Biosciences, Biotechnology Research Asia Vol. **6**(2), 745-750 (2009)

Adhatoda vasica: As a potential nematicidal source against root-knot *Meloidogyne incognita*

JASHODA KUMARI*, ANKITA SHARMA, MADHU RATHORE and KANIKA SHARMA

Microbial Research Laboratory, Depertment of Botany, MLS University, Udaipur- 313001 (India).

(Received: March 17, 2009; Accepted: April 27, 2009)

ABSTRACT

The effect of crude aqueous extract of different parts of *Adhatoda vasica* i.e. fresh leaves, inflorescence and bark on egg hatching and juvenile mortality of *Meloidogyne incognita* was studied *in vitro*. Fresh plant material was used to prepare the extracts. Observations were taken after 24, 48 and 72h. Leaf and inflorescence extract were found to be most effective on inhibition of egg hatching after 48h whereas bark extract was found to be less effective. 100% juvenile mortality was observed with undiluted crude aqueous leaf extract whereas inflorescence and bark extract exhibited only 86.66% and 52.66% mortality respectively after 48h.The preliminary phytochemical screening was done to show the presence of secondary metabolites in crude aqueous extract of *Adhatoda vasica*.

Key words: *Adhatoda vasica*, Plant extract, *Meloidogyne incognita*, Hatching, Mortality, and Phytochemical analysis.

INTRODUCTION

Disease of crop plants cause serious losses and have adverse effect on the agricultural economy of a country. Infection by nematode, especially *Meloidogyne incognita* (Root knot nematode) is one of the reason for severe losses in vegetable plants in all over world (Dawar, 2007). In recent years, the use of botanicals for the control of nematode is gaining importance because of increased awareness of environmental and human health hazards associated with the chemical control. Several plants are known to possess nematicidal and nematostatic properties (*Hassan et al*., 2000 and Pandey *et al.,* 2001). Various organic additives of plant origin including oil- seed cakes, leaves and other plant parts are being effectively used for management of nematode (Patel *et al.,* 2004; Sharma and Trivedi, 2002). In the present study, *in vitro* effect of crude aqueous extract of leaf, inflorescence and bark of *Adhatoda vasica* on egg hatching and larval mortality was studied. Preliminary phytochemical screening was also studied.

MATERIAL AND METHODS

Extract preparation

Healthy leaves, inflorescence and bark of *Adhatoda vasica* used for the study. Fresh plant material was collected from Botanical garden, College of Science, Udaipur (Raj.) and washed with sterile distilled water. 100gm of respective fresh plant material was macerated with 100ml sterile distilled water and the resultant solution was allowed to stand for 48h in the refrigerator, after that the following solution was passed through a four ply muslin cloth and centrifuged for 15 minutes at 3000rpm. The supernatant was filtered through Whatman's filter paper No.-1 (Patel *et al*., 2004) and filtrate was further diluted with sterile distilled water to prepare 5%, 10%, 20%, 40% and 80% extract

concentrations. Undiluted filtrate was taken as 100% concentration.

Inoculum development

Brinjal seedlings were grown in steam sterilized soil and inoculated with pure stock culture of *Meloidogyne incognita* obtained from previous experiment. From galled roots egg masses of *M. incognita* were extracted after 45 days using sodium hypochlorite (Hussey and Barker, 1973) and incubated at 30^{0}C for 24h. The freshly hatched second stage nematode juveniles were collected and used as inoculum. The suspension containing juveniles of *M. incognita* in distill water was concentrated and standardized so that each 1ml suspension contained approximately 100 juveniles.

Nematicidal activity of extract

The nematicidal effect of plant extract was done by following two methods

Hatching assay

M. incognita egg masses of roughly similar sizes were placed in glass cavity blocks (1 egg mass/ cavity block) containing 3ml aqueous extract of respective plant and incubated at room temperature. Three replicates and a distilled water control were maintained simultaneously. Observations were taken at 24, 48 and 72h interval.

