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Evaluation of Nematicidal Activity of Ethanolic Extracts of Medicinal Plants to *Meloidogyne incognita* (Kofoid and White) Chitwood under Lab Conditions

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ABSTRACT

Nematicidal efficacy of various medicinal viz., turmeric (Curcuma longa), marwatulsi (Origanum majorana), mentha(Mentha arvensis), aonla (Phyllanthus emblica) and jatropha (Jatropha curcas) was evaluated in vitro against root-knot nematode, Meloidogyne incognita. Ethanolic extracts of these plants were extracted and tested against root- knot nematode at different dilutions i.e. 1:5, 1:10, 1:20, 1:40 and 1:80. Ethanolic extracts of all plants showed nematicidal effect against M. incognita at varying degree. Percentage larval mortality and inhibition of egg hatching were directly proportional to the concentration of ethanolic extracts and exposure period to each extract. Ethanolic extracts of turmeric (C. longa) was found to be more effective amongst all other plant extracts in increasing mortality and inhibited the egg hatching.

Key words: *Essential oil, Meloidogyne incognita, nematicidal plants, mortality*

INTRODUCTION

Plant parasitic nematodes cause severe damage to almost all the crops. They feed on the roots or above ground parts ectoparasitically as well as endoparasitically. Reduction in the quantity and quality of the crop produces results in decline in net profits. Several control practices of nematode management *viz* cultural methods, physical methods, biological control, chemical control, use of resistant varieties etc. have been found highly effective against different nematodes. But each has its own merits and demerits. Though chemical control provide good control but residue problem, pest resurgence and high dosage are the key factor for their limited use in nematode management program. So it has become essential to go for cheaper, eco-friendly and safer ways of nematodes management. Several plants and their products have been recorded to posses nematicidal and nematostatic properties in their roots, shoots, leaves, flowers etc[3]. Nematicidal phytochemicals are generally safe for the environment and humans[1].

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Organic amendments are not only safe to use but also have the capacity to improve soil structure and fertility. Thus, control strategies are now directed towards the use of natural products. Bioactive products of plants being less persistent in environment and are safe for mammals and other non target organisms. Botanical pesticides are readily available in many places, often cheaper than their synthetic counter parts and their crude extracts are easy to prepare even by farmers. These are also less likely or slow down the development of resistance or resurgence in pests. The benefits of natural pesticides have aroused interest in protection of crop plants. The present paper reports the *in-vitro* nematicidal activity of plants extracts on hatching and mortality of root knot nematode, *M. incognita* larvae.

MATERIAL AND METHODS

Preparation of Extract

Ethanolic extracts of oven dry leaves of marwa tulsi (Origanum majorana), mint (*Mentha arvensis*), rhizome of turmeric (*Curcuma longa*) and fruit of aonla (*Phyllanthus emblica*) and seed of jatropha (*Jatropha curcas*) were prepared by refluxing the dry matter (50g) with ethanol (350 ml) on soxhelt apparatus for 8 hrs. The ethanolic extracts were distilled and the crude extracts obtained after distillation were completely dried and the weight of the solid mass was measured. The solid ethanolic extracts (5 g) were dissolved in minium amount of surfactant (Exalin) was added and the final volume (50 ml) was made with distilled water to make 10 percent(w/v) ethanolic extracts of respective plants. Minimum amount of ethanol and exalin used in extract preparation with final volume (50 ml) with distilled water was taken as control.

In vitro testing for nematode mortality

Five ml. of suspension containing approximately 100 freshly hatched juveniles of root-knot nematode, *M. incognita* were taken in 50 mm size petriplates. Measured quantity of stock solution was added to each petriplate to make the resultant dilutions of 1:5, 1:10, 1:20, 1:40, and 1:80..Water alone was taken as check. Each dilutions was replicated three times. These petriplates were kept in BOD incubator at 25±1^{O}C. Larval mortality after 48 hrs exposure of the larvae to different dilutions of alcoholic extracts were recorded by counting living and dead second stage juveniles under stereoscopic binocular microscope. Juveniles put in distilled water and exalin were treated as control. Larvae that did not respond to touch by a fine needle were counted as dead. Percent larval mortality was calculated and data thus, obtained was subjected to angular transformation and analysed by applying CRD factorial design.

In vitro testing for egg hatching

Egg masses of root-knot nematode, *M.incognita* were collected from infected brinjal roots and washed with sterile distilled water. The similar sizes egg masses were kept in 50mm petri-dish (4 egg masses/ petridish) and measured quantity of stock solution was added to each petriplate to make the resultant dilutions of 1:5 and 1:20.Each dilutions was replicated three times .These petriplates were kept in BOD incubatorat 25±1^{O}C .The number of juveniles hatched after 3, 6, 9 and 12 days of exposure to different concentration were counted under stereoscopic binocular microscope and each interval these egg masses were transferred to new petriplates and fresh extracts of same concentration were poured.. Egg masses put in distilled water and exalin were treated as control. Mean larvae hatched was calculated.

