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Bio-potential of Aqueous Extract of *Verbesina encelioides*, *Moringa oleifera*, *Cassia fistula* against *Meloidogyne incognita*

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ABSTRACT

Background: The *Meloidogyne incognita* is the most destructive pathogen and causes loss of yield of various economically important plants of various families. It is polyphagous in behaviour and has a high reproductive rate, these parameters are a hurdle in management of it. Various chemicals are used to control nematode worldwide that are very expensive and highly toxic to the environment and human beings. For ecologically safe method, three plants *i.e. Verbesina encelioides, Moringa oleifera*, *Cassia fistula* and their various parts such as leaf, stem and flower's cold aqueous extracts were tried *in vitro* to test their nematicidal activity.

Methods: Cold aqueous extracts (10% w/v) of each plant material were prepared by mixing 10g of each plant material in 100 ml of distilled water and kept aside for 48 hours. To estimate inhibition of egg hatching and mortality rate of second stage juveniles of *Meloidogyne incognita*, eggs and juveniles were exposed for 24, 48 and 72 hours in different concentrations (20 ppm to 200 ppm) of plant extracts.

Result: All three tested plant extracts showed promising nematicidal activity against *Meloidogyne incognita* and the leaf extract of *Moringa oleifera* prevents the hatching of eggs. Inhibition of larval hatching from egg and nematode mortality rate were strongly influenced by concentration of extract, plant species and duration of exposure.

Key words: Chemical, Hatching, Management, *Meloidogyne incognita*, Mortality, Nematicidal.

INTRODUCTION

The production of most vegetables crops is affected by soil borne diseases that are caused by soil pathogens such as *Rhizoctonia* spp., *Fusarium* spp., *Phythium* spp., *Phytophthora* spp. and *Meloidogyne* spp. (Panth *et al*., 2020). Above all mentioned soil pathogens, *Meloidogyne* spp is highly devastating pathogen to a wide range of plants and causing US $ 100 billion annual loss in yield globally (Oka *et al*. 2000; Kiewnick *et al*. 2006). Significant yield losses in various crops such as Tomatoes, Okra, Cotton have been observed as a result of soil inhabiting *Meloidogyne incognita* by Du *et al.* 2020; Baheti and Bhati, 2017; Memoona *et al.* 2014 respectively

To control root knot nematode, various chemical such as Methyl Bromide (as soil fumigant), Fenamiphos, Ethoprop, Oxamyl, Aldicarb were used but after the implementation of Montreal Protocol, the application of Methyl Bromide has been banned in majority of countries (US-EPA, 2020) and Aldicarb has been banned for environmental concern (FDACS, 2021). In recent years, the use of nematicides is not considered a useful mean to control pathogens as they being highly toxic cause deleterious effect on human health and environment and are costly too affecting the economy of farmers (Nico *et al*. 2004; Huang *et al*. 2014).

Hence, in order to overcome the drawbacks of nematicides and to safeguard the environment, maintaining ecological balance, managing root knot nematode (here after RKN) population, fulfilling farmers requirement, biological control has emerged as an alternative method, which is cost effective and eco-friendly (Collange *et al*. 2011). A new approach such as the use of plants and their derivatives (phytochemicals) as nematicidal agents against broad range phytoparasitic nematodes is developing recently. (Sardari *et al*. 2015; Kepenekci *et al*. 2016). Insunza *et al*. (2001) evaluated aqueous extract of thirty plants as nematicidal agents against *Xiphinema americanum sensu lato* and Elbadri *et al*. (2008) screened the methanol and hexane extracts of twenty seven plants against *Meloidogyne incognita*. The main objective of this study was to evaluation of nematicidal activity of aqueous extracts of various plant parts (like leaf, stem, root and flowers) of *Verbesina encelioides* (Cav.) Benth. and Hook. f. ex A. Gray, *Moringa oleifera* Lam. and *Cassia fistula* L. against *Meloidogyne incognita in vitro*.

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MATERIALS AND METHODS

Collection of plant materials

All three selected plants were collected from campus of University of Rajasthan in month of March to May (flowering season) and identified in the herbarium of Department of Botany. Plant materials were washed with tap water to

remove dust and debris and then finally washed by distilled water. The Leaves, stems and flowers of each plants were separated and air dried by placing them on blotting paper in dark. Roots of *Verbesina encelioides* plants were also dried. After drying, each plant material was grinded to obtain fine powder at room temparature.

