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Research Article
Phytochemical Investigation, Nematostatic and Nematicidal Potential of Weeds Extract Against the Root-knot Nematode, *Meloidogyne incognita In Vitro*

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Abstract

Background and Objective: Root-knot nematode (RKN), *Meloidogyne incognita* is a catastrophic phytonematodes parasite causing enormous losses to wild as well as cultivated crops particularly vegetables. The objective of the study was to evaluate the nematicidal activity of leaf extracts of six different weed viz., *Argemone mexicana*, *Achyranthes aspera, Ricinus communis, Acalypha indica, Parthenium hysterophorus* and *Trianthema portulacastrum* were tested for phytochemical examination and against egg hatching and mortality of root-knot nematode, *Meloidogyne incognita* under *in vitro* condition. **Materials and Methods:** Leaves of six weeds were thoroughly washed. Weigh 25 g leaf and grind in mortar and pestle with 75 mL of distilled water. The egg masses and second stage juveniles (J2) of *M. incognita* were exposed to 24, 48 and 72 h in various concentrations (S, S/2, S/10, S/100) of leaf extracts of the six different weeds. Phytochemicals analysis of *A. mexicana* and *A. aspera* was done manually. The data were analyzed by one-way analysis of variance (ANOVA) using SPSS 17.00 software. **Results:** During the experiment, almost all the weeds species exhibited nematostatic as well as nematicidal potential. Leaf extracts of *A. mexicana* and *A. aspera* found to be highly toxic against the root-knot nematode. Standard concentration (S) of leaf extracts of *A. mexicana* and *A. aspera* showed highest hatching inhibition and J2 mortality of *M. incognita*. **Conclusion:** It is concluded that the inhibition of egg hatching and mortality of *M. incognita* juveniles may be due to the phytochemicals viz., alkaloids, flavanoids, phenols, tannins, saponins, phytosteroids and mucilage/gum present in the aqueous extract of the weeds. Egg inhibition and J2 mortality decreased with an increase in the dilution of all the extracts. There was a gradual decrease in egg hatching and increase in mortality rate of J2 of *M. incognita* with increasing in concentration of leaves extract and exposure time.

Key words: Egg hatching, juvenile (J2) mortality, *Meloidogyne incognita*, nematicidal activity, phytochemicals

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The root-knot nematode, *Meloidogyne incognita*, is a major plant-parasitic nematode affecting the quantity and quality of various annual and perennial crops in the world[1] and are responsible for approximately 50% of overall damage[2]. *Meloidogyne* spp., cause annual losses of about USD$ 100 billion worldwide[3]. The impact of this nematode genus is enhanced by its wide host range of more than 5,000 plant species and it causes severe economic losses in many agricultural and horticultural crops[4]. Management of nematodes is difficult to control because of their wide host range and having high rate of reproduction, capable of producing thousand of eggs per female[5]. Use of chemical nematicides is usually more effective than other strategies but on the other hand, they have caused significant environmental problems due to their toxic residues and associated environmental damage that resulted in severe restrictions on their use[6]. Several plant products have an eco-friendly and toxicologically safe, selective and effective nematicidal potential[7]. The nematicidal properties of plants due to the presence of compounds such as isothiocyanates, thiophenics, glucosides, alkaloids, phenolics, thianins and fatty acids have been identified and reviewed[8]. Therefore the use of organic soil amendments can provide safe and pollution free management of root-knot nematode. Several examples of naturally occurring compounds which suppressive *Meloidogyne* spp., have been identified. These include a glycoside (asparagusic acid) isolated from *Asparagus officinalis* L.[9] and two novel nematicidal compounds namely nonacosane-10-ol and 23a-homostigmast-5-en-3b-ol recently isolated from the roots of *Fumaria parviflora* Lam.[10]. The potential use of organic amendments, plants extract and biofertilizers is gaining considerable attention because of being safe cheap and ecofriendly nature[11-16]. Phytochemicals have received increasing attention during the last decade due to their biological importance and having potential health effects, such as antioxidant, anticancer, anti-aging, antiatherosclerotic, antimicrobial and anti-inflammatory activities[17]. Because of their ubiquity, found in abundance quantity and low cost, many phytochemicals have been isolated and identified from various plants such as fruits, vegetables, spices, cereals and medicinal herbs[18]. Till now thousands of plants and their parts have been screened for the isolation of various phytochemicals which having the potential against different pests and also use in are in commercial formulation[19,20]. Phytochemicals are non-nutritive obtain from plant that have protective or disease preventive properties[21]. The potential of using different plants extract for the management of root-knot nematode, *M. incognita* has been shown by different studies[22]. The main objective of the present study was to evaluate the effect of leaves of various weeds on hatching and mortality of *M. incognita* and to ascertain their role as a bionematicide to control root-knot nematode. The objectives of this study were also to detect weather:

