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Isolation of flavipin, a fungus compound antagonistic to plant-parasitic nematodes

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Summary – An isolate of the fungus *Chaetomium globosum* produced culture broths that inhibited *in vitro* egg hatch and juvenile mobility of root-knot nematode (*Meloidogyne incognita*) and hatch of soybean cyst nematode (*Heterodera glycines*). Extraction and bioassay-directed fractionation of the culture broth filtrate determined that flavipin, a low molecular weight compound, was the fungus metabolite responsible for most of the nematode-antagonistic activity. Synthesis of flavipin permitted evaluation of the compound as a suppressor of nematode populations on plants in glasshouse studies. Muskmelon (*Cucumis melo*) plants in steamed and unsteamed soil were inoculated with root-knot nematodes and various concentrations of flavipin were applied to the soil. Contrary to expectations from the *in vitro* studies, the number of galls per g of roots increased with flavipin treatment at the 14-day harvest. No effect of flavipin on nematode populations was found at the 55-day harvest. In general, plant growth and nematode populations were greater in plants grown in steamed soil.

Keywords – *Chaetomium globosum*, egg hatch, *Heterodera glycines*, *Meloidogyne incognita*, natural product, nematicide.

Due to environmental and health concerns, the use of several widely applied nematicides such as methyl bromide has or will become restricted, leaving many crops vulnerable to nematode pests unless new technologies are developed. Particularly difficult will be future management of the root-knot nematode, *Meloidogyne incognita* (Kofoid & White) Chitwood, a pest attacking hundreds of crop and ornamental plant species worldwide. Potential sources of new chemicals to manage populations of plant-parasitic nematodes include natural products produced by fungi (Anke & Sterner, 1997). As part of a programme to search for nematode-antagonistic compounds, the culture broths of over 250 fungi isolated from soybean cyst nematode eggs (*Heterodera glycines* Ichinohe) were screened for activity against *M. incognita* and *H. glycines* (Meyer *et al*., 1998). The current study describes the bioassay-guided isolation of a compound responsible for *in vitro* inhibition of *M. incognita* egg hatch and juvenile mobility by the culture broth of one of these fungi, an isolate of *Chaetomium globosum*. This isolate was selected because its culture broth filtrate was inhibitory against egg hatch of both *M. incognita* and *H. glycines*. Once the active compound was identified, it was synthesised and tested in glasshouse experiments to evaluate its potential as a control agent on plants.

Materials and methods

FUNGUS

The fungus was isolated from soybean cyst nematode (*Heterodera glycines* Ichinohe) collected near Anda City, Peoples Republic of China, in cooperation with Dr Xing-Zhong Liu (Chinese Academy of Agricultural Sciences, PRC). The isolate was identified to genus by Dr Richard Humber (USDA, ARS, Plant Protection Unit, NY, USA)

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(Meyer *et al.*, 1998) and to species as *Chaetomium globosum* Kunze *ex* Steud. 1824 by Dr David Farr (Systematic Botany and Mycology Laboratory, USDA, ARS, Beltsville, MD, USA). A culture is maintained in the Nematology Laboratory, USDA, ARS, Beltsville, MD, USA, as isolate L250.

In vitro nematode bioassays

Eggs from root-knot and soybean cyst nematodes cultured in a glasshouse on tomato (*Lycopersicon esculentum* Mill. cv. Orange Pixie) and soybean (*Glycine max* (L.) Merr. cv Essex) plants, respectively, were surface-disinfested with 0.5% sodium hypochlorite and placed in 24-well tissue culture plates with sterile-filtered test solutions (Nitao *et al.*, 1999). Fungus extracts and purified compounds were dissolved in dimethylsulfoxide (DMSO) before mixing in water (final concentration: 0.5% DMSO). Control solution was 0.5% DMSO in water.

For root-knot nematodes, the number of hatched second-stage nematode juveniles (J2) and the number of those J2 that were mobile and immobile were recorded after 1 week. Soybean cyst nematode hatch was recorded 2 weeks after the initiation of the bioassay. Each bioassay trial consisted of five replicate wells per treatment, except for one treatment in the soybean cyst nematode trial with four replicates. Each trial used 110 to 300 eggs per well.

