MANAGEMENT OF PHYTONEMATODES INFECTING TOMATO AND EGGPLANT WITH METHANOL EXTRACTS OF TWO PLANT SPECIES

A. Usman and M.A. Siddiqui

Plant Pathology and Nematology laboratories, Department of Botany, Aligarh Muslim University, Aligarh-202002, U.P, India

Re-submitted: *19 November 2010; Accepted*: *2 March 2012*.

Summary. Leaf extracts of the plants *Jatropha curcas* and *Brassica nigra* were used as bare-root dip treatments for the management of the phytonematodes *Meloidogyne incognita* and *Rotylenchulus reniformis* infecting tomato (*Solanum lycopersicum*) and eggplant (*Solanum melongena*). The extracts of both plants significantly reduced the root-knot development caused by *M. incognita* and the multiplication of *R. reniformis*. Leaf extracts of *J. curcas* inhibited the multiplication of the nematode more than those of *B. nigra*. Plant growth was also improved. Higher concentrations of leaf extracts or longer duration of root dip treatment increased plant weight and reduced disease incidence.

Key words: Bare-root dip treatment, *Brassica nigra*, *Jatropha curcas*, leaf extract**,** phytonematodes.

Worldwide crop yields are reduced by more than 20% annually due to the actions of pests and diseases; however, individual fields may sustain losses of 50-100% from one or more pests (Dhaliwal and Koul, 2007). Phytonematodes are one of the main factors limiting yield of almost all types of crop. In India, yield losses due to root-knot nematodes (*Meloidogyne* spp.) range from 35.0 to 39.7% (Reddy, 1985; Jonathan *et al*., 2001). The control of phytonematodes is difficult because they inhabit the soil and usually attack the underground plant parts. The withdrawal from the market of hazardous broad spectrum pesticides/nematicides has emphasized the need for novel methods to control nematodes. There is now tremendous pressure on growers to use methods of nematode control which do not pollute or cause other undesirable side effects (Duncan, 1991) and that use biodegradable products (Tiyagi and Ajaz, 2004). A number of organic additives of plant origin, including oil-seed cakes, chopped plant parts and seed dressing made from plant extracts, have been used to control nematodes (Muller and Gooch, 1982; Tiyagi *et al*., 1988; Akhtar and Alam, 1993; Tiyagi and Ajaz, 2004). Root-dip treatment with various plant extracts was also found effective for controlling plant-parasitic nematodes (Siddiqui and Alam, 1990; Tiyagi *et al*., 1990) but the use of so-called 'botanicals' made from *Jatropha curcas* L. and *Brassica nigra* L. as bare-root dip treatment had not been reported earlier. Tomato and eggplant are important vegetable crops worldwide. Unfortunately, the productivity of these crops has decreased gradually, partly due to plant-parasitic nematodes, which affect both quality and quantity of yield. A preliminary soil survey of plant-parasitic nematodes, conducted in poor yielding fields of tomato and eggplant, in Aligarh and adjoining districts, revealed the presence of the root-knot nematode *M. incognita* (Kofoid *et* White) Chitw. and the reniform nematode *Rotylenchulus reniformis* Linford *et* Oliveira. Therefore, tests were conducted to evaluate the efficiency of leaf extracts of *J. curcas* and *B. nigra* for control *M. incognita* in tomato and *R. reniformis* in eggplant, used as bare-root dip treatments.

MATERIALS AND METHODS

Methanolic extracts of *J. curcas* and *B. nigra* were prepared by refluxing the dry matter (50 g) of dry leaves with methanol (350 ml) in a soxhlet apparatus for 8 h. The methanolic extracts were distilled and the crude extracts obtained after distillation were completely dried and the weight of the solid mass was measured. Ten per cent (w/v) methanolic extracts, designated as standard ('S'), were prepared for each plant. To do this, five grams of the solid methanolic extracts were dissolved in 15 ml of methanol and 1 ml of a surfactant (Exalin) was added and the volume made up to 50 ml with distilled water. Other dilutions, such as S/2 and S/10, were prepared by adding the required quantity of distilled water to the standard extracts.

Meloidogyne incognita was cultured on eggplant and egg masses were handpicked and placed in a Petri dish containing sterile distilled water, and kept at 28 ± 2 ^{0}C. Second-stage juveniles (J2) that emerged within 24 hours were used as inoculum for the experiments. *Rotylenchulus reniformis* was reared on cowpea and mixed vermiform stages of the nematode were extracted from the soil by the combined screening-funnel technique (Ayoub, 1980) and used within 1 day of extraction.

