1. INTRODUCTION

1.1 Background

Plant parasitic nematodes are unsegmented roundworms, usually microscopic in size, feeding on plant roots which seriously damage crops and reduce the plant productivity. As they live under the soil, it seems very difficult to control them even by using chemical nematicides. One of the most damaging groups of plant nematodes is the root-knot nematodes i.e *Meloidogyne* spp. It is an obligate root parasite of more than 200 plant species including vegetable, horticulture and woody plants (Hussey 1985). It causes conspicuous galls in the roots due to which plants suffer from vascular damages which disturb water and mineral uptake. They are capable of severely damaging a wide range of crops, causing dramatic yield loss. In Nepal, the root-knot nematode is considered as the major problem for many agriculture crops (Manandhar and Amatya 1988, Keshari 2004). Therefore, the control of root-knot nematodes is very important to enhance plant productivity.

In the more intensively grown crops, synthetic nematicide is one of the primary means of nematode control. However, their high cost, unavailability at the time of need and the hazard they pose as environment pollutant discourage most potential users. Many pesticides have now been withdrawn from the use, and thus needing to develop a new, safe, pollution free and effective option. There is an increasing interest in finding nematostatic and nematicidal compounds from the plants or plant products (Chitwood 2002).

Plants are the important sources of naturally occurring pesticides which may contain many compounds with nematicidial activity, known as botanical pesticides. Gommers (1981) and Chitwood (2002), in recent year, a variety of plants including neem (*Azadirachta indica*), garlic (*Ailium sativum*), marigolds (*Tagetes erectus*) etc, and their bi-products have been evaluated for their nematicidal properties and efficacy in the management of plant parasitic nematodes. Plant extracts have been found effective for the control of plant parasitic nematodes (Chatterjee and Sukul 1980). Leaf extracts of certain plants are known to have nematicidal or nematidostatic properties against several plant-parasitic nematodes as they contain active toxic chemicals such as azadirachtin, cameric acid, lantanilic acid, olenolic acid, etc. (Hussain et al. 1996, Khanna 1991, Siddiqui and

Alam 1989). These nematicidal plants could be toxic to the nematode, but neither to the plants nor to the associated beneficial microorganisms.

The control of root-knot nematode using plants and their products could be of greater significance since approaches are eco-friendly, cheaper and locally available in Nepal. There are many plants which might have nematicidal activities. However, these plants are not yet screened against plant nematodes. Therefore, the present study aims to find out the nematicidal property of locally available *Lantana camara* against the root-knot nematode (*Meloidogyne* spp.) in laboratory condition.

The population of plant-parasitic nematodes in the field can be minimized through several approaches such as using natural enemies (Khan and Kim 2007) and Khan et al. 2007) enhancing cultural practices (Okada and Harada 2007) cultivating resistant cultivars (Williamson and Kumar 2006) and applying pesticides (Browning et al. 2006). Since the 1950s, however, farmers have relied mainly on synthetic pesticides rather than on other approaches. This sometimes results in excessive and unsafe use of synthetic pesticides (Taniwiryono et al. 2007). Therefore, it has become an important issue to find alternative control strategies, which are as effective as synthetic pesticides, safer to farmers, consumers, and the environment and relatively easily available at low price (Fernandez et al. 2001). One of possible alternatives is the utilization of pesticides from plant origin, known as botanical pesticides (Javed et al. 2006). These pesticides are generally considered to be non-persistent under field conditions as they are readily transformed by light, oxygen and microorganisms into less toxic products. Therefore no residues are expected on the products or in the environment (Ujvary 2001).

Meloidogyne javanica and *Meloidogyne ethiopica* have been reported to occur in Ethiopia (Stewart and Dagnatchew 1967, Tsedeke 1986, Ahmad et al. 2010, Ziaulhaq et al. 2011). Among them *M. incognita* is the most widespread species (Wondirad and Kifle 2000). Tadele and Mengistu (2000) and Solomon (1987) reported the occurrence of *M. incognita* on tomato in the eastern part of the country, particularly in eastern Hararghe where many vegetable crops were attacked by root-knot nematode. Apart from the Eastern parts of the country, root-knot nematode (particularly *M. incognita*) is the major problem in tomato cultivation in the central and western parts too (Wondirad and Tesfamariam 2002). Root-knot nematode has become a very important constraint in the production of vegetables,

particularly tomato. Nowadays, it has become a bottleneck in tomato production in the glass house cultivation in Hawassaarea.

Botanicals (plant-based pesticidal chemicals) have found favour as alternatives to pesticides in recent times. Some of these botanicals are already being exploited commercially in insect pest management (Agnihotri et al. 1999). Plants are nature's chemical factories which provide the richest sources of organic chemicals on earth (Grainge and Ahmed 1988). Exploration of nematicidal potential of botanicals and their application is on increase. Different plant species are being tested to identify the sources of nematicidal substances and many of them have shown promising results in the control of plant parasitic nematodes (Abdi 1996). In spite of the wide distribution of root-knot nematode on many crops in Nepal, little work has been done on the control of root-knot nematode in the country. So far, no efforts have been made to exploit locally available botanicals for the control of root-knot nematode on crops in Nepal. Therefore, this encouraged me to undertake the present investigation on evaluation of locally available plant species for their effectiveness against root-knot nematodes.

1.2 Introduction of *Meloidogyne* spp. (for detail appendices 4 to 6)

Meloidogyne spp. belongs to order Rhabditida and commonly known as root-knot nematode as they live inside the roots and form galls. *Meloidogyne* was first observed by Berkeley in 1855 and described it as a new genus in 1877. Root-knot nematode, *Meloidogyne* spp. Kofoid and White (Chitwood) (Tylenchida: Heteroderidae) is a major plant-parasitic nematode species affecting the quantity and quality of the crop production in many annual and perennial crops. Infected plants show typical symptoms including root galling, stunting and nutrient deficiency, particularly nitrogen deficiency (Siddiqui et al. 2001). On Bangka Island, South of Sumatra, Indonesia, this nematode is considered to be one of the major problems in black pepper cultivation. In 2003, 4.900 ha of the total of 52.468 ha of pepper plantations were severely infected by this pest. Although, there is no information about the exact impact of nematode infection on the loss of pepper production, it is clear from visible inspection that severely attacked plants have a reduced vitality, which produce less fruits, and finally will die. Davis and May (2004) informed that the yield losses of cotton production caused by *M. incognita* in 2002 were estimated to be between 18.0-47.3%. Therefore, the presence of this pest in plantations has to be controlled.

1.3 Introduction of *Lantana camara* (for detail appendix 7)

The genus *Lantana* (Verbenaceae) contained seven species, six from South America and one from Ethiopia (Munir 1996). *Lantana* (from the Latin lento, to bend) probably derives from the ancient Latin name of the genus Viburnum, which it resembles a little in foliage and inflorescence. *Lantana* is mostly native to subtropical and tropical America, but a few taxa are indigenous to tropical Asia and Africa. It now occurs in approximately 50 countries, where several species are cultivated under hundreds of cultivar names. The recorded number of *Lantana* species varies from 50 to 270 specific and subspecific entities, but it appears that a better estimate is 150 species (Atkin 2004). The genus is a difficult one to classify taxonomically since species are not stable and hybridisation is widespread, the shape of inflorescence changes with age, and flower colours vary with age and maturity (Munir 1996).

1.4 Objectives

The main objective is to evaluate the leaf extract of *Lantana camara* for its nematicidal property against 2^{nd} stage juveniles (J_2) of root- knot nematode (*Meloidogyne* spp.*)* in *in-vitro* condition.

The specific objective was to examine:

- to observe the percentage of mortality of second stage juveniles (J_2) of root- knot nematode (*Meloidogyne* spp.) against different concentrations of aqueous leaf extracts of *L. camara*.
- to compare the mortality of juveniles in different concentrations at 12hrs, 24hrs and 48hrs of incubation period.

