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Efficacy of botanicals on mortality of second stage juveniles and egg hatching of *Meloidogyne javanica* L: An eco-friendly management approach

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Abstract
Root knot nematode is an important pest in many vegetable, fruits and field crops. The extracts of seven different botanicals were tested for their effect on the egg hatching and mortality of *Meloidogyne javanica*. The results revealed that neem (*Azadirachta indica*) had the highest inhibitory effect and recorded lowest egg hatching percentage and highest larval mortality. It was followed by *Carrissa spinarum* (Wild Karonda) root extract. The inhibitory effect of the botanicals on egg hatching and larval mortality was directly proportional to the exposure period and inversely proportional to the concentration.

Keywords: *Meloidogyne javanica*, Neem, *Calotropis procera*, *Tagetes*, egg hatching, larval mortality

Introduction
Nematodes are the lower invertebrates which are highly diversified, bilaterally symmetrical, triploblastic, unsegmented, pseudocoelomic and multicellular animals. Generally the nematodes are free living in soil, and in marine and fresh water, but few genera are known to be parasitic in nature. Plant parasitic genera of nematodes exhibit three different kinds of behaviours: ectoparasitic, semi-endoparasitic and endoparasitic which lead to enormous crop losses. Endoparasites such as the species of *Meloidogyne*, *Globodera* and *Heterodera* are considered as the major agriculturally important pests.
In economic terms, the nematodes cause a loss of about US$157 billion annually to world agriculture production (Abad *et al*., 2008) [1]. The yield losses vary greatly depending upon the type of host species and the amount of inoculum. Several workers in India have attempted to assess the crop losses caused by plant parasitic nematodes. The genus *Meloidogyne* is worldwide considered as one of the most important genera of plant parasitic nematodes (Siddiqi, 2000; Dong *et al*., 2001; Trudgill and Blok, 2001; McK Bird and Kaloshian, 2003) [19, 6, 21, 16] having 97 valid species (Hunt and Handoo, 2009) [8]. Due to root knot nematode infestation yield loss of 35.0- 39.7 per cent was reported by (Reddy, 1985 and Jonathan *et al*., 2001) [17, 12]. Jain *et al*., (2007) [10] estimated the crop losses to Indian agriculture due to root-knot nematode are about Rs. 210 crores annually. The most well-known species of root-knot nematode are *Meloidogyne incognita*, *M. javanica*, *M. arenaria* and *M. hapla* which are responsible for high economic damage to varied crops (Khalil, 2013) [14].
The most effective method of nematode disease management is the use of synthetic chemical nematicides. But, in recent years, due to health hazards, adverse effects on the non-target organisms and the environmental issues, interest has been shifted in discovering nematostatic compounds of the plant origin (Chitwood, 2003) [4]. In nature, plants produce a number of secondary metabolites to defend themselves against various pests and diseases which possess nematicidal properties. These botanicals offer an alternate strategy for the nematode management due to their facile biodegradability, selective toxicity to target organisms and ecofriendly nature. The present study was aimed at identifying the potential botanicals with nematicidal properties which can be effectively used for nematode management.

Materials and Methods
The experiment was conducted to know the effect of botanicals on egg hatching of *M. javanica*.

Preparation of botanical extracts

Leaves of marigold *Tagetes patula* L. (French marigold) and *Tagetes erecta* L. (African marigold), *Calotropis procera* Ait, *Calotropis gigantea* Ait. (Aak) and *Azadirachta indica* A. Juss (Neem), rhizomes of *Acorus calamus* L. (Sweet flag) and roots of *Carissa spinarum* L. (Wild Karonda) were collected from nearby area and washed in running tap water followed by distilled water. These samples were air dried in the room temperature and grounded separately into powder by using electrical blender. The powder of leaves, rhizomes and roots were taken separately and soaked in 100 ml distilled water using an electrical mixer. These mixtures were kept for 24 hrs and filtered through muslin cloth followed by Whatman filter paper. The final volume of each filtrate was measured and considered as standard solution(s) (100 percent concentration) and then kept in the refrigerator until used for experiment.