- Extract of weeds would inhibit root-knot nematode and or may be lethal to the juveniles
- Phytochemical screening of extract
- To examines the main constituents responsible for second stage juvenile (J2) inhibition and morality

MATERIALS AND METHODS

Culture of nematodes: Root-knot nematode, *Meloidogyne incognita*, (Kofoid and White) Chitwood was choosing as the test pathogen. The reproduction of *M. incognita* mainly occur in the month of September-October when temperature range between 25-30°C. The culture of root-knot nematode, *M. incognita* was preserved on potted brinjal plants. The infected plants were uprooted, carefully washed in running tap water 3-4 times. The egg masses were handpicked using sterilized forceps from roots. These eggmasses were washed in distilled water and then placed in 15 mesh sieves (8 cm in diameter) containing crossed layer of tissue paper and placed in petri dishes containing water just deep enough to contact the egg masses.

Preparation of extract: Leaves of six weeds viz., *A. mexicana, A. aspera, Ricinus communis, Acalypha indica, Parthenium hysterophorus* and *Trianthema portulacastrum* were thoroughly washed. Weigh 25 g leaf from each weeds and grind in mortar and pestle with 75 mL of distilled water. After grinding the material taken in to centrifuge tube and centrifuged at 6000 rpm for 10 min. The centrifuged supernatant was filtered through Whatman's No. 1 filter paper and filtrate as termed as standard solution (S). The extract (S) was diluted to S/2, S/10, S/100 with adding desired amount of distilled water in standard solution.

Hatching test: Five healthy, equal sized and freshly picked eggmasses of *Meloidogyne incognita* have been selected from infected roots of brinjal (*Solanum melongena*, L. Family: Solanaceae) for the experiment. The eggmasses were transferred in to petri dishes containing 5 mL of leaf extract of different weeds of various concentrations (S, S/2, S/10 and

Table 1: Effect of aqueous extract of fresh chopped leaves of different weeds species on the second stage juvenile hatching of *M. incognita in vitro*

	No. of juvenile hatched in different concentration after 5 days									
	S		S/2		S/10		S/100		DW	
Treatments	n	%	n	%	n	%	n	%	n	%
Argemone mexicana	0	100	14	95.88	45	86.76	77	77.35	340	0
Achyranthus aspera	0	100	18	94.70	58	82.94	93	72.64	340	0
Ricinus communis	0	100	23	93.23	67	80.29	108	68.23	340	0
Parthenium hysterophorus	0	100	28	91.76	76	77.64	120	64.70	340	0
Acalypha indica	3	99.11	36	89.41	84	75.29	125	63.23	340	0
Trianthema ptulacastrum	5	98.52	44	87.05	93	72.64	138	59.41	340	0
LSD at 5%	1.32		9.60		12.76		13.03		0	
LSD at 1%	18.71		28.63		19.19		17.95		0	

Each value is an average of three replicate, DW: Distilled water (Control), n (%), values for percent inhibition in juvenile hatching over control are given in parentheses and values given in without parentheses represent number of hatched juveniles of *M. incognita* in different concentration of aqueous extract

