EFFECT OF PHENOLIC ACIDS AND AN AROMATIC ALDEHYDE ON INFECTIVITY OF *MELOIDOGYNE JAVANICA* AND COLONIZATION BY *PSEUDOMONAS AERUGINOSA* IN MUNGBEAN

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Summary. Various concentrations of the two phenolic acids (benzoic acid and caffeic acid) used in combination caused greater suppression of the nematode population densities in soil and consequent root-knot development due to *Meloidogyne javanica* in mungbean. With an increase in phenolic concentration, shoot growth was retarded progressively but there was no apparent effect on root growth. However, *Pseudomonas aeruginosa* to some extent reduced any adverse effect of the phenolic acids on plant growth. Combination of phenolic acids at higher concentrations also reduced populations of *P. aeruginosa* in the rhizosphere. Of the two phenolic acids tested, caffeic acid exhibited greater activity against *M. javanica* and was used with benzaldehyde, an aromatic aldehyde. Caffeic acid and benzaldehyde at high concentrations markedly suppressed nematode population densities and root-knot infestation. The two allelochemicals used with *P. aeruginosa* also significantly suppressed root-knot infection and enhanced plant growth. Benzaldehyde applied alone had no significant impact on populations of *P. aeruginosa* whereas caffeic acid used alone or in combination with benzaldehyde markedly reduced bacterial populations.

During the last few decades, nematode control has been based largely on the use of chemicals. However, because of environmental toxicity and cost of these chemicals, alternative means of control have also been investigated. There are numerous reports of nematicidal chemicals in crude plant homogenates, leachates, and decomposing residues, which are usually assumed to be secondary metabolites for chemical defences against disease and parasites (Halbrendt, 1996). The compounds released into the rhizosphere that influence plant growth are known as allelochemicals. 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 Panner, 1976; Worsham, 1989). A major concern regarding the role of phenolic acids as allelochemical agents in no-till systems pertains to the fact that concentrations of individual phenolic acids recoverable from field soils are well below the levels required for inhibition of germination and growth *in vitro* (Waller *et al.*, 1987; Lyu *et al.*, 1990; Blum, 1995, 1996).

Certain aromatic compounds of plant origin have shown potential negative impact on both fungal and phytoparasitic nematodes (Bauske et al., 1997) and they do not reduce colonization of roots by plant growth-promoting rhizobacteria (PGPR). Soler-Serratosa et al., (1996) reported a marked suppression of various plant-parasitic nematodes following combined application of thymol, a phenolic monoterpene and benzaldehyde, an aromatic aldehyde present in nature as a moiety of plant cyanogenic glucoside. As an example, p-hydroxybezaldehyde occurs as a seconadry metabolite in sudan grass which possesses some nematicidal activity (Widmer and Abawi, 2000). The present paper describes the effect of i) various concentrations of the two phenolic acids (caffeic acid and benzoic acid) individually or in combination on root-knot development and population densities of *Meloidogyne javanica*; ii) various concentrations of caffeic acid and benzaldehyde alone or in combination on root-knot nematode; iii) various concentrations of allelochemicals on colonization by *P. aeruginosa* and growth of mungbean.

MATERIAL AND METHODS

Soil (sandy loam, pH 8.1, moisture holding capacity 39%) was obtained from the experimental field of the Department of Botany, University of Karachi. Eight-mungbean *Vigna radiata* (L.) Wilczek seeds were sown in 8 cm diam. plastic pots each containing 350 g soil. Before sowing, the soil was drenched with 20 ml of caffeic acid (a derivative of cinnamic acid) and *p*-hydroxybenzoic acid (a derivative of benzoic acid) to give concentrations of 0, 1, 2 and 4 mg/g soil. Concentrations of the phenolic compounds greater than used here have been found to be phytotoxic to mungbean (Shaukat and Siddiqui, unpublished). In the combination treatments, concentration of each acid was reduced to half (0, 0.5, 1 and 2 mg/g). Soil drenched with 20 ml sterile distilled water served as controls. Treatments were replicated four times and arranged in a randomised block design. One week after seedling emergence, roots were inoculated by adding 2000 freshly hatched juveniles of *M. javanica* (Treub.) Chitw., through four openings made around the plants.

The experiment was terminated 45 days after inoculation and growth parameters (plant height and fresh

weight of shoot and root) were recorded. The numbers of galls produced on the entire root system were counted under low magnification (x 6). The nematode soil population was determined using for extraction the modified Baermann funnel technique.

