Chapter 2

In vitro screening of endophytic *Fusarium* isolates against the banana burrowing nematode, *Radopholus similis* (Cobb) Thorne

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Abstract

Radopholus similis is one of the key pests of banana in the East African Highlands and in the world. Although control of this pest has mainly relied on the use of clean planting material, re-infestation of plants in the field remains a critical concern. Alternative management options such as the use of fungal endophytes may be used to provide protection and extend plant life. In this study, a total of 35 endophytic *Fusarium* isolates were screened for the production of secondary metabolites antagonistic to *R. similis* in culture. Undiluted and diluted culture filtrates were tested against motile stages of *R. similis* and eggs. Tests were conducted using culture filtrates of the fungal isolates grown in banana corm broth. All isolates tested demonstrated some level of *in vitro* antagonistic activity on the mobility of *R. similis* mixed stages (males, females and juveniles). The percentage of immobilized nematodes increased with increase in the length of exposure time to culture filtrates. After 24 hrs exposure, up to 100% of nematodes were immobilized compared to 26.5% in control treatments. Mortality of mixed stages of *R. similis* exposed to culture filtrates for 24 hrs, followed by rinsing with sterile distilled water, demonstrated that the effects of culture filtrate treatment were irreversible, as nematodes did not recover and were considered dead. Nematode mortality rates after 24-hr exposure periods ranged from 76.4% to 100.0%. The effects of culture filtrates on *R. similis* motile stages increased with increasing culture filtrate concentration. Culture filtrates also demonstrated inhibitory effects on hatching of *R. similis* eggs. *Radopholus similis* males were more sensitive to culture filtrate treatment than females. The results obtained demonstrate the potential for using endophytic *Fusarium* isolates as biological control agents against *R. similis* and/or toxic derivatives as potential nematicides against the nematode.

Introduction

The banana burrowing nematode, *Radopholus similis* (Cobb) Thorne, is one of the major constraints to banana production in the world (Sarah, 1989). *Radopholus similis* feeds inside the plant roots, and the damage it causes leads to necrosis and impaired water and nutrient uptake. Root necrosis, in turn, reduces the plant's anchorage in the soil and plants can easily be toppled during strong winds. Yield losses caused by *R. similis* can be as much as 50% (Speijer and Kajumba, 2000). Chemical control of the nematode is not a feasible option to small-scale farmers due to their high cost and lack of availability. Management of *R. similis,* thus, relies on cultural methods, such as the use of healthy, clean planting material obtained from pared and hot water-treated suckers, and tissue culture plants (Speijer *et al*., 1995; Speijer *et al*., 1999). These methods offer temporary control of nematodes only, as reinfestation in the fields occurs readily (Stanton, 1999; Speijer *et al*., 2001).

Biological control of *R. similis* using fungal endophytes offers a novel and promising nematode management option. In the past, fungal endophytes have successfully been used for nematode control in crops such as tomato (Hallman and Sikora, 1994). It was also previously demonstrated that fungal endophytes from healthy banana roots were able to kill nematodes *in vitro* and to protect tissue culture banana plants against nematodes in the screen house (Pocasangre, 2000; Niere, 2001).

Fungal endophytes protect plants against nematodes in several ways. The main method by which nematodes are inhibited *in vitro* is through antibiosis. Antibiosis is the antagonism resulting from the production of secondary metabolites by one microorganism that are toxic to another microorganism (Alabouvette and Lemanceau, 1999). Production of nematode-antagonistic metabolites by fungi and bacteria in culture is a well-documented phenomenon (Anke and Sterner, 1997; Sharon *et al*., 2001), and has been reported for *Paecilomyces lilacinus* (Thom) Samson (Cayrol *et al*., 1989), *Fusarium equiseti* (Corda) Sacc. (Nitao *et al*., 2001), various rhizosphere fungi (Alam *et al*., 1973), *Fusarium solani* (Mart) Sacc. (Mani and Sethi, 1984; Mani *et al*., 1986; Zareen *et al*., 2001), *Penicillium* and *Aspergillus* spp. (Dahiya and Singh, 1985; Molina and Davide, 1986; Ansari *et al*., 2002), *Pseudomonas striata* (Chester) Migula (Ansari *et al*., 2002), *Pseudomonas aeruginosa* (Schroeter) Migula (Ali *et al*., 2002), *Trichoderma virens* Miller, Giddens & Foster (Meyer *et al*., 2000), *Trichoderma*

harzianum Rifai (Ansari *et al*., 2002) and *Chaetomium globosum* Kunze ex Steud. (Nitao *et al*., 2002). As a whole, antagonistic activity by fungi against the root-knot nematodes *Meloidogyne* spp. and the cyst nematodes *Heterodera* spp. has received the greatest attention.

Various non-endophytic *Fusarium* isolates have been shown to produce filtrates toxic to plant-parasitic nematodes *in vitro* (Ciancio, 1995; Anke and Sterner, 1997; Nitao *et al*., 2001, Meyer *et al*., 2004; Mennan *et al*., 2005). Hallman and Sikora (1996) demonstrated production of toxins by an endophytic isolate of *Fusarium oxysporum* Schlecht.: Fries from tomato plants against *Meloidogyne incognita* (Kofoid & White). Similarly, various endophytic *Fusarium* isolates obtained from banana plants have been shown to produce secondary metabolites in culture that immobilized and caused mortality of *R. similis* (Pocasangre, 2000; Niere, 2001; Dubois *et al*., 2004; Niere *et al*., 2004).

The use of fungal culture filtrates to demonstrate toxicity against plant-parasitic nematodes can help to rapidly identify strains with potential for biological control (Nitao *et al*., 1999). The objectives of the current study were, therefore, to screen various endophytic *Fusarium* isolates for production of metabolites antagonistic to *R. similis* motile stages (males, females and juveniles), to determine the effects of fungal culture filtrates on hatchability of *R. similis* eggs, and to determine the effects of different culture filtrate concentrations on motile stages of *R. similis*. These tests could be used for developing a rapid bioassay screening procedure with which different fungal isolates could be discerned.

Materials and methods

Endophytic fungal isolates

Thirty-five endophytic fungal isolates belonging to various *Fusarium* spp. were obtained from the fungal culture collection at the International Institute of Tropical Agriculture (IITA) laboratory in Namulonge, Uganda. These isolates are preserved at 4°C in soil tubes at IITA. Nine of the isolates were previously isolated from the healthy roots and corms of banana plants growing in nematode-infested plantations in Uganda (Schuster *et al*., 1995; Griesbach, 2000) and have been tested both for *in vitro* and *in vivo* activity against *R. similis* (Table 1).

The results of the initial *in vitro* screening, however, were not quantified (Niere, 2001). The other 26 isolates were selected from a collection of *Fusarium* spp. that were originally obtained from mature and healthy banana plants of the cultivar Kibuzi (*Musa* spp., AAA-EA) in 2001 in Uganda (Table 2). Selection of these isolates was based on the developmental stage of the plant and the plant part from which the isolates were obtained. Isolates were, thus, collected either from a flowering plant or a sucker attached to the flowering plant, from roots, or from the outer corm tissues. These isolates had not been screened against *R. similis* before.

