1. INTRODUCTION

Nematode is microscopic unsegmented vermiform, bilaterally symmetrical pseudocoelomates, commonly described as filiform or thread like a characteristic reflected by the greek origin of the taxon nema (=thread) and its nominative plural nemata (Bird and Bird, 1991).

Nematodes are the most numerous metazoan on earth. They are found in all oceans from the Polar regions to the equator, from the littoral zone to the abyssal depths; they colonize fresh water, lakes, rivers and marshes and all types of soil from the Antarctic to the tropics. Nematodes can parasitise on most of the groups of animals including nematodes and a variety of algae, fungi and higher plants (Luc *et al*., 2005).

Plant parasitic nematodes inhabit all parts of plants including developing flowers, buds, leaves, stems and roots and they have a great variety of feeding habits. Some species feed only on the outermost plant tissues, other penetrates to deeper tissues and still others induce their host to produce special nutrients sources upon which the parasite subsist. About 95% of the plant parasitic nematodes live in the soil, in and around the roots of the plants and feed in or on roots while others invade leaves and stems. However on the basis of their feeding habits, plant parasitic nematodes can be broadly categorized as ectoparasitic, semi-endo parasitic and endo parasitic nematodes.

Ectoparasitic nematodes such as *Pratylenchus, Trichodorus, Tylenchorhynchus*, *Helicotylenchus*, *Hemicycliophora*, *Hoplolaimus*, *Longidorus*, *Rotylenchus*, *Xiphinema* etc have a rather long and powerful stylet with the help of which they can penetrate the root tissues. The stylet is used to pierce the epidermal cells of the roots and underlying layers of the cortex to suck out the cell contents.

Two genera *Rotylenchulus* and *Tylenchulus* are semi-endoparasitic that penetrate the root by their anterior body end, the posterior end remaining outside the root surface and

becoming highly swollen due to this phenomenon they loose their motility and become sedentary.

Endoparasitic nematodes are either sedentary or migratory. Some endoparasitic nematodes such as root-knot nematodes (*Meloidogyne*) and cyst nematodes genera (*Heterodera*, *Globodera*, *Cactodera*) can develop their feeding sites inside the root and become sedentary whereas other endoparasitic nematodes of genera *Pratylenchus*, *Radopholus* and *Hirschmanniela* migrate in the roots and form root lesion by burrowing the tissue and again invading other healthy root tissue (Moens, 2005).

Tomato is an important vegetable crop grown by both large and small-scale farmers around the world, remaining second in importance to potato in many countries. Tomato is consumed raw or cooked. Large quantities of tomato are used to produce soup, juice, ketchup, paste and powder. Tomato is also rich in medicinal value. The pulp and juice is digestible, mild aperient promoter of gastric secretion to be intestinal antiseptic. It is also popular because it supplies vitamin c and adds variety of colour and flavour to the food. It can be grown in wide range soils and climates and its culture extends from tropics to a few degrees within the Arctic Circle.

In Nepal, off-season tomato cultivation is one of the important incomes generating enterprise for small-scale farmers (Budathoki *et al*, 2004). Cultivation of this crop is getting popular day by day for quick and high-income generation.

Despite its much importance, the tomato crop is not free from problem. Farmers have been facing a number of problem including disease, insect, cultivars etc. Among them root knot is a major biotic factor which is responsible for low yield of tomato in poly house. However, tomato is also infected by other genera of nematodes such as *Rotylenchus* spp, *Naccobus* spp, *Globodera* spp, *Heterodera* spp, *Ditylenchus* spp, *Pratylenchus* spp, *Radopholus* spp, *Belonolaimus* spp, *Trichodorus* spp, *Paratrichodorus* spp, *Longiduros* spp etc.

In a recent interaction with the commercial tomato growers of Hemja (Pokhara) has revealed that root knot nematode has caused 15-30 percent yield reduction in tomato production. Informal reporting from the farmers in the recent years revealed that the cash income of the farmers has been decreased up to 35%. Problem of this nematode has been increasing like a forest fire especially in poly house tomato cultivation.

In a poll organized by Sasser and Freckman (1987), among the membership of three nematological societies showed a yield loss of 20.6% in tomato due to plant parasitic nematodes. Bhatti and Jain (1977) observed losses worth of 27, 46 and 91% in brinjal, tomato and okra respectively due to root knot nematodes. Reddy (1985) analyzed the crop loss assessment studies and indicated the loss in terms of yield of tomato by 39.77% at a population of 20 juvenile of root knot nematode per gram of soil. Krishnappu *et al*., (1992) found 15 to 60% yield loss in tomato.

The genus *Meloidogyne* includes more then 80 species. However, in tomato only four species i.e. *M. incognita*, *M. arenaria*, *M. javanica*, *M. hapla* have been reported from different districts of Nepal such as Kathmandu, Bhaktapur, Kavre, Lamjung, Pokhara, Chitwan, Dhankuta, Parbat, Palpa and Jhapa.

Management of the plant parasitic nematodes has become an essential regarding the substantial loss in the crop yield and quality. Various strategies have been employed by researchers as well as farmers for the control of plant parasitic nematodes including root knot nematodes such as biological, cultural practices, chemical, organic and inorganic soil amendments and breeding resistant cultivars.

With an objective to find out the efficacy of management strategies for root knot nematode, this dissertation work has been conducted with the attachment on the regular project of Plant Pathology Division under Nepal Agriculture Research Council (NARC).

2. INTRODUCTION OF *Meloidogyne.*

2.1. Taxonomic position of *Meloidogyne* spp

Nematodes are classified within the phylum nematoda with two classes: Enoplea and Chromodorea (Deley and Blaxter, 2002). The order Dorylaimida within the Enoplea and the 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 and Goeldi described it as a new genus in 1877. By the end of the year 1995, the genus *Meloidogyne* included more than 80 species.

The taxonomical position of *Meloidogyne* species is as follows

Phylum: Nematoda
Class: Chromodorea
Order: Rhabditida
Sub-order: Tylenchina
Infra-order: Tylenchomorpha
Super-family: Tylenchoidea
Family: Meloidogynidae
Sub-family: Meloidogyninae
Genus*: Meloidogyne*
Common name: Root knot nematode

2.2. Morphological description of *Meloidogyne* spp

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).

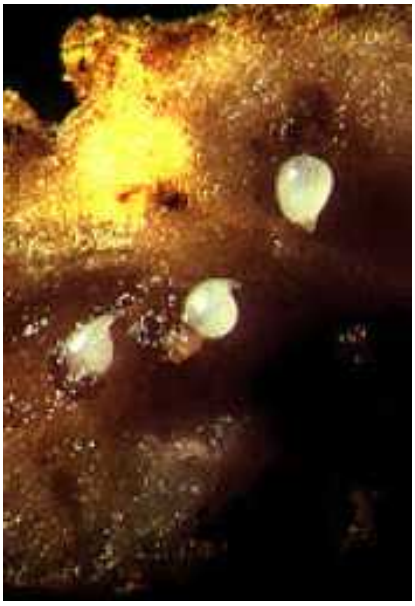
Male

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 an 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).

Egg of *Meloidogyne*

Juvenile

Male



Root knot in tomato roots

Females of *Meloidogyne* in roots

Figure 1.1. Figures showing egg, juvenile, male, female and root galls in tomato

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 conoid 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).

2.3. Host range of *Meloidogyne* spp

Root knot nematodes occur throughout most of the world, infect all major crop plant 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).

2.4. General symptom and Feeding behavior of *Meloidogyne* spp:

Root knot nematode 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 nematodes.

Symptoms also include malfunctioning root system, eg. patchy, leaf chlorosis due to decreased rate of photosynthesis , excessive wilting during dry and hot decreased yields and crop quality and sometimes premature senescence and death (Moens, 2005). 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 cytokineses (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.

2.5. 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. The second stage juvenile molts thrice to become an adult (figure 1).

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

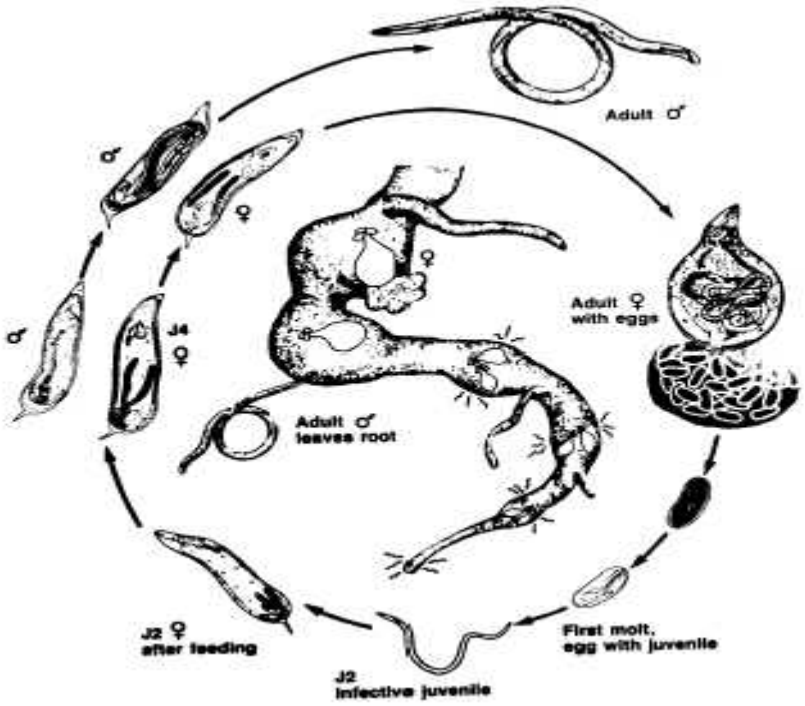


Figure 1.2. Diagram of the life cycle of root-knot nematode, *Meloidogyne* spp. Abbreviation: J2, second-stage juvenile; J4, fourth-stage juvenile.

2.6. 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).

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

Meloidogyne species	Populations studied (number)	Countries of origin (number)	Chromosome number		Mode of reproduction
			n	2n	
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. plantani	1	1		42-44	
M. querciana	1	1		30-32	
Total	584				

Source: Eisenback, J. D., and H. H. Triantaphyllou. 1991. Root-knot nematodes: *Meloidogyne* species and races.

2.7. Sex determination

In facultative and parthenogenetic species sex determination appears to be controlled largely by the environment. Under favourable environmental conditions most 2^{nd} stage juvenile develop into female and the remaining juveniles into males with one testis. However, the unfavorable conditions due to 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).

3. OBJECTIVES

3.1. GENERAL OBJECTIVE

- To test the efficacy of different management strategies on *Meloidogyne* spp. in tomato plant.

3.2. SPECIFIC OBJECTIVES

- To isolate the *Meloidogyne* spp. from root of different vegetables cultivated in natural condition.
- To test phytopathogenicity of *Meloidogyne* eggs in tomato plant.
- To test the efficacy of poultry manure, mixture of cow dung and urine and mustard cake as organic soil amendments against *Meloidogyne.*
- To evaluate the suppressive effect of *Paecilomyces lilacinus* and *Trichoderma harzianum* on *Meloidogyne.*
- To assess the efficiency of chemicals like Furadan on *Meloidogyne*.

4. LITERATURE REVIEW

4.1. General review of plant parasitic nematodes

Plant parasitic nematodes constitute one of the most important groups of organism inhabiting the soil and in and around the roots of the plants. The first known report of the observation of plant parasitic nematode was made by John T. Needham in 1743, when he observed thousands of nematodes within the wheat gall (*Anguina tritici*). In 1855, a second plant parasitic nematode was recorded by M.J. Berkeley. He observed that galls produced on green house grown cucumber contained nematodes; the *Meloidogyne* species.

Plant parasitic nematodes are elongated, cylindrical worm, tapering more or less at the head and tail ends and encased in a very tough and impermeable transparent or semi-transparent cuticle. They are generally vermiform, however in certain cases of nematodes where degree of parasitism has advanced, the males remain vermiform while the females are spherical, kidney shaped, saccate etc. They bear a characteristic feeding apparatus known as stylet or spear which causes damage to host plants by secreting chemicals and also help in feeding on root tissues (Thorn 1961). On the basis of their feeding habits nematodes are classified as ecto; semi-endo; endo parasites etc. Nematodes belonging to genera *Meloidogyne* are sedentary endoparasites.

