Asian J. Exp. Sci., Vol. 16, No. 1&2, 2002, 21-28

Screening of Leaf Extracts of Some Plants for Their Nematicidal and Fungicidal Properties Against *Meloidogyne incognita* and *Fusarium oxysporum*

Nidhi Sharma and P.C. Trivedi
Department of Botany
University of Rajasthan, Jaipur -302 004

Fresh leaf extracts of *Datura stramonium, Calotropis procera, Verbesena enceloides, Parthenium hysterophorus, Morus alba, Phyllanthus amarus, Eichhornea crassipes, Ricinus communis, Jatropha curcas, Azadirachta indica, Tinospora cordifolia, Clerodendron multiflorum, Catharanthus roseus* and *Adhatoda vesica* were tested against root-knot nematode, *Meloidogyne incognita* and wilt fungus, *Fusarium oxysporum* f.sp. *cumini* infesting cumin. In the preliminary studies, almost all the plant species exhibited nematicidal and antifungal property. *Calotropis procera* and *Ricinus communis* gave best results against the nematode and *Datura stramonium* and *Calotropis procera* showed maximum antifungal activity against *Fusarium oxysporum* f.sp. *cumini*.

Key Words : *Fusarium oxysporum* f.sp. *cumini, Meloidogyne incognita, Trichoderma viride, T. harzianum, T. virens, T. hamatum.*

Introduction :

Cumin (*Cuminum cyminum* L.) is one of the most important spice crop of Rajasthan covering 163, 688 hectares with a production of 64,892 MT (Sreekumar, 1994). The root-knot nematode, *Meloidogyne incognita* is an important root parasite infecting cumin and causing about 43% reduction in the yield (Midha and Trivedi, 1991). Another limiting factor is *Fusarium oxysporum* f.sp. *cumini* causing wilt of cumin and losses upto 80% (Mathur and Prasad, 1964a). But management of root-knot nematodes and wilt fungus with chemicals, under field condition is cost prohibitive, hazardous and cause serious environmental pollution. So efforts are being made these days to shift from the conventional use of chemicals to the use of eco-friendly botanicals for the management of plant parasitic nematodes. Organic amendments are not only safe to use but also have the capacity to improve soil structure and fertility. Thus, control strategies are now directed towards the use of natural products.

Bioactive products of plants being less persistent in environment and are safe for mammals and other non target organisms. Botanical pesticides are readily available in many places, often cheaper than their synthetic counter parts and their crude extracts are easy to prepare even by farmers. These are also less likely or slow down the development of resistance or resurgence in pests. The benefits of natural pesticides have aroused interest in protection of crop plants. The present paper reports the in vitro nematicidal and antifungal activity of leaf extracts of fifteen plants against root knot nematode *Meloidogyne incognita* (Kofoid and White) Chitwood and wilt fungus, *Fusarium oxysporum* f.sp. *cumini* obtained from infected cumin roots.

Material and Methods :

For nematicidal activity of plants : The fifteen plants which were screened for nematicidal activity are listed in Table-1. Fresh leaves of the plants were collected and washed in sterile water. Leaf extract was prepared by grinding 2g each of fresh whole leaves in 5ml distilled water using a pestle and mortar. In order to remove plant debris, the extracts were passed through a four ply muslin cloth and centrifuged for 5 minutes at 4000 rpm and filtered through Whatman's filter paper no.-1. The stock solution, thus obtained was used for evaluating their nematicidal activity and it was designated as hundred per cent. From this standard / stock solution(s), required concentrations (25,50,75,100) were prepared by adding distilled water. Distilled water alone served as control. For the hatching experiment special polyvinyl chloride (PVC) tubing (4 cm dia; 0.5 mm high) were cut and mm stainless steel screen was sealed to each ring. Four P.V.C. legs were attached to elevate each ring.

For obtaining population of egg masses, pure culture of *Meloidogyne incognita* was maintained on cumin in sterilized soil. Effect on hatching was evaluated on two mature egg masses of uniform size suspended in the extracts and water (check) replicated three times in cavity blocks. The cavity blocks were kept at 26±1°C. The number of hatched juveniles were counted after 24,48 and 72 hrs of treatments. After every 24 hrs. test solution was discarded after counting the number of hatched larvae and the unhatched eggs in the sieve were placed in freshly made test solution. This was done to eliminate the effect of bacterial action on the unhatched eggs which were removed

from the test solution. The unhatched eggs were placed in distilled water for another 24 hrs to record further hatching, if any.