Differences in egg hatch and J2 mobility between test and control solutions were tested with one-way analysis of variance (ANOVA) followed by Tukey's test for pairwise comparisons of means. When more than one bioassay trial was conducted for a set of treatments, trials were statistically analysed separately, but for brevity, means and standard errors are reported on pooled data since the pattern of means were similar between trials. Data were arcsin transformed if necessary. Kruskal-Wallis test on ranks was used when results from one bioassay trial could not be transformed to satisfy assumptions of normality and homogeneous variances. SigmaStat software (SPSS, Chicago, IL, USA) was used for analyses. A significance level of $P < 0.05$ was used for all tests. Untransformed means and standard errors are reported.

Bioassay-guided isolation of nematode-antagonistic compound

Chaetomium globosum cultures grown on potato-dextrose agar plates for 1 week were used to inoculate potato-dextrose broth (250 ml broth per 1 l flask). After incubating for 1 week at 25°C, shaken at 240 rpm, broth cultures were centrifuged at 13 700 *g* for 20 min, and supernatants were vacuum filtered through diatomaceous earth.

The broth filtrate was extracted with Amberlite XAD-16 resin (Maul *et al.*, 1999) by gently agitating the resin in the broth (1 : 10 resin : broth, v/v) for 1 h on a rotary shaker. The resin was collected in sintered glass Buchner funnels, washed with water, and extracted with methanol (twice the resin volume). A total of 7.2 l broth was processed in two batches, yielding 7.5 g of residue after drying *in vacuo*.

Batches of the dried methanol eluate (3.6 and 3.9 g) were each extracted with small volumes of methanol (3 × 15 ml), resulting in methanol-soluble and methanol-insoluble fractions (Fig. 1). A portion of the methanol-soluble residue (5.5 g) was extracted with chloroform (5 × 25 ml), producing chloroform-soluble and chloroform-insoluble fractions. The methanol-insoluble, chloroform-insoluble, and chloroform-soluble residues were bioassayed against root-knot nematode hatch and mobility at 250 μg/ml (two trials).

The chloroform-soluble material was re-extracted with small volumes of methanol (3 × 5 ml) to prepare a sample for column chromatography, resulting in 0.57 g of mate-

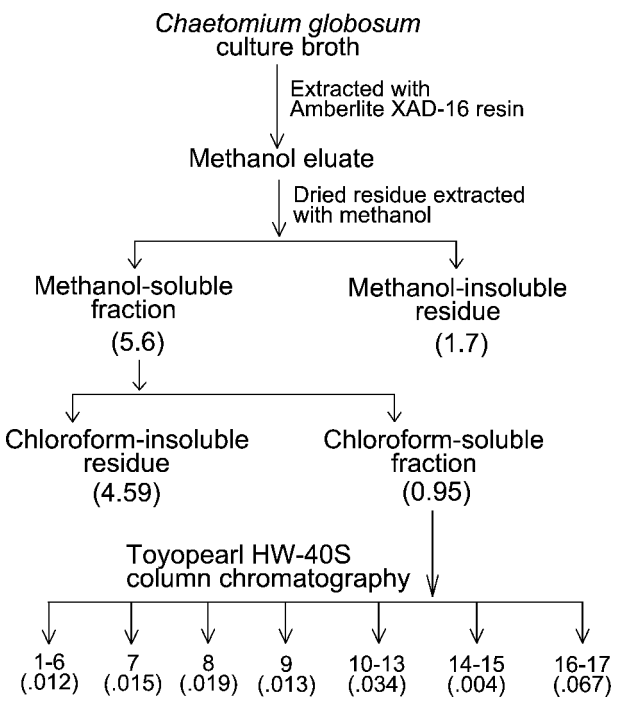

Fig. 1. *Extraction and fractionation of* Chaetomium globosum *culture broth filtrate (numbers in parentheses are dry weights (g) of fractions).*

rial readily soluble in methanol. A portion of this material (0.2 g) was fractionated on a medium-pressure liquid chromatography column (2.5 × 27 cm) containing Toyopearl HW-40S (TosoHaas, Montgomeryville, PA, USA) eluted with methanol (2 ml/min). After a 90 ml void volume, 17 fractions were collected: fraction 1 (30 ml), fractions 2 through 16 (4 ml each), and fraction 17 (122 ml). Fractions containing shared components were combined (Fig. 1) after examination by thin-layer chromatography (silica gel, 8 : 1 $CHCl_3$-MeOH). Combined fractions were bioassayed against root-knot nematode hatch and mobility at 120 μg/ml (two trials), except fraction 14-15 because of its limited quantity.

