EFFECT OF OXAMYL AND FENAMIPHOS ON EGG HATCHING, MOTILITY, AND ROOT PENETRATION OF *TYLENCHULUS SEMIPENETRANS*

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Summary. The effect of oxamyl and fenamiphos on egg hatching, motility and penetration of juveniles of *Tylenchulus semipenetrans* into roots of sour orange (*Citrus aurantium*) rootstock were investigated under controlled conditions in a growth chamber. Both chemicals were used at 10, 50, 100, and 500 µg a.i/ml of water concentrations. Egg hatching between 0.0-5.7 % was obtained when eggs were exposed to concentrations exceeding 100 µg a.i/ml water of oxamyl or fenamiphos for seven or fourteen days, compared to 22.1 and 36.1% in the controls, respectively. However, maintaining the eggs for two weeks in distilled water following the seven- or fourteen-day exposure to all tested concentrations of both pesticides, significantly increased egg hatching, suggesting that temporary inhibition occurred. Similarly, oxamyl and fenamiphos at 100 µg a.i/ml of water reduced (P = 0.05) juvenile motility to 55.3 and 18.8%, respectively, compared with 89.5% in the control. Also, root penetration rate was reduced (P = 0.05) to 3.4 and 2.5% when juveniles were exposed to oxamyl or fenamiphos at 100 µg a.i/ml of water, respectively, compared with 9.4% in the control.

Oxamyl and fenamiphos are ambimobile systemic nematicides (Wright, 1981). When sprayed on the foliage, they appear in root diffusates, whereby they inhibit feeding, temporarily inactivate, repel or kill nematodes in the rhizosphere (Potter and Marks, 1976; Dropkin, 1988; Hsu *et al.*, 1995). When fenamiphos is applied to the soil, it is absorbed by the plant root and kills both ectoparasitic and endoparasitic nematodes (Hafez *et al.*, 1981). It has also been suggested that foliar spray of oxamyl (4.8 g a.i/l water), oxamyl spray plus soil treatment with fenamiphos (11.2 kg a.i/ha), induces narcosis in nematodes that causes slow death or reduced reproduction rather than immediate kill (Atilano and Van Gundy, 1979).

Pree *et al.* (1989) suggested that reduced movement rather than direct mortality may be an important part of the control obtained from fenamiphos treatment. Oxamyl and fenamiphos prevent egg hatching, and root penetration, and hinder development or reproduction after penetration (Miller, 1970; Radewald *et al.*, 1970).

Aqueous solutions of oxamyl and fenamiphos at concentrations up to 1,000 µg a.i/ml inhibited egg hatching of *Heterodera schachtii* Schmidt but permanent suppression of hatching was only obtained with fenamiphos 1000 µg a.i/ml (Steele, 1976). It was found that juveniles of the citrus nematode showed slight movement after ten days in oxamyl 10 µg a.i/ml; whereas 98-100% of those in 50 or 100 µg a.i/ml were dead after ten days (Baines and Small, 1976).

The objective of the research reported here was to ascertain the effect of oxamyl (carbamate) and fenamiphos (organophosphate) on egg hatching, juvenile motility and penetration of the Mediterranean race (local race) of *Tylenchulus semipenetrans* Cobb into the roots of sour orange (*Citrus aurantium* L.) which is a widespread rootstock in Jordan and many other countries of the Middle East.

MATERIALS AND METHODS

Aqueous solutions of oxamyl (methyl carbamoyl oxyimino-1-methylthio-N,N-dimethyl acetamide) and fenamiphos [ethyl 3-methyl- 4-(methylthio) phenyl isopropyl phosphoramidate] were prepared from liquid formulations of the commercial products Vydate 24% and Nemacur 40% EC. Appropriate amounts of oxamyl or fenamiphos were added to the nematode suspension in distilled water containing 1,000 eggs in test tubes to make a total volume of 10 ml with final concentrations of 10, 50, 100, and 500 µg a.i/ml of water. Eggs in distilled water only served as control. Test tubes were incubated for seven to 28 days in a growth chamber maintained at 27±1 °C. The chemical solutions were removed daily and replaced with the respective freshly prepared solutions to avoid aeration problems and degradation of the original chemical (Huang and Van Gundy, 1978).

Treatments were organized in a factorial arrangement, rate x time schedule (A x B) within a completely randomized design of four replicates, where the factors (A) and (B) were in nine and four levels, respectively (Table I).