Radopholus similis *cultures*

Pure sterile cultures of *R. similis* were prepared and are maintained on carrot disks at the IITA laboratory in Namulonge, Uganda according to the method described by Speijer and De Waele (1997). Nematode-infested banana roots were obtained from banana fields, cut into 1-cm-long pieces and macerated in a Waring blender (Waring, Connecticut, USA) with tap water for 15 s. The macerated suspension was collected in a modified Baermann dish and nematodes extracted overnight. Nematodes that collected at the bottom of the dish were transferred into a beaker, concentrated in a 28-µm sieve, and transferred to a counting slide. Individual *R. similis* females were handpicked with a sterile sucking tube from the slide under a dissecting microscope and transferred into a 25 ml sterile glass test tube. The sucking tube was obtained by burning the tips of two Pasteur pipettes pressed against each other. The pipettes were pulled apart after melting, resulting into a tiny opening that was used to suck nematodes from the suspension. The nematode-containing suspension was made up to 2 ml, and the nematodes surface-sterilized by adding 1 ml of a 6000 ppm streptomycin sulphate solution to the suspension for 4 hrs. The streptomycin sulphate solution was siphoned from above the settled nematodes, and the nematodes rinsed with sterile distilled water (SDW) (autoclaved at 121°C for 15 min). The rinsing step was repeated with a 30 min interval between the two rinses.

Fresh carrots were surface-sterilized by dipping them in 96% ethanol, followed by flaming. The outer peel was removed with a flame-sterilized carrot peeler and the process repeated twice. After surface sterilization, the carrots were cut with a sterile scalpel into disks *ca.* 0.5 cm thick and placed into 30-mm-diameter sterile glass Petri dishes. Approximately 25 gravid females were inoculated on the cortex of each carrot disk and the Petri dishes sealed with

parafilm. The Petri dishes were maintained in an incubator at 27°C. Three to 4 weeks later, nematodes were harvested by rinsing the Petri dishes with SDW under laminar airflow and the suspension collected in a sterile beaker. The nematodes were then either sub-cultured onto new carrot disks to replenish the nematode inoculum bank, or used for experiments.

Culturing of fungal isolates and preparation of culture filtrates

Fungal isolates preserved in soil tubes were cultured on synthetic nutrient agar (SNA) (1 g KH_2PO_4, 1 g KNO_3, 0.5 g $MgSO_47H_2O$, 0.5 g KCl, 0.2 g glucose, 0.2 g sucrose, 0.6 ml NaOH (1 M) and 13.2 g agar in 1000 ml SDW) in 55-mm-diameter Petri dishes for 1 week. The isolates were then transferred to liquid banana corm broth (BCB). BCB was used in an attempt to simulate the growth conditions under which the endophytes naturally occur. The broth was prepared as follows: Corms were obtained from approximately 50 cm high suckers of the cultivar Enyeru (syn. Nabusa, *Musa* spp. AAA-EA) in Namulonge, Uganda. The corms were pared to remove roots and adhering soil, and cut into pieces of *ca.* 1 cm^3 in the laboratory. Five hundred grams of corm tissue was boiled in 1 L of tap water until soft. The mixture was then passed through cheesecloth to remove corm pieces and the resultant solution topped up to 1 L with SDW. Aliquots of 100 ml were dispensed into 250-ml Erlenmeyer flasks and sterilized at 121°C for 15 min. The medium was allowed to cool and then aseptically inoculated with 7-day-old agar disks of the different fungal isolates grown on SNA. Inoculated flasks were incubated for 14 days at laboratory conditions of ± 25°C with a natural photoperiod of 12 hrs light and 12 hrs darkness. Control flasks were not inoculated with fungal cultures. The flasks were manually shaken each day to disperse the fungal spores in the medium.

Pure fungal culture filtrates were obtained by centrifuging the fungal cultures in BCB at 6000 rpm for 10 min (Meyer *et al*., 2000). The resultant supernatant was aseptically transferred to sterile 25 ml screw cap glass bottles and filtered through 0.22 µm millipore filters. The pH of the fungal filtrates was measured and the average pH calculated. The pH of the control treatment was also measured and adjusted to equilibrate the average pH of the fungal culture filtrates. An additional control treatment with unadjusted pH was included. Culture filtrates were kept in the fridge at 4°C overnight.

Nematicidal activity of undiluted culture filtrates

One milliliter of 2-wk-old undiluted culture filtrates of each of the nine *F. oxysporum* isolates was transferred to 30-mm-diameter sterile glass Petri dishes. The filtrates were inoculated with approximately 100 sterile mixed stages (females, males and juveniles) of *R. similis* kept in 100 µl SDW. Control Petri dishes had pure BCB inoculated with the mixed nematode culture. Two control treatments were included in the study: one with unadjusted pH and another in which the pH was adjusted to the average pH of the culture filtrates. Three replicate Petri dishes were used for each fungal isolate and for the controls, and the experiment was repeated three times. During the course of each experiment, Petri dishes were left completely randomized and undisturbed on laboratory benches.

For the bioassays with the nine prescreened *F. oxysporum* isolates, the effect of culture filtrates on nematode activity (paralysis) was assessed by recording the number of active and inactive nematodes after exposure times of 3, 6 and 24 hrs. Reversibility of toxic effects of culture filtrates and mortality of nematodes exposed to the culture filtrates was determined after an exposure period of 24 hrs. To assess mortality, the nematodes were first rinsed through a 28-µm sieve with SDW and transferred back into the Petri dishes. The Petri dishes were then incubated under laboratory conditions for a further 24 hrs. Nematodes that remained paralysed after probing with a fine needle were considered dead (Cayrol *et al*., 1989).

Separate bioassays were conducted for the 26 *Fusarium* isolates obtained from roots and corms of banana plants from Masaka district, Uganda, since all isolates could not be handled in one bioassay. The bioassay was performed at an exposure time of 6 hrs, based on results obtained with the nine prescreened *F. oxysporum* isolates. A reference isolate *V5W2*, previously selected as the best of the nine prescreened *F. oxysporum* isolates, was included for comparison. Four replicate Petri dishes were used for each fungal isolate and for the controls, and the experiments repeated three and six times for corm and root isolates, respectively.

Nematicidal activity of different culture filtrate concentrations

Two *F. oxysporum* isolates, *V5W2* and *Eny1.31i*, were selected to test the effect of culture filtrate concentrations on the paralysis and mortality of mixed stages of *R. similis*. Fungal isolates were grown in BCB, and culture filtrates prepared as described above. Six concentrations, 2.5, 5, 10, 25, 50 and 100% of the filtrate were prepared by diluting the culture filtrates with SDW. One milliliter of the diluted culture filtrates of each test isolate was transferred into 30-mm-diameter sterile glass Petri dishes. Control Petri dishes received uninoculated broth, with the pH of the control treatment adjusted to equal the average pH of the test isolates. Approximately 100 *R. similis* (mixed stages) in 100 µl sterile distilled water were transferred to the filtrates. The treatments were replicated four times, and the experiment was repeated once. The activity (mobility) of nematodes immersed in diluted culture filtrates was determined by counting the number of active and inactive nematodes after an exposure time of 6 hrs. Reversibility of the toxic effects of culture filtrates and mortality of nematodes was determined as described above.