1.5 Rational of the study:

Meloidogyne spp. causes serious problem limiting the plant productivity of many economically important crops of Nepal (Manandhar and Amatya 1988). Some works have been done in Nepal regarding the survey and identification of nematodes.

The area still untouched for the nematode research is the proper control of root-knot nematodes in Nepal. Nematodes are no-doubt, spreading day by day and damaging severely the crops. Almost nothing is done beside some chemical control. As we all are aware that the synthetic nematicides cause environment pollution and health hazard, it is very important to explore any other control measures that are effective and safe to the human health.

Nepal is rich in biodiversity and natural plant resources. Many native plants possess nematicidal potential. However, we are not sure about the plants that have nematicidal activities. The farmers are unaware of potential of natural plants and their products that could be used to kill plant nematodes. Therefore, this project can be an initial step to explore the nematicidal plants as an alternative to chemical pesticides.

A few works have been done in Nepal if compared to other nations. The present work is a small step in nematicidal screening. The result will hopefully help the Nepalese farmers to use the nematicidal plants rather than chemical pesticides.

2. LITERATURE REVIEW

In Nepal, despite of the fact that the *Meloidogyne* spp. causes serious problem limiting the plant productivity of many economically important crops of Nepal (Manandhar and Amatya 1988, Keshari 2004) very few work have been done in the management aspect which was made by Dr. H.K. Manandhar of Plant Pathology Department, NARC. Plant parasitic nematodes are the major pests in many countries particularly in the tropic and subtropics, where they are recognized as the cause of dramatic yield losses on the wide range of crops (Luc et al. 2005). Among the plant parasitic nematodes, the most destructive species is the *Meloidogyne incognita* (Chitwood 2002) which cause serious problems to a number of economically important agriculture and hoticultural plants (Tsay et al. 2004). Presently, management of the nematodes has been done by using plant resistance, crop rotation, cultural practices or chemical nematicides (Chitwood 2002). Chemical control is expensive and could create a potential hazard to the environment and human health. Because of these inconvenience, there is an increasing interest in discovering the nematicidal compounds from the plants or plant products (Chitwood 2002).

A number of works have been done about the nematicidal activity of several plants and their products against *Meloidogyne incognita.* Linford et al. (1938) were the first to study nematicidal effect of chopped Pineapple (*Annanus comosus*) leaves used as organic amendment against *Meloidogyne* spp. The plant species antagonistic to *Meloidogyne* spp. are the leaves and flowers of Marigold (*Tagetes* spp. leaves, root and seeds of neem (*Azadirachta indica*) and leaves and seeds of chinaberry (*Melia azedarach*) (Rather et al. 2007). Similarly, essential oils and plant extracts of sweet wormwood (*Artemisia obsinthium*), thymes (*Thymus vulgaris),* peppermint (*Mentha spicata*), fennel (F*oeniculum vulgare*), garlic (*Allium sativum*) and *Eucalyptus* spp. *are* toxic to the nematodes and reduced hatching activity (Ibrahim et al. 2006). Among these plants, marigold (*Tagetes* spp.) is the most commonly studied. As marigold belongs to the Asteraceae, it is possible that other members of the family may also possess antagonistic properties against plant parasitic nematodes (Tsay et al. 2004). Azadirachtin a commercial name of neem in Asteraceae family is one of the most common nematicidal plants that were recognized in the middle of the 20th century. Neem is available in simple homemade powder and the appropriate aqueous extracts made from them (Javed et al.

2006). The products of neem are azadirachtin which is common in more species of Asteracae family (Chitwood 1992).

Significant progress has been made in this area after the year 2000. Adegbite (2005) reported the effect of plant extracts and essential oils on root-knot nematode (*Meloidogyne* spp.) Likewise, (Ayodele 2011) evaluated plant extracts and *Pseudomonas* spp. for the control of *Meloidogyne incognita* on tomato. In 2011, various indigenous plants were screened for nematicidal activities by various scientists such as by (Ayodele 2011 and Katooli et al. 2011).

According to (Tayer et al. 2012) *Lantana* plant species are the best known for having nematicidal properties. This work would make some contribution in this aspect.

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

Barbarola (1982) achieved good control of root-knot nematode with cow dung and poultry manure. Amending soil with animal manure and agricultural byproduct has reduced *Meloidogyne* spp. number on variety of crop (Rodriguez-kabana et al. 1986). The organic amendment tends to alter the host parasite relationship in favour of the crop and also increase 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 buildup of nematode and root galling. Groundnut cake with *P. lilacinus* was most effective (Khan and Saxena 1997).

The nematode population in the soil and severity of population attack decreases in the plants amended with biogas slurry. It helps the plant for more vegetative grow and tended to flower and fruit much earlier (Jothi et al. 2003).

Abubakar et al. (2004) conducted a 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.

The use of various parts of indigenous plants as botanical extracts has become important in pest management in recent years following the environmental hazards caused by chemical control measures (Mangala and Mauria 2006).

Moringa oleifera is widely used in water treatment and in this study was found to be a good inhibitor of nematode egg hatch and juvenile survival. It was also effective in reducing nematode population in plants with a subsequent increase in plant growth and yield. Guzman (1984) found water extracts of *Moringa* to be as toxic to *Meloidogyne incognita* as standard pesticides.

Seed extracts of neem have also been found to be excellent suppressor of *Meloidogyne incognita* (Salawu 1992a) and *Heterodera sacchari* (Salawu 1992b). Salako et al. (2008) found that many farmers in Nigeria use neem for various crop protection practices with a lot of success. Seed dressing of cowpea with neem reduced seedling infestation of disease by as much as common seed dressing pesticides (Salako et al. 2008). Fatoki (2001), Egunjobi and Onayemi (1981) also found that neem leaf extracts compared with carbofuran in reducing nematode population in infected cowpea plant with an accompanied yield increase.

The nematicidal effect of the tested extracts may possibly be attributed to their high contents of certain oxygenated compounds which are characterized by their lipophilic properties that enable them to dissolve the cytoplasmic membrane of nematode cells and their functional groups interfering with the enzyme protein structure (Knobloch et al. 1989). The mechanisms of plant extracts action may include denaturing and degrading of proteins, inhibition of enzymes and interfering with the electron flow in respiratory chain or with ADP phosphorylation (Konstantopoulou et al. 1994).

Qamar et al. (2005) isolated lantanilic acid, camaric acid and oleanolic acid from the methanolic extract of the aerial parts of *Lantana camara* through bio-assay guided fractionation. These compounds exhibited significant mortality against root-knot nematode *Meloidogyne incognita* at 0.5% concentration.

Shervin et al. (2011) reported that neem seed powder at the rate 50g/kg soil decreased root knot index from 4.7 (control) to 0.25 and disease severity from 85% (control) to 12% on tomato inside the glass house. There was also an increase in growth factors such as plant weight and length on treated plants.

Susan et al. (2005) studied the leaf extracts of *Ocimum basilicum, Tagetes erecta, Chrysanthemum cinerariafolium, Melia azadirach* and seed extract of *Azadirachta indica* against the root knot nematode *Meloidogyne incognita* on eggplant. All the extracts were effective against nematode juveniles.

Lantana and Mexican marigold leaves extracts at 5% concentration resulted in a pronounced reduction in nematode numbers followed by *Lantana* 3% concentration and pyrethrum flower extracts with 5% concentration. The reproduction of *M. incognita* was significantly suppressed by all the treatments as compared to untreated plants. Similarly, all the plant extract treatments were highly effective in their ability in reducing root-knot index when compared with untreated plants. The plant extracts might be directly toxic to eggs or juveniles and thus reduced the root-knot nematode population density as well as galling. The decrease in number of the nematode accompanied by increase in growth of tomato suggests nematicidal potential of the tested plant extract. Similar results have been obtained by other workers too (Babatola 1990, Akhtar and Alam 1993, Alam et al. 1994).