4.2. General review on management of *Meloidogyne* spp.

Root knot nematodes are an important limiting factor in vegetable production around the world. Once large population of *Meloidogyne* have developed in a field, it is impossible to eradicate them completely from the soil. It is also difficult to maintain a population at sufficiently low levels without the use of effective management tools used in a logical ordered system. A number of strategic reviews have been published that concentrate on specific regions or on nematode management in vegetable production (Johnson and Faaassuliotis, 1984; Netscher and Sikora, 1990; Noling and Becker, 1994; Johnson, 1998; Sikora, 2002). Some methods for the management of root knot nematode problem in crops can be summarized as chemical, biological, cultural, organic soil amendments and resistant cultivars.

4.3. Management of *Meloidogyne* through Biological agents

Nematodes have also been successfully controlled by the bio-control agent (Davide and Zorilla, 1983; Kerry 1984; Morgan-Jones *et al*., 1984; Jatala 1986; Khan and Hussain, 1988,1990). Strain of *Arthrobotrys irregularis* grown on rye grain reduced root knot galling and increased tomato yields when introduced in the soil at 140g/m^2 (Cayrol and Frankowski, 1979; Cayrol, 1983). *Pasteuria penetrans* is an obligate parasite of nematodes including *Meloidogyne* (Birchfield and Antonpoulos, 1976). Similarly root-knot nematodes can also be controlled by *Pochonia chalamydosporia* (Van damme *et al*., 2005), *Trichoderma*, arbuscular micorrhizal fungi (AMF) (Sampat and Trivedi, 2004), *Paecilomyces lilacinus*, *Glomus mossae* and a group of microorganism like Rhizobacteria (Luc *et al*., 2005).

P. penetrans is a common parasite of *Meloidogyne* and is often found attached to juveniles. The spore forms of *Pasteuria penetans* can resist both draught and exposure to non-fumigant nematicides (Mankau and Prasad, 1972).

The colonization of plants with endomycorrhizal fungi apart from providing plants with nutrients has been reported to have a depressive effect on root knot nematodes. According to Sikora (1978), penetration and development of *M. incognita* in tomato was significantly reduced by *Glomus mosseae* in glass house studies.

Groups of microorganism that may be effective in reducing nematode damage are the plant health promoting rhizobacteria (Sikora, 1988 and Oostendrop and sikora, 1989), which could be applied as seed dressing or as a drench treatment for transplants. Application through drip irrigation system may prove effective method of post planting application (Zavaleta-meija and Van Grundy, 1982).

The co-cultivation of *Concanavolia ensiformis* (leguminous plant) and tomato resulted a significant reduction in galling caused by *Meloidogyne incognita* and *Nacobbus aberrans*, while under the same condition, *Mucuna deeringrana* was less effective (Mendoza *et al.*, 1989).

The inoculation of 10g and 20g of fungus *P. lilacinus* resulted in a threefold and fourfold increase in tomato yields respectively. Greatest protection against *Meloidogyne incognita*

was attained when *P. lilacinus* was delivered into the soil 10 days before and again at planting with twofold increase in yield than the previous experiment (Caniballas and Barker, 1989).

An experiment conducted by Hollanad *et al* in 1999, showed that eggs of all stages including those containing unmatched juveniles, 3rd and 4th stage juveniles and adult females of *Meloidogyne* were readily infected by *P. lilacinus*.

Anwar, (2003) also found the impact of *M. incognita* in incidence of root rot diseases caused by *Rhizoctonia solani* in tomato. He observed highest score (5.50) of root rot disease when inoculated with *M. incognita* than in the plants inoculated with fungus only.

The fungal bioagents like *Aspergillus fumigatus*, *A. terrus*, *P. lilacinus* and *Trichoderma viridae* improved plant growth parameters and reduction in nematode multiplication in Balsam (Goswami *et al*., 2005).

Kumar and Singh (2006), assessed the efficacy of *Arthrobotrys dactyloids* for biocontrol of root knot nematode diseases in tomato and found that all isolates of *A. dactyloids* captured and killed *M. incognita* and *Tylenchorhynchus brassicae* but not *Hoplolaimus indicus*. Its effect as bio control was enhanced when its mass culture was applied with cow dung manure.

The presence of *Pochonia chlamydosporia* reduced the number of plant parasitic nematodes (51-78%) including migratory ectoparasites while the freeliving nematodes, culturable bacterial populations assesed by biolog were unaffected (Tahseen et *al*., 2005 and Van Damme *et al*., 2006).

An experiment was carried out to evaluate the biofumigant effect of different organic materials such as residue from pepper, strawberry, tomato, and cucumber crops, orange juice industry residues, commercial manure and sheep manure on *M. incognita*. The result showed that all biofumigant materials significantly decreased *M. incognita*

population and galling indices in tomato cv. Marmande. A greater effect was observed when applying crop residue together with manure (Piedra Buena *et al.*, 2006).

A research conducted by Mennan *et al*., (2006), demonstrated that *Hirsutella minnesotensis* may be used as potential suppressor of *M. hapla* in vegetable production in the great lakes region.

4.4. Application of different organic amendment for the management of *Meloidogyne*

Plant parasitic nematodes are effectively controlled by the application of soil organic amendments in various parts of the world (Jhonson, 1959; Singh and Sitaramaiah, 1966; Khan, 1976). Oil cakes saw dust, urea and bagasse have also been used to minimize root knot population (Singh and Sitaramaiah, 1966, 1967; Sikora *et al*., 1973). Chitin in combination with waste products from the paper industry has been used to reduce nematodes population densities to different degrees. In addition to their suppressive effect on nematode density, organic amendment improve soil structure and water holding capacity and have also been found to control root diseases of crop plants (Luc *et al*., 2005).

Barbarola (1982) achieved good control of root knot nematode with cow dung and poultry manure. Amending soil with animal manure and agricultural byproducts has reduced *Meloidogyne* spp number on variety of crops (Rodriguez-kabana *et al*., 1987). The organic amendment tends to alter the host parasite relationship in favour of the crop and also increases plant vigor, enabling plant to withstand nematode attack (Singh *et al*., 1986).

The integration of oil cakes (except mahua-cake), bone and horn meals with *Paecilomyces lilacinus,* resulted in increased plant growth and reduced population build up of nematode and root gallings. Groundnut cake with *P. lilacinus* was most effective, Khan and Saxena (1997).

An application of fly ash in soil enhances yield of infected plants by 96% and suppresses the nematode diseases and reproduction by 63 and 73% respectively (Khan *et al*., 1997).

Plant growth promoting rhizobacteria such as strain 569 M^r suppressed *M. phaseolina* in both iron deficient and iron sufficient soil (Siddiqui *et al*., 2002).

The nematode population in the soil and severity of nematode attack decreases in the plants amended with biogas slurry. Also the plants put up more vegetative growth and tended to flower and fruit much earlier (Jothi *et al*., 2003).

The nematode population in the soil and severity of nematode attack decreases in the plants amended with biogas slurry. Also the plants put up more vegetative growth and tended to flower and fruit much earlier (Jothi *et al*., 2003).

Abubakar *et al.,* (2004), conducted an greenhouse experiment to test the efficacy of cow dung and urine separately and in combination and found that the mixture of urine and cow dung showed best result to suppress *M. incognita* race 1.

In an experiment conducted by Trivedi *et al*., in 2004 revealed that neem and mustard cake combination is highly effective against Root knot nematode.

Nicco, *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%, and 100% V/V reduced the root galling and final population of *M. incognita* and *M. javanica* in tomato. They observed increasing rate of 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%).

Lopes *et al*., in 2005, carried out a research in tomato plant, incorporating the dry above ground parts of velvet bean and tomato to the soil. Result showed that the soil amended with velvet bean affected negatively in the reproduction and gall formation of *M. incognita* and *M. javanica* as well.

The use of cover crops and poultry litter compost is an effective method to reduce nematode population only if successively incorporated into rotational cropping sequences (Everts *et al*., 2006).

In an experiment carried out by Kokalis *et al*., in 2005, out of six management systems on root knot nematodes in tomato and cucumber plants in greenhouse, organic, bare ground fallow and conventional production treatments reduced galling.

4.5. Review on screening of cultivars against *Meloidogyne*

Siddiqui, 1992 studied response of 45 chickpea cultivars to *Meloidogyne incognita* and their effect on perioxidase activity and found positive correlation between peroxidase activity and the degree of resistance present in the cultivars.

Montasser, (1995) investigated that out of eighteen screened flower bulb for their susceptibility to *M. incognita* on the basis of root gall index, 8 species were highly resistant, 6 susceptible, 2 species slightly resistant, one species moderately resistant and one species very resistant.

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*, where as only few were resistant to *M. incognita* race 2.

Hussain *et al*., 2001 screened ten cultivars of Chick pea against *M. javanica* and they found that plant shoot in nematode inoculated pots were significantly reduced (at $p<0.01$) compared to nematode free control, maximum suppression in shoot weight in cv. Nes 950174 in response to nematode infection, lower degrees of gall formation per root system in cv. 95004 and higher reproduction factor in cv. 96003. All cultivars were moderately resistant.

In a research conducted by Tsay *et al*., 2004, out of 56 spp. and 43 genera of Astereaceae plants, 9 were highly resistant to *M. incognita* without gall formation, 26 spp and 6 cultivars were moderately resistant with 25% fewer roots.

Similarly Bam, 2005, screened 8 cultivars of Tomato and found that a single cultivar i.e. 'T-597-5' was highly resistant to *Meloidogyne* and rest were susceptible.

4.6. Review on chemical management for *Meloidogyne*

Nematicides used in control of root knot nematodes are either fumigants, which are usually liquids and enter the soil water solution from a gas phase or non-fumigant, granular or liquid compounds which are water-soluble. Fumigant nematicides are generally more effective in controlling root knot nematodes and in increasing crop yield than are non-fumigants nematicides because fumigant nematicides have a broader spectrum of activity controlling soil insects, fungal diseases and weeds in addition to other plant parasitic nematodes. However granular or liquid formulations of contact and or systematic nematicides are more suitable for use on small farms, provided the growers are made aware of proper handling and application technique as well a time of application (Luc *et al*., 2005).

Nematicides such as carbofuran (Akhtar *et al*, 2005), Fosthiozate (Taba. *et. al*., 2006), methylbromide (luc, *et al*., 2005), 1-3 d plus chloropicrin (Hamil *et. al*., 2005), etc have also been found effective in control of the root knot nematode.

Ethylene bromide (15%) @ 20 gallons per acre gave the best control of nematodes (*M. incognita*) with the highest yield of tobacco in Nathalia, Victoria (Meagher *et al*., 1966).
Bhardwaj, *et al*., (1985) evaualated the nematicide, Furadan (3G) to control the root knot nematodes *Meloidogyne* spp infesting Okra and egg plant in Chitwan, Nepal.
Highest yield and canmass was noticed with aldicarb and oxamyl in split application with single treatment and satisfactory result were noticed only with aldicarb applicated in autumn (Lobster and Klerk, 1985).

Lamberti *et al.*, (2000), observed that the preplant application of granular or microincapsulated liquid Fenamiphos gave good result on Cantaloupe and significant yield increase was obtained in tobacco by soil fumigation with 1,3-D 97 or with preplant application of Aldicarb. However the preplant sol fumigation with metam-sodium or 1,3 dicholoropropene (1,3-D 97) increased yields and root gall indices to different extent.
A significant reduction of nematode juveniles and root galling index was observed in plots treated with either metham sodium and cadusfos or 1,3-dichloropropene and

cadasufos. Nematode decrease was observed greater when these three chemicals were applied in the same plots (Giannakou and Anastasiadis, 2005).
Methyl bromide and 1-3-D plus chloropicrin are effective fumigants with higher yields (Hamill *et al*., 2005).

