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# EFFECTS OF TREATED MUNICIPAL WASTE WATER ON *MELOIDOGYNE JAVANICA* EGG HATCH AND PENETRATION[1]

by

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**Summary**. Eggs of *Meloidogyne javanica* were exposed *in vitro* to increasing concentrations of treated municipal waste water (TMWW) over a period of 8 days. Hatched juveniles ($J_2$) were extracted and counted every 2 days. Juveniles that hatched from day 6 to day 8 were used as inoculum to determine their capability to invade tomato roots under greenhouse conditions. TMWW suppressed egg hatch at all concentrations, at least 4 days after incubation. After day 4 the hatch suppression was proportional to TMWW concentrations, or incubation period. Hatch suppressions (% of control) at day 8 were 11.3, 27.8, 34.4 and 51% at concentrations of 25, 50, 75, and 100% TMWW, respectively. Invasion of tomato roots by the hatched juveniles was not adversely affected.

In Saudi Arabia, treated municipal waste water (TMWW) has become widely used in some areas as an additional source of irrigation water on certain crops. Analysis of the chemical quality of TMWW from Riyadh Treatment Plant indicated that such water is suitable for irrigation and could be considered as a source of plant nutrients (El-Mashhady and El-Nennah, 1982).

Research on the effects of TMWW on plant-parasitic nematodes has been limited and results have been inconsistent. Some studies indicated that irrigation with TMWW increased nematode populations (Yeates, 1978; Al-Yahya *et al.*, 1988a) or enhanced their penetration into roots and development (Al-Hazmi *et al.*, 1988a). Other work showed that TMWW had no effect on nematode populations (Al-Hazmi *et al.*, 1991 and 1992), or that egg hatch was suppressed (Lal and Yadav, 1976; Al-Yahya *et al.*, 1988b). TMWW-related materials such as raw and composted sewage sludges are sometimes used as soil conditioners and fertilizers, but their average chemical and biochemical compositions differ from those of TMWW (Habicht, 1975; El-Mashhady and El-Nennah, 1982; Al- Yahya *et al.*, 1988a; Al- Hazmi *et al.*, 1991; Castagnone-Sereno and Kermarrec, 1991). Sewage sludge has been found to suppress nematode infectivity and reproduction (Hunt *et al.*, 1973; Habicht, 1975; Lal *et al.*, 1977; Klinger and Kunz, 1986; Castagnone-Sereno and Kermarrec, 1991), even though opposite effects (Klinger and Kunz, 1986), or no significant effects (Hunt *et al.*, 1973) also were reported.

The variability of these results could be attributed to one or a combination of several factors such as: 1) chemical and biochemical composition of the material used, 2) modification of the rhizosphere environment, 3) method of application, 4) exposure period, 5) nematode species, and 6) life stage of test nematodes.

The objectives of the present study were to determine the effects of TMWW, at increasing concentrations, on egg hatch (*in vitro*) of *Meloidogyne javanica* (Treub) Chitw. and to determine the root penetration by the hatched juveniles under greenhouse conditions.

## Materials and methods

The egg hatch test was conducted *in vitro* to determine the effects of TMWW at increasing concentrations. The TMWW used in this study was obtained from the Agricultural Experiment Station, King Saud University at Riyadh where it is used for irrigation. Chemical analysis showed that concentrations of Pb, Ni, Cr, Co and Cd in the TMWW were present only in trace amounts, as expected in domestic municipal waste water (Table I). Eggs of *M. javanica* were collected from established greenhouse stock culture on tomato (*Lycopersicon esculentum* Mill. cv. Rutgers) by NaOCL (Hussey and Barker, 1973) and a concentrated egg suspension was prepared. The TMWW was thoroughly mixed with distilled water (v/v) to obtain four TMWW