For antifungal activity of plants : As given in Table-2, 15 plant leaves extracts were tested for their fungicidal efficacy by the poisoned food technique (Nene and Thapliyal, 1993). The extraction from plant parts was done with the help of pestle and mortar. By adding equal amount of hot water, the extracted material was then filtered through muslin cloth, further 6ml of extract was mixed with 100 ml of molten PDA cooled to 45°C and @1% (w/v) and sterilized in autoclave. Each treatment was replicated thrice with appropriate untreated controls. These were incubated for 3 days at 28±1°C before recording radial mycelial growth of *Fusarium oxysporum* f.sp. *cumini.*

Results and Discussions :

The efficacy of leaf extracts of various plants on hatching of *M. incognita* eggs have been depicted in Table-1. Amongst the fifteen plants tested, leaf extracts of almost all the plants exhibited a gradual increase in hatching of eggs from their higher concentration to lower concentration treatments. This showed that higher concentration of leaf extracts were inhibitory in action against egg hatching of the nematode. Thus hatching was maximum after 72 hours in lowest concentration (S/100) in almost all plants at varying degrees. All the nematodes in control (dis. water) remained active during the period of observations.

Exposure time played an important role in the mortality of nematode. Nematodes did not revive when transferred to dis. water after exposure to various extracts indicating the toxic effect as irreversible. But hatching of juveniles enhanced after the eggs were transferred from the plant extracts to distilled water. Complete inhibition of hatching was observed in the highest concentration in *Azadirachta indica* after 72 hrs and in *Calotropis procera* after 24 hrs. Maximum inhibition of hatching was in case of *Calotropis procera* (11.28%) and *Ricinus communis* (11.91%).

Several plant extracts are known to possess nematotoxic properties (Sosamma and Jayasree, 2002). Alongwith this several workers Nandal and Bhatti (1993) Trivedi *et al.* (1980) reported the role of Calotropis leaves in

reducing nematode population. The reduction of nematode population may be attributed to the production of nematicidal substances like terthienyl, triterpenoid and other alkaloids by organic compounds. Rastogi and Mehrotra (1995) isolated two triterpene esters with biological activity from *Calotropis* leaves.

According to the results (Table-2) plant leaf extracts of *Datura stramonium* and *Calotropis procera* were found to be highly significant in reducing the radial growth of the pathogen. (72.33% and 67.94% respectively). Leaf extracts of *Parthenium hysterophorus* and *Ricinus communis* (67.52%) and *Phyllanthus amarus* and *Tinospora cordifolia* (65.82%) showed the same per cent inhibition of the growth of the pathogen. The other extracts in order of superiority were *Azadirachta indica, Jatropha gossypifolia, Lawsonia inermis, Eichhornia crassipes, Verbesena enceloides* and *Morus alba*. The aqueous extracts of these plants were found to affect the growth of the fungus. It is therefore, encouraging to identify and characterize the active principle. Moreover, because of the water soluble nature of the toxic principle, it is ideal for developing into herbal pesticides. The inhibitory effect of the plant extracts might be attributed to the presence of some antifungal toxicants.

Several authors have also reported the fungicidal activity in wide variety of taxa. Ravichandar (1987) reported that the growth of *R. solani* was completely inhibited with the leaf extract of *Acacia nilotica*. Neem and akven leaf extracts are also known to reduce the viability of *R. solani* and mycelial growth considerably *in vitro* (Manibhushan Rao *et al*., 1988). Grewal and Grewal (1988) mentioned differential fungicidal properties of leaf extract of *Azadirachta indica, Chrysanthemum indicum* and *Tagetes erecta* against various weed moulds of mushroom. Sarkar *et al*. (1988) used leaf extracts of *Casuarina* and water hyacinth for reducing the incidence of weed fungi in *Pleurotus* beds. The presence of antifungal compounds in higher plants is well recognised and considered valuable for plant disease control (Singh and Dwivedi, 1987). Various plant extracts have been evaluated for their antifungal property against different pathogens (Tripathi *et al*., 2002; Mathur and Gurjar, 2002).

Table 1 : Screening of some plant leaf extracts for toxic effect on larvae of Meloidogyne incognita infecting cumin (Cuminum cyminum L.)

