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NEMATICIDAL ACTIVITY OF WASTE WATER FROM OLIVE OIL-MILLS

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Summary. The effects of waste water from olive oil mills on root-knot nematode (*Meloidogyne spp.*) development on tomato roots and its ethyl acetate-acetone extract effect on egg hatching of *Meloidogyne incognita* were investigated in pot and laboratory experiments, respectively. Separation of phenols was done in the HPLC and individual phenols were tested for their effect on nematodes in the soil. Galling and egg masses on tomato roots were reduced significantly by addition of waste water to the soil. Egg hatch was also significantly reduced by the ethyl acetate-acetone extract at the two concentrations tested. Some of the phenols reduced galling and egg masses on tomato roots when added to the soil.

Root galling induced by *Meloidogyne incognita* on tomato was reduced by soil mulching with flax, lucerne and orchard grass residue (Johnson, 1972). Some organic compounds e.g. oilcakes of castor bean (*Ricinus communis*) and neem (*Azadirachta indica*) have been tested for root-knot nematodes control (Sitamaraiah and Singh, 1978; Rodriguez-Kabana *et al.*, 1987). Leaves and water extract of *A. indica* are nematicidal (Egunjobi and Afolami, 1976; Gupta and Gupta, 1980). Residues from the flax and sugar cane industries were effective against root-knot nematode and *Xiphinema index* infecting tomato and grapevine (Sikora *et al.*, 1973; Choleva and Tzvetkov, 1996).

Waste – products from the olive - oil industry are produced in large quantities in all olive – growing countries. Root galling of tomato induced by *M. arenaria* was suppressed when fresh olive pomace was incorporated into the soil (Rodriguez-Kabana *et al.*, 1992; Rodriguez-Kabana *et al.*, 1995). Cosiderablerable quantities of waste water are also produced which is the subject of the present study to assess its nematicidal effect against nematodes (*Meloidogyne spp.*).

Material and methods

Egg masses of a mixed population of root-knot nematodes, *Meloidogyne incognita* (Kofoid *et* White) Chitw. and *M. javanica* (Treub) Chitw., were collected from heavily galled roots of tomato (*Lycopersicon esculentum* Mill.) and transferred to a 100 µm-pore sieve. The sieve was placed in small dish and water added so that it just touched the mesh before putting in an incubator at 25 °C. Second stage juveniles (J_2s) were collected every other day and kept at 10-15 °C. These nematodes (2-7 days old) were used to inoculate tomato plants.

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Waste water was collected from a commercial olive oil mill. The ethyl acetate-acetone extract of olive oil mill waste water, total phenols and separation of polyphenols with High Pressure Liquid Chromatography (HPLC) were conducted according to Servili and Montedoro (1989) and Montedoro *et al.* (1992).

The effect of oil mill waste water on root-knot nematodes on tomato plants was studied by mixing 1000 second-stage juveniles (J_2) of *Meloidogyne* mixed population (*javanica/incognita*) with 100 ml of tap water or waste water and applying to premoistened soil. After five days a 30-day old tomato plant was transplanted to each pot. Treatments were replicated six times in pots filled with 2 l peatsoil. Plants were maintained in a glasshouse with the temperature fluctuating from 14 to 34 °C. They were watered as needed and fertilized with a 20-20-20 fertilizer every 15 days.

After a 60 day growing period the tomato plants were uprooted, the roots washed free of soil and the number of galls, eggmasses and fresh plant weight were recorded. The number of egg masses was estimated under a dissecting microscope after staining in Phloxine.

Eggs of *M. incognita* were collected from infected tomato roots with NaOCl as described by Hussey and Barker (1973). Roots were chopped into 1-2 cm pieces and vigorously shaken in 1% sodium hypochlorite (NaOCl) for 4 minutes in a bottle. The mixture was passed quickly through 250 µm-pore sieve over a 38 µm-pore sieve. Eggs collected on the 38 µm-pore sieve were rinsed with tap water to remove excess NaOCl.

The effect of the ethyl acetate-acetone extract on hatching was tested by incubating, at 25 °C, 100 eggs in 0, 50 and 100 ppm of the extract. Each treatment was replicated three times. Juveniles were counted after 1, 3, 6, 9 and 13 days. The zero control was distilled water.

Thirty-day-old tomato plants, were transplanted into pots two days after inoculation with 3000 *M. javanica* eggs and moistening-with 30 ml of 15 ppm each of the following phenols: 1) vanillic acid, 2) cafeic acid, 3) tyrosol, 4) OH-benzoic acid, 5) O-coumaric acid, 6) syringic acid, and 7) P-coumaric acid. Control plants received distilled water. The plants were maintained in a glasshouse at a mean temperature of 24 °C for 75 days and were watered and fertilized as previously described. Treatments were replicated five times and arranged in completely randomized design. Plants were harvested after 75 days, fresh plant weights and numer of galls and eggmasses in each plant were recorded.

Results and discussion

Total polyphenols in the olive oil mill waste water were measured colorimetrically at 727 mm were 3,430 ppm.

