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Potential Role of Aqueous Extract of Some Weeds against Egg Hatching and Juvenile Mortality of Root-Knot Nematode *Meloidogyne incognita*

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Abstract: Natural pesticides derived from plants are one of the best alternative approaches for the management of nematodes, as excessive use of chemicals eradicate beneficial organisms in the soil and disturb ecological equilibrium and ultimately cause environmental degradation. Six different weed plants *viz*., *Achyranthes aspera* L., *Solanum xanthocarpum* Schard. & JC wendl. *Amaranthus spinosus* L., *Ranunculus pensylvanicus* L.f., *Cassia tora* L., *Oxalis stricta* L. were collected from in and around the campus of the Aligarh Muslim University, Aligarh. The nematode eggs and juveniles were exposed at 24, 48 and 72 h in different concentrations (S, S /2, S /10, S /100, S is the standard concentration and S/2, S/10, S/100 is the dilution of Standard solution) of weeds extracts. The plant extract of weeds *S. xanthocarpum* and *A. aspera* exhibited highly promising mortality (86-100%) after 72 h exposure period respectively, while the plant extract of *O. stricta* and *C. tora* exhibited minimum promising mortality (48-52%) after 24 h of exposure period respectively. There was a gradual decrease in egg hatching with an increase in the concentration of aqueous extracts of weeds. *A. aspera*, *S. xanthocarpum* and *A. spinosus* were found to be most effective in reducing egghatching and increase in mortality of second stage juveniles of *M. incognita*. Efficacy of treatments improved with increase in their concentration and exposure period. Hatching of larvae and juvenile mortality were strongly influenced by concentration of plants extract.

Keywords: Egghatching, Juvenile mortality, *Meloidogyne incognita*, Plant extract.

1. Introduction

Nematodes are small unsegmented, multicellular and pseudocoelomic worms live in water, soil, plants and animals. Plant parasitic nematodes are capable of reproducing over 2,000 species of plants [1] and are responsible for approximately 50% of overall damage [2]. Root–knot nematode, *Meloidogyne incognita* is major plant parasitic nematodes affecting quantity and quality of the crop production in many annual and perennial crops, causing an estimated yearly crop loss of $100 billion worldwide [3]. Nematodes are difficult to control because of their wide host range and high rate of reproduction, with females capable of producing up to thousand eggs / female [4]. Plant–parasitic nematodes are recognized as the causes of serious yield losses on a wide range of crops [5]. Infected plants show typical symptoms including root galling, stunting and nutrient deficiency, particularly nitrogen deficiency [6]. Synthetic / Chemical nematicides is one of the most fastest and effective nematode control methods, but they are hazard to humans, environment and are relatively unaffordable to the average small scale farmers [7]. Moreover, the use of synthetic chemicals has also been restricted because of their carcinogenicity, teratogenicity, high and acute residual toxicity, ability to create hormonal imbalance, spermatotoxicity, long degradation period and food residues [8-11]. Therefore, it has become an important issue to find alternative control strategies, which are as effective as

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synthetic pesticides, safer to farmers, consumers and the environment and relatively easily available at low price [12]. Application of plant extract as natural pesticide is probably one of the feasible substitute, but the formulation required improvement. Many compounds with nematicidal activity have been found in plants, including alkaloids, diterpenes, fatty acids, glucosinolates, isothiocyanates, phenols, polyacetylenes, sesquiterpenes [13-16]. Large number of compounds with nematicidal activity has also been isolated from species in the family Asteraceae [13, 16]. Extract derived from certain plants kill or repel pests, make unconscious, disrupt their life cycle, or discourage them from feeding. Plant extracts containing volatile compounds [17], especially essential oils, have been found to possess antimicrobial, insecticidal and nematicidal activity [18-20]. Some of these components may be detected at a distance by olfaction and act as attractants or repellents [21]. The application of extracts either enabled the plants to resist the nematode invasion or activated directly the defense mechanisms of plants [22]. Certain plants kill or repel pests, disrupt their life cycle or discourage them from feeding. Botanical extracts induce insecticidal activity, repellence to pests, antifeedant effects and insect growth regulation, toxicity to nematodes, mites and other pests, as well as antifungal, antiviral and antibacterial properties against pathogens [23, 24]. They not only reduce the nematode population but also enhance the plant growth [25].