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TABLE I - *Characteristics of treated municipal waste water used in the study.*

| Component | Unit | Mean ± SD | Component | Unit | Mean ± SD |
|---|---|---|---|---|---|
| pH | | 7.2 ± 0.3 | $SO_4$ | me/l | 6.5 ± 0.5 |
| EC | ds/m | 1.6 ± 0.1 | Fe | µg/l | 221.0 ± 65.9 |
| COD | mg/l | 97.0 ± 22.3 | Zn | µg/l | 47.8 ± 19.8 |
| N | mg/l | 21.1 ± 2.1 | Mn | µg/l | 30.5 ± 13.6 |
| P | mg/l | 6.8 ± 0.5 | Cu | µg/l | 10.5 ± 4.0 |
| K | mg/l | 14.1 ± 0.6 | Pb | µg/l | 3.1 ± 1.8 |
| Ca | mg/l | 123.5 ± 13.4 | Ni | µg/l | 2.5 ± 0.7 |
| Mg | mg/l | 26.1 ± 3.4 | Cr | µg/l | 2.1 ± 0.7 |
| Na | mg/l | 124.7 ± 8.3 | Co | mg/l | 2.7 ± 1.5 |
| Cl | me/l | 4.4 ± 0.2 | Cd | µg/l | 2.7 ± 1.5 |
| $HCO_3$ | me/l | 3.1 ± 0.2 | | | |

Values are means of 12 samples (one sample/month).

concentrations: 25, 50, 75 and 100% TMWW. The control treatment was distilled water. These dilutions plus the control correspond to five incubation-water treatments. One ml of the concentrated egg suspension containing ca. 40,000 eggs was placed on tissue paper in each of 20 perforated plastic cups (9 cm-diam) which were placed in unperforated plastic cups (9 cm-diam) partially filled with the designated incubation water to form an aqueous film around the eggs (Al-Yahya *et al.*, 1988b). The five treatments, each with four replicates, were randomized and maintained in an incubator at 25 °C, RH 65%. Every 2 days, for an 8 day period, the distilled water and TMWW dilutions were changed and any second-stage juveniles ($J_2$) that had hatched were removed, counted, and recorded as the cumulative hatch. However, since $J_2$ that hatched during the first 24 hrs were not exposed to TMWW long enough to have an impact on their behaviour, they were counted and discarded (not included in the cumulative hatch).

A greenhouse test was conducted to determine root penetration by $J_2$ that hatched from day 6 to day 8. Juveniles from the respective treatments were rinsed several times in distilled water, counted, and used separately to inoculate tomato cv. Rutgers seedlings grown in 10 cm-diam plastic pots containing a steam-sterilized sandy soil. Each seedling was inoculated with 1100 $J_2$ in 20 ml water, by pouring the suspension into four holes in the soil near the base of the seedling. The five basic water treatments were replicated four times and arranged in a complete randomized design on a green-house bench (25±3 °C). The seedlings were irrigated with tap water as needed. All seedlings were harvested 14 days after inoculation. Roots were gently removed from the soil, washed in running tap water, and dried of excess water. They were then stained with acid fuchsin in lactophenol (Daykin and Hussey, 1985). After destaining, roots were pressed between glass plates for enumeration of juveniles.

Both egg hatch and penetration tests were repeated six months later, and data from both runs of each test were combined for analysis of variance. Fisher's least significant differences (FLSD) were calculated when F values were significant.

## Results

Egg hatch during the first 2 days was not significantly different among all treatments (Table II). However, at day 4 all TMWW concentrations suppressed egg hatch relative to the control ($P \leq 0.05$) but no significant differences were found among TMWW treatments. After day 4, hatch suppression was proportional to the concentration of TMWW; fewer eggs hatched as the concentration of TMWW was increased. These effects became more evident as the incubation period was extended. Hatch suppressions (% of control) at day 8 (end of the test) were 11.3, 27.8, 34.4 and 51% at TMWW concentrations of 25, 50, 75 and 100%, respectively. No complete suppression was found.

Second-stage juveniles ($J_2$) that hatched from day 6 to day 8 were tested for their capacity to invade tomato roots. Based on the number of juveniles ($J_2$-$J_4$) per root system that were recovered 14 days after inoculation, none of the TMWW concentrations adversely influenced the penetration by the hatched $J_2$ (Table III). No attempt was made to determine the post infection development of *M. javanica* in the different treatments. The recovered stages were mostly juveniles ($J_2$-$J_4$). However, very few young females were found in some replicates.

