Plant Pathology Journal

ISSN 1812-5387

science alert

ANSInet
an open access publisher
http://ansinet.com

Pakistan Journal of Plant Pathology 2 (3): 174-180, 2003
ISSN 1680-8193

Suppression of *Meloidogyne javanica* by *Conyza canadensis*, *Blumea obliqua*, *Amaranthus viridis* and *Eclipta prostrata*

Zarina Begum, [1]S. Shahid Shaukat and [1]Imran A. Siddiqui
National Nematological Research Centre, University of Karachi, Karachi-75270, Pakistan
[1]Soil Biology and Ecology Laboratory, Department of Botany, University of Karachi, Karachi-75270, Pakistan

Abstract: Aqueous shoot extract of four weed species including *Conyza canadensis*, *Blumea obliqua*, *Amaranthus viridis* and *Eclipta prostrata* inhibited egg hatch and caused mortality of *Meloidogyne javanica*, the root-knot nematode juveniles *in vitro* to varying extent with *A. viridis* being the most effective. The efficacy of the powdered shoot material as soil organic amendment was tested against two nematode inoculum levels (2000 and 4000 J_2 pot^{-1}) in a pot experiment. Soil amendment with the powdered shoot material generally reduced nematode population density, root-knot development and reproductive potential of *M. javanica* in brinjal roots. *A. viridis* was most effective in the suppression of root-knot nematode at both the nematode inoculum rates but caused slightly reduction in plant growth presumably owing to its allelopathic activity in soil.

Key words: Plant parasitic nematodes, organic amendments, allelopathy, toxicity

Introduction

Soil amendments have been explored as a method of suppressing plant-parasitic nematodes. It has been shown that the efficacy of organic amendments against nematodes depends on the physical and chemical properties of the amendments (Rodriguez-Kábana, 1986). Nematicidal compounds are released by decomposing organic matter, or the compounds synthesized by the microorganism involved in the decaying process. The use of nitrogenous organic matter as soil amendment is a successful strategy for the control and management of *Meloidogyne* spp. and other plant-parasitic nematodes in vegetables and other root-knot susceptible crops (Mian and Rodriguez-Kábana 1982; Rodriguez-Kábana *et al.*, 1990). Aqueous extract of various plant species reduced mobility of *Xiphinima americana in vitro* (Insunza *et al.*, 2001). Shaukat and Siddiqui (2001a) found that extracts of various weeds, obtained from Karachi University Campus, caused significant mortality of *M. javanica* juveniles *in vitro*. Methanolic extract of *Lantana camara* produced considerable mortality of *M. javanica* juveniles *in vitro* and reduced population densities and galling intensity in mungbean roots (Ali *et al.*, 2001). Similarly, soil amendment with *Argemone mexicana* substantially lowered the populations of *M. javanica* in the roots and rhizosphere of tomato plants under greenhouse conditions (Shaukat *et al.*, 2002). These authors suggested that a reduced pH after soil amendment with *A. mexicana* was related with the inhibition of root-knot nematodes.

The objective of this study was to evaluate I) the ***in vitro*** egg hatch and nematicidal activity of the selected plant species against ***Meloidogyne javanica*** (Treub.) Chitw., ii) the influence of soil amendments with powdered shoot material of weed species including ***Conyza canadensis***, ***Blumea obliqua***, ***Amaranthus viridis*** and ***Eclipta prostrata*** on the development of nematode population densities in soil, root-knot infection and nematode reproductive potential and iii) the effects of such amendments on growth of brinjal (***Solanum melongena*** L.).

Materials and Methods

Plant material and extract preparation

The Laboratory experiments were performed at the Department of Botany, University of Karachi while greenhouse trial was conducted at the National Nematological Research Centre, University of Karachi. The nematode antagonistic weed species including ***Conyza canadensis***, ***Blumea obliqua***, ***Amaranthus viridis*** and ***Eclipta prostrata*** were collected from the experimental field of the Crop Diseases Research Institute, Karachi University Campus. The shoot material of the plants was air-dried in shade and finely powdered. A 50 g powdered shoot was soaked in 200 ml sterile distilled water and left for 72 h at room temperature. The extract was filtered through two layers of Whatman No.1 filter paper and kept at 6°C prior to use. To avoid bacterial contamination in the extract, appropriate quantities of antibiotics were added.