Fractions 7 and 8 were analysed by ^1H and ^{13}C nuclear magnetic resonance (NMR) spectroscopy on a Bruker QE 300 MHz spectrometer; chemical shifts are reported relative to the solvent, acetone-d_6. Fraction 8 was further bioassayed against soybean cyst nematode hatch and root-knot nematode hatch and mobility at concentrations of 30, 60 and 120 μg/ml (two trials).

Flavipin was synthesised and bioassayed against root-knot nematode hatch and mobility at concentrations of 30, 60, and 120 μg/ml (one trial). Flavipin was synthesised by first using formaldehyde and hydrochloric acid to convert trimethylgallic acid into 6-(chloromethyl)-3,4,5-trimethoxyphthalide (King & King, 1942), which was then reduced with lithium aluminium hydride to give 3-methyl-4,5,6-trimethoxyphthalyl alcohol (Bhattacharjee & Popp, 1980). Flavipin trimethyl ether was produced from 3-methyl-4,5,6-trimethoxyphthalylalcohol by Swern oxidation (Farooq, 1994). The choice of Swern oxidation greatly improved the yield of flavipin trimethyl ether compared to previously published methods, as most other oxidations of o-phthalyl alcohols yield lactones instead of the desired dialdehydes (*e.g.*, Al-Mousawi *et al.*, 1979; Bhattacharjee & Popp, 1980). Flavipin trimethyl ether was demethylated with boron tribromide (Al-Mousawi & McOmie, 1981) to produce flavipin, which was purified by flash chromatography on silica gel (33-50% ethyl acetate in toluene, 0.1% trifluoroacetic acid), sublimation *in vacuo*, or recrystallisation from glacial acetic acid.

Glasshouse bioassay of flavipin on muskmelon plants

The effects of flavipin on plant growth and root-knot nematode colonisation were tested using muskmelon plants grown in pots of steamed or unsteamed soil. Muskmelons (*Cucumis melo* L.) cv. Hearts of Gold seeds were germinated in Terra-Lite Redi-Earth Peat Lite mix (Scotts-Sierra Horticultural Products Company, Marysville, OH, USA). After 2 weeks, the Peat Lite mix was gently washed with water from the roots, and the seedlings were transplanted into 10.2 cm diameter 570 cm^3 pots containing sandy soil mix (85 : 12, fine sand : compost; one seedling per pot). One hundred and sixty seedlings were transplanted into soil that had been pre-treated by steaming for 3 h, and an equal number were transplanted into unsteamed soil. Immediately after transplanting, each plant was inoculated with root-knot nematode by applying 5000 eggs in 1 ml water into the soil at the base of the plant. Eggs were from a culture maintained on plants of tomato cv. Orange Pixie. Twenty-five ml of 0, 30, 60 or 120 μg/ml flavipin solution were then poured around the base of each plant (19 to 20 plants for each soil type × flavipin concentration combination). Test solutions consisted of flavipin (synthesised as described above) dissolved in water. The 0 μg/ml control solution was deionised water. Plants were randomly arranged on the bench in the glasshouse with natural and supplemental lighting (18-25°C, 16 h continuous light per day).

Fourteen days after inoculation and application of test solutions, half of the plants from each treatment combination were harvested (20 per treatment). Shoot height (cm) from soil surface to uppermost bud was recorded, and roots were separated from shoots. Soil was washed from the roots, and the number of root-knot nematode galls was counted. Roots were then dried at 60°C to obtain root dry weight (g).

The remaining plants were fertilised with Osmocote Plus 15-9-12 fertiliser (Scott's-Sierra Horticultural Products Company) and allowed to grow until 55 days after inoculation, at which time shoot height (cm) from soil surface to uppermost bud was recorded, and plants were harvested. Shoots were separated from roots, and the former were dried at 60°C for 2 days to obtain shoot dry weight (g). For each plant, the soil was gently washed and collected from the roots and combined with the rest of the soil contained in the pot. Root-knot nematode eggs and J2 were counted in a 1/40 subsample of the soil to estimate the number present in the entire soil volume (Meyer *et al.*, 2000). Roots were drained of excess water and weighed to obtain root fresh weight. Eggs and J2 from the root surfaces were collected by bathing intact roots with 0.5% sodium hypochlorite, sieving, and centrifuging in sucrose solution to remove debris (Meyer *et al.*, 2000). The roots were then macerated, and the eggs and J2 inside the roots were extracted by agitating root pieces in sodium hypochlorite, sieving and centrifuging in sucrose.

