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Viability of *Heterodera glycines* Exposed to Fungal Filtrates[1]

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Abstract: Filtrates from nematode-parasitic fungi have been reported to be toxic to plant-parasitic nematodes. Our objective was to determine the effects of fungal filtrates on second-stage juveniles and eggs of *Heterodera glycines*. Eleven fungal species that were isolated from cysts extracted from a soybean field in Florida were tested on J2, and five species were tested on eggs in vitro. Each fungal species was grown in Czapek-Dox broth and malt extract broth. No toxic activity was observed for fungi grown in Czapek-Dox broth. Filtrates from *Paecilomyces lilacinus, Stagonospora heteroderae, Neocosmospora vasinfecta,* and *Fusarium solani* grown in malt extract broth were toxic to J2, whereas filtrates from *Exophiala pisciphila, Fusarium oxysporum, Gliocladium catenulatum, Pyrenochaeta terrestris, Verticillium chlamydosporium,* and sterile fungi 1 and 2 were not toxic to J2. Filtrates of *P. lilacinus, S. heteroderae,* and *N. vasinfecta* grown in malt extract broth reduced egg viability, whereas *F. oxysporum* and *P. terrestris* filtrates had no effect on egg viability.

Key words: biological control, egg, *Exophiala pisciphila,* fungus, *Fusarium oxysporum, Fusarium solani, Gliocladium catenulatum,* hatching, *Heterodera glycines,* juvenile, nematode, *Neocosmospora vasinfecta, Paecilomyces lilacinus, Pyrenochaeta terrestris,* soybean cyst nematode, *Stagonospora heteroderae,* toxicity, toxin, *Verticillium chlamydosporium,* viability.

Fungi are commonly isolated from cysts and egg masses of Heteroderidae. Some of these fungi may invade living eggs. Others do not invade living eggs but may produce toxic substances to kill nematodes before colonizing them (Morgan-Jones and Rodríguez-Kábana, 1985). A number of fungi isolated from nematodes, soil, and plants have been shown to produce substances that inhibit hatching of eggs or kill nematodes (Alcantara and de Azevedo, 1980; Caroppo et al., 1990; Ciancio et al., 1988; Dahiya and Singh, 1985; Hallmann and Sikora, 1996; Mani and Sethi, 1984; Nitao et al., 1999; Sakhuja et al., 1979; Singh et al., 1991; Walia and Swarup, 1985). A metabolite solution produced by *Fusarium oxysporum* Schlecht, a non-pathogenic endophyte, reduced the number of *Meloidogyne incognita* (Kofoid & White) Chitwood juveniles in the roots in a greenhouse study (Hallmann and Sikora, 1996). Nitao et al. (1999) demonstrated that several different compounds in culture filtrates of *Fusarium equiseti* (Corda) Sac. may be nematode-antagonistic. An antibiotic isolated, purified, and characterized from *Cylindrocarpon olidum* (Wollenweber) (Coosemans, 1991; Yu, 1993) showed high nematicidal activity and had a low toxicity toward vertebrates (Coosemans, 1991). A red pigment produced by *Verticillium suchlasporium* Gams & Dackman also had nematicidal properties (Lopez-Llorca and Boag, 1993). Purified extracts of a *Penicillium* sp., *Penicillium oxalicum* Currie & Thom, *Penicillium anatolicum* Stolk, and *A. niger* showed high nematicidal activity at 100 and 200 ppm (Molina and Davide, 1986). Culture filtrates from *P. lilacinus* grown in different liquid media and under various conditions were inconsistent in their toxicity toward nematodes (Cayrol et al., 1989). The activity of a toxin produced by *Paecilomyces lilacinus* Samson was independent of the pH and specific to species of Heteroderidae. The major active substance in the culture filtrate has been identified as acetic acid (Djian et al., 1991).

More than 40 species of fungi have been isolated from females, cysts, and eggs of *Heterodera glycines* Ichinohe collected from a soybean (*Glycine max* (L.) Merr.) field at the University of Florida, Green Acres Agronomy Farm, Gainesville (Chen et al.,

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1994), and from potting soil used in greenhouse tests (Chen et al., 1996b). The soil in the field was suppressive to *H. glycines* (Chen et al., 1996b). The fungi frequently associated with females, cysts, and eggs of *H. glycines* have been tested for their pathogenicity to the nematode eggs on water agar and in sterile soil (Chen et al., 1996a).