RESULTS AND DISCUSSION

Effect on nemic mortality

The data in table 1 revealed that ethanolic extracts of all the plants were found lethal to

juveniles of *M. incognita.* Rate of mortality was directly proportionate to concentration of extracts. The highest mortality of larvae was observed at 1:5 dilution of essential oils of tested plants while lowest was observed at lowest dilution i.e. 1:80. Per cent mortality were recorded to be (8.71) in control. Among alcoholic extracts maximum per cent larval mortality was recorded with turmeric (90.17) followed by marwa tulsi (86.23) and mentha i.e. (84.17) at 1:5 dilution respectively. The rhizome extract of turmeric was found consistent in terms of larval mortality as it was effective up to 1:40 dilution but showed low mortality at 1:80 dilution.

Effect on hatching of *Meloidogyne incognita*

Effect of alcoholic extracts of different plants on hatching of *Meloidogyne incognita* larvae are presented in Table-2. All plants alcoholic extracts showed inhibitory effect on hatching of *Meloidogyne incognita* . The rate of hatching was inversely proportional to concentration of extracts and exposure period, as it decreased with increase in concentration. The maximum hatching of eggs was observed in 1:20 while lowest rate at 1:5 dilution in all plants alcoholic extracts tested. Among alcoholic extracts, extract obtained from mentha showed most inhibitory effect followed by turmeric and marwa tulsi respectively. Minimum hatching (18.6 larvae) was recorded with turmeric followed by mentha (20.3 larvae) after 12^{th} day. The maximum egg hatching (614 larvae) was recorded at 3^{th}day in 1% ethanol as check It is revealed from table 1 that all three factors *viz*, ethanolic extract, their concentrations and exposure time significantly affected hatching individually as well as in combination with one another as check.

The present investigation are in adjustable conformity with the finding of Prasad and Suverna[5], who while testing four concentrations of root and leaf extracts of *Calotropis procera* and *Parthenium hysterophorus* (0.5, 1.0, 2.0 and 4.0% at exposure periods of 24, 48 and 72 hrs) against reniform nematode reported higher mortality of pre-adults of *Rotylenchulus reniformis* race-A infesting sunflower cv. Morden as compared to the control. Similarly Goel and Gupta[2] also reported the nematicidal potential of onion cv. N-53 extract against second stage juveniles of *M. javanica*. The root extract of onion at 100 per cent concentration with 48 hrs exposure time caused the highest larval mortality followed by leaf and bulb extracts of the same concentration. While evaluating the nematicidal properties of 15 plant and their various parts viz. *Albizzia amara*, *Aristalochia bractiata*, *Tagetes erecta*, *T. patula*, *Origanum majorana*, *Azadirachta indica*, *Butea monosperma* and *Calotropis gigantea* leaves, *Acorus calamus* roots, *Allium sativum* bulbs, *Citrullus lanatus*, *Areca catechu* and *Anona reticulate* seeds, and *C. gigantea* and *Carica papaya* latex against the root-knot nematode, *M. incognita* egg masses, Saravanapriya *et al*.[6], reported that the seed extract of *A. catechu* showed highest inhibition rate at 0.1 per cent concentration. The latex of *C. papaya* caused 98.22 and 100 per cent hatching inhibition at 1.0 and 10.0 per cent concentrations, respectively. The latex of *C. gigantea* also caused 100 per cent inhibition at 10.0 per cent concentration. Nayak *et al*.[4], evaluated the nematicidal effect of methanolic extracts of neem against second stage juveniles of M. *incognita* and revealed that methanolic extract at 5 ml concentration exhibited maximum mortality (81 %) after 24 hours. In the present investigation observations showed the nematicidal activities of plant extracts resulting inhibition of hatching and mortality of larvae of *M. incognita*.