In another experiment, after soil amendment with phenolic acids and their mixtures, a plant growth-promoting rhizobacterium, *Pseudomonas aeruginosa* (Schroeter) Migula (strain IE-6S$^+$) was also introduced in the soil. Inoculum was produced by transferring two loopfuls of the bacterium from a 5 day old culture grown on King's B medium (amended with 100 ppm of streptomycin) at 28 °C to 100 ml King's B liquid medium and incubated at room temperature on a shaker (150 rpm) for 48 h. Bacterial cells were centrifuged (4,500 x *g*, 15 min), at 4 °C, the supernatant was discarded and the pellets resuspended in sterile $MgSO_4$ (0.1 M). The soil was drenched with 25 ml $MgSO_4$ (0.1 M) served as controls. There were four replicates of each treatment and pots were randomised. The experiment was terminated 45 days after the addition of the nematodes and plant growth parameters and numbers of galls on the root system were counted. Rhizosphere populations of *P. aeruginosa* were re-isolated by the modified methods of Pillay and Nowak (1997) in which root samples with adhering soil were placed in a 250 ml Erlenmeyer flask containing 10 ml of 0.1M $MgSO_4$ solution (pH 6.5) plus 0.02% tween-20 and shaken vigorously. Ten-fold serial dilutions of the suspension were prepared and 50 ml aliquots from 10^2, 10^3 and 10^4 dilutions were plated onto KB medium supplemented with streptomycin. The plates were incubated at room temperature (25±3 °C) for 48 h and the number of cfu recorded. Plants grown in soil not inoculated with *P. aeruginosa* were also examined for the presence or absence of the bacterium.

In the previous experiments, caffeic acid caused marked suppression of the root-knot nematode and hence was used in combination with benzaldehyde (an aromatic aldehyde). Before seed sowing, the soil was drenched with 20 ml of caffeic acid and benzaldehyde to give concentrations of 0, 1, 2 and 4 mg/g soil. In the combination treatments, concentration of each component was reduced to half (0, 0.5, 1 and 2 mg/g soil). Soil drenched with 20 ml sterile distilled water served as control. Treatments were replicated four times and arranged in a randomised complete block design. The rest of the procedure was the same as outlined above. In another experiment, in addition to various concentrations of allelochemicals alone or in combination, *P. aeruginosa* was also introduced in the same manner as described above. The rest of the procedure was same as described earlier.

The data were subjected to factorial analysis of variance (FANOVA) followed by least significant differences (LSD) in accordance with Sokal and Rohlf (1995) using STATISTICA ver. 6.0 program. The correlation coefficient was also computed where necessary. Bacterial population was transformed to $\log_{10}$ (x+1) before subjecting to analysis.

RESULTS AND DISCUSSION

Factorial analysis of variance revealed that both benzoic acid and caffeic acid caused significant ($P<0.001$) suppression of nematode population densities in soil and root knot development due to *M. javanica* in mungbean (Table I). Caffeic acid and *p*-hydoxybenzoic acid (both at 4 mg/g soil) in combination caused 60% suppression of galling intensity. Caffeic (4 mg/g) and *p*-hydroxybenzoic (1 mg/g) acids applied together caused 51% suppression in nematode soil population level. Phenolics either used singly or in combination significantly ($P=0.01$) altered plant height and fresh weight of shoot. In general, while high concentrations reduced plant growth, low concentrations enhanced plant growth. On the other hand, root weight remained uninfluenced by the phenolics.

Caffeic acid, *p*-hydroxybenzoic acid and *P. aeruginosa* together significantly ($P<0.001$) suppressed root-knot development. Interactions of *p*-hydroxybenzoic acid and caffeic acid ($P<0.01$), and *p*-hydroxybenzoic acid and *P. aeruginosa* ($P<0.001$) were also significant in this respect (Table II). Phenolics used with *P. aeruginosa* caused greater suppression of the root-knot infection compared to their application alone. Combined application of the two phenolics at high concentration (4 mg/g) in the presence of *P. aeruginosa* resulted in the greatest suppression (>69% over the untreated controls) of root-knot development though plant height and shoot weight were significantly reduced ($P<0.05$). *P. aeruginosa* either alone or used in conjunction with the individual or combination of the two phenolics enhanced plant growth compared to the corresponding treatments without the bacterium. With an increase in phenolic concentration in the soil, rhizosphere populations of *P. aeruginosa* declined progressively and greater reduction was found in the combination of the two phenolics (particularly at higher concentrations) compared to the application of either phenolic acid alone. A significant positive correlation between root-knot development and bacterial populations in the rhizosphere was also recorded ($r=0.512$; $p<0.05$).