An experiment was conducted by Haseeb *et al*., (2005) to compare the efficacy of Carbofuran, Bavistin, *Azadiracta indica* seed powder, green mould (*Trichoderma harzinnum))* and rhizobacteria against root knot nematode and found that Carbofuran and *A. indica* seed powder increased plant growth and yield significantly than bavistin and *P.fluorescens*. Carbofuran was highly effective against nematode, bavistin against fungus, *A. indica* seed powder against both the pathogens and both the bioagents were moderately effective against both the pathogens.

The toxicity of a nematicide Fosthiozate to the nematode trapping fungus *Monacrosprosporium elliosporium* and the control effect of granule formulations containing fungal propagule and the nematicide on *M. incognita* were examined on tomatoes. The nematicide had no effect on spore germination or hyphal growth at 3000 or 300ppm in CMA medium but drying of the granules sometimes decreased the survival rate of the fungus.The control test of *M. incognita* on tomatoes using the fungus nematicide formulations showed a very high effect when mixed with the total soil in pod experiments. The fungus was detected in all soil samples on completion (Taba *et al*., 2006).

Adegbite and Agbaje (2007) confirmed the suppressive effect of Furadan (Carbofuran) application on root knot nematode *M. incognita* race 2 multiplications on yam hybrid.
For *Meloidogyne* spp in tomato and cucurbits, the recommended nematicides are Aldicarb, Ethoprophos, Oxamyl, fenamiphos, Oazomet etc (Gowen, 2007).
Based on summary and comparison of methyl bromide alternative chemical trial in Florida since 1994, Telone c-17 or Telone c-35 (1,3-Dicholoropropene plus 17% or 35% Chloropicrin), in combination with separately applied herbicide for weed control, has

been identified as the best chemical alternative replacement for Methyl bromide for some vegetables row crops such as tomato (Noling, 2003).

4.7. Review on Cultural and other management practices

4.7.1. Crop rotation

Crop that is unsuitable for nematode infection, growth or reproduction or the crops that are detrimental to nematodes or the non-host crops are generally cultivated in crop rotation for the control of nematodes.

Root knot nematodes however are extremely polyphagus therefore relatively few non-host crops are available for control through crop rotation.

A rotation of sesame, maize, groundnut, sorghum, cabbage, velvet bean and then resistant sweet potato was effective in controlling *M. incognita* in Cuba (Fernandez et al., 1992, 1998). Root knot densities on tomato after sesame were reduced upto 75% as compared with rotation with sweet potato.

The use of host differential allows determination of the four main species and races of *Meloidogyne* (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.

4.7.2. Trap crops

In trap cropping a good host is planted for a short duration of time to ensure good nematode penetration and then the developing sedentary juveniles in the root tissues are killed by root removal from the soil or by destruction of the root tissue by physical means or with herbicides.

In Cuba, Lettuce (*Lactuca sativa*) and Radish *(Raphanus sativus*) are used as trap crops (Cuadra *et al*., 2000).

4.7.3. Cover crops

Non-host of the root knot are used mainly to protect the soil from erosion, to suppress weed growth between major vegetable crops to give some nematode control.

A number of non-host crops such as Velvet bean (*Mucuna pruriens*), horse bean (*Canavalia ensiformis)* and joint vetch (*Aeschynomene americana*) have been tested for the use as cover crops in the southern USA for nematode control (Mc Sorley *et al*., 1994 a,b).

The use of Elephant grass, *Pennisetum purpureum*, as mulch or the cultivation of *Brachiaria plantagea* led to significant reduction in galling over continuous tomato. Plant growth was increased the most in the *P. purpureum* treatment (Matsumoto *et al*., 2002).

4.7.4. Antagonistic crops

Plants antagonistic to nematodes are those that are considered to produce anthelminthic compounds (Grainge and Ahmed, 1998; Jairajpuri *et al*., 1990). These crops contain toxic substances with different modes of action (Pandey *et al*., 2003).

Marigold, sun hemp, castor bean, partridge pea, asparagus and sesame have been studied extensively to control nematode activity. Out of six cover crops to control *M. incognita* in tomato, Marigold had the greatest negative effect (Swamy *et al*., 1995).

Ploeg (1999) demonstrated that *Tagetes patula*, *T. erecta, T. signata* and a *Tagetes* hybrid reduced galling in a subsequent susceptible tomato crop compared with tomato-tomato rotation.

4.7.5. Soil solarization

It is as non-chemical technique in which transparent polythene tarps are laid over moist soil for a 6-12 week period to heat non-cropped soils to temperatures lethal to nematodes and other soil borne pathogens. Soil solarization with plastic mulches, which leads to the development of lethal temperatures in the soil, is being used in some countries for the control of root knot nematode and soil borne diseases (Katan, 1981; Whitehead, 1998).

Solarization applied in the summer in Morocco before the next tomato in plastic greenhouse led to a 99% reduction in *M. javanica* densities; when compared with the controls (Eddaoudi and Ammati, 1995). Similar results were obtained in India following solarization for 6 weeks in the summer months, with reduction in *M. incognita* and *Pythium aphanidermatrum* (Reddy *et al*., 2001).

Solarization for 2-4 weeks combined with Cadusafos or Fenamiphos, was considered a sustainable alternative to methyl bromide fumigation in greenhouse tomato in Cyprus (Ioannou *et al*., 2002).

4.7.6. Flooding

Root knot densities drop significantly when soils are flooded for prolonged periods of time. Sikora (1989) showed that the degree of root knot damage to processing tomato crops in the Philippines was less severe in rotation of paddy rice-tomato than in rotation without paddy rice.

Noling (2003) stated that alternating 2-3 weeks cycles of flooding with drying seems to be more effective than long continuous flooding cycles.

According to Padgham (2003), root knot juveniles are killed after exposure to anaerobic conditions that begin in the soil a few days after flooding.

4.7.7. Fallow

Bare fallow is effective in root-knot management especially when it can be used in hot, dry summer months between crops where alternative weed hosts are seldom a problem (Johnson and Fassuliotis, 1984; Brown and Kerry, 1987 and Netscher and Sikora, 1990).

4.7.8. Soil tillage

Repeated tilling of the soil at regular intervals for 30 days during hot and dry seasons between crops can significantly reduce root knot nematode densities in the upper horizons due to dessication of eggs and juveniles. Tillage also eliminates alternative weed hosts and volunteer plants from the previous crops (Johnson and Fassuliotis, 1984; Perez, 1990).

4.7.9. Root destruction

It has been estimated that the when soil temperatures are high, each month that the root system survives causes a tenfold increase in root knot nematode densities so to prevent the spread of the nematode to the follow up crops and to reduce the population density of the nematode, galled roots should be eliminated by uprooting and destruction (Anonymous, 2004).

Beside the above mentioned management practices, other methods such as use of healthy transplants, grafting and weed control have also been found effective in the control of root knot nematode in crops.

4.8. 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 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. This is the first report on the soil nematodes of Nepal.

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 high in number (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.

5. MATERIALS AND METHOD

The plant parasitic nematode used in this study was *Meloidogyne* an obligate endoparasite of roots. A single cultivar (Srijana) of tomato was used. An experiment was established with 7 treatments having 5 replications including one control in randomized complete block design (RCBD). The treatments were based on the application of *T. harzianum, commercial product of Paecilomyces lilacinus*, mustard cake, mixture of cow dung and urine, poultry manure, Furadan, and control. Each treatment was artificially inoculated with an equal density (i.e. 6000 eggs per pot) of *Meloidogyne* eggs. The study was conducted in screenhouse of Plant Pathology Division of National Agricultural Research Council (NARC), during 2007.

5.1. MATERIALS

To conduct the experiment on different management strategies against *Meloidogyne* in tomato, the 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
Acid fuchsin-acetic acid solution
4% Formalin

Furadan

Paecilomyces lilacinus

Trichoderma harzianum

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

Plant material

Compost manure

Chicken manure

Cow dung and urine

Mustard cake

Marker

Sticker

Paper bag

Bamboo stick

Twin ball (Plastic rope)

5.2. METHOD

With an objective of evaluating the efficiency of different management strategies on *Meloidogyne,* a common cultivar of Tomato i.e., Shrijana 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. 22 days old seedlings were transplanted in a pot of diameter 12.5 cm in five replicates with two plants per pot, which were filled with 1500gm (1200gm sterilized soil and 300gm sterilized compost), while mixture of cow dung and urine, and poultry manure were applied instead of compost in respective treatments. The pots were artificially inoculated with 6000 eggs per pot (@ 4 eggs/gm of soil) after two weeks of the transplantation. A total of 35 pots with seven treatments having five replicates were prepared. The experiment was carried out in screenhouse about 36 days from the date of inoculation of eggs. Measurement of gall index, calculation of Reproductive factor *(Rf*) and evaluation of the efficacy of the treatments on the nematode.

5.2.1. Preparation of the inoculum

Meloidogyne was collected directly from the field where the nematode 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.

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

A Tomato cultivar Shrijana was 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).

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.

5.2.6. Treatment details

The experiment was laid down in a RCBD with seven treatments in five replications, including one control treatment. There were a total of 35 pots in the experiment. All pots were artificially inoculated with the equal number of nematode eggs. The different treatments for the management purposes were incorporated as follows:

- Application of *Trichoderma harzianum* inoculated compost @ 300g/pot.
- Application of commercial product of *Paecilomyces lilacinus* @ 10 ml per pot (10 ml stock solution diluted in 50ml water)
- Application of mustard cake @ 30g per kg soil in 1:25 ratio of mustard cake and soil.
- Application of cow dung + urine @ 300g per pot in 1:4 ratio of cow dung + urine and soil.
- Application of Poultry manure @ 250g / pot in 1:5 ratio of poultry manure and soil.
- Application of Furadan (Carbofuran) @ 1g per kg of soil.
- Control.

5.2.7. Inoculation of eggs

The inoculum density was fixed 4 egg/gm of soil. The 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.

5.2.8. 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 few days before sowing the seeds. Manure was added in the seed plots and mulching was done after seedling.
Watering was done every alternate day till germination and every day after germination.
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.

5.2.9. 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 2^{nd} 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). Root galling was indexed from 0-10 scale as described by Bridge & Page (1980). 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.

5.2.10. Data collection and statistical analysis

The total nematodes (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).


Fig. 2 Preparation of nursery bed at HRD, Khumaltar

Fig. 3 Transplanting tomato plants in screen house at PPD, Khumaltar

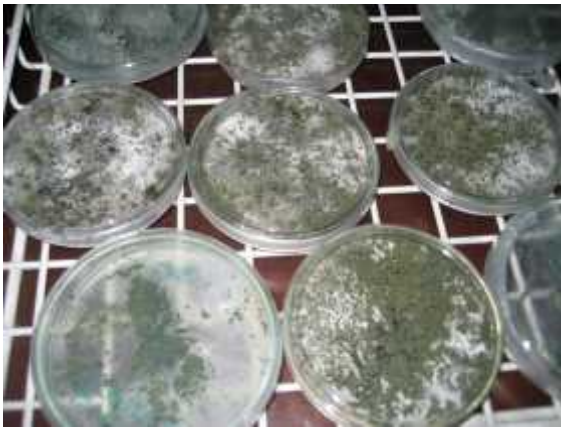


Fig. 4 *T. harzianum* culture in potato dextrose agar (PDA)

Fig..5 Sample collection during field visits at Hemja, Pokhara

Fig. 6 Inoculation of *Meloidogyne* eggs in tomato plants at PPD, Khumaltar

Fig.7 Inoculated plants in screen house at PPD, Khumaltar

Fig. 8 Caring of the plants

Fig. 9 Harvesting of the roots with galls of *Meloidogyne*

Fig. 10 Staining of the roots in the solution of Phloxine B (0.015%)

Fig. 11 Counting of eggs and juveniles in stereoscope microscope at PPD laboratory

6. RESULTS

The effect of different treatments on *Meloidogyne* in the tomato plant cv. Shrijana were observed after 36 days of the inoculation of the eggs, assuming the *Meloidogyne* requires a month to complete its lifecycle.