Table 2: Effect of aqueous extract of chopped leaf of different weeds species on the mortality of *M. incognita* juveniles in *vitro* after 24 h

	S		S/2		S/10		S/100		DW		
Treatments	n	%	n	%	n	%	n	%	n	%	Regression equation
Argemone mexicana	77	81.4	63	63.5	48	45.6	40	27.7	0	9.8	$\tilde{y}$ = 45.6+17.9 (x-2)
Achyranthus aspera	74	80.4	59	62.6	44	44.8	37	27.0	0	9.2	$\tilde{y}$ = 44.8+17.8 (x-2)
Ricinus communis	67	70.32	54	54.86	41	39.4	35	23.94	0	8.48	$\tilde{y}$ = 39.4+15.46 (x-2)
Parthenium hysterophorus	63	66.56	50	51.38	39	36.2	29	21.02	0	5.84	$\tilde{y}$ = 36.2+15.18 (x-2)
Acalypha indica	58	60.9	46	46.9	36	33.4	27	19.9	0	6.4	$\tilde{y}$ = 33.4+13.5 (x-2)
Trianthema portulacastrum	52	54.9	43	42.7	32	30.5	25	18.3	0	6.1	$\tilde{y}$ = 30.5+12.2 (x-2)
LSD at 1%	18.71		28.63		19.19		17.95		0		
LSD at 5%	13.35		20.42		13.68		12.80		0		

Value is an average of three replicates, DW: Distilled water (control), values given in parentheses present percent mortality over control and values given in without parentheses represent number of dead juveniles of *M. incognita* in different concentration of aqueous extract

Table 3: Effect of aqueous extract of chopped leaf of different weeds species on the mortality of *M. incognita* juveniles *in vitro* after 48 h

	S		S/2		S/10		S/100		DW		
Treatments	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)	Regression equation
Argemone mexicana	75	65.40	58	53.32	44	40.80	32	28.28	0	15.40	$\tilde{y}$ = 40.8+12.52 (x-2)
Achyranthes aspera	73	73.40	54	56.30	40	39.20	29	22.10	0	5.00	$\tilde{y}$ = 39.2+17.10 (x-2)
Ricinus communis	65	65.42	50	49.91	32	34.40	25	18.89	0	3.38	$\tilde{y}$ = 34.4+15.51(x-2)
Parthenium hysterophorus	62	61.20	46	46.30	28	31.40	21	16.50	0	1.60	$\tilde{y}$ = 31.4+14.90 (x-2)
Acalypha indica	60	58.00	43	43.50	24	29.00	18	14.50	0	0.00	$\tilde{y}$ =29+14.50 (x-2)
Trianthema portulacastrum	55	52.80	39	39.50	21	26.20	16	12.90	0	-0.40	$\tilde{y}$ =26.2+13.30 (x-2)
LSD at 1%	18.71		28.63		19.19		17.95		0		
LSD at 5%	13.35		20.42		13.68		12.80		0		

Each value is an average of three replicates, DW: Distilled water (control), values given in parentheses present percent mortality over control and values given in without parentheses represent number of dead juveniles of *M. incognita* in different concentration of aqueous extract

S/100). Each treatment having three replicates. After 5 days total number of hatched larvae was counted and compared percent with control (Table 1). In control set hatching is done in distilled water there is no leaf extract (standard solution).

Mortality test: Five milliliter of water suspension containing 100 sec stage juveniles (J2) of *M. incognita*, added in petri dishes containing various concentrations (S, S/2, S/10, S/100) of leaf extracts of different weeds according to the method[23]. Each treatment having three replicates. The petri dishes were kept at 28±2°C. The immobilized second stage juveniles (J2) were counted after 24, 48 and 72 h of exposure period (Table 2-4). The mean percentage of mortality was calculated. However distilled water served as control.