The roots of 21 day old seedlings of tomato cv. 'Pusa Ruby' and eggplant cv. 'Pusa Kranti', previously grown in sterilized micro-plots, were dipped in different concentrations of leaf extracts of *J. curcas* and *B. nigra* separately for 20, 40 or 80 minutes (Tables I-IV). After the dip treatment, the roots of these seedlings were washed several times with distilled water and transplanted immediately in earthen pots (15 cm) containing 1 kg of

Table I. Effects of bare root dip treatments in methanol extracts of leaves of *Jatropha curcas* and *Brassica nigra* on the inhibition of root-knot development of *Meloidogyne incognita* and plant growth of tomato cv. "Pusa Ruby".

Treatment		*Jatropha curcas*					*Brassica nigra*				
Dip duration (min)	Concen-tration of extract	Plant weight (g)			No. fruits per plant	Root-knot index	Plant weight (g)			No. fruits per plant	Root-knot index
		Root	Shoot	Total			Root	Shoot	Total		
20	S	9.89	20.57	30.46	9.0	1.87	9.88	20.12	30.00	9.0	2.0
	S/2	8.98	19.71	28.69	7.0	1.98	8.12	18.51	26.63	6.0	2.2
	S/10	7.84	18.61	26.45	5.0	2.15	7.02	17.52	24.54	5.0	2.5
40	S	11.94	21.71	33.65	10.0	1.20	10.78	21.44	32.22	9.0	1.4
	S/2	9.77	20.63	30.39	8.0	1.40	9.49	19.44	28.93	7.0	1.6
	S/10	8.51	19.63	28.14	5.0	1.85	8.55	18.35	26.90	5.0	1.8
80	S	13.17	23.02	36.19	11.0	0.95	11.64	22.63	34.27	10.0	1.2
	S/2	10.86	21.85	32.71	8.0	1.10	10.44	20.54	30.98	8.0	1.3
	S/10	9.74	20.51	30.25	7.0	1.48	9.49	19.06	28.56	7.0	1.4
Undipped inoculated control		5.52	8.44	13.96	4.0	5.00	5.52	8.44	13.96	4.0	5.0
Undipped uninoculated control		14.32	30.53	44.85	14.0	0.00	14.32	30.53	44.85	14.0	0.0
LSD at 5%		0.317	0.224	0.395	0.635	0.001	0.182	0.218	0.305	0.603	0.179

Initial nematode population = 1000 second stage juveniles per pot.
Each value is the average of five replicates.

Table II. Effects of bare root dip treatments in methanol extracts of leaves of *J. curcas* and *B. nigra* on the inhibition of root-knot development of *M. incognita* and plant growth of eggplant cv. "Pusa Kranti".

Treatment		*Jatropha curcas*					*Brassica nigra*				
Dip duration (min)	Concentration of extract	Plant weight (g)			No. fruits per plant	Root-knot index	Plant weight (g)			No. fruits per plant	Root-knot index
		Root	Shoot	Total			Root	Shoot	Total		
20	S	8.45	21.21	29.66	4.0	1.5	8.74	20.79	29.53	4.0	1.6
	S/2	7.63	19.49	27.12	3.0	1.7	7.48	18.66	26.14	3.0	1.8
	S/10	6.67	18.66	25.33	3.0	2.0	6.69	17.49	24.19	3.0	2.1
40	S	10.54	22.52	33.06	5.0	1.3	9.65	21.49	31.14	5.0	1.4
	S/2	9.63	21.49	31.12	4.0	1.5	8.78	20.30	29.08	4.0	1.7
	S/10	7.59	19.85	27.44	3.0	1.7	7.59	18.68	26.28	3.0	2.0
80	S	12.74	23.73	36.47	6.0	0.8	12.13	22.55	34.68	6.0	0.8
	S/2	10.66	22.56	33.22	5.0	1.1	10.22	21.45	31.67	5.0	1.2
	S/10	8.68	20.53	29.21	4.0	1.4	8.60	19.58	28.18	4.0	1.5
Undipped inoculated control		6.35	9.25	15.60	3.0	5.0	6.35	9.25	15.60	3.0	5.0
Undipped uninoculated control		15.59	27.34	42.93	9.0	0.0	15.59	27.34	42.93	9.0	0.0
LSD at 5%		0.381	0.364	0.464	0.351	0.167	0.393	0.354	0.586	0.351	0.173

Initial nematode population = 1000 second stage juveniles per pot.
Each value is the average of five replicates.