The objective of this study was to evaluate the toxic activity of culture filtrate from selected fungi against *H. glycines* eggs and second-stage juveniles (J2). The fungi chosen either colonized a high percentage of the nematode eggs or were encountered at a high frequency in the cysts in a Florida soybean field (Chen et al., 1994; Chen et al., 1996b). An abstract of this study has been published (Chen et al., 1998).

Materials and Methods

Fungal culture filtrates: Fungi used in this study included *Exophiala pisciphila* McGinnis & Ajello, *F. oxysporum, Fusarium solani* (Mart) Sacc, *Gliocladium catenulatum* Gilm. & Abbott, *Neocosmospora vasinfecta* E. F. Smith, *P. lilacinus, Pyrenochaeta terrestris* (Hansen) Gorenz, Walker & Larson, *Stagonospora heteroderae* Morgan-Jones, *Verticillium chlamydosporium* Goddard, sterile fungus 1 (black, yeast-like fungus), and sterile fungus 2. The fungi were cultured in malt extract broth or Czapek-Dox broth. Flasks (500 ml) containing 300 ml of liquid medium received a 1-cm^2 block from a 2-week-old fungal colony growing on corn meal agar. For the two sterile fungi, the colony block was chopped with a knife before being placed into the flask. The fungi were cultured for 2 weeks (or 3 weeks for the two sterile fungi) in darkness on a linear Eberbach shaker (at a low speed of forward and backward motion; Eberbach Corporation, Ann Arbor, MI) at room temperature (23–24 °C). The filtrates were first passed through a layer of filter paper (Whatman Cat. No 1001 110) and then through a 0.45-μm filter to remove bacteria and fungal spores. The filtrates were used at concentrations of the original preparation (1×) and diluted 5× or 25×. The pH of each culture filtrate was measured.

Effect on juveniles: An isolate of *H. glycines* race 3 cultured on susceptible soybean cv. Cobb in pots in the greenhouse was used in all experiments. The females and cysts on roots and in rhizosphere soil were extracted by a procedure described previously (Chen et al., 1996b). The females and cysts were placed in a solution containing 100 mg of streptomycin, 50 mg of chlortetracycline, and 30 mg of quinolinol (SCQ)/liter and stored at 10 °C until used. The cysts (we assume that all females became cysts after a period of storage) were put on six layers of tissue paper on a screen placed in a jar containing a solution of 4 mM $ZnCl_2$ and incubated at room temperature (23–24 °C). Second-stage juveniles that emerged from cysts within 2 days were collected. A suspension of 500 J2/ml of sterile water was prepared. About 50 J2 in 0.1 ml of water and 1 ml of a culture filtrate were added to each well of a 24-well tissue culture plate. Water and the media without fungal culture were included as controls. Four replicates were used per treatment. The plates were maintained at room temperature (23 to 24 °C). Viability of the J2 was determined after 4, 8, 12, 24, 48, and 72 hours with a technique described by Chen and Dickson (2000). Briefly, two drops of 1 N NaOH were added to each well. The response of J2 to NaOH was monitored with the use of an inverted microscope. The J2 that changed their body shape from straight to curled or hook-shaped within 3 minutes were considered alive, whereas the nematodes not responding to the addition of NaOH were considered paralyzed or dead. Percentages of J2 paralyzed or dead were calculated. The experiment was repeated for *E. pisciphila, G. catenulatum, P. lilacinus, P. terrestris,* and *S. heteroderae.* The data were subjected to analysis of variance (ANOVA), and LSD at $P = 0.05$ was used to compare the means of percentages of J2 paralyzed or dead among the treatments.

Effect on egg hatch: Yellow to light-brown females and cysts were extracted from the soybean roots grown in the greenhouse. The females and cysts were crushed with a tissue grinder to release eggs. The eggs were separated from debris by centrifugation in a 0 to

55% (w/v) sucrose linear gradient at 1,500*g* for 5 minutes. A band containing eggs and J2 was poured onto a sieve with 38-µm openings and rinsed with sufficient water to remove the J2. The eggs retained on the sieve were transferred onto another 38-µm-pore sieve that had been autoclaved, rinsed with sterile water, and treated with a solution containing SCQ for 24 hours at 10 °C. The eggs were rinsed with sterile water, treated with 0.5% chlorhexidine diacetate salt for 15 minutes, and rinsed with sterile water. An egg suspension was made in sterile deionized water (3,000 eggs/ml). One milliliter of the egg suspension was placed on a 1-cm-diam. sieve with 35-µm openings. The sieves with eggs were placed in a 24-well tissue culture plate.