Effect of fungal culture filtrates on hatching of R. similis *eggs*

R. similis egg hatch was determined following treatment with the nine pre-screened *F. oxysporum* isolates. Single eggs of *R. similis* were obtained from 3- to 4-week-old carrot disk nematode cultures. The carrot disk cultures were macerated in a Waring blender, and both motile stages and eggs of *R. similis* collected overnight in a modified Baermann dish (Hooper *et al*., 2005). The suspension was concentrated in a 28-µm sieve and transferred to counting-slides, from where the eggs were hand-picked under a stereomicroscope and transferred to sterile test tubes. The eggs were surface sterilized in a 6000-ppm streptomycin sulphate solution for 1 hour (Speijer and De Waele, 1997) and washed twice with SDW, with a 30 min interval. Undiluted fungal culture filtrates (1 ml) were transferred into 30-mm-diameter glass Petri dishes, and approximately 50 surface-sterilized eggs were added. The eggs were incubated in the fungal filtrate for 48 hrs and then transferred to SDW before incubation for 15 days. The number of juveniles that emerged was recorded at 3-day intervals. Three repeat bioassays were conducted. Two controls were used for comparison: one in SDW, and the other in uninoculated BCB. Three replicate Petri dishes were used for each fungal isolate and the control treatments, and the experiments were repeated three times. In the first bioassay,

the control treatment was SDW only. All bioassays were conducted on laboratory benches in a completely randomized design.

Data analysis

Separate counts for mobile and immobilized males, females and juveniles of *R. similis* were made for all bioassays. From the counts of inactive/active and dead/living nematodes, percentage paralysis or mortality were calculated, respectively, and the means for each treatment calculated. Prior to analysis, all nematode data were tested for normality and homogeneity of variances using Shapiro-Wilkinson, Levene Welch and Kolmogorov-Smirnov tests. Normal probability plots, box plots and stem leaf plots were additionally used to confirm normality of data. If not normally distributed, various transformations were tested until the most suitable transformation was obtained. Percentage paralysis and mortality were arcsine sqrt-transformed to normalize data and homogenize variances.

For all bioassays using motile stages of *R. similis*, one-way ANOVA was conducted to investigate differences among treatment means. If different, means were separated using Tukey's studentized range test (SAS Institute, 1989). Two-way ANOVA was used to demonstrate interaction effects between treatments and exposure time, treatments and sex, and sex and exposure time. When the sex of the nematodes was included as a factor, juveniles were omitted from the analysis, as they represented less than 5% of the total nematodes.

Percentage egg-hatching was calculated as the number of hatched juveniles, divided by number of eggs originally placed in a Petri dish, and multiplied by 100. Before analysis, percentage egg-hatching was arcsine sqrt-transformed to normalize data. One-way ANOVA was conducted to demonstrate variability among treatment means. If different, means were separated using Tukey's studentized range test. From the bioassays with different culture filtrate concentrations, the LC_{50} values for isolates *V5W2* and *Eny1.31i* were calculated using the Calcusyn 1.1 (Biosoft, Cambridge, UK) software package (Chou & Hayball, 1996).

Results

In vitro screening of Fusarium oxysporum *isolates*

Culture filtrates of the nine *F. oxysporum* fungal isolates (Table 1) paralysed all stages of *R. similis in vitro*. Significant differences (P<0.0001) were observed between the control treatments and some of the endophyte treatments in each of the three bioassays (Table 3). Isolate *Eny7.11o* paralyzed nematodes significantly in all but one (bioassay 2, after 24 hrs exposure) of the treatments, while isolates *Eny1.3li* and *1113W3* reduced activity after 6 of the 9 exposure time periods in the three bioassays. The percentage of paralyzed nematodes differed between the three independent bioassays (P=0.0006), indicating that the fungal isolates' effects varied between repeat experiments.

The effect of fungal culture filtrates on nematode paralysis was influenced by the length of exposure of the nematodes to the culture filtrates (Table 3). Bioassays differed significantly (P<0.0001) in the percentage of nematodes paralyzed at each exposure time. The percentage of paralysed nematodes increased with an increase in the duration of exposure to the culture filtrate (Table 3). At 3 hrs exposure time, the percentage nematode paralysis by filtrates of some of the fungal isolates tested (e.g. *Eny7.11o*, *III3W3 V1W7* and *V2W2*) differed from the controls in Bioassays 1 and 3. After 6 hrs of exposure, most of the fungal isolates differed from the control treatments and often from each other, while few differences were observed among isolates after 24 hrs of exposure. Thus, fungal isolates could be best differentiated from each other after 6 hrs of exposure to culture filtrates.

Nematicidal effects on *R. similis* were observed for all the undiluted culture filtrates after exposure for 24 hrs (Table 4). Dead nematodes appeared straight (uncurved) with elongated bodies, while live nematodes retained the normal sigmoid shape and exhibited some slight movement. In all three bioassays conducted with the nine *F. oxysporum* isolates, the percentage of dead nematodes was significantly higher for all fungal treatments compared to the controls (P<0.0001, Table 4). Nematode mortality rates in culture filtrates ranged from 76.4 to 100.0%, with a strong interaction effect observed between treatments and bioassays (P<0.0001; Table 4).

Culture filtrates from the nine *F. oxysporum* isolates exhibited selectivity for *R. similis* sexes. *Radopholus similis* males were more sensitive to culture filtrates than females after 3 and 6 hrs of exposure (Table 5). No differences, however, were observed between males and female *R. similis* response to culture filtrates after 24 hrs of exposure.

In vitro screening of Fusarium *spp. from banana plants*

The percentage nematodes paralyzed in the culture filtrates of different *Fusarium* spp. obtained from banana corms in Masaka differed among the three bioassays ($P<0.0001$), with a strong interaction between treatments and bioassays ($P<0.0001$). This means that the percentage paralyzed nematodes in culture filtrates of the same isolate differed across repeat bioassays. In bioassay 1, the percentage of nematodes paralyzed in the control treatments was significantly lower than that in culture filtrates of isolates *M86* and *M127*. Culture filtrates of these two isolates also resulted in significantly higher nematode paralysis compared to all other isolates with an exception of isolates *M*66 (Table 6). In bioassay 2, all fungal isolates except, isolates *M72* and *M120* caused significantly higher levels of nematode paralysis compared to the controls, while in bioassay 3, culture filtrates of all tested isolates with an exception of isolates *M119*, *M120 and M127* paralyzed significantly more nematodes in comparison to the controls (Table 6). The percentage of paralyzed nematodes did not differ significantly ($P=0.05$) between the two controls in all three bioassays, although the percentage of nematodes paralyzed in the pH-adjusted control (Control 2) was numerically lower than in the control with unadjusted pH in 2 out of the 3 bioassays (Table 6).

The culture filtrates of the different *Fusarium* isolates from corms caused variable levels of nematode mortality after 6 hrs of exposure (Table 6). The interaction between treatments and bioassays was significant ($P<0.0001$). In bioassay 1, isolates *M86* and *M66* resulted in a significantly higher nematode mortality compared to the controls, while in bioassay 2, isolate *M66* caused significantly more death to nematodes than the control treatments. In bioassay, isolates *M121* and *M127* caused significantly higher mortality than the controls. The control treatment with the adjusted pH (control 2) had a lower nematode mortality than the control with unadjusted pH in bioassays 1 and 2, although the difference was not significant ($P>0.05$). In all three bioassays, nematodes recovered from the immobile state after rinsing

with sterile distilled water (Table 6). Nematode recovery rates ranged from 10.8% to 61.9%, 2.7% to 71.3% and 20.2% to 56.6% in bioassay 1, 2 and 3, respectively.