Mortality assay

Second stage juvenile (J_2) of *M. incognita* were used to assay the effect of leaf, inflorescence and bark of *A. vasica* on juvenile mortality. One ml of nematode suspension containing approximately 100 *M. incognita* J2 juvenile was mixed with 3ml of respective plant extract in cavity blocks using sterile distilled water as control. Each treatment, including the control, was replicate 3 times. Observations were taken at 24, 48 and 72h intervals and percent larval mortality was calculated. Larval that did not respond to touch by a fine needle were counted as dead.

Phytochemical screening

Crude aqueous extract of *A. vasica*, leaf, inflorescence and bark was subjected to qualitative test for presence of alkaloids, sterols, tannins, carbohydrates and saponins suggested by Kokate *et al.,* (1990)

Statistical analysis

The significance of the work was analyzed statistically. R (Relation coefficient) and P-value was calculated by linear regression analysis using the software origin (6.0). Standard deviation and Standard error (SD & SE) were also calculated.

RESULTS

Result of effect of crude aqueous extract of different plant parts of *Adhatoda vasica* on egg hatching and juvenile mortality of *Meloidogyne incognita* after 24, 48, and 72 h are listed in table 1

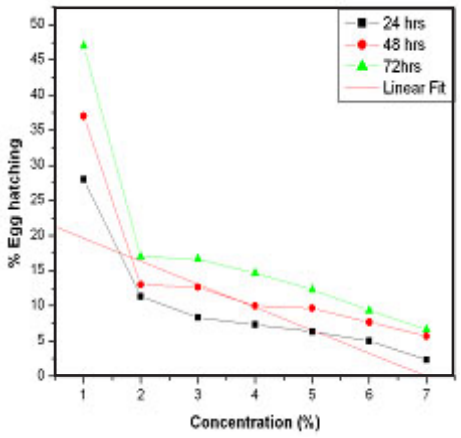

Fig.1: Effect of crude aqueous leaf extract of *A. vasica* on egg hatching of *M. incognita* (R=0.832; P= 0.020)

Fig. 2: Effect of crude aqueous inflorescence extract of *A. vasica* on egg hatching of *M. incognita* (R= -0.954; P= 8.26)

and 2. Since all plant parts inhibit egg hatching and showed juvenile mortality of *M. incognita*, it can be suggested that *A. vasica* possess nematicidal activity. At 24h, 28% egg hatching was observed in control, where as only 2.33 % egg hatching was observed with 100% concentration of leaf extract. Similarly, only 8.66% and 14.33% egg hatching was observed at the same concentration of inflorescence and bark extract after 24h. Results of mortality assay shows that maximum juvenile mortality was observed with leaf extract followed by inflorescence and bark extract. These results suggest that extract concentration and time of exposure are directly proportional to inhibition of egg hatching and juvenile mortality. Of the three plant parts used, aqueous extract of leaf was found to be most effective inhibitor of egg hatching followed by inflorescence and bark. These results are corroborated by statistical analysis. The experiment of inhibitory effect of egg hatching with leaf extract is significant (P= 0.02<0.05, R= 0.83) whereas with inflorescence and bark are not significant (R= -ve) Fig. (1, 2 & 3) In case of juvenile mortality experiment, significant P values were obtained for the crude aqueous

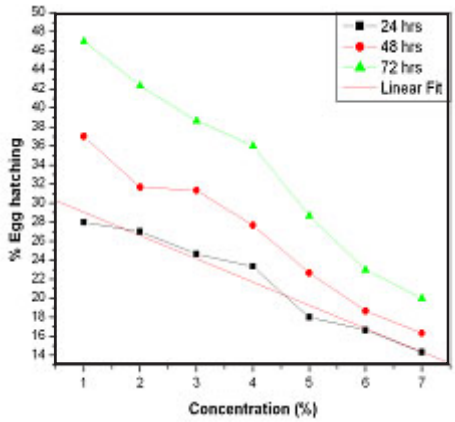

Fig. 3: Effect of crude aqueous bark extract of *A. vasica* on egg hatching of *M. incognita*. (R= -0.983; P= <0.0001)

