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EFFECT OF AQUEOUS SOLUTIONS OF RUTIN ON THE BEET CYST NEMATODE *HETERODERA SCHACHTII*

by
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Summary. Three *in vitro* experiments were undertaken to test the nematicidal activity of rutin on the beet cyst nematode *Heterodera schachtii*. Cysts were immersed in aqueous solutions of rutin at different concentrations (0.02, 0.04, 0.08 and 0.16 mM) during the first four weeks of the hatching test, throughout the hatching test, or immersed in the above solutions for 24, 48, 96, 192, 384, 768 and 1536 hours and then subjected to hatching test. Results did not indicate any nematicidal or nematostatic activity.

Active principles with nematicidal or nematostatic properties are present in many plant species (Gommers, 1981; Grainge and Ahmed, 1988). Leaf aqueous extracts of *Ruta graveolens* have shown greater nematicidal effect than fenamiphos, in particular against *Meloidogyne* spp. (Sasanelli and D'Addabbo, 1993) and the beet cyst nematode, *Heterodera schachtii* (Sasanelli and D'Addabbo, 1992). Chemicals responsible for this nematicidal activity have not yet been identified, although they were supposed to be the same as those possessing insecticidal activity, such as rutin, a flavonoid, xanthotoxin, a coumarine (Holyoke and Reese, 1987) or two alkaloids, kokusaginine and skimmianine (Grainge and Ahmed, 1988).

The present investigation examined the *in vitro* effect of different dilutions of aqueous solutions of rutin on the beet cyst nematode *Heterodera schachtii* Schmidt.

Materials and methods

Soil was collected from a field infested with *H. schachtii* at Avezzano (L'Aquila) and cysts were extracted with the Fenwick can. Batches of 100 cysts of similar size (about 85 eggs and juveniles/cyst) were placed in 2 cm diam sieves (215 µm aperture) arranged in 3.5 cm diam Petri dishes (Greco *et al.*, 1982). They were then subjected to various treatments (see below) and arranged in a complete randomized block design with three replicates for each treatment in a growth cabinet at 24 ± 2 °C.

In the first experiment, 0.04, 0.08, 0.16 and 0.32 mM aqueous solutions of rutin were prepared and then adjusted to 3 mM zinc chloride adding an equal volume of 6 mM of this solution (Clarke and Shepherd, 1966; Greet, 1974) to obtain the four different final concentrations (0.02, 0.04, 0.08 and 0.16 mM) used for the hatching test. Three ml of each test solution, sufficient to cover the cysts, were then added to the batches of cysts. After four weeks the cysts were removed from the rutin solution and the incubation continued for a further 10 weeks in an artificial hatching agent. Cysts in a 3 mM zinc chloride aqueous solution were used as control. Emerged juveniles were removed and counted and solutions were renewed at weekly intervals.

The second experiment was similar to the

first but cysts were maintained in the rutin solutions until the end of the incubation period.

In a third experiment cysts of *H. schachtii* were immersed in the test solutions for 24, 48, 96, 192, 384, 768 or 1536 hours. Cysts in distilled water were the control. At the end of each immersion period cysts were subjected to the hatching test over 12 weeks. Again emerged juveniles were removed and counted and the hatching agent was renewed every week.

At the end of the three experiments cysts were crushed, according to Seinhorst and Den Ouden (1966), and unhatched eggs were counted. The numbers of second stage juveniles emerging weekly were expressed as cumulative percentages of the total egg content of the cysts. Data were statitically analyzed and means compared by Duncan's multiple range test.

Results and discussion

In the first experiment final cumulative egg hatch of control cysts was significantly lower than that of cysts in 0.16 mM rutin solution, although this difference became significant (P = 0.01) only from the twelfth week (Table I). About half of the juveniles had emerged by the first week of incubation and no significant hatch increase was observed after removing the rutin. Emergence in the 0.04 mM rutin solution remained constantly lower (P = 0.01) than that in the 0.16 mM solution, although it could be due to experimental error, while there was no statistical differences among the other concentrations throughout the hatching test.

Maintaining cysts continuously in the rutin solutions (experiment 2), did not reduce final hatch percentage, but emergence of juveniles from cysts seems to have been stimulated significantly (P = 0.01) in 0.02 mM rutin compared to the control (Table II). In this experiment also at least half of the juveniles emerged during the first week of incubation.

In the third experiment immersion of cysts in rutin solutions for different times resulted in significantly (P = 0.01) fewer juveniles emerging from cysts in 0.16 mM rutin, only for 24 and 96 hrs exposures, and for cysts in 0.02 mM rutin for 96 hrs (Table III).

Results obtained with the three experiments were not clearly explainable. From the first experiment it appears that juvenile emergence could have been stimulated by the higher concentrations of rutin. Conversely, in the second experiment emergence of juveniles seems to have been stimulated only in 0.02 mM rutin. The third experiment showed that emergence

TABLE I - *Weekly cumulative percentages of juveniles emerged from cysts of* Heterodera schachtii *immersed in rutin solutions at different concentrations for the first four weeks of the incubation period.*

Concentration (mM)	Weeks													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
0.02	25.3 AB	28.1 AB	29.6 AB	31.8 AB	32.6 AB	33.3 AB	35.2 A	36.6 A	38.6 A	40.4 A	41.7 A	48.3 AB	51.7 AB	58.1 AB
0.04	20.7 A	23.5 A	24.8 A	27.8 A	28.8 A	29.9 A	31.7 A	32.5 A	33.8 A	35.7 A	36.7 A	41.3 A	45.1 A	49.5 A
0.08	27.3 AB	30.0 AB	31.6 AB	34.5 AB	36.1 AB	37.0 AB	39.3 AB	40.2 AB	41.8 AB	43.9 AB	44.9 AB	47.8 A	51.0 A	53.8 A
0.16	36.3 B	39.8 B	41.6 B	43.9 B	44.8 B	45.4 B	48.8 B	50.3 B	55.3 B	55.4 B	56.7 B	63.4 B	67.0 B	69.0 B
Control	25.4 AB	29.8 AB	31.6 AB	34.3 AB	36.0 AB	36.9 AB	38.8 AB	40.0 AB	41.7 AB	44.0 AB	44.9 AB	47.8 A	51.2 AB	53.8 A

Data followed by the same letters on the same column are not statistically (P = 0.01) different according to Duncan's multiple range test.