The culture filtrates of *F. oxysporum, N. vasinfecta, P. lilacinus, P. terrestris,* and *S. heteroderae* from malt extract broth were used. The filtrates were added to the tissue culture plates in a quantity (about 2 ml) that just reached the bottoms of the sieves. Solutions of 4 mM $ZnCl_2$, malt extract broth, and water were used as controls. $ZnCl_2$, a stimulant for *H. glycines* hatch, was included to indicate viability and degree of dormancy of eggs. It was also an important positive control if any of the fungal filtrates would stimulate hatch. Four replicates of each concentration were used. The eggs were incubated in the solutions at 23 to 24 °C. The hatch solutions were replaced by fresh ones at days 3 and 7. The numbers of nematodes hatched at days 3, 7, and 14 were counted, and percentages of cumulative hatch were calculated. The experiment was repeated. In the repeated test (Test 2), all solutions were replaced by a fresh $ZnCl_2$ solution at day 14 and the eggs were incubated for another week. The additional week of incubation in $ZnCl_2$ was aimed at determining the viability of the eggs treated with the test solutions. The data were subjected to ANOVA, and LSD at $P = 0.05$ was used to compare means of percentage hatch among treatments.

Results

Effects on J2: The effects of culture filtrates on J2 viability varied among the fungal species and between the two culture media. Culture filtrates of all fungi grown in Czapek-Dox broth exhibited no nematicidal activity against J2 (data not presented). Culture filtrates of *F. solani, N. vasinfecta, P. lilacinus,* and *S. heteroderae* in malt extract broth showed the greatest nematicidal activity toward *H. glycines* J2 (Table 1). Among the filtrates, *P. lilacinus* in malt extract broth was the most toxic to the J2. In Test 1, 47% of J2 were paralyzed after 4 hours of exposure to the 1× filtrate, and all of the nematodes were paralyzed when the exposure time was more than 24 hours. The 25× dilution of the *P. lilacinus* filtrate showed low toxicity to the nematode in Test 1. In Test 2 (repeated test), however, the 25× dilution of the *P. lilacinus* filtrate showed a significant toxic effect on the J2, paralyzing 97% of J2 after 72 hours (Table 1). Culture filtrate of *S. heteroderae* in 1× malt extract broth paralyzed all J2 after an exposure period of 48 hours (Table 1). In Test 2, a 5× dilution of *S. heteroderae* culture filtrate paralyzed all J2 after an exposure period of 72 hours (Table 1). A 25× dilution of *S. heteroderae* culture filtrate did not paralyze the nematodes within the exposure period of 72 hours (Table 1). Culture filtrates of *N. vasinfecta* and *F. solani* in malt extract broth exhibited similar toxic activities to J2 and paralyzed 100% and 87% of J2, respectively, after 72 hours at 1× concentration. Culture filtrates of *E. pisciphila, F. oxysporum, G. catenulatum, P. terrestris, V. chlamydosporium,* and sterile fungi 1 and 2 in malt extract broth did not significantly reduce J2 viability (Table 1).

There was a wide range of pH in culture filtrates among fungi in this study (Table 2). The pH of malt extract broth and fungal culture filtrates in malt extract broth was lower than the pH of Czapek-Dox broth and corresponding fungal culture filtrates in Czapek-Dox broth. There also was variation of pH in fungal culture filtrates between the two tests (Table 2).

Effects on hatching: All culture filtrates at 1×, 5×, or 25×, and the culture medium (malt extract broth) inhibited egg hatch as compared with water (Figs. 1 and 2). *Paecilomyces lilacinus* was the most toxic to the

TABLE 1. Percentage of second-stage juveniles of *Heterodera glycines* paralyzed after a period of incubation in filtrates of fungal cultures in malt extract broth.