Two trials of the experiment were conducted, and the trials were combined for analyses with individual pots used as replicates. The variables analysed for plants harvested after 14 days were shoot height, root dry weight, and the number of galls per g root dry weight. The variables analysed for plants harvested after 55 days were shoot height, shoot dry weight, and root fresh weight. Measures of root-knot nematode populations from 55-day plants were the number of eggs and J2, expressed per g root fresh weight, occurring in the soil, on the root surface, and inside the root. The total number of eggs and J2 per g root fresh weight in each pot (*i.e.*, the sum of eggs and J2 from the roots and soil) was also analysed.

Two-way ANOVA was used to test for significant effects of soil type (unsteamed *vs* steamed), flavipin concentration (0, 30, 60, 120 μg/ml), and the interaction of soil type and flavipin concentration. Data were transformed when necessary to satisfy normality and homogeneity of variances requirements. For 14-day data, root dry weight and galls per root weight were square-root transformed. For 55-day data, root fresh weight was ln-transformed; eggs and J2 on the root surface and the total in each pot were square-root transformed, and eggs and J2 in the soil and inside the root were transformed by $x^{1/3}$. Tukey's test was used for multiple comparison of means for effects found to be significant by two-way ANOVA. For 14-day shoot height, transformations failed to correct departures from normality and variance homogeneity; therefore, Kruskal-Wallis one-way ANOVA on ranks across all treatment combinations was used, followed by Dunn's method for all pairwise comparisons. Results are reported as back-transformed means except for 14-day shoot height, which is reported as untransformed means. A significance level of $P < 0.05$ was used for all tests.

Results

Bioassay-guided isolation of the nematode-antagonistic compound

Bioassays of the methanol-insoluble, chloroform-soluble and chloroform-insoluble fractions revealed that the methanol-insoluble fraction of the culture broth extract did not significantly reduce root-knot nematode hatch or J2 mobility at 250 μg/ml (Table 1). In contrast, both chloroform-soluble and chloroform-insoluble fractions derived from methanol-soluble material significantly reduced hatch and mobility. Of these two active fractions, the chloroform-soluble fraction affected egg hatch significantly more than the chloroform-insoluble fraction (Tukey's test), reducing hatch to 30 to 40% of the control hatch, and the percentage of J2 that were mobile was reduced to *ca* 5% of the control.

Table 1. Meloidogyne incognita *egg hatch (%) and hatched second-stage juvenile (J2) mobility (%) after 1 week* in vitro *in test solutions (250 μg/ml) of* Chaetomium globosum *fractions (means ± SE, n = 10; 290 to 300 eggs per replicate).*

Chaetomium extract fraction	Egg hatch (%)	Mobile J2 (%)
Control	52 ± 3.4	97 ± 0.4
Methanol insoluble	44 ± 1.6	96 ± 0.7
Chloroform soluble	10 ± 0.9	3.4 ± 1.5
Chloroform insoluble	22 ± 1.6	23 ± 3.8

