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EFFECT OF *CINERARIA MARITIMA, RUTA GRAVEOLENS* AND *TAGETES ERECTA* LEAF AND ROOT EXTRACTS ON ITALIAN POPULATIONS OF *MELOIDOGYNE* SPECIES

by
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Summary. Extracts from *Tagetes erecta* and *Cineraria maritima* showed good nematicidal activity against *Meloidogyne arenaria, M. hapla,* and *M. javanica,* but not on *M. incognita. Ruta graveolens* had a greater nematicidal effect on all species tested, and in particular the leaf extract was more efficient than fenamiphos. Root leachates showed no nematicidal activity.

Several plants belonging to different botanical families contain active principles with nematicidal or nematostatic properties (Gommers, 1981; Grainge and Ahmed, 1988). Their use as a green manure could be a control measure alternative to/or integrated with chemical nematicides.

Nematicidal activity against *Heterodera schachtii* was shown by extracts of *Tagetes erecta* L., *Cineraria maritima* DC. and *Ruta graveolens* L. (Sasanelli and D'Addabbo, 1992). But there is no information on the effect of these plants on *Meloidogyne* species. Therefore, an experiment was undertaken to investigate the *in vitro* effect of leaf and root aqueous extracts and of root leachates of these three plants on egg hatch of Italian populations of *M. arenaria* (Neal) Chitw., *M. hapla* Chitw., *M. incognita* (Kofoid *et* White) Chitw. and *M. javanica* (Treub) Chitw.

Materials and methods

Aqueous extracts of leaves and roots, alone or in combination, and root leachates of *C. maritima, R. graveolens* and *T. erecta* were prepared as described before (Sasanelli and Di Vito, 1991), except that 100 g green leaves or roots, or 50 g when used in combination, were soaked in 400 ml distilled water. Distilled water or an aqueous solution containing 5 µg/ml fenamiphos were used as control (Greco and Thomason, 1980).

The nematode populations were reared on tomato cv. Roma in a glasshouse at 20-25°C. Batches of twenty egg masses of similar size (averaging 20,000 eggs) of each nematode species were placed on 2 cm diam sieves (215 µm aperture) and each sieve was put in a 3.5 cm diam Petri dish. Two ml of each test solution, sufficient to cover egg masses, were then added to four batches of egg masses. The dishes were arranged in a complete randomized block design with four replicates of each treatment and incubated in a growth cabinet at 20°C (Ekanayake and Di Vito, 1984).

Emerged juveniles were removed and counted every week, renewing the hatching solutions at the same time, over a nine weeks period. After the first four weeks the egg masses were removed from extracts, root leachates and fenamiphos, and the incubation continued for five more weeks only in distilled water, according to the methodology of previous experiments (Sasanelli and Di Vito, 1991; Sasanelli and D'Addabbo, 1992).

At the end of the experiment the egg masses were immersed in a 1% sodium hypochlorite aqueous solution (Hussey and Barker, 1973) and the unhatched eggs were counted. Numbers of juveniles emerging weekly were expressed as cumulative percent of the total initial population.

Data were statistically analysed and comparisons made with LSD's.

Results

Meloidogyne arenaria (Table I). Emergence of juveniles from egg masses was suppressed in all the aqueous extracts during the first four weeks. There was no significant difference between hatch in all leaf extracts (3-7%), root extract of *C. maritima* (2%), combined root and leaf extracts of *C. maritima* (5%) and *R. graveolens* (4%) and that in the fenamiphos. Hatch in root and combined extracts of *T. erecta* and in root extract of *R. graveolens* resulted significantly higher than in fenamiphos (38%) but less than in distilled water (81%). Cumulative hatch per-

TABLE I - *Effect of root and leaf extracts and root diffusates of* Cineraria maritima, Ruta graveolens *and* Tagetes erecta *on the per cent cumulative hatch of* Meloidogyne arenaria.

Treatment (aqueous extracts)	Incubation periods (weeks)									L S D	
	In test solutions				In distilled water						
	1	2	3	4	5	6	7	8	9	0.05	0.01
Leaves											
C. maritima	0	0	3	5	11	26	40	43	43	11	14
R. graveolens	0	7	7	7	7	8	8	11	12	6	8
T. erecta	2	2	3	3	3	6	28	34	34	5	7
Roots											
C. maritima	1	2	2	2	2	4	13	27	29	5	7
R. graveolens	9	20	23	25	34	52	58	59	59	13	17
T. erecta	24	26	35	43	48	51	53	53	53	20	27
Roots and leaves											
C. maritima	1	3	5	5	5	6	13	20	21	7	10
R. graveolens	1	4	4	4	7	12	19	20	20	7	10
T. erecta	3	16	16	20	30	38	40	41	41	14	19
Root leachates											
C. maritima	20	58	76	79	79	79	79	79	79	5	7
R. graveolens	21	56	80	82	82	82	82	83	83	9	12
T. erecta	16	53	78	81	81	81	81	81	81	4	5
Fenamiphos (5 µg/ml)	3	3	3	3	4	16	26	35	38	12	16
Distill. water	22	56	78	80	80	81	81	81	81	9	12
L S D 0.05	7	8	9	9	11	11	11	11	11	–	–
0.01	10	10	12	13	14	15	15	15	15	–	–

