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Efficacy of certain botanicals for the management of *Rotylenchulus reniformis* infecting okra and cowpea

Usman A.* and Mansoor A. Siddiqui

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

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Leaf extracts of two plants such as *Murraya koenigii* L. Spreng and *Vitex negundo* L. were used as bare-root dip treatment for the management of phytonematode, *Rotylenchulus reniformis* infecting Okra (*Abelmoschus esculentus* (L.) Moench) cv. "Pusa Sawani" and Cowpea (*Vigna unguiculata* (L.) Walp). cv.'Pusa Komal' Plants. Significant reduction was observed in the nematodes multiplication of *R. reniformis* on the experimental plants. Leaf extracts of *Murraya* caused relatively higher inhibition in nematode multiplication than *Vitex*. Improvement in plant growth was noted. The efficacy of root-dip treatment with respect to improvement in plant weight and reduction in disease incidence increased with the increase in concentration of leaf extracts and root dip duration.

Key words: Bare-root dip treatment, cowpea, Murraya koenigii, leaf extracts, phytonematode, Vitex negundo.

INTRODUCTION

Phytonematodes reduce crop yields worldwide each year by more than 20% on an average, however, individual fields may sustain losses up to 50 to 100% from one or more pests (Dhaliwal and Koul, 2007;Agrios, 1997). Phytonematodes are one of major yield limiting factors around the world in almost all types of crop plants. Yield losses in India due to root-knot nematodes (*Meloidogyne* spp.) range from 39.7 to 46.0% (Bhatti and Jain, 1977; Reddy, 1985). The control of Phytonematodes is more difficult than other pests because they inhabit the soil and usually attack the underground plant parts. Since withdrawal of more hazardous broad spectrum pesticides/nematicides has emphasized the need for novel methods to control nematodes. There is now tremendous pressure on growers to use several methods of nematode control which do not pollute or otherwise it led to the undesirable side effect such as deterioration of environment, hazard to human health and harmful effects on non-target organisms (Duncan, 1991) and biodegradable in nature (Tiyagi and Ajaz, 2004). A number of organic additives of plant origin including oil-seed cakes, chopped plant parts and seed dressing with plant extracts have been used as nematode controlling agents (Akhtar and Alam, 1993; Muller and Gooch, 1982; Tiyagi et al., 1988; Tiyagi and Ajaz, 2004;Mahmood et al., 2007; Bunt, 1975). Root-dip treatment with various plant extracts was also found effective for controlling the population of plant-parasitic nematodes (Siddiqui and Alam, 1990; Tiyagi et al., 1990). Utilization of medicinal plants such as *Murraya koenigii* L. Spreng and *Vitex negundo* L. were not reported earlier as bare-root dip treatment. Since time immemorial okra and cowpea considered as important vegetable crops and grown across the world to meet the demand of different groups of population. But their production decreased gradually, partly because of plant-parasitic nematodes causing damage in the form of quality and quantity of yield and partly due to fluctuation in seasons. Keeping in view the importance of vegetable crops and their lower production seems to be due to phytonematodes, it is therefore

*Corresponding author. E-mail: usumadeena@gmail.com.

Table 1. Effect of bare-root dip treatment in the methanol extract of leaves of Murraya koenigii and Vitex negundo on inhibition of population of reniform nematode, *Rotylenchulus reniformis* and plant growth of okra cv. "Pusa Sawani".

Treatment		*Murraya koenigii*						*Vitex negundo*					
Dip duration (min)	Concentration of extract	Plant weight (g)			Rf= Pf/Pi	No. of fruits per plant	Final nematode population	Plant weight (g)			Rf= Pf/Pi	No. of fruits per plant	Final nematode population
		Root	Shoot	Total				Root	Shoot	Total			
20	S	9.46**	32.68**	42.14**	1.84**	8.00	1845.0**	8.56**	30.42**	38.98**	1.559**	8.00	1559.3**
	S/2	7.66ns	26.63**	34.29**	1.94**	6.00	1944.3**	7.38*	27.66**	35.05**	2.512**	6.00	2512.0**
	S/10	5.44**	18.68**	24.13**	2.44**	5.00	2436.7**	4.32**	19.58**	23.90**	1.626**	5.00	1626.0**
40	S	9.69**	33.35**	43.04**	1.63**	10.00	1627.0**	9.68**	34.60**	44.28**	1.745**	10.00	1744.7**
	S/2	8.59**	29.67**	38.26**	1.76**	8.00	1760.7**	6.42**	30.31**	36.73**	2.557**	8.00	2556.7**
	S/10	6.49**	26.61**	33.11**	2.45**	7.00	2450.0**	5.42**	27.45**	32.87**	1.767**	8.00	1767.0**
80	S	13.61**	38.56**	52.17**	1.72**	13.00	1715.7**	13.46**	37.53**	51.00**	1.957**	13.00	1956.7**
	S/2	12.50**	31.57**	44.06**	1.83**	10.00	1830.0**	12.71**	30.43**	43.14**	1.761**	11.00	1761.0**
	S/10	9.75**	27.66**	37.41**	1.95**	9.00	1946.7**	9.56**	26.63**	36.19**	1.987**	9.00	1986.7**
Undipped inoculated control		7.67	9.60	17.27	4.30	5.00	4250.0	7.67	9.60	17.27	4.300	5.00	4250.0
Undipped uninoculated control		13.38	39.41	52.79	0.00	16.00	0.00	13.38	39.41	52.79	0.000	16.00	0.00
LSD at 5%		0.221	0.318	0.402	0.019	NS	19.06	0.26	0.34	0.48	0.013	NS	12.99