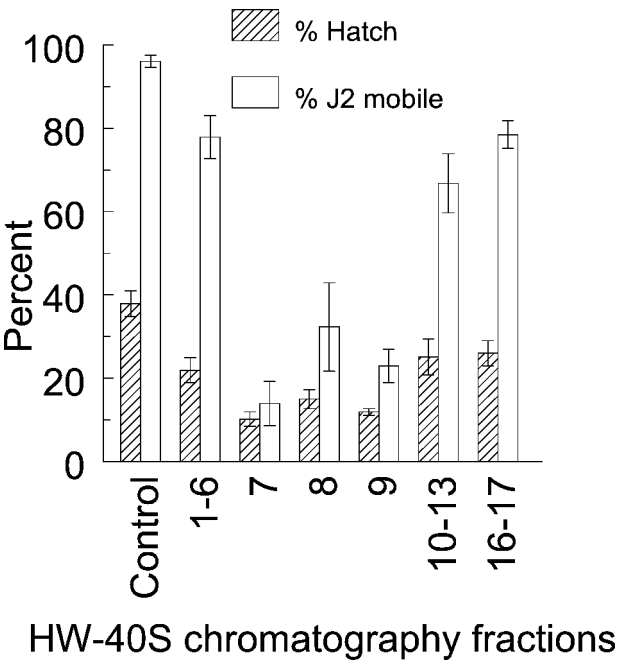

Fig. 2. Meloidogyne incognita *egg hatch (%) and hatched second-stage juvenile (J2) mobility (%) after 1 week* in vitro *in solutions of compounds in medium pressure liquid chromatography fractions of* Chaetomium globosum *culture broth (mean ± SE, n = 10; 110 to 200 eggs per replicate). Compounds within fractions were tested at a concentration of 120 μg/m; fraction 14-15 was not tested due to limited quantity.*

After separation of the chloroform-soluble fraction by chromatography on the HW-40S column, greatest nematode-antagonism was found to reside in fractions 7, 8 and 9 (Fig. 2). These fractions appeared on thin-layer chromatography to consist primarily of a single UV-active compound that intensified in yellow colour after spray-

ing with H_2SO_4 and charring. The ^1H NMR spectra of fractions 7 and 8 corresponded to that reported by Sekita *et al.* (1982) for flavipin (1,2-benzenedicarboxaldehyde-3,4,5-trihydroxy-6-methyl) (Fig. 3): δ 2.51 (3H, s), 10.39 (1H, s), 10.58 (1H, s). The ^{13}C NMR spectrum was consistent with this structure: δ 17.0 (C-7), 120.0 (C-2), 131.4 (C-6), 135.9 (C-1), 143.4 (C-4), 157.2 (C-3), 158.5 (C-5), 199.6 (C-9), 204.3 (C-8).

Fraction 8, which NMR indicated was nearly pure flavipin, was bioassayed at various concentrations against root-knot nematode to determine the activity range *in vitro* (Fig. 4). Significant effects on hatch and J2 mobility com-

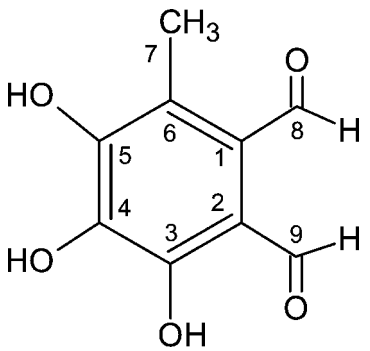

Fig. 3. *Chemical structure of flavipin (1,2-benzenedicarboxaldehyde-3,4,5-trihydroxy-6-methyl).*

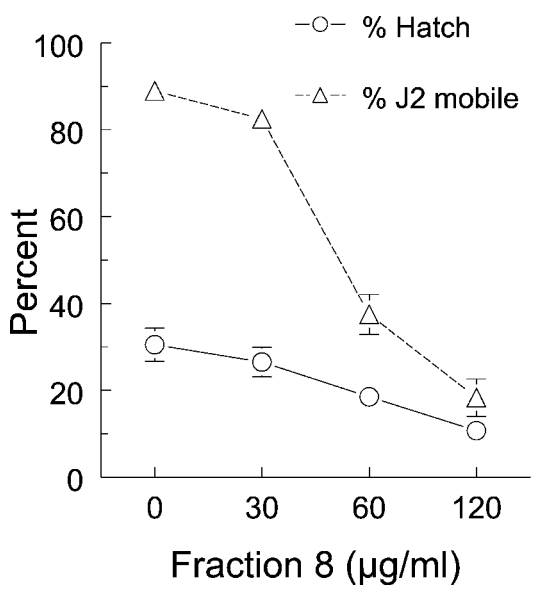

Fig. 4. Meloidogyne incognita *egg hatch (%) and hatched second-stage juvenile (J2) mobility (%) after 1 week* in vitro *in three concentrations of test solutions containing* Chaetomium globosum *fraction number 8 (means ± SE, n = 10; 190 eggs per replicate).*