Fungal filtrate	Dilution	Incubation period (hours)				
		4	8	24	48	72
				Test 1		
Exophiala pisciphila	1	4.7	3.4	5.6	3.9	7.6
	5	0.3	0.2	0.2	0.6	0.9
	25	0.2	0	0.6	0.4	0.8
Fusarium oxysporum	1	0.9	1.2	0	1.2	4.1
	5	0.8	0.4	0.5	2.1	2.1
	25	0	0.6	0.7	0	1.5
Fusarium solani	1	13.9	0.4	14.4	83.4	87.2
	5	0	0	1.3	0.8	0
	25	0	0	0	0	0
Gliocladium catenulatum	1	0	0	1.0	14.2	7.9
	5	0	0	1.0	5.0	5.5
	25	0	0	0	6.3	3.8
Neocosmospora vasinfecta	1	0	1.0	4.1	99.0	100.0
	5	1.3	0	0	0	3.3
	25	0	0	0	0	0
Paecilomyces lilacinus	1	46.9	87.7	100.0	100.0	100.0
	5	3.5	4.9	10.6	12.6	9.0
	25	0.8	1.0	0.8	0.0	0.4
Pyrenochaeta terrestris	1	0.6	0	0.4	1.7	6.3
	5	1.5	0.3	0.6	0.5	0
	25	1.3	0.8	1.1	0	0
Stagonospora heteroderae	1	4.4	12.1	81.6	100.0	100.0
	5	0.0	0.0	13.7	21.2	5.1
	25	0.0	0.0	1.7	2.0	1.5
Verticillium chlamydosporium	1	0.2	1.9	1.3	2.6	1.3
	5	0.4	0	0.8	1.3	1.6
	25	0	0.3	0	0	0
Sterile fungus 1	1	0	0	3.2	0	0
	5	0	0	0	0	0
	25	0	0	0	0	0
Sterile fungus 2	1	0	0	1.0	0	0
	5	0	0	0	0	0
	25	0	0	0	0	0
Malt extract broth	1	0.9	0	0.1	0.2	0
Water		0	0	0.1	0.2	0.2
LSD ($P = 0.05$)		6.5	3.2	3.6	5.9	3.0
				Test 2		
Exophiala pisciphila	1	0	0	0	0.6	0
	5	0	0	0	0	0
	25	0	0	0	0	0
Gliocladium catenulatum	1	0	0	0	0	0
	5	0	0	0	0	0
	25	0	0	0	0	0
Paecilomyces lilacinus	1	80.6	100.0	100.0	100.0	100.0
	5	29.2	18.4	25.7	75.8	100.0
	25	8.2	42.6	48.8	86.0	97.3
Pyrenochaeta terrestris	1	0	0	0	0	0
	5	0	0	0	0	0
	25	0	0	0	0	0
Stagonospora heteroderae	1	34.8	58.6	97.3	100.0	100.0
	5	12.2	12.8	19.0	29.6	100.0
	25	0	0	0	2.8	5.5
Malt extract broth		0	0	0	0	0.6
Water		0	0	0.1	0.2	0
LSD ($P = 0.05$)		10.2	16.2	7.2	8.2	2.2

TABLE 2. The pH value of filtrates from fungal cultures in two liquid media at 23 to 25 °C in the dark for 2 weeks.

Fungus	Malt extract	Czapek-Dox
Exophiala pisciphila	4.7 (7.0)[a]	4.9 (5.5)
Fusarium oxysporum	5.3 (5.0)	7.7 (7.5)
Fusarium solani	4.0	6.9
Gliocladium catenulatum	4.8 (4.9)	5.5 (6.1)
Neocosmospora vasinfecta	4.2 (4.8)	5.8 (6.5)
Paecilomyces lilacinus	3.6 (3.6)	7.0 (7.2)
Pyrenochaeta terrestris	7.5 (6.5)	7.9 (7.4)
Stagonospora heteroderae	4.1 (4.5)	7.2 (7.0)
Verticillium chlamydosporium	4.7	7.3
Sterile fungus 1 (Black yeast-like fungus)	5.8	6.7
Sterile fungus 2	4.3	7.3
Medium	4.8 (4.9)	7.6 (7.6)

[a] Numbers outside the parentheses are data from Test 1, and numbers in the parentheses are data from Test 2.

nematode eggs. In Test 1, egg hatch in 1× and 5× dilutions of *P. lilacinus* culture filtrates was lower than in controls of malt extract broth, water, and $ZnCl_2$ (Fig. 1C). In Test 2, the nematode hatch in all dilutions of *P. lilacinus* culture filtrates was less than 1% in the first 2 weeks of incubation, which compares to 37% and 35% of hatch in water and $ZnCl_2$, respectively (Fig. 2C). The egg hatch in the *P. lilacinus* filtrates was also lower than that in 5× or 25× malt extract broth (Fig. 2C). The 1× and 5× dilutions of *P. lilacinus* culture filtrates killed most eggs, as indicated by the fact that most eggs treated with the filtrates for 2 weeks did not hatch even after being transferred to the $ZnCl_2$ solution (Fig. 2D). Egg hatch after transfer to $ZnCl_2$ solution was similar in the 25× dilution of *P. lilacinus* culture filtrate and the broth control, indicating that the eggs were not killed but hatch was inhibited by the 25× dilution of *P. lilacinus* culture filtrate.