The percentage nematodes immobilized in culture filtrates of *Fusarium* isolates obtained from banana roots in Masaka differed across repeat bioassays (P<0.0001). A strong interaction effect between treatments and bioassays (P<0.0001) was observed. In all the six bioassays conducted with these isolates, the percentage nematodes immobilized by some *Fusarium* culture filtrates were significantly higher than the control treatments (Table 7). In bioassay 1, for example, culture filtrates from all tested isolates with an exception of isolates *M321*, *M360* and *M388* caused significantly higher nematode immobilization than the controls. The percentage of immobilized nematodes did not significantly differ between the two control treatments. Culture filtrates from several isolates (e.g. *M*387, *M*343, *M*291, *M*325, *M*419 and *M*365) resulted in nematode immobilization rates similar to that of the reference isolate *V5W2* (Table 7) in some of the bioassays in which isolate *V5W2* was tested.

Percentage nematode mortality in culture filtrates of some *Fusarium* isolates obtained from banana roots in Masaka was significantly higher than the nematode mortalities obtained in the controls (Table 8). The percentage nematodes killed in the fungal culture filtrates also differed across repeat bioassays (P<0.0001) with a strong interaction between treatments and bioassays (P<0.0001). No significant differences were observed between the control treatments in the percentage of nematodes killed in bioassays 1, 3, 4 and 6. In these bioassays, however, the control with adjusted pH (control 2) tended to cause lower nematode mortality than the control with unadjusted pH. Culture filtrates of isolates *M*290, *M*304, *M*291, *M*325, *M*304, *M*329 and *M*379 resulted in nematode mortalities similar to those of the reference isolate, *V5W2*.

Effect of different culture filtrate concentrations on Radopholus similis *motile stages*

The percentage nematodes immobilized by culture filtrates of both isolates *V5W2* and *Eny1.31i* increased with an increase in filtrate concentration (Table 9). Differences in the percentage of immobilized nematodes as the culture filtrate concentration increased from 2.5% to 100% were observed for both isolates. This difference was significant when the concentration of the filtrate was reduced from 100 to 50% for isolate *Eny1.31i* (P=0.0001),

but not for isolate *V5W2* (*P*=0.1682) in bioassay 1. For the repeat bioassay, the reduction was significant for both isolates (*P*<0.0001). No significant differences, however, were observed when the filtrate concentration was reduced from 50 to 25%, or among filtrate concentrations of 2.5, 5% and 10% (Table 9).

Percentage mortality of nematodes differed significantly among the different culture filtrate concentrations for both isolates *V5W2* and *Eny1.31i* (*P*<0.0001), and increased as the filtrate concentration increased (Table 9). Significant differences in the percentage of dead nematodes were evident only between the 50% and 100% filtrate concentrations. Once a dilution rate of 25% or less was obtained, the percentage of nematodes killed in culture filtrates were not significantly reduced any more (Table 9). The LC_{50} values for isolate *V5W2* was 21.9 ± 2.5% and 20.4 ± 6.4% in bioassay 1 and 2, respectively, while the LC_{50} for isolate *Eny1.31i* was 18.5 ± 5.2 and 9.6 ± 1.7% in bioassays 1 and 2, respectively.

Effect of fungal culture filtrates on Radopholus similis *egg hatch*

Hatching of *R. similis* eggs was significantly reduced by fungal culture filtrates and the culture filtrate control when compared to the treatment with sterile distilled water (P<0.0001) (Fig. 1). The number of eggs that hatched increased with the increase in incubation days. In bioassay 1, percentage egg hatch in SDW was >70.3% (Fig. 1A) while in the culture filtrates, egg hatch was <15% after 15 days of incubation. In bioassays 2 (Fig. 1B) and 3 (Fig. 1C), percentage egg hatch in SDW was lower than in bioassay 1 (30.3% and 40.2%, respectively), but still significantly lower than the culture filtrate treatments. Eggs appeared normal in shape in both the control and culture filtrate treatments.

Discussion

In this study, culture filtrates of *Fusarium* endophytes from banana roots and corms resulted in the *in vitro* paralysis and mortality of motile stages of *R. similis*. Endophytic *F. oxysporum* isolates that resulted in consistently good paralysis and mortality of *R. similis* included *V5W2*, *V4W5*, *Eny1.31i* and *Eny7.11o*. Similarly, several *Fusarium* isolates (*M*66, *M*86, *M*127 and *M*387) from roots and corms of banana plants in Masaka caused consistently high paralysis and mortality. Despite the great variation that occurred within treatments and the absence sometimes of significant differences among isolates, results obtained in this study may be considered when selecting candidate isolates for *in planta* studies in the greenhouse and the field. Nitao *et al.* (1999) have previously used in vitro screening using fungal filtrates to provide a simple and rapid method of identifying potential biological control agents. In the current study, culture filtrates from various *Fusarium* spp. isolates have demonstrated the potential for using the isolates as possible biological control agents of *R.similis*.

All isolates of *F. oxysporum* studied by Niere (2001) reduced the mobility of nematodes in the current study. This demonstrated the ability of the isolates to retain *in vitro* nematicidal activity even after long periods of storage. Culture filtrates of *Fusarium* isolates from roots and corms of plants from Masaka demonstrated the capacity to immobilize motile *R. similis* stages after 6 hrs of exposure. Similarly, isolates *M*66 (*Fusarium chlamydosporium* Wollenw. & Reinking), *M*86 (*F. solani*) and *M*127 (an unidentified *Fusarium* sp.) from Masaka district exhibited good *in vitro* inhibition of *R. similis* motile stages. These species should, therefore, be considered as potential biological control agents against *R. similis* in future studies.

Different levels of nematode immobilization and mortality were obtained for the same *Fusarium* isolates despite efforts to maintain the same experimental conditions for repeat bioassays. Several factors may have contributed to these inconsistencies, such as the type of culture medium (Cayrol *et al*., 1989), the age of the fungal culture filtrates (Mankau, 1969), the culture filtrate concentration and the nematode incubation period (Mani and Sethi, 1984; Meyer *et al*., 2000; Ali *et al*., 2002). Cayrol *et al*. (1989) reported that the culture medium and cultural conditions, such as aeration and pH, affected production of inhibitory compounds by some microorganisms. The culture medium used for the current study was made from banana

corms, and the age of the banana suckers and the length of boiling might have influenced results. However, the pH of fungal filtrates on nematode inhibition in this study seems negligible, as no significant differences were found between the control with unadjusted pH and the one with pH adjusted to the average pH of the culture filtrates except in a few bioassays. The metabolites produced in the culture filtrates by the various *Fusarium* isolates were, therefore, primarily responsible for immobilizing and killing *R. similis* motile stages. Cayrol *et al*. (1989) also reported that, although toxin production by fungi may be influenced by pH, the toxins act in a wide range of pH values and are independent of the culture filtrate pH.