Preparation of aqueous extract of plants

Cold aqueous extracts (10% w/v) of each plant material were prepared by mixing 10g of each plant material in 100ml of distilled water and kept aside for 48 hours. All the extracts were filtrated and each filtrate was evaporated using a hot plate at a temperature 50°C. The concentrated solution each plant was stored in refrigerator at 4°C as stock solution. At the time of experiment 20-200 ppm concentration from stock solutions was prepared by dissolving filtrate into distilled water. Distilled water was used as control.

Preparation of inoculum

To establish pure culture of *Meloidogyne incognita,* a single mass was removed from infected plant and surface sterilized with 0.5% NaOCl subsequently rinsed with double distilled water for five times. Then after single egg mass was inoculated into a pot containing okra plant in sterilized soil and kept at room temperature for 2-3 months to maintain pure culture. Pure egg masses were teased out from infected plant of okra manually under the microscope and cleaned with distilled water.

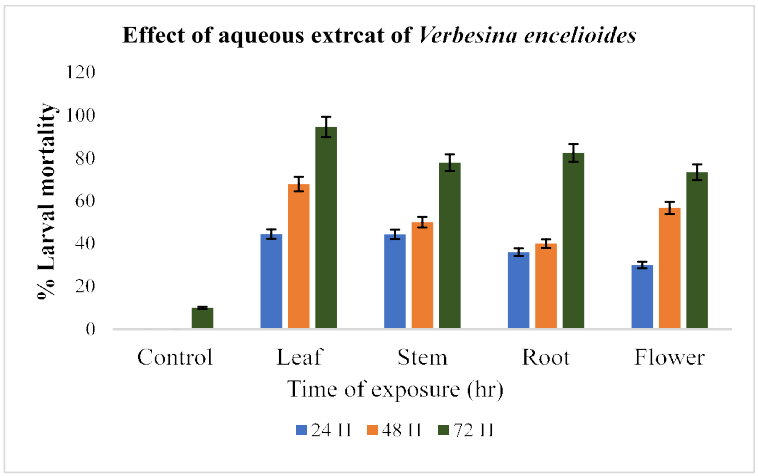
***In-vitro* evaluation of nematicidal property of plant extracts**

Effect of each plant extracts on hatchability of egg was determined by suspending 5 mature egg masses in each plant extracts of different concentration in cavity blocks. For each plant material three replicates were prepared and all cavity blocks were kept at room temperature. These blocks were observed after 24, 48 and 72 hr and the readings were recorded.

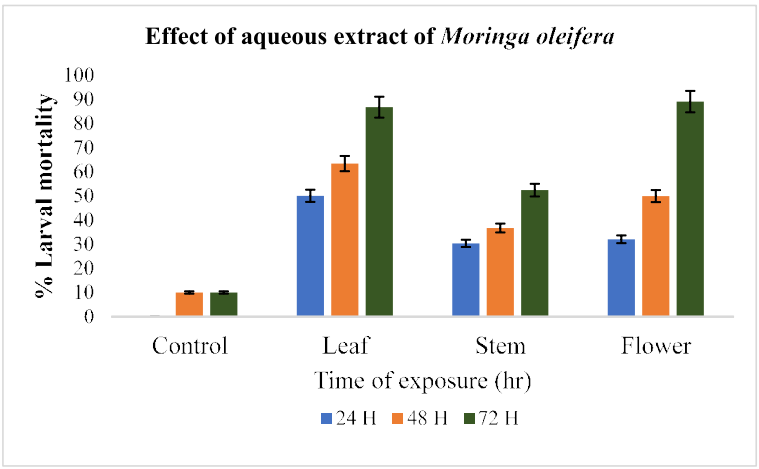
Percentage mortality of each plant aqueous extract were determined by placing newly hatched 30 J_2 larva in 20-200 ppm concentration of each plant extracts and observed after 24, 48 and 72 hours. Analysis of variance (ANOVA) was used to analysis variationnnnn and data analysis in the experiment and means were separated from

Table 1: Effect of different concentration of plant extracts on egg hatchability in the RKN, *Meloidogyne incognita*.