A wide variety of plant species, representing 57 families have been shown to nematicidal compounds [26]. Consequently, a large number of plants/ plant parts have been screened for their nematicidal activities [27, 28]. Davis and May [29] reported that the yield loss of cotton production caused by *M. incognita* in 2002 was estimated to be between 18.0 – 47.3%. A number of researchers reported about the nematicidal properties of many plants products against *M. incognita* [26]. However, the list of plant materials with ovicidal and larvicidal potentials seems to be inexhaustible [30]. Hence, an experiment was conducted to evaluate leaves extract of some weed plants *viz.*, *Achyranthes aspera*, *solanum xanthocarpum*, *Amaranthus spinosus*, *Ranunculus pensylvanicus*, *Cassia tora* and *Oxalis stricta* to ascertain potential toxicity against juveniles of the root-knot nematode against *Meloidogyne incognita.*

2. Materials and Methods

2.1. Culture for the Nematode Inoculum

Eggmasses were handpicked using sterilized forceps from heavily infected roots of brinjal/ eggplant (*Solanum melongena* L.) family- Solanaceae and 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 having water just deep enough to contact the eggmasses which can help in juvenile hatching that can be used in experiment.

2.2. Preparation of Extract

Leaves of six different plants species *viz.*, *Achyranthes aspera*, *Solanum xanthocarpum*, *Amaranthus spinosus*, *Ranunculus pensylvanicus*, *Cassia tora* and *Oxalis stricta* were collected, thoroughly washed with alcohol and chopped. Twenty five gram chopped leaves of each sample were soaked in 75 mL distilled water and kept overnight. The extract was prepared by grinding mortar and pestle, passed through muslin cloths so that all the plant debris can be removed and then filtered through Whatman filter paper No.1. The filtrate was termed as sample solution (S) designated as (100%). From this sample solution desired amount of dilution S/2, S/10, S/100 was made by adding proper amount of distilled water. Distilled water served as control.

2.3. Mortality Test

For mortality experiment, 5 mL of water suspension containing 100 second stage juveniles (J2) of *Meloidogyne. Incognita* were transferred to 3 mm petridish having different concentrations (S, S/2, S/10 and S/100) of leaf extract of different plant species separately [31]. Each treatment contains three replicates. The petridishes were kept at 28 °C in Biological Oxygen Demand (BOD). The immobilized juveniles were counted after 24, 48 and 72 h of the exposure period. The death of juveniles was confirmed by transferring immobilized juveniles into water for 1 h and mean percentage mortality was calculated.

2.4. Hatching Test

For hatching experiment 5 healthy and uniform size eggmasses was taken from thoroughly washed roots of brinjal infected with root-knot nematode, *M. incognita*. The eggmasses were transferred to 40 mm petridishes containing 5 ml of leaf extract of different dilutions (S, S/2, S/10, S/100) separately. Each treatment was replicated three times. Total number of hatched juveniles was counted after 3 and 6 days with the help of counting dish under stereoscopic microscope and percent inhibition over distilled water was calculated. Distilled water served as control for hatching.

3. Results

Effect of aqueous extracts from leaves of six different locally available plants viz., *Achyranthes aspera*, *Solanum xanthocarpum*, *Amaranthus spinosus*, *Ranunculus pensylvanicus*, *Cassia tora* and *Oxalis stricta* on eggs and J2 of *M. incognita* was shown in Table 1 & 2. Data presented in Table 1 clearly revealed that all the tested plant extracts showed deleterious effect on juvenile mortality. Mortality of juveniles appeared to be due to toxic effect of chemicals present in the leaf extracts of test plants [32]. Efficacy of plant extracts, however, depends on its

concentration and the duration of exposure of the nematode, [33-35]. Highly deleterious effect of leaf extract of *A. aspera* was found against J2 of *M. incognita* which shows 68% of the mortality with 24 hrs of exposure period.

Table-1. Effect of aqueous extract of chopped leaf of different plant species on the mortality of *Meloidegyne incognita* juveniles *in vitro* after 24, 48 and 72 hours.