Little information is available on the nematode-inhibiting components of fungal culture filtrates and on specific phytotoxins produced by the fungi. The fact that the culture filtrates in this study were free of spores and mycelia, however, strongly implies that the compounds in the filtrates were toxic to *R. similis*. It has been demonstrated before that *Fusarium* spp. can produce toxic secondary metabolites such as zearalanone, fumonisins, tricothecenes and fusaric acid (Vey *et al*., 2001). Ciancio (1995) further demonstrated that *Fusarium* mycotoxins, such as T2-toxin, monilliformin, verrucarin A, cytochalasin B and Enniatin B, caused significant mortality of *Meloidogyne javanica* (Treub) Chitwoodi juveniles in the laboratory. Similarly, culture filtrates of non-endophytic strains of *Penicilliun oxalicum* Currie & Thom, *Penicillium anatolicum* Stolk and *Aspergillus niger* Van Tieghem caused mortality to *R. similis* after 48 hrs immersion (Molina and Davide, 1986).

Fungal culture filtrates demonstrated an increase in *in vitro* activity as the length of exposure time increased. A mortality rate of up to 100.0% was obtained when nematodes were exposed to *F. oxysporum* culture filtrates for 24 hrs. When exposed to culture filtrates for shorter periods, nematodes underwent partial paralysis that was reversible after rinsing off the filtrates with SDW. This was demonstrated in the bioassays conducted with the 26 *Fusarium* spp., whereby a certain percentage of nematodes exposed to culture filtrates for 6 hrs recovered from the immobile state after the culture filtrates were rinsed out, indicating that the toxin activity was neurotropic. The toxic effects of the culture filtrates were not reversible after nematodes had been exposed to it for 24 hrs, indicating that these nematodes could be considered dead. Reversibility of toxic effects has also been demonstrated with culture filtrates of *P. lilacinus* against different nematode species (Cayrol *et al*., 1989) when exposed

for less than 48 hours. The fact that male *R. similis* was immobilized and killed more rapidly than the females is of little significance, as male nematodes are not infective on banana (Speijer and De Waele, 1997).

The use of diluted culture filtrates was intended to mimic the natural conditions encountered by *R. similis* in the banana plants. As expected, the percentage immobilized nematodes decreased with a reduction in culture filtrate concentration. This is in agreement with findings by others regarding the effects of fungal filtrate concentrations on the immobilization of nematodes (Mankau, 1969; Mani and Sethi, 1984; Zareen *et al*., 2001). The low rates of nematode immobilization at low culture filtrate concentration might be due to the dilution of the toxic compounds. If the fungal isolates produce the toxins inside the plant, that concentration would probably be lower than in the bioassays conducted with undiluted culture filtrates. Thus, the levels of nematode control in the plants due to toxins can be expected to be lower than in the laboratory. Production of toxins in the plant may, however, act as an additional form of armory that would help the plant guard itself from invasion by the nematode. Production of toxic metabolites in the plant by *Fusarium* endophytes may inhibit not only nematode mobility, but also host searching and the infection processes (Sikora *et al*., 2003). While the production of toxic metabolites may be the main mechanism of action in the laboratory, the value of nematicidal compounds in the plant, and the mechanism whereby endophytes protect banana plants against *R. similis*, should be further investigated. It is important to recognize that isolates not showing *in vitro* activity against nematodes may be able to induce systemic resistance in the plant.

Culture filtrates of endophytic *F. oxysporum* isolates substantially inhibited egg hatch of *R. similis*. Inhibition of egg hatch of nematodes by fungal culture filtrates has been demonstrated for root-knot nematodes before. Ansari *et al*. (2002) demonstrated inihibitory effects of *P. striata*, *T. harzianum* and *T. viride* culture filtrates on egg hatch of *M. javanica* after 7 and 14 days of incubation. Reduction of egg hatch of *M. incognita* by microbial extracts of *P. oxalicum*, *P. anatolicum* and *A. niger* was also reported by Molina and Davide (1986). In contrast to these studies, some fungal filtrates have been shown to stimulate nematode egg hatch (Meyer *et al*., 2004). As *R. similis* eggs are reported to hatch within 4 to 10 days (Gowen *et al*., 2005), the eggs that did not hatch after 15 days of incubation in the current study were considered dead. The mechanism whereby fungal culture filtrates inhibited egg

hatch of *R. similis* was not investigated in this study, but may be due to toxic effects on eggshell permeability that inhibits or suppresses larval development (Bone *et al*., 1987; Samaliev *et al*., 2000). A low molecular weight compound, flavipin, was isolated from *C. globosum* which inhibited both egg hatch and juvenile mobility of *M. incognita* and *Heterodera glycines* Ichinohe (Nitao *et al*., 2002).

From the current study it was evident that *Fusarium* endophytes may be a rich source of bionematicides that can be harnessed for the management of *R. similis* and possibly other nematode species. Purification and identification of such compounds may also be useful in identifying genes responsible for their production, and the potential generation of transgenic banana plants expressing such genes. Using this technology, Atkinson *et al*. (2004) have produced transgenic Grande Naine banana plants, modified with a proteinase inhibitor gene, that reduced damage caused by *R. similis* with 70%.

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Table 1: The identity and origin of nine endophytic *Fusarium oxysporum* isolates obtained from banana (*Musa* spp.) plants in Uganda that were screened against the motile stages and eggs of *Radopholus similis* in the laboratory.

Isolate	Species	Cultivar	Plant part	*In-vitro* activity*
Eny1.31i	*F. oxysporum*	Enyeru (AAA-EA)	Corm	nt
Eny7.11o	*F. oxysporum*	Enyeru (AAA-EA)	Corm	nt
III3W3	*F. oxysporum*	Unknown AAA-EA	Root	++
III4W1	*F. oxysporum*	Unknown AAA-EA	Root	++
V5W2	*F. oxysporum*	Unknown AAA-EA	Root	++
V4W5	*F. oxysporum*	Unknown AAA-EA	Root	+
V2W2	*F. oxysporum*	Unknown AAA-EA	Root	(+)
V1W7	*F. oxysporum*	Unknown AAA-EA	Root	+
Emb2.4o	*F. oxysporum*	Embiire (AAA-EA)	Corm	nt

**In vitro* inactivation of *R. similis* with *F. oxysporum* culture filtrates, as cited in Niere (2001), with ++ constantly good inactivation; + good inactivation; (+) moderate/varying inactivation; nt, not tested.

Table 2: The identity and origin of 26 endophytic Fusarium isolates obtained from banana (Musa spp.) plants in Masaka District, Uganda that were screened against the motile stages of *Radopholus similis* in the laboratory.