Importance of organic horticultural production, which avoids synthetic pesticides applications, increased the research on botanical pesticides with potential use for nematode management. In the present field studies application of plant extract was found to reduce root-knot indices on tomato root system, final nematode population density in the soil significantly over untreated control. With the increase in level of plant extract concentration, a corresponding significant reduction was observed in the number of galls and nematode population over untreated control. Root-knot infestation stunted all untreated plants and reduced leaf production as well as tomato yield (Rao et al. 1998, Sasser and Carter 1982).

The effects of the tested plant extracts against infestation of root-knot nematode and yield of tomato plant were different, in some cases. The differences in the toxicity of different plant extracts could be due to the differences in the chemical compositions and concentrations of toxic components. The extract might also have affected plant growth differently due to difference in chemical composition. Such results have also been reported by (Firoza and Maqbool 1996) when they used different plant seeds and leaves extract against *Helicotylenchus dihystera* in tomato.

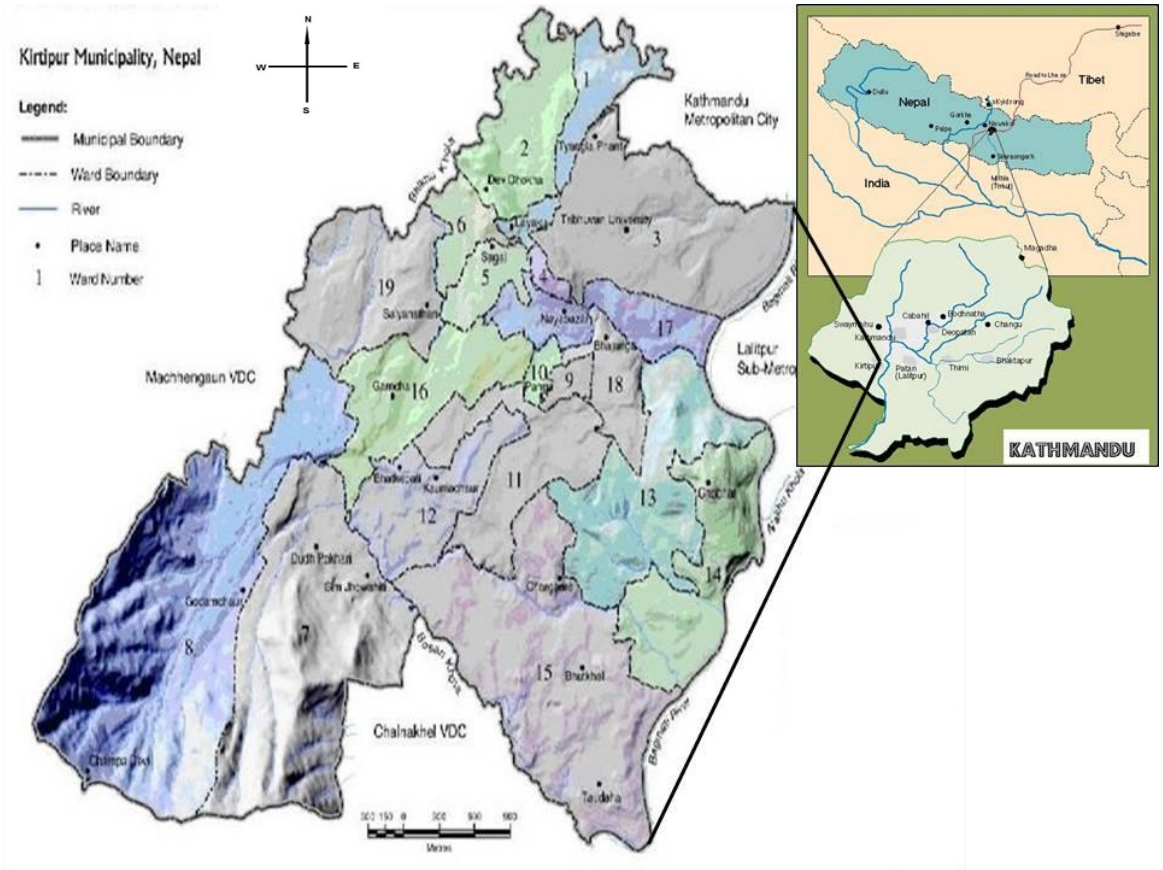
Akhtar (1999) found that decomposed and un-decomposed extracts of neem cake and leaves as bare root dip treatment caused significant reduction in root knot development of pre-infected tomato seedlings as well as those inoculated with juvenile of *Meloidogyne incognita* after dip treatment. Banu and Vadivelu (1995) observed that seeding dip with extracts of Castor, suppressed the root-knot nematode *Meloidogyne incognita* but neem seed kernel extracts and neem cake extracts were found to be least in reducing nematode population. The chemicals viz., hostathion which possesses rugby, carbifuran, phorate and neem leaves and oil cake were used to conduct bare-root dip experiment, were found highly toxic to root-knot nematode *Meloidogyne incognita* in tomato and eggplant (Siddhiqui 1998).

Jiskani et al. (2005) observe that the efficacy of different doses of neem seed decoction and neem leaf extracts on the reproductive activity of *M. incognita* infesting tomato showed that plant growth parameter significantly increased and root gall decreased.

3. MATERIALS AND METHODS

3.1 STUDY AREA

The propose study area for the research is Kirtipur Municipality of the Kathmandu district. Kirtipur is one of the recently urbanized city of Kathmandu valley located to South-west of the central Kathmandu. It is declared as municipality in 2053 B.S and is divided into 19 wards. It extends from 27º 41' 36" – 27º 38' 37" N to 85º 18' 00" – 85º 14' 64" E. It has 1300 to 1402 meter of altitudinal range from sea level. It is surrounded by the Bagmati River in the east, Tinthana and Machhegaon VDC in the west, ward no.14 of the Kathmandu Metropolitan in the north and Chalnakhel VDC and Shesnarayan VDC in the south. The shape of the municipality resembles almost a square, the area being 14.76 sq km and the study area covers an overall area of the municipality (fig 1).



(Source: ICIMOD)

Figure 1. Map showing the location of Kirtipur.

3.2 Plant material

Lantana camara used for the experiment was collected from natural habitats of Kirtipur. This plant belongs to the family Verbenaceae. Leaves were used for the experiment. The study was conducted in laboratory of Central Department of Biotechnology and Central Department of Zoology. The study was conducted from December 2012 to September 2013.

3.3 Preparation and storage of aqueous leaf extract

A fresh mature healthy leaves of *Lantana camara* were plucked from their branch, washed and spread inside the chamber of hot air oven at temperature of 28^{o}C overnight. The dried leaves were ground to fine powder using classic Maraja Electric Glinder. 250 gm. fine powder of *L. camara* was dissolved in 1000 ml (1gm/4 ml basis). Aqueous suspensions were kept in conical flask, allowed to soak on Stvart Orbital Shaker at room temperature for 24 hrs for the extraction of active ingredients. The suspension was then passed through two folds of muslin cloth followed by filtration through Whatman no.1 filter paper. The filtrate obtained so far was centrifuged at 2400 rpm for 10 min and clear supernatant was stored at 4^{o}C in a plastic container which was considered as standard solution 'S'. Other different concentrations i.e. 10% and 50%, respectively were prepared by adding required amount of sterilized distilled water for laboratory experiments.

3.4 Source of root-knot nematode (*Meloidogyne* spp.*)*

Root-knot nematode, *Meloidogyne* spp. was collected from the roots of heavily infected tomato plants of Kirtipur. After the identification, cultures of *Meloidogyne* spp. were maintained on tomato roots in the greenhouse at the Central Department of Botany, Kirtipur.