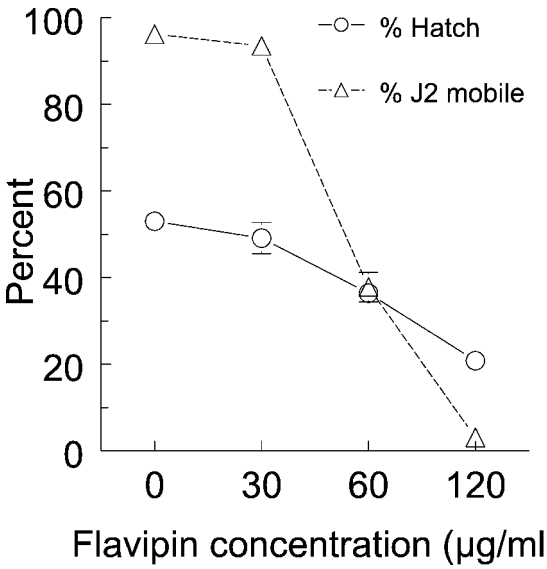

Fig. 5. Meloidogyne incognita *egg hatch (%) and hatched second-stage juvenile (J2) mobility (%) after 1 week* in vitro *in three concentrations of flavipin (means ± SE, n = 10; 230 eggs per replicate).*

pared to the control after 7 days were found at 60 and 120 μg/ml, but not at 30 μg/ml, the lowest concentration tested. Fraction 8 also significantly reduced soybean cyst nematode hatch after 14 days, egg hatch at 0, 30, 60 and 120 μg/ml were 12.3 ± 1.2, 7.1 ± 0.8, 4.6 ± 0.7 and 3.4 ± 0.4%, respectively (180 eggs per replicate). Soybean cyst nematode egg hatch was significantly less than the control at 30 μg/ml in one of the two trials, and at 60 and 120 μg/ml in both trials. Bioassay of synthetic flavipin against root-knot nematode confirmed the nematode-antagonistic activity of this compound (Fig. 5). As with fraction 8, synthetic flavipin caused a significant reduction in hatch and J2 mobility compared to controls at 60 and 120 but not at 30 μg/ml.

Glasshouse bioassay of flavipin on muskmelon plants

Muskmelon growth as measured by root weight at 14 and 55 days and by shoot height at 55 days was significantly greater in steamed than in unsteamed soil (Table 2) (two-way ANOVA). Average root dry weight at 14 days was 25-50% greater in steamed soil. At 55 days, shoot height was 0-15%, and root fresh weight 10-20% greater in steamed than unsteamed soil. Shoots at 14 days also tended to be longer in steamed than unsteamed soil by 15-50%, depending on the treatment group; Kruskal-Wallis test on ranks detected a significant

Table 2. *Size of glasshouse-grown muskmelon (*Cucumis melo*) plants in steamed soil or unsteamed soil at 14 and 55 days after inoculation with* Meloidogyne incognita *(5000 eggs per plant) and treatment with three concentrations of flavipin (means, n = 20 except n = 19 for unsteamed soil at 0 and 60 μg/ml flavipin).*

Flavipin	14 day				55 day					
(μg/ml)	Shoot height (cm)*		Root dry weight (g)**		Shoot height (cm)**		Shoot dry weight (g)		Root fresh weight (g)**	
	Steamed	Unsteamed	Steamed	Unsteamed	Steamed	Unsteamed	Steamed	Unsteamed	Steamed	Unsteamed
0	7.68	6.72	0.138	0.088	164	150	6.78	6.39	33.9	27.8
30	7.58	6.10	0.137	0.101	172	148	5.96	6.23	30.7	27.8
60	8.56	5.82	0.136	0.093	152	141	5.89	5.85	31.2	27.5
120	9.40	6.42	0.138	0.103	147	149	6.13	6.56	32.7	26.7

* = significant difference among treatments according to Kruskal-Wallis test on ranks of all treatment combinations.
** = significant effect of soil in two-way analysis of variance; flavipin and flavipin × soil effects were not significant.

overall difference among treatments, but Dunn's multiple comparison test failed to detect specific differences between groups. Of the plant variables measured, only shoot weight at 55 days did not show a detectable soil treatment effect. Although variation in shoot and root growth was observed among flavipin concentrations and between soil types within flavipin concentrations, no statistically significant flavipin or soil × flavipin effects were detected by two-way ANOVA.

