



NEMATICIDAL EFFICACY OF BOTANICALS EXTRACTED FROM *ADHATODA VASICA* AND *ANDROGRAPHIS AFFINIS* AGAINST *MELOIDOGYNE INCOGNITA*

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ABSTRACT

Laboratory and screen house experiments were carried out to evaluate the effect of two botanicals extracted from *Adhatoda vasica* and *Andrographis affinis* in the control of root knot nematode (*M. incognita*) on tomato. Approximately 100 juveniles were dispensed into petridishes containing both the crude and diluted extracts of the botanicals except the control which contained only distilled water. In the screen house 5, 10 and 15gm of powdered plant material was applied on top of soil in form of mulch to each pot except the control which received no treatment. Triazophos a chemical nematicide was used as a check and applied at the rate of 1 ml/cm³ of soil. The results in both experiments indicated that *Adhatoda vasica* crude extract gave the best and recorded highest juvenile mortality of 100 % after 72hrs and recorded the highest growth parameters and least nematodes population. It is recommended that *Adhatoda vasica* extracts and powder be tried in the field before recommending to tomato farmers for the control of *M. incognita*.

KEYWORDS: *Adhatoda vasica*, *Andrographis affinis*, *Lycopersicon*, *Meloidogyne*, Nematicidal, Botanicals

INTRODUCTION

Tomato (*Lycopersicon esculentum*, Mill) is the second most cultivated vegetable crop in the world, after potato, with an annual production of nearly 100 million tons of fresh tomato in 3.7 million hector worldwide, China, USA and Turkey are the leading producers (FAO). It is a short duration crop, high yielding, economically attractive and its area of cultivation is increasing daily (Sharkara *et al.*, 2005). The fruit contain antioxidants, vitamins and minerals, and can be processed into juices, ketchup, and puree, eaten raw in salads or cook into stew (Beutner *et al.*, 2001). Root knot nematodes (*Meloidogyne* species) are one of the major root pests of plants including tomato worldwide and limit its production (Davis & May, 2005; Sikora *et al.*, 2005, Melakeberhan *et al.* 2012). Crops infected by nematodes especially vegetables such as tomato record yield losses of up to 80 % on heavily infested soils (Kaskavalci, 2007, Singh and Kumar, 2015). More than 2000 plants species were affected by plant parasitic nematodes (Azhagumurugan *et al.*, 2014). Chemical Nematicide is one of the most fastest and effective nematode control methods, but they are detrimental to both humans and the environment and are relatively unaffordable to the average small scale farmer (Washira *et al.*, 2009). There is therefore the need to develop alternative methods of control that are cheap, environmentally friendly and not harmful to humans. The use of botanicals is one of the alternative methods suggested by nematologist for nematode control. Botanicals such as *Azadirachta*, *Eucalyptus*, *Chrommelina*, *Sidaacuta* and *Citrus aurantifolia* have been found to be effective in the control of nematodes in cowpea, tomato, egg plant and chich pea fields (Umar *et al.*, 2010, Singh, 2015). Many botanical extracts have been

found to contain phytochemical such as alkaloids, tannins, saponins, flavonoids, diterpenes, glucosinolates, acetylenes and thienyls (Gommers, 1981, Chitwood, 2002). Which are effective against plant parasitic nematodes (Goswan *et al.*, 1986, Adegbite 2003).

MATERIALS & METHODS

Experimental Site

The experiment was carried out in the laboratory and screen house of the Nematology Research Lab, PG Department of Zoology, Bareilly College Bareilly, U.P., and India. Bareilly lies between latitude 28.35°N altitude 79.42°E, Elevation: 166 meters (544 feet).

Botanicals Preparation

The leaves of the plant, *Adhatoda vasica* and *Andrographis affinis* were collected from the College Campus. The leaves were collected, shade dried and powdered with the help of a mixer grinder. The leaf powder was extracted by using Soxhlet apparatus with 200 ml of acetone as a solvent. The extracted leaf material was then dissolved in acetone (1:10) w/v to prepare stock solution. Different concentrations of plant extracts were prepared from the stock solution using distilled water.

Phytochemical Analysis of Plant Materials

Phytochemical analysis of the plant materials was carried out in the laboratory using the methods described by Sawant *et al.* (2013).

Extraction of Eggs

For obtaining the egg masses and nematode larvae, pure culture of *M. incognita* maintained on tomato plants in sterilized soil. Root-knot nematode infected tomato plant (cv. Pusa) from the pure culture pot was up-rooted and washed gently under running tap water. Egg masses of *M.*

incognita were picked up from the root using dissecting needle and forceps. The collected egg masses were kept in water at 10 C in a refrigerator to prevent hatching before application of treatments.

