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Evaluation of Botanicals Toxicants against Root-knot Nematode, *Meloidogyne incognita in vitro*

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Authors' contributions

This work was carried out in collaboration between all authors. Author MAS designed the study and guided. Author AK performed the experiment, wrote the protocol and wrote the first draft of the manuscript. Authors MT and MA managed the analyses of the study and literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The present experiment was carried out to investigate the nematostatic and nematicidal potential of aqueous extracts of six weed leaves *viz*., *Ageratum conyzoides, Eichornia crassipes, Ipomoea carnea, Nicotiana plumbaginifolia, Acalypha indica* and *Trianthema portulacastrum* against root-knot nematode, *M. incognita in vitro*. The eggs and second stage juveniles (J2) of *M. incognita* were exposed to various concentrations *viz*., 1500 ppm, 1000 ppm, 500 ppm and 250 ppm of aqueous extracts of leaves. All the aqueous extracts damaged the eggs, reduced the hatching and caused paralysis and death of second stage juveniles (J2) of *M. incognita*. The highest inhibition in hatching and maximum mortality occurred by using *A. conyzoides* followed by *E. crassipes, I. carnea, N. plumbaginifolia, A. indica* and the least was observed by using *T. portulacastrum*. It can be concluded that the degree of effectiveness was found directly proportional to the dilutions of extract. The 1500 ppm concentration of aqueous extract of all weeds was found to be highly effective against hatching and mortality of *M. incognita* as compared to other concentrations.

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1. INTRODUCTION

The root-knot nematode, *Meloidogyne* spp. is a major agricultural pest. It damages a broad range of crops particularly vegetable crops, causing dramatic yield loss mainly in tropical and subtropical agriculture [1]. This pest is responsible for approximately 50% of overall damage [2]. Root-knot nematode attacks over 2000 plant species causing stunted growth and finally low yield [3]. The pest is the most pathogenic nematode in agriculture, causing an estimated yearly crop loss of $100 billion worldwide [3]. The root-knot nematode is controlled through the use of chemical nematicides, crop rotation, organic amendments and biocontrol agents. Though, the use of nematicides has been the most effective method for the root-knot nematode management, but they are hazardous in nature. Therefore, there is a necessity to search new, cheap and eco-friendly methods of nematode management. Hence, the use of plant extracts is gaining attention due to their availability, cost effectiveness, pollution-free and enivironmently safe. Many botanical extracts have been found to contain phytochemicals such as alkaloids, tannins, saponins, flavonoids, diterpenes, glucosinolates, acetylenes and thienyls [4] which are effective against plant-parasitic nematodes [5]. Botanical extracts that contain alkaloids and flavonoids were found to have ovicidal property against *Meloidogyne* eggs [5]. Hence, the aim of this study was to determine the effect of aqueous extract of leaves obtained from six weeds *viz*., *A. conyzoides, E. crassipes, I. carnea, N. plumbaginifolia, A. indica* and *T. portulacastrum* on egg hatching and mortality of second stage juveniles of *M. incognita* under *in vitro* and to ascertain their role as a bionematicide to control root-knot nematode. The main aim of the present research use to prove;

1) Extract of weeds would inhibit the hatching of root-knot nematode and or may be lethal to the juveniles.
2) The potentials of aqueous extract for the control of root-knot *Meloidogyne incognita.*

2. MATERIALS AND METHODS

2.1 Collection of Root-knot Nematode

The roots of brinjal (*Solanum melongena* L., Family- Solanaceae) heavily infected with root-knot nematode (*Meloidogyne incognita*) were collected from the agricultural field, Panjipur Village located near the district Aligarh, Uttar Pradesh. The plants were gently uprooted, removed the soil particles adhering to the roots and brought to laboratory for further examination. The infected roots were gently washed with running tap water and observed for the presence of eggmasses.

2.2 Preparation of Nematode Inoculum

Eggmasses were handpicked using sterilized forceps from heavily infected roots. Collected eggmasses were washed in Double Distilled Water (DDW) and then, placed in mesh sieves (8 cm in diameter) containing a cross layer of tissue paper, placed in petridishes containing water just deep enough to cover the eggmasses and left for 1-3 days. The second stage juveniles (J2) hatched out from eggmasses were collected in a beaker. The identification of root-knot nematode, *M. incognita* was done on the basis of parenial pattern. The inoculum was homogenized by counting the number of second stage juveniles (J2) per ml of DDW in counting dish under stereoscopic microscope.