Different parameters such as root gall index (*GI*) and reproduction factor (*Rf*) were taken for the determination of the efficacy of the treatments. The root gall index and reproductive factor were determined according to Tayler and Sasser (1978), Bridge and Page (1980).

6.1. Determination of Gall index (*GI*)

A single cultivar of tomato (Shrijana) was inoculated with 6000 eggs and seven different management strategies were applied including one control with five replicates of each treatment. The roots were collected from the soil 36 days after inoculation and rating of the gall index was done by counting the number of galls present in each root. The result was analyzed by gall index method as described by Bridge and Page (1980) at $p<0.05$.

The data in the Table 3 shows the effect of various treatments on root knot index or root gall index (*GI*). All the treatments except Furadan (Carbofuran) had lower root gall index as compared to the control treatment (inoculated untreated plants). However Furadan (*GI*=6.17a) had more or less similar gall index to that of the control. The application of *P. lilacinus* also did not have significant difference in root galling (*GI*=4.79ab) as compared to the control (Table 3). The phytopathogenecity of the *Meloidogyne* was higher in case of the application of Furadan followed by biological agents than others.

The lowest root galling index was observed in the application of mustard cake @ 30g per kg soil (*GI*=1.0d), followed by the mixture of cowdung and urine @300g per pot (*GI*=2.79c), poultry manure @ 250g per pot (*GI*=3.06c). Three different treatments such as *T. harzianum* @ 300g per pot (*GI*=3.87bc), *P. lilacinus* @ 10ml per pot (*GI*=4.8ab) and Furadan @ 1g per kg soil (*GI*=6.17a) did not show any significant difference from one another in case of *GI*. However *T. harzianum* and poultry manure showed significant difference with the control at $P<0.05$, while the treatments mustard cake and cow dung

and urine showed highly significant difference with all the other treatments at P<0.05 shown in figure 12.

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

S.N.	Treatments details	Gall index (*GI*)	Remarks
1	*Trichoderma harzianum* inoculated compost @ 300g/pot	3.87 **bc**	Significant
2	*Paecilomyces lilacinus* @ 10 ml per pot	4.79 **ab**	Non - Significant
3	Mustard cake @ 40g per kg soil	1.0 **d**	Highly significant
4	Cow dung + urine @ 300g per pot	2.79 **c**	Highly significant
5	Poultry manure @ 250g / pot	3.06 **c**	Significant
6	Furadan (Carbofuran) @ 1g per kg of soil	6.17 **a**	Non - Significant
7	Control	6.06 **a**	Check
LSD (P< 0.05)			1.46
CV (%)			30.94

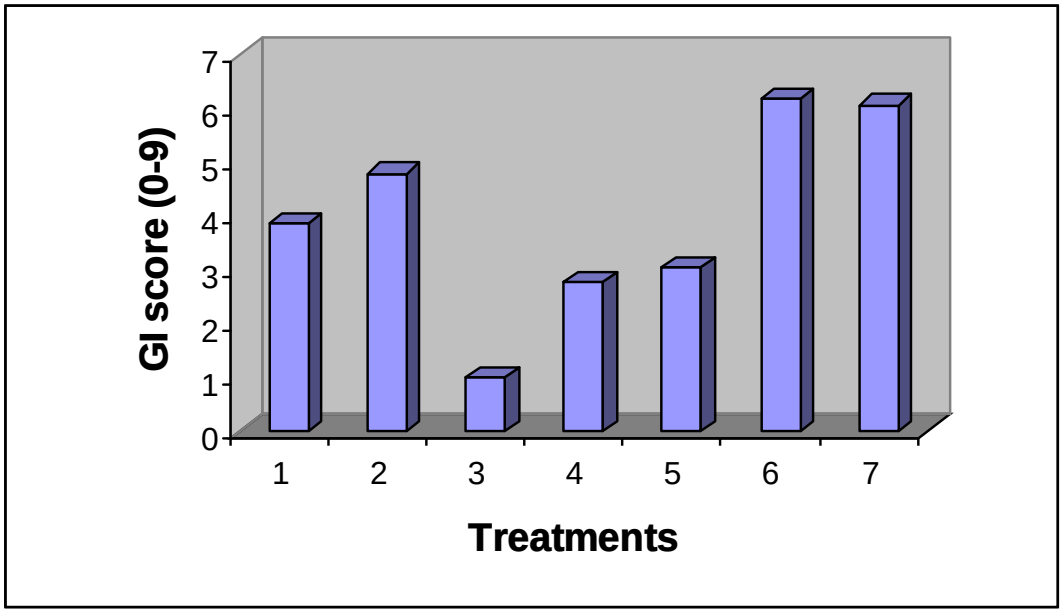


Figure 12. Bar diagram representation of gall index of different treatments against *Meloidogyne* gall formation in root of tomato (cv. Shrijana) at screen house experiment at Khumaltar.

6.2. Determination of Reproduction factor (*Rf*)

After rating the gall index, eggs were extracted from the whole root system and juveniles were also extracted from the 100g soil samples and the final population of the nematodes eggs and juveniles (*Pf*) was calculated. Then *Rf* was determined as follows:

Reproduction factor (*Rf*)= *Pf / Pi*

Here,

Pi is the initial population of the *Meloidogyne* eggs inoculated.

Pf is the final population of the nematode at the time of harvesting.

Data (Table 4) shows that the effect of different treatments on Reproduction factor were mostly significant (at $P<0.05$) as compared to the control plants.

In figure 13, the greatest suppression of the nematode population i.e., Reproduction factor (*Rf*) was achieved by the application of the mustard cake application (*Rf* = 0.062d), followed by the mixture of cowdung and urine (*Rf*= 0.38c), poultry manure (*Rf*= 0.76c), and *T. harzianum* (*Rf*=2.94 b). While in case of the treatments *P. lilacinus* (Rf=5.09ab) and Furadan (Carbofuran) (*Rf*=7.6a), the results were similar as mentioned in the gall index. These treatments did not show any significant difference from each other as well as in comparison to control (*Rf*=7.96a).

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

S.N.	Treatments details	Initial Population (*Pi*)	Final Population (*Pf*)		Reproduction factor (*Rf*)
			Eggs in root	J_2 in soil	
1	*Trichoderma harzianum* inoculated compost @ 300g/pot	6000	17700	0	2.94 b
2	*Paecilomyces lilacinus* @ 10 ml per pot	6000	30600	258	5.09 ab
3	Mustard cake @ 40g per kg soil	6000	376	0	0.062 d
4	Cow dung + urine @ 300g per pot	6000	2027	0	0.38 c
5	Poultry manure @ 250g / pot	6000	4562	0	0.76 c
6	Furadan (Carbofuran) @ 1g per kg of soil	6000	45800	859	7.6 a
7	Control	6000	47800	1659	7.96 a
	LSD (P<0.05)				0.38
	CV (%)				16.35

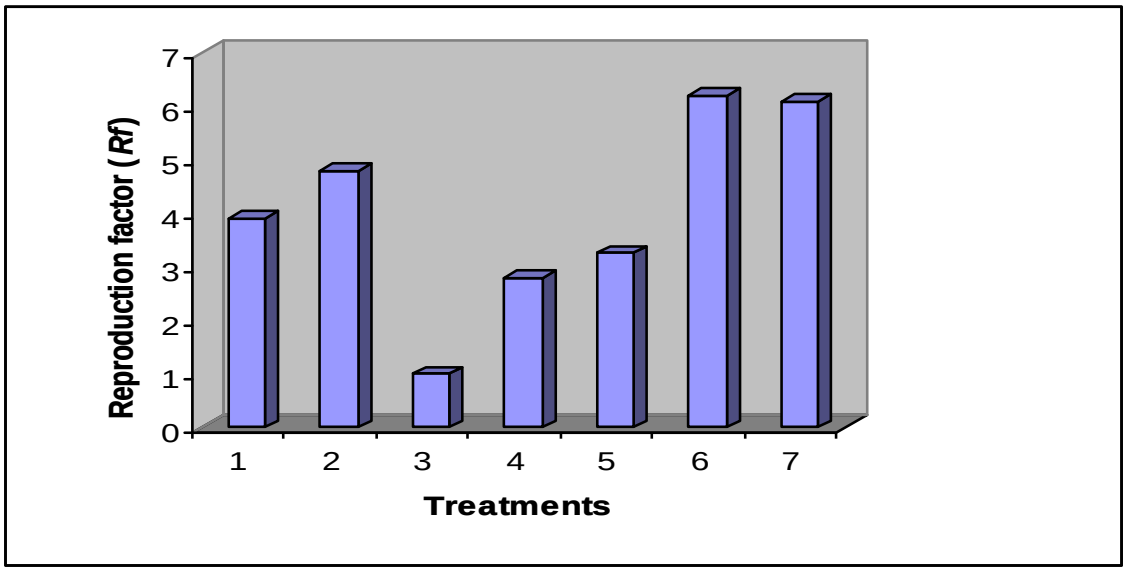


Figure 13. Effect of treatments on the reproduction factor of *Meloidogyne* spp. in tomato (cv. Shrijana) on the screen house pot experiment in Khumaltar.

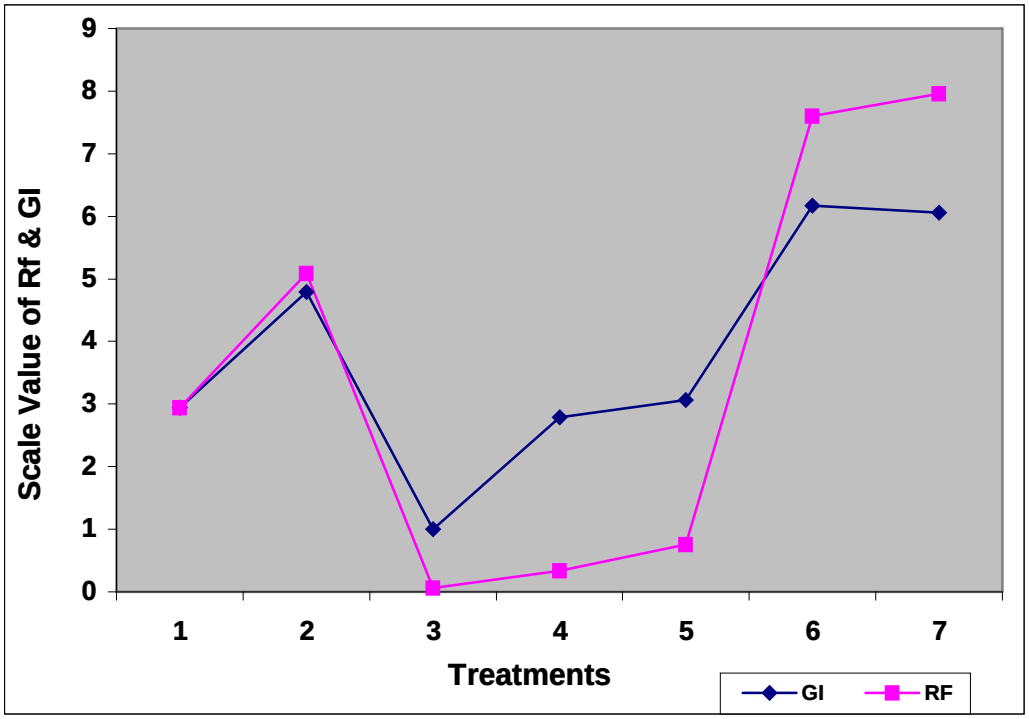


Figure 14. Interaction of gall index with reproduction factor of *Meloidogyne* spp. in tomato (cv. Shrijana) in screen house condition in Khumaltar.

Figure 14 shows the relation between gall index and reproduction factor. With an increase in the root gall index the reproduction factor has also increased however in some cases there has been inconsistency such as in case of the cow dung and urine mixture and the application of poultry manure the *GI* shows higher value than the *Rf*. Thus showing a negative correlation between *Rf* and *GI* in these two cases.

