EFFECT OF FOUR FUNGI ISOLATED FROM TREATED SEWAGE WATER ON POPULATION DEVELOPMENT OF *TYLENCHULUS SEMIPENETRANS*

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ABSTRACT

Al-Hazmi, A. S., F. A. Al-Yahya, and M. A. El-Seady. 1988. Effect of four fungi isolated from treated sewage water on population development of *Tylenchulus semipenetrans*. Nematrópica 18: 93–97.

Four fungi, *Paecilomyces variotii, Mucor hiemalis, Aspergillus petrakii,* and *Penicillium simplicissimum* were isolated frequently from secondary-treated sewage water used for irrigation. The effect of these fungi on population development of the citrus nematode (*Tylenchulus semipenetrans*) on lime (*Citrus aurantifolia*) seedlings was tested. Nematode populations recovered from roots and soil were greater when seedlings also were inoculated with *P. variotii* and *M. hiemalis* than when seedlings also were inoculated either with *A. petrakii* and *P. simplicissimum* or when no fungus was added.

Key words: Aspergillus petrakii, Citrus aurantifolia, citrus nematode, interrelationship, irrigation, lime, *Mucor hiemalis, Paecilomyces variotii, Penicillium simplicissimum,* sewage water, *Tylenchulus semipenetrans.*

RESUMEN

Al-Hazmi, A. S., F. A. Al-Yahya y M. A. El-Saedy. 1988. Efecto de cuatro hongos aislados de aguas de alcantarillado sobre el desarrollo de poblaciones de *Tylenchulus semipenetrans*. Nematrópica 18: 93–97.

Se aislaron con frequencia de aguas regadío provenientes del tratamiento secundario de desagües de alcantarillado, las especies fungosas *Paecilomyces variotii, Mucor hiemalis, Aspergillus petrakii* y *Penicillium simplicissimum*. Los efectos de estos hongos sobre el desarrollo de poblaciones del nematodo de los cítricos (*Tylenchulus semipenetrans*) se estuadiaron con plántulas de limón (*Citrus aurantifolia*). Las poblaciones del nematodo tanto en las raíces como en el suelo fueron mayores cuando las plántulas fueron inoculadas con *P. variotii* y *M. hiemalis* que cuando se inocularon con *A. petrakii* o con *P. simplicissimum* o aún cuando no se inocularon.

Palabras claves: Aspergillus petrakii, Citrus aurantifolia, desagües de alcantarillado, encaldo, *Mucor hiemalis,* nematodo de los cítricos, *Paecilomyces variotii, Penicillium simplicissimum, Tylenchulus semipentrans.*

INTRODUCTION

During investigations on the effects of secondary-treated sewage water on *Tylenchulus semipenetrans* Cobb, we isolated four fungal species

from sewage water. These fungi were identified as: *Paecilomyces varioti* Bain., *Mucor hiemalis* Wehmer, *Aspergillus petrakii* Voros, and *Penicillium simplicissimum* (Oudem.) Thom. Studies of the interrelationships of these fungi with *T. semipenetrans* might help in understanding the effects of sewage water on the citrus nematode. The present study was conducted to determine the effect of these four fungi isolated from sewage water on population development of *T. semipenetrans* on lime seedlings *(Citrus aurantifolia* L.)

MATERIALS AND METHODS

Several fungi were isolated from secondary-treated sewage water used for irrigation in the Agriculture Experiment Station, King Saud University, located at Dirab. Potato dextrose agar (PDA) (Difco Laboratories, Detroit, Mich., U.S.A.) with or without streptomycin, and water agar were used to isolate fungi. The four fungi listed earlier were isolated frequently on PDA at 30 C. Cultures were purified and maintained by frequent transfer to fresh PDA. A slant culture of each fungus on PDA was sent to the Commonwealth Mycological institute to confirm species identification.

To prepare inocula, fungi were cultured on PDA in petri plates (9-cm-diam) at 30 C for 10 days. Using a cork borer, fungal disks (0.4-mm-diam) were taken from each culture and tranferred to 250-ml conical flasks containing 50 ml potato dextrose broth. Cultures were maintained at room temperature (25 C ± 2 C). After 7 days, mycelial mats were recovered from the broth by pouring cultures onto Whatmam No. 1 filter papers supported on a Buchner funnel. Fungal mats from four flasks of each culture were macerated in 200 ml of distilled water in a blender for 30 sec and used as inocula. Fifty ml of each inoculum suspension (mycelial mat of one flask) was poured around the roots of individual 3-mo-old lime seedlings. The seedlings were grown in 20-cm-diam pots filled with equal parts of a steam-sterilized mixture of sand and clay.