Egg masses of *Meloidogyne* spp. were hand-picked up using sterilized forceps from heavily infected tomato roots. These egg masses were washed with distilled water and incubated at 28± 2^{0}C for 24 hrs for hatching. The hatched juveniles was collected after placing the juvenile suspension through a course sieves (8 cm in diameter) containing tissue paper and kept in the petridish with water just deep enough to contact the tissue paper to collect second stage juveniles, so called (J_2).

3.5 Laboratory (*in vitro*) experiment

The aqueous leaf extract prepared as above was evaluated for nematicidal activity against second-stage juvenile (J_2) of *Meloidogyne* spp. under laboratory condition in order to assess the larval mortality. For this experiment, 100 freshly hatched J_2 larvae were transferred to plastic petridish of 2.5cm in diameter containing 10 ml of different concentrations i.e. 10%, 50%, and 100%, respectively of leaf extract. The petridish with 100 juveniles and 10 ml of distilled water (without plant extract) was considered as a control. All the petridishes were maintained at 25± 2^0C in an incubator.

After 12 hrs, 24 hrs and 48 hrs of incubation, mobile and immobile J_2 larvae were counted under stereoscopic microscope in order to record larval mortality. Immobilized larvae were confirmed by using needle as dead larvae failed to respond to stimulation with a needle (Cayrol et al. 1989). Each treatment was replicated three times. Both mobile and immobile nematodes of each treatment were counted.

3.6 Statistical analysis

On the basis of laboratory experiment, the data were recorded as larval mortality (dead or alive). All the data were analyzed according to analysis of variance (ANOVA) using SPSS 17.0 program.

4. RESULTS

The nematicidal effect of aqueous extract of leaf of *Lantana camara* on mortality of second stage juveniles (J_2) of *Meloidogyne* spp. in laboratory condition is given below.

4.1 Effect of different concentrations of aqueous extracts of *Lantana camara* on mortality of juveniles of *Meloidogyne* species after 12 hours treatment

Figure 1 shows that the percentage of mortality of juveniles of root-knot nematode (100 juveniles used for each experiment) was found to be different with different concentration (S = 100%, S/2 = 50%, and S/10 = 10%) and duration of treatment (12hrs, 24hrs, and 48hrs). Among all the treatment, 100% of leaf extract proved highly toxic to juveniles followed by 50% and 10% concentrations.

In the present data, mortality of J_2 of *Meloidogyne* spp. in *Lantana camara* was 82.6%, 36.6% and 3.3% in 100%, 50%, 10% concentrations of leaf extract, respectively as compare to distilled water control after 12hrs (fig 2).

In other way, out of 100 larvae used in each experiment 17.4%, 63.4% and 96.7% larvae remained active in 100%, 50% and 10% concentration of leaf extract of *Lantana camara* as compared to the distilled water control.

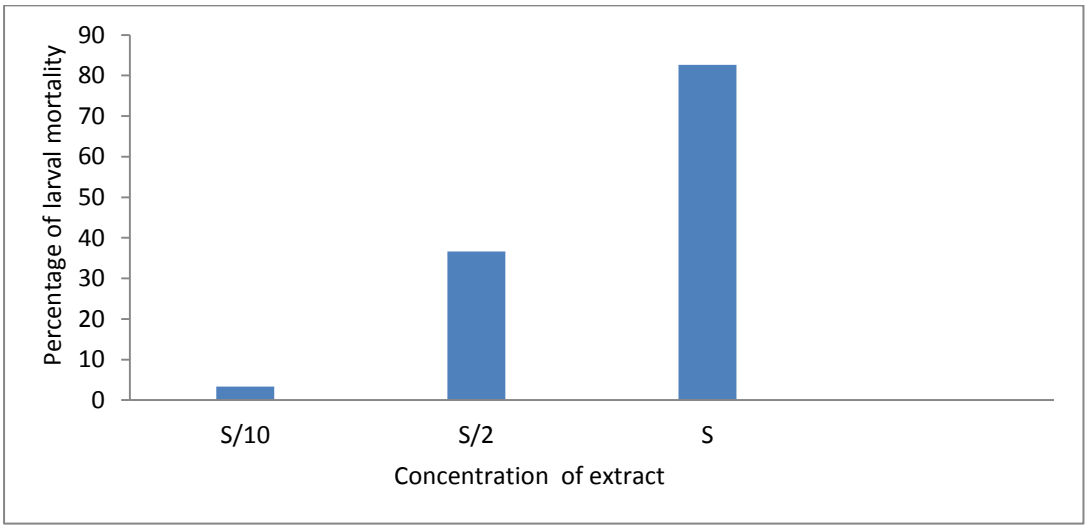


Figure 2. Percentage mortality of juveniles of *Meloidogyne* spp. in different concentration of aqueous extract of *Lantana camara* leaf *in vitro* at 12hrs (S=100%, S/2=50%, S/10=10%).

Each value is the mean of three replicates.

Number of second stage juvenile used =100 (for each treatment).

4.2 Effect of different concentrations of aqueous extracts of *Lantana camara* on mortality of juveniles of *Meloidogyne* species after 24 hours treatment

Figure 2 shows mortality of J_2 of *Meloidogyne* spp. in *Lantana camara* was 89.3%, 50.0% and 16.0% in 100%, 50%, 10% concentrations, respectively as compared to distilled water control after 24hrs(fig 3).

In terms of mobilization of larvae, out of 100 larvae used in each experiment 10.7%, 50.0 % and 84.0% larvae were found to be active in 100%, 50% and 10% concentration of leaf extract of *Lantana camara* as compared to the distilled water control.

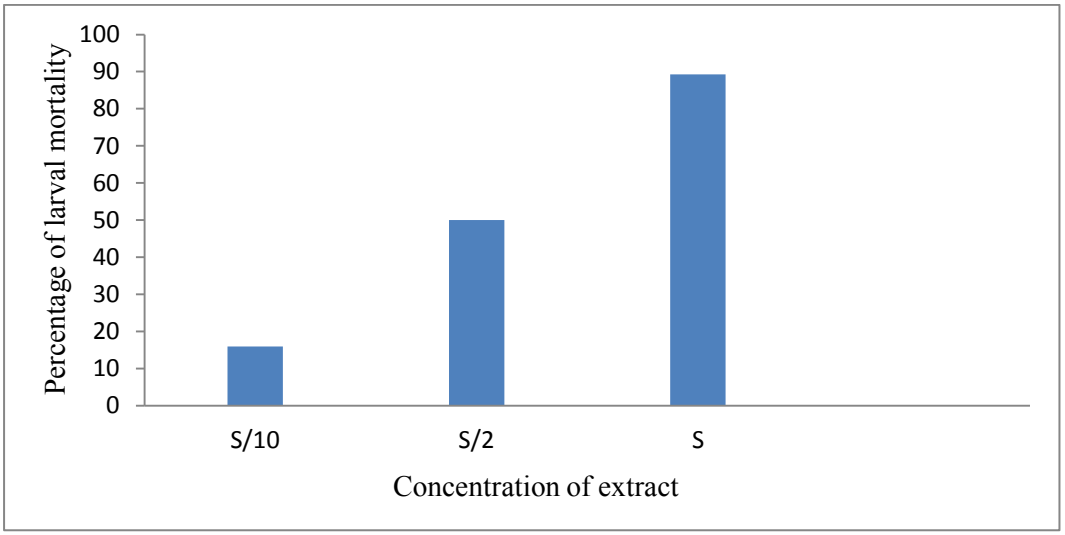


Figure 3. Percentage mortality of juveniles of *Meloidogyne* spp. in different concentration of aqueous extract of *Lantana camara* leaf *in vitro* at 24 hrs (S=100%, S/2=50%, S/10=10%).

Each value is the mean of three replicates.

Number of second stage juvenile used =100 (for each treatment).