Treatment	Exposure period (Hours)	Percent Mortality in Different Extract					Regression Equation
		S	S/2	S/10	S/100	DW	
Achyranthes aspera	**24**	68 (78.8%)	65 (64.3%)	60 (49.8%)	56 (35.3%)	0 (20.8%)	Ȳ= 49.8+14.5 (x-2)
	48	90 (99.2%)	78 (78.2%)	70 (57.2%)	48 (36.2%)	0 (15.2%)	Ÿ=57.2+21(X-2)
	72	100 (110.8%	88 (87.6%)	78 (64.4%)	56 (41.2%)	0 (18%)	Ȳ=64.4+23.2(x-2)
Solanum xanthocarpum	**24**	64 (74.5%)	61 (60.5%)	58 (46%)	47 (31.5%)	0 (17.6%)	Ȳ=46 +14.2 (x-2)
	48	84 (90%)	72 (70.4%)	54 (50.8%)	44 (31.2%)	0 (11.6%)	Ÿ=50.8+19.6(X-2)
	72	86 (96.2%)	80 (76.2%)	63 (56.2%)	52 (36.2%)	0 (16.2%)	Ȳ=56.2+20(x-2)
Amaranthus spinosus	**24**	62 (71.3%)	59 (57.9%)	55 (44%)	44 (30.1%)	0 (16.7%)	Ȳ= 44+13.9 (x-2)
	48	70 (74.72%	58 (58.96%)	48 (43.2%)	40 (27.44%)	0 (11.68%)	Ÿ=43.2+15.76(X-2)
	72	80 (85.6%)	65 (67.4%)	58 (49.2%)	43 (31%)	0 (12.8%)	Ȳ=49.2+18.2(x-2)
Rannunculus pensylvanicus	**24**	56 (61.72%	54 (50.66%)	49 (39.6%)	39 (28.5%)	0 (17.48%)	Ÿ=39.6+11.06(X-2)
	48	65 (68.6%)	54 (53.4%)	40 (38.2%)	32 (23%)	0 (7.8%)	Ÿ=38.2+15.2(X-2)
	72	72 (76.2%)	60 (59.2%)	45 (42.2%)	34 (25.2%)	0 (8.2%)	Ȳ=42.2+17(x-2)
Cassia tora	**24**	52 (58.8%)	47 (47.3%)	44 (35.8%)	36 (24.3%)	0 (12.8%)	Ÿ=35.8+11.5(X-2)
	48	60 (59.92%	45 (45.86%)	30 (31.8%)	24 (17.74%)	0 (3.68%)	Ÿ=31.8+14.06(X-2)
	72	68 (70.4%)	54 (54.4%)	40 (38.4%)	30 (22.4%)	0 (6.4%)	Ȳ=38.4+16(x-2)
Oxalis stricta	**24**	48 (55%)	45 (44.2%)	41 (33.4%)	33 (22.6%)	0 (11.8%)	Ÿ=33.4+10.8(X-2)
	48	46 (47.2%)	36 (36.6%)	26 (26%)	22 (15.4%)	0 (4.8%)	Ÿ=26+10.6(X-2)
	72	60 (61.6%)	48 (47.4%)	32 (33.2%)	26 (19%)	0 (4.8%)	Ȳ=33.2+14.2(x-2)

It was followed by the aqueous extract of *S. xanthocarpum* having 64% mortality. Juvenile mortality shows direct relationship with the exposure period and concentration of the extract. S concentration of all weed extract at 72 h of exposure showed highest mortality of juveniles while S/100 concentration of leaf extract at 24 h dipping revealed minimum mortality. Moreover S concentration of leaf extract of *S. xanthocarpum, A. spinosus, R. pensylvanicus, C. tora* and *O. stricta* showed 64, 62, 56, 52, 48% juvenile mortality. It is evident from the results that time period play most prominent role in increasing nematode mortality. Our results are in conformation with Ajayi, *et al.* [36]; Oyedunmade [37] and Abolusoro [38][39] reported inhibitory effects of some plant extracts on nematode egg hatch and juvenile survival. Among all the tested extract *A. aspera* showed maximum 100% mortality at 72 h which was followed by *S. xanthocarpum, A. spinosus, R. pensylvanicus, C. tora* and *O. stricta* showing 86, 80, 72, 68, 60 % juveniles mortality respectively. Minimum mortality was shown by *O. stricta* 48 at 24 hrs of exposure. Similar results were obtained by Jain, *et al.* [40]; Hussaini, *et al.* [41]; Asif, *et al.* [39]. As the time period increase from 24 to 72 h, mortality increase in successive manner as depicted in Table 1. Ansari, *et al.* [42] reported that aqueous extract of the plant helps in increment of juvenile mortality and percentage inhibition in hatching in *in vitro* test viz., *E. hirta*, *C. esculenta* and *W. chinensis*. While in the pots experiment as organic amendments they causes to increase in the nutrient status of the soil thereby improvement in crop yield and reduction in nematode potential. So from the above results a direct conclusion can be drawn that exposure time period and concentration of the extracts have direct impact on juvenile's mortality. Nematodes that do not regain their motility after transferring them in distilled water were considered as dead. Several plant extracts have been shown to inhibit acetyl cholinesterase activity in insects [43]. Since acetylcholine also serves as a neurotransmitter in nematodes, essential oil components may adversely affect their nervous system. Another possibility is that plant

