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Plant Protection Department, College of Agriculture
King Saud University - Riyadh, Saudi Arabia

EFFECT OF TREATED SEWAGE ON EGG HATCH AND INFECTIVITY OF *TYLENCHULUS SEMIPENETRANS*

by
F.A. AL-YAHYA, A.S. AL-HAZMI and M.A. EL-SAEDY

Summary. When eggs of *Tylenchulus semipenetrans* were incubated in sewage, stored, autoclaved or boiled-condensed sewage water, egg hatch was suppressed. Number of juveniles that hatched at 2, 9 or 15 days incubation were not significantly different among all treatments. After 15 days incubation (end of the test), the cumulative (total) numbers of hatched juveniles were significantly less in all sewage water treatments than in distilled or tap water. Over 50% of the juveniles that hatched after 2 weeks incubation in sewage water treatments were immobile. Percent infectivity of juveniles hatched after one week was significantly higher than those hatched after 2 weeks. No significant differences in infectivity were found among all treatments at one or two weeks.

Recently, many countries have established plants for the treatment of sewage water and have used the treated water for agricultural irrigation. Saudi Arabia has established several plants since 1982, and treated sewage water from the Riyadh plant has been pumped to nearby agricultural areas for irrigation of crops such as citrus, alfalfa and date palms.

There is little published information on the effect on plant-parasitic nematodes after irrigation with treated sewage water. In India, Lal and Yadav (1976) found that sewage water reduced egg hatch of *Meloidogyne incognita* and *Rotylenchulus reniformis* by about 70%. Motility of the sting nematode, *Belonolaimus longicaudatus*, was greatly suppressed when it was exposed for 12 hr to water extracts from saturated composted municipal refuse (Hunt *et al.*, 1973).

This study was conducted to determine the effect, *in vitro*, of treated sewage water, used in irrigation, on egg hatch of the citrus nematode, *Tylenchulus semipenetrans* Cobb, and to test the infectivity of hatched juveniles on lime (*Citrus aurantifolia* L.) seedlings in a growth chamber.

Materials and methods

The secondary-treated sewage water was obtained from the Agricultural Experiment Station, K.S.U. at Dirab, Riyadh (Al-Yahya *et al.*, 1987). Sewage water was autoclaved at 1.2 kg/cm^2 for 20 min, or boiled-condensed using soxhlet apparatus as needed.

Citrus nematode eggs were extracted from sour orange (*Citrus aurantium* L.) infected roots grown on the College of Agriculture Farm at Olyisha, Riyadh. They were extracted using NaOCl (Hussey and Barker, 1973) for 3 min, and an aqueous suspension was prepared.

To determine the effect of sewage water on egg hatch, six treatments: 1) distilled water, 2) tap water, 3) sewage water, 4) sewage water stored for 7 months, 5) autoclaved sewage water, and 6) boiled-condensed sewage water were prepared and replicated four times.

Twenty four units each consisting of two 9 cm diam plastic cups one placed inside the other were used. The bottom of the inside cup was perforated with small holes (3 mm diam.) and a nematode-filter paper (Brocades-Verbandstoffen Nijinegen) was placed over the perforated bottom. Thirty five ml of the treatment water was added to the lower cup to just cover the nematode filter paper. To each unit 8,000 eggs of the citrus nematode in a suspension were poured on to the nematode filter paper. Units were arranged in a randomized complete block design in an incubator at 27 ± 2°C and relative humidity of 65 ± 5%. At 2, 7, 9, 13 and 15 days incubation, hatched juveniles were counted and cups received fresh treatment solutions. The percentage of cumulative (total) hatch was calculated for each treatment.

Juveniles that hatched 2 weeks after egg incubation in the different treatments were counted, and their motility percent was calculated.

The influence of treatments on the hatched juveniles was examined on thirty six lime seedlings, grown for four months in 10 cm diam plastic pots filled with a steam-sterilized sandy soil. Eighteen seedlings were inoculated with equal numbers of juveniles that hatched at 7 days in the six hatching treatments. Eighteen other seedlings were similarly inoculated with juveniles hatched at two weeks. Treatments were thus replicated three times. Inoculated seedlings of each set of the test were arranged in a randomized complete block design in a growth chamber (27°C, and 30×10^3 lux light intensity for 12 hr per day). Seedlings were irrigated with their respective treatment as used for egg hatch until termination of the experiment. Seedlings from each inoculation date were harvested after 26 days. Roots were removed from the soil, washed gently with running tap water, dried of excess moisture, and stained with acid fuchsin-lactophenol (Daykin and Hussey, 1985). Numbers of juveniles and adults attached to roots were counted and recorded. Percent infectivity was calculated for each treatment.

Results

Numbers of juveniles that hatched at 2, 9 or 15 days incubation were not significantly different among all treatments (Table I). By the end of the test (15 days of incubation), the total (cumulative) numbers of hatched juveniles were significantly (P=0.05) less in all sewage water treatments than in distilled or tap water treatments. Total hatch in sewage water was significantly less than in autoclaved sewage water (Table I).

When eggs were incubated for two weeks, motility of juveniles in the sewage water treatments was greatly reduced. Over 50% of the juveniles were immobile. Sewage and boiled-condensed sewage water resulted in 82.5% and 76.9% immobility which was significantly higher than in distilled, tap or stored sewage water (Table II).

Juveniles that hatched in all treatments after one or two weeks incubation were infective. However, percent juvenile infectivity (based on distilled water infectivity) after one week incubation was significantly higher in all sewage treatments, except stored sewage, than at two weeks (Table III). No significant differences in infectivity were found among treatments at one or two weeks. However, percent infectivity at two weeks in all sewage water treatments, except stored sewage, were much less than those of distilled and tap water.