Second-stage juveniles (J2) and males of *T. semipenetrans* were extracted using a combination of sieving and Baermann trays (7) from soil around infected field-grown sour orange trees *(Citrus aurantium* L.) at the College of Agriculture Farm, King Saud University, Olyisha, Riyadh. One wk after fungal inoculation, each lime seedling was inoculated with 20 000 J2 by adding nematodes to the soil around the roots. The five treatments citrus nematode alone (control), citrus nematode + *P. variotii*, citrus nematode + *M. hiemalis*, citrus nematode + *A. petrakii*, and citrus nematode + *P. simplicissimum* were replicated four times in a randomized complete block design in a growth chamber at 27 C with a 12 hr photoperoid of 30×10^3 lux. Lime seedlings were watered regularly

with tap water and fertilized once with a complete (18-18-5-1.5: N, P, K, trace elements) fertilizer (N = 20.6% ammonia).

The experiment was terminated 9 wk after the nematodes were added. Plants were removed from pots and J2, males, females, and eggs were extracted from infected roots using the NaOCl method (2) for 7 min and counted. Males and J2 in soil were extracted using a combination of sieving and Baermann trays and counted. Numbers of the different nematode life stages extracted from lime roots and soil were transformed to $\log_{10}(X)$ for statistical analysis. An analysis of variance was conducted and means separated using Fisher's protected LSD procedure.

RESULTS AND DISCUSSION

Numbers of each *T. semipenetrans* life stage extracted from roots and soil are shown in Table 1. The addition of the fungi *P. variotii* and *M. hiemalis* increased the population development of *T. semipenetrans*. The numbers of J2, males, and the total nematodes recovered from roots and soil to which these two fungi were added were significantly higher than populations either recovered from the control or from treatments involving *A. petrakii* and *P. simplicissimum* (Table 1). This increase probably was due either to a direct influence on *T. semipenetrans* or to some change in the physiology and/or morphology of lime roots. The results obtained with *P. variotii* and *M. hiemalis* might explain, in part, the increase of *T. semipenetrans* populations on lime seedlings irrigated with treated sewage water which was found in a previous test (1). Yeates also reported an increase in poplulations of certain plant-parasitic (*Ditylen-*

Table 1. Numbers and life stage of *Tylenchulus semipenetrans* extracted from lime roots and soil infested with *Paecilomyces variotii, Mucor hiemalis, Aspergillus petrakii* and *Penicillium simplicissimum* isolated from treated sewage water.

Treatment	No./stage[y]				
	J2	Males	Females	Eggs	Total
T. semipenetrans (Control)	8 735 b[z]	1 837 a	79 a	4 551 a	15 220 a
T. semipenetrans + *P. variotii*	22 010 c	4 643 b	170 a	9 374 b	36 197 b
T. semipentrans + *M. hiemalis*	16 571 c	4 301 b	122 a	6 320 ab	27 314 b
T. semipenetrans + *A. petrakii*	9 218 b	842 a	102 a	5 831 ab	15 993 a
T. semipenetrans + *P. simplicissimum*	5 648 a	2 496 a	88 a	4 394 a	12 626 a

[y]Means of four replications.
[z]Means in a column followed by the same letter are not significantly different according to Fisher's protected LSD procedure ($P = 0.05$).

chus, Heterodera, Tylenchus) and free-living nematode genera in pasture soil irrigated with dairy shed effluent compared to dryland pasture (8). *Pratylenchus* was not found at the dryland site but reached high levels at the effluent-irrigated site.

Increase of nematode populations in the presence of certain fungi is not uncommon (5). *Verticillium dahliae* Kelb. was reported to increase *Tylenchorhynchus capitatus* Allen on tomato and *Pratylenchus penetrans* (Cobb) Chitwood & Oteifa on eggplant and tomato (4). *Fusarium oxysporum* f. sp. *vasinfectum* (Atk.) Snyd. and Hans also was found to increase populations of *P. brachyurus* (Godfrey) Filipjev & Schuurmans Stekhoven (3).

Addition of the other two fungi, *A. petrakii* and *P. simplicissimum*, did not significantly change nematode populations. These normally non-pathogenic fungi may become pathogenic in the presence of the citrus nematode and therefore fewer infection sites were available to nematodes. *Aspergillus ochraceus* Wilhelm and *Penicillium martensii* Biourge were reported to increase root necrosis when introduced to tobacco plants previously inoculated with *Meloidogyne incognita* (Kofoid & White) Chitwood (6). However, in that paper data on nematode populations were not reported. Since the main objective of this study was to determine changes in nematode populations, no attempt was made to determine the disease severity or population changes of the four fungi we investigated.

In conclusion, *P. variotii* and *M. hiemalis* recovered from treated sewage water used for irrigation facilitated population increase of *T. semipenetrans*. The mechanism(s) by which these fungi caused the population increases is not known but may be due either to a direct effect on the nematodes or to a morphological change in the root system which provided more infection sites. Future research to elucidate the mechanism(s) involved should prove fruitful.

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