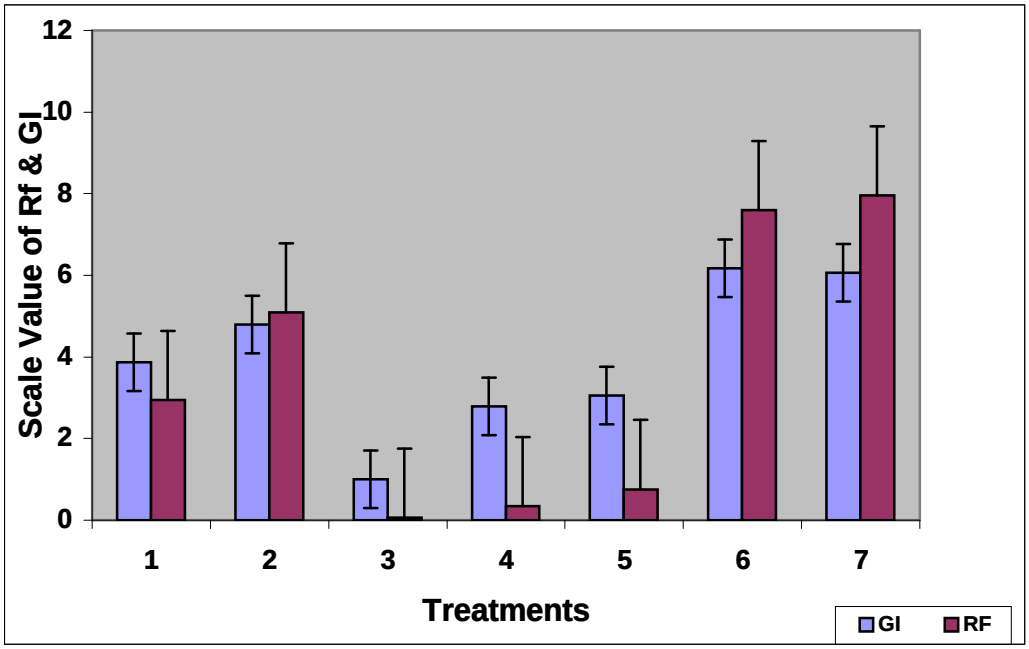


Figure 15. Relation between gall index and its level of reproduction factors in different treatments against root knot nematode in screen house experiment at Khumaltar. (Mean in standard error).

In the above figure 15, higher the gall index, higher is its reproduction factor (*Rf*), and lower the *GI*, lower is the *Rf*. But there was not positive correlation between *Rf* and *GI* in case of the treatments cow dung and urine mixture and poultry manure. In case of cow dung and urine the *GI* value is higher while the *Rf* value is lower and similarly in case of poultry manure also the Rf value is lower but the *GI* is higher.

7. DISCUSSION

Root knot nematodes are most important and cosmopolitan pest of vegetables distributed worldwide and infesting more than 2500 kinds of host plants (Siddiqui, 1986). Although over 90 species of *Meloidogyne* have been described to date, four species are of particular economic importance to vegetable production, *M. incognita, M. javanica, M. arenaria and M. hapla.* More than 90% of the damage to the crop plants is caused by these nematodes in the tropical region (Sasser, 1980).

Estimation of vegetable crop losses in the tropics (Sasser, 1979) ranged from 17 to 20% on aubergine, 18 to 33% on melon and 24 to 38% on tomato. Similarly, Reddy (1985), estimated an annual loss of 39.77% in tomato production.

Regarding the substantial loss caused by the *Meloidogyne* spp. researchers and the farmers are being attentive towards its management and considerable progress has been made in the field of biological, chemical, organic amendment, cultural and other management strategies since the recognition of morphologically and physiologically discrete species of *Meloidogyne* (Chitwood, 1949) and a number of strategic reviews have also been published on nematode management in vegetable production.

In this study, the efficacy of six different management strategies on *Meloidogyne* in tomato plants such as the application of *T. harzianum, P. lilacinus,* poultry manure, mixture of cow dung and urine, mustard cake and Furadan was tested. The experiment results indicated that the mustard cake and mixture of cow dung and urine were the most effective against the *Meloidogyne* spp. among the treatments with *Rf* = 0.062d and *GI*=1.0d at $P<0.05$.The phytopathogenecity of *Meloidogyne* was lower in the application of mustard cake and cow dung as compared to the application of biological agents and Furadan.This significant suppression of the reproduction of the nematode and reduced galling in this treatment could have been due to a number of factors such as: toxic metaboloic released in soil, the addition of the manure to the soil leads to better environment for the growth and development of the roots, enhancing the utilization of

soil nutrients and consequently nematode damage is reduced (Vander-Borgett *et al*., 1994).

The control in organic amendments may be contributed due to (i) toxic compounds present in the organic materials as in neem. (ii) non-toxic compounds such as residual sugar in bagasse (iii) toxic metabolites produced during microbial degradation or (iv) enhancement of nematode antagonists (Luc *et al*., 2005).

Organic amendments also increase plant vigour, enabling plants to withstand nematode attack (Singh *et al*., 1986). However the reduction in galling in the application of poultry manure probably resulted from the production of the ammonical nitrogen following its addition. (Rodríguez-kabana, 1986; Rodríguez-Kabana, *et al.,* 1987). In addition to their effects on nematode density, organic amendments also improve soil structure and water holding capacity, reduce diseases and limit weed growth, which ultimately leads to a stronger plant and improved tolerance to nematode attack. Organic amendments have been found to be significantly effective when combined with biocontrol agents such as *Pochonia chlamydosporia, T. harzianum and G.s fasciculatum.* Neem based oil cakes and related products have also been used in combination with several bio-control agents (Naik *et al*., 1998).

The reduction in the reproduction factor of the nematode may be responsible for the observed decrease in root galling indices. The decrease in the number of nematodes suggested the nematicidal potential of the cow dung and urine. Similar observations had been made by the other researchers also (Babalola, 1990; Akhtar and Alam, 1990, 1992, Alam *et al*., 1994). This result is also in conformity with the result obtained by Abubakar *et al*., 2004.

Application of mustard cake was also found to be effective in reducing gall formation in the root system following cow dung and urine mixture *Rf*= 0.38c and *GI*=2.79c which was again followed by the application of poultry manure with *Rf*=0.76c and *GI*=3.06c. Thus these two treatments were also significant in reducing the gall formation and the suppression of the nematodes reproduction.

The experiment result also showed that the bio-control agents such as *T. harzianum* was slightly significant with the control while *P. lilacinus* did not suppress the nematodes reproduction and gall index to significance level however these two biocontrol agents were not significantly different from each other. The treatment of *T. harzianum* (*Rf*=2.94b and *GI*=3.87bc) and *P. lilacinus* (*Rf*=5.09ab and *GI*=4.79ab) at P<0.05 were less significant than the organic amendments such as cow dung and urine, poultry manure and mustard cake application. The increase in root galling and reproduction factor in both the above cases were unexpected as both of the above bio-control agents were shown to be effective in controlling root knot nematodes.

T. harzianum, which is known to be effective against fungal diseases, also has activity towards root knot nematodes. Control of root knot with an Indian strain of *T. harzianum* was enhanced by adding the antagonist to the soil amended with neem cake (Rao *et al*., 1997). Both *T. harzianum* and *T. lignorum* increased plant growth and reduced *M. javanica* galling in tomato and aubergine in soil treated with the fungi 18 days prior to planting in greenhouse tests (Sharon *et al.,* 2001). Single treatments of *T. harzianum* and *T. virides* were effective at low initial root knot densities in one cycle vegetable crops grown in organoponics (Perez, 2001).

The result also showed that *T. harzianum* inoculated compost @ 300g per pot was significant in controlling root knot nematodes and reduced galling index. However, they were less significant than the organic amendments. Similarly *P. lilacinus*, which is predominantly a fungal egg pathogen, has been marketed for use in the Philippines and South Africa (Kiewnick, 2004). Effective bio-control of root knot nematodes in the field has been reported on vegetable and other crops in a number of countries (Holland *et al*., 2003). The effectiveness of *P. lilacinus* was confirmed by Khan and Saxena (1997) in combination with the organic materials. In addition to parasitism, other factors may also be involved in the nematode management (Isogai *et al*., 1980). The ability of *P. lilacinus* increased when it is integrated with the organic materials. It is assumed that the decomposition of organic matter increase released nematicidal principal (s) and the residual organic matter increase fungal activity and persistence (Alam *et al*., 1979; Kerry,

1984; Rao and Reddy 1994). *P. lilacinus* and soil amended with ground nut oil cake seems the best combination (Rao and Reddy,1994).

Thus the application of *P. lilacinus* alone might be the reason behind the comparatively less effectiveness of the treatment in our experiment. However, in the experiment commercial *P. lilacinus* was used. In general, the efficacy of biocontrol agent (antagonists) to suppress the nematodes is very low as compared to chemical due to its life duration, its formulation which is affected by several factors on their survival in new environment. There can also be some latent parameters because many physical, chemical and microbial factors affect the establishment of newly introduced organisms.

The experiment result showed that Furadan (Carbofuran) was least effective amongst the six treatments with *Rf*=7.6a and *GI*=6.17a indicating that this chemical did not work properly as the reproduction factor nearing to that of the control treatment and the *GI* even greater than that of the control treatment.

This result is totally in disagreement with the previous works done so far. The suppressive effect of Furadan was confirmed by Adegbite and Adbage, (2007) on *M. incognita* race 2 multiplications on hybrid yam varieties. Similarly, Akhatar *et al.*,(2005), reported Furadan to be most effective against nematodes, which was in conformity with Butool *et al*. (1998), who also found Carbofuran effective in suppressing *M. incognita* on *Hyoscyomus muticus.*

In the present experiment, the result obtained is unexpected as Furadan did not show any significance in nematode suppression as its *Rf* and *GI* were more or less similar to that of the control. Whereas Furadan has been reported as one of the effective chemical to control the root knot nematodes (Akhtar *et al.,* 2005; Adegbite and Agbage, 2007). Carbofuran impairs nematode neuromuscular activity by inhibiting the function of enzyme acetyl cholinesterase resulting in reduced movement and multiplication (Evans, 1973). The nematodes may also be killed while feeding on root tissues by the systemic

action of these nematicides when they are absorbed by the plant roots and translocated in the plant system (Van Berkum and Hoestra, 1979).

However, in some cases, the trend of result showed inconsistency. It might be due to biotic and abiotic factors. Some possible reasons may include: nematode may not be adopted to the full range of soil environment conditions conducive to root (eg: low oxygen or high carbondioxide concentrations, dry or high moisture content), root damage caused by the nematodes could result apparently low biomass in strata of high nematode abundance (Forge *et al*., 1998), adverse affect of soil micro-organisms (Timper and Brodie, 1993), population of nematode consisting of a mixture of pathotypes (Cook and Evans, 1987) and error during the lab exercise (Verschoor and De Goede, 2000).

8. CONCLUSIONS

The present experiment on management strategies for root knot nematodes helped us to understand and demonstrate the efficacy of different management practices. The experiment was done for the first time in tomato cultivar Shrijana.

Very little work has been done on *Meloidogyne* in Nepal, which is mostly limited to distribution and diversity while interests are being given by the researchers and agriculturist towards the management of the root knot nematodes. Works have been reported on screening of tomato cultivars against root knot nematodes and different cultural and other management practices are also being practiced by the farmers as well.

Our present study concentrates on the management strategies that can be applied against root knot nematode in tomato plants. The whole experiment was carried out in the screenhouse of Plant Pathology Division of National Agriculture research Council (NARC).

Based on the results obtained in this study, it is concluded that:

- Mustard cake applied at @ 30g per kg soil was most effective against root knot nematode.
- A mixture of cow dung and urine applied in the ratio of 1:4 with soil (i.e., 300g cow dung and urine mixture with 1200g of soil) is also effective in suppressing the reproduction factor of root knot nematode and reducing the gall index among the treatments applied. The *Rf* and *GI* were significantly lower at P>0.05.
- Application of poultry manure also suppressed the nematode reproduction and root galling significantly.
- Bio-control agents such as *T. harzianum* and *P. lilacinus* were less effective in controlling the root knot nematode in comparison to the organic amendments. Thus reconfirmation test is required
- The chemical Furadan was not effective in suppression of the nematodes thus indicating that this chemical did not function in our experiment. This chemical also needs to be reconfirmed.

