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Efficacy of Botanical Extracts on Hatching of *Meloidogyne incognita* Eggs under *in vitro* Study

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A B S T R A C T

In vitro study was conducted to study the effect of botanicals against hatching of *Meloidogyne incognita* egg masses. The aqueous extract of neem (*Azadirachta indica)* had the highest level of inhibition on hatching of nematode eggs (60.50 and 71.5% after 72 hr and 120 hr respectively). Significant increase in egg hatching inhibition as compared to neem was also observed at 120 hr interval (66.5 and 54.25 %) with Thulasi (*Ocimum sanctum*) and Calotropis (*Calotropis gigantea*) respectively. In control 100 % of egg hatching was found upto 48hrs.

Introduction

Chilli (*Capsicum annum* L.) is considered as one of the most important commercial spice crops and is widely used universal spice, named as wonder spice. India is the world leader with a production of 13.76 million tons of chillies contributes 36 per cent to world's production followed by China, Thailand and Pakistan (FAO Stat., 2013).

Root-knot Nematodes (RKNs) belonging to the genus *Meloidogyne* are considered the most important group of plant-parasitic nematodes worldwide attacking nearly every crop. These nematodes inflict great losses to various horticultural crops (Sasser and Freckman, 1987). About 5 per cent of the total world crop yield is destroyed due to root knot nematodes (Sasser, 1987). Chemical nematicide is one of the most fastest and effective nematode control methods, but they are detrimental to both humans and the environment and are relatively unaffordable to the average small scale farmers (Washira *et al.*, 2009). Therefore, there is a need to

develop alternative methods of control that are cheap, environmentally friendly and not harmful to humans. Many botanical extracts have been found to contain phytochemical such as alkaloids, tannins, saponins, flavonoids, diterpenes, glucosinolates, acetylenes and thienyls (Gommers, 1981; Chitwood, 2002) which are effective against plant parasitic nematodes (Goswani *et al* (1986); Adegbite, 2003). Thus, the present investigation was done to evaluate the Nematicidal efficacy of different botanical extracts on hatching of *Meloidogyne incognita* eggs under *in vitro* condition.

Materials and Methods

Collection of botanicals

The botanicals *viz*., Kathivel (*Acacia auriculiformis*), Calotropis (*Calotropis gigantea*), Neem (*Azadirachta indica*), Thulasi (*Ocimum sanctum*) and Kolinji (*Tephrosia purpurea*) were collected from in and around Agricultural College and Research Institute, Eachangkottai farm.

Preparation of aqueous extracts of botanicals

Healthy leaves of Kathivel, Calotropis, Neem, Thulasi and Kolinji were used for aqueous extracts preparation. It was prepared separately by grinding 50g of leaves with 200ml of distilled water. To obtain a clear and transparent extract, the aqueous extract was filter through a muslin cloth and then centrifuged at 4000 rpm for 10 minutes. The supernatant solution was considered as stock solution and stored it in a refrigerator for laboratory studies.

Egg mass collection

Root-knot nematode infected Chilli plant (cv. PKM 1) from the pure culture pot was up-rooted and washed gently under running tap water. Egg masses of *M. incognita* were picked up from the root using dissecting needle and forceps. The collected egg masses were kept in water at 10°C in a refrigerator to prevent hatching before application of treatments.

In vitro study

The effect of different botanical extracts on the hatching of *M. incognita* eggs was evaluated under *in vitro* by using the following procedure. The experiment was conducted in a 7.5 cm diameter petri plates taking 10 ml stock solution from each botanicals and maintaining 4 replications. Sterilized distilled water was taken as control. Five uniformly sized egg masses of *M. incognita* were transferred to each botanical extracts in sterilized petri plates (7.5 cm diameter), while egg-masses in distilled water only served as control. The experiment was laid out in completely randomized design (CRD). The petri plates containing the suspension and the egg masses were kept at room temperature on laboratory bench to allow eggs hatch. The number of hatched second stage juveniles was counted after 24, 48, 72 and 120 hrs. The suspension from each petri plate was first transferred to nematode counting dish retaining the egg masses in petri plate. The number of juveniles was counted under stereoscopic microscope. Fresh plant extract/distilled water was added to petri plates and kept again at the laboratory bench.

Statistical analysis

The data were analysed by ANOVA and least significant differences were calculated at p = 0.1.

Results and Discussion

Nematicidal efficacy of botanical extracts *viz*., *T. purpurea, A. auriculiformis, C. gigantea* and *O. sanctum* on hatching of *M. incognita*

eggs was made *under in vitro* condition. The results of nematode egg hatching test showed that all tested botanical extracts were found to decrease the hatching rate in egg masses of *M. incognita*. The aqueous extract of *A. indica* had the highest level of inhibition on hatching of nematode eggs (60.50 and 71.5 per cent after 72 hr and 120 hr respectively). Significant increase in egg hatching inhibition as compared to *A. indica* was also observed at 120 hr interval (66.5 and 54.25 per cent) with *O. sanctum* and *C.gigantea* respectively. In control 100 per cent of egg hatching was found upto 48hrs (Table 1).

Table.1 Effect of different botanicals on inhibition of egg hatching under *in vitro* study

Treatments	Per cent Inhibition of egg hatching at different time interval			
	24h	48h	72h	120h
Calotropis (*Calotropisgigantea*)	36.75	42.25	50.5	54.25
Thulasi (*Ocimum sanctum*)	41.75	46.5	53	66.5
Neem (*Azadirachtaindica*)	43.5	53.75	60.5	71.5
Kathivel (*Acaciaauriculiformis*)	26.25	34.5	41.5	38.5
Kolinji (*Tephrosiapurpurea*)	26.75	38	47	45.25
Control	0.00	0.00	0.25	0.36
CD = 0.05	0.602	0.473	0.602	2.126
SEd	0.286	0.225	0.286	1.012

Plate.1 Botanicals used to evaluate the hatching of *Meloidogyne incognita* eggs

Plate.2 *In vitro* study on efficacy of botanical extracts on hatching of root knot nematode, *Meloidogyne incognita* eggs

A number of wild plants growing throughout the world produce compounds having an immobilizing effect on *M. incognita*. These compounds are likely secondary metabolic products and while not involved in primary metabolism contribute to the defense of plants. Enhanced inhibition on egg hatching was obtained in neem extract followed by ocimum and calotropis indicating that they possessed nematostatic properties, presence of toxic chemicals in the botanicals might have acted as prohibitors inhibiting emergence of juveniles (Sarosh and Hussain, 1986). The effect of different extracts on egg hatching could be due to the presence of tannins, alkaloids and flavonoids which have been reported to kill nematodes.

The nematicidal effect of Ocimum extract is 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 (Knoblock *et al.,* 1989).

In conclusion, experimental results from this study revealed that the nematicidal potential in test plants and identified neem, ocimum and calotropis highly effective in controlling *M. incognita*. These findings proved that botanicals possess a nematicidal activity. Thus, the plant extracts can be used for the management of root knot nematodes. Its application is expected to be cheap, easily available and ecofriendly.

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