Each concentration of the tested chemicals was placed in sixteen tubes. Tubes of each treatment were divided into two equal batches and each batch was further subdivided into two groups, four tubes each. After one week, one group in the first batch (seven days in

Table I. Effect of different oxamyl and fenamiphos treatments on egg hatching (%) of *Tylenchulus semipenetrans.*

Treatment (µg a.i/ml water)	Seven days in chemical solution	Seven days in chemical solution and fourteen days in distilled water	Fourteen days in chemical solution	Fourteen days in chemical solution and fourteen days in distilled water
Control	22.1 A[2] d[3]	42.1 A b	36.1 A c	49.7 A a
Oxam. (10)	8.9 B b	17.4 B a	11.9 B b	20.4 B a
Oxam. (50)	5.6 BC c	10.6 CD ab	7.1 C bc	3.0 CD a
Oxam. (100)	2.3 CD b	7.5 DEF a	2.6 DE b	0.2 DE a
Oxam. (500)	0.0 D b	4.9 F a	1.1 E b	6.1 F a
Fenam (10)	6.1 BC b	14.0 BC a	9.5 BC b	15.1 C a
Fenam (50)	5.5 BC b	10.7 DE a	6.3 CD b	12.0 CD a
Fenam (100)	3.8 CD c	8.0 DEF ab	5.7 CD bc	11.3 CD a
Fenam (500)	2.6 CD b	6.1 F a	3.1 DE b	7.3 EF a

[1] Approximately 1,000 eggs per test tube were initially used in the different treatments.
[2] Different capital letters in the same column indicate significant difference in Duncan's Multiple Range Test at (P = 0.05).
[3] Different small letters in the same row indicate significant difference in Duncan's Multiple Range Test at (P = 0.05).

chemical suspension) was fixed in warm 5% formaldehyde, and the numbers of eggs and hatched juveniles were counted. Eggs and hatched juveniles in the tubes of the other group were collected on a 500-mesh sieve and re-suspended in distilled water for a further fourteen days period, with daily changes in distilled water, and then examined for hatched juveniles. The other two groups of the second batch were similarly treated, but after two weeks in chemical solution.

The effect of different concentrations of oxamyl and fenamiphos on the motility of juveniles of *T. semipenetrans* was studied in a growth chamber following the Moje technique (Moje, 1959). Suspensions of approximately 1,000 newly hatched juveniles in 5 ml distilled water and chemical solutions of oxamyl or fenamiphos were mixed in the bottles to have a total volume of 10 ml with final concentrations of 10, 50, 100 and 500 µg a.i/ml of water. Juveniles exposed to 10 ml distilled water served as control.

The exposure period of all chemical treatments in the bottles was 24 hours at 27±1 °C. The mouth of the bottle was tightly covered with two-layers of tissue paper and then the bottle was turned upside down in 8 cm diameter Petri dishes containing 12 ml of the same test chemical solution, for another 24 hours. Total exposure to chemicals was 48 hours. Bottles were arranged in a completely randomized design, with four replicates in each of the nine treatments. Motility was expressed as percentage of active juveniles migrated into each Petri dish divided by the total number of juveniles exposed to the test chemical.

The effect of oxamyl and fenamiphos on the infectivity of juveniles of *T. semipenetrans* was determined following Moje's (1959) technique. Approximately 1,000 newly hatched juveniles and chemical solutions of oxamyl or fenamiphos were mixed in the 20 ml-bottles to have a total volume of 10 ml with final concentrations of 10, 50, 100, and 500 µg a.i/ml of water. Juveniles exposed to 10 ml distilled water only served as control. The period of exposure to the treatments was 24 hours at growth chamber temperature (27±1 °C). The treated juveniles were introduced around the roots of three-month-old sour orange seedlings grown in pots containing sterilized soil (35% sand, 10% silt, 55% clay; pH 7.5; organic matter 2.05%) and perlite 2:1 v/v. The plants were maintained for 70 days in the growth room before harvesting (Van Gundy *et al.,* 1967). Treatments were arranged in a completely randomized design with four replicates. Adult females were extracted from the roots (Duncan, 1986; Greco *et al.,* 1993) and the data were expressed as the percentage of females developing from the original number of juveniles.

RESULTS

The factorial analysis showed a highly significant (AB) interaction.