Phytochemical screening: Phytochemicals analysis of two weed namely *A. mexicana* and *A. aspera* was done because these two weed are highly toxic to juveniles and eggmasses of *M. incognita in vitro* test. That's why phytochemical analysis of *A. mexicana* and *A. aspera* was done to determine the various components which are toxic to root-knot nematode, *Meloidogyne incognita*. The plant extracts were screened to

Table 4: Effect of aqueous extract of chopped leaf of different weeds species on the mortality of *M. incognita* juveniles *in vitro* after 72 h

	S		S/2		S/10		S/100		DW		
Treatments	n	%	n	%	n	%	n	%	n	%	Regression equation
Argemone mexicana	85	98.92	79	79.56	72	60.20	65	40.84	0	22.20	$\tilde{y}$ = 60.2+19.36 (x-2)
Achyranthes aspera	80	92.36	75	74.48	68	56.60	60	38.72	0	20.84	$\tilde{y}$ = 56.6+17.88 (x-2)
Ricinus communis	76	88.20	69	69.68	63	52.60	55	35.52	0	17.00	$\tilde{y}$ = 52.6+17.08 (x-2)
Parthenium hysterophorus	71	79.20	62	63.60	59	48.00	48	32.40	0	16.80	$\tilde{y}$ = 48+15.60 (x-2)
Acalypha indica	66	75.20	58	59.60	53	44.00	43	28.40	0	12.80	$\tilde{y}$ = 44+15.60 (x-2)
Trianthema portulacastrum	61	66.84	51	53.32	49	39.80	38	26.28	0	12.76	$\tilde{y}$ = 39.8+13.52 (x-2)
LSD at 1%	18.71		28.63		19.19	17.95		0			
LSD at 5%	13.35		20.42		13.68	12.80		0			

Each value is an average of three replicates, DW: Distilled water (control), values given in parentheses present percent mortality over control and values given in without parentheses represent number of dead juveniles of *M. incognita* in different concentration of aqueous extract

Table 5: Phytochemical screening of leaves extract of *Argemone mexicana* L., in different solvent

Phytochemicals	Methanol	Chloroform	Petroleum	Water	Ethanol	Acetone
Carbohydrate	+	+	-	-	+	+
Protein/amino ninhydrin	-	+	-	+	+	-
Flavonoids	+	-	-	+	-	+
Tannin/phenol ferric	+	+	-	+	+	+
Carbohydrate	+	-	-	+	+	+
Saponins	-	-	-	+	-	-
Phytosteroid	-	+	+	-	+	-
Triterpenoids	-	-	-	-	-	+
Mucilage/gum	-	+	+	+	+	+
Phlabotanins	-	-	-	-	+	-

+: Presence of phytochemicals, -: Absence of phytochemicals

Table 6: Phytochemical screening of leaves extract of *Achyranthes aspera* L., in different solvent

Phytochemicals	Ethanol	Methanol	Water	Chloroform	Petroleum	Acetone
Alkaloids	-	+	+	+	-	+
Flavonoids	+	+	+	-	-	-
Terpinoids	-	+	-	-	-	-
Saponins	+	-	+	-	-	-
Protein	+	+	+	-	-	-
Carbohydrate	+	+	+	+	+	-
Phenol/tannins	+	+	+	-	-	+
Glycosides	+	+	-	+	-	+
Steroids	-	+	-	+	+	+
Gum/mucilage	+	+	+	-	-	-
Phlabotanins	+	-	-	+	-	-

+: Presence of phytochemicals, -: Absence of phytochemicals

qualitative phytochemical examination for identification of various classes of active chemical constituents through the standard methods under various solvents.

Test for alkaloids (Mayers test): Approximately 1 mL of leaf extract, 6 drops of Mayer's reagent was added. The formation of yellowish creamish precipitate confirmed the presence of alkaloids (Table 5, 6).

Test for saponins (Foam test): One milliliter of leaf extracts was mixed with 5 mL of distilled water. The contents were heated in a boiling water bath. Frothing indicated the presence of saponins (Table 5, 6).

