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SUPPRESSION OF *MELOIDOGYNE JAVANICA*, THE ROOT-KNOT NEMATODE BY SOME ASTERACEOUS PLANTS IN PAKISTAN

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ABSTRACT

The influence of shoot and root extracts of nine plant species including *Gaillardia aristata, Cosmos bipinnatus, Helianthus annuus, Tagetes erecta, Tagetes patula, Chamomilla recutita, Matricaria discoidea, Calendula officinalis* and *Zinnia elegans* belonging to the family Asteraceae was tested towards egg hatch and mobility of *Meloidogyne javanica*, the root-knot nematode juveniles *in vitro*. In general, root extracts of the plant species were more effective in the inhibition of nematode compared to the corresponding shoot extracts. When plant species were compared, shoot extract of *Z. elegans* inhibited egg hatch most while shoot extract of *T. erecta* caused greatest mortality of *M. javanica* juveniles. When compared with the controls, soil amendment with *Z. elegans* significantly reduced *M. javanica* population densities in soil and subsequent root-knot development in tomato while *T. erecta* failed to produce such effects. Similarly, soil amendment with *Z. elegans* resulted in a significant increase in plant height. Whereas both amendments enhanced fresh weight of shoot compared to the controls, none of the amendments had an influence on root growth of tomato plants.

Keywords: allelopathy, soilborne pathogens, plant-parasitic nematodes, organic amendments

INTRODUCTION

Plant-parasitic nematodes cause yield loss of approximately $100 billion worldwide each year (Sasser and Frecman, 1987) and, of this damage, 70% is considered to be due to root-knot nematode (*Meloidogyne* spp.). These nematodes have worldwide distribution but they are more abundant in warm temperate and tropical soils where they cause heavy crop losses.

Nematode control generally involves the use of nematicides, which present potential risks to non-target organisms and the environment. In the search for more benign and acceptable alternatives to chemicals, possibilities are being investigated to exploit nematode-antagonistic plants for *Meloidogyne* control. For instance, Ali *et al*. (2001) observed that powdered shoot extract of *Lantana camara*, a cosmopolitan weed, caused significant mortality of *Meloidogyne javanica* juveniles *in vitro.* These authors further demonstrated that soil amendments with *L. camara* markedly reduced nematode population densities and subsequent root-knot infection in mungbean. Similarly, *Argemone mexicana* produced nematicidal effects *in vitro* and reduced *M. javanica* population densities in the rhizosphere and roots of tomato (Shaukat *et al*., 2002).

Of the nematode antagonistic plants, *Tagetes* spp. (Asteraceae) has received considerable attention. *Tagetes* spp., produce ∝-terthienyl which have nematicidal qualities (Fassuliotis and Skucas, 1969; Gommers and Bakker, 1988). Methanolic extract of *T. patula* reduced egg hatch and caused substantial mortality of *M. javanica* juveniles *in vitro* (Husun-bano *et al*., 1999). The aim of the present study was to determine the influence of i) shoot and root extracts of nine plant species belonging to the family Asteraceae towards egg hatch and mobility of *M. javanica* juveniles *in vitro* and ii) soil amendment with shoot material of *T. erecta* and *Z. elegans* on the development of nematode population densities in soil, root-knot infection caused by *M. javanica* and growth of tomato plants.

MATERIALS AND METHODS

Collection of plant material and preparation of extracts

The work was conducted at the Department of Botany, University of Karachi. Extracts were derived from 9 plant species belonging to the family Asteraceae (Table 1). The plants were obtained from various sources including farms, glasshouses, nurseries (in particular, Karachi University nursery), and herbal shops. Aqueous extracts were prepared from air-dried plant material, with aerial parts and roots separately. The plant material (50 g) was finely chopped and 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^{o}C prior to use.

***In vitro* egg hatch test**

To study the effects of the plant extract on egg hatch of *M. javanica*, two medium-sized egg masses with 2 ml of the aqueous extract of each species were transferred into a 1 cm diameter cavity glass slide. The egg masses placed in sterile distilled water served as controls. Each treatment was replicated four times and the cavity glass slides were randomized on laboratory bench. The numbers of hatched juveniles were counted after 48 h. The egg masses were then transferred into cavity glass slides containing 2 ml sterile distilled water to ascertain whether the egg masses kept in the extract had been temporarily or permanently inactivated. The emergence of juveniles from egg masses was counted again after a further 48 h period.

***In vitro* juvenile mortality test**

To study 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 42±5 second stage 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. The nematodes were considered to be dead when they did not move on probing with a fine needle.