TABLE I - *Effects of different sewage water treatments on hatch of* Tylenchulus semipenetrans[1].

Treatment (water)	Mean no. hatched juveniles					Total hatch (cumulative)	Total % hatch
	Incubation period (days)						
	2	7	9	13	15		
Distilled	889a	3491a	643a	450bc	215a	5688a	71.1a
Tap	1011a	2791ab	640a	960a	343a	5745a	71.8a
Sewage	905a	1655d	444a	239c	154a	3397c	42.5c
Stored sewage	945a	2160bcd	531a	394bc	121a	4151bc	51.9bc
Autoclaved sewage	731a	2580abc	503a	443bc	163a	4420b	55.3b
Boiled-condensed sewage	831a	1782cd	532a	607ab	217a	3969bc	49.6bc

[1] Original inoculum was 8000 eggs/unit replicated 4 times. Data were transformed to square root before analysis. Means in a column followed by the same letter(s) are not significantly different at P=0.05.

TABLE II - *Effects of different sewage water treatments on immobility of hatched juveniles of* Tylenchulus semipenetrans *after two weeks incubation*[1].

Treatment (water)	Mean no. hatched juveniles used	% immobility
Distilled	450	24.2a
Tap	960	24.3a
Sewage	239	82.5b
Stored sewage	394	50.7a
Autoclaved sewage	443	56.1ab
Boiled-condensed sewage	607	76.9b

[1] Values are means of 4 replicates. Data were transformed to arcsin before analysis. Means in a column followed by the same letter(s) are not significantly different at P=0.05.

TABLE III - *Percent infectivity of* Tylenchulus semipenetrans *juveniles hatched at one and two weeks from eggs incubated in different sewage water treatment.*

Treatment (water)	% relative infectivity[1]	
	Weeks after egg incubation	
	One	Two
Tap	56.0	42.7
Sewage	74.1	10.8
Stored sewage	85.2	40.8
Autoclaved sewage	90.7	2.4
Boiled-condensed sewage	94.9	4.2
Average	82.2	20.2

[1] Percent based on 100% infectivity in distilled water; data were transformed to arcsin before analysis. Means in a row underscored by the same line are not significantly different at P=0.05.

Discussion

The present results suggest that chemical and, to a less extent, biological suppressive factors might be involved in the egg hatch. The suppressive mechanism is as yet unknown. Eggs of *T. semipenetrans* are of relatively large size, thin-shelled, and usually not deposited until after segmentation begins (Cobb, 1914). Our study suggests that treatment by sewage water might influence or alter the regulatory mechanism involved in hatch initiation which is determined by physiological maturation at a certain stage of development.

Our results are similar to those reported by Lal and Yadav (1976) on the suppressive effects of sewage water on *M. incognita* and *R. reniformis*. However, autoclaved sewage water gave less suppression than sewage water whereas Lal and Yadav (1976) found that boiled sewage water resulted in higher suppression. This discrepancy might be due to differences in sewage water composition, nematode species or both. Hatch suppression *in vitro*, as found in this study, might be equilibrated or altered when sewage water is applied *in vivo* as irrigation water (Al-Yahya *et al.*, 1987).

Motility of juveniles that hatched at two weeks incubation was significantly reduced by sewage water or boiled-condensed sewage water. However, since juveniles of *T. semipenetrans* are usually slow moving (Cobb, 1914) and since immobility was found to be reversible in some nematodes (Hunt *et al.*, 1973) immobility, reported here, should not be considered as a measure of death but rather a measure of inactivity.

The fact that infectivity of juveniles hatched after one week incubation was higher than at two weeks might be due to the higher immobility of juveniles hatched at two weeks. Lack of significant differences obtained among treatments in the infectivity at two weeks is probably due to an inoculum level (300 juv/plant) insufficient to cause detectable differences, and/or to the large variation of infectivity found in some replicates. In fact, most replicates in sewage, autoclaved, and boiled-condensed sewage water treatments showed no infection at all. Since juveniles hatched at one week were much more infective than those hatched at two weeks, juvenile infectivity appeared to be adversely affected after one week of egg incubation.

Literature cited

Al-Yahya F.A., Al-Hazmi A.S. and El-Saedy M.A., 1987 - Effect of irrigation with treated sewage water on the citrus nematode, *Tylenchulus semipenetrans. J. Coll. Agri., King Saud Univ., 10*, in press.

Cobb N.A., 1914 - Citrus-root nematode. *J. Agric. Res., 2*: 217-230.

Daykin M.E. and Hussey R.S., 1985 - Staining and histopathological techniques in nematology, pp. 39-48. *In* Barker K.R., Carter C.C. and Sasser J.N., eds, An Advanced Treatise on *Meloidogyne*, vol. *2*: Methodology. North Carolina State University Graphics. Raleigh, North Carolina, U.S.A. 223 pp.

Hunt P.G., Smart G.C., Jr. and Eno C.F., 1973 - Sting nematode, *Belonolaimus longicaudatus*, immotility induced by extracts of a composted municipal refuse. *J. Nematol., 5*: 60-63.

Hussey R.S. and Barker K.R., 1973. A comparison of methods of collecting inocula of *Meloidogyne* spp., including a new technique. *Pl. Dis. Reptr, 57*: 1025-1028.

Lal A. and Yadav B.S., 1976 - Inhibitory effect of sewage on hatching of eggs of root-knot and reniform nematode. *Indian J. Mycol. and Plant Pathol., 5*: 17-18.

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