Test for tannins (Braymer's test): Mixed 1 mL of the leaf extract with 2 mL of water. To these 2 drops of 5% ferric chloride solution was added. Appearance of dirty green precipitate indicated the presence of tannins (Table 5, 6).

Test for steroids (Salkowski test): Approximately 2 mL of chloroform was added to 2 mL of the leaf extract, followed by concentrated sulphuric acid. Appearance of reddish brown ring at the junction showed the presence of steroids (Table 5, 6).

Test for terpenoids: To 2 mL of the leaf extract 2 mL acetic acid was added. Then concentrated sulphuric

acid was added. Deep red color development showed the presence of terpenoids (Table 5, 6).

Test for phenols: One milliliter of the leaf extracts was treated with 3% ferric chloride. Formation of deep blue color shows the presence of phenol (Table 5, 6).

Test for flavonoids: One milliliter of the leaf extracts was added with 1 mL of sulphuric acid. Orange color appearance confirmed the presence of flavonoids (Table 5, 6).

Test for carbohydrates (Molisch's test): Few drops of Molisch's reagent were added to 2 mL portion of the various extracts. This was followed by addition of 2 mL of concentration H_2SO_4 down the side of the test tube. The mixture was then allowed to stand for 2-3 min. Presence a red or dull violet colour at the interphase of the two layers shows the positive test (Table 5, 6).

Test for phlobatannins (Precipitate test): When 2 mL of extract was boiled with 1 mL of 1% aqueous hydrochloric acid was taken deposition of a red precipitate confirmed the presence of phlobatannins (Table 5, 6).

Test for amino acids and proteins: Two milliliters of filtrate was treated with 2-5 drops of ninhydrin solution placed in a boiling water bath for 1-2 min and noticed the appearance of purple colour (Table 5, 6).

Test for glycosides (Keller-Killani test): To 2 mL of extract, glacial acetic acid, one drop 5% ferric chloride and concentrated sulphuric acid were added. Display of reddish brown colour at the junction of the two liquid layers represented the presence of cardiac glycosides (Table 5, 6).

Test for proteins: Various extracts were dissolved in few milliliters of water and treated with.

Millon's reagent: Appearance of red colour shows the presence of proteins and free amino acids (Table 5, 6).

Biuret test: Equal volume of 5% solution of sodium hydroxide and 1% copper sulphate were added. Appearance of pink or purple colour indicated the presence of proteins and free amino acids (Table 5, 6).

Test for gums and mucilage: About 10 mL of various extracts were added separately to 25 mL of absolute alcohol with constant stirring and filtered (Table 5, 6).

Statistical analysis: The data were analyzed by one-way analysis of variance (ANOVA) using SPSS 17.00 software (SPSS Inc., Chicago, IL, USA)[24]. Least significant differences (LSD) were calculated at p = 0.05 and 0.01 to test for significant differences between the treatment means.

RESULTS

Table 1 shows that aqueous extracts of six weeds inhibited the hatching of second stage juvenile (J2) of *M. incognita in vitro*, indicating clearly that all the weeds having nematostatic as well as nematicidal activity against root-knot nematode. The percent inhibition of second stage juveniles hatching in comparison to distilled water decreased with increased of the dilution of extract (S, S/2, S/10 and S/100). The data suggested that standard concentration of leaves extracts of *A. mexicana* and *A. aspera* showing 100% inhibition of J2 hatching. Other dilutions viz., S/2, S/10 and S/100 were less effective as compared to standard concentration (S). Distilled water (C) showed no mortality. Results showed that aqueous extract of above six weed weeds viz., *A. mexicana, A. aspera, R. communis, A. indica, P. hysterophorus* and *T. portulacastrum* was found to be toxic in nature in causing mortality of second stage juveniles (J2) of *Meloidogyne incognita*. Table 2 shows that *A. mexicana* and *A. aspera* display highest percent mortality of J2 of *M. incognita* and least was observed in *T. portulacastrum* in different concentration viz., S, S/2, S/10, S/100 as compare to distilled water (DW) control 0 after 24 h of exposure time. *Meloidogyne incognita* mortality varied in time dependent and concentration dependent manner. Table 3 shows that highest mortality of second stage juveniles (J2) was found in the aqueous extract of *A. mexicana* and *A. aspera* and lowest percent mortality was noticed in *T. portulacstrum* in the different concentration viz., S, S/2, S/10, S/100 as compare to distilled water (DW) control 0 after 48 h of exposure time. Maximum mortality was found in aqueous extract of *A. mexicana* followed by *A. aspera*, *R. communis, P. hysterophorus*, *A. indica* and *T. portulacastrum* respectively in different concentration viz., S, S/2, S/10, S/100 after 72 h of exposure time (Table 4). Standard (S) concentration of the extracts and 72 h exposure period represent maximum toxicity compare to the all the dilution of the extract and time period. Aqueous extract at of S/100 concentration did not affect egg hatch and caused only a trifling suppression of J2 activity. However, concentrations of S and S/10 were effective at reducing J2 hatch from eggs. It has been concluded from this experiment that certain