For plants harvested at 14 days, flavipin significantly affected the number of galls per root weight in plants (Fig. 6). However, contrary to the negative effects seen *in vitro*, the number of galls increased with increasing flavipin concentration up to 60 μg/ml. Multiple comparison of means between flavipin concentrations across soil types indicated that there were more galls per root weight at 60 μg/ml than at 0 and 30 μg/ml. Soil and soil × flavipin effects were not significant in the two-way ANOVA. The number of galls per plant, not adjusted for root weight, showed a similar pattern; the mean numbers of galls per plant in steamed soil were 107, 102, 148 and 139 at 0, 30, 60 and 120 μg/ml flavipin, respectively, and in unsteamed soil were 76, 99, 103 and 96 galls per plant. There were more galls in steamed soil at 60 and 120 than at 30 μg/ml (Kruskal-Wallis one-way ANOVA on ranks; Dunn's method for multiple comparisons).

At 55 days, no significant flavipin or soil × flavipin effects on nematode populations were detected. Soil treatment did significantly affect nematode populations at this harvest date. The numbers of eggs and J2 per g root weight, in the soil, on the root surface and inside the root were all significantly greater in steamed than in unsteamed soil (Table 3). The total nematode population per root weight followed the patterns observed separately in the soil and root samples (Fig. 7); total number of eggs and J2 per g root weight was 25-130% greater in steamed than in unsteamed soil. An analysis of the total number of eggs and J2 per pot, unadjusted for root weight, exhibited a similar pattern of results, with total nematode populations per pot ranging between 56 000 to 79 000 for plants grown in steamed soil compared to 20 000 to 33 000 in unsteamed soil (significant soil effect, two-way ANOVA).

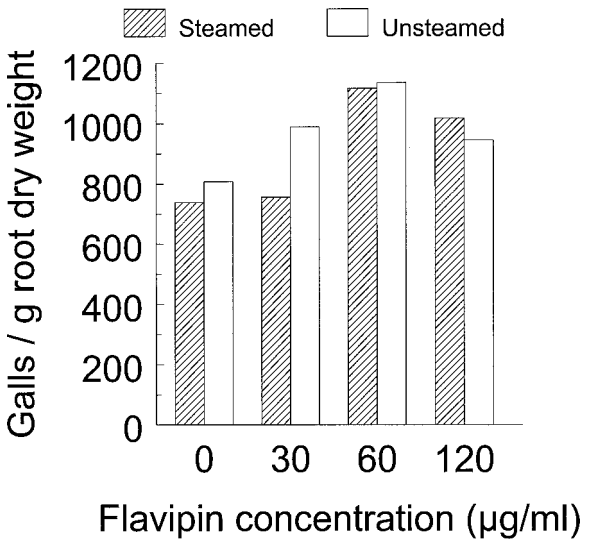

Fig. 6. Meloidogyne incognita *infection (galls per g root dry weight) on glasshouse-grown muskmelon (*Cucumis melo*) in steamed or unsteamed soil at 14 days after inoculation (5000 eggs per plant) and treatment with three concentrations of flavipin solutions (means, n = 20).*

Table 3. Meloidogyne incognita *eggs and juveniles (J2) on glasshouse-grown muskmelon (*Cucumis melo*) plants in steamed or unsteamed soil at 55 days after inoculation (5000 eggs per plant) and treatment with three concentrations of flavipin solutions (means, n = 20, except n = 19 for unsteamed soil at 0 and 60 μg/ml flavipin).*

Flavipin (μg/ml)	55-day (eggs + J2)/root weight					
	Inside root		On root surface		In soil	
	Steamed	Unsteamed	Steamed	Unsteamed	Steamed	Unsteamed
0	557	283	979	633	354	305
30	635	236	1150	492	485	224
60	621	344	1150	906	467	243
120	473	280	848	769	301	248

Each variable was significantly ($P \leq 0.05$) affected by soil type; flavipin and flavipin × soil effects were not significant (two-way analysis of variance).

