	
	11	8		43-48	Obligatory
M. hispanica	4	4		33-36	
M. incognita	6	6		32-38	
	215	64		41-46	mitotic
M. javanica	126	45		42-48	
M. microcephala	3	2		36-38	
M. oryzae	2	1		51-55	parthenogenesis
M. platani	1	1		42-44	
M. querciana	1	1		30-32	
Total	584				

Source: Eisenback, and Triantaphyllou. 1991

2.8 Sex determination

In facultative and parthenogenetic species sex determination appears to be controlled largely by the environment. Under favourable environmental conditions most 2nd stage juvenile develop into female and the remaining juveniles into males with one testis. Unfavorable conditions brought about by nutritional deficiencies in the host, injury to the host plants, high temperatures or crowding of juveniles in the root lead to the development of many male populations. Sometimes male intersexes are derived from female destined juveniles, which experience unfavorable conditions at an advanced stage of development (Triantaphyllou1960; Davide and Triantaphyllou, 1967).

2.9 Host-Parasite Relationships (Feeding behaviour)

Root-knot nematodes usually cause the formation of knots or galls on roots of susceptible host plants. The second-stage infective juveniles are attracted to host roots. They accumulate in the regions of the apical mertistem, cell elongation, and near points of emergence of lateral roots. The juveniles usually enter the roots behind the root cap. After penetration, the second-stage juveniles migrate intercellularly in the cortex to the region of cell differentiation where they settle and begin feeding. Their heads lie at the periphery of the vascular parenchyma tissue, and the remainder of their bodies in the cortex parallel to the long axis of the root. Preferred feeding sites are primary phloem or adjacent undifferentiated parenchyma-like cells of the pericycle. In response to the feeding of the second-stage juvenile, the host tissue undergoes pronounced morphological and physiological changes. Some parenchyma cells develop into permanent nurse cells for the nematode. They become hypertrophied, multinucleate "giant cells", possibly as a result of the introduction of secretions produced by the subventral esophageal glands cells of the feeding second-stage juvenile. The nematodes derive nourishment from these specialized nutritive cells and cannot continue their development to adulthood without them. Males apparently do not feed as adults and leave the roots, but females continue feeding at the same giant cells for the remainder of their life spans.

Giant cells are essential for a successful host-parasite relationship. Their active metabolism is maintained either through secretions of the dorsal esophageal gland (Bird, 1968) or the removal of solutes by the adult female. Photosynthetic products are mobilized to the giant cells in the roots and, as a result, plant growth and yield may be suppressed. Other above-ground symptoms of infected plants include chlorosis of foliage and temporary wilting during periods of water stress. Nutrient and water absorption are greatly reduced by the damaged, galled root system.

Root tissues around the nematode and the giant cells undergo hyperplasia and hypertrophy resulting in the characteristic root gall. Galls usually develop 1-2 days after juvenile penetration. Gall size is commonly related to the number of nematodes present in the tissue but may also depend on the plant species parasitized. Galls induced by most *Meloidogyne* species are similar in their morphology. Some species, however, such as *M. exigua, M. hapla*, and *M. kikuyensis*, produce characteristic galls which may be useful in species identification.

2.10 Ecology

Dormancy

Survival of *Meloidogyne* eggs and juveniles is less persistent than in the closely related genus *Heterodera* where eggs in cysts remain viable for 1-5 years. Development of the genital primordium (diagrammatic) of *M. incognita* juveniles 5, 10, 14 and 18 days after initiation of feeding illustrates the anatomical changes that lead to the development of females, sex-reversed male, or normal males. Adverse environmental conditions lead to sex reversal and the development of males with variable number of testis.

Eggs of *Meloidogyne* are deposited in a gelatinous matrix (white or brown). White egg masses are usually formed early in the host growing cycle; the eggs they contain hatch immediately making different generations per growing cycle possible. Brown masses are formed later or in adverse conditions; the eggs they contain are in dormancy and do not hatch immediately. These latter egg masses ensure some carry over from one season to another.

Temperature

Temperature is an important factor in several stages of the development of nematodes. Temperature influences distribution, survival, growth and reproduction. Within the genus *Meloidogyne* two distinct groups can be distinguished: thermophils and cryophils, which can be separated on their ability to survive lipid-phase transitions that occur at 10^oC. *M. hapla, M. chitwoodi and M. incognita, M. javanica* and *M. exigua* are thermophils and do not have extended survival at temperature below 10^oC. Like survival, hatching is controlled by temperature.

Within a species one may consider thermotypes. These populations can be distinguished from each other by small differences in temperature minimums. This is due to adaptation of geographical populations to local temperatures.

Soil texture, moisture, aeration and osmotic potential

These interacting factors are difficult to separate from each other. *Meloidogyne* species are active in soils with moisture levels 40-60% of field capacity. As the soils either dry or increase in moisture, nematode activity decreases.

In drying environments, the gelatinous matrix of the egg sac appears to maintain a high moisture level and provide a barrier to water loss from eggs. Embryos and first stage

juveniles are more resistant to water loss than unhatched second stage juveniles because changes in the egg membrane after the first moult.

In drying soils, nematodes may be submitted to high osmotic pressures, especially after fertilizer applications. Even small changes in osmotic potential can be important to nematode behaviour.

Increased crop damage is often associated with alkaline soils. This seems associated with stress on the host plant.

3

OBJECTIVES OF THE STUDY

3.1. General Objective:

- Z To screen varieties or cultivars of tomato plant to *Meloidogyne* to reveal their resistance.

3.2. Specific objective:

- Z To isolate the *Meloidogyne* species from root of tomato plant cultivated in natural condition
- Z To isolate and inoculate the *Meloidogyne* eggs to the test plant.

4

LITERATURE REVIEW

4.1. General review on Plant parasitic nematodes in Nepal

The first investigation on plant nematodes for soil pathogens including root-knot nematode, in Nepal, began in 1963 along with the establishment of Plant Pathology Division, Khumaltar. For the first time Bhatta (1967) reported eleven different species of plant parasitic nematodes. Later Amatya and Shrestha did an extensive survey of plant parasitic nematodes in different parts of the country and reported 23 genera along with the *Meloidogyne*. Then in 1973, Zulini had collected nematodes from high altitude Khumbu area and reported some 21 genera of soil and fresh water nematodes with the description of new species.

Hogger reported *M. incognita* associated potato crop in 1981 and Bhardwaj (1982) and Bhardwaj and Shrestha (1983) reported some naturally infested hosts of *M. arenaria*, *M. incognita* and *M. javanica* in Chitwan district.

Khan (1983) found thirteen genera of plant parasitic nematodes including one genera, *Psylenchus* from Nepal associated with pineapple crop in Chitwan. 12 genera of plant parasitic nematodes associated with 7 vegetable crops from Kathmandu valley among which the population of *Meloidogyne incognita* were reported higher (Keshari, 1986).

Manandhar, and Amatya (1988) identified *M. javanica* and *M. incognita* race 2 infesting chickpea at national grain legume improvement programme, Rampur Chitwan by performing North Carolina host differential test.

Four genera of plant parasitic nematodes i.e., *Helicotylenchus*, *Meloidogyne*, *Tylenchorhynchus*, and *Pratylenchus* associated with the rhizosphere of papaya plant from Chitwan, Nepal (Yadav *et al*., 1989).

An experiment in winter crops (potato, tomato, coriander, spinach, pea, gram, prickly amaranthus, edible amaranthus, radish, dill, broccoli, cabbage, fenugreek, pea, gram, lentil and cowpea) and summer crops (brinjal, pointed gourd, pumpkin, French bean, bitter gourd, okra, cucumber, sponge gourd, bean, board bean) was conducted at IAAS. The results revealed that *M. incognita* was most common and predominant species and

56% of crops were infested, followed by *M. arenaria* and *M. javanica* with 37% and 23% crop infestation respectively. In some cases it was also observed that more than one species of *Meloidogyne* infested in the single species of plant (Rana and Ali, 1992).

Rana, (1995) first surveyed on the infestation of plant parasitic nematodes in Chyotes. Among 36 samples only one soil sample was infested with *Helicotylenchus* while remaining soil sample were infested by *Meloidogyne* spp.

Pokhrel, (1998) described Entomopathogenic nematodes useful for biocontrol agent.

4.2. Review on screening research in global context

- Hendy *et al.*, (1983) identified two pungent capsicum (from central America) and Pm 678(from India) that were resistant to three of the main Meloidogyne spp; *Meloidogyne arenaria*, *M. incognita* , and *M.javanica*.
- Aarts *et al.*, (1991) developed a molecular probe for the tightly link acid phosphates-1 (APS-1) locus to cloning the root-knot nematode resistance gene Mi in tomato.
- Mercer *et al*., (1992) observed the resistance and tolerance cultivars of white cloves to root-knot nematode. They have selected eighteen cultivars which were naturally infested with nematodes derived by crossing of plant for improved tolerance or resistance to root- knot nematode over unselected controls.
- Marban-Mendoza (1992) studied the control of *M.incognita* on tomato by two tropical legumes *Pueraria phaseoloides* and *Arachis pintol* cultivated with tomato. They found four other legumes had no effect of root galling. Presence of soluble lectins, i.e phaseolus vulgar is responsible for the antinematodal property of legumes.
- Siddiqui (1992) studied response of 45 chickpea cultivars to *Meloidogyne incognita* and their effect on perioxidase activity. He determined the percentage of reduction in dry shoot weight and nematode reproduction. He found positive correlation between peroxidase activity and the degree of resistance present in the cultivars.
- Walters (1993) evaluate ℑ degree of resistance in cucumber (*Cucumis sativus* and horned cucumber *(C.metuliferus)* to *Meloidogyne incognita*. All twenty four *C.metuliferus* cultigens were found to be resistant to all root knot nematodes while out of 884 *C. sativus* cultigens, only fifty were somewhat resistant to *M. arenaria* and *M.incognita*. All 884 *C.sativus* cultigens retested were resistant to *M. hapla*. All *C. sativus* cultigens retested, including Lj 90430, were highly susceptible to *M.incognita* races 1 and 3, two *C.metuliferus* cultigens retested were highly resistant to all root knot nematodes.