Extraction of eggs from plant tissues

The experiment was conducted to know the effect of botanicals on mortality of *M. javanica*. Eggs of *M. javanica* were collected from two months old infected tomato plant using 2 per cent sodium hypochloride solution (Hussy and Barker, 1973) [9]**.** Galled roots with egg masses were washed free of soil, cut into 2 cm pieces and after placing in 2 per cent sodium hypochloride solution were shaken for 2 minutes. To separate the organic debris from eggs, this suspension was poured through a series of sieves. The eggs were collected on 38 µm-pore sieve and washed carefully with tap water. The egg suspension was poured on to a cotton-wool filter paper (Modified Baermann) and incubated at 27±1 ºC. The hatched second stage juveniles (J2) collected within 48 hrs.

Effect of botanical extracts on egg hatching of *M. javanica*.

The evaluation was carried out in tissue culture cavity blocks. There were seven treatments, three dosages and each experiment was replicated thrice. The Petri dishes with distilled water was taken as control. One hundered eggs of *M. javanica* were suspended in 1, 2 and 5 per cent concentration of stock solution of each botanical extract. All the Petri dishes were arranged in a completely randomized design and kept at ambient temperature 27±1ºC in BOD incubator. After 12, 24, 48 and 72 hours exposure period, the per cent hatched eggs were counted under microscope at magnification 40X (10 x 4).

Effect of botanical extracts on mortality of second stage larvae of *M. javanica*

Hundred freshly hatched second stage juveniles (J2) of *M. javanica* were placed into a tissue culture cavity blocks each containing 1 ml of the diluted botanical extracts of 1, 2 and 5 per cent and distilled water served as control. These were arranged in a completely randomized design (CRD) and replicated thrice on the laboratory bench. A count of dead juveniles was done at various time period of 12, 24, 48, and 72 hours using stereomicroscope. Nematodes that assumed the characteristic dead straight in position were trapped with a picking instrument to check if they would respond and when they did not, they were picked out of the extract. They were then placed in distilled water to confirm that they were dead.

Results and Discussion

Effect of botanical extracts on egg hatching of *M.javanica*.

Aqueous extracts of T. patula, T. erecta, C. spinarum, A. calamus, C. procera, C. gigantean and *A. indica* were applied in three concentrations (1, 2 and 5%) and exposure time for 12, 24, 48, 72, and 96 hours. Data on effect of plant extracts on hatching of *M. javanica* eggs are presented in Table 1. All the extracts showed inhibitory effect on the hatching of *M. javanica* eggs when overall effect was analysed. The proportion of egg hatching was directly proportional to exposure period and inversely proportional to concentration of extracts. Among plant extracts, *A. indica* showed most inhibitory effect followed by *C. spinarum*, *A. calamus*, *T. erecta*, *T. patula*, *C. procera*, and *C. gigantea*, respectively. It was observed that when compared to control, initiation of hatching was delayed in botanical treatments with increase in concentration of the botanicals. The highest rate of hatching was observed in 1 per cent while lowest rate at 5 per cent concentration in all plant extracts tested. The maximum hatching was recorded in control (93%) after end of the 96 hours. Among the botanicals, egg hatching were found maximum in *C. gigantea* followed by *C. procera*, *T. patula*, *T. erecta*, *A. calamus*, *C. spinarum*, and *A. Indica* at 1 per cent after the 96 hours, respectively.

Table 1: Effect of aqueous extracts of botanicals on the egg hatching of *Meloidogyne javanica*.