Extraction of Juveniles of *M. incognita*

Root knot nematode (*M. incognita*) was identified using the head and stylet morphology and perennial pattern as described by Eiseback *et al.* (1981). It was then maintained on tomato cv Pusa and second stage juveniles were extracted from galled roots using a 2% NaOCl solution and the eggs released from the roots were collected using the modified technique described by McClure *et al.*, (1973). The egg suspension was poured on a cotton-wool filter paper and incubated at 28±2 C to obtain freshly hatched juveniles (J₂). Juveniles were collected and used within 48 hrs.

Effect of Extract on Juvenile Mortality of *M. incognita*

100 juvenile suspensions of *M. incognita* contain in a 10 ml syringe were dispensed into Petri dishes. 5, 10 and 15 ml of the crude extract and diluted form were separately added into Petri dishes except the control which contain distilled water and juveniles only. There were 9 treatments replicated three times (T1– *A. vasica* crude extract, T2- *A. vasica* crude extract + 5 ml of distilled water, T3- *A. vasica* extract + 10 ml of distilled water, T4- *A. vasica* crude extract + 15 ml distilled water, T5- *A. affinis* crude extract, T6- *A. affinis* crude extract + 5 ml distilled water, T7- *A. affinis* crude extract + 10 ml distilled water, T8- *A. affinis* crude extract +15 ml distilled water, Con-Control). Petri dishes were arranged in a complete randomized design in the laboratory. Percentage juvenile mortality was observed over a period of 24, 48, 72 and 96 hrs.

Screen House Experiments

2 kg sterilized sandy loam soil was filled into 10cm diameter perforated earthen pots with depth 15cm. Four week-old susceptible tomato seedlings cv Pusa raised in sterilized soil in the nursery were transplanted into each pot. 1000 juveniles' suspensions of *M. incognita* contain in a syringe were inoculated into each pot by exposing the

roots and emptying the syringe. 5, 10 and 15g of powdered plant material was applied on top of soil in form of mulch to each pot except the control which received no treatment. Triazophos a chemical nematicide was used as a check and applied at the rate of 1 ml/cm³ of soil. Super hydro-solution organic fertilizer containing macro and micro elements was applied thrice at the rate of 1ml/ liter of water every forth night using 5 liter sprayer. There were 8 treatments replicated thrice. Pots were arranged in a complete randomized design in the screen house. Data were collected on growth parameters and yield. Galls were rated using the rating scheme described by Sasser *et al.*, (1984). Nematode were extracted and counted using the methods described in Barker (1985).

Statistical analysis

The data were analyzed statistically for ANOVA (Analysis of Variance), Standard Deviation (SD) and Turkey's Multiplication (TM) by using Graph Pad Prism 5.0 software, USA. Tukey's multiplication comparison test and one-way ANOVA was employed to test for significant differences between the treatments.

RESULTS & DISCUSSION

Phytochemical Analysis

Phytochemical screening of leaves extract of *A. affinis* indicates the presence of glycosides triterpenoids, flavonoids, alkaloids and steroids in different organic solvents like methanol, ethanol, acetone and chloroform. Presence of active chemicals like carbohydrates, amino acids, glycosides, terrapins, tri-terpenoids, flavonoids, alkaloids and steroids and lack of saponins and tannins was confirmed in methanolic leaves extract of *A. vasica* (Table 1). Nematicidal potency of botanicals obtained from *A. affinis* and *A. vasica* is possibly due to such active compounds. Prior to this work such active metabolites, alkaloids and flavonoids of plants were reported to have ovicidal and nematicidal properties against root knot nematode by Knobloch *et al.*, (1989), Adegbite (2003) and Trifonovo *et al.*, (2009).

TABLE 1: Preliminary photochemical screening of the leaves of *Andrographis affinis* and *Adhatoda vasica*

Constituents	Photochemical screening of <i>A. affinis</i> through following extraction				Photochemical screening of <i>A. vasica</i> through methanolic extraction
	Methanol	Ethanol	Acetone	Chloroform	Methanol
Carbohydrate	-	-	-	-	+
Amino acid	*	*	*	*	+
Glycosides	-	+	+	+	+
Terrapins	*	*	*	*	+
Triterpenoid	+	+	+	+	+
Flavonoids	+	+	+	+	+
Saponins	*	*	*	*	-
Alkaloids	-	-	-	-	+
Steroids	+	+	+	+	+
Tannins	*	*	*	*	-

(Keys: + presence, - absent and * not attended.)