- Suguna (1993) evaluated the invitro response of various explants on Murashige and Skoog medium with growth regulators by inoculating *M.incognita* on tomato leaf, root and on root culture in invitro condition. Tomato leaf callli of 32 day did not seem to favour *M. incognita*. Only 45.33% Juvenile penetrated the root callus and 27.94% reached the adult female stage. In invitro root culture 66% larvae penetrated and 75.76% reached adult female stage.
- Khan (1994) surveyed incidence and intensity of root-knot nematode associated with vegetable crops; about 50% vegetable fails examined were infested by nematodes and some incidence of disease also recorded. They isolated the M. *javanica, M.incognita* and *M.arenaria* and found that egg plant and cucumbers were was affected plants. The survey was conducted in 9 districts of Agra and Bareilly Division of UP India.
- Singh (1994) studied relative performance of five root-knot nematode resistant tomato lines derived from five different crosses through pedigree; were evaluated in replicated yield trial for four years. The highest performing line 1-6-1-4 gave an average yield of 574 Q/ha which is 4% higher than checks, bearing medium sized pear shaped fruits.
- Montasser *et al.,* (1995) investigated that out of eighteen screened flower bulb species for their susceptibility to *M. incognita* on the basis of root gall index, eight species were highly resistant, six species susceptible, 2 species slightly resistant, one species moderately resistant and one species very resistant.
- Cho (1996) screened 33 carnation cultivars cultured in Korea for resistant to *M. incognita*; seven cultivars found to highly resistant, 12 cultivars were moderately resistant while remaining 14 cultivars found to be highly susceptible.
- Potenza (1996) compared the response of *M. incognita* in resistant in resistant cultivar (Moapa 69) and susceptible cultivar(Lohantan) by inoculation of 2nd stage juvenile on 10 day old seedlings. By 72 hours after inoculation majority of J2 were amassed inside the vascular cylinder in roots of susceptible cultivars, while J2 had not entered to the vascular cylinder of resistant cultivar. There was minor difference in gene expression observed in inoculated and uninoculated root of resistant cultivars while greatest difference between inoculated and uninoculated roots of susceptible cultivar.
- Maluf (1996) studied heritability of root-knot nematode in a population of 226 sweet potato clones of diverse origin; most of the genotype showed resistance to *M. javanica*, whereas only a few were resistant to *M. incognita* race 2.
- Mayer, (1998) evaluated suppression of *Meloidogyne incognita* population by using fungus verticillium in one pot, while the controlled plant pot was treated with water.

Forty five days after transplanting the eggs counted from fungus treated plant did not differ significantly from the number on water controlled plants.

- Walker *et al.,* (2001) examined the effect of various population densities of *M. incognita* and *Thielaviopsis basicola* on cotton by infesting the plots with *T. basicola* chalamydospores per gram and 0,5 or 10 *M .incognita* eggs and juveniles per cm· of soil. They observed combination of *M. incognita* and *T. basicola* reduced plant survival compared to the non infested control to either pathogen alone. Same observation was done by Anwar, (2003)by examining the impact of *Meloidogyne incognita* in incidence of root rot disease caused by *Rhizoctonia solani* in tomato. He observed highest score bb(5.50) of root rot disease when inoculated with *M. incognita* than in the plant inoculated.
- Coyne *et al.*, (2003) surveyed eight commonly grown root and tuber crops from 430 fields in Uganda *Meloidogyne* & they extracted 69 species of plant parasitic nematodes among which species were the most frequently recovered across crops with the major species *M. arenaria, M. hapla, M. incognita,& M. javanica* observed on cassava.
- Morais *et al*., (2003) identified 93 plant nematode species among them the genera *Meloidogyne* along with *Helicotylenchus*, *Paratrichodorus* were most frequently encountered.
- Divito *et al.,* (2003) studied the reaction of potato clones to Italian population of *Meloidogyne arenaria*, *M .hapla , M. incognata & M. javanica* in a glass house. They observed that the one clone each of *S. cacoense and S. tuberosum* were resistant to *Meloidogyne* spp. tested, one clone each of *S. commersonii, S. tarijense & S. tuberosum* were resistant only to *M. javanica*. The remaining clones were susceptible to all of four species of root-knot nematode.
- Nico *et al*., (2004) studied the control of root-knot nematode by composted agro industrial wastes such as composted dry cork, dry grape mare and 1:1 mixture of dry olive mare + dry husks as an amendment to potting mixture. Amending the potting mixture with composted dry cork at rates of 0%, 25%, 50%, 75%&100% V/V reduced the rot galling & final population of *M. incognita & M. javanica* in tomato. They observed increasing rate of the amendment reduced the root galling of tomato caused by *M. javanica* (51.3%) and final population (82.6%) while reduced root galling caused by *M. incognita* (40.8%) and final population (81.9%).

4.3. Host resistance and tolerance

In plant nematology, resistance is defined as the ability of a host plant to prevent multiplication of the nematode (Cook and Evans, 1987; Trudgill, 1991). Tolerance describes the amount of injury caused by the nematode (Cook and Evans, 1987; Wallance, 1987). It refers to the ability of the plant to withstand or to recover from the injury caused by the nematode (Roberts, 1990; Trudgill, 1991; Roberts 2002;). Due to increasing concern about environmental contamination by pesticides, both plant resistance and tolerance to plant-parasitic nematodes have increased in importance during the past decades. Resistant cultivars also needs to be tolerant, those that are intolerant will suffer extreme damage if grown in heavily infested soil. Equally intolerant cultivars that are not resistant tend to increase nematode population densities to damagingly high level (Trudgill, 1991). To be economically interesting, resistant cultivars should yield as much as high-yielding susceptible cultivars treated with nematicides, they have no need of specialized equipment and growing techniques and hence incur no additional costs (Epps *et al*., 1981; Boerma and Hussey, 1992; Young, 1998). So using resistant cultivars is advantageous in integrated control programs (Thies *et al*., 1992). Although resistance is not inherently better than the other approaches to manage nematodes, neither are other approaches universally superior to resistance (Starr *et al*., 2002. Where resistance is lacking, tolerance offers acceptable alternatives (Table 2).

Table 2. Differential host test identification of the most common *Meloidogyne* species and races (Hartman and Sasser, 1985).

Meloidogyne species and races	Tobacco	Cotton	Pepper	Watermelon	Groundnut	Tomato
M. incognita						
Race 1	-	-	+	+	-	+
Race 2	+	-	+	+	-	+
Race 3	-	+	+	+	-	+
Race 4	+	+	+	+	-	+
M. arenaria						
Race1	+	-	+	+	+	+
Race 2	+	-	-	+	-	+
M. javanica	+	+	-	+	-	+
M. hapla	+	-	+	-	+	+

Cotton, cv. Deltapine; tobacco, cv. N.C. 95; Pepper cv. Early California Wonder; water melon cv. Charleston Gray; groundnut cv. Florunner; tomato, cv. Rutgers.

(-) Indicates a resistant host; (+) indicates a susceptible host.

Resistance to nematodes can be monogenic, oligogenic or polygenic. When resistance genes express large affects and can easily be identified they are said to be major genes, where as minor genes individually have only a small effect on expression of resistance (Cook and Evans, 1987). Van Der Plank (1963) described two main types of resistance, horizontal effective against all genetic variants of a particular parasite, and vertical, effective against certain variants only. Vertical resistance is generally monogenic while horizontal resistance is usually oligogenic or polygenic. Active or post-infectional resistance concerns those reactions of the host which occur in response to attack by a parasite where as passive or pre-infectional resistance involves mechanisms already present before attack (Cook and Evans, 1987).

The pre-infectional resistance may be conferred by physical, chemical and physiological properties of the plant (Cook and Evans, 1987). The natural presence of nematicidal compounds such as polythienyls, alkaloids, phenolics, polyacetylens, fatty acids, terpenoids was thoroughly reviewed by Gommers (1981) and Chitwood (1993). Resistance is generally expressed by post-infectional responses including a hypersensitive reaction (Robberts *et al*., 1999), production of phytoalexins (Huang and Barker, 1991), protease inhibitors (Vrain *et al*., 1995), accumulation of pathogenesis related proteins (Rahimi *et al*., 1996), cell wall strengthening and other reactions (Robberts *et al*., 1999). To migratory endo-parasitic nematodes, the thickened tissues or strengthening of cell wall is specially important as resistance mechanism, conferring a barrier to the penetration or migration and the accessibility of abundant nutrition whereas deployment of cell wall degrading enzymes may be important for migration and infestation.

Resistant cultivars differ from susceptible ones in the rate of invasion, in nematode reproduction as well as in the damage caused by the nematode (Griffin, 1998). Although susceptible plants are invaded by more nematodes, similar ultra structural changes occur within the tissue of both plant types. Susceptible plants demonstrate a greater degree of swelling and disorganization of the cellular structure. As resistant plants age, they overcome the damage and grow normally (Griffin, 1968, 1987). Resistance is most frequently linked with biochemical events taking place after nematode infection or by particular morphological features differentiating the resistant plant from susceptible ones (Peng and Moens, 2003).

4.4. Screening techniques

According to Boerma and Hussay (1992), the first step to the use of resistant cultivars by classical techniques is to locate a source of resistance by evaluating thousands of genotypes in a reliable manner. The screening procedure includes the planning and establishing experiments, preparation of plants and inoculums, inoculation, maintenance of inoculated plants, and data collection (Kalpan, 1990; Harris, 1990). Plants with little or no nematode infection should be evaluated again to confirm their resistance and then further tested for their yielding and other economic characters (Brodie and Plasisted, 1993; Young, 1998).

4.5. Screening conditions

Screening experiments for resistance can be established in the field, micro plots, green house, shade house, environmental growth chamber and in vitro (Starr, 1990). Since cultural conditions can greatly influence, on one hand, the growth of plants and, on the other, the survival, development and reproduction of nematodes, particularly migrating plant parasitic nematodes exposed to the soil environment, it is necessary to conduct screening experiments under carefully defined conditions (Peng and Moens 2002). While setting up experiment to evaluate resistance, care must be taken to avoid or to compensate for, edge effects and cross contamination (Harris, 1990).

The glasshouse is often advocated for a screening procedure in order to obtain uniform nematode infestations between screenings and prevent seasonal restrictions (Boerma and Hussay, 1992). In the glasshouse soil can be mixed to give any desired texture, pH, nutrient status and water content or biological compositions. However environmental conditions often vary sufficiently in green houses between seasons to appreciably affect results (Young, 1998).