2.3 Preparation of Extracts

Leaves of six weeds *viz*., *A. conyzoides, E. crassipes, I. carnea, N. plumbaginifolia A. indica* and *T. portulacastrum* were washed and weighed 25 g, was air dried and were grinded in mortar and pestle to make the powder form. Then, take 1.5 g of dried leaves powder of each weed mixed with 1000 ml DDW. After mixing, the material put out in centrifuge tube and centrifuged at 10000 RPM for 10 minutes. The centrifuged supernatant was filtered through Whatman,s No-1 filter paper and filtrate was termed as 1500 ppm solution and diluted to 1000 ppm, 500 ppm and 250 ppm concentration by adding required amount of DDW.

2.4 Mortality Test

Approximately, 100 freshly hatched second stage juveniles (J2) of *M. incognita* were transferred separately in to petridishes containing 10 ml of different concentrations (1500 ppm, 1000 ppm, 500 ppm, 250 ppm) of aqueous extract of leaves. Petridish containing DDW served as control. Each treatment was replicated five times. The petridishes were kept at 28°C. The dead nematodes were counted

after 24, 48 and 72 hours of exposure period and the mean percentage of mortality was calculated. Mortality of nematodes was counted after the juveniles (J2) were transferred to DDW. Regression Equation was calculated to.....?

2.5 Hatching Test

For hatching experiments, five medium size and healthy eggmasses of root-knot nematode, *M. incognita* (with approximataly 380 eggs) were handpicked with the help of sterile forceps and transferred separately into petridishes containing 10 ml of aqueous extract of different concentration (1500 ppm, 1000 ppm, 500 ppm, 250 ppm) of each weed. Each treatment was replicated five times including DDW which served as control. The numbers of hatched juveniles were counted after 5 days and the percentage inhibition was calculated.

Percentage inhibition in hatching =

$$\frac{\text{Control (Distilled Water)} \times \text{Treated}}{\text{Control (Distilled Water)}} \times 100$$

2.6 Statistical Analysis

The data of the experiments were analyzed statistically using the Statistical Package for the Social Sciences SPSS 12.00 Software (SPSS Inc., Chicago, IL, USA) for analysis of variances (ANOVA). Duncan's Multiple Range Test was employed to test for significant difference between the treatments.

3. RESULTS

The results of the present study showed the nematicidal effect of aqueous extracts of six weeds *viz*., *A. conyzoides, E. crassipes, I. carnea, N. plumbaginifolia, A. indica* and *T. portulacastrum* on hatching and mortality of *M. incognita in vitro*. The data presented in tables 1-4 revealed that all the weed extracts have nematicidal potential. Among all the treatments, maximum percentage inhibition of second stage juveniles (J2) was observed by using *A. conyzoides* followed by *E. crassipes, I. carnea, N. plumbaginifolia A. indica* and *T. portulacastrum* (Table 1) at different tested concentrations after 5 days. The control showed 100% egg hatchability (340) because it contained only Double Distilled Water (DDW). Aqueous extract of all the tested weeds showed gradual decrease in egg hatching from their lower concentration to higher concentration. Aqueous extract of 250 ppm concentration did not affect egg hatching and caused only a trifling suppression of second stage juvenile's hatching. However, concentration of 500 ppm and 1000 ppm were effective in reducing juveniles hatching from eggs (Table 1). It was observed that with increase in the dilution extract the nematicidal properties of extract were decreased.

The results presented in Tables 2, 3 and 4 showed that the weed extract of *A. conyzoides, E. crassipes, I. carnea, N. plumbaginifolia, A. indica* and *T. portulacastrum* cause toxic effect on second stage juvenile of *M. incognita.* The aqueous extract of *A. conyzoides* showed the highest mortality. The least was observed in *T. portulacastrum* at different concentrations. In DDW (control) there was no mortality after 24 hours of exposure time (Table 2). The highest mortality of second stage juveniles (J2) after 48 hours of the exposure was depicted in the aqueous extract of *A. conyzoides* and the lowest percentage mortality was noticed in *T. portulacastrum* at different concentrations as compare to DDW (control). Other aqueous extracts of weeds *viz*., *E. crassipes, I. carnea, N. plumbaginifolia* and *A. indica* showed minimum percent mortality (Table 3).