Treatment	Percent concentration	Egg hatching of nematodes and exposure time (hrs.)					Mean
		12	24	48	72	96	
T. patula	1	8	16	42	59	83	41.60
	2	6	14	34	52	76	36.39
	5	4	11	27	43	62	29.40
T. erecta	1	8	14	40	59	78	39.80
	2	5	11	32	51	70	33.80
	5	4	10	25	41	59	27.80
C. procera	1	9	17	45	64	85	44.00
	2	6	14	36	56	79	38.20
	5	4	9	28	47	66	30.80
C. gigantea	1	9	18	49	66	88	46.01
	2	7	15	41	60	82	41.00
	5	4	10	29	54	73	34.00
A. indica	1	7	12	28	51	68	33.20
	2	4	9	21	38	60	26.40
	5	2	5	15	26	46	18.80
C. spinarum	1	7	13	31	53	70	34.80
	2	5	11	25	40	64	29.00
	5	2	7	18	29	51	21.40
A. calamus	1	8	14	38	56	74	38.00
	2	5	11	27	49	68	32.00
	5	3	9	21	34	53	24.00
Control	In water	13	27	61	79	93	54.60
		SEm ±			C. D. at 5%		
Treatment (A)		0.19			0.54		
Concentration (B)		0.11			0.33		
Hours (C)		0.15			0.42		
Interaction (A X B)		0.33			0.93		
Interaction (A X C)		0.43			1.21		
Interaction (B X C)		0.26			0.74		
Interaction (A X B X C)		0.75			2.01		
CV		3.62					

Similar findings were obtained by Saravana Priya *et al*., (2004) [18], Kaur and Katoch (2012) [13], Chedekal (2013) [3], and Kumar and Nandlal (2016) [15]. Siddiqui and Alam (1985) [20] reported that the fresh extracts of fruits, leaf bark, root and gum of neem inhibited hatching of *Meloidogyne incognita*. Hatching of *M. incognita* juveniles was significantly reduced in root extracts of *Azadirachta indica* (Neem), *Ricinus communis* (Castor bean) and *Tagetes erecta* (Marigold) as well (Adegbite & Adesiyan, 2005) [2]. Alpha-terthienyl is believed to be the main allelopathic compound in marigold

responsible for nematode suppression (Gommers and Bakker, 1988) [7]. The neem leaves gave maximum inhibition over all the botanicals after 96 hours at 1, 2 and 5 per cent concentration, respectively (Javed *et al*., 2007) [11]**.** Similar results were observed when egg masses were placed in extracts of neem crude formulations (leaves and cake) and a refined product (Aza) formulations. Hatching did not occur at all the concentrations (10%, 5%, 2.5% and 1.25% w/v). But, when the treated egg masses were returned to water, the eggs resumed hatching.