weeds extracts are a source of cheap and effective nematicides for the management of root-knot nematodes.

Phytochemical screening of leaves extract of *A. mexicana* and *A. aspera* in different solvent viz., petroleum ether, chloroform, ethanol, distilled water and acetone extracts revealed the presence alkaloids, flavonoids, phenols, tannins, steroids, terpenoids, quinines carbohydrates, amino acid/proteins and saponins (Table 5, 6) by positive reaction with the respective test reagent. Phytochemical screening showed that maximum presence of phytoconstituents in aqueous extracts. Analysis was done to examine the presence and nature of the chemical present in the extract and powder.

DISCUSSION

Finding of the research clearly suggested that weeds extract of the plants viz., *A. mexicana, R. communis, A. aspera, P. hysterophorus, T. portulacastrum* and *A. indica* were potential sources of bionematicides against root-knot nematode. These results revealed that flavonoides, terpenoids and polyphenolic compounds of these weeds might possess the nematistatic as well as nematicidal activity. Phytochemical analysis was done to examine the presence and nature of the chemical present in the extract. This study indicated that, leaves extract of *A. mexicana* and *A. aspera* have various chemical constituents viz., alkaloids, flavonoids, phenols, tannins, saponins, phytosteroids and mucilage/gum. Phytochemical analysis also revealed that plant is rich in alkaloids, phenols terpenoids and flavonoids etc., have high rate of nematicidal activity[25,26]. It might be possible that maximum inhibition of egg hatching and mortality of second stage juveniles of *M. incognita* may be due to the presence of these pytochemicals in leaves extract of *A. maxicana* and *A. aspera*. The bioactivity of *A. mexicana* and *A. aspera* against nematodes may be attributed to the presence of the alkaloids in its leaves[27]. Nematicidal property of some phytochemical (saponins, flavonoids and glycocides) content extracted by these plant leaf or oxygenated compounds which have been characterized by their lipophytic properties that enable them dissolve the cytoplasmic membrane of the nematode cells and their functional groups interfering with enzyme protein structures of nematodes[28]. During the last decade, research on nematode control was focused on proposing strategies for inhibition of egg hatch[29], enhanced juvenile mortality[30]. It has been observed that nematicidal activity was directly related to concentration of extract. The result of this study is in agreement with other previous work[31,32]. *Ageratum conyzoides* show nematicidal as well antibacterial activitydue to the abundant presence of phytocompounds[33], including alkaloids, flavonoids, tannin, saponins and phenol. The nematostatic effects of tannins from chestnut (*Castanea sativa*) against the juveniles of *M. javanica* are well documented[34]. The flavone-C-glycoside and lantanoside also found significantly effective against *M. incognita*[9].