extracts or essential oils cause inhibition in membrane permeability and disruption of the cell membrane of nematodes. Another explanation for the high mortality might be that juveniles died from lack of oxygen if the levels of oxygen were low in the amended dishes [44].

Table-2. Effect of aqueous extracts of fresh chopped leaves of different plants species on the juvenile hatching of *M. incognita in vitro* at 3 and 6 days.

Treatment	Exposure periods (Days)	Number of juvenile hatched in different concentration after 3 and 6 days				
		S	S/2	S/10	S/100	DW
Achyranthes aspera	**3**	0 (100%)	21 (94.16%)	54 (85%)	88 (75.55%)	360 (0%)
	6	0 (100%)	18 (95%)	53 (85.27%)	87 (75.83%)	360 (0%)
Solanum xanthocarpum	**3**	0 (100%)	26 (92.77%)	61 (83.05%)	103 (71.38%)	360 (0%)
	6	0 (100%)	25 (93.05%)	58 (83.88%)	97 (73.05%)	360 (0%)
Amaranthus spinosus	**3**	3 (99.16%)	34 (90.55%)	77 (78.61%)	119 (66.94%)	360 (0%)
	6	0 (100%)	32 (91.11%)	76 (78.88%)	117 (68.88%)	360 (0%)
Ranunculus pensylvanicus	**3**	5 (98.61%)	39 (89.16%)	82 (77.22%)	129 (64.16%)	360 (0%)
	6	0 (100%)	36 (90%)	81 (77.50%)	128 (64.44%)	360 (0%)
Cassia tora	**3**	8 (97.77%)	45 (87.50%)	89 (75.27%)	136 (62.22%)	360 (0%)
	6	4 (98.88%)	43 (88.05%)	88 (75.55%)	132 (63.33%)	360 (0%)
Oxalis stricta	**3**	10 (97.22%)	49 (86.38%)	99 (72.50%)	148 (58.88%)	360 (0%)
	6	6 (98.33%)	48 (86.66%)	99 (72.50%)	147 (59.16%)	360 (0%)

DW= Distilled water (Control).

Values for percent inhibition in juvenile hatching over control are given in parenthesis.

Leaf extract of all the tested plants depicts gradual decrease in egghatching from their lower concentration to higher concentration. This revealed that the high concentration of extract shows toxic effect on juvenile hatching. Maximum egghatching was observed in control although among the treated ones maximum hatching was found in S/100 concentration of all the extracts at 3 days exposure. While minimum number was observed in S concentration of all extracts at 6 days. Largest average number of juvenile hatching was observed in S, S/2, S/10 and S/100 concentrations of leaf extract of *O. stricta* was 10, 49, 99 and 148 respectively. In descending order it was followed by *Cassia tora* showing 8, 45, 89 and 136 followed by *R. pensylvanicus* 5, 39, 82 and 129 followed by *A. spinosus* 3, 34, 77 and 119 followed by *S. xanthocarpum* 0, 26, 61 and 103 followed by *A. aspera* 0, 21, 54, 88 at 3 days exposure and lowest juvenile was observed *A. aspera* and *S. xanthocarpum* 0, 18, 53 and 87 and 0, 25, 58, 97 and at 6 days respectively. While 360 juveniles were hatched in distilled water control. The results presented in Table 2 clearly represents that Sample concentration S of *A. aspera*, *S. xanthocarpum*, *A. spinosus* and *R. pensylvanicus* showed the maximum inhibition of egghatching (100%), followed by *Cassia tora* 98.88% and *O. stricta* 98.33% at 6 days. On dilutions i.e. S/2, S/10 and S/100, also showed inhibitory effect but found to be less effective as compared to the standard concentration of extracts. Inhibition in juvenile hatching increases with increase in the concentration of extract and exposure period. In our result inhibition in juvenile hatching increase with increase in time period from 3 to 6 days of exposure and on decrease with the dilution. Maximum inhibition in egghatching at S/100 concentration was observed in aqueous extracts of *A. aspera*, *S. xanthocarpum*, *A. spinosus*, *R. pensylvanicus*, *C. tora*, and *O. stricta* showing 75.83%, 73.05%, 68.88%, 64.44%, 63.33% and 59.16% respectively at 6 days. The results in our study showed that all the plant extracts tested have nematicidal activity and are in agreement with the findings of [39, 42, 45-47]. During the study increase in inhibition in egg hatching (100%) and increase in mortality(99.2%) of juvenile caused by higher concentration of *A. aspera* may be due to the presence of some nematicidal and nematotoxic compounds present in the crude extracts. Inbaneson, *et al.* [48] found that *A. aspera* extract showing antiplasmodial activity and also observed that extract contain Alkaloids, Glycosides, Saponins and triterpenoids. The inhibition in egghatching may be due to presence of some chemical in plant extracts.

