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Effect of Plant Extracts on Hatching and Mortality of Root-Knot Nematode, *Meloidogyne incognita* Larvae (*in-vitro*)

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This research was conducted to find out the effect of aqueous extracts of different medicinal plants against the root-knot nematode , *Meloidogyne incognita* in laboratory at CCSHAU , Hisar. Aqueous extracts from plant leaves were screened for egg hatchability and nemic mortality against second stage juveniles (J_2) of *M. incognita.* The nematode eggs and juveniles were exposed to 24, 48 and 72 hrs to different dilutios of extracts . The dilutios as 1:5, 1:10, 1:20, 1:40 and 1:80 were prepared by diluting stock solution. As a result, *M. arvesis* (mentha)1:10 (v/v) dilution significantly reduced the egg hatchability and it was followed by *C.longa*(turmeric). At the end of 48 hrs incubation, 1:5 and 1:10 (v/v) dilutions of *M. arvensis* extract and *C.longa* produced maximum mortality in juveniles of the nematode. The results of our investigation show that five plants contain nematicidal compounds. It seems that the use of plant extracts might have increasing popularity in future as a component or ingredient of biopesticides. Therefore, further research is necessary to found out toxic compounds released by species and carried out experiments *in vivo* for the control of root-knot nematode , *M. incognita*.

Keywords: Hatching, Mortality, *in vitro*, Nematode.

Root-knot nematode, (*Meloidogyne* spp) are among the serious and wide spread nematode pests attacking various economically important crops. 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 control, 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 But farmer mainly relied on chemical nematicides because it effectively kill nematodes in soil . Chemical control, though gives quick control but these chemicals are not only expensive but also caused severe environmental hazards. Some fumigant nematicides have been banned since year 1979 (DBCP) by Environmental Protection Agency as environmental toxins 2005. Therefore there is an urgent need to replace pesticides with alternative means of control that are less toxic and more eco friendly. Other convential methods for nematode control like cultural, physical, biological and plant resistance, which too have technical and operational limitations of one kind or other. The use of non-hosts and antagonistic plants having allelopathic effect offers an promosing area for combating and managing root-knot nematodes. Plant parts and their products have been found to posses nematicidal and nematostatic properties against plant parasitic nematodes. Nematicides of plant origin contains iso-thiocyanates, thiophenics, glucosides, alkaloids, phenolics and fatty acids in their different plant parts which are fatal to nematodes. Thus, the present investigation was done to evaluate the nematicidal effect of aqueous

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extract of medicinal against egg hatching and larval mortality of *Meloidogyne incognita*.

MATERIAL AND METHODS

Preparation of aqueous plant extracts

Healthy leaves of one year old plant of turmeric (*Curcuma longa*), marwa tulsi (*Origanum majorana*), mint(*Mentha arvensis*), aonla (*Phyllanthus emblica*) and jatropha (*Jatropha curcas)* were collected from herbal park of CCSHAU, Hisar (Haryana) These collected plant leaves were washed with steriled distilled water.The aqueous extract of these plants were prepared separately by grinding and dissolving 2 g of ground material in 5 ml of distilled with the help of pestle and mortar. To obtain a clear and transparent extract, free of all plant debris , the water extracts were filtered through a four ply muslin cloth and then centrifuged for five minutes at 4000 rpm and then filtered through a Whatman's filter paper .The aqueous extracts thus prepared were kept in covered flasks in refrigerator and were taken as stock solution for evaluating their nematicidal effect on hatching and larval mortality under *in vitro* condition.

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. Each dilution 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 different oils were recorded by counting living and dead second stage juveniles under stereoscopic binocular microscope. Juveniles put in distilled water 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.

Testing for egg hatching

Egg masses of root-knot nematode, *M.incognita* were collected from infected brinjal roots and washed with steriled distilled water. The similar sized 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 dilution 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 dilutions were counted under stereoscopic binocular microscope. Egg masses put in distilled water were treated as control.