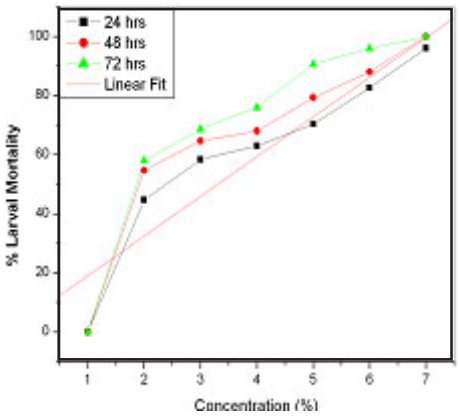

Fig. 4: Effect of crude aqueous leaf extract of A. vasica on juvenile mortality of *M. incognita.* (R=0.936; P= 0.0019)

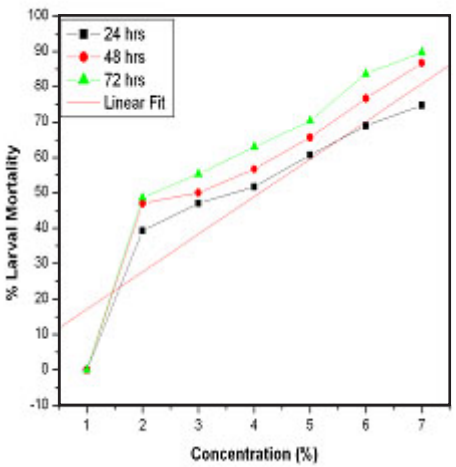

Fig. 5: Effect of crude aqueous inflorescence extract of *A. vasica* on juvenile mortality of *M. incognita*. (R=0.923; P= 0.0029)

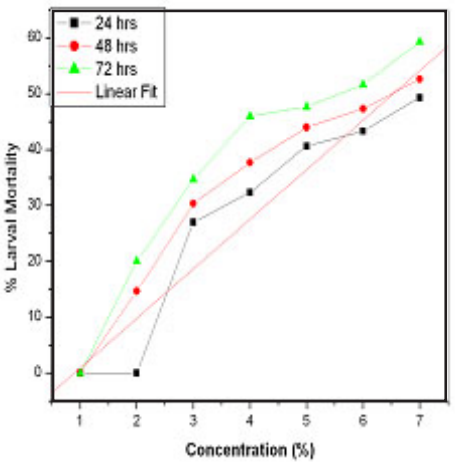

Fig. 6: Effect of crude aqueous bark extract of *A. vasica* on juvenile mortality of *M. incognita* (R=0.950; P= 0.0010)

Table 1: Effect of crude aqueous extract of different plant parts of *Adhatoda vasica* on egg hatching of root-knot nematode *Meloidogyne incognita*

Plant part	Time duration In hours	Control (%)	% Egg Hatching						SD (±1)	SE (±1)
			5%	10%	20%	40%	80%	100%		
Leaf	24	28	11.33(59.53)	8.33(70.25)	7.33(73.82)	6.33(77.39)	5.00(82.14)	2.33(91.67)	8.5	3.20
	48	37	13.00(64.86)	12.66(65.78)	10.00(72.97)	9.66(73.89)	7.66(79.29)	5.66(84.70)	10.6	4.01
	72	47	17.00(54.05)	16.66(64.55)	14.66(68.80)	12.33(73.76)	9.33(80.14)	6.66(85.82)	13.5	5.09
Inflorescence	24	28	19.00(32.14)	17.66(36.92)	16.33(41.67)	13.66(51.21)	9.66(65.5)	8.66(69.07)	6.5	2.46
	48	37	22.00(40.54)	19.33(47.75)	17.33(53.16)	15.33(68.06)	11.33(76.39)	10.33(78.48)	8.9	3.39
	72	47	24.66(47.53)	21.66(53.91)	19.33(58.87)	17.33(63.12)	14.00(70.21)	12.66(73.06)	11.6	4.39
Bark	24	28	27.00(3.57)	24.66(11.92)	23.33(16.67)	18.00(35.71)	16.66(40.50)	14.33(48.82)	5.3	2.02
	48	37	31.66(14.43)	31.33(15.32)	27.66(25.24)	22.66(38.75)	18.66(49.56)	16.33(55.86)	7.5	2.85
	72	47	42.33(9.93)	38.66(17.74)	36.00(23.40)	28.66(39.02)	23.00(51.06)	20.00(57.44)	11.9	4.51

