Nematicidal Activity of *Lantana Camara* L. for Control of Root-Knot Nematodes

Ganesh Ghimire[1], Ranjana Gupta[1] and **Arvind K Keshari[2]**

1 Central Department of Zoology, Tribhuvan University, Kirtipur, Kathmandu, Nepal

2 Patan Multiple Campus, Tribhuvan University, Lalitpur, Kathmandu, Nepal

Abstract

Various concentrations of aqueous leaf extract of *Lantana camara* L. were assessed against second stage juveniles (J_2) of *Meloidogyne* spp. (Goeldi, 1982) for its nematicidal potency *in vitro* conditions. Study showed 50% concentration of *Lantana camara* leaf extract at 48 hrs of incubation period and above showed effective in immobilizing second stage of larvae (J_2) of *Meloidogyne* spp. The standard concentration 'S' (100%) of leaf extract was found to be highly nematostatic, 98.66% of nematode were found dead in 48 hrs. Similarly, 57.66% of nematode juveniles were found dead when applied 50% concentration in 48 hrs. Mean number of (J_2) dead at 100% concentration for three time period was statistically significant highest at 48 hrs. So far, 50% concentration in 48 hrs and above was appropriate for controlling the root-knot nematode which seems as an alternative to chemical pesticides.

Keywords: Concentration, Leaf extract, *Meloidogyne*, Nematostatic, Second stage juveniles (J_2)

Introduction

One of the most damaging groups of plant nematodes is the root-knot nematode (*Meloidogyne* spp.). It is an obligate root parasite of more than 200 plant species including vegetable, horticulture and woody plants (Hussey 1985). Root-knot nematode, *Meloidogyne* spp. (Goeldi, 1982) (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). 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. Since, the chemical pesticides cause hazard to the biodiversity, therefore, the use of botanical pesticides can be better option to control root-knot nematodes.

Materials and Methods

Study Area

The proposed study area for the research was Kirtipur Municipality of the Kathmandu district. Kirtipur is one of the recently urbanized cities of Kathmandu valley located to South-west of the central Kathmandu. It extends from 27º 41' 36" – 27º 38' 37" N to 85º 18' 00" – 85º 14' 64" E and has 1300 to 1402 meter of altitudinal range from sea level (Fig. 1).

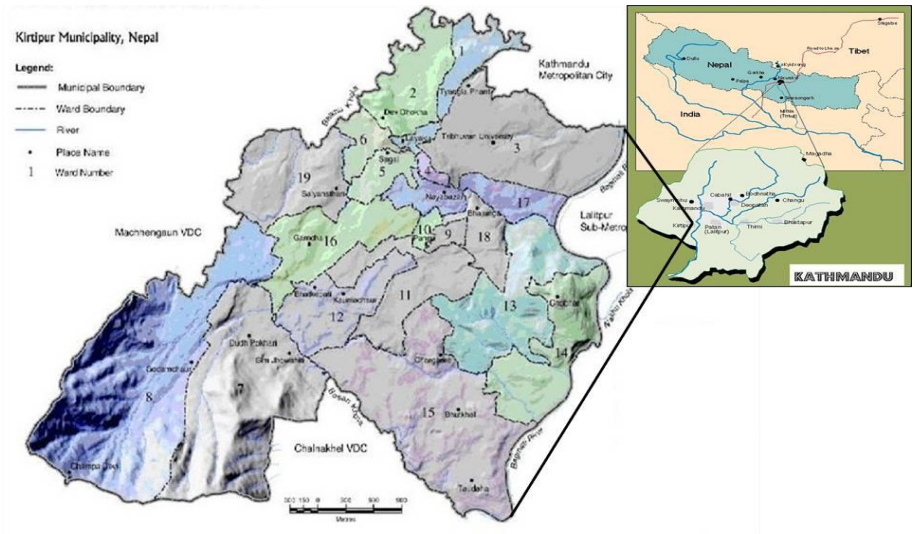

Figure 1. Map of Nepal and study area.

Plant material

Lantana camara L.used for the experiment was collected from natural habitats of Kirtipur. 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.

Preparation and storage of aqueous leaf extract

Fresh mature healthy leaves of *Lantana camara* were washed and air dried using an oven at 28ºC. The dried leaves were ground using a Classic Maraja Electric Blinder. A 250 gm of fine powder was dissolved in 1000 ml sterilized DH_2O (1gm/4 ml basis). Aqueous suspension was allowed to soak on Stvart Orbital Shaker at room temperature for 24 hrs for the extraction of active ingredients and 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 4ºC in a plastic container as standard solution 'S'. Other different concentrations i.e. 10%, 50%, and 100% respectively were prepared by adding required amount of sterilized DH_2O for laboratory experiments.