centage was always higher for root and combined extracts of *T. erecta* and root extracts of *T. erecta* and *R. graveolens* than for the other extracts. Root leachates and water control gave the highest hatch percentages: these increased significantly (P = 0.01) in the second and third week, then remaining substantially stationary (about 80%).

Meloidogyne hapla (Table II). All the aqueous extracts inhibited juveniles emergence from eggs during the first four weeks and, except for the leaf extract from *T. erecta* (23%), there were no significant differences when compared with fenamiphos. Final cumulative percentages were significantly (P = 0.05) higher than fenamiphos only for the extracts of *T. erecta* and combined extract of *C. maritima*. Root leachates apparently had no effect on egg hatch, significantly larger and quicker (P = 0.01) than in distilled water.

Meloidogyne incognita (Table III). At the end of the fourth week hatch in aqueous extracts was significantly (P = 0.01) higher than in fenamiphos (7%), except for leaf (3%) and combined (16%) extracts of *R. graveolens*. Emergence in all root leachates and the root extract of *T. erecta* was similar to the water control. The final cumulative hatch percentages of all *T. erecta* extracts, and the combined extract of *C. maritima* and root extract of *R. graveolens*, was not different from distilled water.

Meloidogyne javanica (Table IV). The hatch pattern was similar in root leachates of all plants and in water, although the final percentage was above 90%. Hatch was greatly suppressed in all plant extracts except in leaf extracts of *C. maritima*. After transferring to distilled water, the emergence of juveniles recovered completely with egg masses that had previously been incubated in root extract from *T. erecta* (73%) and partially for those incubated in leaf extract of *C. maritima* (64%) or *T. erecta* (41%) and in the combined extract of *T. erecta* (45%).

TABLE II - *Effect of root and leaf extracts and root diffusates of* Cineraria maritima, Ruta graveolens *and* Tagetes erecta *on the per cent cumulative hatch of* Meloidogyne hapla.

Treatment (aqueous extracts)	Incubation periods (weeks)									L S D	
	In test solutions				In distilled water						
	1	2	3	4	5	6	7	8	9	0.05	0.01
Leaves											
C. maritima	4	5	6	7	11	25	52	56	58	7	10
R. graveolens	1	2	2	2	2	4	6	6	6	4	6
T. erecta	2	5	13	23	39	57	66	67	68	18	24
Roots											
C. maritima	4	5	6	7	10	16	40	47	49	12	16
R. graveolens	4	4	4	5	5	16	37	42	43	9	12
T. erecta	6	8	9	11	15	36	67	70	70	9	12
Roots and leaves											
C. maritima	4	4	4	4	4	7	60	62	63	7	10
R. graveolens	5	5	6	6	8	13	19	20	21	10	14
T. erecta	2	3	6	9	18	43	59	60	60	5	7
Root leachates											
C. maritima	22	46	72	91	95	96	96	96	96	5	6
R. graveolens	26	47	75	87	90	91	91	91	91	5	6
T. erecta	23	42	75	84	93	94	94	94	94	5	7
Fenamiphos (5 µg/ml)	4	5	6	6	23	41	45	46	47	18	24
Distill. water	22	45	57	67	74	83	86	86	87	17	23
L S D 0.05	4	6	7	10	12	13	13	12	12	–	–
0.01	5	8	9	13	17	18	17	16	15	–	–

Hatch was inhibited in leaf extract of *R. graveolens*, while there was little recover in the other extracts from this plant (21-23%).

Discussion

Emergence in root leachates and water control was always similar and exhausted within four weeks, thus confirming previous findings (Ekanayake and Di Vito, 1984), although it was quicker for *M. incognita* and *M. javanica*. Egg masses of these two species were probably in a more advanced developmental stage than those of *M. arenaria* and *M. hapla* and this could have masked the effect of some extracts, particularly those that induced a large hatch after the first week, without any increase until the fourth.

Aqueous extracts of *T. erecta* were effectively nematicidal against *M. arenaria* and *M. javanica*, and better than against *M. hapla*, with no effect on *M. incognita*, confirming the findings of Hackney and Dickerson (1975). The inhibiting effect of α-terthienyl (Uhlenbroek and Bijloo, 1958), the active principle in the extracts of this plant, although variable, confirms the results of previous experiments (Muñoz *et al.*, 1982).