The 1× solution of *N. vasinfecta* culture filtrate killed some eggs because there was limited increase of hatch in $ZnCl_2$ solution after 2-week treatment with the culture filtrate (Fig. 2D). There was no obvious lethal effect of 5× and 25× dilution of *N. vasinfecta* filtrate on nematode eggs. The toxic activity of *S. heteroderae* to the eggs was similar to that of *N. vasinfecta*.

Culture filtrates of *F. oxysporum* and *P. terrestris* reduced egg hatch at days 3 and 7 in Test 1 (Fig. 1A,B) and days 3, 7, and 14 in Test 2 (Fig. 2A,B,C) as compared with water and $ZnCl_2$. The eggs, however, were not killed by the filtrates because they hatched readily when transferred into the $ZnCl_2$ solution (Fig. 2D). A similar effect of culture medium on the nematode egg hatch was observed, indicating that the inhibitory effect of culture filtrates of *F. oxysporum* and *P. terrestris* was mainly from the culture medium rather than the fungi. Indeed, egg hatch was higher in all dilutions of culture filtrates of *F. oxysporum* and *P. terrestris* than in malt extract broth, except that 1× dilution of *P. terrestris* culture filtrates reduced egg hatch in Test 1 (Figs. 1C, 2C).

DISCUSSION

In our study, several fungi produced substances toxic to J2 and eggs. The non-toxic activity of culture filtrates of *F. oxysporum* and *V. chlamydosporium* in this study, however, contrasted with the results in previous reports, in which culture filtrates of these fungi were toxic to nematodes (Caroppo et al., 1990; Hallmann and Sikora, 1996; Sakhuja et al., 1979). There are many possible reasons for this, including differences in fungal strains, nematode species, culture media, and experimental conditions. Toxin production was influenced by a number of factors such as culture medium, aeration of media, and pH (Cayrol et al., 1989). The

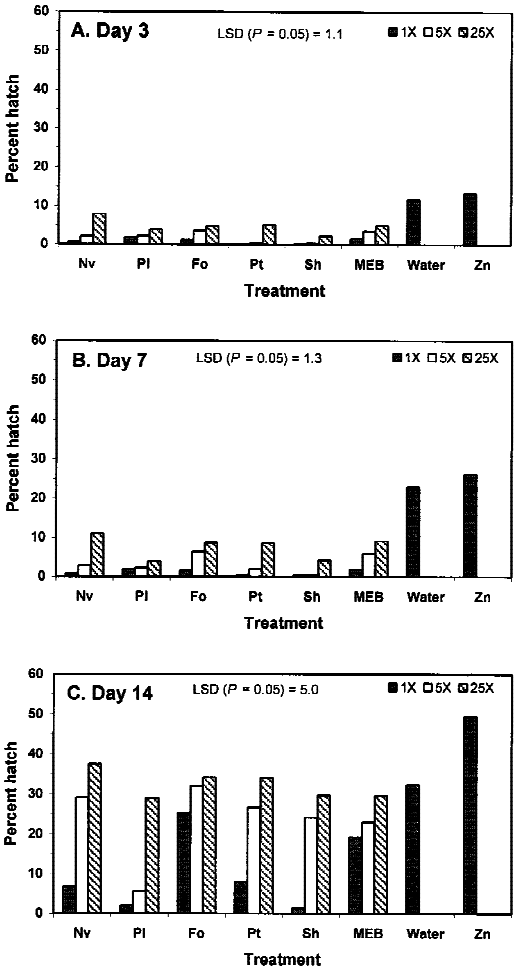

FIG. 1. The effect of filtrates of fungal cultures on *Heterodera glycines* hatch – Test 1. Fo = *Fusarium oxysporum,* Nv = *Neocosmospora vasinfecta,* Pl = *Paecilomyces lilacinus,* Pt = *Pyrenochaeta terrestris,* Sh = *Stagonospora heteroderae,* MEB = malt extract broth (culture medium), Zn = 4 mM $ZnCl_2$ solution. The fungi were cultured in MEB for 2 weeks at 23–24 °C in darkness on a shaker. The culture filtrates were tested at original concentration (1×), 5× dilution (5×), and 25× dilution (25×). The hatch solutions were replaced by fresh ones at days 3 and 7. Cumulative percentage hatch was determined at days 3 (A), 7 (B), and 14 (C).