Azadirachtin is the major nematotoxic compound present in neem and they are released through volatilization, exudation, leaching and de-composing of the plant parts[25,35] reported that methanol and hexane extracts of the 27 samples were screened for nematicidal activity against second stage juveniles of *M. incognita* in the laboratory. Prior to this study such active metabolites, alkaloids and flavonoids of plants were reported to have ovicidal and nematicidal properties against root knot nematode[28]. Piperamides is produced by certain species of piper genus, such as capsaicin which belong to *Capsicum* genus, (*Capsicum frutescens*) has nematicidal properties[36]. Botanicals such as *Polyaithia longifolia, Datura stramonium, Thuja orientalis, Targetes minita, Citrus medica* and *Peruvian zinna* were found to be effective on nematodes both *in vivo* and *in vitro*[37-42]. Ferreira *et al*.[37] reported that aqueous extracts of *Zinnia peruviana* and *Wedelia* species inhibited egg hatched of *Meloidogyne incognita* when compared to the control by 92.72% and 97.48% respectively. Also Kumari *et al*.[43] reported that plant extracts of neem, *Clitoria teratea* and *Passiflora foetida* were effective in reducing egg hatch of *M. incognita* in mulberry. It was reported that extracts of plants containing tannins, alkaloids and flavonoids were effective against root knot nematodes both invivo and *in vitro*[44,45]. Some pairs of terpenes have synergistic impact on *M. incognita* which cause paralysis and those pairs are trans-anethole/geraniol, trans-anethole/eugenol, carvacrol/eugenol and geraniol/carvacrol[46]. The various organic compounds found in a single plant tissue can confer synergistic nematicidal properties, leading to high nematode mortality[9]. The bioactivity of *Peganum harmala* against nematodes may be attributed to the presence of the alkaloids in its leaves including β-carboline, harmine, harmaline, harmalol, harman and quinazolines as vascine and vasicinone[27]. The findings of present research experimental work advocate a new and novel natural pathway in evaluating potent nematicidal and nematostatic agents against *M. incognita* from plants like *Argemone mexicana, Achyranthe saspera, Ricinus communis Parthenium hysterophorus*, *Acalypha indica* and *Trianthema portulacastrum*. Results of the study demonstrated that plants containing nematicidal compound can further established as bionemticide and phytomedicine for the therapy of several

infections. Identification of natural organic compounds and characterization of potentially active phytoconstituents is the needs of the hour as successfully utilization of this molecule may finally pay off a way for the development of natural biopeticidesfor *in vivo* trials as they may be asset for the sustainable nematode management programme in the form of climate smart agriculture approach. The results of the study showed that like *Argemone mexicana* and *Achyranthes aspera* showed highest toxic effect against *M. incognita* as compare to other viz., *Ricinus communis Parthenium hysterophorus*, *Acalypha indica* and *Trianthema portulacastrum*. It is recommended that field trials be carried out to determine its efficacy before recommending to farmers. Therefore it was concluded that the severe infection caused by *Meloidogyne 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. Plant products proved the way for the healthy and pollution free sustainable environment.

CONCLUSION

The result of present study clearly showed that the leaves extract of six weeds viz., *A. mexicana, A. aspera, Ricinus communis, Acalypha indica, Parthenium hysterophorus* and *Trianthema portulacastrum* may be one of the best ways for nematode management. The aqueous extract of *A. mexicana* showed highest nematistatic and nematicidal efficiency against the egghatching and juveniles mortality of *M. incognita in vitro* due to the presence of various phytochemicals which are toxic to nematode survivality. The use of various plants extracts having nematicidal properties against *M. incognita* may contribute to minimize the hazardous effect of nematicides on flora and fauna. Hence the impressive outcome of the results revealed that the addition of crude extract or product may provide safe and environmentally reliable alternative method against root-knot nematode, *Meloidogyne incognita*.

SIGNIFICANCE STATEMENTS

This study discovered that among the selected weeds used, *A. mexicana* and *A. aspera* was found to be most effective against the second stage juveniles (J2) and hatching inhibition of *Meloidogyne incognita in vitro*. Utilization of natural exudates is one of the safe and protective methods for the management of nematode without disturbing the equilibrium of soil and environment.

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