Table III. Effects of bare root dip treatments in methanol extracts of leaf of leaves of *J. curcas* and *B. nigra* on the inhibition of population of the reniform nematode *R. reniformis* and plant growth of tomato "Pusa Ruby".

Treatment		*Jatropha curcas*						*Brassica nigra*					
Dip duration (min)	Concentration of extract	Plant weight (g)			Final nematode population	No. of fruits /plant	Rf = Pf/Pi	Plant weight (g)			Final nematode population	No. of fruits /plant	Rf = Pf/Pi
		Root	Shoot	Total				Root	Shoot	Total			
20	S	8.55	28.55	37.11	1950	7.0	1.95	8.71	27.47	36.18	1970	7.0	1.97
	S/2	7.44	25.56	33.00	2150	6.0	2.15	6.61	25.57	32.18	2250	6.0	2.25
	S/10	6.61	22.92	29.53	2368	5.0	2.37	6.54	17.53	24.07	2450	5.0	2.45
40	S	9.44	29.90	39.34	1740	9.0	1.74	9.58	30.41	39.99	1750	10.0	1.75
	S/2	8.55	24.31	32.87	1850	7.0	1.85	8.62	25.64	34.25	1900	7.0	1.90
	S/10	7.30	19.50	26.79	2611	7.0	2.61	8.57	18.58	27.16	2250	7.0	2.25
80	S	12.56	32.51	45.07	1650	11.0	1.65	11.54	31.51	43.05	1550	10.0	1.55
	S/2	11.42	29.58	41.00	1830	9.0	1.83	10.34	28.49	38.83	1750	8.0	1.75
	S/10	9.65	21.53	31.18	1950	7.0	1.95	9.58	20.32	29.90	1850	8.0	1.85
Undipped inoculated control		7.67	9.60	17.27	4250	5.0	4.30	7.67	15.99	23.66	4250	5.0	4.25
Undipped uninoculated control		13.38	39.41	52.79	0.00	16.0	0.00	13.38	39.41	52.79	0.00	16.0	0.00
LSD at 5%		0.311	4.847	4.966	182.7	0.642	0.183	0.273	5.694	5.670	180.5	0.643	0.181

Rf = reproduction factor
Pi = (initial nematode population) = 1000 young females per pot.
Pf = final nematode population.
Each value is the average of five replicates

Table IV. Effects of bare root dip treatments in methanol extracts of leaves of *J. curcas* and *B. nigra* on the inhibition of population of the reniform nematode *R. reniformis* and plant growth of eggplant cv. "Pusa Kranti".

Treatment		*Jatropha curcas*						*Brassica nigra*					
Dip duration (min)	Concentration of extract	Plant weight (g)			Final nematode population	No. of fruits /plant	Rf = Pf/Pi	Plant weight (g)			Final nematode population	No. of fruits /plant	Rf = Pf/Pi
		Root	Shoot	Total				Root	Shoot	Total			
20	S	10.41	26.70	37.12	1950	8.0	1.95	9.42	27.50	36.92	1850	8.0	1.85
	S/2	9.61	24.61	34.22	2200	7.0	2.20	8.45	23.60	32.05	2000	7.0	2.00
	S/10	8.49	18.51	27.00	2550	6.0	2.55	7.59	19.58	27.17	2430	5.0	2.43
40	S	12.56	29.59	42.16	1750	8.0	1.75	12.48	28.52	41.00	1650	8.0	1.65
	S/2	10.55	26.52	37.06	1900	7.0	1.90	10.53	24.49	35.02	1870	7.0	1.87
	S/10	9.61	24.51	34.12	2300	6.0	2.30	8.69	22.52	31.21	2100	7.0	2.10
80	S	14.60	31.50	46.10	1450	10.0	1.45	13.57	29.60	43.17	1350	10.0	1.35
	S/2	12.53	28.58	41.12	1750	9.0	1.75	11.57	27.53	39.10	1430	8.0	1.43
	S/10	10.51	26.58	37.09	1950	7.0	1.95	9.66	24.77	34.43	1750	7.0	1.75
Undipped inoculated control		8.60	10.45	19.05	4152	4.0	4.15	8.60	10.45	19.05	4150	4.0	4.15
Undipped uninoculated control		19.66	34.44	54.10	0.00	11.0	0.00	19.66	34.44	54.10	0.00	11.0	0.00
LSD at 5%		0.277	0.200	0.302	180.0	0.588	0.180	0.315	0.198	0.326	171.1	0.584	0.171