activities of the toxin products varied among different nematode species (Cayrol et al., 1989; Hallmann and Sikora, 1996). Variations of toxin production among strains within a species have also been observed (Hallmann and Sikora, 1996).

All fungal culture filtrates with toxic activity toward the nematode juveniles had a lower pH than culture filtrates of other fungi. Cayrol et al. (1989) reported that higher toxin production occurred at lower pH, but pH had no effect on the nematicidal activity of culture filtrate of *P. lilacinus.* Further study is needed to determine whether pH has an effect on toxin production and toxic activity of *N. vasinfecta, S. heteroderae,* and *F. solani.*

The role of toxins in infection of nematodes is not fully understood. Morgan-Jones et al. (1983) observed that a strain of *V. chlamydosporium* isolated from Alabama inhibited hatch of *M. arenaria* when the fungus was near eggs on water agar, suggesting an exogenous effect possibly involving the presence of a translocatable toxin or exogenous enzymes. They speculated that both toxin and exoenzymes might be involved in infection of *M. arenaria* eggs by the fungus. The strain of *V. chlamydosporium* used in our study was highly pathogenic to the eggs of *H. glycines* and was able to colonize the nematode juveniles in cysts (Chen et al., 1996a). The results of non-toxic activity of *V. chlamydosporium* in this in vitro study cannot be extrapolated to in situ infection of nematodes by the fungus. *Neocosmospora vasinfecta* and *S. heteroderae* were encountered at high frequencies in cysts of *H. glycines* collected in the Florida soybean field (Chen et al., 1994) but colonized lower numbers of *H. glycines* eggs than *V. chlamydosporium* (Chen et al., 1996a). Further study is needed to determine whether the substances in the culture filtrates of *N. vasinfecta* and *S. heteroderae* play a role in colonization of eggs and whether the substances have anti-microbial activities that may enable them to compete with other fungi in soil and cysts. Some strains of *P. lilacinus* have antagonistic activity against fungi and bacteria (Brian and Hemming, 1947; Cartwright and Benson, 1995), and toxins may enable the fungus to compete with soil microorganisms. *Paecilomyces lilacinus* appears to be a good root colonizer and rhizosphere competitor (Cabanillas et al., 1989).

Because toxin production by fungi was influenced by culture media (also see Cayrol et al., 1989), the effects of toxins on nematodes may not become clear until toxin pro-