The most prominent mortality of juveniles was observed in the aqueous extract *of A. conyzoides* and least in *T. portulacastrum* after 72 hours of exposure time (Table 4). The 24 hours duration showed minimum mortality, while 72 hours of the exposure showed maximum. The extract of 1500 ppm concentration after 72 hours of exposure period recorded maximum toxicity. During the experiment, the *A. conyzoides* and *E. crassipes* leaves extracts showed the most prominent effect on second stage juvenile's mortality and hatching of *M. incognita*. It might be due to the presence of various types of phytocompounds in aqueous extract of weeds.

4. DISCUSSION

The present study revealed that the all the aqueous extracts of weeds *viz*., *A. conyzoides, E. crassipes, I. carnea, N. plumbaginifolia, A. indica* and *T. portulacastrum* showed inhibition in hatching and caused mortality of *M. incognita.* Our results indicate that aqueous extract of *A. conyzoides* was the most effective against *M. incognita*. The nematicidal activity of aqueous extract of weeds against *M. incognita* may be due to the presence of various phytochemicals *viz*., alkaloids, flavonoids, glycosides, saponins, tannin and terpinoids.

Table 1. Effect of aqueous extract of some weed species on the egg hatching of *M. incognita in vitro* after 5 days

Treatment	Number of juvenile hatched in different concentration				
	1500 ppm	1000 ppm	500 ppm	250 ppm	DW
Ageratum conyzoides	55^{c} ±4.93 (85.52%)	76^{e} ±4.61 (80.00%)	87^{d} ±3.51 (77.10%)	98^{e} ±4.58 (74.21%)	380 (0%)
Eichornia crassipes	69^{c} ±5.85 (81.84%)	79de ±4.93 (76.57%)	101^{c} ±4.58 (73.42%)	116^{d} ±2.30 (69.47%)	380 (0%))
Ipomoea carnea	85^{b} ±4.04 (77.63%)	98^{cd} ±5.85 (74.21%)	112^{c}±4.61 (70.52%)	128^{cd} ±3.51 (66.31%)	380 (0%)
Nicotiana plumbaginifolia	93^{ab} ±4.35 (75.52%)	107^{c} ±2.64 (71.84%)	126^{b} ±3.21 (66.84%)	142^{c}±4.61 (62.63%)	380 (0%)
Acalypha indica	98^{ab} ±4.16 (74.21%)	117^{ab} ±3.60 (69.21%)	139^{b} ±3.78 (63.42%)	167^{b} ±4.00 (56.05%)	380 (0%)
Trianthema portulacastrum	109^{a} ±5.56 (71.31%)	124^{a} ±3.05 (67.36%)	154^{a} ±5.50 (59.47%)	187^{a} ±3.60 (50.78%)	380 (0%)

Each value is an average of five replicates, DW=Distilled Water (Control)
Values are given in parentheses represent percentage inhibition in juvenile hatching over control.
Values are given without parentheses represent number of the hatched juveniles of Meloidogyne incognita at different concentrations.
Means in each column followed by the same letter are not significantly different according to Duncan Multiple Range Test ($p \leq 0.05$)

Table 2. Effect of aqueous extracts of some weed species on the mortality of *M. incognita* juveniles *in vitro* after 24 hours

Treatment	Percentage mortality in different extract					Regression equation
	1500 ppm	1000 ppm	500 ppm	250 ppm	DW	
Ageratum conyzoides	62^{a} ±4.61 (64.80%)	50^{a} ±4.93 (50.30%)	38^{a} ±3.78 (35.80%)	29^{a} ±4.72 (21.30%)	0 (6.80%)	ỹ =35.8+14.5(x-2)
Eichornia crassipes	53^{ab} ±4.04 (57.20%)	44^{ab} ±3.05 (63.60%)	31^{ab} ±4.50 (30.00%)	22^{ab} ±3.78 (16.40%)	0 (4.67%)	ỹ =30+13.6(x-2)
Ipomoea carnea	44^{bc} ±4.35 (45.80%)	36^{bc} ±3.46 (35.30%)	25^{bc} ±2.51 (24.80%)	19^{b} ±2.30 (14.30%)	0 (3.80%)	ỹ =24.8+10.5(x-2)
Nicotiana plumbaginifolia	36^{cd} ±3.46 (35.60%)	27^{cd} ±3.00 (27.20%)	19^{cd} ±3.60 (18.80%)	13^{bc} ±3.46 (10.40%)	0 (2.00%)	ỹ =18.8+8.4(x-2)
Acalypha indica	30de ±5.03 (29.60%)	20de ±3.21 (21.80%)	12de ±3.51 (14.00%)	8^{c}±2.08 (6.20%)	0 (1.60%)	ỹ =14+7.8(x-2)
Trianthema portulacastrum	22^{e} ±4.16 (19.80%)	13^{e} ±2.64 (14.60%)	7^{e} ±1.73 (9.40%)	5^{c} ±1.52 (4.20%)	0 (1.00%)	ỹ =9.4+5.2(x-2)