Caffeic acid used singly or in combination with benzaldehyde caused significant ($P<0.001$) suppression of *M. javanica* in mungbean (Table III). Population densities of *M. javanica* in soil also declined significantly with the increasing concentration of caffeic acid. Benzaldehyde (applied at 2 mg/g of soil or 4 mg/g of soil) in combination with caffeic acid resulted in the synergistic effects in suppressing nematode population densities and subsequent galling intensity. The greatest suppression of nematode population densities in the soil (>50% over the controls) and root knot infection (>66%) was achieved when caffeic acid and benzaldehyde were used together (both at

Table I. Effect of mixture of phenolic acids on root-knot nematode development, population densities and growth of mungbean.

Treatments	Galls/ root system	Nematode in 250 cc soil	Plant height (cm)	Shoot weight (g)	Root weight (g)
Caffeic acid 0 µg/g + Benzoic acid 0 µg/g	102	4290	16.9	0.4	0.4
Caffeic acid 0 µg/g + Benzoic acid 1 µg/g	87	3960	23.1	0.7	0.3
Caffeic acid 0 µg/g + Benzoic acid 2 µg/g	77	3590	23.4	0.8	0.3
Caffeic acid 0 µg/g + Benzoic acid 4 µg/g	71	3060	18.0	0.5	0.3
Caffeic acid 1 µg/g + Benzoic acid 0 µg/g	88	3540	18.5	0.6	0.4
Caffeic acid 1 µg/g + Benzoic acid 1 µg/g	88	3740	22.0	0.5	0.3
Caffeic acid 1 µg/g + Benzoic acid 2 µg/g	71	3470	16.8	0.5	0.3
Caffeic acid 1 µg/g + Benzoic acid 4 µg/g	73	3130	18.6	0.5	0.4
Caffeic acid 2 µg/g + Benzoic acid 0 µg/g	81	2570	19.3	0.7	0.4
Caffeic acid 2 µg/g + Benzoic acid 1 µg/g	60	3220	19.0	0.5	0.4
Caffeic acid 2 µg/g + Benzoic acid 2 µg/g	64	3050	16.8	0.5	0.3
Caffeic acid 2 µg/g + Benzoic acid 4 µg/g	57	2490	15.5	0.4	0.3
Caffeic acid 4 µg/g + Benzoic acid 0 µg/g	68	2870	18.3	0.4	0.3
Caffeic acid 4 µg/g + Benzoic acid 1 µg/g	50	2056	18.8	0.5	0.3
Caffeic acid 4 µg/g + Benzoic acid 2 µg/g	50	2140	14.6	0.3	0.3
Caffeic acid 4 µg/g + Benzoic acid 4 µg/g	41	2080	12.7	0.3	0.2
$LSD_{0.05}$ Caffeic acid	5	10	411	0.9	0.05
p-hydroxybenzoic acid	5	10	411	0.9	0.05

Table II. Effect of mixture of phenolic acids on root-knot nematode development, growth of mungbean and populations of *Pseudomonas aeruginosa* in the rhizosphere.