S.No.	Plant used	Duration in Hours	Number of juveniles hatched control				Control (D.W.)
			S/25	S/50	S/75	S/100	
1.	*Datura stramonium*	24	1.81	3.62	5.50	7.81	29
	Linn. (Solanaceae)	48	2.60	3.79	5.00	8.19	38
		72	3.29	5.77	7.89	11.42	47
		Total % hatched	3.86	6.59	9.19	13.71	57
2.	*Calotropis procera*	24	0.00	2.33	4.50	6.33	29
	(Ait) R.Br.	48	1.50	2.50	4.66	7.23	38
	(Asclepiadaceae)	72	3.22	5.17	6.11	9.00	47
		Total % hatched	2.36	5.00	7.63	11.28	57
3.	*Adhatoda vesica*	24	7.30	9.00	11.51	13.00	29
	Nees. (Acanthaceae)	48	6.50	7.33	9.33	11.47	38
		72	9.83	11.24	13.56	16.99	47
		Total % hatched	11.81	13.78	17.2	20.73	57
4.	*Verbesena encaloides*	24	6.33	10	14.66	16.23	29
	Benth. and Hook	48	7.00	11.33	17.66	19.55	38
	(Verbenaceae)	72	9.66	13.33	22.33	25.33	47
		Total % hatched	11.49	17.33	19.99	30.55	57
5.	*Parthenium*	24	8.3	10	14.5	16.80	29
	hysterophorus	48	10	12	18	20.11	38
	Linn. (Compositae)	72	11	15	24	27.44	47
		Total % hatched	14.65	18.50	28.25	32.17	57
6.	*Morus alba* Linn.	24	13.66	23	29	32.33	29
	(Moraceae)	48	16.66	21.66	27.66	30.14	38
		72	24.33	26.33	31.00	33.66	47
		Total % hatched	27.32	35.49	43.83	48.06	57
7.	*Phyllanthus amarus*	24	20	22.33	25.33	27.00	29
	(Euphorbiaceae)	48	22	26	31.66	33.66	38
		72	24.66	27.33	34	36.50	47
		Total % hatched	33.33	37.83	45.49	48.58	57
8.	*Eichhornea*	24	8.33	12	16.66	21.70	29
	crassipes Solms.	48	10	14.66	18.66	23.03	38
	(Pontederiaceae)	72	12.33	17.33	21.00	27.82	47
		Total % hatched	15.33	13.33	28.16	36.27	57

Contd.....

S.No.	Plant used	Duration in Hours	Number of juveniles hatched control				Control
			S/25	S/50	S/75	S/100	(D.W.)
9.	*Jatropha curcas*	24	3.00	6.24	9.15	11.79	29
	Linn.	48	3.12	5.46	7.32	12.70	38
	(Euphorbiaceae)	72	5.66	10.99	12.50	13.00	47
		Total % hatched	5.89	11.34	14.48	18.74	57
10.	*Ricinus communis*	24	2.50	4.01	5.13	7.82	29
	Linn.	48	2.66	3.19	5.00	7.00	38
	(Euphorbiaceae)	72	4.00	5.33	7.66	9.00	47
		Total % hatched	4.58	6.26	8.89	11.91	57
11.	*Azadirachta indica*	24	2.5	2.5	11	22.5	29
	A. Juss. (Meliaceae)	48	0.5	3.0	4.5	8.5	38
		72	0.0	0.0	0.5	0.5	47
		Total % hatched	1.5	2.7	8.0	15.7	57
12.	*Clerodendron*	24	4.66	6.13	7.91	11.12	29
	multiflorum (Burm.f.)	48	3.77	5.44	6.22	10.50	38
	O. Ktze (Verbenaceae)	72	5.42	6.50	7.11	11.00	47
		Total % hatched	6.92	9.03	10.62	16.31	57
13.	*Catharanthus roseus*	24	16.8	25.2	27.3	30.1	29
	L. (Apocynaceae)	48	11.00	14.66	17.33	19.50	38
		72	7.8	18.50	24.00	28.50	47
		Total % hatched	17.8	29.18	34.31	39.05	57
14.	*Tinospora cordifolia*	24	5.33	7.12	9.52	11.77	29
	(Willd.) Miers.	48	4.81	6.33	8.00	10.50	38
	(Menispermaceae)	72	6.66	7.00	9.05	11.00	47
		Total % hatched	8.4	10.22	13.28	16.63	57
15.	*Lawsonia inermis*	24	3.00	5.79	7.82	9.00	29
	Roxb. (Lythraceae)	48	4.05	6.50	9.11	11.79	38
		72	5.66	7.24	8.15	9.32	47
		Total % hatched	6.35	9.76	12.54	15.05	57
	CD at 5%		0.58	1.29	1.32	0.77	

Table 2 : Screening of some plant leaf extracts for antifungal effect against *Fusarium oxysporum* f. sp. *cumini*.