Isolate	Species	Cultivar	Plant No	Stage of plant	Plant part
M-66	*F. chlamydosporium*	Kibuzi (AAA-EA)	2	Sucker	Outer corm
M-72	*F. subglutinans*	Kibuzi (AAA-EA)	2	Sucker	Outer corm
M-86	*F. solani*	Kibuzi (AAA-EA)	3	Sucker	Outer corm
M-87	*F. oxysporum*	Kibuzi (AAA-EA)	3	Sucker	Outer corm
M-110	*F. anthophilum*	Kibuzi (AAA-EA)	3	Flowering	Outer corm
M-119	*F. solani*	Kibuzi (AAA-EA)	1	Sucker	Outer corm
M-120	*Fusarium* sp.	Kibuzi (AAA-EA)	1	Sucker	Outer corm
M-121	*Fusarium* sp.	Kibuzi (AAA-EA)	1	Sucker	Outer corm
M-122	*Fusarium* sp.	Kibuzi (AAA-EA)	1	Sucker	Outer corm
M-127	*Fusarium* sp.	Kibuzi (AAA-EA)	5	Sucker	Outer corm
M-290	*F. solani*	Kibuzi (AAA-EA)	1	Sucker	Root
M-291	*F. oxysporum*	Kibuzi (AAA-EA)	1	Sucker	Root
M-304	*F. oxysporum*	Kibuzi (AAA-EA)	2	Sucker	Root
M-321	*F. anthophilum*	Kibuzi (AAA-EA)	3	Sucker	Root
M-325	*F. oxysporum*	Kibuzi (AAA-EA)	3	Sucker	Root
M-337	*F. oxysporum*	Kibuzi (AAA-EA)	4	Sucker	Root
M-343	*Fusarium* sp.	Kibuzi (AAA-EA)	4	Sucker	Root
M-360	*Fusarium* sp.	Kibuzi (AAA-EA)	5	Sucker	Root
M-365	*F. oxysporum*	Kibuzi (AAA-EA)	5	Sucker	Root
M-379	*F. oxysporum*	Kibuzi (AAA-EA)	1	Flowering	Root
M-385	*F. oxysporum*	Kibuzi (AAA-EA)	3	Flowering	Root
M-387	*F. oxysporum*	Kibuzi (AAA-EA)	2	Flowering	Root
M-388	*F. anthophilum*	Kibuzi (AAA-EA)	2	Flowering	Root
M-419	*F. oxysporum*	Kibuzi (AAA-EA)	4	Flowering	Root
M-428	*Fusarium* sp.	Kibuzi (AAA-EA)	4	Flowering	Root
M-459	*F. oxysporum*	Kibuzi (AAA-EA)	5	Flowering	Root

Table 3: Percentage paralysis (mean ± S.E.) of *Radopholus similis* mixed stages (males, females and juveniles) in culture filtrates of nine endophytic *Fusarium oxysporum* isolates after exposure times of 3, 6 and 24 hrs.

Treatment	Exposure time in culture filtrate								
	Bioassay 1			Bioassay 2			Bioassay 3		
	3 hrs	6 hrs	24 hrs	3 hrs	6 hrs	24 hrs	3 hrs	6 hrs	24 hrs
Control 1	33.8 ± 4.9^{bc}	62.4 ± 0.5^{cd}	93.0 ± 0.7^{b}	34.0 ± 0.9^{bc}	38.0 ± 0.7^{b}	98.4 ± 0.9^{ab}	13.8 ± 3.1^{cd}	18.1 ± 3.5^{d}	20.8 ± 3.9^{c}
Control 2	33.4 ± 2.9^{c}	43.3 ± 1.1^{e}	21.1 ± 2.9^{c}	27.6 ± 3.2^{bc}	30.8 ± 3.3^{b}	29.9 ± 1.1^{d}	18.7 ± 4.9^{d}	25.9 ± 3.9^{d}	32.5 ± 4.9^{c}
Emb2.4o	29.8 ± 3.2^{c}	63.6 ± 7.1^{c}	96.5 ± 1.2^{ab}	40.3 ± 7.2^{bc}	72.3 ± 4.9^{a}	99.3 ± 0.7^{a}	18.7 ± 2.8^{d}	46.5 ± 7.4^{cd}	83.6 ± 1.6^{ab}
Eny1.31i	37.1 ± 6.1^{bc}	80.8 ± 4.4^{ab}	100.0 ± 0.0^{a}	42.6 ± 2.5^{bc}	83.0 ± 3.7^{a}	100.0 ± 0.0^{a}	63.5 ± 3.7^{a}	77.7 ± 1.7^{a}	88.2 ± 4.4^{ab}
Eny7.11o	68.4 ± 4.9^{a}	82.9 ± 2.9^{ab}	100.0 ± 0.0^{a}	71.6 ± 9.1^{a}	71.3 ± 7.1^{a}	100.0 ± 0.0^{a}	50.3 ± 1.1^{ab}	66.8 ± 5.0^{abc}	91.1 ± 2.7^{ab}
1113W3	67.4 ± 2.7^{a}	86.1 ± 1.5^{a}	99.1 ± 0.5^{ab}	39.4 ± 4.4^{bc}	36.3 ± 4.9^{b}	73.5 ± 4.7^{c}	43.1 ± 8.8^{ab}	72.8 ± 5.9^{ab}	91.8 ± 2.6^{ab}
1114W1	55.3 ± 4.7^{ab}	84.1 ± 1.7^{a}	100.0 ± 0.0^{a}	53.1 ± 6.9^{ab}	72.0 ± 3.6^{a}	100.0 ± 0.0^{a}	33.2 ± 3.6b^{cd}	51.1 ± 4.7^{c}	90.0 ± 2.2^{ab}
V1W7	66.5 ± 4.6^{a}	60.6 ± 2.3^{cd}	94.6 ± 2.7^{ab}	50.5 ± 6.5^{ab}	39.8 ± 5 .7^{b}	90.1 ± 2.0^{b}	47.1 ± 1.9^{ab}	63.9 ± 0.7^{abc}	93.6 ± 4.4^{a}
V2W2	28.6 ± 5.0^{c}	48.5 ± 1.8^{de}	98.1 ± 0.5^{ab}	72.5 ± 5.2^{a}	39.5 ± 7.3^{b}	100.0 ± 0.0^{a}	16.2 ± 1.9^{d}	58.8 ± 2.3^{abc}	100.0 ± 0.0^{a}
V4W5	31.8 ± 3.2^{c}	90.6 ± 0.2^{a}	97.9 ± 0.4^{ab}	49.1 ± 4.0^{ab}	72.6 ± 4.4^{a}	100.0 ± 0.0^{a}	41.2 ± 6.4^{bc}	52.8 ± 2.5^{bc}	72.4 ± 8.8^{b}
V5W2	27.2 ± 3.6^{c}	68.8 ± 2.0^{bc}	99.2 ± 0.8^{ab}	17.7 ± 3.5^{c}	76.6 ± 2.8^{a}	100.0 ± 0.0^{a}	14.5 ± 1.4^{d}	54.8 ± 1.4^{bc}	88.4 ± 2.3^{ab}
P-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

In control 1, the pH was unadjusted (5.89, 6.31 and 6.46), and in control 2 to the average pH of the culture filtrates (9.16, 7.37 and 7.68) in bioassay 1, 2 and 3, respectively. In each column, the means followed by the same letter (superscript) are not significantly different at $P>0.05$ according to Tukey's studentized range test. n=3.

Table 4: Percentage mortality (mean ± S.E.) of *Radopholus similis* mixed stages (males, females and juveniles) in culture filtrates of nine endophytic *Fusarium oxysporum* isolates after an exposure time of 24 hrs, n=3.