9. RECOMMENDATION

On the basis of the study, *Meloidogyne* species were found to be highly destructive to tomato plants and other vegetable as well causing a great loss in the country's economy due to substantial loss in the production of tomato and other vegetables of farmers and small-scale growers. Thus management of nematode has become an essential in our country. There are various methods that can be implied by the farmers as well as small-scale growers to control the *Meloidogyne* spp.

Among the six management strategies implied in the experiment, the organic soil amendment seemed to suppress the nematode population by suppressing the *Rf* and reducing the *GI* as well. Therefore following suggestions have been recommended for the management of the *Meloidogyne* spp.

- The mustard cake was highly significant in suppressing the root knot nematode. Thus, this can be recommended as one of the possible methods for reducing the population of root knot nematodes.
- The mixture of cow dung and urine was also found to be highly effective against *Meloidogyne*. Hence, this can be recommended for wide use in tomato cultivation.
- Poultry manure also reduced root galling and nematode population. Hence can also be recommended for minimizing the *Meloidogyne* population in tomato cultivation.
- In Nepal, very little work has been done to control the root knot nematodes in tomato as well as other vegetables crops that are economically important. Thus further researches are necessary regarding the control of nematode problem in Nepal.
- Agricultural nematology plays important role for the better production of crops. Hence the courses should be allocated in the University level with better equipment facilities in our country.
- Awareness programmes should also be launched among the farmers regarding the knowledge of the nematodes and the serious damage and loss caused by them to the crops.

- The farmers and small scale growers should be encouraged to apply organic soil amendments as it increases fertility adding nutrient to the soil and does not have any adverse effect on the environment as the broad spectrum nematicides have. However in case of the severe infestation of the nematodes chemicals can also be used.
- Cultural practices such as use of cover crops, trap crops, non-host plants, or antaginist plants and crop rotation should also be encouraged as it helps to reduce the nematode population.

REFERENCES

1. Abubakar U., Adamu T. and Manga S. B. (2004). Control of *Meloidogyne incognita* (Kofoid and white) chitwood (rootknot nematode) of *Lycopersicon esculentum* (tomato) using cow dung and urine. African Journal of Bio technology 3 (8): 379 -381.
2. Adegbite, A.A. and Agbaje G.O. (2007) Efficacy of Furadan (carbofuran) in control of root knot nematode (*M. incognita* race 2*)* in hybrid yam varieties in south- western Nigeria. World Journal of Agricultural Sciences 3(2): 256-262.
3. Akhtar, H., Sharma, A. and Shukla P.K. (2005). Effect on management of root knot nematode, *M. incognita*- wilt fungus, *Fusarium*- oxysporium disease complex of green gram, *Vigina radiata* cv Mc-1108. Department of plant protection faculty of Agricultural sciences. Aligarh Muslim University, Aligarh, India 6(8): 736-742.
4. Akhtar, M. and Alam, M.M. (1992). Effect of crop residue amendments to soil for the control of plant parasitic nematodes. Bioresour. Technol. 14:81-83.
5. Akhtar, M., Alam, M.M. (1990). Control of Plant Parasitic nematodes with Agro-wastes Soil amendment. Pakistan J. Nematol. 8:52-28.
6. Alam, M.M., Khan, A.M. and Saxena, S.K. (1979). Mechanism of control of Plant Parasitic nematodes as a result of the application of organic amendments to the soil. V-Role of phenolic compounds. Indian J. Nematol., 9:136-142.
7. Anonymuos (2004). Institute offood and agriculture sciences. IFAS Florida Nematode Control Guide. Entomology and Nematology department and Cooperative Extension Service, University of Florida, Gainesville, Florida. Available at: edis. ifas. ufl. edu/ cv 112.
8. Anwar, A. (2003). Impact of *Meloidogyne incognita* in incidence of root rot disease caused by *Rhizoctonia solani* in tomato. Agricultural and Biological Research 19(1): 22-28.

9. Babalola, J.O. (1990). Pathogenicity of the root knot nematode, *Meloidogyne incognita* on okra, *Abemochus esculentus* and effect of some organic pests on okra. Nig. J. Plt. Proct. 1:23-29.
10. Babarola, J.O. (1982). Effect of soil amendments on nematodes in tomato cultivation. In: Proceedings of the Vth annual conference of the horticultural society of Nigeria, 21-25 November, Zaria, Nigeria, p.155.
11. Baidya, S. (2006). Interaction between *Pratylenchus penetrans* and *P. crenatus. In:* Moens, M., (2005). Introduction to the biology and management of nematodes in maize with Agro- ecosystems. International nematology course, University of gent, Belgium, Belgium class notes 58pp.
12. Bam, A. (2005). Screening of tomato germplasm against *Meloidogyne incognita.* M.Sc. thesis. Central Department of Zoology, Kirtipur, Kathmandu, Nepal.
13. Berkeley, M.J. (1855). Vibrio forming exrescenece on the roots of cucumber plants. Gard Chhoo (14): 220.
14. Bhardwaj, L. N. and Shrestha, S.M. (1983). Root knot nematode of Chitwan district of Nepal. II. J. inst. Agric. Anim. Sci. (Nepal) 4 (I and II): 27- 30.
15. Bhardwaj, L.N. (1982). Root knot nematodes of Chitwan district of Nepal. I.J. Inst. Agric. Anim. Sci. (Nepal) 2(1): 1-5.
16. Bhardwaj, L.N., Shrestha, S.M. and Mishra, N.K. (1985). Evaluation of Furadan for the control of root knot nematodes infesting okra and eggplant in Chitwan, Nepal. J. Inst. Agri. Anim. Sci. 6: 43-49.
17. Bhatta, D.D. (1967). A note on plant parasitic nematodes of the Kathmandu valley. Indian phytopathology 20(1): 73-74.
18. Bhatti,D.S., and Jain, R.K. (1977). Estimation of loss in okra, tomato and brinjal yield due to *Meloidogyne incognita*. Indian J. Nematol 7:37-41.
19. Birchfield, W. and Antonpoulos, A.A. (1976). Scanning electron microscope observation of *Duboscquia penetrans* parasitizing root-knot larvae. Journal of Nematology 8:272-283.
20. Bird, A.F. and Bird, J. (1991). The Structure of Nematodes. Academic Press, Inc. San Diego, California 92101.

21. Bridge, J. and Page, S.L.J. (1980). Estimation of root knot nematode infestaion levels on roots using a rating chart. Tropical Pest Management 26: 296-298.

22. Brown, R.H. and Kerry, B.R (eds) (1987). Principles and Practices of Nematode Control. Academic Press, New York.

23. Budatthoki, K., Pradhan, N.G., Regmi, H.N. and Bhurtyal, P.R. (2004). Advance of Horticulture Research in Nepal. Proceeding the fourth National Workshop on Horticulture 2-4.

24. Butool, F., Haseeb, A. and Shukla, P.K. (1998). Management of root-knot nematode, *M. incognita*, infesting Egyptian henbane, *Hyoscyamus muticus* L., by the use of nematicides and oilcakes. International Journal of Pest management 44:199-202.

25. Cabanillas, E., Barker, K.R. (1989). Impact of *Paecilomyces lilacinus* inoculum level and application time on control of *Meloidogyne incognita* on tomato. Journal of Nematology 2 (1): 115-120.

26. Cayrol, J.C. (1983). Lutte biologique contre les *Meloidogyne* au Moyen d' *Arthrobotrys irregularis* Revue de nématologie 6: 265-273.

27. Cayrol, J.C. and Frankowski, J.P. (1979). Une mithod de lutte biologique contre les nematodes á galles des racines apartenant au genre *Meloidogyne*. Pépiniéristes Horticulteurs maraîchers. Revue horticole 143:15-23.

28. Cook, R. and Evans, K. (1987). Resistance and Tolerance. *In*: Brown, R.H. and Kerry, B.R. (ed). Principles and practices of nematode control in crops. Academic Press, New York, USA. Pp.179-231.

29. Cuadra, R.M., Cruz, X. and Fajardo, J.F. (2000). Cultivos de ciclo coprto camo plantas trampas para el control del nematodo agallador. nematropica 30: 241-246.

30. Davide, R. G.and Triantaphyllou, A. C. (1967). Influence of the environment on development and sex differentiation of root-knot nematodes. II. Effect of host nutrition. *Nematologica* 13:111-117.

31. Davide, R.G., and Zorilla, R.A. (1983). Evaluation of a fungus, *Paecilomyces lilacinus* (Thom) Samson, for the biological control of the potato cyst nematode *Globodera rostochinensis* Woll as com parul with some nematicides. Phillipine Agriculturist 66:397-404.

32. Deley, P. and Blaxter, M.L. (2002). Systemic position and phylogeny. In: Lee D.L (ed). The Biology of Nematodes. Taylor and Francis, London, Newyork 1-30.

33. Eddaouti, M. and Ammati, M. (1995). Study on the effects of single and combined soil solarization and the use of resistant tomato varieties on the populations of *Meloidogyne javanica* and the production of tomato in South morocco. Afro-Aasian Journal of Nematology 5:28-33.

34. Eisenback, J. D., and H. H. Triantaphyllou. 1991. Root-knot nematodes: *Meloidogyne* species and races. Pp. 191-274, *In*: W. R. Nickle, ed., Manual of Agricultural Nematology. Marcell Dekker: New York.

35. Evans, A.A.F. (1973). Mode of action of nematicides. Annals of Applied Biology 75:469-473.

36. Everts, K.L., Sardanelli, S., Kratochvil, R.J., Armentrout, D.K., and Gallaghar, L.E. (2006). Root knot and root- lesion nematode suppression by cover crops, poultry litter and poultry litter compost. Plant Disease 90(4) : 487-492.

37. Fernández, E., Pérez, A., Lorenzo, E. and Vinent, E. (1992). Efectividad del uso del ajonjolí (*Sesanum indicum*) como cultivo intercosecha contra *Meloidogyne incognita*. Revista de Protección Vegetal 7:39-42.

38. Fernánndez, E., Pérez, M., Gandarilla, H.,Vázquez, R., Fernández, M., Acosta, O.and Basterrechea, M. (1998). Guia para disminuir infestaciones de *Meloidogyne* spp mediante el emplea de cultivos no susceptibles. Boletín Técnico. INISAV, Cuba 4, no.3.

39. Forge, A.T., Deyoung, R. and Vrain, T.C., (1998). Temporal changes in the vertical distribution of *Pratylenchus* under Raspberry. Journal of nematology 30(2): 179-198.

40. Giannakou, I.O. and Anastasisdis, I. (2005). Evaluation of chemical strategies as alternative to methyl bromide for the control of root knot nematodes in greenhouse cultivated crops. Crop Protection 24(6): 499-506.

41. Goswami, B.K., Mittal, A. and Singh, S. (2005). Management of disease complex cause by root knot nematode, *Meloidogyne incognita* and root rot fungus, *Rhizoctonia solani* on Balsam, *Impatiens balsamina*, by fungal bio agent. Pesticide Research Journal 17 (1): 19-22.

42. Gowen, S.R. (2007). Chemical control of nematodes: - efficiency and their side effects. Plant nematode problems and their control in near east regions. Agriculture Department.

43. Grainge, M. and Ahmed, S. (1998). Handbook of Plants with Pest-control Properties. John Wiley and Sons, New York.

44. Hamill, J.E. and Dickson D.W. (2005). Effect of application of fumigant and non-fumigant nematicides on cantaloupe grown in deep sand soils in Florida. Journal of Nematology 37(3): 281-284.

45. Hartman, K.M. and Sasser J.N. (1985). Identification of *Meloidogyne* species on the basis of differential hosts test and perennial pattern morphology. *In*: Barker, K. R., Carter, C.C. and Sasser, J.N. (eds.) Advance treatise on *Meloidogyne* Volume II Methodology. North Carolina University Graphics, Raleigh, North Carolina, Pp 69-78.