Separation of polyphenols in the HPLC gave the following phenols in the extract, with ppm in parenthesis: OH-tyrosol (296.3), tyrosol (56.4), vanillic acid (24.0), cafeic acid (43.8), P-coumaric (19.3), O-coumaric acid (11.1), syringic acid (24.2), feluric acid (19,5), and OH-benzoic acid (25.5).

The waste water treatment significantly reduced galling and egg masses on plant roots in pots inoculated with *M. incognita/javanica* (Table I). These results indicate that there are nematicidal substances in the olive oil mill waste water.

In our experiment, application of phenols to the soil, significantly reduced the number of galls and egg masses, except P-coumaic acid (Table II). Polyphenols from other plant residues have also been found to be toxic to nematodes (Sitamaraiah and Singh, 1978).

The extract of olive oil mill waste water inhibited hatching of *M. incognita* eggs, and it was similar at 50 and 100 ppm (Fig. 1). A reduction in hatching and penetration of *Meloidogyne* of tomato roots of certain plant materials have been reported by Johnson and Shamiyeh (1976) and Prot and Kornprobst (1985); hatch-

TABLE I - *Number of galls, egg masses and fresh plant weight of tomato 80 days after inoculation with 1000 2nd stage juveniles of* Meloidogyne incognita/javanica.

Treatment	Galls/ plant	Egg masses/ plant	Plant weight g
Water only	150 (2.17)	85 (1.93)	160
Oil-mill wastewater	5 (0.72)	5 (0.72)	161
LSD	0.165	0.165	NS

Numbers in parenthesis are Log_{10}.

TABLE II - *Effect of phenols on plant weight and* M. javanica *galls and egg masses on tomato cv. Carouso.*

Treatment	Fresh plant weight, g	Galls/ groot	Egg masses/ g root
Vanillic acid	182	51 (1.67)	33 (1.48)
Cafeic acid	204	34 (1.51)	21 (1.28)
Thyrosol	246	33 (1.50)	24 (1.33)
OH-benzoic acid	177	48 (1.63)	39 (1.55)
O-Coumaric acid	206	43 (1.61)	31 (1.44)
Syringic acid	218	46 (1.65)	36 (1.53)
P-Coumaric acid	186	67 (1.81)	46 (1.65)
Water	205	103 (1.99)	76 (1.87)
LSD (P«0.05)	84	0.21	0.23

Numbers in parenthesis are Log_{10}.

ing of *Meloidogyne incognita* eggs was significantly inhibited by the extract and was reduced to 20% compared to 80% hatching in water (Fig. 1). Extracts from olive leaves also have been found to reduce egg hatching of *Meloidogyne* (Vouyoukalou, 1994). In the chromatogram there are additional phenols that we did not test, as well as unidentified substances that may make the extract more effective than the phenols which were tested, or their action in mixture is different (Blum, 1996).

The increase in nematotoxicity of different extracts might be due either to the release of

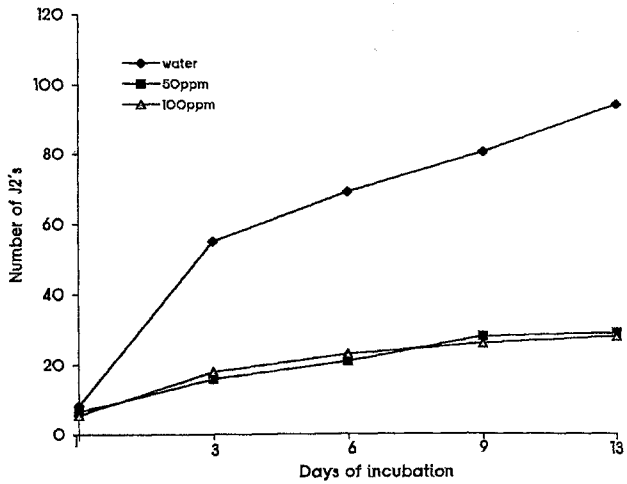

Fig. 1 - Number of *Meloidogyne incognita* second stage juveniles (J2) hatched from 100 eggs treated with olive oil-mill waste water extract at 50 ppm and 100 ppm and water at 25 °C.

toxic chemicals or to the accumulation of toxic metabolites of microorganisms which become active during decomposition. Olive pomace when incorporated into the soil increases microbial activity (Rodriguez-Kabana *et al.*, 1995).

Results obtained in pot experiments provide evidence of the nematicidal properties of oil mill waste water and confirm previous findings on the nematicidal properties of oil mill waste products (Rodriguez-Kabana *et al.*, 1995). Phenols are nematicidal constituents of oil mill waste water.

Fresh plant weight was not affected (P=0.05) by the addition of the waste water from olive oil mill compared with the control (Table I). Also, application of phenols in the soil did not affect plant fresh weight (Table II). Nematode control was obtained without phytotoxicity at the concentrations used in our experiment and this nematicidal action should be taken into consideration in recycling this product into the agricultural environment.

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