Name of plant	Parts used	Exposure time in hr	Egg hatchability at different concentration (ppm)										
			Control	20	40	60	80	100	120	140	160	180	200
Verbesina encelioides	Leaf	24	20	32	29	26	22	19	15	13	09	06	01
		48	27	26	21	18	14	11	08	07	04	00	00
		72	36	23	17	12	10	09	06	03	00	00	00
	Stem	24	20	34	28	23	20	17	14	10	09	04	00
		48	27	30	25	21	17	13	13	09	07	03	00
		72	36	25	20	18	16	14	11	08	07	02	00
	Root	24	20	20	16	14	11	09	07	07	05	04	01
		48	27	18	13	12	10	08	07	04	03	01	00
		72	36	10	06	06	04	02	01	00	00	00	00
	Flower	24	20	35	30	28	25	21	19	15	13	08	05
		48	27	27	25	22	20	16	12	09	06	05	01
		72	36	21	20	17	15	11	08	05	03	01	00
Moringa oleifera	Leaf	24	20	16	15	13	10	08	05	04	02	01	00
		48	27	10	09	08	06	03	01	00	00	00	00
		72	36	02	01	00	00	00	00	00	00	00	00
	Stem	24	20	36	32	27	23	23	19	17	15	12	11
		48	27	24	22	18	16	13	10	09	09	05	03
		72	36	19	17	14	11	07	07	05	04	03	02
	Flower	24	20	31	29	23	21	18	15	12	11	09	05
		48	27	28	26	23	20	17	14	11	09	06	03
		72	36	24	21	20	17	15	13	10	07	04	00
Cassia fistula	Leaf	24	20	39	39	37	36	33	25	20	17	13	07
		48	27	33	30	34	32	27	22	17	13	09	04
		72	36	27	25	22	18	18	15	15	12	11	03
	Stem	24	20	30	30	26	23	22	17	14	13	10	08
		48	27	26	25	21	21	18	15	12	10	07	05
		72	36	18	17	15	13	10	09	07	05	04	04
	Flower	24	20	32	30	28	22	19	15	11	09	09	07
		48	27	34	30	26	21	17	13	10	08	08	05
		72	36	29	26	24	18	15	11	08	06	04	03



A



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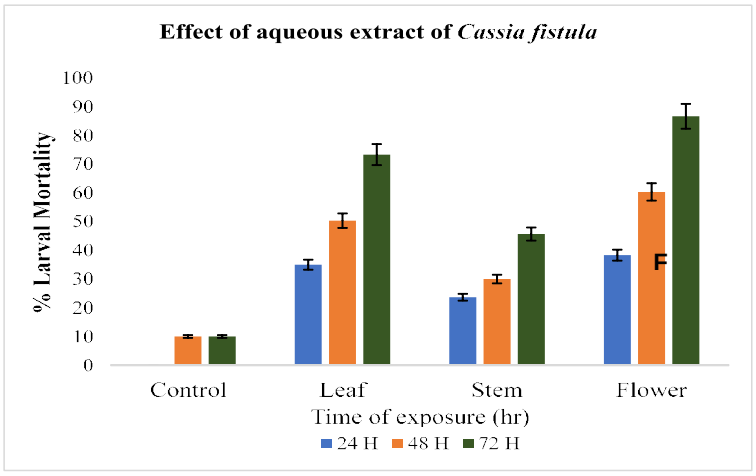

C

Fig 1: Effect of aqueous extract of (A) *Verbesina encelioides* (B) *Moringa oleifera* (C) *Cassia fistula* on mortality of larva.

Duncan multiple test range. Lethal concentration (hereafter LC) 30, 50 and 90 were also determined by Probit analysis. Distilled water was used as control.

The hatchability was determined by following formula =

hatched larvae /5

The percentage mortality was calculated by following formula =

$$\frac{\text{Total dead larvae}}{\text{Total larvae}} \times 100$$

RESULTS AND DISCUSSION

Hatchability

Egg hatchability decreased with increase in extract concentration and time of exposure. All tested plants showed effective nematicidal properties. The root and leaf extract of *Verbesina encelioides* was more lethal to *Meloidogyne incognita* than flower and stem extract. Among all extracts the most effective extract was leaf extract of *Moringa oleifera*. It showed 95-97% inhibition of hatching egg after 72 hours ($P<0.005$). Effect of different concentration of plant extracts on egg hatchability in the RKN, *Meloidogyne incognita* is mentioned in Table 1.

Mortality

Percentage of larval mortality increases with concentration of extracts and exposure time. Results of *Verbesina encelioides*, *Moringa oleifera* and *Cassia fistula* are presented in Fig 1 A,B,C respectively.