4.3 Effect of different concentrations of aqueous extracts of *Lantana camara* on mortality of juveniles of *Meloidogyne* species after 48 hours treatment

Figure 3 shows that the percentage of mortality of juveniles of root-knot nematode differed with different concentration and duration of treatment (12hrs, 24hrs, and 48hrs). Among all the treatment, 100% of leaf extract proved highly toxic to juveniles followed by 50% and 10% concentrations.

In the present data, mortality of J_2 of *Meloidogyne* spp. in *Lantana camara* was 98.6%, 57.6and 28.6% in 100%, 50%, 10% concentration, respectively as compared to distilled water control (fig 4).

In terms of mobilization of J_2 larvae, out of 100 larvae used in each experiment 1.4%, 42.4% and 71.4% larvae were found to remain active in 100%, 50% and 10% concentration of leaf extract of *Lantana camara* as compared to distilled water control.

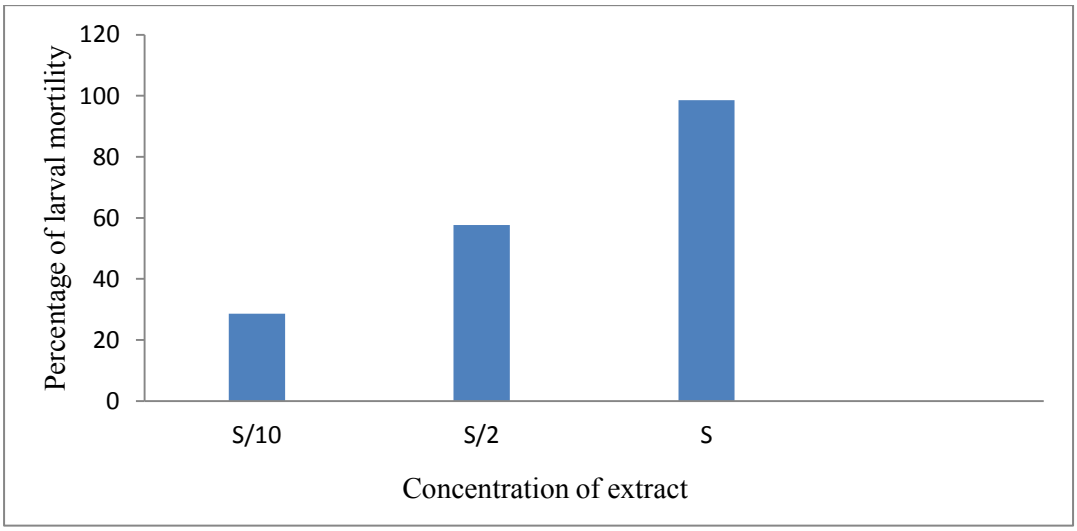


Figure 4. Percentage mortality of juveniles of *Meloidogyne* spp. in different concentration of aqueous extract of *Lantana camara* leaf *in vitro* at 48 hrs (S=100%, S/2=50%, S/10=10%).

Each value is the mean of three replicates.

Number of second stage juvenile used =100 (for each treatment).

4.4 Comparative view of different concentration of aqueous extract of *Lantana camara* on mortality of juveniles of *Meloidogyne* species after 12hrs, 24hrs and 48hrs, respectively.

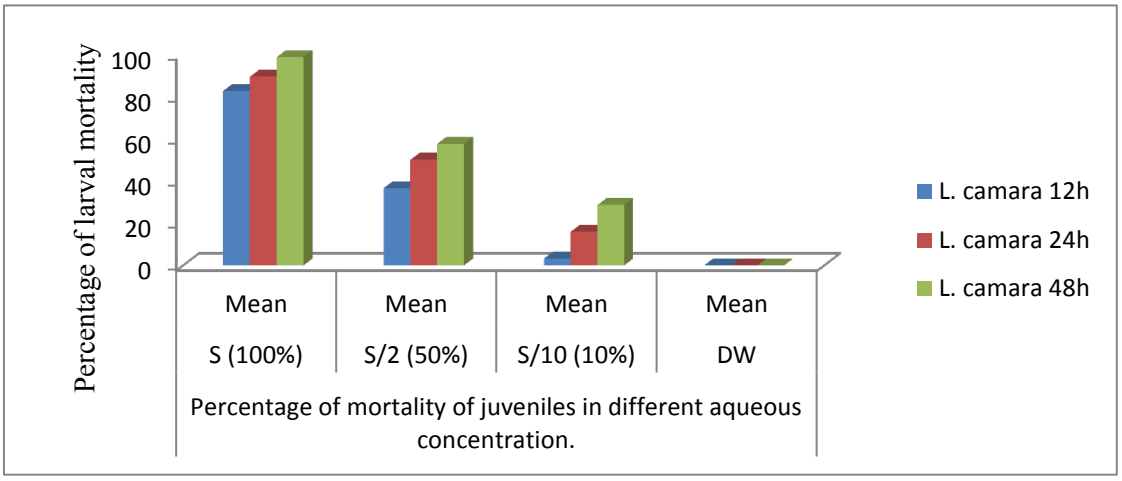


Figure 5. Comparative percentage mortality of juvenile of *Meloidogyne* spp. in different concentrations of aqueous plant extract of *Lantana camara* at 12hrs, 24hrs and 48hrs, respectively.

While comparing all the above data together second stage juvenile (J_2) of *Meloidogyne* species exhibited the nematostatic effect *in vitro* for *Lantana camara* leaf extract (fig 5). The standard aqueous solution of leaf which gave up to 98.6% immobilization activity when exposed for 48hrs while juvenile J_2 were immobilized upto 82.6% when exposed for 12hrs in the same aqueous extract. The immobilization rate of juvenile J_2 was too low in 10% of aqueous extract. The mortality of juvenile J_2 was more significant with increasing extract strength of leaf from 10% to 100%. Therefore, 100% of the leaf extract showed the significant result in controlling the juveniles of *Meloidogyne* spp.

Table 1. Mean number of juvenile mortality in different aqueous concentration at 12hrs, 24hrs and 48hrs.

Plant	Incubation Period (hrs)	Concentration			LSD(0.05)
		S (100%)	S/2 (50%)	S/10 (10%)	
Lantana camara	12hrs	8.27[a]	3.67[b]	0.33[c]	0.39
	24hrs	8.93[a]	5.00[b]	1.60[c]	0.53
	48hrs	9.87[a]	5.77[b]	2.86[c]	0.45

Degree of freedom (df) = 58
Tabulated value = 2.0017

Result in table 1 also showed that effect of various concentrations of leaf extracts of *Lantana camara* on larval mortality over exposure time i.e 12hrs, 24hrs and 48hrs. In general, larval mortality increased with the increase in exposure period but decrease with dilution. Comparison of treatment mean regarding period of leaf extract of *Lantana camara* indicated that test plant gave the maximum mortality at 100% concentration at 48 hrs of exposure showing each treatment significantly different than that of other. Mortality in distilled water (control) was negligible.

5. DISCUSSION

In the present study, various concentrations of aqueous leaf extract of *Lantana camara* were assessed for nematicidal activity in the laboratory condition. All the treatments exhibited natural nematicidal potential to varying degree. The result showed significant juvenile mortality potential of plant extract against *Meloidogyne* juveniles. The nematicidal effect of leaf extract on juvenile mortality of *Meloidogyne* spp. was concentration dependent i.e the juvenile mortality increased with the increased extract concentration. Similar finding that the efficacy of the plant extract depends on the concentration and duration of exposure of juveniles to the extract was given by (Mahmood et al. 1979, Laki and Gupta 1980).