Egg hatch activity of the plant extract

To examine the influence of plant extracts on egg hatch of ***M. javanica***, 200±21 eggs were transferred into cavity glass slide containing 2 ml extract of a plant species. The eggs kept in sterile distilled water served as controls. Each treatment was replicated three times and the cavity glass slides were randomized. After a 48 h exposure, the hatched juveniles were counted. The eggs with intact juveniles were then transferred into cavity glass slides containing 2 ml sterile distilled water to ascertain whether the eggs kept in the extract had been temporarily or permanently inactivated. The juveniles that emerged from the eggs were recounted after a further 48 h period.

Nematicidal activity of the plant extract

To assess the effects of aqueous extract of each plant species on mortality of ***M. javanica***, two ml of each extract was poured in a glass cavity slide and about 38±7 freshly hatched surface sterilized juveniles of ***M. javanica*** placed in each glass slide. Juveniles kept in sterile distilled water served as controls. Treatments were replicated three times and dead nematodes in each cavity slide were counted after 24 and 48 h.

Greenhouse experiments

Powdered shoot material of nematode antagonistic plants was thoroughly mixed with sandy loam soil (72% sand, 17% silt and 11% clay; pH 8.1 and organic matter of 0.3%) to make 25 or 50 g kg^{-1} (3 or 5% w/w) concentrations and put into 8-cm-diam. plastic pots at 400 g pot^{-1}. Soil without amendments served as control. The pots were watered daily to promote microbial

activity so as to partially decompose the plant tissues. Following three weeks after soil amendments, three brinjal (***Solanum melongena*** L.) seedlings (about 8 cm tall and at two-leaf stage), raised in steam sterilized soil were planted in each pot. The seedlings were allowed to establish for one week before soil in each pot was inoculated by adding a total of 2000 or 4000 freshly hatched juveniles (< one week-old) of ***M. javanica*** through four soil openings made around each plant. Treatments and controls were replicated five times and randomized within blocks on a greenhouse bench.

Plants were harvested at 52 days after transplant and growth parameters including plant height and fresh weight of shoot and root were recorded. Number of galls and egg masses produced on the entire root system was counted using a hand lens. Root-knot nematodes were extracted from soil (250 cc) using a modified Baermann funnel technique and counted (Rodríguez-Kábana and Pope, 1981).

Statistical analysis

Data were subjected to analysis of variance (ANOVA) and factorial analysis of variance (FANOVA) depending upon the experimental design followed by least significant differences (LSD) test and Duncan's multiple range test using STATISTICA ver. 5.0 software (Statsoft Inc. Tulsa, Oklahoma, USA, 1995). Percentage data were transformed by an arcsine transformation prior to analysis.

Results

Egg hatch activity of the plant extract

Exposure of ***M. javanica*** eggs to shoot extract of taxonomically different plant species resulted in a significant ($p<0.05$) reduction of egg hatch compared to the controls (Table 1). When efficacy of shoot extract of such nematode antagonistic plant species was tested, ***Amaranthus viridis*** was found to cause maximum inhibition of egg hatch (32.46%) over the controls. When eggs with intact juveniles were transferred from shoot extract to sterile distilled water, hatching activity of the nematode was generally lower (except ***Eclipta prostrata***) in distilled water compared to the controls. ***Amaranthus viridis*** (>32% over the controls) caused greatest permanent inactivation of eggs followed by ***Conyza canadensis*** (>24% compared to the controls).

Egg hatch activity of the plant extract

Shoot extract of plant species caused significant ($p<0.05$) mortality of ***M. javanica*** juveniles, ***in vitro***, at both the exposure periods (Table 2). However, the extent of nematode mortality varied with plant species. With respect to the nematicidal activity, ***A. viridis*** exhibited greatest nematicidal effects followed by ***C. canadensis*** at both the time periods.