Each value is an average of five replicates; **, significance; NS,for non-significance; Initial inoculum level of nematode =1000 specimens per pot.

considered worthwhile, a preliminary soil survey for the presence of plant-parasitic nematode was conducted in the infected fields of okra and cowpea in Aligarh and adjoining districts, revealed the presence of reniform nematode, *Rotylenchulus reniformis* which are found associated with poor and unthrifty crop growth. It was necessary to evaluate the efficiencies of *M. koenigii* and *V. negundo* against *R. reniformis* on okra and cowpea with respect to plant weight by using bare-root dip treatment. The objective of this research was to determine the efficacious nature of these botanicals and to explore the safe and eco-friendly management practices of phytonematodes causing damage to okra and cowpea.

MATERIALS AND METHODS

Methanolic extracts of dry leaves of *M. koenigii* and *V. negundo* were prepared by refluxing the dry matter (50 g) with methanol (350 ml) on soxhlet apparatus for 8 h. The methanolic extracts were distilled and crude extracts obtained after distillation were completely dried and the weight of the solid mass was measured. The solid methanolic extracts (5 g) were dissolved in minimum amount of methanol and small amount of surfactant (Exalin) was added and the final volume (50 ml) was made with distilled water. Thus 10% (w/v) methanolic extracts were obtained for each plant and these extracts were designated as standard 'S'. Other dilutions were prepared such as S/2 and S/10 by adding the required quantity of distilled water to the standard extracts. The roots of twenty one day old seedlings of Okra and Cowpea previously grown in sterilized micro-plots were dipped in different concentrations of leaf extracts of *M. koenigii* and *V. negundo* separately for the duration of 20, 40 and 80 min as per procedure described in Tables 1 and 2. The roots of these seedlings after dip treatment were washed several times with distilled water and transferred immediately to 6 inch diameter earthen pots containing 1 kg steam sterilized soil-manure mixture (3:1). Seedlings of okra and cowpea were then inoculated with 1000 IV stage infective young females of reniform nematode, *R. reniformis* according to inoculation schedule given in tables. Undipped inoculated and uninoculated plants served as control. There were five replications for each treatment. These experimental pots kept at glasshouse benches in randomized block design and necessary caring and watering done whenever required for whole duration. After 100 days of nematodes inoculation, the experiment was terminated and the plant roots were gently washed and length of root and shoot of okra and cowpea were measured. Soil population of reniform nematode, *R. reniformis* was determined by processing the soil with the help of Cobb's sieving and

Table 2. Effect of bare-root dip treatments in the methanol and extracts of leaves of *Murraya koenigii* and *Vitex negundo* on the inhibition of population of reniform nematode, *Rotylenchulus reniformis* and plant growth of cowpea cv.' Pusa Komal'.