Among all the treatments with 100%, 50% and 10% concentrations of leaf extract of *Lantana camara* incubated for 12 hrs, 24 hrs and 48 hrs, the treatment with 100% concentration incubated at 48hrs was found significantly effective in controlling *Meloidogyne* juveniles. This result indicated that the standard concentration 'S' of leaf extract was found to be highly nematostatic in which 98.66 % of juveniles were dead in 48 hrs. This result agrees with the result obtained by (Ahmad et al. 2010) in which the standard concentration 'S' of leaf extract of *L. camara* was found highly nematostatic, in which juveniles were completely paralyzed after 12 hrs and after 48 hrs. Similar result was obtained by (Akhtar and Mahmood 1994) who reported that water extracts from leaves and root of Mexican marigold and leaves of *Lantana* reduced the hatching of *M. incognita* eggs significantly. Similarly, (Raoet et al. 1998) found that with the increase in level of plant extract concentration of *Lantana* and Marigold, a corresponding significant reduction was observed in the number of galls and nematode population over untreated control proving nematicidal property.

The nematicidal activity of *Lantana camara* against juveniles of *Meloidogyne* spp. have also been reported by many reseearchers (Begum et al. 2008, Qamar et al. 2005, Shaukat and Siddiqui 2001). Similarly, the copped leaves of *L. camara* reduced root galling caused by *M. incognita* on papaya (Reddy et al. 1993). The mortality of juveniles might be due to active ingredients present in the leaf extract, which has been shown to act as potent nematicide. The active ingredients present in the leaf extracts of *Lantana camara* such as lantanoside, lantanone, camaric acid and olenolic acids (Begum et al. 2000 and Qamar et al. 2005) which may have larvicidal or ovicidal properties. These chemicals

either denatured or degraded the proteins and inhibited the enzymes responsible for electron transport system in respiratory chain and for ADP phosphorylation (Susan et al. 2005).

Root-knot nematode is one of the major pests of many crops and causes significant reduction in yield and quality. The present study was conducted to explore the potentials of some locally available plant species for the control of root-knot nematode, *Meloidogyne* spp. According to present study, *Lantana* appeared to be a promising botanical and can be used as an attractive alternative to synthetic pesticides. Although, the findings of the present investigation are not conclusive, therefore, further studies should be conducted in greenhouse and field conditions to assess the nematicidal activity. As compare to the other countries, a few works on nematicidal activity have been done in Nepal despite the fact that *Meloidogyne* spp. cause serious problem limiting the plant productivity of many crops. This work will hopefully fill the gap in the research.

6. CONCLUSION AND RECOMMENDATIONS

It was found that 50% concentration of *Lantana camara* at 48 hrs treatment and above the concentrations of leaf extract was deleterious to root-knot nematode. This finding could be important from the point of view of controlling the root-knot nematode without the use of chemical pesticides in view of environmental pollution likely to cause. The control of *Meloidogyne* spp. by the leaf extracts used in this study might be probably based on a complex mode of action involving multiple mechanisms. Therefore, future studies are needed to characterize the active compounds in the test plant parts that are nematicidal and possessing complex modes of action. The effectiveness of non-chemical pest management techniques such as use of plant resistance, crop rotations, biological control and other integrated pest management techniques can be improved if they are carefully planned after considering the parasitizing species (Hussey 1990).

In Nepal, very little research has been conduct to control the root-knot nematode in tomato as well as other important vegetables crops. Thus appropriate research work is needed for the proper control of root-knot nematode problem. Awareness programs should be conducted among the farmers regarding the knowledge of root-knot nematode and the serious damage and loss caused by them to vegetables and different other crops.

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APPENDICES

1. Number of nematode immobilize and mobilize during the test by 10% plant extract in three different replication.

12Immobilized	12Mobilized	24Immobilized	24Mobilized	48Immobilized	48Mobilized
1	9	2	8	3	7
0	10	1	9	2	8
0	10	2	8	3	7
0	10	1	9	2	8
0	10	1	9	2	8
0	10	1	9	1	9
1	9	2	8	2	8
0	10	1	9	2	8
0	10	0	10	1	9
0	10	1	9	1	9
0	10	3	7	5	5
1	9	2	8	4	6
1	9	1	9	2	8
0	10	3	7	5	5
1	9	1	9	3	7
1	9	2	8	4	6
0	10	1	9	3	7
1	9	2	8	3	7
0	10	1	9	3	7
0	10	0	10	3	7
1	9	3	7	4	6
0	10	2	8	3	7
0	10	1	9	3	7
0	10	1	9	3	7
1	9	3	7	4	6
0	10	1	9	2	8
0	10	1	9	2	8
0	10	2	8	3	7
1	9	4	6	5	5
0	10	2	8	3	7

2. Number of nematode immobilize and mobilize during the test by 50% plant extract in three different replication.

12Immobilized	12Mobilized	24Immobilized	24Mobilized	48Immobilized	48Mobilized
3	7	4	6	4	6
4	6	5	5	6	4
3	7	4	6	5	5
3	7	3	7	4	6
4	6	4	6	4	6
3	7	4	6	5	5
3	7	5	5	6	4
2	8	3	7	4	6
2	8	2	8	5	5
3	7	4	6	5	5
4	6	6	4	6	4
5	5	5	5	7	3
5	5	5	5	6	4
4	6	4	6	5	5
5	5	6	4	7	3
5	5	6	4	7	3
5	5	5	5	5	5
5	5	5	5	6	4
4	6	6	4	6	4
3	7	4	6	5	5
3	7	5	5	7	3
4	6	6	4	6	4
4	6	5	5	6	4
3	7	6	4	7	3
3	7	6	4	6	4
4	6	5	5	6	4
4	6	6	4	6	4
3	7	7	3	7	3
3	7	7	3	7	3
4	6	7	3	7	3

3. Number of nematode immobilize and mobilize during the test by 100% plant extract in three different replication.

12Immobilized	12Mobilized	24Immobilized	24Mobilized	48Immobilized	48Mobilized
7	3	7	3	9	1
9	1	10	0	10	0
8	2	9	1	10	0
8	2	8	2	10	0
8	2	8	2	10	0
9	1	10	0	10	0
8	2	8	2	10	0
6	4	7	3	9	1
7	3	8	2	9	1
8	2	8	2	9	1
8	2	8	2	10	0
8	2	9	1	10	0
9	1	10	0	10	0
8	2	8	2	10	0
9	1	10	0	10	0
9	1	10	0	10	0
9	1	10	0	10	0
9	1	9	1	10	0
8	2	9	1	10	0
9	1	9	1	10	0
6	4	9	1	10	0
8	2	9	1	10	0
8	2	9	1	10	0
9	1	10	0	10	0
9	1	10	0	10	0
9	1	10	0	10	0
9	1	9	1	10	0
8	2	9	1	10	0
9	1	9	1	10	0
9	1	9	1	10	0

4. Taxonomic position of *Meloidogyne* spp.:

Phylum: Nematoda

Class: Chromodera

Order: Rhabditida

Sub-order: Tylenchina

Infra-order: Tylenchomorpha

Super-family: Tylenchoidea

Family: Meloidogynidae

Sub-family: Meloidogyninae

Genus: *Meloidogyne*

Common name: Root-knot nematode

5. Morphological description of *Meloidogyne* spp.:

Female : Length = 0.44-1.30mm, width = 0.35-0.700mm, stylet = 10-24µm, usually 14-15µm, dorsal oesophgeal gland orifice (DEGO) = 2-10µm (Eisenback and Traintophyllou 1991). Mature females are swollen to pear shape or nearly spherical shape except for an elongate anterior end. Its body remain 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 region forms a characteristic pattern. The female stylet is shorter with a small basal bulb. The stylet is moved by protactor muscles and functions like hypodermic needle. The paired gonads have extensive convoluted ovaries that fill most of the swollen body cavity (fig 6). 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 Traintophyllou 1991).