Egg hatching was significantly inhibited in all treatments, as compared with the untreated control (Table I). There was a decrease in egg hatching with every increase in the concentration of oxamyl or fenamiphos. Effective inhibition of egg hatching (between 94.3 and 100%) was obtained when the eggs were exposed to the high rates (100 and 500 µg a.i/ml water) of oxamyl and fenamiphos for one or two weeks with no significant differences between the two exposure periods. However, oxamyl tended to be more inhibitive than fenamiphos (Table I). Hatching of eggs transferred to distilled water following the seven- or fourteen-day nematicide treatments, was significantly improved. Egg hatching increased significantly with every increase of the exposure time in dis-

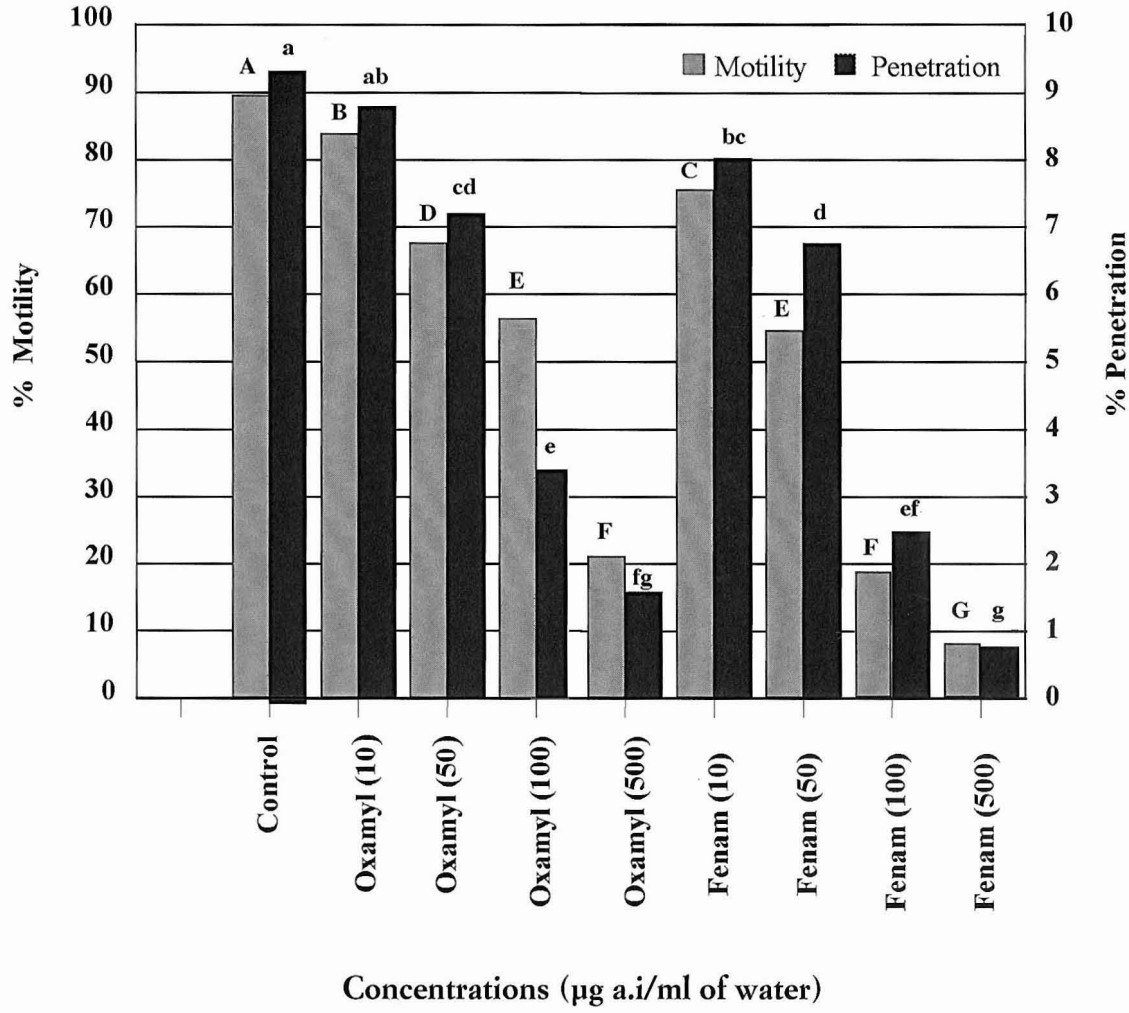

Fig. 1. Motility and root penetration of juveniles of *Tylenchulus semipenetrans* exposed to different concentrations of oxamyl and fenamiphos.

tilled water (control), and reached 49.7% when the eggs were held for four weeks in fresh water.