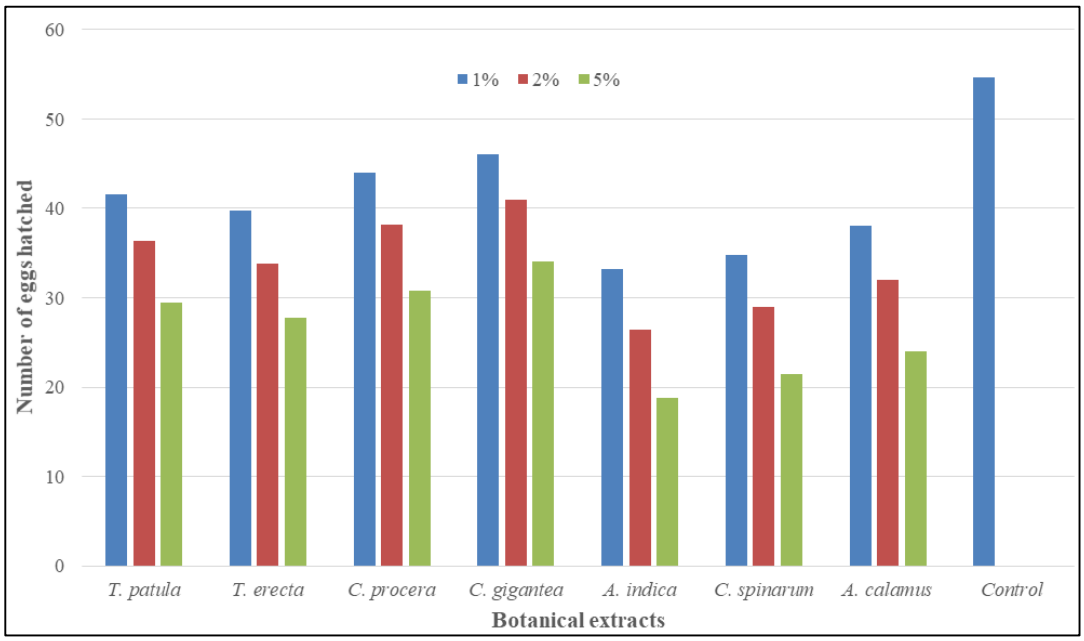

Fig 1: Effect of botanical extracts on egg hatching of *M. javanica*

Effect of aqueous extracts of botanicals on the mortality of second stage juveniles of *M. javanica*.

The effect of seven botanicals *viz. T. patula*, *T. erecta*, *C. spinarum*, *A. calamus*, *C. procera*, *C. gigantea* and *A. indica* at three concentrations (1, 2 and 5%) on mortality of second stage juveniles of *M. javanica* after 12, 24, 48, 72, and 96 hours of exposure were investigated. The results revealed that treatment of second stage juveniles of *M. javanica* with all the tested botanical extracts caused highly significant mortality of larvae, when overall effect was analysed (Table 2). *Azadirachta indica* extract was found to be the most effective whereas *C. gigantea* was least effective in causing mortality of second stage larvae of root-knot nematode. The inhibitory (killing) effect of all botanical extracts increased with increase in the concentration from 1 to 5 per cent and with increase in time of exposure of the nematode larvae from 12 to 96 hours. Neem leaves extract at 1, 2 and 5 per cent concentration caused significantly higher mortality of J2 larvae at exposure period of 96 hours compared to check. The increase in the mortality of larvae after 96 hours exposure at all the concentration was higher as compare to 12, 24, and 72 hour exposure. *A. indica* extract caused 68 per cent mortality of larvae at the end of 96 hours exposure (Table 2). The next best treatment was *C. spinarum* which showed highest mortality at 5 per cent concentration after 96 hours of exposure (36.79%). It was followed by A. *calamus*, *T. erecta*, *T. patula*, *C. procera* and *C. gigantea* with 34.60, 33.40, 31.60, 30.00, and 28.60 at 5 percent concentration after 96 hours of exposure, respectively.

Table 2: Effect of aqueous extracts of botanicals on the mortality of second stage juveniles of *Meloidogyne javanica.*

Treatment	Percent concentration	Percent immobilized nematodes and exposure time (hrs.)					Mean
		12	24	48	72	96	
T. patula	1	2	8	15	25	31	16.20
	2	5	11	22	29	39	21.20
	5	9	18	33	43	55	31.60
T. erecta	1	3	9	16	26	32	17.19
	2	6	12	23	30	41	22.40
	5	10	18	34	46	59	33.40
C. procera	1	3	7	15	21	29	15.20
	2	5	10	21	28	36	20.00
	5	9	16	31	41	52	30.00
C. gigantea	1	2	6	14	19	28	13.80
	2	5	9	20	26	36	19.20
	5	8	15	30	39	51	28.60
A. indica	1	4	12	19	29	34	19.60
	2	8	15	28	37	43	26.20
	5	12	23	45	52	68	40.00
C. spinarum	1	3	11	18	28	36	19.20
	2	7	14	26	34	47	25.60
	5	11	21	38	48	66	36.79
A. calamus	1	3	10	17	27	34	18.20
	2	7	12	24	32	43	23.60
	5	10	19	35	47	62	34.60
Control	In water	0	0	0	2	4	1.20
		SEm ±			C. D. at 5%		
Treatment (A)		0.18			0.52		
Concentration (B)		0.11			0.31		
Hours (C)		0.14			0.41		
Interaction (A X B)		0.32			0.90		
Interaction (A X C)		0.41			1.16		
Interaction (B X C)		0.25			0.71		
Interaction (A X B X C)		0.72			2.02		
CV		5.84					