S. No.	Name of Plants	Colony growth of *Fusarium oxysporum* f.sp. *cumini* (mm)		Growth inhibition of *Fusaruim oxysporum* f.sp. *cumini* (%)
		Control (mm)	Interaction (mm)	
1.	*Datura stramonium* Linn.	78	21.66	72.23
2.	*Calotropis procera* (Ait) R.Br.	78	25	67.94
3.	*Verbesena enceloides* Benth. Hook	78	35	55.12
4.	*Parthenium hysterophorus* Linn.	78	25.33	67.52
5.	*Morus alba* Linn.	78	37.33	52.14
6.	*Phyllanthus amarus*	78	26.66	65.82
7.	*Eichhornea crassipes* (Mart.) Solms.	78	33	57.69
8.	*Jatropha curcas* Linn.	78	30	61.53
9.	*Ricinus communis* Linn.	78	25.33	67.52
10.	*Azadirachta indica* A. Juss.	78	29	62.82
11.	*Tinospora cordifolia* (Willd.) Miers	78	26.66	65.82
12.	*Lawsonia inermis* Roxb.	78	30.33	61.11
13.	*Adhatoda vesica* Nees.	78	41	47.43
14.	*Clerodendron multiflorum* (Burm. f.) O. Ktze	78	44	43.58
15.	*Catharanthus roseus* L.	78	37	52.56
	CD at 5%		6.93	8.87

Values are mean of 3 replicates.

References :

Grewal P.S. and Grewal S.K. (1988) : Selective fungicidal properties of some plant extract to mushroom weed moulds. *Phytopathol Medit.* **27,** 112-114.

Manibhushanrao K., Baley U.I. and Joe Y. (1988) : Influence of various amendments on soil microflora in relation to sheath blight of rice. 5th Int. Cong. Pl. Pathol. Kyota, Japan.

Mathur B.L. and Prasad N. (1964) : Variation in Fusarium oxysporum f.sp. cumini. *Indian J. Agric. Sci.* **34(4),** 273-277.

Mathur Kamlesh Singh R.D. and Gurjar R.B. (1995) : Evaluation of different fungal antagonists, plant extracts & oil cakes against Rhizoctonia solani causing stem rot of chilli. *Ann. Pl. Protec. Sci.* **10(2),** 319-322.

Midha R.L. and Trivedi P.C. (1991) : Estimation of losses caused by Meloidogyne incognita on coriander, cumin and fennel. *Current Nematology.* **2,** 159-162.

Nandal S.N. and Bhatti D.S. (1993) : Preliminary screening of some weeds and shrubs for their nematicidal activity against Meloidogyne javanica. *Indian J. Nematol.* **13,** 123-127.

Nene Y.L. and Thapliyal, P.N. (1983) : Fungicides in Plant Disease Control Oxford and IBH Publ. Co., New Delhi, 507 pp.

Rastogi P.M. & Mehrotra, B.N. (1995) : In compendium of Indian Medicinal Plants IV. 137.

Ravichandar R. (1987) : Studies on antifungal activity of some plant extracts. II M.Sc. (Ag.). Thesis Tamil Nadu Agric. Univ. Coimbatore, pp. 90.

Sarkar B.B., Bhattacharjee A.K. and Chakraborti D.K. (1988) : Effect of hot water treatment and plant materials on the control of weed fungi in tray culture of oyster mushroom. *Indian J. Mush.* **10-11,** 82-86.

Singh R.K. and Dwivedi R.S. (1987) : Effect of oil on Sclerotium rolfsii, causing root rot of barley. *Indian Phytopath.* **40,** 531-533.

Sosamma V.K. and Jayasree D. (2002) : Effect of leaf extracts on the mortality of root-knot nematode, Meloidogyne incognita juveniles, *Indian J. Nematol.* **32(2),** 183-233.

Sreekumar B. (1994) : Production and export of seed spices with special reference to Rajasthan. *Spices Indica*. **7,** 6-8.

Tripathi A.K., Verma K.P., Agrawal K.C. and Rao S.S. (2002) : Effect of plant extracts on mycelial growth, sporulation & spore germination of Alternaria lini under in vitro condition. *J. Mycol. Pl. Pathol.* **32(2),** 268-269 (Abstr.).

Trivedi P.C. Bhatanagar, A. and Tiagi B., (1980) : Effect of decomposed green leaves on the incidence of root-knot of chilli. Univ. *Studies in Botany*. **9,** 8-13.