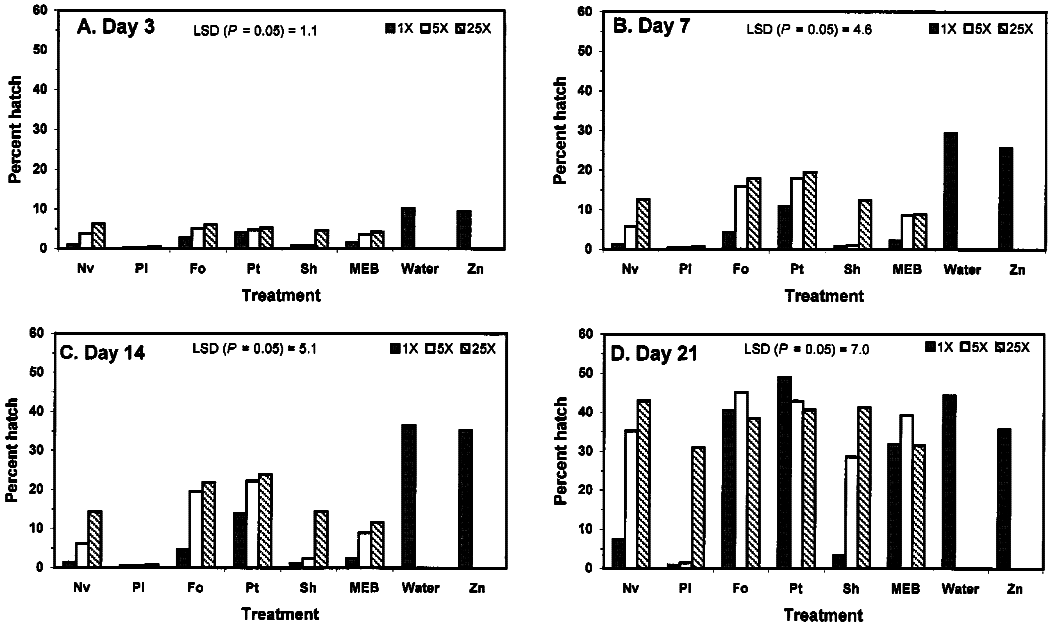

FIG. 2. The effect of filtrates of fungal cultures on *Heterodera glycines* hatch – Test 2. Fo = *Fusarium oxysporum,* Nv = *Neocosmospora vasinfecta,* Pl = *Paecilomyces lilacinus,* Pt = *Pyrenochaeta terrestris,* Sh = *Stagonospora heteroderae,* MEB = malt extract broth (culture medium), Zn = 4 mM $ZnCl_2$ solution. The fungi were cultured in MEB for 2 weeks at 23–24 °C in darkness on a shaker. The culture filtrates were tested at original concentration (1×), 5× dilution (5×), and 25× dilution (25×). The hatch solutions were replaced by fresh ones at days 3 and 7. At day 14, all of the solutions were replaced by a fresh $ZnCl_2$ solution that served as a check for viability of the eggs by stimulating hatching, and percentage hatch was determine after 1 week (day 21). Cumulative percentage hatch was determined at days 3 (A), 7 (B), 14 (C), and 21 (D).

duction by fungi is evaluated in situ. The toxin production within a cyst in soil or on a root may be different from that in culture media. A previous study indicated that fungi with little or no pathogenicity to *H. glycines* eggs could reduce the number of viable eggs within the nematode cysts (Chen et al., 1996a). More study is needed to determine whether these fungi can produce toxins within the cysts and reduce viability of eggs.

In this study, *F. oxysporum* and *P. terrestris* appeared to increase egg hatch as compared with malt extract broth. The mechanism involved in this increase is unclear. One of the possible reasons for the increase of egg hatch might be that the fungi consumed the nutrients in the culture media and reduced concentrations of hatching inhibitors in the media, as reduction of hatch in culture media has been observed in several studies (also see Khan and Husain, 1989; Mehta et al., 1990; Nitao et al., 1999). However, the possibility that the fungi produced hatching stimulants cannot be ruled out.

LITERATURE CITED

Alcantara, V. S. B., and J. L. de Azevedo. 1980. Toxicity of fungal filtrates to the nematode *Rotylenchulus reniformis.* Fitopatologia Brasileira 5:201–206.

Brian, P. W., and H. G. Hemming. 1947. Production of antifungal and antibacterial substances by fungi, preliminary examination of 166 strains of Fungi Imperfecti. Journal of General Microbiology 1:158–167.

Cabanillas, E., K. R. Barker, and L. A. Nelson. 1989. Survival of *Paecilomyces lilacinus* in selected carriers and related effects on *Meloidogyne incognita* on tomato. Journal of Nematology 21:121–130.

Caroppo, S., B. Perito, and O. Pelagatti. 1990. In vitro evaluation of nematicide activity by several fungi against *Meloidogyne incognita* eggs. Redia 73:451–462.

Cartwright, D. K., and D. M. Benson. 1995. Biological control of Rhizoctonia stem rot of poinsettia in polyfoam rooting cubes with *Pseudomonas cepacia* and *Paecilomyces lilacinus.* Biological Control 5:237–244.

Cayrol, J. C., C. Djian, and L. Pijarowski. 1989. Study of the nematocidal properties of the culture filtrate of the nematophagous fungus *Paecilomyces lilacinus.* Revue de Nématologie 12:331–336.

Chen, S. Y., and D. W. Dickson. 2000. A technique for determining live second-stage juveniles of *Heterodera glycines.* Journal of Nematology 32:117–121.

Chen, S. Y., D. W. Dickson, J. W. Kimbrough, R. McSorley, and D. J. Mitchell. 1994. Fungi associated with

females and cysts of *Heterodera glycines* in a Florida soybean field. Journal of Nematology 26:296–303.