Highest percentage larvae mortality (94.47±0.44) was shown in leaf extract of *Verbesina encelioides*. Stem extracts of each plant showed lowest mortality rate. Percentage mortality of stem extract of *Verbesina encelioides, Moringa oleifera* and *Cassia fistula* were 77.77±0.77, 52.35±0.09 and 45.67±0.77, respectively.

Flower extracts also showed prominent nematicidal activity on J_2 juveniles. Percentage mortality of flower extract of *Verbesina encelioides*, *Moringa oleifera* and *Cassia fistula* were 73.33±0.49, 88.96±0.33 and 86.67±0.17, respectively.

Table 2: Effect of different concentration of plant extracts on larval mortality of the RKN, *Meloidogyne incognita*.

Name of plant	Parts used	Exposure time in hr	% of larval mortality at different conc. (in ppm) of plant extracts										
			Control	20	40	60	80	100	120	140	160	180	200
Verbesina encelioides	Leaf	24	0.00	0.00	0.00	2.20	6.67	14.43	18.87	23.33	27.93	33.33	44.43
		48	10.00	0.00	4.44	21.11	25.56	30.00	32.22	40.00	47.78	57.78	67.78
		72	10.00	10.00	12.20	22.23	28.13	31.90	40.00	50.00	67.77	75.57	94.47
	Stem	24	0.00	0.00	0.00	0.00	11.10	22.23	28.90	31.10	33.33	38.87	44.43
		48	10.00	0.00	0.00	12.20	14.43	18.90	28.90	30.00	37.77	40.00	50.00
		72	10.00	0.00	0.00	11.10	22.23	33.33	40.00	50.00	55.57	66.67	77.77
	Root	24	0.00	0.00	2.00	3.67	7.33	10.67	15.67	19.67	18.67	24.33	36.00
		48	10.00	1.00	3.33	6.67	8.33	11.67	18.66	20.00	23.33	26.67	40.00
		72	10.00	3.33	6.67	16.66	20.00	26.33	33.66	53.33	60.32	75.67	82.33
	Flower	24	0.00	0.00	0.00	0.00	3.33	13.33	16.67	20.00	23.67	26.33	30.00
		48	10.00	0.00	3.33	6.67	16.67	20.00	26.67	36.67	43.33	53.33	56.67
		72	10.00	0.00	3.33	8.33	22.67	24.57	33.33	40.00	53.33	60.00	73.33
Moringa oleifera	Leaf	24	0.00	0.00	0.00	3.33	8.89	15.56	15.56	21.11	32.22	41.11	50.00
		48	10.00	0.00	0.00	6.67	10.00	20.00	23.33	36.67	46.67	50.00	63.33
		72	10.00	3.33	6.67	16.67	23.33	36.67	53.33	60.00	66.67	73.33	86.67
	Stem	24	0.00	0.00	0.67	3.33	6.67	9.33	13.67	19.33	22.00	27.00	30.33
		48	10.00	3.33	6.67	8.00	13.33	15.00	20.00	23.67	26.67	33.67	36.67
		72	10.00	6.67	11.33	16.67	23.36	27.00	32.58	37.56	42.33	49.45	52.35
	Flower	24	0.00	0.00	2.33	3.00	6.33	8.67	14.00	18.00	23.67	27.00	32.00
		48	10.00	3.22	6.67	9.00	13.33	15.00	21.33	26.67	33.33	40.67	49.86
		72	10.00	6.67	11.37	19.38	23.67	33.35	42.57	53.67	68.33	74.58	88.96
Cassia fistula	Leaf	24	0.00	0.00	0.00	0.33	2.67	5.33	10.33	16.33	22.33	28.67	35.00
		48	10.00	1.00	3.00	9.33	9.33	12.67	17.33	23.67	33.00	42.33	50.33
		72	10.00	6.67	9.33	16.67	16.67	20.00	24.00	33.33	46.67	53.67	73.33
	Stem	24	0.00	0.00	0.00	0.00	3.33	6.00	10.00	13.33	16.67	20.00	23.67
		48	10.00	0.00	0.00	3.33	6.67	6.67	13.33	16.67	23.33	26.67	30.00
		72	10.00	0.00	0.00	10.00	13.33	15.00	23.33	26.67	33.33	37.67	45.67
	Flower	24	0.00	0.00	0.00	1.00	2.33	4.67	8.33	9.67	18.33	25.67	38.33
		48	10.00	0.00	2.67	4.67	10.33	18.33	26.33	36.67	43.67	51.33	60.33
		72	10.00	3.33	10.00	23.33	35.67	43.67	56.67	66.67	75.00	86.67	86.67

In-vitro effect of different concentration of all plant extracts with incubation time on Second stage juvenile of *Meloidogyne incognita* is represented in Table 2 and LC with 30, 50 and 90 was determined by Probit analysis and also mentioned in Table 3.

