ECO-FRIENDLY MANAGEMENT OF *ROTYLENCHULUS RENIFORMIS* AND *MELOIDOGYNE INCOGNITA* ON CASTOR BY USING BOTANICAL AMENDMENTS WITH POTENTIAL ANTIHELMINTHIC PROPERTIES

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Summary. The efficacy of applications of bark powder obtained from ten plant species, amla (*Phyllanthus emblica*), arjuna (*Terminalia arjuna*), asoca (*Saraca asoca*), babul (*Acacia nilotica*), bottle brush (*Callistemon lanceolatus*), eucalyptus (*Eucalyptus citriodora*), jamun (*Syzygium cumini*), mango (*Mangifera indica*), neem (*Azadirachta indica*), and tamarind (*Tamarindus indica*), incorporated into the soil was assessed in a pot experiment to manage a castor decline induced by root-knot (*Meloidogyne incognita*) and reniform (*Rotylenhulus reniformis*) nematodes. Treatments with bark powder from neem, asoca, jamun and babul improved castor seedling growth and suppressed nematode reproduction and number of galls/root system. Neem bark powder was the most effective. The rest of the bark products had no significant effect on nematode population levels and plant growth. The residual soil population levels, at the end of the experiment, remained at high damaging levels for a new crop in all of the nematode infested pots regardless of the amendments that were applied.

Key words: Botanical compounds, reniform nematode, *Ricinus communis*, southern root-knot nematode, soil amendments.

The recent withdrawal from the market of many effective nematicides due to environmental concern has prompted the search for new nematode management strategies in agriculture. Organic soil amendments have been found to be highly beneficial in many situations, although application of large quantities of these products can pose difficulties in their procurement and application. Some recent investigations have indicated that many organic materials, including the extracts of selected plants, have nematicidal potential (Siddiqui and Alam, 1988, 1989a, b; Jabri *et al*., 1991). Many plant extracts in oil formulation from neem, quillaja and sesame are available in the market and have been used with variable results in many countries. In India, the naturally sloughed-off barks from many medicinal trees are generally considered as wastes causing disposal problems. Recent regulations against bark incineration to manage wood waste has complicated the disposal problems of these products and has generated interest for the agricultural use of timber by-products. The potential nematicidal properties of some of these wood by-products applied as soil amendments could benefit greatly Indian agriculture. In order to provide growers and agricultural specialists in India with additional data on the efficacy of these products in suppressing nematode populations and improving plant growth, a study was conducted to assess the effect of these bark products on castor (*Ricinus communis* L.) seedlings grown in pots infested with the root knot nematode, *Meloidogyne incognita* (Kofoid *et* White) Chitw., in association with the reniform nematode, *Rotylenchulus reniformis* Linford *et* Oliveira.

Castor was selected for this study because of its economic importance as a non-edible oilseed crop in arid and semi-arid regions of India (Damodaran and Hegde, 2005) and also for its susceptibility to *M. incognita* and *R. reniformis*, which in concomitant infestations induce serious crop losses in India (Alam *et al*., 1979; Khan *et al*., 1986).

Pure cultures of *M. incognita* and *R. reniformis* originating from a single egg mass collected from an infested eggplant (*Solanum melongena* L.) were raised and maintained on the same host separately.

Bark pieces were removed from the following ten medicinally important plants: amla (*Phyllanthus emblica* L.), arjuna [*Terminalia arjuna* (Roxb. ex DC). Wight *et* Am.], asoca [*Saraca asoca* (*Roxb*.) de Wilde], babul [*Acacia nilotica* (L.) Willd. e*x*. Delile], bottle brush [*Callistemon lanceolatus* (Sm.) Sweet], eucalyptus (*Eucalyptus citriodora* Hook.), jamun [*Syzygium cumini* (L.) Skeels], mango (*Mangifera indica* L.), neem (*Azadirachta indica* A. Juss.), and tamarind (*Tamarindus indica* L.). The bark was collected in autumn after the plants had shed their leaves, as this is the time of the year when the flow of sap is maximum and bark is easily radially detached from the wood. Large bark pieces were stripped off longitudinally from a selected portion of the trunk and branches and dried in an oven at 80 °C for about 12 hours. Thereafter, the barks were cut into small pieces and finally ground into powder using an electric grinder. Later, the bark powder of each plant was thoroughly mixed in the already autoclaved mixture of loam soil : river sand : farmyard manure (3:1:1) at the rate of 50 g/kg soil. Each of the 25-cm-diameter pots used was filled with 4 kg of bark amended soil. The soil was watered daily to keep it moist and left as such for two weeks.