Greenhouse experiments

Soil amendments with plants caused significant ($p<0.05$) influence on nematode populations (Table 3). Greatest reduction in soil nematode population was produced by ***A. viridis*** followed

Table 1: Effects of four plant species on egg hatch of *Meloidogyne javanica*

Plant species	Number of eggs hatched		Total no. of eggs hatched	Inhibition % over control
	Extract	Distilled water[a]		
Control	191	77	268	-
Conyza canadensis (L.) Cronquist	145	59	202	-24.62
Blumea obliqua (L.) Druce	163	69	236	-11.94
Amaranthus viridis (L.)	129	51	180	-32.83
Eclipta prostrata (L.) L.	154	83	237	-11.56
$LSD_{0.05}$	27	12	-	-

[a]After a 48 h exposure to plant extract, the egg masses were transferred to sterile distilled water

Table 2: Effects of four plant species on mortality of *Meloidogyne javanica*

Plant species	Mortality % Exposure time (h)	
	24	48
Control	6	11
Conyza canadensis	39	57
Blumea obliqua	22	27
Amaranthus viridis	61	74
Eclipta prostrata	33	49
$LSD_{0.05}$	18	21

Table 3: The influence of soil amendments with powdered shoot of four plant species on nematode population densities in soil, root-knot and egg mass development due to *Meloidogyne javanica* on brinjal

Treatments	Conc. (%)	Nematode populations 250 g soil		Galls per root system		Egg masses per root system	
		Nematode inoculum levels					
		2000	4000	2000	4000	2000	4000
Control	-	1875	2835	126	195	52	78
Conyza canadensis	2.5	1630	2325	96	123	44	61
	5.0	1540	2080	75	113	24	46
Blumea obliqua	2.5	1755	2315	113	175	49	72
	5.0	1760	2205	102	157	31	44
Amaranthus viridis	2.5	1425	2025	70	101	27	41
	5.0	1245	1895	59	87	19	27
Eclipta prostrata	2.5	1790	2855	124	206	67	81
	5.0	1695	2735	111	192	41	57
$LSD_{0.05}$							
Treatment		289		34		15	
Concentration		211		26		11	
Nematode density		211		26		11	

Table 4: The influence of soil amendments with powdered shoot of four plant species on growth of brinjal plants

		Plant height (cm)		Shoot weight (g)		Root weight (g)	
		Nematode inoculum levels					
Treatments	Conc. (%)	2000	4000	2000	4000	2000	4000
Control	-	15.7	13.3	2.8	2.1	1.1	1.3
Conyza canadensis	2.5	16.9	14.1	2.6	2.2	1.4	1.5
	5.0	13.4	10.9	1.8	1.9	1.0	1.0
Blumea obliqua	2.5	18.9	16.7	3.1	2.7	1.6	1.8
	5.0	19.1	18.4	3.5	3.0	1.4	1.6
Amaranthus viridis	2.5	14.1	13.0	2.5	1.8	1.1	1.4
	5.0	13.9	12.1	2.2	1.8	0.9	1.0
Eclipta prostrata	2.5	16.7	15.4	3.3	2.6	1.4	1.2
	5.0	16.2	15.8	3.7	2.5	1.2	1.4
$LSD_{0.05}$							
Treatment		2.5		0.8		0.6	
Concentration		2.1		0.5		0.4	
Nematode density		2.1		0.5		0.4	

by *C. canadensis* and *E. prostrata* while *B. obliqua* did not reduce the nematode population significantly. Generally, the nematode density in soil was observed to be greater at higher (4000 J_2 pot^{-1}) nematode inoculum. Regardless of nematode application rate, amendment with 5% shoot material resulted in greater reduction of nematode densities than did 2.5% dosage. With respect to nematode inoculum density of 2000 J_2 pot^{-1}, galling intensity was significantly lowered following soil amendment with *A. viridis* at both the dosages and by *C. canadensis* at 5% application rate only. However, at a higher inoculum density (4000 J_2 pot^{-1}), with a few exceptions, root-knot development was markedly reduced by the plant species at both the application rates. With the exception of *E. prostrata* at both concentrations and *C. canadensis* and *B. obliqua* at 2.5% application rate, soil amendment with shoot material of plant species significantly (p at the most 0.05) reduced egg-mass production by *M. javanica* on brinjal roots, at both the nematode inoculum levels.

