Nematicidal activity of some phenolic compounds against *Meloidogyne incognita*

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SUMMARY

A number of phenolic compounds including monohydroxy, dihydroxy and trihydroxy compounds, quinones and aromatic acids such as transcinnamic acid have been studied for their nematicidal activity and their effect on egg hatch of *Meloidogyne incognita*. Maximum nematicidal activity was exhibited by transcinnamic acid followed by pyrogallol, 2-OH napthoic acid and ethyl gallate. After autoxidation, certain compounds like α-resorcylic acid, 3,4 dihydroxybenzoic acid (protocatechuic acid), ferulic acid and caffeic acid were found to be highly toxic to the nematode larvae though they showed poor activity before autoxidation. Transcinnamic acid and 2-OH napthoic acid also markedly suppressed the egg hatch of *M. incognita*.

RÉSUMÉ

Activité nématicide de quelques composés phénoliques envers Meloidogyne incognita

Un certain nombre de composés phénoliques comprenant des composés mono-, di- et trihydroxylés, des quinones et des acides aromatiques tels l'acide transcinnamique ont été testés pour leur action nématicide et leur effet sur l'éclosion, en utilisant *Meloidogyne incognita*. L'action nématicide maximale a été montrée par l'acide transcinnamique, suivi par le pyrogallol, l'acide 2-OH naphtoïque et le gallate d'éthyle. Après autooxidation certains composés tels les acides α-résorcylique, 3,4 dihydroxybenzoïque (acide protocatechuique), ferulique et caféique, se sont montrés hautement toxiques envers les larves du nématode bien que leur activité ait été faible avant autooxidation. Les acides transcinnamique et 2-OH naphtoïque inhibent également de façon nette l'éclosion de *M. incognita*.

A number of phenolic compounds such as chlorogenic and caffeic acids are reported to be involved in the defence mechanisms operating in plants against nematode infections (Epstein, 1972; Hung & Rohde, 1973; Bajaj & Mahajan, 1977; Giebel, 1982), but there is little experimental evidence to indicate that orthodihydroxy phenolic compounds and quinones are directly involved in the inactivation of plant nematodes. The levels of preformed phenols in roots have been correlated with resistance in certain plant cultivars to nematodes (Cohn, 1974; Narayana & Reddy, 1980). Certain compounds like acetylenes, terpenoid aldehydes, sesquiterpenoids and phenoxypropionic acid derivatives are known to have nematicidal activity against some nematodes (Veech, 1979; Mori *et al.*, 1982; Hayashi, Wada & Munakata, 1983). The present investigation was undertaken to check the *in vitro* activity of some phenolic compounds against the second stage juveniles of *Meloidogyne incognita* (Kofoid & White) Chitwood and their effect on its egg hatch. As oxidation products of certain phenolic acids are believed to be more toxic to nematodes (Hung & Rohde, 1973), ten phenolic compounds were oxidized and assayed for nematicidal activity.

Material and methods

Egg masses or freshly hatched second stage juveniles were obtained from cultures maintained on egg plant. The nematicidal activity of the chromatographically pure compounds was assayed according to Kimura *et al.* (1981) with slight modification. Two ml of aqueous solution of each test compound was taken and 1 ml of water suspension with a definite number of infective juveniles added to it to obtain a final product concentration of 1,100 ppm. The mouth of the glass vial was secured with tissue paper using a rubber band. Three replications of each compound were maintained alongwith control. The vials were then placed in dark at 27-28° for 48 h before being inverted in small beakers containing 5 ml of 1,000 ppm streptocycline. A small piece of glass was placed under the rim of the vial to allow nematodes to egress. Nematodes recovered in the beaker after 24 h were counted and percent mortality calculated by the formula $\frac{B - A}{B} \times 100$, where B is the number of nematodes recovered after in the control and A is the number of nematodes recovered in the treatment.

Ten phenolic compounds were oxidized by passing air through their aqueous solution at pH 8 and pH subsequently adjusted to 7.0 after the reaction was over. These were also then assayed for nematicidal activity.

To check the effect of the test compounds on the hatching of the egg masses of *M. incognita*, 2 ml of 500 ppm solution of the test compound was taken in a cavity block and one egg mass, previously checked for the presence of eggs was placed in it. Three replications were maintained. Hatched juveniles were counted every 24 h for 7 days. The data were then subjected to the analysis of variance.

Results

Out of the 28 compounds tested, ten gave a mortality percentage of 75 or above. Four of these — transcinnamic acid, pyrogallol, 2-OH napthoic acid and ethyl gallate were highly toxic with a mortality greater than 95 %. Five compounds (2,6-dibromoquinone chloroimide, catechin hydrate, β-resorcylic acid, m-hydroxy benzoic acid and 3,4-dihydroxy benzoic acid) exhibited moderate activity with mortality ranging from 52-66 % (Table 1, A).

With the oxidized phenolic acids, the greatest percent mortality was observed with α-resorcylic acid (97.6). Ferulic acid, 3,4-dihydroxy benzoic acid and caffeic acid also showed high activity. Lower activity was indicated by chlorogenic acid and catechin hydrate, while vanillic acid, phloroglucinol and p-hydroxy cinnamic acid induced negligible nematode mortality.