Each value is an average of three replicates. Value in ( ) parentheses are percent decreasing over control

Table 2: Effect of crude aqueous extract of different plant parts of *Adhatoda vasica* on juvenile mortality of root-knot nematode *Meloidogyne incognita*

Plant part	Time duration In hours	Control (%)	% Larval Mortality						SD (±1)	SE (±1)
			5%	10%	20%	40%	80%	100%		
Leaf	24	00	44.66	58.33	63.00	70.33	82.66	96.00	30.9	11.71
	48	00	54.66	64.66	68.00	79.33	88.00	100.00	32.4	12.24
	72	00	58.00	68.66	76.00	90.66	96.00	100.00	34.3	12.98
Inflorescence	24	00	39.33	47.33	51.66	60.66	69.00	74.66	24.9	9.41
	48	00	47.00	50.00	56.66	65.66	76.66	86.66	27.9	10.57
	72	00	48.66	55.33	63.00	70.33	83.66	89.66	29.7	11.22
Bark	24	00	00	27.00	32.33	40.66	43.33	49.33	20.1	7.61
	48	00	14.66	30.33	37.66	44.00	47.33	52.66	18.9	7.17
	72	00	20.00	34.66	46.00	47.66	51.66	59.33	20.7	7.82

Each value is an average of three replicates.

Table 3: Preliminary phytochemical screening of *Adhatoda vasica*

Plant part	Alkaloid	carbohydrate	Flavonoid	Volatile oil	Tannin	Saponin	Steroid
Leaf	+	-	-	+	+	+	+
Inflorescence	+	-	-	+	-	+	-
Bark	-	-	-	-	+	+	-

+ Present, – absent

extract of all three plant parts. Higher value of R (Correlation coefficient) in linear regression plots gave good linearization between concentration of extracts and percent mortality rate of *M. incognita*. (Fig 4, 5 & 6) Preliminary phytochemical screening are presented in Table no. 3

DISCUSSION

Plants are a source of naturally occurring pesticides. Plants contain several secondary metabolites which are responsible for their antimicrobial activity (Mcgow anf Eloff, 2005; Baswa *et al.,* 2001). Most of these secondary metabolites like alkaloids, flavonoids, saponins, tannins volatile oil etc. are soluble in water as well as organic solvent (Harborne,1984). Nematicidal activity of aqueous extract of several plants has been reported earlier (Singh *et al*., 2001; Sharma and Patel 2001; Sosamma and Jayasree, 2002). In present study significant larval mortality and inhibition of egg hatching was observed with crude aqueous extract of leaf, inflorescence and bark of *A. vasica*. The presence of some toxic chemical compounds having the nematicidal/ nematotoxic activity may be responsible for the larval mortality and inhibition of egg hatching (Shngh and Sitaramaiah, 1973; Khan 1974). Several nematicidal compounds like alkaloids, flavonoids, terpinoids and phenolic compounds have been isolated from plants (David J. Chitwood, 2002). The nematicidal activity of aqueous extract of *A. vasica* is due to presence of biologically active compounds. Wide spectrum of antimicrobial activity such as antibacterial, antifungal activity, pesticidal activity etc (Prema, 2004; Inee Gogai *et al.,* 2005; Sadek, 2003) of *A. vasica* are reported. Results of preliminary phytochemical screening also suggest the presence of alkaloids, volatile oils saponins, tannins and steroid in *A. vasica*. Thus it can be suggested that inhibition of hatching and juvenile mortality of *M. incognita* may be attributed to the presence of these secondary metabolite in the crude aqueous extract of *A. vasica*.