Larvicidal Efficacy of Botanicals

Different concentrations of botanicals prepared from leaves extracts of *Adhatoda vasica* and *Andrographis affinis* show a significant larval mortality in *in-vitro* screening. The results indicated in Table 2 show that except T8 and Control treatment all the treatments show

more than 50% of larval mortality just after 24 hrs of exposure. Strong nematicidal potency recorded with crude extract of *Adhatoda vasica* (T1) where 99.3% followed by 100% juvenile mortality was recorded after 48 and 72 hrs of exposure respectively. Gradual percent decrease in juvenile mortality was observed with gradual increase in

dilution of all the treatments. Except T7 and T8 treatments all other doses were effective to kill larvae 100% after 96 hrs of exposure. Among 8 different treatments, T8 treatment of *A. affinis* crude extract with 15 ml distilled water was found to be least effective which gives 40.43, 55.86, 65.73 and maximum 79.62 percent larval mortality

after 24, 48, 72 and 96 hrs of exposure respectively. Statistical analysis and comparative nematocidal efficacy of two tested plants indicates that treatments of *Adhatoda vasica* are more potent in than *Andrographis affinis* (Table 3).

TABLE 2. Percent larval (J_2) mortality by different treatments and exposure of leaves extract of *A. vasica* and *A. affinis*

Exposure period (in hrs)	Treatments								SD†	
	T1†	T2†	T3†	T4†	T5†	T6†	T7†	T8†		Con.†
24	92.65	72.86	63.32	45.03	73.24	63.73	56.46	40.34	0.33	26.22
48	99.3	92.06	73.86	67.05	93.34	83.58	71.77	55.86	0.66	29.79
72	100	98.1	85.62	75.55	99.46	92.75	81.11	65.73	1.33	30.95
96	100	100	100	100	100	100	93.67	79.62	2	32.27

†Keys: T1- *A. vasica* crude extract, T2- *A. vasica* crude extract + 5 ml of distilled water, T3- *A. vasica* extract + 10 ml of distilled water, T4- *A. vasica* crude extract + 15 ml distilled water, T5- *A. affinis* crude extract, T6- *A. affinis* crude extract + 5 ml distilled water, T7- *A. affinis* crude extract + 10 ml distilled water, T8- *A. affinis* crude extract +15 ml distilled water, Con-Control- Only distilled water, SD- Standard Deviation.

TABLE 3. Showing the column statistics of the effect of different concentrations of *A. vasica* and *A. affinis* crude extracts on juvenile mortality

S. No.		Treatments								
		T1	T2	T3	T4	T5	T6	T7	T8	Con.
1.	No. of values	4	4	4	4	4	4	4	4	4
2.	Minimum	92.65	72.86	63.32	45.04	73.24	63.73	56.47	40.34	0.333
3.	25% Percentile	94.31	77.66	65.96	50.54	78.27	68.69	60.29	44.22	0.416
4.	Median	99.65	95.08	79.74	71.30	96.41	88.17	76.45	60.80	1.000
5.	75% Percentile	100.0	99.53	96.41	93.89	99.87	98.19	90.53	76.15	1.833
6.	Maximum	100.0	100.0	100.0	100.0	100.0	100.0	93.67	79.63	2.000
7.	Mean	97.99	90.76	80.70	71.91	91.51	85.02	75.76	60.39	1.083
8.	Std. Deviation	3.574	12.40	15.76	22.72	12.55	15.70	15.68	16.54	0.739
9.	Std. Error	1.787	6.200	7.882	11.36	6.275	7.851	7.840	8.271	0.369
10.	Lower 95% CI	92.30	71.03	55.62	35.76	71.54	60.03	50.81	34.07	-0.09
11.	Upper 95% CI	103.7	110.5	105.8	108.1	111.5	110.0	100.7	86.71	2.259

The potential of using plant extracts in controlling plant parasitic nematodes has been shown earlier by several workers like Pandey *et al.*, 2000; Orisajo *et al.*, 2007; Abbasi *et al.*, 2008, Pavraj *et al.*, 2012; Oka *et al.*, 2012; Singh and Kumar, 2015. Phytochemical analysis also indicates that the botanicals extracted from tested plants, *A. vasica* and *A. affinis* in present study have potential nematocidal properties against root-knot nematodes.