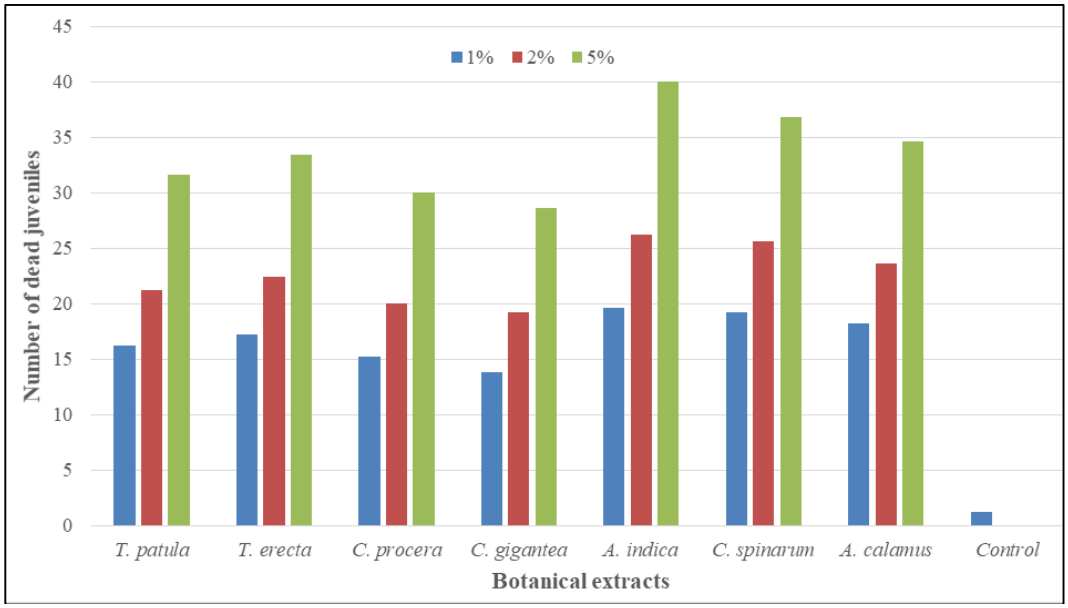

Fig 2: Effect of botanical extracts on mobility of *M. javanica juveniles*

Similar observations were reported by Javed *et al*. (2007) [11]. He observed that when second stage juveniles of *Meloidogyne javanica* was exposed to aqueous extracts of neem crude formulations (leaves and cake), the 10 per cent extract was most effective with 83 and 85 per cent mortality, respectively. According to Devi *et al*. (2011) [5], *Acorus calamus* L. was highly effective against second stage larvae of *Meloidogyne incognita*. Cent per cent larval mortality was obtained with aqueous extracts of rhizome of *A. calamus* at 5 and 10 percent concentration in 96 hours and 72 hours of treatments respectively. Chedekal (2013) [3] exposed the second stage juveniles of *Meloidogyne incognita* to aqueous extracts from fresh leaves of *C. procera*, *A. indica*, *Clerodendrum inerme* and *Lantana camara*. The highest mortality of second stage juveniles was observed in leaf extracts of *A. indica* (90.17%) whereas least mortality was observed in *C. procera* (60.33%).

Conclusion

Thus, it can be concluded that the botanical extracts were highly effective for the management of root knot nematode. The extracts were found to reduce the egg hatching and cause the mortality of the larvae. The inhibitory effect increased with increase in concentration and exposure time. It can be considered as an alternative for the chemical nematicides. The botanicals thus offer an effective and eco-friendly strategy for the management of these hidden enemies of the crop.

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