The seeds of castor were surface sterilized with 0.1% $HgCl_2$ solution and five of these seeds were sown in

Table I. Effect of ten bark products of medicinal plants on the plant growth components of castor seedlings kept, for four months, in pots containing soil infested concomitantly with initial populations (Pi) of 2000 J_2 and 1000 immature females of *Meloidogyne incognita* and *Rotylenehulus reniformis*/kg, respectively.

Treatment	Plant length				Dry weight				Yield	
	Shoot (cm)	Root (cm)	Total (cm)	% reduction[1] over control	Shoot (g)	Root (g)	Total (g)	% reduction[1] over control	Seed weight (g)	% reduction[1] over control
Control I (uninoculated + unamended)	95.0	31.6	126.6	-	60.6	17.8	78.4	-	248.0	-
Control II (Mi + Rr) (inoculated + unamended)	53.3	16.8	70.1	44.6	30.9	8.4	39.3	49.9	110.3	55.5
Amla bark + (Mi + Rr)	50.8	16.6	67.4^{ns}	46.8 (-2.2)	29.8	8.2	38.0^{ns}	51.5 (-1.6)	118.2^{ns}	52.3 (3.2)
Arjuna bark + (Mi + Rr)	55.4	17.0	72.4^{ns}	42.8 (1.8)	29.2	8.1	37.3^{ns}	52.4(-2.5)	107.8^{ns}	56.5 (-1)
Ashok bark + (Mi + Rr)	65.5	21.8	87.3**	31.0 (13.6)	39.7	11.3	51.0**	34.9 (15.0)	153.0**	38.3 (17.2)
Babul bark + (Mi + Rr)	57.4	18.8	76.2**	39.8 (4.8)	33.2	10.0	43.2**	44.9 (5.0)	127.9**	48.4(7.1)
Bottle brush bark + (Mi + Rr)	52.1	17.2	69.3ns	45.3 (-0.7)	31.8	9.0	40.8^{ns}	48.0 (1.9)	114.0^{ns}	54.0 (1.5)
Eucalyptus bark + (Mi + Rr)	55.7	16.4	72.1^{ns}	43.1 (1.5)	30.3	8.6	38.9^{ns}	50.4 (-0.5)	116.8^{ns}	52.9 (2.6)
Jamun bark + (Mi + Rr)	62.4	20.3	82.7**	34.7 (9.9)	36.5	11.0	47.5**	39.4 (10.5)	139.8**	43.6 (11.9)
Mango bark + (Mi + Rr)	51.2	17.2	68.3^{ns}	46.1 (1.5)	30.1	8.6	38.7ns	50.6 (-0.7)	112.0^{ns}	54.8 (0.7)
Neem bark + (Mi + Rr)	69.5	22.4	91.9**	27.4 (17.2)	42.3	12.0	54.3**	30.7 (19.2)	162.1**	34.6 (20.9)
Tamarind bark + (Mi + Rr)	54.5	16.5	71.0^{ns}	43.9(0.7)	31.4	8.8	40.2^{ns}	48.7 (1.2)	106.1^{ns}	57.2 (-1.7)
C.D. (P = 0.05)	3.87	2.18	4.72		2.16	1.64	2.60		8.75	

Mi = *Meloidogyne incognita*; Rr = *Rotylenchulus reniformis*.
Values are means of three replicates.
[1]Outside parenthesis % over control I, in parenthesis % over control II (= prevented damage).
* Significant over control II at 5.0% level.