46. Hewlett, T. E., and A. C. Tarjan 1993. Synopsis of the genus *Meloidogyne* Goeldi, 1887. Nematropica 13:79-102.

47. Hogger, C.H. (1981). Root knot nematodes in Nepal pp 148 in proc. third int. *Meloidogyne* project plan. cong. on root- knot nematodes, *Meloidogyne* spp; 20-24.

48. Holland, R.J., Williams, K.L. and Khan, K. (1999). Infection of *Meloidogyne javanica* by *Paecilomyces lilacinus.* Earth and environmental science. Journal of Nematology 1(2): 131-139.

49. Holland, R.J., Williams, K.L. and Nevalainen, K.M.H. (2003). *Paecilomyces lilacinus* strain Bioact 251 is not a plant endophyte. Australian Plant Pathology 32:473-478.

50. Hussain, S., Zareen, A., Javed, M.Z. and Abid, M. (2001). Response of ten chickpea cultivars against *M. javanica* and disease control by fungal filtrates. Pakistan Journal of Biological Sciences 4(4): 421-432.

51. Ioannou, N., Ionnou, M. and Paroussi, G. (2002). Integrated management of soil borne pathogens of greenhouse tomato in Cyprus. *In*: Voyotzis, D. and Paroussis, E. (Eds). Proceedings of the second Balkan symposium on vegetable and potatoes. Thessaloniki, Greeece 2000. Acta Horticulturae 579: 433-438.

52. Isogai, A., Suzuji, A., Higashikawa, S. and Tanura, S., (1980). Constiuents of peptidal antibiotic p-168 produced by *Paecilomyces lilacinus* (Thom) Samson. Agri. Biol. Chem., 44:3029-3031.

53. Jairajpuri, M.S., Alam, M.M. and Ahmed, I. (1990). Nematode Biocontrol. CBS Publishers, New Delhi.

54. Jatala, P. (1986). Biological Control of Plant Parasitic Nematodes. Annual Review of Phytopathology 24: 453-489.

55. Johnson, A.W. (1998). Vegetable Crops. In: Baker, K.R., Pederson, G.A. and Windham, G.L. (eds). Plant and Nematode Interaction. American Society Of Agronomy, Madison, Wisconsin, PP 595-636.

56. Johnson, A.W. and Fassuliotis, G. (1984). Nematode parasites of vegetable crops. In: Nickle, W.R. (eds). Plant and Insect Nematodes. Marcel Dekker Inc., New York. Pp323-372.

57. Johnson, L.F. (1959). Effect of the addition of organic amendment to the soil on root knot of tomato. Preliminary Report. Plant Disease Reporter 43: 1059-1062.

58. Jothi, G., Pugalendhi, S., Poornima, K. and Rajendran, G. (2003). Management of root-knot nematode in tomato *Lycopersicon esculentum,* Mill., with biogas slurry. Department of Nematology, Tamilnadu Agricultural University, Coimbatore 641003, India. Bioresource Technology 89:169-170.

59. Katan, J. (1981). Solar heating (solarization) of soil for control of soilborne pests. Annual Review of Phytopathology 19:211-236.

60. Kerry, B.R. (1984). Nematophagus fungi and the regulation of nematode populations in soil. Helminthological Abstracts, Series B 53:1-14.

61. Kerry, B.R. (1987). Biological control. In: Brow, R. H and Kerry, B.R (eds). Principles and practice of nematode control in crops. London Academic Press Inc: 233-263.

62. Keshari, A.K. (1999). Surveillance of plant parasitic nematodes of different vegetable crops of Kathmandu valley. III National conference on science and technology abstract. Royal Nepal Academy Of Science and Technology. Naya Baneshwar, Kathmandu, Nepal.

63. Khan, A.M. (1976). Control of disease caused by nematode by the application of oil-cake manures. Final technical report. Botany dept., Aligarh Muslim University, Aligarh, India p.94.

64. Khan, M.M. (1983). Plant parasitic nematodes associated with pineapple in Rampur, Chitwan. J. Inst. Agri. Anim. Sci. (Nepal). 4 (I and II): 85-86.

65. Khan, M.R., Khan, M.W., and Singh K (1997). Management of root knot disease of tomato by the application of fly ash in soil. Plant Pathology. Institute of Agricultural and Department of Botany, Aligarh Muslim University 46: 33-43.

66. Khan, T.A and Hussain, S.I. (1988). Studies on the efficacy of *Paecilomyces lilacinus* as biocontrol agent against a disease complex caused by the interaction of *Rotelynchus reniformis*, *Meloidogyne incognita* and *Rhizoctonia solani* on cowpea. Nematol. Medit., 16:229-231.

67. Khan, T.A and Hussain, S.I. (1990). Biological control of root knot and reniform nematodes and root rot fungus on cowpea, Bioved 1:19-24.

68. Khan, T.A. and Saxena, S.K. (1997). Integrated management of root-knot nematode *M. javanica* infecting tomato using organic materials and *Paecilomyces lilacinus.* Bioresource technology Botany dept., Aligarh Muslim University, Aligarh-202002. India. 61: 247-250.

69. Kiewnick, S. (2004). Biologocal control of plant parasitic nematodes with *Paecilomyces lilacinus*, strain 251. *In*: Sikora, R.A., Gowen, S., Hauschild, R. and Kiewnick, S. (eds). Multitrophic Interactions in soil and Integrated control. Proceedings of the International organization for control of noxious Animals and Plants, bad Honnef, Germany 2003. IOBC- WPRS Bulletin 27:133-136.

70. Kleyhans, K. P. N. (1991). The root-knot nematodes of South Africa. Republic of South Africa. Department of Agricultural Development. Technical Communication no. 231.

71. Kokalis-Buerelle, N., Chellemi, D.O. and Peries, X. (2005). Effect of soils from six management systems on root-knot nematodes and plant growth in greenhouse Assays. Journal of Nematology 37(4): 467-472.

72. Krishnappu, K., Ravichandra, N.G and Reddy, B.M.R. (1992). Nematode pests of national importance and their management. pp 57-66. In Pest management and

pesticides: Indian scenario (Ed) B.V. David, Namrutha, Pub Madras, India. 384.pp.

73. Kumar, D. and Singh, K.P. (2006). Assessment of predacity and efficacy of *Arthrobotrys dactyloides* for biological control of root-knot disease of tomato. Journal of Phytopathology (Berlin) 154 (1): 1-5.

74. Lamberti, F., Addabbo, T.D', sasanelli, N., Carella A. and Greco, P. (2000). Chemical control of root knot nematode. International society for Horticulture science. (Online).

75. Lopes, E.A., Ferraz, S., Ferreira, P.P. and Amora, D.X. (2005). Effect of incorporation of the dry above ground parts of Velvet bean and tomato to the soil on *Meloidogyne incognita* and *M. javanica*. Nematologia Brasileira 29(1): 101-104.

76. Loubser, J.T., Klerk cAde (1985). Chemical control of root knot nematode in established vineyards. South African journal for enology and viticulture; Viticulture and Oenological research institute stellenbosch, Republic of South Africa. H- plant pathologys 631-633.

77. Luc, M., Sikora, R.A. and Bridge, J. (2005). Plant Parasitic Nematodes in Subtropical and Tropical Agriculture. CABI Bioscience, Egham, UK.

78. Maluf, W.R., Azeviedo, S.M. and Campus, V.P. (1996). Heritability of root knot nematode resistance in sweet potatoes. Journal of Genetics and Breeding 50(2): 161-165.

79. Manandhar, H.K. and Amatya, P. (1988). Chickpea varietal screening against root knot nematodes. Paper presented at working group meeting for winter grain legumes Sept. 7-8, National wheat development programme, Bhairahawa, Nepal.

80. Mankau, R. and Prasad, N. (1972). Possibilities and Problems in the use of a sporozoan endoparasite for biological control of plant parasitic nematodes. Nematropica 2: 7-8.

81. Matsumoto, M.N., Goulart, A.M.C., Homechin, M., Sakamoto, M.M.Y. and Santiago, D.C. (2002). Mulching with *Pennisetum purpureum* and other cultural practices for the management of *Meloidogyne javanica* on tomato under greenhouse conditions. Nematologia Brasileira 26:101-104.

82. McSorley, R., Dickson, D.W. and de Brito, J.A. (1994a). Host status of selected tropical rotation crops to four populations of root-knot nematodes. Nematropica 24: 45-53.

83. McSorley, R., Dickson, D.W., de Brito, J.A. and Hochmuth, R.C., (1994b). Tropical rotation crops influence nematode densities and vegetable yield. Journal of Nematology 26: 308-314.

84. Meagher, J.W., French, F.L. and Paddick, R.G. (1966). The occurance and chemical control of root knot nematode (*Meliodogyne incognita*) on flue cured tobacco in Victoria. Australian Journal of Experimental Agriculture and Animal Husbandry 6(22): 339-343.

85. Mendoza, N.M., Dicklow, M.B. and Zuckerman, B.M. (1989). Evaluation of control of *Meloidogyne incognita* and *Nacobus aberrans* on tomato by two leguminous plants. Revue nématol 12(4): 409-412.

86. Mennan, S., Chen, S. and Melakeberhan, H. (2006). Suppression of *M. hapla* population by *Hirsutella minnesotensis*. Biocontrol Science and Technology 16(2): 181-193.

87. Moens, M. (2005). Interaction to the biology and management of nematodes interacting with Agro- Ecosystems. International Nematology course, University of Gent, Belgium. *Class notes* 58 pp.

88. Montasser, S.A. (1995). Reaction of certain flower bulb to root knot nematode, *Meloidogyne incognita*. Pakistan Journal of Nematology 32(2): 99-102.

89. Morgan-Jones, G., White, J.E. and Rodriguez-Kabana, R. (1984). Phytonematodes pathology. Ultrastructural studies .II. Parasitism of *Meloidogyne arenaria* eggs and larvae by *paecilomyces lilacinus*. Nematopica 14:57-71.

90. Naik, B.G., Nanjegowda, D., Ravi, K. and Reddy, P.P. (1998). *In:*Kumar, N.K.K. and Verghese, A. (eds) (1997). Advances in IPM for Horticultural Crops. Proceedings of the first national symposium on pest management in Horticultural crops: Environmental implications and Thrusts, Bangalore, India Pp.321-422.

91. Nebra, S. and Trivedi, P. C. (2004). Proceedings of the National academy of science. India section B. Biological Sciences 79(2): 143-152.

92. Netscher, C. (1981). Preliminary studies on the control of *Meloidogyne* species. Proceedings of the 3rd Research Planning Conference on Root knot nematodes *Meloidogyne* species. Regions IV and V, Ibadan, Nigeria, pp.247-253.

93. Nicco, A. I., Katael, M. J - Diaz, and Casatillu, P. (2004). Control of root knot nematodes by composted agroindustrial waste in potting mixture. Crop Protection 23(7): 581-587.

94. Noling, J.W. (2003). Nematodes and their management. Horticultural science Department. UF/IFAS, Florida. Extension service, ENY 4507178. Available at: edu. ifas. ufl. edu/ cv 112p.

95. Noling, J.W. and Becker, J.O. (1994). The challenge of research and extension to define and implement alternatives to methyl bromide. Journal of Nematology Supplement 26: 573-586.

96. Oostendrop, M., Sikora, R.A. (1989). Utilization of antagonistic rhizobacteria as a seed treatment for biological control of *Heterodera schachtii* in sugarbeet, Revue de Nematology 12:77-73.

97. Orion, D., Wergin, W.p., Chitwood, D.J. and Erbe, E.F. (1994). Low temperature scanning electron microscope observation of *Meloidogyne incognita* egg mass: The gelatinous matrix and embryo development. Journal of Nematology 26:402-411.

98. Pandey, R., Sikora, R.A., Kalra, A., Singh, H.B. and Pandey, S. (2003). Plants and their products act as major nematode inhibitory agents. *In*: Trivedi, P.C (ed) Nematode Management in Plants. Scientific Publishers, Jodhpur, India 103-131.