Root system of most of the vegetables crops is affected by obligate endoparasite RKN, *Meloidogyne incognita* and cause of knot like formation on roots. To control RKN various methods such as chemical (known as nematicides) physical, biological and resistant cultivars are applied. Among all of methods, application of chemical as fumigants, sprayer, liquid is very effective and gives quick results to the farmers but most of the chemicals were banned due to their toxic and carcinogenic nature not only to human being but also for animals and also harmful to environment (Wachira *et al*. 2009; Dubey *et al*. 2011).

Present study is related to above mentioned approach. It was observed that all three tested plants showed nematicidal activity against *Meloidogyne incognita in vitro* and confirmed that plants have nematicidal properties. The nematicidal properties of a plant extract such inhibition of egg hatch and mortality to J_2 larva was depend upon time of exposure and concentration of extract. Similar result was obtained by Elbadari *et al*. 2008; Wiranto *et al.* 2009; Khan *et al*. 2019.

Aqueous leaves extract of each selected plant species *viz Jatropha pandurifolia, Polyalthia longifolia, Wedelia chinensis, Nerium indicum, Duranta repens*, *Cassia fistula* (Asif *et al*. 2014) and *Amygdalus scoparia, Arctium lappa* (Farzaneh *et al*. 2019) showed 99-100% mortality rate and had nematicidal activity after 48 hours of exposure. The leaf extract of *Moringa oleifera* was the highly active in decreasing egg hatchability. The leaf extract of *Verbesina encelioides* was the highly active in increasing mortality of juveniles. In previous study, leaves extract of Neem (*Azadirachta indica*) showed 100% mortality rate against RKN *in vitro* condition (Oka *et al*. 2007), 95-99% mortality of juveniles of RKN was observed in leaves extract of six plants after 72 h of exposure (Elbadri *et al*. 2008).

Flower and stem extract of tested plants also affected the juveniles of *Meloidogyne incognita. Verbesina encelioides*, *Moringa oleifera*, *Cassia fistula* have potential to control Root Knot Nematode, *Meloidogyne incognita*. Highest mortality was recorded in leaf extract of *Verbesina*

Table 3: Lethal concentration of all tested plant extracts.

Name of plant	Part used	Exposure time in hours	LC 30	LC 50	LC 90
Verbesina encelioides	Leaf	24	190.55	151.36	323.59
		48	107.15	38.04	263.63
		72	66.37	107.40	363.08
	Stem	24	152.05	184.50	299.23
		48	135.52	169.04	290.40
		72	105.93	138.36	221.82
	Root	24	165.20	225.42	484.17
		48	247.74	363.08	1671.09
		72	83.56	129.12	378.44
	Flower	24	176.60	217.77	366.44
		48	121.90	159.22	305.49
		72	110.92	141.91	261.22
Moringa oleifera	Leaf	24	147.57	183.65	316.96
		48	130.32	160.32	266.69
		72	75.68	113.76	309.03
	Stem	24	168.27	227.51	478.63
		48	181.97	396.28	2691.53
		72	101.16	216.27	1406.05
	Flower	24	167.49	228.03	487.53
		48	147.91	291.07	1534.62
		72	70.47	112.46	356.45
Cassia fistula	Leaf	24	183.23	226.46	381.94
		48	153.46	262.42	979.49
		72	101.62	194.98	974.99
	Stem	24	197.24	246.60	428.55
		48	169.43	215.28	389.05
		72	142.23	178.24	309.74
	Flower	24	183.23	232.81	419.76
		48	124.45	161.81	307.61
		72	65.46	96.38	249.46

encelioides and *Moringa oleifera* and also in root extract of *Verbesina encelioides*. These plants can be used for management of *Meloidogyne incognita.*

CONCLUSION

Extracts and dried material of above mentioned plants can be used to control root knot nematode, *Meloidogyne incognita* and a good alternative of chemical nematicides. However, further research on their mechanism of action and formulation of a chemical that is toxic to root knot nematode are required for the best result.

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