Male : Length =1000-1500µm i.e. 1-1.5mm, stylet = 13-30µm, spicules length = 19-40µm, DEGO = 2-13µm posterior to style knob base (Eisenback and Traintophyllou 1991). They are vermiform. Their lip region has a distinct head cap, which includes a labial disc surrounded by lateral and medial lips. The esophagus has developed procorpus, metacarpus with a valve, narrow isthmus and a ventrally overlapping glandular basal bulb. Its stylet is strongly developed with a large basal knob. Spicules and gubernaculums are nearly terminal and the blunt rounded tail, which has no bursa. The tail is short and hemispherical (fig 6). 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 Traintophyllou 1991).

2nd stage juvenile : Length = 280-500µm, stylet = 10µm (13-30µm), DEGO = 2-8µm, tail length = 15-100µm (Eisenback and Traintophyllou 1991). It is the infective stage and often found free living in the soil (fig 9). The stylet is slender and bear rounded basal knobs. The median esophageal glands are extensive, overlapping the intensive 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 Traintophyllou 1991).

Egg and egg sac : Length = 80µm and width = 35µm (Orion et al. 1994). The egg of *Meloidogyne* spp. 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 juvenile and hatches into second stage juvenile. The eggs and the GM from the egg mass, which is generally found at the interface between the gall surface and the soil (fig 8). The GM is produced by six rectal glands, during egg lying which are arranged radially around the female anal opening. The density of the layered material in the GM appeared to change 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).

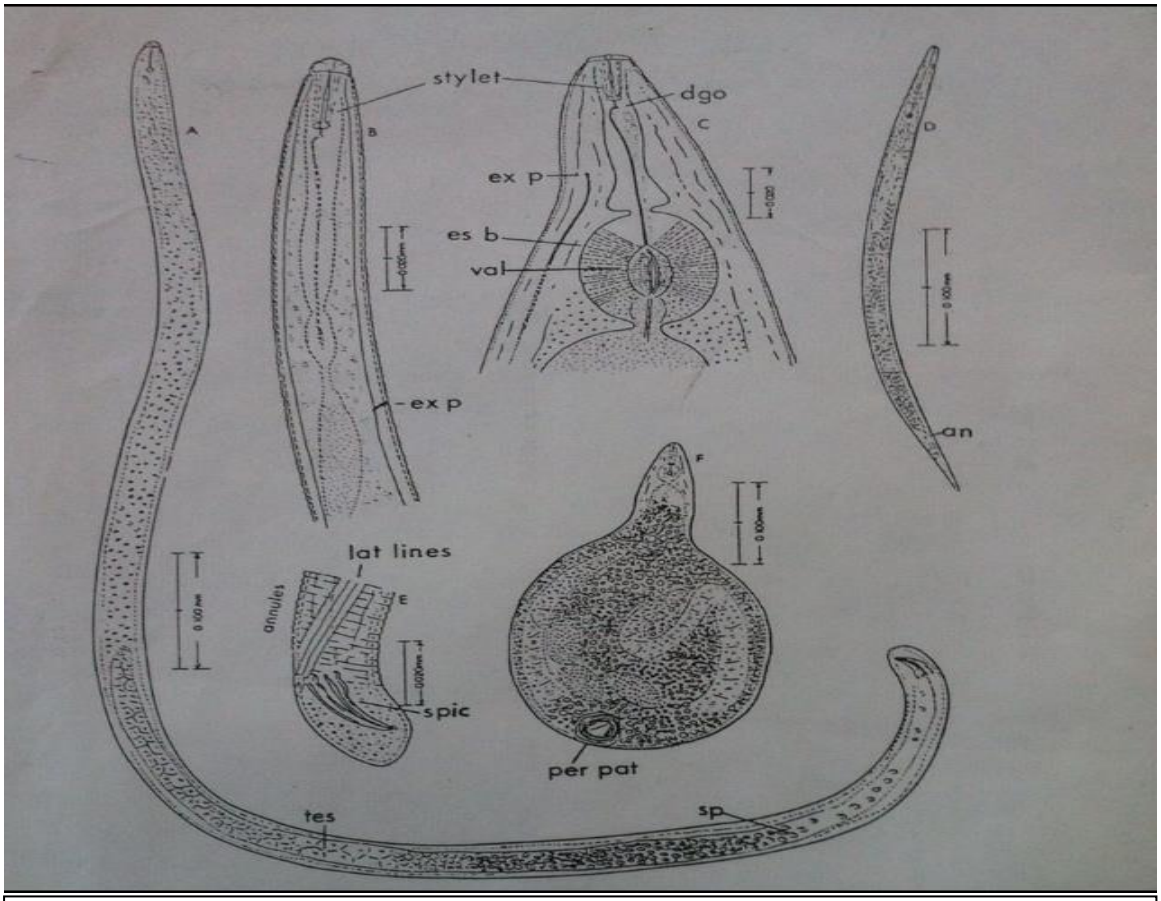

Figure 6. Anatomy of *Meloidogyne* species. A: Male, Full length, Showing stylet, testis (tes), sperm (sp) and spicules. B: Male anterior, showing stylet, esophagus and excretory pore (ex p). C: Female anterior, showing stylet, dorsal gland orifice (dgo), excretory pore (ex p), esophageal bulb (es b), and valve (val). D: Larva. E: Male posterior, F: Female showing esophagus, ovaries, and perineal pattern. (Taylor 1967).

Figure 7. Showing root infected by root-knot nematode.

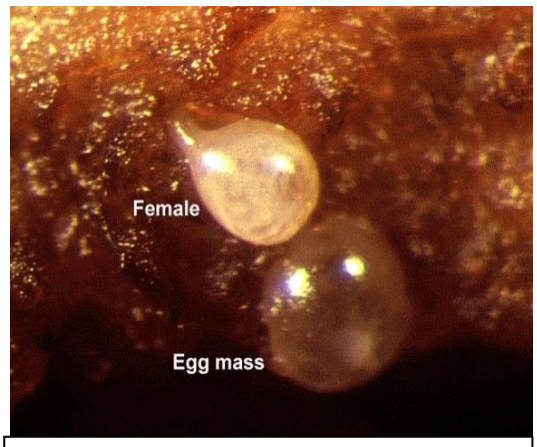

Figure 8. Showing female root-knot nematode along with egg mass.

Figure 9. Showing second stage juvenile (J_2) larva of *Meloidogyne* spp.

6. Life cycle of *Meloidogyne* spp : The life cycle of *Meloidogyne* spp. completes within four weeks at 25^{o}C (Luc et al. 2005). One moult occurs in the egg, leading to hatching of infective 2nd stage juvenile (J_2). 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 moult occurs. Females then continue to grow nearly spherical in form. After the last moult, however, males are seen coiled looped within the "sausage" cuticle, from which they emerge and migrate towards a female. Mating may occur but is not essential to the development since parthenogenesis occur in this genus. Eggs form a single female number form 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 juvenile prior to hatching and emergence. The second stage juvenile (J_2) moult thrice to become an adult (fig 10).