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

Root-knot nematode, *Meloidogyne* spp. was collected from the roots of heavily infected tomato plants. 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 using sterilized forceps from heavily infected tomato roots, washed in distilled water and incubated at 28± 2^0C for 24 hrs for hatching. The hatched juveniles were 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).

***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 conditions in order to assess the larval mortality. For

this experiment, 100 freshly hatched J_2 larvae were transferred to 2.5 cm in diameter plastic petridish containing 10 ml of different concentrations of leaf extract i.e. 10%, 50%, and 100% respectively. The petridish with 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 microscopic in order to record larval mortality. Immobilized larvae were confirmed by using needle as dead larvae failed to respond to stimulation with a needle. Each treatment was replicated three times.

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.

Results

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

The percentage mortality of J_2 of *Meloidogyne* spp. was 82.6%, 36.6% and 3.3 in 100%, 50%, 10% concentrations of leaf extract respectively as compared to control after 12 hrs. In other way, out of 100 J_2 used in each experiment 17.4%, 63.4% and 96.7% larvae were remained active in 100%, 50% and 10% concentration of leaf extract of *L. camara* as compared to control. Among all the treatment, 100% of leaf extract proved highly toxic to juveniles followed by 50% and 10%. Similarly, the percentage mortality of J_2 was 89.3%, 50.0% and 16.0% in 100%, 50%, 10% concentrations of leaf extract, respectively as compared to control after 24 hrs. In terms of mobilization of larvae, out of 100 larvae used in each experiment 10.7%, 50% and 84% larvae were remain active in 100%, 50% and 10% concentration of leaf extract. Likewise, the percentage mortality of J_2 was 98.6%, 57.6 and 28.6% in 100%, 50%, 10% concentration respectively as compared to control after 48 hrs. In terms of mobilization of J_2 larvae, out of 100 larvae, 1.4%, 42.4% and 71.4% larvae were found active in 100%, 50% and 10% concentration of leaf extract of *L. camara*. In general, percentage mortality was proportionally correlated with the concentrations and exposure periods of extract. The highest mortality (98.6%) was recorded in 100% concentration of leaf extract at 48 hrs of exposure period followed by 89.6% in 24 hrs and 82.6% in 12 hrs (Table 1).

Table 1. Effect of different concentration of plant extract of *L. camara* on larval mortality after 12, 24 and 48 hrs

Plant	Incubation period (hrs)	% of mortality of nematode in different concentrations			
Lantana camara		S (100%)	S/2 (50%)	S/10 (10%)	Control
	12 hrs	82.6	36.6	3.3	0
	24 hrs	89.3	50.0	16.0	0
	48 hrs	98.6	57.6	28.6	0

Each value is the mean of three replicates.

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

Result in table 2 also showed that effect of various concentrations of leaf extracts of *Lantana camara* on larval mortality over exposure time i.e 12 hrs, 24 hrs and 48 hrs. In general, percentage of larval mortality increased with the increase in exposure period but decrease with dilutions. 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 other. Mortality in control treatment was negligible.

Table 2. Mean number of larval mortality in different concentration at 12, 24 and 48 hrs

Plant	Incubation period (hrs)	Concentrations				LSD (0.05)
Lantana Camara		S (100%)	S/2 (50%)	S/10 (10%)		
	12 hrs	8.26[a]	3.67[b]	0.33[c]		0.39
	24 hrs	8.93[a]	5.00[b]	1.60[c]		0.53
	48 hrs	9.86[a]	5.77[b]	2.86[c]		0.45

Degree of freedom (df) = 58

T-value = 2.0017

Discussion

In the present studies, various concentrations of aqueous leaf extract of *L. camara* were assessed for nematicidal activity in the laboratory conditions. 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 decreases with increased extract concentration as the efficacy of the plant extract depends on the concentration and duration of exposure of juveniles to the extract (Mahmood et al. 1997). Among all the concentrations of leaf extract of *L. camara* tested, 100% concentration at 48 hrs was found effective in controlling *Meloidogyne* juveniles. This result agrees with the result 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. The nematicidal activity of *L. camara* against juveniles of *Meloidogyne* spp. has also been reported by many authors (Begum et al. 2008, Qamar et al. 2005, Shaukat and Siddiqui 2001). The mortality of juveniles might be due to nematicidal chemicals present in the leaf extract as *L. camara* contains camaric acid and olenolic acids which may have larvicidal or ovicidal properties.

The findings of the present investigation are not conclusive. Further studies should be conducted in greenhouse and field conditions to assess the nematicidal activity. In comparison to the other countries, very limited work on nematicidal treatment is 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 this research.

Conclusion

It was found that 50% concentration of *L. camara* leaf extract at 48 hrs and above was found 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, further studies are needed to characterize the active compounds in the test plant that are nematicidal and possessing complex modes of action.

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