A growth chamber can effectively avoid the variation in the environment conditions between different seasons (Peng, 2001). However, there may still be variation between different places inside the chamber. The limited space in growth chamber will limit the number of plants screened every time, so the size of containers and plants are important factors to consider.

4.6. Inoculums preparation and inoculation

To realize the fast screening of an as large number of plants as possible, the production of adequate inoculum and effective techniques are essential. Infested roots or soil in the field

or green house can be the most convenient source of inoculums for Root-knot nematode. The amount of inoculums must be optimized for differentiation of the plant response among genotypes (Fassuliotis, 1985). There is an inverse relationship between the growth of the host and level of nematode inoculum (Goodey, 1965; Griffin, 1993a, 1993b). when screening for resistance, the amount of inoculum should be enough to establish, but limited so as not to cause too much injury and mask potentially useful genetic material (Fassuliotis, 1985; Kaplan, 1990; Townshend, 1990; Young, 1998; Peng and Moens, 2002). In long-term experiments with many generations, the initial nematode density is not as important because the population will reach an equilibrium density when food supply becomes limiting (Aycock *et al*., 1976).

Experimental soil can be inoculated with *Meloidogyne* after the test plants have been planted (Townshend, 1990). For inoculation nematode suspension are often transferred to holes made in the soil near the plant base by large bore-needles and syringes (Seinhorst and Kozlowsca, 1977; Vrain and Daubeny; Townshend, 1990; Thies *et al*., 1992; Melakeberhan, 1998). To allow the nematodes to move and feed, inoculated soil or plants should be kept moist within 24 hours after inoculation and during the maintenance period (Griffin and Krusber, 1990; Baujard, 1995). Physiological age of the plants at the time of inoculation should be standardized. Nematode reproduction rate are related to the density and growth rate of the roots, which can change during development (Harris, 1990).

4.7. Host nutrition and chemical application

The effects of host nutrition are known to affect the development and reproduction of plant parasitic nematodes (Melakeberhan *et al*., 1997). Slow-release fertilizers are generally considered harmless to nematodes and are excellent for providing adequate nutrition for plants (Peng and Moens, 2003). Systemic pesticides, particularly insecticides, should be avoided because of their potential harm to nematodes if washed into the soil or translocated into the roots (Harris, 1990; Kaplan, 1990).

The duration of experiment is probably more important than the age of the plant at inoculation (Harris, 1990). If food supply is not limiting, the longer the plant is exposed to the nematodes, the higher the final population. However, a prolonged feeding can lower the reproduction rate, until the nematode population reaches an equilibrium density (Aycock *et al*., 1976). Sufficient time must be allowed for nematodes to complete at least one life cycle before the plant to be harvested. More generations of nematode can reduce the effects of sampling extraction errors on the detection of reproduction rate of nematode. This also takes into consideration the development rate and mortality, as well as the fecundity of the nematode (Harris, 1990). If necessary, additional host plants can be planted into the pots or the field inoculated with nematodes to allow for sufficient nematode generations (Bernard and Keyserling, 1985; Norton *et al*., 1985).

4.8. Extraction and estimation of nematode density

At the end of the screening period, nematode levels in both plants and growing medium are estimated by extraction by Baermann funnels, elutriation or centrifugal-flotation (Olthof, 1986; Vrain and Daubeny, 1986; Brodie and Plaiste, 1993; Griffin. 1993a; Vrain *et*. *al*., 1994). Failure to consider both components of the population can result in gross errors in the estimate of population density (Twonshend, 1990).

The extractable population density at the end of the incubation and its ratio to the initial population density of nematodes (Pf/Pi) are common measurements for comparison of host suitability of plants (Westcott and Zehr, 1991). However, the result is not always clear-cut: some plants may support reproduction of just a few nematodes and this may be due to a number of causes, such as the test population of nematodes consisting of a mixture of pathotypes, the resistance may be polygenic and partial, or local failure of the resistance mechanism within a plant (Cook and Evans, 1987). Also, a large initial population may cause much competition between nematodes and result in a small final population (Cook and Evans, 1987). Host can be designed on the basis of reproduction factor (*Rf*) i.e. final population/initial population. A *Rf* of over 10 indicates an excellent

host; an *Rf* of 1 to 10 indicates a good host; an *Rf* of about 1 indicates a maintenance host; and an *Rf* of between 1 and 0 indicates a poor host or nonhost (Ferris *et al*., 1993). When cultivars with known resistance or susceptibility are used, the evaluated materials can then be rated by index of susceptibility (Brodie and Plaisted, 1993).

Tolerance is identified by comparing plant growth parameters on both nematode – inoculated and non-inoculated plants. Differences in tolerance are usually determined ultimately by comparing the relative yield losses of a range of genotypes growing in uniformly infested soil in the field or micro plots (Cook and Evans, 1987; Harris, 1990).

5

MATERIALS AND METHODS

The plant parasitic nematode used in this study was *Meloidogyne* an obligate endoparasite of roots. An experiment was established with 14 cultivars in randomized complete block design (RCBD). Each cultivar was artificially inoculated with an equal density (i.e. 6000 eggs per pot) of *Meloidogyne* eggs. The study was carried in screenhouse of Plant Pathology Division of National Agricultural Research Council (NARC).

5.1. Materials

For the screening of tomato cultivars against *Meloidogyne*, following materials were used.

5.1.1. Lab Equipments

Soil sterilizing machine, Weighing balance, Sieve, Compound microscope Counting disc, Photographic microscope, Stereoscope, Counter, Incubator, Fridge, Inoculation chamber.

5.1.2. Glassware

Measuring cylinder, Beaker, Pipette, Slide cover slip, Scalpel, Petridish, Forceps, Scissor, Brush, Needle, Filter paper, Clips, Funnel, Plastic pipe, Conical flask, Water bottle, Vial, Aluminum foil, Inoculation needle, Stand, Parafilm, Cotton.

5.1.3. Chemicals

1% NaOCl solution, 4% Formalin, Phloxine B, NaCl, Ethyl alcohol, Methyl alcohol, Lactic acid, Blotting paper, Filterpaper.

5.1.4. Farm Materials

Bucket, Polythene bag, Plastic pots, Plastic plates, Sterilized soil, Marker, Sticker, Paper bag, Bamboo stick, Twin ball (Plastic rope).

5.2 Method

For screening of tomato cultivars against *Meloidogyne,* fourteen cultivars of tomato seedlings were germinated in nursery bed of $1m^2$. The nursery bed was drenched with 2% formalin and covered with polythene sheet tightly. Tomato seed were sown after 4 days of drenching, in Horticultural Research division of NARC. Twenty-two days old seedlings were transplanted in a pot of diameter 12.5 cm. 5 replicates with two plants per pot, which were filled with 1500gm (1200gm sterilized soil and 300gm sterilized compost). The pots were artificially inoculated with 6000 eggs per pot (@ 4 eggs/gm of soil) after two weeks of the transplantation. A total of 70 pots having five replicates were prepared. The experiment was carried out in screen house about 36 days from the date of inoculation of eggs. Measurement of Gall index, calculation of Reproductive factor *(Rf)* and rating of plant cultivars to the susceptibility on *Meloidogyne* was done according to the method given by Taylor and Sasser (1978).

Figure 2. Land preparation for nursery establishment in HRD, Khumaltar

5.2.1. Preparation of the inoculum

Meloidogyne was collected directly from the field where the nematodes had been reported previously i.e., from the field at Hemja, Pokhara in Kaski District. The field was situated about 15 kms from the heart of Pokhara valley. The galled samples were collected in March 2007 and preserved in the pot of tomato plants in the screenhouse. The collected samples were washed well and chopped into pieces of about 1-2cm. The roots were then mixed with total 200 ml of 0.5% NaOCl (25 ml of 4% NaOCl and 175 ml of distilled water) with 30 gm of chopped roots in a conical flask and then shaken vigorously for 4

minutes to dissolve the gelatinous matrix of the egg sac and release the eggs. The roots were then poured into the sieve of 125μ placed over sieve of 30μ. Then root tissues and solution was extensively rinsed immediately to remove all NaOCl and to collect the eggs from the sieve. Roots were collected in the upper sieve of 125μm and the nematode eggs were collected in the lower sieve of 30μm. The eggs were collected from the sieve in the beaker.

5.2.2. Counting of the nematode eggs

A 0.5 ml sample was taken with the help of a pipette after homogenizing the solution and added 1 ml of tap water in a clear counting disc. The aliquots were counted till the cv below 15% and the final number of eggs estimated from the total suspension.

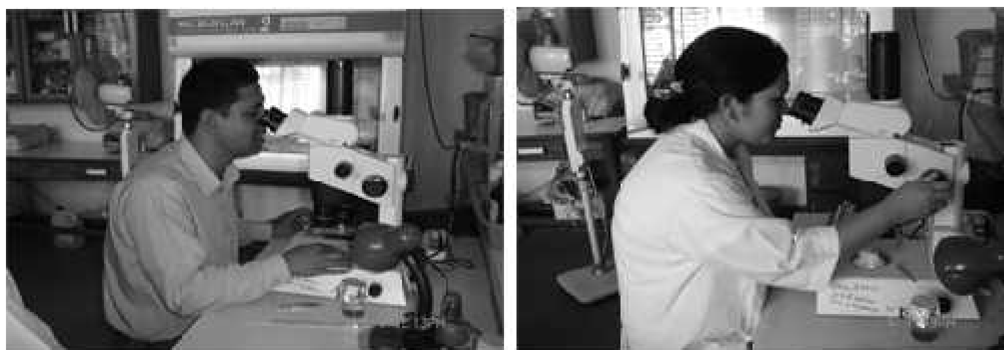

Figure 3. Nematode identification and counting in laboratory at PPD, Khumaltar

5.2.3. Preparation of the soil

Soil was collected from the field and plant debris and other materials were removed. The soil was sterilized for 6 hours with the sterilizing machine and the texture of the soil was tested in the Soil Science Division (NARC). The texture was found to be 69.3% sand, 22% silt and 8% clay i.e., the texture of sandy loam soil. The sterilized soil was then filled in the plastic pots.