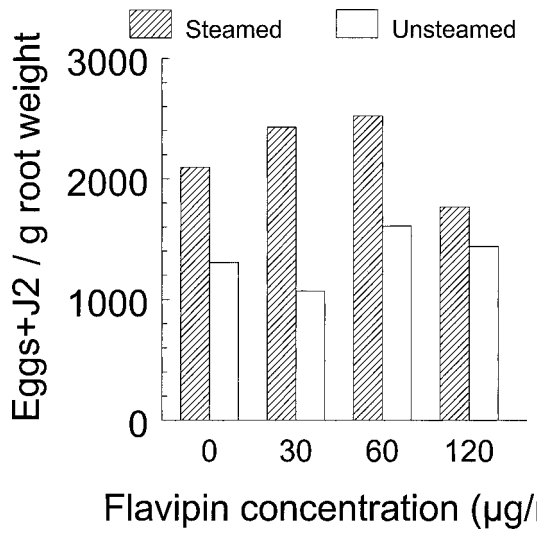

Fig. 7. Meloidogyne incognita *eggs and juveniles (J2) on glasshouse-grown muskmelon (*Cucumis melo*) plants (total per g root fresh weight) in steamed or unsteamed soil at 55 days after inoculation (5000 eggs per plant) and treatment with three concentrations of flavipin solutions (means, n = 20, except n = 19 for unsteamed soil at 0 and 60 μg/ml flavipin).*

Discussion

Chaetomium globosum produces a variety of biologically active compounds (Cole & Cox, 1981). The *in vitro* nematode-antagonistic activity of the isolate tested herein was mainly due to flavipin. Fractions that reduced root-knot nematode egg hatch and J2 mobility consisted primarily of this compound, and bioassays of the synthesised compound confirmed the activity. Flavipin is produced by several *Aspergillus* and *Epicoccum* species as well as by *Chaetomium globosum* (Raistrick & Rudman, 1956; Bamford *et al*., 1961; Sekita *et al*., 1982). The compound is toxic to microorganisms, particularly to fungi (Raistrick & Rudman, 1956; Bamford *et al*., 1961; Baráthová *et al*., 1969; Burge *et al*., 1976; Brown *et al*., 1987; Mallea *et al*., 1991; Madrigal & Melgarejo, 1995), and was implicated as the mechanism responsible for the biocontrol of *Monilinia laxa*, a fungal plant pathogen, by *Epicoccum nigrum* (Madrigal *et al*., 1991). The effects against whole organisms other than fungi and bacteria are poorly studied except for a few reported bioassays on plants (Bamford *et al*., 1961; Burge *et al*., 1976). Biochemically, flavipin inhibits electron transport, oxidative phosphorylation and protein synthesis, possibly as a result of the aromatic orthodialdehyde functionality cross-linking lysine residues in the proteins of cytochrome C and mitochondrial membranes (White & Elliott, 1972, 1980; Madrigal & Melgarejo, 1994). Other biochemical effects include inhibition of interleukin-1 receptors and glucose-6-phosphate translocase (Stefanelli *et al*., 1997; Vertesy *et al*., 1999).

Compounds other than flavipin may have contributed to the *in vitro* bioactivity. Fraction 7, which consisted primarily of flavipin, also contained very low concentrations of unidentified compounds, and tended to reduce hatch and mobility slightly more than fraction 8, which was nearly pure flavipin. However, this difference was statistically significant only in J2 mobility in one of two bioassay trials. Flavipin also inhibited egg hatch of soybean cyst nematode, indicating that nematode-antagonistic activity is not specific to the root-knot nematode.

Despite having *in vitro* nematode-antagonistic activity, flavipin did not suppress root-knot nematode populations on muskmelon plants in glasshouse experiments, and contrary to expectations, increased the number of galls per g root weight after 14 days in both steamed and un-

steamed soil. Flavipin may have altered root physiology, making the plants more susceptible to nematode colonisation or improving the nutritional quality of the host. Alternatively, the antimicrobial activity of the compound may have outweighed its nematode-antagonistic properties, changing the microbial community of the rhizosphere to one more favourable to the nematodes. The importance of the microbial community was apparent from the positive effects of steaming the soil on both plant growth and nematode populations. The interaction between flavipin and the nematodes may have been limited by the binding of the compound to the soil and its marginal solubility in water. Preliminary observations indicating that flavipin does not leach out of the soil were consistent with the hypothesised low mobility of the compound in the soil. Improving delivery of flavipin by addition of a solubiliser could result in improved effects on nematode populations.

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Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

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