Treatment		*Murraya koenigii*						*Vitex negundo*					
Dip duration (min)	Concentration of extract	Plant weight (g)			Rf=Pf/Pi	No. of fruits per plant	Final nematode population	Plant weight (g)			Rf= Pf/Pi	No. of fruits per plant	Final nematode population
		Root	Shoot	Total				Root	Shoot	Total			
20	S	10.36**	30.62**	40.98**	1.75**	8.00	1751.00	11.39**	28.42**	39.81**	1.85	7.00	1850.00
	S/2	8.45ns	24.63**	33.08**	1.97**	6.00	1968.00	9.28**	23.63**	32.91**	1.95	6.00	1950.00
	S/10	8.18**	16.51**	24.69**	2.33**	4.00	2325.00	8.60ns	15.57**	24.17**	2.45	6.00	2450.00
40	S	12.60**	30.58**	43.19**	1.53**	9.00	1528.00	13.57**	31.58**	45.15**	1.55	8.00	1550.00
	S/2	9.45**	27.61**	37.06**	1.67**	8.00	1672.00	9.41**	27.33**	36.74**	1.75	7.00	1750.00
	S/10	8.37ns	23.60**	31.97**	2.31**	7.00	2312.00	8.35*	24.67**	33.02**	2.44	7.00	2440.00
80	S	13.53**	33.58**	47.11**	1.65**	10.00	1650.00	16.47**	33.56**	50.03**	1.70	9.00	1700.00
	S/2	10.46**	28.44**	38.90**	1.72**	8.00	1715.00	11.49**	28.72**	40.21**	1.75	8.00	1750.00
	S/10	7.73**	25.55**	33.28**	1.89**	8.00	1892.00	8.55ns	26.69**	35.24**	1.90	7.00	1900.00
Undipped inoculated control		8.60	10.45	19.05	4.15	4.00	4152.00	8.60	10.45	19.05	4.15	4.00	4152.00
Undipped uninoculated control		19.66	34.44	54.10	0.00	11.00	0.00	19.66	34.44	54.10	0.00	11.00	0.00
LSD at 5%		0.273	0.295	0.274	0.001	NS	NS	0.194	0.235	0.221	NS	NS	NS

Each value is an average of five replicates; **, significance; NS,for non-significance; Initial inoculum level of nematode =1000 specimens per pot.

decanting method along with Baermann's funnel technique (Southey, 1986). Statistical analysis of the data for critical difference (C.D.) at *P*=0.05 and *P*=0.01 probability was done according to the procedure described by Pansey and Sukhatme (1978).

RESULTS

The data presented in Tables 1 and 2 clearly revealed that root-dip treatment in different concentrations (S, S/2, S/10) and dip durations (20, 40 and 80 min) of leaf extracts of *M. koenigii* and *V. negundo* brought about significant reduction in nematode multiplication of *R. reniformis* infecting okra and cowpea. The maximum reduction in nematode population was observed in those plants treated with S concentration for 80 min dip duration in both the plants. Plant weight was reduced due to *R. reniformis* by (42.67% in okra and (56.25%) in cowpea plant in undipped inoculated plants as compared to undipped uninoculated plants. However, significant improvement was noted in all the plants treated with leaf extracts along with inoculations by the nematodes. The reproduction factor of *R. reniformis was* determined as maximum in undipped inoculated plants in both the test plants. *Vitex* showed comparatively less efficacy against plant-parasitic nematodes than *Murraya.* Plant weight of okra and cowpea was found to be higher with higher concentration of leaf extracts of *M. koenigii* and *V. negundo* and with longer dip duration. The multiplication rate of *R. reniformis* was found gradually decreased with an increase in the concentration of leaf extracts along with duration of dip treatment (Tables 1 and 2).

DISCUSSION

The different concentrations (S, S/2 and S/10) of leaf extracts of *M. koenigii* and *V. negundo* showed significant reduction in multiplication rate of *R. reniformis* on okra and cowpea. The maximum inhibition in disease incidence development was observed in plants dipped in 'S' concentration for 80 min. Nematode development may have been affected by the leaching of chemicals coating the roots of seedlings into the rhizosphere which repelled or killed the nematode juveniles that attacked the roots. Similar

observation has been 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 responses. The plant growth of undipped plants was reduced significantly due to inoculation of *R. reniformis* but the reduction was controlled by the root-dip treatment in leaf extracts which seems to be due to the reduction in disease incidence. This may be explained due to some substances already present in the leaf extracts of *M. koenigii* and *V. negundo* plants that exhibited highly nematicidal activity which seems to be deleterious to the root-knot development. Alam et al. (1977, 1978, 1979) have reported that ammonia, fatty acids, H2S, aldehyde, formaldehyde, phenolic compounds are released on decomposition of organic additives and he also observed that these chemicals have been found detrimental to the population build-up of nematodes *in vitro* studies. Leaf extracts in the form of organic additives also released some phenolic compounds/nutrients which accelerated rapid root development and overall plant growth thus help the plants to develop resistance against nematode attack. Our results are also in accordance with those of Tiyagi et al. (2001). Thus, it is evident from the present studies where significant improvement in plant weight of okra and cowpea was noticed due to significant reduction in the root-knot development and nematodes multiplication. The inferences drawn from the present investigation that the bare-root dip treated plants clearly show tolerance against the disease incidence caused by nematodes. This also confirms the induced defense mechanisms reported by Kast (1985). Tiyagi et al. (1986) and Siddiqui and Alam (1989) suggested systemic activity of some plant products against nematodes when given root-dip treatment to the plants. The treated plants also showed increased root-growth, which may be due to better vegetative growth, non-inhibitory effects and suppression of soil pathogenic nematodes. Thus, it appears from the present study that these plants may offer a basis for finding and developing new botanical biopesticides/ nematicides.

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