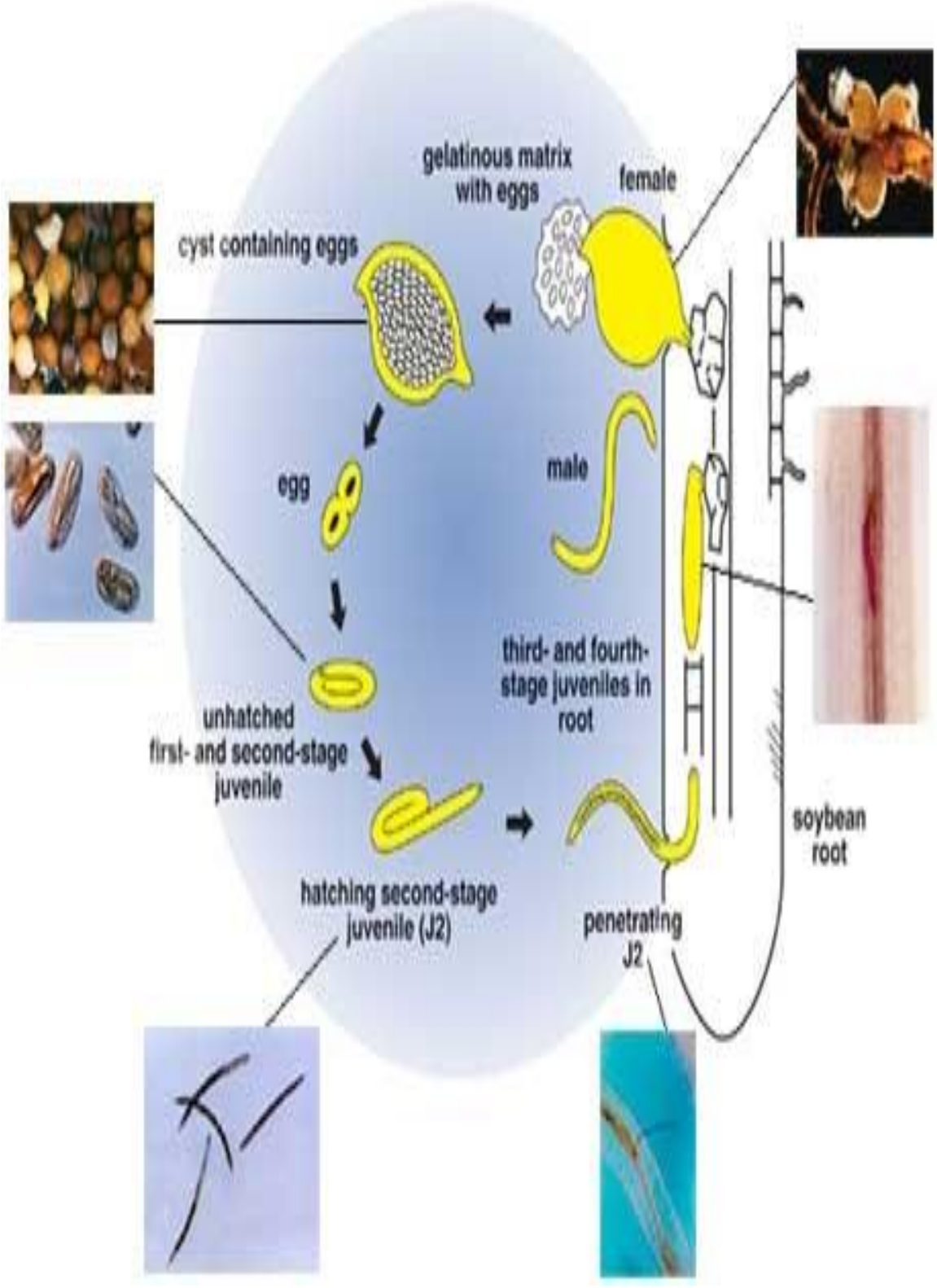

Figure 10. Diagram of the life cycle of root-knot nematode, *Meloidogyne* spp.

7. About *Lanatana camara*

Leaves are ovate or ovate oblong, 2-10 cm long and 2-6 cm wide bright green, rough, finely hairy, with serrate margins and emit a pungent odour when crushed. The stem in cultivated varieties is often non-thorny and in weedy varieties with recurved prickles. It is woody, square in cross section, hairy when young, cylindrical and up to 15 cm thick as it grows older. *Lantana* spp. is able to climb to 15 m with the support of other vegetation. Flower heads contain 20-40 flowers, usually 2.5 cm across; the colour varies from white, cream or yellow to orange pink, purple and red. Flowering occurs between August and March, or all year round if adequate moisture and light are available (fig 11). Pollinators include lepidopteron species and thrips. The fruit is a greenish blue-black colour, 5-7 mm in diameter, drupaceous, shining, with two nut lets; seed setting takes place between September to May with 1 - 20 seeds on each flower head. Mature plants produce up to 12,000 seeds annually. Seed germination occurs when sufficient moisture is present; germination is reduced by low light conditions. The root system is very strong with a main taproot and a mat of many shallow side roots. According to (Tayer et al. 2012) *Lantana* plant species are the best known for having nematicidal properties.

Lantana camara a native of tropical America is widely naturalized in many tropical and sub- tropical regions. Various parts of the plant are used in folklore and indigenous systems of medicine for the treatment of cuts, ulcers, swellings, eczema, malaria and tumors (Sastri, 1962, Kiktikak and Basu 1981). Although literature is available on the allopathic potential of *L. camara* (Casado 1995, Rajbansi and Inubushi 1997) little attention has been given to the possibility that it may also be toxic to nematodes attacking crop plants (Sharma 1996 and Jomati et al. 1998). Recently (Begum et al. 2008) isolated four different compounds, lantanoside, linoroside, camarinic acid and lantanone.

Lantana camara has been used in many parts of the world to treat a wide variety of disorders (Ross 1999). *Lantana camara* has been used in folk remedies for cancers and tumors. A tea prepared from the leaves and flowers was taken against fever, influenza and stomach-ache. In Central and South America, the leaves were made into a poultice to treat sores, chicken pox and measles. Fevers, colds, rheumatism, asthma and high blood pressure were treated with preparations from the plant (Irvine 1961). In Ghana, an infusion of the whole plant was used for bronchitis and the powdered root in milk was given to children for stomach-ache (Irvine 1961). In Asian countries, leaves were used to

treat cuts, rheumatism, ulcers and intestinal worms. Decoctions were applied externally for leprosy and scabies. It has been claimed that a steroid, lancamarone, from the leaves, exhibited cardiotonic properties (Sharma & Kaul 1959) and that lantamine, an alkaloid from the stem, bark and roots showed antipyretic and antispasmodic properties comparable to those of quinine (Sastri 1962) but the validity of these claims has not been confirmed. An alkaloid fraction which lowered blood pressure, accelerated deep respiration and caused shivering in dogs was isolated from the leaves, and it was suggested that it may be useful in reducing fevers and as a treatment for asthma and hypertension (Sharaf and Naguib 1959).

Figure 11. Showing apical part of *Lantana* spp.

8. Photoplates

Figure 12. Hand picking of *Lantana camara*.

Figure 13. Leaves of *Lantana camara* to dry up in hot air oven.

Figure 14. Grinding apical part of *Lantana camara* using a grinder.

Figure 15. Weighting of *Lantana camara*.

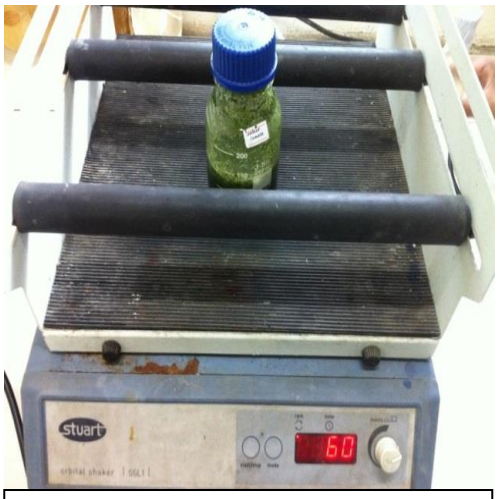

Figure 16. Shaking of extract for 24 hrs using a shaker.

Figure 17. Centrifuging of *Lantana camara*.

Figure 18. Storing the stock solution of plant extract in refrigerator.

9. Laboratory test

Figure 19. Source of root-knot nematode collected from kirtipur area.

Figure 20. Maintaining suitable temperature for hatching in J_2 larvae.

Figure 21. Allowing aqueous extract of *Lantana camara* over *Meloidogyne* spp.

Figure 22. Counting mobile and immobile J_2 under microscope.

Figure 23. Consulting with co-supervisor in lab.