Treatments	Galls/ Root System		Plant height (cm)		Shoot weight (g)		Root weight (g)		Bacterial rhizosphere colonization (Log cfu g^{-1} fresh wt.)
	Pa-	Pa+	Pa-	Pa+	Pa-	Pa+	Pa-	Pa+	
Caffeic acid 0 µg/g + Benzoic acid 0 µg/g	127	71	15.3	18.1	0.5	0.6	0.4	0.5	4.36
Caffeic acid 0 µg/g + Benzoic acid 1 µg/g	91	80	19.5	20.6	0.7	0.6	0.3	0.3	4.73
Caffeic acid 0 µg/g + Benzoic acid 2 µg/g	74	69	21.2	21.9	0.7	0.7	0.4	0.4	4.40
Caffeic acid 0 µg/g + Benzoic acid 4 µg/g	68	44	22.4	22.5	0.5	0.7	0.4	0.4	3.86
Caffeic acid 1 µg/g + Benzoic acid 0 µg/g	101	79	18.0	18.8	0.6	0.6	0.2	0.4	4.68
Caffeic acid 1 µg/g + Benzoic acid 1 µg/g	93	69	20.5	20.9	0.6	0.6	0.3	0.3	4.58
Caffeic acid 1 µg/g + Benzoic acid 2 µg/g	60	57	14.9	15.4	0.5	0.5	0.3	0.2	4.08
Caffeic acid 1 µg/g + Benzoic acid 4 µg/g	62	65	17.9	17.5	0.5	0.6	0.3	0.3	3.90
Caffeic acid 2 µg/g + Benzoic acid 0 µg/g	83	58	18.5	18.8	0.5	0.7	0.4	0.4	4.09
Caffeic acid 2 µg/g + Benzoic acid 1 µg/g	63	72	18.2	18.8	0.6	0.6	0.4	0.3	3.78
Caffeic acid 2 µg/g + Benzoic acid 2 µg/g	71	43	16.1	15.5	0.5	0.6	0.3	0.3	4.27
Caffeic acid 2 µg/g + Benzoic acid 4 µg/g	74	79	15.5	15.8	0.4	0.5	0.3	0.3	3.75
Caffeic acid 4 µg/g + Benzoic acid 0 µg/g	81	61	17.9	17.7	0.5	0.5	0.4	0.3	3.76
Caffeic acid 4 µg/g + Benzoic acid 1 µg/g	56	56	17.8	16.8	0.5	0.6	0.3	0.3	3.58
Caffeic acid 4 µg/g + Benzoic acid 2 µg/g	49	57	14.9	15.9	0.3	0.5	0.3	0.3	3.64
Caffeic acid 4 µg/g + Benzoic acid 4 µg/g	54	39	14.8	15.1	0.3	0.5	0.2	0.3	3.54
$LSD_{0.05}$ Caffeic acid	6		0.33		0.03		0.02		0.22
p-hydroxybenzoic acid	6		0.33		0.03		0.02		0.22
P. aeruginosa	4		0.23		0.02		0.01		–

Pa- = soil without *P. aeruginosa*; Pa+ = soil with *P. aeruginosa*.

Table III. Effect of caffeic acid and benzaldehyde on root-knot nematode development, population densities and growth of mungbean.

Treatments	Galls/ root system	Nematode in 250 cc Soil	Plant height (cm)	Shoot weight (g)	Root weight (g)
Caffeic acid 0 µg/g + Benzoic acid 0 µg/g	118	3775	17.3	0.4	0.4
Caffeic acid 0 µg/g + Benzoic acid 1 µg/g	102	3880	21.4	0.6	0.4
Caffeic acid 0 µg/g + Benzoic acid 2 µg/g	94	3450	21.5	0.7	0.3
Caffeic acid 0 µg/g + Benzoic acid 4 µg/g	85	3290	22.4	0.7	0.4
Caffeic acid 1 µg/g + Benzoic acid 0 µg/g	98	3180	19.7	0.7	0.4
Caffeic acid 1 µg/g + Benzoic acid 1 µg/g	75	3240	22.6	0.7	0.3
Caffeic acid 1 µg/g + Benzoic acid 2 µg/g	82	3075	19.3	0.6	0.3
Caffeic acid 1 µg/g + Benzoic acid 4 µg/g	57	2980	21.8	0.6	0.5
Caffeic acid 2 µg/g + Benzoic acid 0 µg/g	73	2375	18.9	0.6	0.4
Caffeic acid 2 µg/g + Benzoic acid 1 µg/g	69	2825	19.2	0.7	0.4
Caffeic acid 2 µg/g + Benzoic acid 2 µg/g	49	2460	17.4	0.5	0.4
Caffeic acid 2 µg/g + Benzoic acid 4 µg/g	43	2390	15.8	0.5	0.4
Caffeic acid 4 µg/g + Benzoic acid 0 µg/g	55	2550	16.9	0.4	0.3
Caffeic acid 4 µg/g + Benzoic acid 1 µg/g	63	2380	17.8	0.4	0.3
Caffeic acid 4 µg/g + Benzoic acid 2 µg/g	47	2090	15.8	0.4	0.4
Caffeic acid 4 µg/g + Benzoic acid 4 µg/g	39	1860	14.9	0.3	0.3
$LSD_{0.05}$ Caffeic acid	5.4	389	0.7	0.1	0.07
p-hydroxybenzoic acid	5.4	389	0.7	0.1	0.07

Table IV. Effect of caffeic acids and benzaldehyde on root-knot nematode development, growth of mungbean and populations of *Pseudomonas aeruginosa* in the rhizosphere.