There were well marked differences among various phenolic compounds for suppression of hatching of eggs of *M. incognita*. Naringenin was found to be the most

Table 1

Nematicidal (A) and anti-hatching (B) activities of phenolic compounds on *Meloidogyne incognita*

Compound	(A)* *Percent mortality*		(B)** *Percent hatching*
	Unoxidized	*Oxidized*	
Transcinnamic acid	99.7	—	0.07
Pyrogallol	99.4	—	—
2-OH napthoic acid	96.4	—	0.07
Ethyl gallate	96.1	—	—
3,4-dihydroxy phenylacetic acid	89.7	—	19.34
Protocatechuic aldehyde	88.8	—	21.45
Hydroquinone	81.9	—	—
p-hydroxy cinnamic acid	76.1	8.2	74.40
Gentisic acid	75.5	—	58.55
2,3-dihydroxy benzoic acid	74.9	94.0	14.24
2,6-dibromoquinone chloroimide	65.7	—	—
Catechin hydrate	56.0	24.0	48.45
β-resorcylic acid	55.3	—	62.48
m-hydroxy benzoic acid	54.9	—	—
3,4-dihydroxy benzoic acid	52.1	—	59.96
Phloroglucinol	42.5	3.4	88.23
Naringenin	39.9	—	0.29
3,4-dihydroxy phenylalanine	39.7	—	—
Vanillin	32.0	—	16.79
Ortho coumaric acid	27.5	—	—
Chlorogenic acid	27.2	29.7	37.83
Vanillic acid	26.1	8.3	31.62
Tyrosine	25.3	—	—
Gallic acid	24.5	44.9	74.49
Caffeic acid	22.6	85.8	52.08
Ferulic acid	18.3	89.5	94.77
Rutin	12.6	—	—
α-resorcylic acid	10.2	97.6	72.45

(A)* In percent of the control (average of three replicates).
(B)** Hatching after 7 days, in percent of the control (average of three replicates).

effective, with total suppresion of hatching. 2-OH napthoic acid and transcinnamic acid were also highly effective in suppressing the egg hatch. The maximum hatch in control was observed in the first two days and some of the compounds like vanillin and 3,4-dihydroxyphenyl acetic acid reduced egg hatch during this period but later on this effect was not evident (Table 1, B).

Discussion

Maximum nematicidal activity was observed in case of transcinnamic acid, pyrogallol, 2-OH napthoic acid and ethyl gallate followed by 3,4-dihydroxy phenyl acetic acid, protocatechuic aldehyde and hydroquinone. Vicinal trihydroxy group as present in pyrogallol and ethyl gallate was important for nematicidal activity. Orientation of OH group in aromatic ring of phenolic acids considerably affected the nematicidal activity. This could be seen from the fact that gentisic acid and 2,3-dihydroxy benzoic acid had lower activity as compared to protocatechuic aldehyde, 3,4-dihydroxy phenyl acetic acid and ethyl gallate. All these compounds have orthodihydroxy phenolic group, which is very readily oxidized to orthoquinones, which have an inhibiting effect on nematodes. Recently, Hayashi, Wada and Munakata (1983) have shown that p-methoxy isomer of propionic acid derivatives is more active than the 0-and m-methoxy isomers. In the present investigations, 3,4-dihydroxyphenyl acetic acid revealed high activity whereas caffeic acid and chlorogenic acid having unsaturated double bond in the side chain had comparatively less activity. Chang and Rohde (1969) have reported that chlorogenic acid acts as repellant to *Pratylenchus penetrans*. This phenolic compound has been associated in the mechanism of disease resistance (Clark *et al.*, 1959). *In vitro* studies did not reveal any nematicidal activity by the oxidized chlorogenic acid also. The *in vivo* activity, as reported by Clark *et al.* (1959), can most probably be due to the quinic acid or caffeic acid parts of chlorogenic acid which are released by the action of hydrolytic enzymes such as esterases. It appears likely that chlorogenic acid is hydrolysed to quinic acid and caffeic acid, the latter being further oxidized to orthoquinone, which has been shown to be toxic to nematodes.

Most of the phenolic acids studied had poor nematicidal activity. Similar was the case with flavonoid compounds like catechin hydrate, rutin and naringenin. Monohydroxy phenolic compounds such as vanillin and vanillic acid also evidenced low activity. High activity shown by transcinnamic acid, which is a non-phenolic aromatic acid is in confirmation to the earlier reports of Sitaramaiah and Singh (1978) who reported that tomato roots infected with *M. javanica* when exposed to aromatic acids, benzoic acid, phenylacetic acid and cinnamic acid had fewer galls and egg masses than the roots in the untreated control.

The autoxidized products of phenolic compounds such as 3,4-dihydroxy benzoic acid, ferulic acid and α-resorcylic acid also showed high nematicidal activity. This could be due to the stabilization of quinones in these phenolic compounds. High activity shown by hydroquinone and the oxidized products of the abovementioned phenolic compounds indicate that quinones are involved in imparting nematicidal activity.

From the investigations on the effect of phenolic compounds on the hatching of the egg masses, it was evident that monohydroxy phenolic compounds such as naringenin and 2-OH napthoic acid and the non phenolic aromatic acids like transcinnamic acid are more effective in suppressing the hatching of *M. incognita* eggs as compared to orthodihydroxy phenolic compounds. The precise mechanism by which this suppression occurs is, however, not clear. In addition to suppressing the egg hatch, both transcinnamic acid and 2-OH napthoic acid have shown remarkable nematicidal activity against the larvae.

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