Root and combined extracts of *C. maritima* had a greater nematicidal effect than leaf extracts, probably because of the higher content of alkaloids (jacobine, jacodine and senecionine) in the roots (Grainge and Ahmed, 1988) than in the leaves. The nematicidal activity was particularly efficient against *M. arenaria* and *M. javanica*, less on *M. hapla* and nil on *M. incognita*. Nematicidal activity against *M. arenaria* and *M. hapla* was the same as that of fenamiphos.

The nematicidal effect of leaf and combined extracts of *R. graveolens* was always significantly greater or similar to that of fenamiphos. The chemicals responsible for this nematicidal activity have not been identified, but it can be

TABLE III - *Effect of root and leaf extracts and root diffusates of* Cineraria maritima, Ruta graveolens *and* Tagetes erecta *on the per cent cumulative hatch of* Meloidogyne incognita.

Treatment (aqueous extracts)	Incubation periods (weeks)									L S D	
	In test solutions				In distilled water						
	1	2	3	4	5	6	7	8	9	0.05	0.01
Leaves											
C. maritima	33	35	35	35	41	53	56	57	58	18	25
R. graveolens	2	3	3	3	3	4	5	10	12	4	6
T. erecta	6	35	35	42	62	78	81	83	84	23	31
Roots											
C. maritima	32	33	33	33	38	51	53	54	54	22	29
R. graveolens	39	43	43	45	56	60	60	60	61	21	28
T. erecta	42	71	73	76	83	83	83	83	83	10	13
Roots and leaves											
C. maritima	39	41	41	41	50	67	71	72	73	14	18
R. graveolens	10	15	15	16	23	41	43	47	49	17	23
T. erecta	20	42	42	43	62	71	73	74	74	13	17
Root leachates											
C. maritima	39	76	79	80	80	80	80	80	80	5	7
R. graveolens	33	72	79	80	80	80	80	80	80	6	8
T. erecta	34	76	80	80	80	80	80	80	80	9	13
Fenamiphos (5 µg/ml)	6	6	7	7	7	9	10	21	29	8	11
Distill. water	42	69	74	74	75	75	75	75	75	12	16
L S D 0.05	11	15	15	14	14	14	14	15	15	–	–
0.01	15	20	20	19	19	19	19	20	20	–	–

assumed that are the same as those possessing insecticidal activity, such as the rutin, a flavonoid, the xanthotoxin, a coumarine (Holyoke and Reese, 1987) and two alkaloids, kokusaginine and skimmianine (Grainge and Ahmed, 1988).

Of the three plant species tested, *R. graveolens* was the most effective; it had high nematicidal activity against the four *Meloidogyne* species, better than fenamiphos. This is the first record of the nematicidal action of *R. graveolens* on *Meloidogyne* spp. However, further investigations are required both under laboratory and field conditions to identify the nematicidal compounds, and to ascertain whether this plant can be effectively used as a green manure for the control of nematodes.

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TABLE IV - *Effect of root and leaf extracts and root diffusates of* Cineraria maritima, Ruta graveolens *and* Tagetes erecta *on the per cent cumulative hatch of* Meloidogyne javanica.

Treatment (aqueous extracts)	Incubation periods (weeks)									L S D	
	In test solutions				In distilled water						
	1	2	3	4	5	6	7	8	9	0.05	0.01
Leaves											
C. maritima	23	55	56	58	61	63	64	64	64	19	25
R. graveolens	3	3	3	3	3	3	3	3	3	2	3
T. erecta	4	28	30	32	35	39	41	41	41	28	38
Roots											
C. maritima	6	11	12	12	12	12	12	12	13	6	8
R. graveolens	19	21	22	22	22	23	23	23	23	9	12
T. erecta	13	17	23	24	61	72	73	73	73	8	11
Roots and leaves											
C. maritima	7	13	13	14	15	16	17	17	17	16	21
R. graveolens	9	10	10	11	12	18	21	21	21	14	19
T. erecta	2	16	19	22	35	44	45	45	45	23	31
Root leachates											
C. maritima	41	74	87	89	89	92	92	92	92	7	9
R. graveolens	42	69	74	84	87	88	89	89	89	10	14
T. erecta	39	61	82	92	92	93	93	93	93	5	7
Fenamiphos (5 µg/ml)	26	28	29	29	29	30	31	31	31	4	5
Distill. water	38	58	79	87	91	92	92	92	92	5	7
L S D 0.05	7	12	13	13	14	14	15	15	15	–	–
0.01	10	16	17	17	19	19	20	20	20	–	–

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