Table 1. Effect of aqueous extracts of plants against nemic mortality

Aqueous Extracts (p)	Per cent mortality of second stage larvae at concentrations (C) 1:5	1:10	1:20	1:40	1:80	Water (control)	Mean for P
Turmeric	81.00	64.10	36.53	12.50	4.77	0.70	
	(64.15)	(53.17)	(37.16)	(20.66)	(12.59)	(4.74)	(32.51)
Marwa tulsi	80.97	55.00	43.60	12.97	5.50	0.70	
	(64.14)	(47.85)	(41.29)	(21.03)	(13.52)	(4.74)	(32.10)
Mentha	82.87	72.90	45.67	19.27	11.17	0.70	
	(65.73)	(58.64)	(42.49)	(26.00)	(16.38)	(4.74)	(34.79)
Aonla	37.22	28.70	10.33	7.90	1.80	0.70	
	(37.56)	(32.32)	(18.64)	(16.31)	(7.60)	(4.74)	(19.32)
Jatropha	65.9	40.87	31.47	14.67	2.73	0.70	
	(46.14)	(39.71)	(34.10)	(22.44)	(9.41)	(4.74)	(26.01)
Mean for C	(55.5)	(46.34)	(34.74)	(20.75)	(11.66)	(4.74)	

C.D. at 5 % Aqueous extract of plants (P) =(1.31)
Concentrations (C.) =(1.43)
P x C = (3.20)

Figures in parenthesis are angular transformed values

Table 2. Effect of aqueous extracts of plants on the egg hatching of root –knot nematode , *Meloidogyne incognita* (Average of three replicates)

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

Aqueous extract(P)	Mean PxT				
	3	6	9	12	POOLED
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)

RESULTS AND DISCUSSION

Experiments were conducted to study the effect of aqueous extracts of different plants on hatching and mortality of *M.incognita* larvae under laboratory conditions.

Effect of aqueous extracts on nemic mortality

Aqueous 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 extracts of tested plants while lowest was observed at lowest dilution i.e. 1:80(table1). Among extracts maximum per cent larval mortality was recorded with mentha i.e. (83.83) followed by turmeric (80.56) marwa tulsi (80.25) at 1:5 dilution respectively. The extract of mentha 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 egg hatching

All plants extracts showed inhibitory effect on egg hatching . 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 extracts tested (table 2). Among aqueous extracts , extracts obtained from mentha showed most inhibitory effect followed by turmeric and marwatulsi respectively. Minimum hatching was recorded with mentha followed by turmeric and marwa tulsi after 12th day. The maximum egg hatching was recorded at 3thday in water. It is revealed from table 2 that all three factors *viz,* aqueous extracts, their concentrations and exposure time significantly affected hatching individually as well as in combination with one another.

The present investigation are in adjustable conformity with the finding of Prasad and Suverna (2005), who while testing four concentrations of root and leaf extracts of *C.procera* and *P. 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 *R..reniformis* race-A infesting sunflower *cv*. Morden as compared to the control. Similarly, Goel and Gupta (2004) 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 sameconcentration. 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*. (2004) 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. In the present investigation observations showed the nematicidal activities of plant extracts resulting inhibition of hatching and mortality of larvae of *M.incognita*.

CONCLUSION

Nematicides of plant origin include iso-thiocyanates, thiophenics, glucosides, alkaloids, phenolics and fatty acids(Grainge & Ahmed **,**1988).. Use of botanicals is now emerging as one of the important means to be used in protection of crop produce and the environment from pesticides pollution, which is a global problem Therefore it is important to examine new plants or new varieties of plants for their efficacy in immobilizing, retarding development or killing nematodes.The intensive use of synthetic pesticides and their environmental and toxicological risks have generated increased global interest in developing alternative sources of chemicals to be used in safe management of plant pests . These compounds can be developed for use as nematicides themselves, or can serve as model compounds for the development of chemically synthesized derivatives with enhanced activity and reduced environmental impacts. These antagonistic plants are excellent candidates because they can be used for developing as nematicides themselves, or they can serve as model

compounds for the development of chemically synthesized derivatives which enhanced activity or environmental friendliness (Chitwood, 2002). Nematicidal bio-active products of plants, being less persistent in environment, are safer for mammals and other non target organisms. Botanical pesticides are often readily available, cheaper than the synthetic counter parts and their crude extracts and are easy to be prepared even by farmers. They reduce the chances of development of resistance or resurgence in pests. Hence, in light of the the above information, the present investigation were proposed to be undertaken to investigate the role of a few nematoxic plants in the management practices which are economically and recofriendly in nature ensuring environmental safety

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