REFERENCES

1. Baswa M Rath C C, Dash S K and Mishra R K., Antibacterial activity of Karanj (*Pongamia pinnata*) and Neem (*Azadirachta indica*) seed oil; *a preliminary report. Microbs* **105**: 183-189 (2001).
2. David J Chitwood. Phytochemical based strategies for nematode control; *Annu. Rev. Phytopathol* **40**: 221-249 (2002).
3. Dawar S, Younus S M And Zaki M J. Use of *Eucalyptus sp,* In the control of *Meloidogyne javanica* root- knot nematode; *Pak. J. Bot*. **39**(6): 2209-2214 (2007).
4. Harborne J B. Method of plant analysis, In: Phytochemical methods , *Chapman and Hall, London, New Yark*, 05-06 (1984).
5. Hassan S M E, Rahman M Sq, Amin M R, Majumdar U K and Ahmad M U, Effect of Neem (*Azadirachta indica*) on the root-knot nematode (*Meloidogyne jananica*) of sweet gourd; *Pak. J. Bio. Sci*. **3**(11): 1853-1854 (2000).
6. Hussey R S and Barker R K, A comparison of methods of collecting inocula of *Meloidogyne spp*. including a new technique;

Plant Dis. Rep. **57**: 1025-1028 (1973).

7. Inee Gogoi, Rahman I, Dolui A K, Preference of alternate host plants by *Helopeltis theivora* water house and their susceptibility to plant extract; *Proc. of the National Acad. of Sci. India. section B, Biological Science*; **75**(3): 181-185 (2005).
8. Khan M W, Alam M M, Khan A M & Saxena S K, Effect of water soluble fractions of oil cakes and bitter principles of neem on some fungi and nematodes; *Acta Bot. Indica* **2**: 120-128 (1974).
9. Kokate C K, Purohit A P and Gokhle S B Pharmacognocy. In Analytic pharmacogocy, 7th (Ed.) *Nirali Prakashan, Pune;* 122-124 (1990).
10. Mcgaw L J and Eloff J N, Screening of 16 poisonous plants for antimicrobial, anthelmintic and cytotoxic activity *in vitro*; *South African J. Bot.* **71**: 302-306 (2005).
11. Pandey R, Kalra A, Katiyar N and Kumar S Nematicidal activity in flowers of some medicinal and aromatic plants; *Indial J. Nematol* **31**(1): 79-98 (2001)
12. Patel A D, Patel D J and Patel N B, Effect of aqueous leaf extract of botanicals on egg hatching and larval penetration of *Meloidogyne incognita* in banana; *Indian J Nematol* **34**(1): 37-39 (2004).
13. Prema P, Antimicrobial activity of selected medicinal plants; *Journal of Ecobiology* **16**(5): 333-337 (2004).
14. Sadek M M, Antifeedant and toxic activity of *Adhatoda vasica* leaf extract against *Spodoptera littoralis* (Lep., Noctuidae); *Journal of Applied Entomology* **127**(9): 396-404 (2003).
15. Sharma D N P and Patel H R, Effect of various exposure periods and concentrations of crude leaf extract of Bidi tobacco on egg hatching of root- knot nematode; *Indian J. Nematol* **31**(2): 129-132 (2001)
16. Sharma N and Trivedi P C, Screening of leaf extracts of some plants for their nematicidal and fungicidal properties against *Meloidogyne incognita* and *fusarium oxisporium*; *Asian J. Exp. Sci.* **16**(122): 21-28 (2002).
17. Singh R A and Sitaramaiah K, Control of plant parasitic nematode with organic amendments of soil; *G.B. Pant University of Agric. & Tech. Pantngar, Research bulletin* **6**: 289 (1973).
18. Singh R, Chhabra H K and Kaul V K, Effect of aqueous extract of plant products on hatching and penetration of *Meloidogyne incognita* infecting sunflower; *Indian J Nematol* **31**(1): 34-37 (2001).
19. Sosamma V K and Jayasree D, Effect of leaf extract on the mortality of root-knot nematode, *Meloidogyne incognita* juveniles; *Indain J. Nematol.* **32**(2): 183-233 (2002).