5.2.4. Crop cultivar

Fourteen tomato cultivars i.e. Neeldhari, Yashwant, HRD-1506, Yumi, Lehar, Avinash-2, CLN 2545 B, C-315, CLN 2026 D, HRD-2, HRD-7, Nayak-B-SS-422, Pusa

Ruby, T-597-5 were sown in the seedbed at Horticulture Research Division (NARC). Two plants were maintained in each plot. After one month of transplantation the plants were staked with bamboo sticks and tied by plastic rope (thread).

Figure 4. Different tomato cultivars being planted in pots at screen house PPD, Khumaltar

5.2.5. Screen house condition

In screenhouse, the temperature was recorded with an average of 30ºC during cropping period. The moisture was adjusted to 40 -50% of the field capacity.

Figure 5. Establishment and caring of plant in screen house at PPD, Khumaltar

5.2.6. Inoculation of eggs

The inoculum density was fixed 4 egg/gm of soil. The homogenized aliquots were counted maintaining the coefficient of variance (CV) below 15%. The inoculum's density was maintained at around 600 eggs per ml. Shallow holes were made close to each plant and nematode eggs were inoculated using a glass pipette. The amount of inoculum inoculated in each pot was 10 ml. Thus the total inoculum density was 6000 eggs per pot. After inoculation the holes were covered with the surrounding soil.

5.2.7. Caring of the plants

From the germination of seedling great care of soil on watering and nutrition was done. For that purpose the seedbed was drenched with 2% Formalin a few days before sowing the seeds.

In the pots the sterilized soil and compost were mixed in the proportion of 1:4 i.e. 300g of compost and 1200g of soil. After transplantation, watering was done daily. The required amount of water was measured by weighing 5-6 pots randomly each day. The moisture level was maintained at 40%-50% of the field capacity. Staking was done to support the weak plants. The plants were also sprayed with Karathine @2ml/l of water to control the Powdery mildew.

5.2.8. Extraction and estimation of final density of *Meloidogyne* spp

The final population of *Meloidogyne* was estimated from the soil and root systems of each pot. At the end of the experiment, all the plants were cut from the soil and the root system was collected from each pot and weighed separately. Root stubbles and fine roots were separated by sieving the soil. From each pot all the soil was collected and mixed thoroughly. A 100g of soil sub sample was taken from each well-mixed soil sample, to extract the 2nd stage juveniles by modified Bearmann tray method. The eggs and the juveniles were extracted from suspension with the help of the sieve of 125μ placed over another sieve of 35μ and collected in a beaker. For the extraction of nematodes from the roots, the entire root was collected, washed and chopped into 1-2cm pieces. The chopped roots were then weighed and a total of 30g of root was mixed with 200ml of 0.5% NaOCl and then shaken vigorously for 4 minutes. The suspension was then poured into the sieve of 125μ placed upon sieve of 35μ. The root tissues and the solution were then rinsed

immediately 4-5 times and the eggs were collected from the sieve. The extracted egg suspension was then stored at 5ºc until counting.

The aliquots were counted till the CV below 15% with the help of a stereoscope and the number of eggs in each beaker estimated. The final population of nematode in each treatment was observed by counting the number of nematode egg per plant and obtained the reproductive factor (*Rf*), dividing the final population of nematode (*Pf*) by original inoculum (*Pi*), (Canto- saenz's, 1983). Final J_2 and egg population were counted as described by Hussey and Barker (1973). Root galling was indexed from 0-10 scale as described by Bridge & Page (1980) and Barker (1978). GM of root galls were stained to make egg masses of root knot nematodes more visible for counting as well as easy for grading root gall severity. Roots were washed thoroughly without soil or other debris and soaked in the stain (Phloxine B solution of 0.015%= 15g per liter of water) for 15 minutes. Then the roots were rinsed in the beaker with water and dried in blotting paper. GM of root galls were stained and examined the galls having egg masses.

5.2.9. Data collection and statistical analysis

The total nematode (eggs and juveniles) population was calculated from both root and soil. The nematodes were extracted from whole root system and 100g of soil sub sample of each pot. Hence, the count figure of nematodes from 100g soil was multiplied by 15 (1500/100=15) to get number for the whole soil. For all experiments, the final population (*Pf*) was calculated as the sum of nematode numbers (eggs and juveniles) extracted from both root and soil respectively. Reproduction factor (*Rf*) for nematode was calculated by dividing the number of nematodes recovered at the end by initial number of nematode eggs inoculated.

The collected data was inserted in excel sheet and mean, standard deviation and standard error were calculated. Homogeneity of variances and fit to normal distribution were checked for with MINI Tab. Data of reproduction factor of nematodes, gall index of root were square root transformed, if necessary to fulfill the assumption of ANOVA. Finally data analysis was performed with the MSTAT for significant test at 0.05 level.

During the data analysis, reproduction factor, gall index of root were considered as the dependent variables and seven different treatments were the independent variables. Means

were compared by the DMRT (P<0.05). In the bar diagram, the mean values are shown as untransformed data and as means ± standard error (SE).

6

RESULTS

Fourteen tomato cultivars (Nildhari, Yashwant, Hybrid 1506, Yumi, Lehar, Avinash-2, CLN 2545B, CLN 2026D, HRD-2, HRD-7, C-315, Nayak-B-SS-422, Pusa ruby and T-597-5 were tested against root knot nematodes. 6000 eggs were inoculated in each of the cultivar with the completion of 36 days of inoculation, the roots were collected from the soil and rating of the gall index was done by counting the number of galls present in each root. The result of gall index and *Rf* was analysed at P< 0.05.by as described by Bridge and Page (1980).

6.1. Determination of gall index (*GI*)

Cultivars showed variation on the gall formation in the root. T-597-5 showed the lowest gall index followed by C-315, Nayak-B-SS-422, whereas, CLN 2545B showed highest gall index followed by Yashwant, HRD-2, Yumi, CLN 2026D, Hybrid 1506, Nildhari, Avinash-2, and HRD-2 respectively. The cultivars Pusa ruby and Lahar were found significantly lower gall index compared with Yashwant, Hybrid 1506, Yumi, CLN 2545B, CLN 2026D and HRD-2 (Table 3).

Table 3. Response of different tomato cultivars on the gall index (*GI*) of *Meloidogyne* spp. in screen house pot experiment at Khumaltar during 2007

S.N.	Cultivars	Gall index	Remarks
1	Nildhari	4.47abcd	Highly susceptible
2	Yashwant	5.17abc	Highly susceptible
3	Hybrid 1506	4.76abc	Highly susceptible
4	Yumi	4.86abc	Highly Susceptible
5	Lehar	3.29d	Moderately Susceptible
6	Avinash – 2	4.26bcd	Susceptible
7	CLN 2545 B	5.56a	Highly susceptible
8	CLN 2026 D	4.83abc	Highly susceptible
9	HRD – 2	4.95abc	Highly susceptible
10	HRD – 7	4.08cd	Susceptible
11	C-315	2.21e	Moderately resistant
12	Nayak - B - SS - 422	2.6e	Moderately resistant
13	Pusa Ruby	3.31d	Moderately susceptible
14	T - 597 - 5	1.5f	Resistant
LSD (P > 0.05)		0.30	
CV (%)		11.42	

Note: Mean value with same alphabet in column are not significantly different at<0.5 level in DMRT (Duncons's Multiple range test).

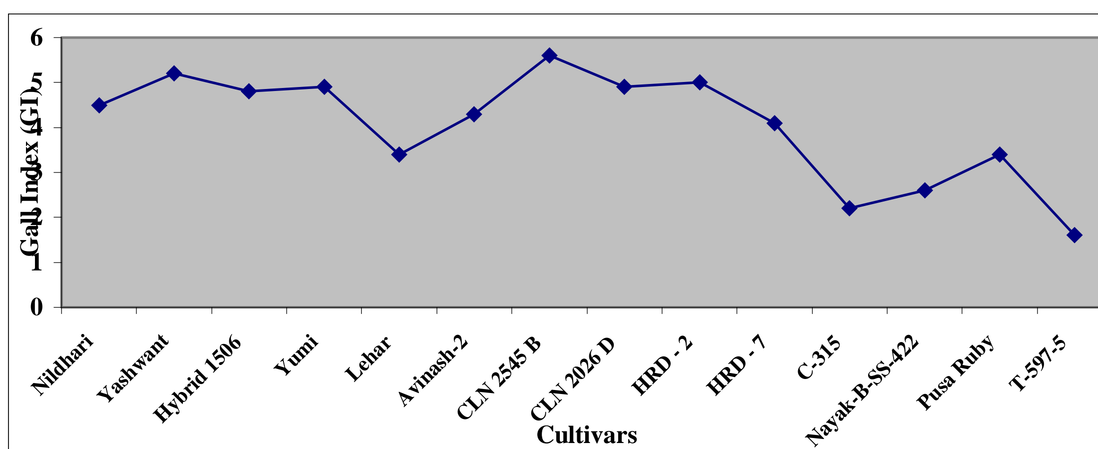

Fig 6. Influence of different cultivars of tomato on the gall index on root of *Meloidogyne* spp. on the screen house pot experiment in Khumaltar.

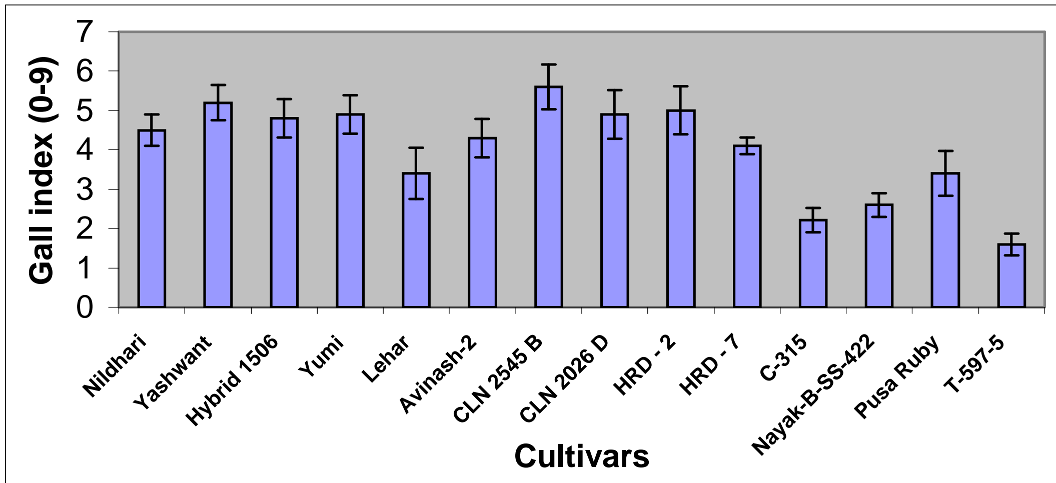

Fig 7. Gall formation rate of *Meloidogyne* spp. on the different tomato cultivars in screen house condition at Khumaltar (mean with ± standard error).

