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THE EFFECT OF *CINERARIA MARITIMA*, *RUTA GRAVEOLENS* AND *TAGETES ERECTA* EXTRACTS ON THE HATCHING OF *HETERODERA SCHACHTII*

by
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Summary. The effect of leaf and root extracts, alone or in combination, and root leachates from *Cineraria maritima*, *Ruta graveolens* and *Tagetes erecta* on the hatching of an Italian population of *Heterodera schachtii* was investigated *in vitro*. All the aqueous extracts showed a nematicidal effect except the mixture of leaf and root extracts of *R. graveolens* and root extracts of *T. erecta*, which showed only a nematostatic effect.

The use of alternatives to chemicals for the control of plant parasitic nematodes is a response to present and future environmental requirements. Many plant species possess nematicidal or nematostatic properties (Gommers, 1981; Grainge and Ahmed, 1988), among them *Tagetes* spp., the leaf and root extracts of which have been shown to be effective against *Globodera rostochiensis* (Woll.) Berhens (Hills, 1960; Omidvar, 1961 and 1962; Sasanelli and Di Vito, 1991). Also the sugar beet cyst nematode, *Heterodera schachtii* Schmidt has been reported to be sensitive to the alkaloids and tannins from *Ricinus communis* L. (Grainge and Ahmed, 1988).

The present investigation examined the nematicidal properties of aqueous extracts from leaves and roots, and root leachates of *Cineraria maritima* DC., *Ruta graveolens* L. and *Tagetes erecta* L. against the beet cyst nematode, *H. schachtii*.

Materials and methods

Heterodera schachtii was collected from a field at Avezzano (L'Aquila). Cysts were extracted by the Fenwick can. Batches of 100 cysts of similar size (averaging 123 eggs/cyst) were placed on 2 cm diam sieves (215 µm aperture). Each sieve was put in a 3.5 cm diam Petri dish, and all dishes were arranged according to a complete randomized block design. There were four replicates for each treatment.

Aqueous extracts from leaves and roots, alone or in combination, and root leachates of *C. maritima*, *R. graveolens* and *T. erecta* were obtained by the method described by Sasanelli and Di Vito (1991). However, in our investigation only 50 g green leaves or roots, or 25 g of each when used in combination, were soaked in 200 ml distilled water. Each plant extract or root leachate was adjusted to 3 mM adding an equal volume of 6 mM of zinc sulphate.

There were two controls: cysts in a 3 mM zinc sulphate aqueous solution, which is reported to give the highest hatch percentage for *H. schachtii* (Clarke and Shepherd, 1966; Greet, 1974) and cysts in the same solution with 5 µg/ml phenamiphos. The latter solution had previously been found to suppress the hatch of *H. schachtii* eggs (Greco and Thomason, 1980).

Three ml of each test solution were added to each batch of cysts, which were then incubated at 25 °C. Juveniles emerging from eggs were removed and counted weekly over a 10 week period. The solutions were renewed weekly but after four weeks the test solutions were removed and for the remaining six more weeks the incubation continued only in the zinc sulphate solution. At the end of the experiment cysts were crushed, according to Seinhorst and Den Ouden (1966), and unhatched eggs and juveniles were counted. Numbers of second stage juveniles emerging weekly were expressed as cumulative percentages of the total egg content of the cysts.

Results

During the first four weeks, emergence of juveniles from cysts incubated in both leaf and root aqueous extracts of the three plant species, alone or in combination, was negligible, although egg hatch in root extracts of *T. erecta* gave significantly ($P = 0.05$) higher hatch (5.7%) (Table I). Juveniles from cysts incubated in root leachates were significantly ($P = 0.01$) more than those in all other treatments, but less than those in the 3 mM zinc sulphate. However, hatch in *C. maritima* leachate after four weeks (11.21%), was larger than in root leachates of *R. graveolens* and *T. erecta*. Maximum hatch was in zinc sulphate, while in phenamiphos hatch of eggs was similar to that in aqueous extracts.