Soil amendment with plant species did not result in any marked changes in plant height with the exception of *B. obliqua* where it was significantly ($p<0.05$) enhanced at both the inoculum levels though shoot and root weights remained uninfluenced (Table 4). In general, there was a tendency for plant height to decrease with increasing application rates of shoot material in soil. In addition, *A. viridis* and *C. canadensis* tended to reduce plant growth, particularly at higher application rate (5%) but at lower application rate (2.5%) shoot and root growth were not affected.

Discussion

Our results indicate that aqueous shoot extract of four weed species including *Conyza canadensis*, *Blumea obliqua*, *Amaranthus viridis* and *Eclipta prostrata* reduced considerable egg hatch and induced juveniles deaths of *M. javanica in vitro*. The weed species differed with

respect to nematicidal activity suggesting that plant species posses nematicidal principle(s) that varied qualitatively and quantitatively. Since plant extracts that showed nematicidal activity, were prepared in water, the active compound(s) seem to be polar in nature. However, whether the inhibition of egg hatch and nematicidal activity found here was due to a single compound or a number of compounds can not be stated with certainty. Further investigations are needed in this regard. Similar to our study, Wang ***et al.*** (2001) observed that root leachates of ***Tegetes erecta*** caused mortality of ***Rotylenchulus reniformis***. Husan-Bano ***et al.*** (1999) observed that methanolic extract of powdered shoot of ***T. patula*** inhibited egg hatch of ***M. javanica in vitro***. Shaukat and Siddiqui (2001a) observed that methanolic extracts of several plant species caused significant morality of ***M. javanica*** juveniles. The observed nematicidal activity could be related with the release of phenolic and other secondary metabolites from powdered shoots of weed species. In a previous study, phenolic compounds such as caffeic acid, benzoic acid and ***p***-coumaric acid induced juveniles deaths in ***M. javanica in vitro*** (Shaukat and Siddiqui 2001b).

In the present study, soil amendment with ***Amaranthus viridis*** reduced nematode population densities in soil, nematode reproductive potential and root-knot infection induced by ***M. javanica*** in brinjal, compared to the controls. These differences with reference to control could either be due to the changed nutrient status of the soil following amendments with plant material or because of the allelochemicals that were added to the soil either directly through the shoot material or through their products of microbial degradation. However, the observed soil suppressiveness to host plant against nematodes via changes in the fungal communities of the soil and rhizosphere can not be ruled out (Shaukat and Siddiqui, 2001c). In the current study, ***Blumea obliqua*** and ***Eclipta prostrata***, which caused some juvenile mortality ***in vitro***, failed to reduce nematode densities and galling in brinjal. It is plausible that ***B. obliqua*** and ***E. prostrata*** produce active nematicidal compounds that are activated only in the presence of light. Amendment of soil with organic toxicants has been used in various pathosystems by several workers under greenhouse conditions (Mankau and Minteer, 1962; Sitaramaiah and Singh, 1978; Rodríguez-Kábana, 1986; Ritzinger and MacSorley, 1998; Shaukat ***et al.***, 2002). Kheir ***et al.*** (2000) studying the effects of 18 ornamental plants, found that soil amendment with two composites, i.e., ***Tegetes erecta*** and ***Zinnia elegans*** resulted in a considerable reduction in reproductive potential and gall development by ***M. incognita*** on sunflower roots.

It is interesting to note that ***C. canadensis*** and ***A. viridis*** when applied at a rate of 5% w/w, reduced plant growth. In addition to competition for resources, some weeds interfere with the crop plants through production of chemical substances (allelochemicals) that inhibit their growth and development. The inhibitory effect of weeds may be due to a variety of allelochemicals, including phenolic acids, terpenes, terpenoids, glycosides, alkaloids and flavonoids (Blum, 1996). Alleopathic potential of ***A. viridis*** and ***C. canadensis*** has been previously reported (Narweal, 1994; Munir, 2001).