The highest gall index on root was seen in cultivar CLN 2545B followed by Yashwant. However, in cultivar HRD-2, Yumi, Hybrid 1506, Nildhari, gall index was moderately less than the former cultivars and all were equally susceptible to the nematode. While cultivars Pusa ruby and Lehar were found to be moderately susceptible with lesser gall index compare to the former mentioned cultivars. But in cultivars C-315, Nayak-B-SS-422 were quite resistant to fewer gall index and the cultivar T-597-5 had most lowest gall index and can be called as resistant cultivar for root knot nematode (Fig 6 and 7).

6.2. Determination of reproduction factor (*Rf*)

Cultivars also differed significantly on their reproduction factor (*Rf*). Among the cultivars, T-597-5, and C-315 had the lowest reproduction factors and were also significantly different with each other. The cultivars CLN 2545B had higher multiplication rate followed by CLN 2026D, Hybrid 1506, Yumi, Nildhari, Yaswant respectively. Nayak-B-SS-422, Pusa Ruby, and HRD-2 showed moderate multiplication rate than others. However, these cultivars did not show significantly different with cultivars Avinash-2, HRD-7 and Lahar but they were significantly lower reproduction rate than Hybrid 1506, Nildhari, Yashwant, Yumi, CLN 2026D, and CLN 2545B (Table 4).

Table 4. Response of different tomato cultivars on the reproduction factor (*Rf*) of *Meloidogyne* spp. in screen house pot experiment at Khumaltar during 2007.

S.N.	Cultivars	Initial Population (Pi)	Final population(*Pf*)		Reproduction factor (*Rf*)
			Eggs in soil	J_2 in soil	
1	Nildhari	6000	20460	600	3.31cd
2	Yashwant	6000	21900	0	3.65cd
3	Hybrid 1506	6000	30240	360	5.1bc
4	Yumi	6000	25320	720	4.34cd
5	Lehar	6000	15700	320	2.61def
6	Avinash - 2	6000	19722	900	3.44de
7	CLN 2545 B	6000	55140	600	9.3a
8	CLN 2026 D	6000	37680	600	6.38b
9	HRD - 2	6000	12900	0	2.15ef
10	HRD - 7	6000	15600	1500	2.85def
11	C- 315	6000	7320	0	1.22g
12	Nayak - B - SS - 422	6000	11460	0	1.91ef
13	Pusa Ruby	6000	13080	0	2.18ef
14	T - 597 - 5	6000	1620	0	0.27h

Note: Mean value with same alphabet in column are not significantly different at<0.5 level in DMRT (Duncons's Multiple range test).

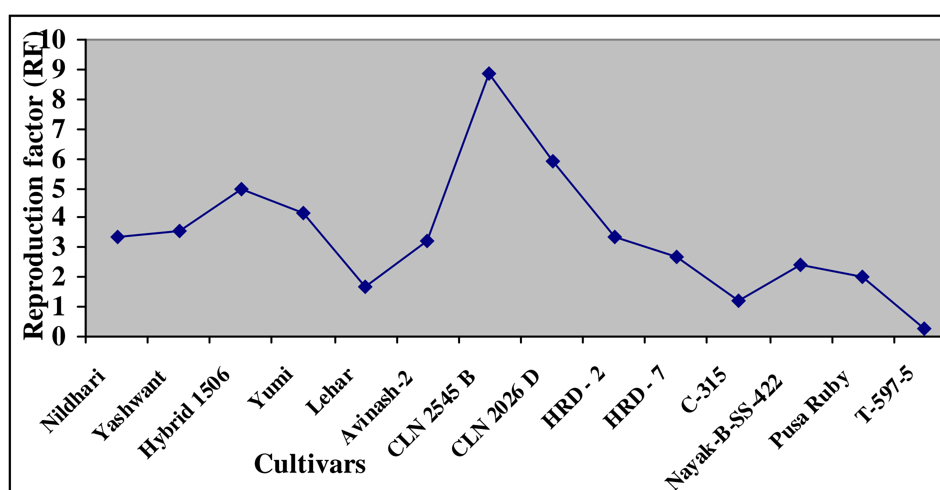

Fig 8. Influence of different cultivars of tomato on the reproduction factor of *Meloidogyne* spp. on the screen house pot experiment in Khumaltar.

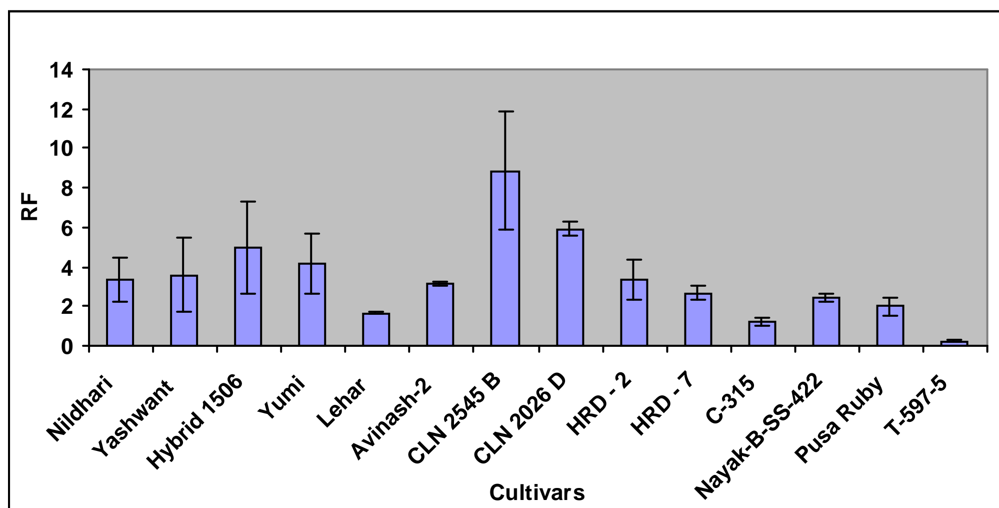

Fig 9. Multiplication rate of *Meloidogyne* spp. on the different tomato cultivars in screen house condition at Khumaltar (mean with ± standard error).

The multiplication of nematode was maximum in CLN 2545 B followed by hybrid 1506, CLN 2026D Yashwant and Yumi showed to some extent rate of *Meloidogyne* spp. Lehar and C-315 showed less multiplication rate of Meloidogyne spp as compare to the rest of the cultivars except the cultivar T-597-5. Multiplication rate of *Meloidogyne* spp. is very minute in the T-597-5. It was found that the final population was higher in all cultivars except in the T-597-5 but the rate of multiplication was varied with cultivars which can be due to their different physio-chemical reaction between host-pathogen and tolerance levels (Fig 8 and 9).

Generally, in most of the cultivars the trend of multiplication was proportionate to the gall index. If the gall index was scored higher then the *Rf* value of the nematode was also increased. But in the some cultivars such as Nildhari, Pusa ruby, Yumi, HRD-2 and Yashwant did not followed the trend. They showed lower Rf value even though the gall index was recorded higher. However, the trend of multiplication did not show linear correlation with gall index (Fig 10).

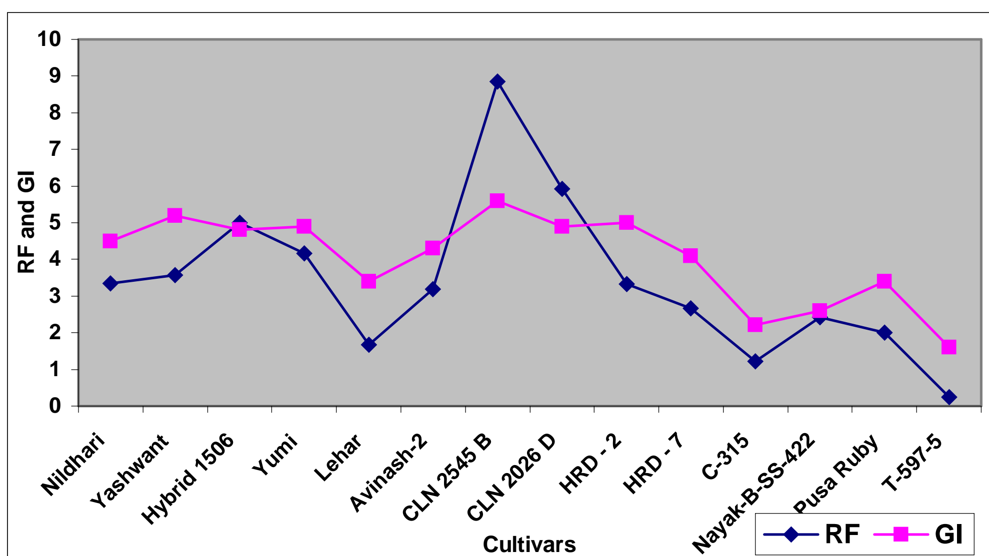

Fig 10.Interaction of gall index with reproduction factor of *Meloidogyne* spp. in different tomato cultivars in screen house condition at Khumaltar (mean with ± standard error).

7

DISCUSSION

Root knot nematodes are most important and cosmopolitan pest of vegetables distributed worldwide and infesting more than 2500 kinds of host plants. Although over 90 species of *Meloidogyne* have been described to date, four species are particular economic importance to vegetables production, *M. incognita*, *M. javanica*, *M. arenaria* and *M. hapla* (Siddiqui, 1986). More than 90% of the damage to crop plants in the warmer latitude is caused *by M. incognita*, *M. javanica*, *M. arenaria* (Sesser, 1980). Fieldmarshal, *et al.,* (1971) estimated the annual losses to nematodes in 240 vegetable crops to the loss of 266,989,100 dollar per year.

