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EFFECT OF SOME PHENOLIC COMPOUNDS ON SURVIVAL, INFECTIVITY AND POPULATION DENSITY OF *MELOIDOGYNE JAVANICA* IN MUNGBEAN

by
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Summary. Effect of various concentrations of three phenolic compounds including benzoic acid, p-coumaric acid and caffeic acid were tested against *Meloidogyne javanica* under laboratory and greenhouse conditions. Phenolic acids at 2000 ppm produced significant mortality of *M. javanica* juveniles *in vitro*. Caffeic acid was more effective against juveniles compared to benzoic acid or p-coumaric acid. Soil application of caffeic acid at 10 µg/g caused complete inhibition in germination of mungbean seedlings. Plant height and fresh weight of shoots progressively decreased with increasing concentrations of the phenolic compounds in soil. However, with increased concentrations of phenolic acids, there was a corresponding decrease in nematode population densities in soil and root and subsequent galling due to *M. javanica*.

In addition to competition for resources, some weeds interact with crop plants through the production of allelochemicals that inhibit germination or growth of other plants. Narwal, (1994) listed 129 weed species having allelopathic capabilities. A variety of allelochemicals have been reported from living and decomposing tissues of various plant species including phenolic acids, hydroxamic acids, terpenes, terpenoids, glycosides, alkaloids and flavonoids (Whittaker and Feeny, 1971; Patrick, 1971; Willard and Penner, 1976; Worsham, 1989). Among these, phenolic acids are the most frequently identified phytotoxins. Recent studies have demonstrated the phytotoxic effects of phenolic compounds (Blum, 1996; Shafer *et al.*, 1998; Burhan and Shaukat, 2000). The addition of organic materials to soil infested with plant-parasitic nematodes has been demonstrated as a satisfactory control method against many phytonematodes (D'Addabbo, 1995). Reports exist where some decomposing plant species release compounds including phenols, tannins, azadirachtin and ricinin toxic to plant-parasitic nematodes (Mian and Rodriguez-Kabana, 1982; Rossner and Zebitz, 1987; Rich *et al.*, 1989). However, there is no previous report of phenolic allelochemical effects on *Meloidogyne javanica*. The present investigation focuses on phenolic allelochemicals as potential nematicidal agents.

Materials and methods

Various concentrations (100, 250, 500, 1000 and 2000 ppm) of three different phenolic compounds including benzoic acid, p-coumaric acid and caffeic acid were prepared in deionized distilled water. To determine the effect of phenolic acids on *Meloidogyne javanica* (Treub) Chitw., 1 ml of the allelochemical of each concentration was put (separately) in a cavity-slide to which a 1 ml suspension of freshly hatched surface sterilized-juveniles (containing 30-40 J2/ml) was added. A glass slide containing 1 ml sterile distilled water served as the control. Each dilution (effectively reduced to half) was replicated four times at room temperature (28±2 °C). After a 48 h incubation period, the number of dead juveniles were counted. Nematodes were considered dead if they did not move when probed with a fine needle (Cayrol *et al.*, 1989).

Eight mungbean [*Vigna radiata* (L.) Wilczek] seeds were sown in 8 cm-diam., plastic pots containing 350 g sandy-loam soil. Before planting, the soil was drenched with 20 ml of the phenolic compounds (p-coumaric acid, benzoic acid or caffeic acid) to give concentrations of 2.5, 5 and 10 µg/g soil. Soil drenched with 20 ml sterile distilled water served as the control. Treatments were replicated four times and arranged in a randomized complete block design. After one week, the percentage of seed germination was recorded and the roots in each pot were inoculated with 2000 freshly hatched juveniles of *M. javanica*. No growth of mungbean seedlings was recorded in caffeic acid at 10 µg/g, therefore, this concentration was excluded from the experiment.

The experiment was terminated 45 days after inoculation and growth-parameters (plant height and fresh weight of shoot) were recorded. The number of galls on the entire root system were counted under low magnification (x 6). To determine the nematode population in roots, 1 g samples were washed in running tap water after which 1 g root portions were wrapped in muslin cloth and dipped for 3-5 min. in boiling acid fuchsin-lactic acid solution. Stained roots were homogenated in an electric blender for 1 min. The homogenate was suspended in 100 ml water and the number of juveniles that had penetrated the roots were counted in five samples of 5 ml each. The nematode soil population was determined using for extraction the modified Baermann funnel technique.

The data were subjected to analysis of variance (ANOVA) followed by least significant difference (LSD) in accordance with Sokal and Rohlf (1995).

Results and discussion

The phenolic compounds were toxic to the juveniles of *M. javanica* and the percentage mortality increased with increasing concentration (Table I). Caffeic acid induced the greatest juvenile mortality while p-coumaric acid showed least toxicity in this respect.

Applications of benzoic acid, p-coumaric acid and caffeic acid to sandy loam soil resulted in a significant ($p<0.001$) reduction in galling due to *M. javanica* over controls (Table II).

TABLE I - *Effect of various concentrations of phenolic compounds on mortality of* Meloidogyne javanica *juveniles in vitro.*

Treatments	Concentrations (ppm)	Mortality %
Benzoic acid	0	1
	100	1
	250	3
	500	3
	1000	13
	2000	32
p-coumaric acid	0	0
	100	2
	250	2
	500	5
	1000	7
	2000	20
Caffeic acid	0	1
	100	3
	250	2
	500	2
	1000	21
	2000	37
$LSD_{0.05}$		8.9

TABLE II - *Effect of phenolic compounds on root-knot nematode development, population densities and growth of mungbean.*

Treatments	Galls/ root system	Nematode population		Germination Percentage	Plant height (cm)	Shoot weight (g)
		Root g^{-1}	Soil 250 cc			
Control	79	90	3375	91	18.6	0.56
Benzoic acid 2.5 µg/g	60	67	3075	81	22.4	0.76
Benzoic acid 5 µg/g	48	55	2525	66	19.5	0.59
Benzoic acid 10 µg/g	50	62	2075	72	14.7	0.37
p-coumaric acid 2.5 µg/g	65	71	2575	81	18.2	0.63
p-coumaric acid 5 µg/g	52	74	2625	88	17.6	0.53
p-coumaric acid 10 µg/g	50	66	2212	75	15.0	0.43
Caffeic acid 2.5 µg/g	50	63	2550	66	16.3	0.50
Caffeic acid 5 µg/g	46	56	2525	53	13.5	0.32
$LSD_{0.05}$	7	22	566	17	3.5	0.16

Maximum inhibition of gall formation (41% of controls) was achieved following application with 0.5 µg/g caffeic acid. The nematode populations in soil varied significantly ($p<0.01$) between treatments, phenolic compounds markedly reducing soil nematode densities, particularly at the highest concentrations. P-coumaric acid at 10 µg/g resulted in the greatest reduction in nematode density. In general, there was no significant difference between treatments with respect to nematode invasion of the mungbean roots with the two exceptions of 2.5 and 5 µg/g p-coumaric acid (Table II). Higher concentrations of phenolic compounds suppressed seed germination, plant height and shoot weight of mungbean, although benzoic acid at 2.5 µg/g enhanced ($p<0.05$) plant growth.

The results suggest that the phenolic compounds could be systematically searched for the nematicidal acitvity for commercial application in the field. In this study efforts were directed towards impact of phenolic compounds on nematode but the effects of such compounds on the rhizosphere microorganisms, especially the beneficial ones, need investigation.

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