Table II In vitro *hatch of* Meloidogyne javanica *in selected concentrations of treated municipal waste water.*

| Conc. (%) | Cumulative hatched juveniles Days after incubation | | | | Total hatch suppression (% of control) |
|---|---|---|---|---|---|
| | 2+ | | | 8 | |
| 0 | 2,322 a | 6,914 a | 11,996 a | 16,340 a | |
| 25 | 1,808 a | 5,441 b | 10,056 b | 14,500 b | 11.3 |
| 50 | 1,874 a | 5,034 b | 8,131 c | 11,792 c | 27.8 |
| 75 | 2,273 a | 4,833 b | 7,712 c | 10,717 d | 34.4 |
| 100 | 2,028 a | 4,733 b | 5,980 d | 8,005 e | 51.0 |

Means are the average of eight replications of two tests. Means within a column followed by the same letter are not different ($P \leq 0.05$) according to Fisher's protected least significant difference (FLSD).
+ This column represents the juveniles that hatched during the second 24 hrs; juveniles that hatched during the first 24 hrs were discarded.

Table III - *Number of* Meloidogyne javanica *juveniles recovered from tomato roots 14 days after inoculation with juveniles hatched from day 6 to day 8 in selected concentrations of treated municipal waste water.*

| Conc. (%) | Recovered juveniles+ Plant | Penetration (% of control) |
|---|---|---|
| 0 | 560 | |
| 25 | 645 | 115.2 |
| 50 | 657 | 117.3 |
| 75 | 569 | 106.4 |
| 100 | 601 | 107.3 |

Means are the average of eight replications of two penetration tests. Means of juveniles are not different ($P \leq 0.05$) according to F-test.
+ Developmental stages were mostly J2-J4; negligible number of young females were observed in some replicates.

## Discussion

Our results on egg hatch agree with those in which exposure of the citrus nematode (*Tylenchulus semipenetrans*) eggs (Al-Yahya *et al.*, 1988b) and eggs of root-knot (*M. incognita*) and reniform (*Rotylenchulus reniformis*) nematodes (Lal and Yadav, 1976) to sewage water suppressed egg hatch. Infectivity of the citrus nematode juveniles that hatched 1 or 2 weeks after exposure to sewage water was also not adversely affected (Al-Yahya *et al.*, 1988b).

The suppressive mechanism is not known, but TMWW might influence or alter the regulatory mechanism involved in hatch initiation which is determined by physiological maturation at a certain stage of development. Since the penetration by the hatched juveniles was not significantly affected, the effects of TMWW–incubation treatments appear to be restricted to the hatching process. However, longer exposure of the eggs might lead to suppression of root penetration. TMWW may damage or kill some juveniles within eggs because of its chemical composition and the organic matter in TMWW could be directly toxic to eggs. Previous studies (Johnson and Shamiyeh, 1975; Habicht, 1976; Mian and Rodriguez-Kabana, 1982; Castagnone-Sereno and Kermarrec, 1991) have shown that organic matter content in sewage sludge or other soil organic amendments have toxic effects on eggs and juveniles of *Meloidogyne* species. The micro-organisms present in TMWW may also affect hatching. Al-Hazmi *et al.* (1988b) isolated four fungal species from TMWW and showed that two of them adversely affected the development of the citrus nematode on lime seedlings.

*In vitro* experiments provide some indication of the effects of TMWW on egg hatch and subsequent infectivity of hatched juveniles, but it does not elucidate what actually occurs at the soil-nematode interface that takes place in the soil. In a 2-year field experiment with four different crops, Al-Hazmi *et al.* (1992) found that irrigation with TMWW has no effect on nematode populations in the soil and similar results were also obtained in a greenhouse study with *M. javanica* on tomato (unpubl. data). A more thorough understanding of the effects of TMWW in the long run on the ecology and host-parasite relationships of nematodes is still needed.

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