Several works have been carried out on tomato cultivars resistant to the root knot nematode. Such as Zaginailo, (1970) breed 'Allround', 'Eurocross' cultivars resistant to *M.*

incognita. Mallo, (1964) observed. Cultivars 'florida', 'Hawaiian crosi', 'Kolea', 'Merbein canner', 'Meebein early', 'Merbe in mid season' were resistant to *M. incognita* & *M. javanica* while the cultivars 'florida 22' resistant to *M. incognita* only and 'PD 611' susceptible to *M. javanica*, *M. hapla* & *M. arenaria*. Montasser, (1995) also worked to screening of flower cultivars resistance to root knot nematode. He found that out of eighteen screened flower bulb species 8 species were highly resistant to *M. incognita*. Singh, *et al*., (1994) observed 4% higher production from resistant cultivars of tomato.

The suitability of a host for plant –parasitic nematodes is expressed as the ability of the nematode to multiply on the host. Nematode multiplication is a standard measurement of resistance which, in nematology, is defined as the ability of a host plant to prevent multiplication of the nematodes (Cook and Evans, 1987; Trudgill, 1991).

The extractable population density at the end of the incubation and its ratio to the initial population density of nematode (*Pf*/P*i*) are common measurements for comparision of host suitability of plants (Wescott and Zehr, 1991). However, the result is not always clear-cut: some plants may support reproduction of just a few nematodes and consisting of a mixture of pathotypes, the resistance may be polygenic and partial, or local failure of the resistance mechanism within a plant (Cook and Evan, 1987). Also, a large initial population (*Pi*) may cause much competition between nematodes and result in a small final population (*Pf*) (Cook and Evan, 1987).

Lower nematode per gram of root indicates the suppression of nematode development in the root. Suppression in the population development could be caused by several factor, including difference in penetration rate, death rate, egression into the soil, or rate of multiplication (Brodie, and Plaisted, 1993). Resistant cultivars differ from susceptible ones in the rate of invasion, in nematode reproduction as well as in the damage caused by the nematode (Griffin, 1998). Resistance is most frequently linked with biochemical events taking place after nematode infection or by particular morphological features differentiating the resistant plant from susceptible ones (Peng and Moens, 2003). The pre-infectional resistance may be conferred by physical, chemical and physiological properties of the plant (Cook and Evans, 1987). The natural presence of nematicidal compounds such as polythienyls, alkaloids, phenolics, polyacetylens, fatty acids, terpenoids was thoroughly reviewed by Gommers (1981) and Chitwood (1993). Resistance is generally expressed by post-infectional responses including a hypersensitive reaction (Robberts *et al.,* 1999), production of phytoalexins (Huang and Barker, 1991),

protease inhibitors (Vrain *et al.,* 1995), accumulation of pathogenesis related proteins (Rahimi *et al.,* 1996), cell wall strengthening and other reactions (Robberts *et al.,* 1999). Host plant resistance has become an important strategy in the management of plant parasitic nematodes. Resistant cultivars have several advantage over other methods of reducing nematodes population such as their use require little or no technology and is cost effective, they allow rotation to be shortened and best use to made of the land and they don't leave toxic residues.

In this present study fourteen tomato cultivars were screened out against *Meloidogyne* spp. Tomato is one of the most popular and widely grown vegetable in the world because of its medicinal as well as its variety of colours, flavours to food. It's adaptation to variable climatic conditions, its culture extends from tropics to a few degree within arctic circle.

The screened tomato cultivars varied to their degree of resistance to *Meloidogyne* spp. Cultivars showed variation in the gall formation and their multiplication in the root. T-597-5 showed the lowest gall index followed by Yashwant, HRD.-2 Yumi, CLN 2026D, Hybrid 1506, Nildhari, Avinash - 2.

Cultivars also differed significantly on their reproduction factor. Among the cultivars, T-5975-5, and C -315 had the lowest reproduction factors and were also significantly different with each other. Bam (2005), also found that T-597-5 resistant to *Meloidogyne* and others cultivars.The cultivars CLN 2545B had higher multiplication rate followed by CLN 2026D, Hybrid 1506, Yumi, Nildhari, Yashwant respectively. Nayak B- SS-422, Pusa Ruby, and HRD-2 showed moderate multiplication rate than others. However, these cultivars did not show significantly different with cultivars Avinash-2 HRD-7 and Lehar but they have significantly lower reproduction rate than Hybrid 1506, Nildhari, Yashwant, Yumi, CLN 2026D, and CLN 2545B.

The rate of multiplication and damage level of nematodes can be varied with the cultivars of the crop. Harris, *et al.,* (2003) evaluated 608 Soyabean PIS imported from China for resistant to *M. incognita* and *M. arenaria*. After three sequential levels of green house screening they had observed seven resistant PIS for *M. incognita* and seven for *M. arenaria*. In fourth level screening they found PI 594753 A and PI 594775A had *M. incognita* gall equal to the highly resistant check PI 96354 and three other PIS had more galls than PI 96354 but fewer than 'Haskell', the resistant check egg. However the

numbers of nematode eggs produced on PI 59475 A and PI 594775A were equal to the highly susceptible 'Bossier'.

The result of the experimental screening helped us to understand the behaviour and response of different cultivar of tomato against root knot nematode. Planting resistant cultivars and other controlling measures are the primary practical methods of suppressing nematode damage to crop of low economic value.

8

CONCLUSION

The study showed that among fourteen tomato cultivars T-597-5 was found to be the resistant against *Meloidogyne*. This result matches with the result obtained by Bam (2005). While the other cultivars were found to be moderately resistant to highly susceptible to *Meloidogyne* spp.

Based on the result obtained it is concluded that:

- T- 597-5 is the resistant cultivars among the fourteen cultivars.
- Next to T- 597-5 cultivars C-315 supported the less population of *Meloidogyne*. So, it is also to some extent resistant to *Meloidogyne* spp.
- Pusa ruby, Nayak-B-SS-422, and Lehar were seen moderately resistant to *Meloidogyne* spp.
- The cultivars Avinash-2, and HRD-7 were observed susceptible to *Meloidogyne* spp.
- Cultivars CLN 2545 B, CLN 2026 D, Hybrid 1506, HRD-2, Nildhari, Yashwant and Yumi were found highly susceptible to *Meloidogyne* spp.

9

RECOMMENDATIONS

On the basis of this study *Meloidogyne* species were found highly destructive to the tomato. High density of nematode recorded in seven tomato cultivars, which will cause loss in productivity of tomato. But the cultivar T - 597-5 was found highly resistant to *Meloidogyne*, in which nematode neither could damage the root system nor multiply in the root system.

Screening for the resistance against nematode is only the method which overcome and other control measures. Therefore the following suggestions have been recommended for improvement of plant cultivars and their productions in relations to plant parasitic nematodes.

- The tomato cultivar T -597-5 was found highly resistant against *Meloidogyne* species followed by C-315, Pusa ruby Nayak-B-SS-422 and Laher. Hence these tomato cultivars can be recommended for widely cultivation.
- In case of Nepal, study on screening of resistant cultivar is still lacking till data. Hence, screening of resistant varieties of different economic plants is most necessary besides tomato.
- By giving the first preference in agricultural field micro level study is needed on plant nematodes which may clear either *Meloidogyne* itself is the main factor for the crop loss.
- Awareness programme should be launched in farmers level regarding proper use of nematicides for the better production of tomato and other crops.

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APENDICES

Annex 1.

CLASSIFICATION AND SPECIES OF *MELOIDOGYNE* GOELDI, 1887

The nominal species of *Meloidogyne* are:
M. acrita Chitwood, 1949 (*M. incognita acrita* Chitwood, 1949)
M. acronea Coetzee, 1956
M. africana Whitehead, 1960
M. aquatilis Ebsary & Eveleigh, 1983
M. ardenensis Santos, 1968
M. arenaria (Neal, 1889) Chitwood, 1949
M. artiellia Franklin, 1961
M. bauruensis Lordello, 1956 (*M. javanica bauruensis* Lordello, 1956)
M. brevicauda Loos, 1953
M. californiensis Abdel-Rahman & Maggenti, 1987 (*M. californiensis* Abdel-Rahman, 1981 nomen nudum)
M. camelliae Golden, 1979
M. caraganae Shagalina, Ivanova & Krall', 1984
M. carolinensis Eisenback, 1982 (*M. carolinensis* Fox, 1967 nomen nudum)
M. chitwoodi Golden, O'Bannon, Santo & Finley, 1980
M. christiei Golden & Kaplan, 1986
M. coffeicola Lordello & Zamith, 1960
M. cruciani Garcia-Martinez, Taylor & Smart, 1982
M. decalineata Whitehead, 1968
M. deconincki Elmiligy, 1968
M. elegans da Ponte, 1977
M. enterolobii Yang & Eisenback, 1983
M. ethiopica Whitehead, 1968
M. exigua Goeldi, 1887
M. fanziensis Chen, Liang & Wu, 1988
M. fujianensis Pan, 1985
M. grahami Golden & Slana, 1978
M. graminicola Golden & Birchfield, 1965
M. graminis (Sledge & Golden, 1964) Whitehead, 1968
M. hapla Chitwood, 1949
M. hispanica Hirschmann, 1986
M. incognita (Kofoid & White, 1919) Chitwood, 1949
M. indica Whitehead, 1968
Kleynhans, K.P.N., 1991. 7
M. inornata Lordello, 1956
M. javanica (Treub, 1885) Chitwood, 1949
M. jinanensis Zhang & Su, 1986