Table 1: Effect of ethanolic extracts of plants against nemic mortality

Ethanolic extracts (P)	Per cent mortality of second stage larvae at concentrations (C)					(Control)	Mean for P
	1:5	1:10	1:20	1: 40	1:80		
Turmeric	90.17 (71.73)	84.03 (66.50)	62.97 (52.49)	43.73 (41.38)	33.63 (35.42)	2.4 (8.81)	(41.70)
Marwa tulsi	86.23 (68.25)	71.73 (57.93)	53.43 (46.95)	34.60 (35.97)	17.40 (24.62)	2.4 (8.81)	(38.25)
Mentha	84.87 (67.17)	71.67 (57.82)	41.53 (40.04)	32.67 (34.82)	15.83 (23.42)	2.4 (8.81)	(37.68)
Aonla	68.77 (56.09)	40.77 (39.66)	31.77 (34.28)	11.80 (20.07)	3.67 (10.94)	2.4 (8.81)	(28.30)
Jatropha	70.33 (56.98)	44.50 (41.81)	35.33 (36.45)	11.57 (19.81)	5.40 (13.42)	2.4 (8.81)	(29.54)
Mean for C	(64.04)	(52.76)	(42.83)	(30.31)	(21.61)	8.81 (8.81)	

C.D. at 5 % Ethanolic extracts of plants (P) =(1.40),
Concentrations (C.) =(1.53),
E x C= (3.44)
Figures in parenthesis are angular transformed values

Table 2: Effect of ethanolic extracts of plants on the egg hatching of root -knot nematode, *Meloidogyne incognita* (Average of three replicates)

	Numberof juveniles hatched													
Ethanolic extract (P)	**1:5**				PxC mean	**1:20**				PxC mean	**Distilled water**				PxC Mean
	3	**6**	**9**	**12**		**3**	**6**	**9**	**12**		**3**	**6**	**9**	**12**	
Turmeric	106.6 (10.3)	68.0 (8.3)	30.6 (5.6)	18.6 (4.4)	(7.1)	158.0 (12.6)	82.6 (9.1)	52.3 (7.30)	29 (5.4)	(8.82)	614.0 (24.7)	397.6 (19.9)	198.33 (14.11)	156.0 (12.5)	(17.8)
Marwa tulsi	142.0 (11.9)	91.6 (9.6)	50.0 (7.1)	21.6 (4.7)	(8.3)	214.3 (14.6)	148.3 (12.2)	84.6 (9.2)	52.3 (7.3)	(10.8)	614.0 (24.7)	397.6 (19.9)	198.3 (14.1)	156.0 (12.5)	(17.8)
Mentha	133.0 (11.5)	88.6 (9.4)	47.3 (6.9)	20.3 (4.6)	(8.1)	189.3 (13.1)	119.0 (10.9)	65.6 (8.1)	38.0 (6.2)	(9.5)	614.0 (24.7)	397.6 (19.9)	198.3 (14.1)	156.0 (12.5)	(17.8)
Aonla	287.6 (16.9)	189.3 (13.7)	112.3 (10.6)	80.6 (9.0)	(12.1)	417.6 (20.4)	315.3 (17.7)	157.6 (12.5)	100 (10.)	(15.2)	614.0 (24.7)	397.6 (19.9)	198.33 (14.11)	156.0 (12.5)	(17.8)
Jatropha	193.0 (13.9)	150.6 (12.3)	98.3 (9.9)	83.3 (9.1)	(11.3)	282.0 (16.8)	197.0 (14.0)	119.6 (10.8)	96.3 (9.8)	(12.9)	614.0 (24.7)	397.6 (19.9)	198.33 (14.11)	156.0 (12.5)	(17.8)
Mean CxT	(12.9)	(10.7)	(8.0)	(6.4)		(15.6)	(12.8)	(9.66)	(7.7)		(24.7)	(19.9)	(14.11)	(12.5)	
POOLED MEAN T	(17.1)	(14.5)	(10.6)	(8.9)											
POOLED MEAN C					(9.5)					(11.4)					(17.8)

Ethanolic extract(P)	Mean PxT				
	3	**6**	**9**	**12**	POOLED MEAN
Turmeric	(16.7)	(13.4)	(9.7)	(7.5)	(11.8)
Marwa tulsi	(17.1)	(13.9)	(10.1)	(8.1)	(12.3)
Mentha	(15.9)	(12.4)	(9.0)	(7.7)	(11.2)
Aonla	(20.7)	(17.1)	(12.4)	(10.5)	(15.2)
Jatropha	(18.5)	(15.4)	(11.6)	(10.5)	(14.0)

C.D. at 5% Ethanolic extracts (P)=(0.11), Conc(C).=(0.09), Exposure period(T)= (0.10) , PxC=(0.02), PxT= (0.23), CxT=(0.18), PxCxT=(0.40)
Figures in parenthesis are √n transformed values

CONCLUSION

It is therefore concluded that the incorporation of plant products such as oils of pre selected plants could provide a suitable and cheaper alternative for management of root knot nematode *M. incognita* which infesting large number of crops. These plants after testing rigorously and repeatedly under field conditions can be a better choice to manage nematodes successfully, effectively and in a safer manner. The toxic principle responsible for the nematicidal action may further be characterized, isolated and exploited as an ideal nematicide of plant origin.

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