Greenhouse experiments

Since powdered shoot material of *T. erecta* and *Z. elegans* caused substantial reduction of egg hatch and caused mortality of *M. javanica* juveniles, therefore these species were selected for greenhouse trial. Powdered shoot material of *T. erecta* or *Z. elegans* was mixed with sandy loam soil (72% sand, 17% silt and 11% clay; pH 8.1 and organic matter 0.3%) to make 30 g kg^{-1} (3% 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 sprinkled with 100-ml water and left for three weeks for the microbial activity to partially decompose the tissues. Subsequently, three-tomato cv SUN '6002' (PVP) seedlings about 5-7 cm tall and at two leaf stage, raised in sterile 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 freshly hatched juveniles 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 produced on the entire root system was counted at a low magnification (x 6). 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) followed by least significant differences (LSD) test and Duncan's multiple range test using STATISTICA ver. 5.0 software (1995; Statsoft Inc. Tulsa, Oklahoma, USA). Percentage data were transformed by an arcsine transformation prior to analysis.

RESULTS

Effects of shoot and root extracts on egg hatch of *M. javanica*

Shoot and root extracts of nine plant species belonging to the family Asteraceae caused significant ($p<0.05$) inhibition of egg hatch of *M. javanica in vitro*, compared to the control (Table 1). In general, root extract was more effective in the inhibition of egg hatch compared to the shoot extracts. When efficacy of the shoot and root extracts of different plant species was compared, shoot extract of *Z. elegans* caused greatest inhibition of egg hatch. After exposure to the extracts, the egg masses were transferred to sterile distilled water to ascertain whether inhibition was temporary or permanent. Whereas emergence of the juveniles from egg mass was greater in distilled water compared to its corresponding shoot or root extracts, with few exceptions, egg hatch was still lower in distilled water transferred from extracts, compared to the controls.

Effects of shoot and root extract on mortality of *M. javanica* juveniles

In general, shoot and root extract of plant species caused significant ($p<0.05$) mortality of *M. javanica* juveniles *in vitro* at both the exposure periods (Table 2). Nematicidal activity of the extracts increased with the increase in exposure period; nematode mortality was higher at 48 h compared to 24 h. Like hatch inhibition activity, root extract of plant species had greater nematicidal activity compared to the corresponding shoot extracts. Comparative efficacy of the plant species revealed that shoot extract of *T. erecta* was most effective in causing juveniles deaths.

Table 1. Effects of nine plant species belonging to Asteraceae family on egg hatching of *Meloidogyne javanica*.

Plant species	Plant part	Number of eggs hatched		Total no. of eggs hatched	Inhibition % over control
		Extract	Distilled water[a]		
Control	-	108	137	245	-
Gaillardia aristata Pursh.	Shoot	88	149	237	-3.26
	Root	79	123	202	-17.55
Cosmos bipinnatus Cav.	Shoot	102	131	233	-4.89
	Root	94	116	210	-14.28
Helianthus annuus L.	Shoot	119	127	246	+0.40
	Root	81	122	203	-17.14
Tagetes erecta L.	Shoot	63	96	159	-35.10
	Root	74	109	183	-25.30
Tagetes patula nana L.	Shoot	92	128	220	-10.20
	Root	101	117	218	-11.02
Chamomilla recutita (L.) Rausch.	Shoot	123	147	270	+10.20
	Root	112	132	244	-0.40
Matricaria discoidea DC.	Shoot	95	146	241	-1.63
	Root	86	122	208	-15.10
Calendula officinalis L.	Shoot	117	137	254	+3.67
	Root	102	141	243	-0.81
Zinnia elegans Jacq.	Shoot	59	91	150	-38.77
	Root	55	102	157	-35.91
$LSD_{0.05}$	-	16	18	-	-

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

Table 2. Effects of nine plant species belonging to Asteraceae family on mortality of *Meloidogyne javanica*.

Plant species	Plant part	Mortality % Exposure time (hours)	
		24	48
Control	-	3	5
Gaillardia aristata	Shoot	23	33
	Root	31	41
Cosmos bipinnatus	Shoot	25	38
	Root	41	51
Helianthus annuus	Shoot	55	67
	Root	57	70
Tagetes erecta	Shoot	64	87
	Root	55	61
Tagetes patula	Shoot	38	51
	Root	47	54
Chamomilla recutita	Shoot	20	29
	Root	36	42
Matricaria discoidea	Shoot	42	57
	Root	47	66
Calendula officinalis	Shoot	29	45
	Root	31	38
Zinnia elegans	Shoot	61	88
	Root	52	81
$LSD_{0.05}$		21	24

Effects of soil amendment with *Tegetes erecta* and *Zinnia elegans*

Soil amendments with powdered shoot of *Z. elegans* significantly ($p<0.01$) reduced nematode population densities in soil and subsequent root-knot development in tomato (Table 3). On the other hand, *T. erecta* neither reduced nematode populations nor did it affect nematode infectivity in tomato. Plant height was markedly enhanced following soil treatment with *Z. elegans* while both amendments increased shoot weights compared to the controls. None of the amendment had an influence on root growth of tomato seedlings.