each pot. After germination, the plants were thinned to leave one healthy seedling per pot. Two-week-old, well-established and healthy seedlings in pots of amended soil were inoculated with *M. incognita* (2000 J_2/kg soil) and *R. reniformis* (1000 immature females/kg soil). Non-infested plants without amendments served as absolute control I, and infested plants also in absence of amendments served as control II. The plants were lightly watered after inoculation and thereafter whenever required. The pots were arranged on a bench in glasshouse at 30 ± 5 °C according to a randomized block design and treatments were replicated thrice. The experiment was run for 4 months and then the plants were uprooted and brought into the laboratory for observation and recording of the data.

For extraction of the nematodes, the soil of each pot was mixed thoroughly and a sub-sample of 200 g was processed according to Cobb's sieving and decanting method followed by the Baermann funnel technique. Each nematode suspension was collected in a beaker and the volume made up to 100 ml. The suspension was agitated by bubbling through a pipette, to ensure a uniform distribution of the nematodes, and 10 ml were drawn off and transferred to a counting dish. The number of nematodes was counted in three replicate subsamples per sample (Southey, 1986). The mean of the three counts was calculated and the final population of the nematodes was referred to 1 kg soil.

To estimate the nematode population in the roots, a 1 g root sample from each replicate was blended with water in an electrically operated Waring blender for about 30-40 seconds. The resulting suspension was collected in a beaker and the volume made up to 100 ml. The nematode population was counted in three subsamples, as for the soil samples. Identification of *M. incognita* and *R. reniformis* was done on the basis of characteristic features of the nematodes given by Eisenback (1985) and Siddiqi (2000), respectively.

Plant growth components recorded included length and dry weight of roots and shoots, and yield/plant, expressed as seed weight/plant. The reproduction factor (Rf) for both nematodes was calculated by dividing the final nematode population (Pf) by the initial population (Pi) (Oostenbrink, 1966).

The data were subjected to analysis of variance and the means compared by the least significant difference at P = 0.05 (Panse and Sukhatme, 1989).

The bark additives did not significantly affect Pf and Rf values, which were comparable with those of control II. High residual soil population levels of both *M. incognita* and *R. reniformis* remained in all infested pots. The association of the two nematodes suppressed significantly the length, dry weight and yield of castor (by 44.6, 49.9 and 55.5%, respectively) in nematode infested and amendment-free pots (control II) as compared to the absolute control I (untreated + unamended soil) (Table I). Amendments with neem, asoca, jamun and babul bark powder mitigated nematode damage. Neem provided the least nematode induced growth suppression, although the growth components (plant length, dry weight and seed production) were still adversely affected by the nematode infestation. These growth components were, respectively, 27.4, 30.7 and 34.6% smaller than those of the absolute control. Asoca, jamum and babul bark amendments produced slightly inferior beneficial effects. The significant and cumulated average amounts of nematode damage for the three growth components that neem, asoca, jamum and babul were able to prevent compared to the infested and non-amended control II were 19, 15, 11 and 5.6%, respectively (Table I). The other bark products used did not provide any protection from the nematode damage.

The final population densities (Pf) and reproduction rates (Rf) of *M. incognita* and *R. reniformis* were reduced in the pots amended with neem, asoca, jamun and babul barks as compared to the infested and amendment-free control II (Table II). The greatest suppression of Pf and Rf values of both nematode species was observed in pots amended with neem bark, followed by those amended with asoca, jamun and babul barks. Root systems were galled by *M. incognita* in all treatments and control II but the gall numbers were smaller (38, 46, 50 and 54) in the treatments with barks of neem, asoca, jamun and babul, respectively, than in control II (72).

The inhibitory action of the bark of neem against *M. incognita* and *R. reniformis* observed in this study may be due to the chemicals present in the tissues of this plant (Bell, 1981; Giebel, 1982; Devakumar *et al.*, 1983). Compounds with similar inhibitory effects against nematodes have been reported also in asoca (Pradhan, *et al.,* 2009), jamum (Daniel, 2006) and babul (McGrady and Cotter, 1989). These compounds may have played an important role in suppressing partially the Pf of the two nematodes.