Effect on Host Plant Growth

The application of the powdered test materials as mulch in screen house pots gives significantly different results among the various rates applied. The results indicated in table 4 shows that plants treated with powder of *A. vasica* provide best result in all growth parameters like plant height (PH), number of leaves (NL), number of fruits (NF) and fruit weight (FW) measured and higher dry matter accumulation, fewer galls and low population of nematodes recorded compared to *A. affinis* treated plants. The *A. vasica* powder applied as mulch might have been lethal to nematodes larvae. The powder probably acted directly on the second stage juveniles in the soil, thus reducing the number of motile juveniles available to penetrate the roots of tomato plants in the screen house. This result was similar to those obtained by Agbenin, *et al.* (2005) on tomato when they used some botanicals to control *M. incognita*. The control recorded the lowest

growth rates, high galling due to nematode activity at root zone resulting in giant cell formation, high population of nematodes because the nematodes larvae were able to penetrate roots freely and reproduce without any inhibition. The low growth parameter performance by the control plants could be as result of the combined effect of nematodes and availability of nutrients.

Results of the present study reveal that soil amendment with mulch of *Adhatoda* and *Andrographis* are effective to reduce nematode infestation and to increase plant growth and fruit yield of tomato. Similar findings have been reported by many other workers. Faruk *et al.*, 2011; Choi *et al.*, 2006; Klein *et al.*, 2012 were also found effectiveness of different organic amendments to reduce the populations of plant parasitic nematodes including *M. incognita*. Soil amendment with oilcakes of mustard, neem and castor has also been reported to be effective against root-knot nematode of okra (Alam *et al.*, 1980; Radson *et al.*, 2006).

The objective of the study was to evaluate the effectiveness of *Adhatoda vasica* and *Andrographis affinis* on the control of *M. incognita* in laboratory and screen house. *Andrographis affinis* is a highly reputed plant used in Ayurvedic system of medicine for the treatment of various ailments of respiratory systems like bronchitis, asthma and it is also used in the treatment of malaria,

dysentery and diarrhea (Jain *et al.*, 1984) and also has anti-inflammatory, analgesic, diarrhea, dysentery, antioxidant, hepatoprotective, Sedative, antispasmodic, anthelmintic properties (Mulla *et al.*, 2010), antimicrobial activity (Sheeba *et al.*, 2012), antidiabetic activity (Bhatt *et al.*,

2011). The leaves are found to activate the digestive enzyme trypsin and significant antifungal activity against ringworm (Khare, 2007). The botanicals are cheap, easily available and environmentally friendly form the basis of the researches in agricultural interest.

TABLE 4. Effect of different concentrations of leaves extract of *A. vasica* and *A. affinis* on plant growth parameter of tomato var. pusa against *M. incognita*

Growth Parameters	Treatments of <i>A. vasica</i>					Treatments of <i>A. affinis</i>				
	TZP	CRD-15	CRD-10	CRD-5	Control	TZP	CRD-15	CRD-10	CRD-5	Control
Plant Height (PH in cm)	41.4	37.4	34.0	27.6	20.4	41.4	36.4	31.7	28.2	20.2
Number Of leaves (NL)	36	33	28.7	23.6	18.3	36	32	28.3	23.6	18.3
Number of Fruits (NF)	14	12.3	8.6	7	2.6	14	12	9	6.6	2.6
Fruit Weight (FW in gm)	54.6	53.2	48.8	45.5	41.3	54.6	51.4	45.7	44.2	41.3
Fresh Shoot Weight (FSW in gm)	88.5	84.5	82.1	78.0	74.2	88.5	84.1	81.7	76.6	74.2
Dry Shoot Weight (DSW in gm)	18.5	17.8	14.7	13.6	10.3	18.5	14.9	13.3	12.6	10.3
Fresh Root Weight (FRW in gm)	24	23.2	19.7	17.5	14.1	24.0	22.5	18.9	17.1	14.1
Dry Root Weight (DRW in gm)	8.4	8.2	7	6.4	5	8.4	7.8	6.7	5.3	5
Number of Gall (NG)	0.4	1	2	2.5	11	0.8	3	3.6	4	10
Number of Parasites (NP)	0	45	72	274.6	2900	0	64	93.3	441	2900

†Values are mean of three replicates, **Keys:** TZP- Triazophos, CRD- Crude Powder (in gm).

CONCLUSION & RECOMMENDATION

The results of the study showed that *A. vasica* crude extract and powdered mulch in the screen house was able to inhibit egg hatch, caused larval mortality and reduced the population of nematodes in potted tomato plants. It is recommended that field trials be carried out to determine its efficacy before recommending to tomato farmers. Therefore it was concluded that the severe infection caused by *Meloidogyne* spp. could be lowered by the plant products in view of eco-friendly environment. This has an advantage against expensive and hazardous chemical Nematicides. Plant products proved the way for the healthy and pollution free sustainable environment.

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