4. Discussion

All the plant extracts studied in the research showed nematicidal properties but among them *A. aspera*, *S. xanthocarpum* and *A. spinosus* are best against *M. incognita* while *A. aspera* showed maximum lethality. The saponin isolated from the leaf of *A. aspera* was assessed for cancer chemo preventive activity [49], as a potential

mosquito larvicidal compound against *A. aegypti* and *C. quinquefasciatus* [50]. The inhibitory effects of leaf extracts of various weed plants has been reported by a number of researchers [51]. The use of leaf extract is suggested as a good substitute for nematicide used in the management of root-knot disease of cacao seedlings [52].The study shows that locally available test plants can be used as natural pesticides which are easily available on low input, have potential to reduce nematode infestation below economic threshold level and sustainable nematode management. Ahmad, *et al.* [53] reported that incorporation of Ficus leaves (*Ficus benghalensis* L.) with compost, NPK and nematicides in soil significantly reduced root-knot development caused by *Meloidogyne incognita*. Other researchers [3, 39, 54, 55] have reported successes in using various plant extracts in nematode management. Chedekal [56] reported *M. incognita* juvenile mortality of 90.17%, 81.33% and 74.00% water extracts of *A. indica, C. inerme* and *L. camara* respectively at 72hours time of exposure. Pakeerathan, *et al.* [57] reported that results *Gliricidia* maculate is most effective followed by *T. populnea* and *A. indica* in managing root knot nematode *M. incognita*. Elbadria, *et al.* [58] earlier reported that methanol extracts of *Solenostemma argel, Aristolochia bracteat* and *Ziziphus spinachristi* leaves and seeds of *Aregimone mexicana, Datura stramonium* and *Azadirachta indica* caused 80-90% juvenile mortality of *M. incognita* juveniles tested. It has been found [59, 60] that extracts contained alkaloids, flavonoids, saponins, amides including benzamide and ketones that singly and in combination inhibited hatching. The mechanism of plant extracts action may include denaturing and degrading of proteins, inhibition of enzymes and interfering with the electron flow in respiratory chain or with ADP phosphorylation. Eucalyptus spp. and its leaves have shown antibiotic activity [61]. Ardakani, *et al.* [62] also reported that essential oil from dried leaves of true myrtle (*Myrtus communis* L.) at the rates of 8000, 4000, 2000, 1000, 500, 250, 125, 62.5 and 0 mg/l were tested for its nematicidal activity against the second stage juvenile (J2) of root knot nematode, *M. incognita*. Meyer, *et al.* [63] revealed that the presence of toxic chemicals in the extracts of *Plantago lanceolata* and *P. rugelii* against *M. incognita*, might have acted as prohibitors inhibiting emergence of juveniles [64]. There are reports of induction of resistance and/or defense reactions in host plants against plant pathogens by compounds produced by biocontrol agents and chemicals contained in extracts of antagonistic plants [65, 66].

5. Conclusion

The result of present study demonstrates that the plant extract of *A. aspera, S. xanthocarpum, and A. spinosus* may be one of the best ways for nematode related problems. Several studies are going on to find out the potential of crude extracts as nematicidal agents This method of nematode management may contribute to minimize the toxicity and hazardous nature of nematicides and further research on these extracts may lead to the identification of active ingredients of new class of natural pesticides which may manufacture at commercial scale for global use. Hence the impressive outcome of the results revealed that the addition of crude extract or product may provide safer and environmentally reliable alternative for the root-knot nematode management.

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