TABLE II - *Weekly cumulative percentages of juveniles emerged from cysts of* H. schachtii *immersed in rutin solutions at different concentrations during all the incubation period.*

Concentration (mM)	Weeks													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
0.02	43.7 B	47.1 B	48.4 B	50.5 B	51.2 B	52.0 B	52.6 B	53.8 B	54.5 B	55.7 B	58.1 B	59.9 B	63.7 B	65.2 B
0.04	31.7 AB	34.2 AB	34.9 AB	36.5 AB	37.1 AB	38.5 AB	40.0 AB	42.5 AB	43.8 AB	45.4 AB	48.3 AB	50.7 AB	52.6 AB	55.7 A
0.08	25.6 AB	28.4 A	30.3 A	33.2 A	34.8 A	36.1 A	37.2 A	38.8 A	39.6 A	40.0 A	43.1 A	45.9 A	47.6 A	49.0 A
0.16	26.6 AB	30.3 AB	32.4 AB	35.7 AB	38.1 AB	39.6 AB	41.6 AB	43.8 AB	44.7 AB	45.5 AB	48.0 AB	50.5 AB	54.2 AB	55.2 AB
Control	21.8 A	24.4 A	26.5 A	28.7 A	30.1 A	30.7 A	31.4 A	32.5 A	35.6 A	38.4 A	40.2 AB	44.7 A	49.6 A	52.4 A

Data followed by the same letters on the same column are not statistically (P = 0.01) different according to Duncan's multiple range test.

TABLE III - *Final cumulative hatch from cysts of* H. schachtii, *previously immersed in rutin solutions at different concentrations for different times, over a twelve weeks incubation period.*

Concentration (mM)	Exposure times (hours)						
	24	48	96	192	384	768	1536
0.02	49.0 AB	46.7 A	34.8 A	48.5 B	73.9 A	64.7 A	79.5 A
0.04	48.1 AB	49.3 A	42.9 AB	43.2 AB	71.1 A	58.1 A	79.5 A
0.08	51.1 AB	50.7 A	48.9 B	34.5 AB	70.5 A	60.9 A	85.0 A
0.16	43.9 A	44.4 A	32.7 A	33.7 A	69.3 A	56.4 A	84.9 A
Control	58.4 B	51.0 A	51.5 B	43.0 AB	64.4 A	58.2 A	82.7 A

Data followed by the same letters on the same column are not statistically (P = 0.01) different according to Duncan's multiple range test.

of cysts in rutin solutions for as long as 1536 hours did not suppress hatching.

These experiments clearly demonstrate that rutin has no nematicidal or nematostatic activity. This suggests that the nematicidal activity of *R. graveolens* aqueous extracts (Sasanelli and D'Addabbo, 1992; 1993) should be attributed to the other active principles present in the plant (Holyoke and Reese, 1987; Grainge and Ahmed, 1988). Further experiments are needed to ascertain which of the active principles possess nematicidal activity.

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Literature cited

CLARKE A. J. and SHEPHERD A. M., 1966. Inorganic ions and hatching of *Heterodera* spp.. *Ann. appl. Biol., 58*: 497-508.

GOMMERS F. J., 1981. Biochemical interaction between nematodes and plants and their relevance to control. *Helminthol. Abst. Series B, Plant Nematology, 50*: 9-24.

GRAINGE M. and AHMED S., 1988. *Handbook of Plants with Pest-Control Properties*. J. Wiley and Sons, New York, pp. 238-248.

GRECO N., BRANDONISIO A. and DE MARINIS G., 1982. Investigations on the biology of *Heterodera schachtii* in Italy. *Nematol. medit., 10*: 201-214.

GREET D. N., 1974. The response of five round cyst-nematodes (Heteroderidae) to five artificial hatching agents. *Nematologica, 20*: 363-364.

HOLYOKE C. W. and REESE J. C., 1987. Acute insect toxi-

cants from plants. *In* "CRC Handbook of Natural Pesticides". Vol. III. Insect Growth Regulators. Part B. (E. D. Morgan and N. B. Mandava Eds.), CRC Press, Boca Raton, Florida, pp. 67-118.

SASANELLI N. and D'ADDABBO T., 1992. The effects of *Cineraria maritima*, *Ruta graveolens* and *Tagetes erecta* extracts on the hatching of *Heterodera schachtii*. *Nematol. medit.*, *20*: 49-51.

SASANELLI N. and D'ADDABBO T., 1993. Effect of *Cineraria maritima*, *Ruta graveolens* and *Tagetes erecta* leaf and root extracts on Italian populations of *Meloidogyne* species. *Nematol. medit.*, *21*: 21-25.

SEINHORST J. W. and DEN OUDEN H., 1966. An improvement of Bijloo's method for determining the egg content of *Heterodera* cysts. *Nematologica*, *12*: 170-171.

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