The results obtained here suggest that the application of a specific organic amendment could be exploited to keep densities of nematodes at a safe threshold level, where they may not cause any harm to the economically important crops.

References

Ali, N.I., I.A. Siddiqui, M.J. Zaki and S.S. Shaukat, 2001. Nematicidal potential of ***Lantana camara*** against ***Meloidogyne javanica*** in mungbean. Nematol. Medit., 29: 99-102.

Blum, U., 1996. Allelopathic interactions involving phenolic acids. J. Nematol., 28: 259-267.

Husan, B., I.A. Siddiqui, W. Ahmed and S. Ehteshamul-Haque, 1999. Effects of some medicinal plants on egg hatching of ***Meloidogyne javanica***, root-knot nematode. Pak. J. Biol. Sci., 2: 1364-1365.

Kheir, A.M., A.A. Al-Sayed and H.I. El-Naggar, 2000. Nematicidal potential of some dry ground ornamental plants against ***Meloidogyne incognita*** on sunflower. Egypt J. Agronematol., 4: 31-40.

Mankau, R. and R.M. Minteer, 1962. Reduction of soil population of citrus nematode by addition of organic matter. Plant Dis. Reptr., 46: 375-378.

Mian, I.H. and R. Rodriguez-Kábana, 1982. Soil amendments with oil cakes and chicken litter for control of ***Meloidogyne arenaria***. Nematropica, 12: 235-246.

Munir, N., 2001. Allelopathic potential of ***Conyza canadensis*** L. and its interaction with the herbicide atrazine. M.Sc. Thesis, University of Karachi, Karachi, Pakistan, pp: 48.

Narweal, S.S., 1994. Allelopathy in Crop Production. Scientific Publishers, Jodhpur, India, pp: 288.

Ritzinger, C.H.S. and R. MacSorley, 1998. Effect of castor and valvetbean organic amendments on ***Meloidogyne arenaria*** in greenhouse experiments. J. Nematol., 30: 624-631.

Rodriguez-Kábana, R., 1986. Organic and inorganic amendments to soil as nematode suppressant. J. Nematol., 18: 129-135.

Rodríguez-kábana, R. and M.H. Pope, 1981. A simple method for the extraction of nematodes from soil. Nematropica, 11: 175-186.

Rodriguez-Kábana, R., D. Boube and R.W. Young, 1990. Chitinous material from crab for control of root-knot nematode. II. Effect of soybean meal. Nematropica, 20: 153-158.

Shaukat, S.S. and I.A. Siddiqui, 2001a. Effect of some phenolic compounds on survival, infectivity and population density of ***Meloidogyne javanica*** in mungbean. Nematol. Medit., 29: 123-126.

Shaukat, S.S. and I.A. Siddiqui, 2001b. Effect of some weed extract on mortality and egg hatching of ***Meloidogyne javanica*** (Treub.) Chitwood. Pak. J. Biol. Sci., 4: 1251-1252.

Shaukat, S.S. and I.A. Siddiqui, 2001c. ***Lantana camara*** in the soil changes the fungal community structure and reduces impact of ***Meloidogyne javanica*** on mungbean. Phytopathol. Medit., 40: 245-252.

Shaukat, S.S., I.A. Siddiqui, G.H. Khan and M.J. Zaki, 2002. Nematicidal and allelopathic potential of ***Argemone mexicana***, a tropical annual weed. Plant and Soil, 245: 239-247.

Sitaramaiah, K. and R.S. Singh, 1978. Effect of organic amendments on phenolic content of soil and plant and response of ***Meloidogyne javanica*** and its host to release compounds. Plant and Soil, 50: 671-679.

Wang, K.H., B.S. Sipes and D.P. Schmiu, 2001. Suppression of ***Rotylenchulus reniformis*** by ***Crotalaria juncea, Brassica napus*** and ***Tagetes erecta***. Nematropica, 31: 237-251.