Rf = reproduction factor
Pi = (initial nematode population) = 1000 young females per pot.
Pf = final nematode population.
Each value is the average of five replicates

steam sterilized soil-manure mixture in the following proportions: sand 60%, manure 17% and clay 23%. The seedlings of tomato and eggplant were then inoculated with 1000 freshly hatched second stage juveniles of *M. incognita,* or 1000 fourth stage infective young females of *R. reniformis*, according to the inoculation schedule given in the tables. Undipped inoculated and uninoculated plants served as controls. There were five replicates per treatment. The pots were arranged, in a randomized block design, on benches of a glass-house and held at 30 ± 2 °C; whenever necessary, any weeds were removed by hand and the plants were watered. One hundred days after the inoculation of the plants by the nematodes, the experiment was terminated. The plant roots were gently washed and the lengths of roots and shoots of the tomato and eggplants were measured. Root-knot indices were assessed on a 0-5 scale (Sasser *et al*., 1984). The soil population of *R. reniformis* was determined by processing the soil (250 g soil) using Cobb's sieving and decanting method and the Baermann's funnel technique (Southey, 1986).

Statistical analysis of the data for critical difference (C.D.) at P = 0.05 was done according to the procedure described by Pansey and Sukhatme (1978).

RESULTS

The data presented in Tables I to IV show that the root-dip treatment in different concentrations (S, S/2, S/10) of leaf extracts of *J.curcas* and *B.nigra* for different durations (20, 40 and 80 min) significantly reduced root-knot development incited by *M. incognita* and caused a significant reduction in multiplication of *R. reniformis* infecting tomato and eggplant. With *M. incognita,* the greatest reduction in root-knot index was observed in the plants treated with the S concentration for 80 min dip duration in both plants. Root-knot indices were greatest at 0.95 and 1.15 in the leaf extracts of *J. curcas* and *B. nigra* in respectively in tomato, and 0.80 and 0.80 in *J. curcas* and in *B. nigra* extracts, respectively in eggplant. With *R. reniformis*, the greatest reduction in nematode population was observed in both host plants when the seedlings had been dipped in the S concentration for 80 minutes. Plant weight of undipped inoculated plants was reduced by the root-knot nematode by 61.4% in tomato and 59.3% in eggplant and by *Rotylenchulus reniformis* by 42.7% in tomato and 56.3% in eggplant, as compared to undipped uninoculated plants. However, significant improvement in plant growth was noted in all plants treated with leaf extracts and inoculated with the nematodes. The reproduction factor of *R. reniformis* and root-knot index were greatest in undipped inoculated specimens of both test plants. *Brassica nigra* extracts were comparatively less effective against the nematodes than those *J. curcas*. Plant weight of tomato and eggplant increased with the increase in concentration of leaf extracts and the dipping duration, whilst the multiplication rate of *R. reniformis* and the root-knot index decreased with the increase in the concentration of the leaf extracts and the duration of the dipping treatment (Tables I-IV).

DISCUSSION

Nematode development in the roots dipped in leaf extracts of both *J. curcas* and *B. nigra* may have been affected by the leaching of chemicals coating the roots of the seedlings into the rhizosphere, which repelled or killed the nematode juveniles that attacked the roots. Such observations were reported by Akhtar and Alam (1990). In another study, Akhtar and Mahmood (1993) reported that the neem-based product Nemin, applied as a bare-root dip treatment, resulted in a decrease in nematode population and an improvement in plant growth. The growth of our undipped plants was reduced significantly by both *M. incognita* and *R. reniformis*; however this reduction was limited by the root-dip treatment with leaf extracts as a consequence of the reduction in disease incidence. This may be due to the presence of some substances in the leaves of *J. curcas* and *B. nigra* that exhibited high nematicidal activity. Alam *et al*. (1977, 1978, 1979) reported that ammonia, fatty acids, hydrogen sulphide, aldehyde, formaldehyde, and phenolic compounds are released following the decomposition of organic additives in the soil. These compounds were found detrimental to the population build-up of nematodes *in vitro*. Organic additives in the form of leaf extracts also released some phenolic compounds/nutrients that decreased root knot development. These treatments may also have resulted in the increase of induced defense mechanisms as reported by Kast (1985). Tiyagi *et al*. (1986) and Siddiqui and Alam (1989) suggested there may be systemic activity of some plant products when applied as root-dip treatment against nematodes. Thus, it appears that these plants offer promise for finding and developing new botanical-based biopesticides/nematicides.