Treatment	Bioassay		
	1	2	3
Control 1	79.1 ± 3.6 [b]	41.2 ± 2.7 [b]	47.7 ± 12.3 [b]
Control 2	14.8 ± 1.2 [c]	14.2 ± 2.1 [c]	36.9 ± 4.8 [b]
Emb2.4o	97.7 ± 0.6 [a]	97.4 ± 1.5 [a]	96.1 ± 1.1 [a]
Eny1.31i	99.0 ± 0.5 [a]	99.1 ± 0.9 [a]	77.6 ± 5.1 [a]
Eny7.11o	99.5 ± 0.4 [a]	100.0 ± 0.0 [a]	84.2 ± 3.0 [a]
1113W3	100.0 ± 0.0 [a]	76.4 ± 1.1 [a]	88.2 ± 2.6 [a]
1114W1	98.6 ± 0.7 [a]	98.5 ± 0.8 [a]	84.8 ± 4.8 [a]
V1W7	94.2 ± 0.5 [a]	95.3 ± 1.3 [a]	97.5 ± 0.4 [a]
V2W2	97.9 ± 1.3 [a]	96.1 ± 1.6 [a]	100.0 ± 0.0 [a]
V4W5	99.6 ± 0.4 [a]	100.0 ± 0.0 [a]	89.9 ± 2.5 [a]
V5W2	99.2 ± 0.8 [a]	100.0 ± 0.0 [a]	99.0 ± 1.0 [a]
P-value	<0.0001	<0.0001	<0.0001

In control 1 the pH was unadjusted (5.89, 6.31 and 6.46), and in control 2 the pH was adjusted to the average pH of the culture filtrates (9.16, 7.37 and 7.68) in bioassay 1, 2 and 3, respectively. Mortality rates were determined after rinsing out nematodes from the culture filtrates and incubating them in sterile distilled water for 24 hrs. In each column, the means followed by the same letter (superscript) are not significantly different at $P>0.05$ according to Tukey's studentized range test.

Table 5: Effect of exposure time to culture filtrates of endophytic *Fusarium oxysporum* isolates on *Radopholus similis* males and females, indicated by the percentage paralysis.

	R. similis paralysis (%)								
	Bioassay 1			Bioassay 2			Bioassay 3		
Exposure time (hrs)	F	M	*P*-value	F	M	*P*-value	F	M	*P*-value
3	44.5[a]	64.3[b]	<0.0001	36.0[a]	58.8[b]	<0.0001	27.6[a]	51.7[b]	<0.0001
6	34.7[a]	81.2[b]	<0.0001	50.1[a]	63.8[b]	<0.0001	47.9[a]	75.7[b]	<0.0001
24	82.3[a]	86.9[a]	0.0011	88.5[a]	93.2[a]	0.0700	76.6[a]	85.4[a]	<0.0001

F= female *R. similis*, M=male *R. similis*. In each row per bioassay, means followed by the same letter (superscript) are not significantly different at $P>0.05$ according to Tukey's studentized range test. n=3.

Table 6: Percentage paralysis and mortality of mixed stages of *Radopholus similis* (males, females and juveniles) following treatment with culture filtrates of nine endophytic Fusarium isolates obtained from corms of banana plants in Masaka district, for 6 hrs.

	Bioassay 1		Bioassay 2		Bioassay 3	
Treatment	% Paralysis	% Mortality	% Paralysis	% Mortality	% Paralysis	% Mortality
Control 1	39.5 ± 3.1^{bc}	40.4 ± 2.2^{b}	19.8 ± 3.3^{d}	30.9 ± 3.8^{bc}	38.2 ± 4.2^{gf}	40.4 ± 3.2^{ef}
Control 2	31.6 ± 3.4^{c}	17.6 ± 3.7^{b}	15.1 ± 2.7^{d}	14.2 ± 2.5^{d}	33.7 ± 1.6^{g}	53.1 ± 5.3^{cde}
M-66	61.9 ± 4.2^{ab}	43.7 ± 2.8^{ab}	75.3 ± 6.9^{a}	52.1 ± 5.2^{a}	67.4 ± 3.8^{cde}	29.2 ± 2.8^{f}
M-72	33.3 ± 1.5^{c}	18.3 ± 1.0^{b}	34.9 ± 5.6^{bcd}	13.9 ± 1.2^{d}	73.2 ± 6.4^{bcd}	41.8 ± 6.2^{def}
M-86	87.7 ± 5.2^{a}	33.4 ± 9.9^{ab}	70.5 ± 1.9^{a}	34.5 ± 2.8^{bc}	90.2 ± 2.9^{a}	61.1 ± 5.7^{bcd}
M-87	36.7 ± 2.4^{bc}	25.1 ± 3.7^{ab}	38.1 ± 4.6^{bc}	17.9 ± 3.2^{cd}	89.8 ± 0.9^{ab}	57.1 ± 1.2^{cde}
M-110	38.9 ± 3.0^{bc}	31.3 ± 6.6^{ab}	41.6 ± 4.5^{b}	18.2 ± 5.4^{cd}	58.8 ± 4.5^{de}	46.9 ± 1.6^{def}
M-119	45.5 ± 2.6^{bc}	38.5 ± 12.3^{ab}	39.3 ± 2.0^{bc}	38.2 ± 4.3^{ab}	53.8 ± 1.1^{ef}	72.4 ± 3.8^{bc}
M-120	45.3 ± 6.9^{bc}	26.5 ± 7.3^{ab}	34.9 ± 4.0^{bcd}	23.1 ± 1.5^{bcd}	37.9 ± 2.1^{gf}	46.7 ± 4.8^{def}
M-121	47.8 ± 6.9^{bc}	38.6 ± 5.4^{ab}	67.3 ± 2.6^{a}	19.3 ± 2.8^{cd}	80.3 ± 1.6^{abc}	80.4 ± 3.6^{ab}
M-127	83.3 ± 2.3^{a}	56.4 ± 12.7^{a}	69.3 ± 3.8^{a}	32.2 ± 2.0^{bc}	53.2 ± 5.1^{ef}	95.7 ± 0.9^{a}
V5W2	nt	nt	nt	nt	87.3 ± 1.4^{ab}	69.5 ± 5.2^{bc}
P-value	<0.0001	0.0159	<0.0001	<0.0001	<0.0001	<0.0001

In control 1 the pH was unadjusted (5.78, 5.91 and 5.71) and in control 2, the pH was adjusted to the average pH of the culture filtrates (7.84, 7.49 and 7.65) in bioassay 1, 2 and 3 respectively. Mortality rates were determined after rinsing out nematodes from the culture filtrates and incubating them in sterile distilled water for 24 hrs. In each column, the means followed by the same letter (superscript) are not significantly different at P>0.05 according to Tukey's studentized range test. nt, not tested. n=4.

Table 7: Percentage paralysis of mixed stages of *Radopholus similis* (males, females and juveniles) following treatment with culture filtrates of seventeen endophytic Fusarium spp. isolates obtained from roots sin Masaka for 6 hrs.