Results in Fig. 1 indicate that motility of juveniles was significantly reduced in all treatments, as compared with the control. Among all treatments, fenamiphos 500 µg a.i/ml of water most effectively reduced the juvenile motility to 8.2%, followed by fenamiphos 100 (18.8%) and oxamyl 500 µg a.i/ml of water (21.0%), compared to 89.5% motility in the untreated control. Percent motility of fenamiphos 50 and oxamyl 100 mg a.i/ml was reduced to nearly 55%.

Root penetration was significantly reduced in all treatments, except for oxamyl 10 µg a.i/ml of water, as compared with the control (Fig. 1). Also, significantly less penetration was detected with every increase in the concentration of each chemical. Among all treatments fenamiphos and oxamyl at (500 µg a.i/ml) most effectively reduced juvenile penetration into roots, but with no significant differences observed between them (Fig. 1). This high effectiveness was followed by fenamiphos and oxamyl at the rate of 100 µg a.i/ml of water that reduced penetration by 2.5 and 3.4 %, respectively.

DISCUSSION

Egg hatching was significantly higher in the control than in the nematicide-treated eggs. This was a clear indication that both nematicides inhibited egg hatching of *T. semipenetrans*. Higher concentrations were more inhibitive than lower ones, also there were no significant differences between the inhibitive effects of oxamyl and fenamiphos at the same concentrations.

The results showed that 49.7% of *T. semipenetrans* eggs hatched during 28 days in distilled water. Similar results were obtained by Huang and Van Gundy (1978) who found that 48% of the eggs hatched in eighteen days in distilled water. Also, it was found that holding the eggs in distilled water following the seven- or fourteen-day exposure to all tested concentrations of oxamyl or fenamiphos, significantly increased egg hatching, suggesting that temporary inhibition has occurred.

It has been suggested that oxamyl acts by preventing egg hatching (Miller, 1970; Radewald *et al.*, 1970). Steele (1976) found that aqueous solutions of oxamyl and fenamiphos at concentrations up to 1,000 µg a.i/ml

inhibited egg hatching of *H. schachtii*, while permanent suppression of egg hatching was obtained only with fenamiphos at 1,000 µg a.i/ml. The non-significant differences between exposure to the nematicides for one or two weeks, implies that inhibition had reached its maximum level at that concentration within one week's duration. This experiment illustrates the importance of avoiding irrigation after nematicide application to the soil, possibly for one week, since water will reduce the effect of the nematicide and enhance egg hatching.

Likewise, juvenile motility was reduced with every increase in the concentration of both oxamyl and fenamiphos, but the latter proved to be more effective than the former.

The impairment of nematode movement by nematicides was used as a criterion in the *in vitro* tests for assessing their nematicidal activity (McBeth and Bergeson, 1953; Taylor *et al.,* 1957; Marban-Mendoza and Viglierchio, 1980; Huang *et al.,* 1983). It was found that juveniles of the citrus nematode showed slight movement after ten days in oxamyl 10 µg a.i/ml, and became active when placed in fresh water for four hours; whereas 98-100% of those in 50 or 100 µg a.i/ml were dead (Baines and Small, 1976).

The present results showed that 9.4% of the inoculum (juveniles) initiated root infection of sour orange seedlings and developed into adults, while fenamiphos and oxamyl (500 µg a.i/ml), allowed only 0.8-1.6 % penetration in fibrous roots. The study showed that high concentrations of both oxamyl and fenamiphos that effectively reduced juvenile motility also reduced root penetration (Fig. 1). Also, there were no significant differences between oxamyl and fenamiphos at the same concentrations.

The highest percentage of infection observed on sour orange roots was near 10% (O'Bannon, 1968). Also, it was suggested by Wilcox (1995), that less than 10% of the young juveniles of *T. semipenetrans* will survive to infest the host. It was also reported by Miller (1970) and Radewald *et al.* (1970) that oxamyl acts by preventing root penetration, hindering development or reproduction after penetration. Van Gundy *et al.* (1967) indicated a close relationship between juvenile motility and infectivity of *T. semipenetrans*.

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