M. kikuyensis de Grisse, 1960
M. kirjanovae Terenteva, 1965
M. kongi Yang, Wang & Feng, 1988
M. kralli Jepson, 1983
M. lini Yang, Hu & Xu, 1988
M. litoralis Elmiligy, 1968
M. lordelloi da Ponte, 1969
M. lucknowica Singh, 1969
M. mali Itoh, Ohshima & Ichinohe, 1969
M. maritima Jepson, 1987
M. marylandi Jepson & Golden, 1987
M. mayaguensis Rammah & Hirschmann, 1988
M. megadora Whitehead, 1968
M. megatyla Baldwin & Sasser, 1979
M. megriensis (Pogosyan, 1971) Esser, Perry & Taylor, 1976
M. microcephala Cliff & Hirschmann, 1984
M. microtyla Mulvey, Townshend & Potter, 1975
M. naasi Franklin, 1965
M. nataliei Golden, Rose & Bird, 1981
M. oryzae Maas, Sanders & Dede, 1978
M. oteifae Elmiligy, 1968
M. ottersoni (Thorne, 1969) Franklin, 1971)
M. ovalis Riffle, 1963
M. partityla Kleynhans, 1986
M. pini Eisenback, Yang & Hartman, 1985
M. platani Hirschmann, 1982
M. poghossianae Kir'yanova, 1963
M. propora Spaull, 1977
M. querciana Golden, 1979
M. salasi Lopez, 1984
M. sewelli Mulvey & Anderson, 1980
M. sinensis Zhang, 1983
M. spartinae (Rau & Fassuliotis, 1965) Whitehead, 1968
M. subarctica Bernard, 1981
M. suginamiensis Toida & Yaegashi, 1984
M. tadshikistanica Kir'yanova & Ivanova, 1965
M. thamesi Chitwood Chitwood, Specht & Havis, 1952 (*M. arenaria thamesi* Chitwood Chitwood, Specht & Havis, 1952)
M. turkestanica Shagalina, Ivanova & Krall’, 1985
M. vandervegtei Kleynhans, 1988
M. wartellei Golden & Birchfield, 1978 (*M. incognita wartellei* Golden & Birchfield, 1978)

Key to Species of *Meloidogyne* Goeldi, 1887

1. Perineal pattern shape rectangular, distinctly circular,
   star-shaped, or with two rope-like striae 2

Perineal pattern shape not as above 8

2. (1) Perineal pattern with rounded arch and two separated, rope-like striae

.... *M. nataliei* Golden, Rose, & Bird, 1981

Perineal pattern not as above 3

3.(2) Perineal pattern shape circular 4

Perineal pattern not circular 7

4.(3) Mean female stylet length greater than 15 µm 5

Mean female stylet length less than 15 µm 6

5.(4) Mean juvenile "c" measurement greater than 15................. *M. propora* Spaull, 1977

Mean juvenile "c" measurement less than 15..............................*M. ovalis* Riffle, 1963

6.(4) Mean juvenile length greater than 415 µm .... *M. ottersoni* (Thorne, 1969) Franklin, 197]

Mean juvenile length less than 415 µm, ...*M. oteifae* Elmiligy, 1968

7.(3) Perineal pattern star-shaped to rectangular,

striae rope-like .... *M. camelliae* Golden, 1979

Perineal pattern rectangular, never star-shaped,

striae not rope-like .. ..*M. brevicauda* Loos, 1953

8.(1) Mean juvenile length greater than 500 µm 9

Mean juvenile length less than 500 µm 11

9.(8) Mean juvenile length greater than 600 µm,

....*M. spartinae* (Rau & Fassuliotis, 1965) Whitehead, 1968

Mean juvenile length less than 600 µm 10

10.(9) Male with 10 lateral incisures. Mean juvenile "c"

measurement 1.2 ....*M. decalineata* Whitehead, 1968

Male with 8 or less lateral incisures. Mean juvenile

"c" measurement less than 9

....*M. oryzae* Maas, Sanders, & Dede, 1978

.... *M. sewelli* Mulvey & Anderson, 1980

11.(8) Mean juvenile "c" measurement 13 or higher

(*M. lucknowica* 12.2) 12

Mean juvenile "c" measurement 10.5 or less

(*M. carolinensis* 10.9) 14

12.(11) Mean juvenile length less than 375 µm

.... *M. artiella* Franklin, 1961

Mean juvenile length greater than 375 µm .... …...13

13.(12) Mean juvenile "c" measurement greater than 20

.... *M. indica* Whitehead, 196

Mean juvenile "c" measurement less than 20

.... *M. mali* Ito, Ohshima, & Ichinohe, 1969

....*M. Iucknowica* Singh, 1969

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14.(11)Mean juvenile length 410 µm or greater........... ....I.r)

Mean juvenile length 400 µm or less 29

15.(14)Mean juvenile "c" measurement below 7.0 ....1(Z

Mean juvenile "c" measurement above 7.5 19

16.(15)Mean male stylet length greater than 20 µm

....*M. arenaria* (Neal, 1889) Chitwood, 1949

M. thamesi Chitwood in Chitwood, Specht, & Havis, 1952

Mean male stylet length less than 20 µm 17

17.(16) Position of juvenile hemizonid posterior to excretory pore 18

Position of juvenile hemizonid anterior to excretory pore

....*M. graminicola* Golden, 1965

18.(17)Juvenile stylet length range 13-15 µm....................*M. naasi* Franklin, 1965

Juvenile stylet length range 11.7-13.4 µm

.....................................*M. graminis* (Sledge & Golden, 1964) Whitehead, 1968

19.(15)Mean male stylet length 19.6 µm or less 20

Mean male stylet length 20 µm or more 22

20.(19)Female stylet length 17-19 µm ....*M. querciana* Golden, 1979

Female stylet length 11-14 µm ....2]

21.(20) Female with posterior protuberance, no stippled

zone near anus ....*M. acronea* Coetzee, 1956

Female without posterior protuberance, usually stippled

zone between anus and tail terminus

....*M. hapla* Chitwood, 1949

....*M. chitwoodi* Golden, O'Bannon, Santo, & Finley, 1980

....*M. subarctica* Bernard,]981

22.(19)Mean male stylet length 23-25 µm 23

Mean male stylet length 20-22 µm 24

23.(22)Juvenile stylet length range 14-17 µm, mean male stylet

length 24 µm ....*M. megatyla* Baldwin & Sasser, 1979

Juvenile stylet length range 10-12 µm mean male stylet

length 25 µm ....*M. grahami* Golden & Slana, 1978

24.(22)Excretory pore posterior to base of female stylet 25

Excretory pore anterior or even with base of female stylet 28

25.(24) Adult female with posterior protuberance

....*M. africana* Whitehead, 1960

Adult female without posterior protuberance 26

26.(25)Juvenile stylet length]].fi-12.6 µm, female stylet length

15.8-17.3 µm...*M. platani* Hirschmann, 1982

Juvenile stylet length 9.1-12.1 µm, female stylet length

11.0-16.2 µm 27

27.(26) Perineal pattern with lateral incisures and punctations

around anus ....*M. cruciani* Garcia-Martinez, Taylor, & Smart, 1982

Perineal pattern without lateral incisures or anal

punctations ....*M. ethiopica* Whitehead, 1968

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28.(24)Mean juvenile length 417 µm, hemizonid posterior to excretory pore

....*M. ardenensis* Santos, 1968

Mean juvenile length 451 µm or greater, hemizonid anterior

to excretory pore ....*M. megadora* Whitehead, 1968

....*M. carolinensis* Eisenback, 1982

29.(14)Mean juvenile body length 340 µm or greater 30

Mean juvenile body length 320 µm

.... *M. kikuyensis* De Grisse, 1961

30.(29) Juvenile stylet length 9 µm, female stylet 11 µm

....*M. exigua* Goeldi, 1887

Juvenile stylet length 9 µm or greater, female

stylet length 13 µm or greater ....……….................................31

31.(30) Male stylet length 13-18 µm long, female with

posterior protuberance ....

............………….. *M. megriensis* (Poghossian, 1971) Esser, Perry, & Taylor, 1976

Male stylet length 18 µm or more, female without

posterior protuberance.......................................……….....................................32

32.(31)Female excretory pore posterior to base of stylet ....……….................33

Female excretory pore anterior to base of stylet.........................………............35

33.(32)Juvenile stylet mean length 12 µm or greater

.... *M. tadshikistanica* Kirjanova & Ivanova, 1965

Juvenile stylet mean length less than 12 µm...34

34.(33)Juvenile "c" measurement 5.8-6.6

……...…........*M. javanica* (Treub, 1885) Chitwood, 1949

……... *M. bauruensis* Lordello, 1956

……..*M. lordelloi* da Ponte, 1969

Juvenile "c" measurement 9.5-13.9 ……....... *M. coffeicola* Lordello & Zamith, 1960

35.(32) Female perineal pattern with distinct punctations present at body terminus above anus ....………... *M. deconincki* Elmiligy, 1968

Female perineal pattern without punctations present at body terminus above anus ....…...................36

36.(35)Male stylet length range 18-26 µm, spicules 28-36 µm...............................

.......……..*M. acrita* Chitwood & Oteifa, 1952

……....................................*M. incognita* (Kofoid & White, 1919) Chitwood, 1949

……..*M. incognita wartelli* Golden & Birchfield, 1978

....*M. inornata* Lordello, 1956

....*M. kirjanovae* Terenteva,] 1965

……...*M. Iitoralis* Elmiligy, 1968

..............……........................ *M. microtyla* Mulvey, Townshend & Potter, 1975

**M. elegans* da Ponte, 1977 keys to this couplet but its description lacks male measurements and female protuberance data. It cannot be further separated in this key.

***Source*:** Hewlett, and Tarjan, (1993). Synopsis of the genus Meloidogyne Goeldi, 1887.

Annex 3. Distribution of root-knot nematodes, *Meloidogyne* species, by continent and order of economic importance.