Chen, S. Y., D. W. Dickson, and D. J. Mitchell. 1996a. Pathogenicity of fungi to *Heterodera glycines*. Journal of Nematology 28:145–158.

Chen, S. Y., D. W. Dickson, and D. J. Mitchell. 1996b. Population development of *Heterodera glycines* in response to mycoflora in soil from Florida. Biological Control 6:226–231.

Chen, S. Y., D. W. Dickson, and D. J. Mitchell. 1998. Viability of *Heterodera glycines* exposed to fungal filtrates. Journal of Nematology 30:490–491 (abstr.).

Ciancio, A., A. Logrieco, F. Lamberti, and A. Bottalico. 1988. Nematicidal effects of some *Fusarium* toxins. Nematologia Mediterranea 16:137–138.

Coosemans, J. 1991. Antagonistic activity of a *Cylindrocarpon olidum* (Wollenw.) isolate against nematodes. Mededelingen van de Faculteit Landbouwwetenschappen Rijksuniversiteit Gent 56:223–228.

Dahiya, J. S., and D. P. Singh. 1985. Inhibitory effects of *Aspergillus niger* culture filtrate on mortality and hatching of larvae of *Meloidogyne* spp. Plant and Soil 86:145–146.

Djian, C., L. Pijarowski, M. Ponchet, N. Arpin, and J. Favre-Bonvin. 1991. Acetic acid: A selective nematicidal metabolite from culture filtrates of *Paecilomyces lilacinus* (Thom) Samson and *Trichoderma longibrachiatum* Rifai. Nematologica 37:101–112.

Hallmann, J., and R. A. Sikora. 1996. Toxicity of fungal endophyte secondary metabolites to plant-parasitic nematodes and soil-borne plant pathogenic fungi. European Journal of Plant Pathology 102:155–162.

Khan, T. A., and S. I. Husain. 1989. Effect of culture filtrates of soil fungi on the mortality and hatching of reniform nematode (*Rotylenchulus reniformis*). Indian Journal of Nematology 19:35–40.

Lopez-Llorca, L. V., and B. Boag. 1993. Biological properties of a red pigment produced by the nematophagous fungus *Verticillium suchlasporium*. Nematologia Mediterranea 21:143–149.

Mani, A., and C. L. Sethi. 1984. Some characteristics of culture filtrate of *Fusarium solani* toxic to *Meloidogyne incognita*. Nematropica 14:121–129.

Mehta, N., K. K. Valia, and D. C. Gupta. 1990. Effect of culture filtrates of *Rhizoctonia solani* and *R. batalicola* cultured on different media on hatching of *Meloidogyne javanica* larvae. Plant Disease Research 5:96–99.

Molina, G. C., and R. G. Davide. 1986. Evaluation of microbial extracts for nematicidal activity against plant-parasitic nematodes *Meloidogyne incognita* and *Radopholus similis*. Philippine Agriculturist 69:173–186.

Morgan-Jones, G., and R. Rodríguez-Kábana. 1985. Phytonematode pathology. Fungal modes of action. A perspective. Nematropica 15:107–114.

Morgan-Jones, G., J. F. White, and R. Rodríguez-Kábana. 1983. Phytonematode pathology: Ultrastructural studies. I. Parasitism of *Meloidogyne arenaria* eggs by *Verticillium chlamydosporium*. Nematropica 13:245–260.

Nitao, J. K., S. L. F. Meyer, and D. Chitwood. 1999. In-vitro assays of *Meloidogyne incognita* and *Heterodera glycines* for detection of nematode-antagonistic fungal compounds. Journal of Nematology 31:172–183.

Sakhuja, P. K., I. Singh, and S. K. Sharma. 1979. Effect of some fungal filtrates on the hatching of *Meloidogyne incognita*. Indian Phytopathology 31:376–377.

Singh, K. P., K. Bihari, and V. K. Singh. 1991. Effect of culture filtrates of fungi colonizing neem cake on *Heterodera cajani*. Current Nematology 2:9–14.

Walia, K., and G. Swarup. 1985. Effect of some fungi on nematode hatching and larval root penetration. Indian Journal of Nematology 15:174–176.

Yu, Q. 1993. Comparison of the antinematode activity of compounds isolated from *Cylindrocarpon olidum* and *Cannabis sativa*. Ph.D. dissertation, Katholieke Universiteit te Leuven. Leuven, Belgium.