When plant extracts and root leachates where removed and the incubation continued in the zinc sulphate solution, egg hatch resumed for those cysts previously incubated in phenamiphos solution, but it was still negligible (1.4-2.6%) for two more weeks for those cysts incubated in all leaf extracts, in root (1.2%) or in root and leaf extract (1.8%) of *C. maritima.* Thereafter the emergence of juveniles increased in all treatments. However, the ultimate egg hatch of cysts incubated for four weeks in all plant extracts was significantly less than in the 3 mM zinc sulphate, except cysts incubated in root extract of *T. erecta*, in the combination of leaf and root extract of *R. graveolens*, and in root leachates of *C. maritima* and *R. graveolens.*

Discussion

The experiment has demonstrated the nematicidal properties of aqueous extracts of *C. maritima.* This is probably associated with the high content of alkaloids (jacobine, jacodine and senecionine) in the plant and especially in the roots (Grainge and Ahmed, 1988).

Root and leaf extracts of *R. graveolens* were nematicidal when used separately, but only nematostatic when combined; the combined extracts suppressed emergence of juveniles from cysts, but emergence resumed when these were removed. This explains a possible antagonistic effect of extracts from leaves and roots, that could be attributable to an interaction of the active principles responsible for the inhibitory effect, the rutin, a flavonoid, the xanthotoxin, a coumarine (Holyoke and Reese, 1987) and two alkaloids, kokusaginine and skimmianine (Grainge and Ahmed, 1988).

Leaf extracts or a combination of leaf and root extracts of *T. erecta* have been shown to be nematicidal. However, in an experiment with *G. rostochiensis* (Sasanelli and Di Vito, 1991), only a nematostatic effect was demonstrated.

The results obtained in this experiment, especially those with *C. maritima* and *R. graveolens* are interesting and need confirmation. It would be useful to ascertain whether the cultivation of these plants for short periods or their use as a green manure could be used successfully for the control of nematodes in the field.

TABLE I - *The effect of root and leaf extract and root diffusates of* Cineraria maritima, Ruta graveolens *and* Tagetes erecta *on the hatching of* Heterodera schachtii

TREATMENT (aqueous extracts)	Cumulative percentages of juveniles emerging weekly											
	Incubation periods (weeks)											
	In test solutions				In zinc sulphate solution						LSD	
Weeks (see text)	1	2	3	4	5	6	7	8	9	10	0.05	0.01
Leaves												
Cineraria maritima	0.17	0.18	0.19	0.20	0.31	1.73	7.71	11.97	15.61	18.08	4.00	5.39
Ruta graveolens	0.18	0.37	0.37	0.41	0.96	1.30	8.91	13.53	17.26	21.72	2.35	3.17
Tagetes erecta	0.21	0.54	0.58	0.59	1.88	2.60	6.63	8.99	11.05	13.05	2.85	3.84
Roots												
Cineraria maritima	0.32	0.37	0.39	0.40	0.41	1.16	3.51	5.15	8.68	11.60	1.00	1.34
Ruta graveolens	1.32	1.86	2.12	2.16	4.86	8.31	12.27	13.71	14.60	15.86	4.13	5.56
Tagetes erecta	4.25	5.45	5.63	5.67	7.69	13.64	21.45	26.34	32.01	36.83	8.74	11.78
Roots and leaves												
Cineraria maritima	0.30	0.31	0.31	0.31	0.44	1.85	4.77	9.27	12.37	15.45	2.48	3.34
Ruta graveolens	1.35	1.62	1.71	1.71	3.70	9.67	20.31	24.00	26.39	28.53	4.28	5.77
Tagetes erecta	0.50	0.56	0.62	0.69	1.90	5.46	7.76	10.41	13.02	15.72	1.29	1.73
Root leachates												
Cineraria maritima	9.71	10.19	10.72	11.21	13.79	20.85	23.26	29.35	32.76	35.34	5.07	6.84
Ruta graveolens	6.59	7.14	7.49	8.11	12.93	18.48	22.25	25.08	27.95	31.00	2.24	3.01
Tagetes erecta	6.10	6.79	7.28	7.78	9.59	12.78	15.51	18.19	21.41	24.61	1.67	2.24
Control A (5 µg/ml Phenamiphos + 3mM Zinc Sulphate	0.50	0.55	0.60	0.61	14.31	26.53	36.19	42.83	47.70	52.29	8.81	11.88
Control B (3mM Zinc Sulphate)	14.11	15.71	16.89	19.13	21.36	24.41	26.29	29.21	31.50	33.96	4.42	5.96
LSD 0.05	2.51	2.41	2.31	2.31	3.48	4.45	5.30	5.56	6.22	6.66		
LSD 0.01	3.36	3.23	3.08	3.08	4.65	5.94	7.08	7.43	8.32	8.90		

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