Table 3. The influence of soil amendments with powdered shoot of *Tegetes erecta* and *Zinnia elegans* on nematode population densities, root-knot development due to *Meloidogyne javanica* and growth of tomato.

Treatments	Nematode populations 250 g soil	Galls per root system	Plant height (cm)	Shoot weight (g)	Root weight (g)
Control	1875[a]	82[a]	15.9[a]	2.6[a]	1.3[a]
Tegetes erecta	1760[a]	72[a]	16.2[a]	3.5[b]	1.5[a]
Zinnia elegans	1585[b]	59[b]	17.4[b]	3.8[b]	1.1[a]
$LSD_{0.05}$	221	13	1.1	0.8	0.3

Means followed by same letters in each column are not significantly different in accordance with Duncan's multiple range test.

DISCUSSION

Organic amendments, such as animal manures and composts, were commonly used in agricultural production for their fertility value prior to the availability of chemical fertilizers. It is likely that these amendments also provided other benefits such as improved plant health due to reduction of nematode population densities and subsequent root infection (Shaukat *et al*., 2002). In the present study, shoot and root extract of nine plant species, belonging to the Asteraceae family, inhibited egg hatch and caused substantial mortality of *M. javanica* juveniles *in vitro*. The plant species exerted a differential influence on eggs and juveniles of *M. javanica*. These results suggest that shoots and roots of the plants contain potential nematicidal compounds, which are different both qualitatively and quantitatively. Furthermore, shoot and root extracts were prepared in distilled water, it is possible that these inhibitory compounds were water-soluble and, hence, could be polar in nature. Since only one solvent (water) was used here, solubility of the inhibitory compounds in other solvents, with varying polarity, may yield interesting results. In the present study, root extract of most plant species gave inhibitory effects better than their corresponding shoot extracts indicate that quantity of phytoalexins (possibly phenols) and other secondary compounds were different in these plant parts. Our results are consistent with the findings of Wang *et al*. (2001) who observed that root leachates of *T. erecta* caused substantial 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*.

In the current study, soil amendment with *Z. elegans* reduced nematode population densities in soil and subsequent root-knot development due to *M. javanica* in tomato seedlings under greenhouse conditions. Soil amendments with botanical toxicants have been used successfully by several workers against plant-parasitic nematodes under greenhouse trials (Mankau and Minteer, 1962; Sitaramaiah and Singh, 1978; Rodríguez-kábana 1986: Ritzinger and Macsorley, 1998; Shaukat and Siddiqui 2001; Shaukat *et al*., 2002). The nematode suppressive activity by *Z. elegans*, observed in this study, could be related with the release of toxic compounds in the rhizosphere. Kheir *et al*. (2000), studying the effects of 18 ornamental plants, found that soil amendment with two composites including *T. erecta* and *Z. elegans* resulted in a considerable reduction in reproductive potential and gall development by *M. incognita* on sunflower roots, with *Z. elegans* being the most efficacious.

Alteration of microbial community structure and composition, antagonistic to nematodes, following soil amendment with *Z. elegans* may have had an indirect role in the reduction of nematode density and infectivity. In a previous study, soil amendments with *L. camara* markedly enhanced populations of fungi antagonistic to root-knot nematode in mungbean rhizosphere and roots (Shaukat and Siddiqui, 2001). Induction of systemic resistance against nematode, in tomato roots, following soil amendment with *Z. elegans* is another area which needs further investigation. In this study, whereas shoot and root extract of *T. erecta* inhibited egg hatch and caused mortality of *M. javanica* juvenile *in vitro*, soil amendments with *T. patula* failed to produce nematode suppressive effects in

our plant assay. It is likely that *T. patula* shoots contains nematicidal compounds which are activated only in the presence of sunlight. Our results corroborate with the findings of Marles *et al*. (1992), who found that toxicity of ∝-terthienyl from *T. erecta* results from photoactivation, but the compounds are completely devoid of nematicidal activity when mixed in soil. It is also possible that *T. erecta* failed to stimulate microbial community in tomato rhizosphere antagonistic to nematodes.

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