Each value is an average of five replicates, DW=Distilled Water (Control).
Values are given in parentheses represent percentage juvenile mortality over control.
Values are given without parentheses represent number of the dead juveniles of Meloidogyne incognita at different concentrations.
Means in each column followed by the same letter are not significantly different according to Duncan Multiple Range Test ($p \leq 0.05$)

Table 3. Effect of aqueous extracts of some weed species on the mortality of *M. incognita* juveniles *in vitro* after 48 hours

Treatment	Percentage mortality in different extract					Regression equation
	1500 ppm	1000 ppm	500 ppm	250 ppm	DW	
Ageratum conyzoides	75^{a} ±5.48 (82.6%)	65^{a} ±4.61 (64.5%)	58^{a} ±5.68 (47.0%)	40^{a} ±4.35 (29.5%)	0 (12%)	ỹ =47+17.5(x-2)
Eichornia crassipes	70^{a} ±4.93 (77.0%)	61^{a} ±7.37 (60.5%)	53^{a} ±4.35 (44.0%)	36^{ab} ±3.78 (27.5%)	0 (11.0%)	ỹ =44+16.5(x-2)
Ipomoea carnea	61^{ab} ±6.55 (72.2%)	54^{ab} ±5.19 (54.4%)	43^{ab} ±6.52 (37%)	27^{abc} ±5.85 (19.4%)	0 (7.9%)	ỹ =37+17.6(x-2)
Nicotiana plumbaginifolia	53^{bc} ±4.04 (55.4%)	42^{bc} ±6.50 (42.8%)	34^{bc} ±4.16 (30.2%)	22^{bcd} ±6.42 (18.76%)	0 (5.0%)	ỹ =30.2+12.6(x-2)
Acalypha indica	45^{c}±5.12 (47.6%)	35^{c} ±4.93 (36.7%)	30^{bc} ±5.77 (25.8%)	18^{cd} ±4.58 (14.9%)	0 (4.0%)	ỹ =25.8+10.9(x-2)
Trianthema portulacastrum	37^{c} ±3.60 (38.2%)	30^{c} ±5.13 (29.1%)	22^{c} ±3.21 (20.0%)	11^{d} ±2.08 (10.7%)	0 (1.8%)	ỹ =20+9.1(x-2)

Each value is an average of five replicates, DW=Distilled Water (Control)
Values are given in parentheses represent percentage juvenile mortality over control.
Values are given without parentheses represent number of the dead juveniles of Meloidogyne incognita at different concentrations.
Means in each column followed by the same letter are not significantly different according to Duncan Multiple Range Test ($p \leq 0.05$)

Table 4. Effect of aqueous extracts of some weed species on the mortality of *M. incognita* juveniles *in vitro* after 72 hours

Treatment	Percentage mortality in different extract					Regression equation
	1500 ppm	1000 ppm	500 ppm	250 ppm	DW	
Ageratum conyzoides	88^{a} ±5.77 (97.2%)	78^{a} ±5.68 (76.9%)	66^{a} ±5.03 (56.6%)	51^{a} ±5.85 (36.3%)	0 (16%)	ỹ =56.6+20.3(x-2)
Eichornia crassipes	84^{ab} ±6.11 (92.4%)	74^{ab} ±4.61 (72.9%)	62^{ab} ±4.61 (53.4%)	47^{a} ±4.04 (33.9%)	0 (14.4%)	ỹ =53.4+19.5(x-2)
Ipomoea carnea	78^{abc} ±4.61 (85.2%)	68^{ab} ±6.11 (66.6%)	56^{ab} ±3.46 (48.0%)	38^{ab} ±6.08 (29.4%)	0 (10.8%)	ỹ =48+18.6(x-2)
Nicotiana plumbaginifolia	69^{bc} ±5.85 (74.4%)	59^{bc} ±7.09 (57.8%)	47^{bc} ±5.29 (41.2%)	31^{bc} ±5.19 (24.6%)	0 (8.0%)	ỹ =41.2+16.6(x-2)
Acalypha indica	61^{cd} ±6.24 (63.6%)	48^{cd} ±4.61 (49.1%)	39^{cd} ±4.35 (34.6%)	25^{bc} ±3.60 (20.1%)	0 (5.6%)	ỹ =34.6+14.5(x-2)
Trianthema portulacastrum	49^{d} ±4.72 (50.0%)	38^{d} ±5.29 (38.0%)	27^{d} ±5.19 (26.0%)	16^{c} ±2..64 (14.0%)	0 (2.0%)	ỹ =26+12(x-2)