Treatments	Galls/ root System		Plant height (cm)		Shoot weight (g)		Root weight (g)		Bacterial rhizosphere colonization (Log cfu g^{-1} fresh wt.)
	Pa-	Pa+	Pa-	Pa+	Pa-	Pa+	Pa-	Pa+	
Caffeic acid 0 µg/g + Benzoic acid 0 µg/g	100	75	16.7	18.8	0.4	0.7	0.5	0.6	4.49
Caffeic acid 0 µg/g + Benzoic acid 1 µg/g	104	64	17.3	21.2	0.6	0.7	0.5	0.5	4.46
Caffeic acid 0 µg/g + Benzoic acid 2 µg/g	91	75	19.4	23.3	0.6	0.8	0.5	0.6	4.53
Caffeic acid 0 µg/g + Benzoic acid 4 µg/g	74	60	19.6	22.9	0.7	0.8	0.6	0.6	4.49
Caffeic acid 1 µg/g + Benzoic acid 0 µg/g	95	67	20.2	19.4	0.6	0.6	0.4	0.5	4.52
Caffeic acid 1 µg/g + Benzoic acid 1 µg/g	119	64	20.6	22.3	0.7	0.6	0.4	0.5	4.29
Caffeic acid 1 µg/g + Benzoic acid 2 µg/g	83	83	20.6	21.9	0.7	0.6	0.5	0.5	4.18
Caffeic acid 1 µg/g + Benzoic acid 4 µg/g	80	59	19.7	21.6	0.6	0.7	0.5	0.6	4.00
Caffeic acid 2 µg/g + Benzoic acid 0 µg/g	75	58	17.9	17.4	0.5	0.5	0.4	0.6	3.97
Caffeic acid 2 µg/g + Benzoic acid 1 µg/g	78	63	18.2	18.2	0.5	0.6	0.4	0.4	3.63
Caffeic acid 2 µg/g + Benzoic acid 2 µg/g	61	51	16.6	17.9	0.6	0.6	0.4	0.5	3.95
Caffeic acid 2 µg/g + Benzoic acid 4 µg/g	61	55	15.8	18.1	0.4	0.6	0.3	0.5	3.68
Caffeic acid 4 µg/g + Benzoic acid 0 µg/g	51	58	14.9	15.3	0.5	0.5	0.3	0.3	3.52
Caffeic acid 4 µg/g + Benzoic acid 1 µg/g	47	48	14.7	15.8	0.4	0.6	0.3	0.4	3.58
Caffeic acid 4 µg/g + Benzoic acid 2 µg/g	55	42	14.4	14.3	0.4	0.4	0.4	0.4	3.52
Caffeic acid 4 µg/g + Benzoic acid 4 µg/g	37	30	13.5	13.9	0.3	0.4	0.3	0.3	3.52
$LSD_{0.05}$ Caffeic acid	7.2		0.42		0.04		0.03		0.19
p-hydroxybenzoic acid	7.2		0.42		0.04		0.03		0.19
P. aeruginosa	5.3		0.19		0.03		0.02		–

Pa- = soil without *P. aeruginosa*; Pa+ = soil with *P. aeruginosa*.

4 mg/g of soil). Whereas increasing concentrations of caffeic acid resulted in a progressive decrease of plant growth, an increase in benzaldehyde concentration (used alone) enhanced plant growth. Caffeic acid and benzaldehyde used together had a pronounced negative impact on growth of mungbean plants. There was no inhibitory effect of the two allelochemicals (either used individually or in combination) on root growth.

The two allelochemicals used with *P. aeruginosa* showed greater suppression of the root-knot infection when compared without the bacterium. *P. aeruginosa* used alone caused >33% suppression ($P<0.05$) of root-knot infection (table IV). The two allelochemicals (both at 4 mg/g of soil) used in combination with *P. aeruginosa* caused maximum suppression of the root-knot infection (>70% suppression compared with untreated controls). Plant growth progressively decreased when caffeic acid was used individually or in combination with benzaldehyde. However, the two allelochemicals mixed with *P. aeruginosa* showed enhanced plant growth compared to their respective treatments in the absence of *P. aeruginosa*. Benzaldehyde used alone had no significant impact on populations of *P. aeruginosa* in the rhizosphere of mungbean whereas, the two allelocehmicals used together caused significant inhibition of the rhizosphere populations of the bacterium.

Phenolics and benzaldehyde, which often are present in the soil in significant amounts, could be combined with some other methods of nematode control in an integrated system to keep the populations of plant-parasitic nematodes with acceptable limits.

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