LITERATURE CITED

Akhtar M. and Alam M.M., 1990. Effect of bare-root dip treatment with extracts of castor on root-knot development and growth of tomato. *Nematologia Mediterranea*, *18*: 53-54.

Akhtar M. and Alam M.M., 1993. Utilization of waste materials in nematodes control: a review. *Bioresource Technology*, *45*: 1-7.

Akhtar M. and Mahmood I., 1993. Control of plant parasitic nematodes with neemin and some plant oils by bare-root dip treatment. *Nematologia Mediterranea, 21*: 89-92.

Alam M.M., Khan A.M. and Saxena S.K., 1979a. Mechanism of control of plant-parasitic nematodes as a result of the application of organic amendments to the soil. IV. Role of formaldehyde and acetone. *Indian Journal of Nematology*, *8*: 172-174.

Alam M.M., Khan A.M. and Saxena S.K., 1979b. Mechanism of control of plant-parasitic nematodes as a result of the application of organic amendments to the soil. V. Role of phenolic compounds. *Indian Journal of Nematology*, *9*: 172-174.

Alam M.M., Siddiqui S.A. and Khan A.M., 1977. Mechanism of control of plant-parasitic nematodes as a results of application of organic amendments to the soil. III. Role of phenols and aminoacids in host roots. *Indian Journal of Nematology*, *7*: 27-31.

Ayoub S.M., 1980. *Plant nematology: An agricultural training aid*. Nema Aid Publications, Sacramento, Calif, USA, 195 pp.

Dhaliwal G.S. and Koul O., 2007. *Biopesticides and pest management: Conventional and biotechnological approaches*. Kalyani Publishers, New Delhi, India, 455 pp.

Duncan L.W., 1991. Current options for nematode management. *Annual Review of Phytopathology*, *29*: 467-490.

Jonathan E.I., Kumar S., Devarajan K., Rajendran G., 2001. Nematode pests of commercial flower crops. Pp. 20-48. *In*: Fundamentals of Plant Nematology (Jonathan E.I., ed.). Devi Publication, Trichy, India.

Kast W.K., 1985. Wirkung alternativer spritzfolgen auf pilzliche Schadetreger bei Reben 1984. *Gesunde Pflanzen*, *37*: 494-501.

Muller R. and Gooch P.S., 1982. Organic amendments in nematode control: An examination of literature. *Nematropica*, *12*: 319-326.

Pansey V.G. and Sukhatme P.V., 1978. *Statistical methods for agricultural workers*. Indian Council of Agricultural Research (IARI), New Delhi, India, 347 pp.

Reddy D.D.R., 1985. Analysis of crop losses in tomato due to *Meloidogyne javanica*. *Indian Journal of Nematology*, *15*: 55-59.

Sasser J.N., Carter C.C. and Hartmann K.M., 1984. Standardization of host suitability studies and reporting of resistance to root-knot nematode. Crop Nematode Research and Control Project. North Carolina State University Press, Raleigh, USA, 7 pp.

Siddiqui M.A. and Alam M.M., 1989. Control of stunt nematode by bare-root dip in leaf extracts of margosa and Persian lilac. *Pakistan Journal of Nematology*, *7*: 33-38.

Siddiqui M.A. and Alam M.M., 1990. Further studies on the use of water hyacinth in nematode control. *Biological Waste*, 33: 71-75.

Southey J.F., 1986. *Laboratory methods for work with plant and soil nematodes*. Ministry of Agriculture, Fisheries and Food, Her Majesty's Stationery Office, London, UK, 402 pp.

Tiyagi S.A. and Ajaz S., 2004. Biological control of plant-parasitic nematodes associated with chickpea using oil-cakes and *Paecilomyces lilacinus*. *Indian Journal of Nematology*, *34*: 44-48.

Tiyagi S.A., Siddiqui M.A. and Alam M.M., 1986. Toxicity of an insect repellant plant to plant-parasitic nematodes. *International Nematology Network Newsletter*, *3*: 16-17.

Tiyagi S.A., Bano M. and Alam M.M., 1988. Evaluation of nematicidal potential in some plant species belonging to the family Compositae. *Indian Journal of Nematology*, *18*: 288-231.

Tiyagi S.A., Ahmad A. and Alam M.M., 1990. Control of root-knot, reniform and stunt nematodes by root dip in leaf extract of lemongrass. *International Pest Control*, *32*: 70-71.