99. Padgham, J.L., Abawai, G.S. and Duxbury, J.M. (2003). Survival and infectivity of *Melidogyne graminicola* in flooded and non flooded soils. Nematologia Mediterranea 31:225-236.

100. Pérez, J. (1990). Determinatción de los períodos de laboreo necesarios para disminuir la infestación de *Meloidogyne incognita* (Kofoid et white) Chitwood en suelos arcillosos. Agrotecnia de Cuba 15:57-63.

101. Pérez, J.M. (2001). *TRichoderma,* Altrenative parael control Biológico de Nematodos en el Marco de una Agricultura Sostenible. Msc. Thesis. Universidad. Central 'Martha Abrees', Faculated de Ciencias Agropecuarias, Villa clara, Cuba.

102. Piedra Buena, A., Garcia-Alveraz, A., Diaz-Rojo, M.Á. and Bello, A. (2006) Use of crop residues for the control of *Meloidogyne incognita* under laboratory condition. Pest Management Science (Online) 62(10): 919-926.

103. Ploeg, A.T. (1999). Greenhouse studies on the effect of marigolds (*Tagetes* spp) on four *Meloidogyne* species. Journal of Nematology 31: 6-69.

104. Pokarel, N. (1998). Entomopathogenic nematodes. The parasites useful for biological control strategy. Tribhuvan University Journal. Vol. XXI, (1).

105. Pokharel R.R. (1993). Plant Parasitic nematodes associated with some fruit plants in Chitwan. Institute of Agriculture and Animal Science (IAAS). 14:121-122.

106. Rana, H.B., and Ali, Z. (1992). Incidence of root knot nematode on vegetables and legumes in Chitwan. Institute of Agriculture and Animal Science (IAAS) 3: 117-119.

107. Rana, H.B., Pokharel, R.R. and Dangol, D.R. (1993). Weed host of root knot nematode (*Meloidogyne* spp) at Chitwan. Institute of Agriculture and Animal Science (IAAS) 14: 117-119.

108. Rana, H.B., Sharma, M.D. and Neupane, K.R. (1995). Plant parasitic nematodes in different landraces of Nepalese chayotes. J. Inst. Agri. Anim. Sci. 16: 91-93.

109. Rao, M.S. and Reddy, R.P., (1994). A method for conveying *Paecilomyces lilacinus* to the soil for the management of root knot nematodes on eggplant. nematol. Medit. 22:265-267.

110. Rao, M.S., Kerry, B.R., Gowen, S.R., Bourne, J.M. and Reddy, P.P. (1997). Management of *Meloidogyne incognita* in tomato nurseries by integration of *Glomus desrrticola with Verticillium chalamydosporium.* Zeitschrift fiir Pflanzenkrankheiten and Pflanzenschulz 104: 419-422.

111. Reddy, P.P. (1985). Estimation of crop losses in peas due to *Meloidogyne incognita.* Indian Journal of Nematology 15:226.

112. Reddy, G.S., Rao, V.K., Sitaramaiah, K. and Chalan, R.V. (2001). Soil Solarization for control of the soil borne pathogen complex due to *Meloidogyne incognita* and *Pythuim aphanidermatum*. Indian Journal of Nematology 31:136-138.

113. Rodríguez- Kábana, R. (1986). Organic and inorganic nitrogen amendment to soil as nematode suppressants. Journal of Nematology 18:129-135

114. Rodríguez- Kábana, R., Morgan- Jones, G., and Chet, I. (1987). Biological control of nematodes. Soil amendments and microbial antagonists. Plant and Soil 100: 237-247.

115. Rodriguez-Kabana, R.G., Morgan-Jones, and Chet. I., (1987). Biological control of nematodes. Soil amendments and microbial antagonists. Plant and Soil 100:237-247.

116. Sasser, J.N. (1979). Pathogenicity, host ranges and variability in *Meloidogyne* spp. *In*: Lamberti, F. and Taylor, C.E. (Eds). Root knot nematodes (*Meloidogyne* spp.) : Systematics, Biology and Control. London, New york, San Francisco, Academic press 257-268.

117. Sasser, J.N. (1980). Root knot nematodes. A global means to crop production plant diseases 64: 36-41.

118. Sasser, J.N. and Freckman, D. (1987). A world perspective on nematology. The role of society. *In*: Veech, J. A and Dickson, D. W (Eds). Vistas on Nematology. Hyattsville, MD, U>S>A< Society of Nematologists 7-14.

119. Sharon, E., Bar-Eyal, M., Chet, I., Herrere-Estrella, A., Kleifeld, O. and Spiegel, Y. (2001). Biological control of the root knot nematode, *Meloidogyne javanica* by *Trichoderma harzianum.* Phytopathology 91:687-693.

120. Siddique, I.A. and Shaukat, S.S. (2002). Mixture of plant disease suppressive bacteria enhances biological control of multiple tomato pathogens. Biology and Fertility of Soils 36(4): 260-268.

121. Siddiqui, M.K. (1986). *Tylenchida* parasites of plants and insects. Commonwelth institute of parasitology.jkvk

122. Siddiqui, Z. and Mahmood, I. (1992). Response of chickpea cultivars to *Meloidogyne incognita* race 3 and their effect on peroxidase activity. Pak. J. Nematol 10(2): 113-117.

123. Sikora, R.A. (1978). Einfluss der endotrophen Mykorrhiza (*Glomus mosseae)* auf das Wirt- Parasit Verhältinis von *Meloidogyne incognita* in Tomaten. Zeitschrift fiir planzenkrankheiten and pflanzensxhuitz 85:197-202.

124. Sikora, R.A. (1988). Inter-relatonship between plant health promoting rhizobacteria, plant parasitic nematodes and soil micro-organisms. Communications in Agriculture and Applied Biological sciences 53: 867-878.

125. Sikora, R.A. (1989). Importance of plant parasitic and entomopathogenic nematodes to vegetable production in the Philippines. GTZ Report, Eschborn, Germany.

126. Sikora, R.A. (2002). Strategies for biological system management of nematodes in horticultural crops: fumigate, confuse or ignore them. Communications in Agricultural and Applied Biological Sciences 67: 5-18.

127. Sikora, R.A., Singh, R.S and Sitaramaiah, K. (1973). Control of root knot through organic and inorganic soil amendments. 3. Effect of rice husk and sugarcane bagasse. Haryana Journal of Horticultural Science 2:123-127.

128. Singh, R.S and Sitaramaiah, K. (1967). Effect of decomposing green leaves, sawdust and urea on the incidence of root knot to okra and tomato. Indian Phytopathology, 20:349-355.

129. Singh, R.S., Sitaramaiah, K. (1966). Incidence of root-knot nematodes on okra and tomato in oil cake amended soil. Plant Disease Reporter, 50:668-672.

130. Singh, S.P., Veena Pant Khan A.M., and Saxena S.K. (1986). Changes in the phenolic contents, related rhizosphere mycoflora and nematode population in tomato inoculated with *Meloidogyne incognita*, as a result of soil amendment with organic matter. Indian Journal of Nematology 115: 197-201.

131. Singh, S.P., Veena Pant Khan, A.M. and Saxena, S.K. (1986). Changes in the Phenolic contents, related rhizosphere mycoflora and nematode population in tomato inoculated with *Meloidogyne incognita*, as a result of soil amendment with organic matter. Indian Journal of Nematology 15:129-135.

132. Swamy, S.D.R., Reddy, P.P., Jegowda, D.N. and Swamy, B.C.N. (1995). Mangement of *Meloidogyne incognita* in tomato nursery by growing trap/antagonistic crops in rotation. Current Nematology 6: 9-12.

133. Taba, S., Moromizato, Z., Takesu, K., Ooshiro, A. and Nasu, K. (2006). Control of the southern root knot nematode, *M. incognita* using granule formulations containing nematode trapping fungus, *Monacrosporium ellipsporum* and a

nematicide. Japanese Journal of Applied Entomology and Zoology 50(2): 115-122.

134. Tahseen Q., Clark, I, M., Atkins, S.D., Hirscg, P.R. and Kerry, B.R. (2005). Impact of the nematophagus fungus *Pochonia chlamydosporia* on nematode and microbial population. Communication in Agricultural and Applied biological sciences 70(1): 81-86.

135. Thorn, G. (1961). Principal of Nematology, Mc Grow Hill book co. Inc. Newyork.

136. Thorn, G., (1949) and Schuster, M.C. (1956). Proc helmints. foe.wash. 23:128-134.

137. Timper, P. and Brodie, B.B. (1993). Infection of *Pratylenchus penetrans* by nematode pathogenic fungi. Journal of Nematology 25(2): 297-302.

138. Triantaphyllou, A. C. (1960). Sex determination in *Meloidogyne incognita* Chitwood, 1949 and intersexuality in *M. javanica* (Treub, 1885) Chitwood, 1949. *Annales de l'lnstitut Phytopathologique Benaki* 3: 12-31.

139. Triantaphyllou, A. C. (1985). Cytogenetics, cytotaxonomy and phylogeny of root-knot nematodes. *In:* An Advanced Treatise On *Meloidogyne*. Vol. I. *Biology and Control,* Eds J. N. Sasser, C. C. Carter, pp. 113-126. A co-operative publication of the Department of Plant Pathology, North Carolina State University and the United States Agency for International Development, Raleigh, USA.

140. Vab berkum, J.A., Hoestra, H. Practical Aspects of the chemical control of Nematicides in soil. *In*: Molde, D., editor. Soil Disinfestation. Amsterdam: Elsevier; 1979. Pp. 53-134.

141. Van Damme, V., Hoedekie, A. and Viaene, N. (2005). Long term efficacy of *Pochonia chlamydosporia* for management of *Meloidogyne javanica* in glasshouse crops. Nematology 7 (5): 727-736.

142. Verschoor, B.C. and De Goede, R.G.M. (2000). The nematode extraction efficiency of Oostenbrink elutriator cottonwool filter method with special reference to nematode body size and life strategy. Brill Academic Publishers, Journal of Nematology 2(3): 325-342.

143. Whitehead, A.G. (1998). Plant Nematode Control. CAB International, Wallingford, UK.

144. Yadav, C.N.R, Shrestha, G.K and Keshari, A.K. (1989). Plant parasitic nematodes in papaya field at Rampur. J. Agri. Anim. Sci. 10: 129-132.

145. Zulini, A. (1973). On some nematodes collected at high altitudes in Nepal, Khumbu Himal. J. Agri. Anim. science 4(3): 401-402.

146. Zavelta-mejia, E. and van Grundy, S.D. (1982). Effects of rhizobacteria on *Meloidogyne* infection. Journal of Nematology 15: 475-476.

APPENDICES

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 *in* Chitwood, Specht & Havis, 1952 (*M. arenaria thamesi* Chitwood *in* 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)

Annex 2.

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
Hewlett, T. E., and A. C. Tarjan, 1983 18
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

Hewlett, T. E., and A. C. Tarjan, 1983 19

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 tohigh,rounded or squared	15-23(19)	flattened laterally extended	distinct, close to rectum	usually conspicuous	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=38-39 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, roundec squared	or 15-25 (19)	slightly flattened	indistinct, close to rectum	conspicuous	2n=39-42
M. vardervegtei	16-20 (18)	cylindrical or expands then narrows	ovoid	indistinct	high, roundedor squared	11-29 (19)	slightly flattened	indistinct	absent	unknown

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; indicated 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. artiella	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 dorsally, 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	11-13	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* (µm)
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. arvensis	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	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. graminicola	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. 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. incognita	Flat to concave, labial disk raised above the medial lips	Not set off, usually marked by 2-3 incomplete 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. 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. ulmi	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. axtgua	Anteriorly flattened, elongate	Usually, smooth	Moderately sized cone and shaft	Set off, small, and rounded	9 10	2.5 4	44 46	12 14	334 358
M incognita	Anteriorly 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	346-463
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, post-eriorly 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, taper-ing 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, taper-ing onto shaft	14-16	2.5-4.5	18-26	2-7	334-370

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