Each value is an average of five replicates, DW=Distilled Water (Control).
Values are given in parentheses represent percentage juvenile mortality over control.
Values are given without parentheses represent number of the dead juveniles of Meloidogyne incognita at different concentrations.
Means in each column followed by the same letter are not significantly different according to Duncan Multiple Range Test ($p \leq 0.05$)

The hatching rate was directly proportionate to exposure period. When eggs were transferred into the DDW (control), the inhibition in hatching decreased which mean that weed extracts have nematostatic effects. As the exposure period increased from 24 to 72 hours, mortality of *M. incognita* and reduction in hatching was also increased in a successive manner. The percentage mortality and hatching was highly influenced by concentration of extract and duration of exposure period.

Among the identified phytochemicals in *E. crassipes,* n-hexadecanoic, ethyl ester and palmitic acid have the antifungal, nematicidal and pesticidal property [6]. Phytochemical analysis also revealed that plant is rich in alkaloids, phenols, terpenoids and flavonoids which have high rate of nematicidal activity [7]. During the last decade, research on nematode control was focused on proposing strategies for inhibition of egg hatching [8] and enhanced juvenile mortality [9]. *Ageratum conyzoides* showed nematicidal as well antibacterial activity due to the abundant presence of phtyocompounds [10], including alkaloids, flavonoids, tannin, saponins and phenol. *Datura* species contains rich source of alkaloids like atropine, meteloidine, nicotine, scopolamine, hyoscyamine, terpenoids and flavonoids which have high rate of nematicidal activity [7]. It was reported that extracts of neem leaf and garlic bulb completely inhibited hatching of egg masses of *M. incognita* and were lethal to larvae [11].

It was reported that lantanilic acid, camaric acid and oleanolic acid were isolated from the methanolic extract of the aerial parts of *Lantana camara* and exhibited significant mortality against root-knot nematode, *Meloidogyne incognita* [12]. It has been reported that aqueous extract of the plant *viz., Argemone Mexicana, Achyranthus aspera* and *Ricinus communis* cause juvenile mortality and inhibition in hatching of second stage juveniles of *M. incognita in vitro* [13]. The findings of present experimental work advocate a new and novel alternative to evaluating nematicidal effect against *M. incognita.* Therefore, it was found that the infestation caused by *M. incognita* could be lowered by the plant products in view of eco-friendly environment. This has an advantage against expensive and hazardous chemical nematicides which have toxic effect on flora and fauna of environment.

5. CONCLUSION

From the above study it may be concluded that all the aqueous extracts of weeds showed nematicidal activity against *M. incognita in vitro.* It may be due to the presence of various phytochemicals in aqueous extract of weeds which showed toxic effect on survivality of root-knot nematode. This method of nematode control may contribute to minimize toxicity and the hazardous nature of chemical nematicides on environment and human. Hence, the outcome of the results revealed that the aqueous extract of several weeds may provide safe and environmentally reliable alternative for the root-knot nematode management programme.

SIGNIFICANCE STATEMENT

Among all the weeds *Ageratum conyzoide* and *Eichornia crassipes* was found to be most effective against the second stage juveniles (J2) and hatching inhibition of *Meloidogyne incognita in vitro*. Utilization of weeds extract is one of the safe and eco-friendly methods for the management of root-knot nematode without any toxic effect on human and environment.

The use of chemical nematicides is one of the most effective method for the management of *M. incognita* but due to their hazardous effect on environment, lead to the search out of strategy that may be positive and eco-friendly for the sustainable nematode management.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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