Continent	North America	South America	Africa	Europe	Asia	Australia
Major pests	*M. incognita* *M. javanica* *M. arenaria* *M. hapla*	*M. incognita* *M. javanica* *M. arenaria* *M. hapla*	*M. incognita* *M. javanica* *M. arenaria* *M. hapla*	*M.incognita* *M.javanica* *M. arenaria* *M. hapla*	*M. incognita* *M. javanica* *M. arenaria* *M. hapla*	*M. incognita* *M. javanica* *M. arenaria* *M. hapla*
Important pests	*M. chitwoodi* *M. graminicola*	*M. exigua* *M. kikuyensis*	*M. acronea* *M. artiellia*	*M. naasi*	*M. graminicola*	*M. naasi*
Important pests in some locations	*M. microtyla* *M. graminis* *M. naasi*	*M. coffeicola* *M. oryzae* *M. salasi*	*M. africana* *M. decalineata* *M. litoralis*	*M. ardenensis*	*M. brevicauda* *M. mali* *M. camelliae*	

Annex 4. TABULAR KEY TO *MELOIDOGYNE* FEMALE

	Stylet length µm	Procorpus lumen lining	Metacorpus lumen lining	Excretpory duct punctations	Dorsal arch above tail terminus	Phasmids apart µm	Rectum at anus	Rectal punctation	Perineal ridge	Chromosome number
M. acronea	12-14 (13)	expands, then narrows	ovoid	indistinct	low, rounded	19-33 (26)	slightly flattened	indistinct	absent	unknown
M. arenaria	16-18(17)	cylindrical or expands then narrows	spheroid or ovoid	indistinct	low, rounded rarely squared	20-34 (26)	flattened laterally extended	usually distinct scattered	rarefy present inconspicuous	2n=35-37 2n=50-53
M. chitwoodi	13-15(14)	expands, then narrows	ovoid	distinct	low tolugh,rounded or squared	13-23(19)	flattened laterally extended	distinct, close to rectum	usually conspicious	n=14-17
M. graminicola	14-18(16)	cylindrical or expands, then narrows	ovoid tended	distinct	low,rounded	11-21(16)	flattened laterally extended	distinct scattered	absent	n=18
M. hapla	12-17(15)	expands, then narrows	spheroid or ovoid	distinct	low, rounded	13-27(21)	slightly flattened laterally extended	indistinct close to rectum	usually conspicuous	n=13-18 2n=30 37
M. incognita	15-17(16)	expands, then narrows	usually spheroid	indistinct	high, rounded or squared	18 - 28 (23)	slightly flattened extended	distinct or indistinct close to rectum	absent	2n=30-39 2n=41-43.
M. javanica	16-18 (17)	cylindrical, rarely narrowed	usually ovoid	sometimes distinct	low to high, rounded or squared	17-27 (21)	flattened laterally extended	distinct scattered	sometimes present	2n=42-47
M. kikuyensis	15-16 (16)	expands, then narrows	ovoid	indistinct	low to high, rounded or squared	15-29 (21)	slightly flattened	indistinct	absent	n=7
M. partityla	15-20 (17)	cylindrical or expands then narrows	spheroid	indistinct	high, rounded squared	or 13-25 (19)	slightly flattened	indistinct, close to rectum	conspicuous	2n=39-42
M. vandervegtei	16-20 (18)	cylindrical or expands then narrows	ovoid	indistinct	high, roundedor squared	11-29 (19)	slightly flattened	indistinct	absent	unkown

Source: Kleynhans, 1991. The root knot nematodes of South Africa.

Annex 5. Summary of important diagnostic of perineal patterns of the agriculturally most important root-knot nematodes (*Meloidogyne*spp.).

Species	Dorsal arch	Lateral field	Striae	Tail terminus
M. exigua	Low, rounded to high and squansh	Inner regions with coarse, raised, looped and folded striae	Coarse, smooth	Whorl absent
M. incognita	High, squansh	Distinct lateral lines smooth to wavy, absent, marked by breaks and forks in striae	Fine to coarse, distinct, sometimes zigzaggy	Often with whorl
M. javanica	Moderately high,	Distinct lateral lines	Coarse, smooth to slightly wavy	Often with distinct whorl
M. arenaria	Low, rounded to high and squarish	Lateral lines absent, marked by short, irregular, forked striae	Coarse, smooth to slightly wavy	Usually without distinct whorl
M. hapla	Low, rounded	Lateral lines inconspicuous	Fine, smooth to slightly wavy	Whorl absent marked by subcuticular punctations
M. chitwoodi	Low, oval to rounded	Indistinct of forking dorsal and ventral striae	Coarse, smooth to wavy and twisted near perineum, often fused	Whorl present
M. graminicola	High, squarish to low and rounded	Not clearly defined; in-dicated by breaks in striae	Smooth and continuous a round pattern	Large terminus, usually free of striae
M. naasi	Low,rounded	Absent	Coarse, smooth; broken and irregular near phasmids	Large terminus, usually free of striae
M. artiellia	High, angular	Absent, or marked by irregularities in thick striae	Fine, smooth, to wavy, very coarse and thick near perineum	Whorl absent

***Source*:** Kleynhans, 1991. The root knot nematodes of South Africa

Annex 6. Summary of important diagnostic characters of stylet of females of the agriculturally most important root-knot nematodes (Meloidogyne spp.).

Species	Stylet cone	Stylet shaft	Stylet knobs	Length (µm)	DEGO[a](µm)
M. exigua	Straight to slightly curved dorsally	Cylindrical, occasionally narrows near junctions with knobs	Small, rounded; slightly indented anteriorly	12-14	4-8
M. incognita	Anterior half distinctly curved dorsally	Slightly wider posteriorly	Set off, rounded to transversely elongate, some times indented anteriorly	15-17	2-4
M. javanica	Slightly curved dorsally	Cylindrical	Set off, transversely elongate	14-18	2-5
M. arenaria	Straight, broad and robust	Wider posteriorly	Not set off, sloping posteriorly, merging with shaft	13-17	3-7
M. hapla	Slightly curved dor-sally, narrow and delicate	Slightly wider posteriorly	Set off, small end rounded	14-17	5-6
M. chitwoodi	Slightly curved dorsally	Cylindrical to slightly wider posteriorly	Not set off, irregular in outline, indented medially	11-12.5	4-5.5
M. graminicola	Slightly curved dorsally	Cylindrical to slightly wider posteriorly	Set off, transversely elongate	10.5-11	3-4
M. naasi	Slightly curved dorsally	Slightly wider posteriorly	Not set off, large and rounded, sloping posteriorly	11-15	2-4
M. artiellia	Slightly curved dorsally	Slightly wider posteriorly	Not set off, large and rounded; sloping posteriorly	12-16	4-7

[a]DEGO is the distance from the base of the stylet to the dorsal esophageal gland orifice.

***Source*:** Kleynhans, 1991. The root knot nematodes of South Africa

Annex 7. Summary of important diagnostic characters of head shapes and stylets of males of *Meloidogyne* spp

Species	Head cap	Head region	Stylet cone	Stylet shaft	Stylet knobs	lgth (µm)	DEGO[a] (µm)
M. exigua	High, rounded, set off from head annule	Not set off, smooth, lateral lips present	Bluntly pointed	Cylindrical, narrows at its base	Small rounded, in some distinctly angular	18-20	3-5
M. incognita	Flat to concave, labial disk raised above the medial lips	Not set off, usually marked by 2-3 incom-plete annulations	Tip blunt, bladelike	Usually cylindrical, often narrows near knobs	Set off, rounded to transversely elongate, sometimes indented anteriorly	23-25	2-4
M. javanica	High rounded, set off from head annule	Set off, smooth or marked by 2-3 incomplete annulations	Tip pointed, cone straight	Usually cylindrical	Set off, low; and transversly very elongated broad	18-22	2-4
M. arenaria	Low, to moderately raised, sloping posteriorly	Not set off, smooth or marked by 1-2 incomplete annulations	Tip pointed, cone broad and robust	Usually cylindrical, often broadens near knobs	Not set off, sloping posteriorly merging with shaft	20-28	4-8
M. hapla	High and narrow	Set off, smooth, larger in diameter than first body annule	Tip pointed, cone narrow, delicate at its base	Cylindrical, often wider or narrower	Set off, small, end round	17-23	4-5
M. chitwoodi	High, rounded, set off from head annule	Not set off, smooth, large lateral lips present	Tip pointed, cone narrow, delicate	Cylindrical to conical	Anteriorly indented, irregular in outline	16-19	3
M. grami-nicola	High, rounded, set off from head annule	Not set off, smooth	Tip pointed often narrows near base	Cylindrical, to distinctly angular	Set off, rounded	16-17	3-4
M. naasi	High, rounded, set off	Not set off, smooth or marked by few annulations	Tip often pointed, cone straight	Cylindrical may narrow near base	Set off, rounded	16-19	2-4
M. artiellia	High, rounded, setoff	Lateral lip present, not set off	Straight, narrow	Cylindrical, wider at its base	Rounded, set off to sloping posteriorly	17-27	5-7

aDEGO is the distance from the base of the stylet to the dorsal esophageal gland orifice.

Annex 8. Summary of important diagnostic characters of second-stage juveniles of the agriculturally most important root-knot nematodes (*Meloidogyne* spp.).

Species	Head cap	Head region	Stylet width	Sylet knobs	Stylet (µm)	DEGO (µm)	Tail (µm)	Terminus (µm)	Body (µm)
M. exigua	Anteriorly flattened, elongate	Usually, smooth	Moderately sized cone and shaft	Set off, small, and rounded	9 10	2.3 4	44 46	12 14	334 358
M incognita	Anteriorly 346-463 flattened, elongate	Usually marked by 1-3 incomplete annulations	Moderately sized cone and shaft	Set off, posteriorly rounded, sloping backward	10 12	2 3	42 63	6 13.5	
M. javanica	Anteriorly flattened, elongate	Usually smooth	Moderately sized cone and shaft versely elongate	Set off; posteriorly rounded, sloping backward, trans	1~12	3.5	51-63	9-18	402-560
M. arenaria	Anteriorly flattened, elongate	Usually smooth	Broad cone and shaft merging with shaft	Not set off, posteriorly rounded,	1~12	3-5	44-69	13	398-605
M. hapla	Rounded, narrow	Rounded, usually smooth	Narrow cone and shaft	Set off, small, and rounded	10 12	3 4	46 69	12 19	357 517
M. chitwoodi	Anteriorly flattened, elongate	Rounded, usually smooth	Narrow cone and shaft	Not set off, outline irregular	10	3 4	39 47	9 14	336 417
M graminicola	Anteriorly flattened, elongate	Rounded, usually smooth	Narrow cone and shaft	Set off, small, and rounded	11-12	2.8-3.4	67-76	14-21	415~84
M. naasi	Anteriorly flattened, elongate	Usually smooth	Narrow cone and shaft	Not set off, tapering onto shaft	13-15	2-3	52-78	17-27	418-435
M. artiellia	Anteriorly flattened elongate	Usually smooth	Moderately sized cone and shaft	Not set off, tapering onto shaft	14-16	2.5-4.5	18-26	2-7	334-370

***Source*:** Kleynhans, 1991. The root knot nematodes of South Africas