The rest of the barks (mango, amla, eucalyptus, arjuna, bottle brush, and tamarind) were not effective in improving plant growth. However, our results contradict those of Jabri *et al.* (1991), who reported that egg hatching of *M. incognita* was inhibited by the nematicidal potential of bark extract of bottle brush.

The results of our investigation agree with Ismail (1998), who observed better growth of plants in soil amended with hardwood bark due to the reduction in the nematode population. The roots of plants grown in bark amended soil undergo various physiological changes, which make them unfavourable for nematode penetration and feeding and thus induce a certain degree of resistance to nematode attack (Malek and Gartner, 1975; McGrady and Cotter, 1989). The nemato-toxic effects of neem, ashok, jamun and babul barks might be due to the accumulated toxicity of compounds from the decomposition of the barks (Bell, 1981; Giebel, 1982; Haseeb and Alam, 1984), an increase in the predation and parasitic activity of the soil biota, or stimulation and selection of microflora capable of producing

Table II. Effect of ten bark products of medicinally important plants on the final populations (Pf) of *M. incognita* and *R. reniformis* on castor seedlings grown for four months in pots containing soil infested concomitantly with initial populations (Pi) of 2000 J2 and 1000 immature females/kg, respectively.

Treatment	*Rotylenchulus reniformis*				*Meloidogyne incognita*				
	Female/root system	Juveniles/ kg soil	Total	Rf = Pf/Pi	Female/root system	Juveniles/ kg soil	Total	Rf = Pf/Pi	No. of galls/ root system
Control II (Mi + Rr) (inoculated+unamended)	310	7214	7524	7.52	266	12602	12868	6.4	72
Amla bark + (Mi + Rr)	309	7035	7344	7.34	274	13286	13560	6.7	68
Arjuna bark + (Mi + Rr)	298	7407	7705	7.70	261	12439	12700	6.3	76
Ashok bark + (Mi + Rr)	206*	3467*	3673*	3.67*	207*	6473*	6680*	3.3*	46*
Babul bark + (Mi + Rr)	234*	4933*	5167*	5.16*	224*	9496*	9720*	4.8*	54*
Bottle brush bark + (Mi + Rr)	299	7343	7642	7.64	264	13056	13320	6.6	69
Eucalyptus bark + (Mi + Rr)	306	7188	7494	7.49	272	12528	12800	6.4	70
Jamun bark + (Mi + Rr)	221*	4034*	4255*	4.25*	216*	7804*	8620*	4.0*	50*
Mango bark + (Mi + Rr)	302	7090	7392	7.37	258	12102*	12360	6.1	74
Neem bark + (Mi + Rr)	182*	2904*	3086*	3.08*	195*	5165*	5360*	2.6*	38*
Tamrind bark + (Mi + Rr)	318	7408	7726	7.72	260	12320	12580	6.2	71
C.D. (P=0.05)	14.56	217.19	566.89	0.48	15.48	713.38	986.85	0.41	4.71

Values are mean of three replicates

* Significant over control II (inoculated and unamended) at 5.0% level of significance

organic material toxic to the nematodes (Alam *et al*., 1975; Mian and Rodriguez-Kabana, 1982; Siddiqui and Alam, 1989a, b). In conclusion, the findings of our study confirm that some of the tested barks can improve the yield of castor, although they do not have a very marked activity in suppressing the Pf and leave soil residual nematode population levels that would be damaging for a new crop. In spite of their incomplete effect on nematode populations, these bark products should be taken into consideration by growers in India and other regions where castor is grown because of their safety, relatively low cost, favourable effects on soil structure and fertility and minimum impact on the environment. The application rate of the barks used was rather high (50 g/kg soil) and it is suggested that more investigations be conducted to explore the possibility of reducing the rate of application of the barks and their suitability for use under field conditions.

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