	Bioassay number*					
Treatment	1	2	3	4	5	6
Control 1	22.9 ± 5.1^{bc}	20.4 ± 1.2^{c}	55.8 ± 3.8^{def}	21.2 ± 3.5^{g}	44.1 ± 0.9^{fg}	55.1 ± 3.0^{c}
Control 2	19.4 ± 2.9^{c}	25.1 ± 3.7^{c}	34.4 ± 2.1^{f}	31.6 ± 0.6^{fg}	37.2 ± 4.4^{g}	25.6 ± 2.3^{d}
M122	89.9 ± 3.9^{a}	24.2 ± 1.6^{c}	36.1 ± 1.3^{f}	nt	nt	nt
M290	80.8 ± 2.0^{a}	61.2 ± 4.0^{ab}	61.5 ± 3.4^{de}	nt	nt	nt
M304	97.7 ± 1.1^{a}	35.8 ± 2.0^{c}	68.1 ± 2.7^{cde}	64.8 ± 2.3^{abc}	81.9 ± 0.6^{bcd}	62.4 ± 3.6b^{c}
M321	39.3 ± 5.6^{b}	66.6 ± 4.2^{a}	53.1 ± 9.8^{def}	nt	nt	nt
M343	91.9 ± 3.5^{a}	59.8 ± 7.4^{ab}	73.8 ± 1.7^{bcd}	nt	nt	nt
M360	33.1 ± 2.4^{b}	41.5 ± 8.6^{bc}	93.1 ± 0.8^{ab}	nt	nt	nt
M379	92.3 ± 4.3^{a}	66.7 ± 4.6^{a}	50.5 ± 1.2^{ef}	32.5 ± 3.4^{fg}	67.8 ± 5.6^{e}	50.3 ± 2.1^{c}
M387	87.2 ± 4.1^{a}	65.9 ± 2.1^{a}	89.7 ± 1.7^{abc}	70.4 ± 3.1^{ab}	77.3 ± 2.1^{cde}	90.7 ± 3.0^{a}
M388	22.8 ± 4.6^{bc}	57.1 ± 3.7^{ab}	86.3 ± 2.0^{abc}	nt	nt	nt
M428	91.4 ± 2.8^{a}	34.3 ± 2.8^{c}	73.7 ± 7.2^{bcd}	nt	nt	nt
M459	94.5 ± 1.3^{a}	60.5 ± 1.9^{ab}	70.7 ± 3.0^{cde}	35.1 ± 6.2^{efg}	76.4 ± 2.4^{de}	52.5 ± 5.0^{c}
M291	nt	nt	nt	53.8 ± 2.7^{bcd}	99.4 ± 0.3^{a}	53.7 ± 3.9^{c}
M325	nt	nt	nt	28.5 ± 6.2^{fg}	90.9 ± 1.5^{abc}	57.3 ± 2.9^{c}
M329	nt	nt	nt	73.6 ± 1.3^{a}	51.1 ± 3.8^{f}	66.1 ± 4.8^{bc}
M365	nt	nt	nt	29.2 ± 1.0^{fg}	99.0 ± 0.7^{a}	79.8 ± 3.6^{ab}
M385	nt	nt	nt	30.9 ± 4.1^{fg}	91.3 ± 1.2^{ab}	50.7 ± 4.8^{c}
M419	nt	nt	nt	38.5 ± 2.9^{def}	87.7 ± 4.0^{abcd}	77.7 ± 7.2^{ab}
V5W2	nt	nt	97.3 ± 1.4^{a}	50.5 ± 1.8^{cde}	92.9 ± 1.1^{ab}	64.1 ± 1.2^{bc}
P-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

* Bioassays 1 – 6 are repeat bioassays. In control 1 the pH was unadjusted (5.83, 5.62, 6.54, 6.02, 6.01 and 5.71) and in control 2, the pH was adjusted to the average pH of the culture filtrates (7.71, 8.58, 7.06, 8.52, 7.86 and 7.65) in bioassay 1, 2, 3, 4, 5 and 6, respectively. In columns, means followed by the same letter are not significantly different at $P>0.05$ according to Tukey's Studentized Range Test. nt, not tested. n=4.

Table 8: Percentage mortality of mixed stages of *Radopholus similis* (males, females and juveniles) following treatment with culture filtrates of seventeen endophytic Fusarium spp. isolates obtained from roots sin Masaka for 6 hrs.

	Bioassay number*					
Treatment	1	2	3	4	5	6
Control 1	46.1 ± 5.1^{b}	80.8 ± 3.5^{a}	15.3 ± 3.3^{c}	26.9 ± 3.6^{b}	18.7 ± 2.4^{f}	80.6 ± 2.8^{a}
Control 2	41.5 ± 5.6^{b}	9.2 ± 2.4^{h}	19.8 ± 3.3^{c}	25.6 ± 2.5^{b}	40.6 ± 1.4^{de}	74.8 ± 2.1^{a}
M122	81.8 ± 1.2^{a}	8.9 ± 1.2^{h}	24.2 ± 2.3^{c}	nt	nt	nt
M290	88.8 ± 3.4^{a}	33.1 ± 4.4^{defg}	80.8 ± 14.6^{ab}	nt	nt	nt
M304	46.5 ± 4.6^{b}	76.1 ± 1.4^{ab}	59.5 ± 2.1^{b}	87.6 ± 1.9^{a}	50.7 ± 1.4^{a}	12.9 ± 2.4^{cd}
M321	38.9 ± 2.7^{b}	42.9 ± 1.2^{de}	30.1 ± 7.1^{c}	nt	nt	nt
M343	30.4 ± 4.3^{b}	17.8 ± 1.7^{gh}	10.8 ± 1.8^{c}	nt	nt	nt
M360	31.6 ± 4.8^{b}	63.2 ± 5.7^{bc}	33.1 ± 1.5^{c}	nt	nt	nt
M379	51.1 ± 3.4^{b}	48.8 ± 4.7^{cd}	70.1 ± 2.3^{b}	27.2 ± 4.9^{b}	48.6 ± 1.9^{a}	31.6 ± 6.4^{bc}
M387	44.3 ± 4.7^{b}	32.3 ± 3.5^{efg}	8.1 ± 1.5^{c}	28.4 ± 1.6^{b}	52.7 ± 3.3^{a}	98.7 ± 0.7^{d}
M388	28.1 ± 5.6^{b}	38.9 ± 2.6^{def}	10.9 ± 2.4^{c}	nt	nt	nt
M428	41.8 ± 6.0^{b}	23.5 ± 3.2^{fgh}	8.7 ± 2.3^{c}	nt	nt	nt
M459	42.1 ± 6.7^{b}	35.4 ± 2.7^{def}	12.2 ± 1.5^{c}	26.4 ± 5.7^{b}	37.9 ± 3.5^{cd}	88.7 ± 2.0^{a}
M291	Nt	nt	nt	85.4 ± 3.4^{a}	47.6 ± 6.9^{a}	5.0 ± 3.1^{d}
M325	Nt	nt	nt	40.2 ± 15.4^{b}	45.5 ± 3.4^{ab}	90.5 ± 0.7^{a}
M329	Nt	nt	nt	74.5 ± 4.6^{a}	53.4 ± 4.9^{a}	24.3 ± 4.6^{bc}
M365	Nt	nt	nt	21.9 ± 4.5^{b}	39.0 ± 4.9^{cb}	88.9 ± 2.6^{a}
M385	Nt	nt	nt	20.1 ± 3.8^{b}	28.9 ± 5.6^{ef}	86.5 ± 2.5^{a}
M419	Nt	nt	nt	27.0 ± 0.8^{b}	42.2 ± 3.2^{cd}	35.5 ± 8.8^{b}
V5W2	Nt	nt	96.2 ± 1.7^{a}	27.7 ± 4.4^{b}	62.1 ± 2.9^{ab}	19.1 ± 1.1^{cd}
P-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

* Bioassays 1 – 6 are repeat bioassays. In control 1, the pH was unadjusted to 5.83, 5.62, 6.54, 6.02, 6.01 and 5.71 and in control 2, the pH was adjusted to the average pH of the culture filtrates (7.71, 8.58, 7.06, 8.52, 7.86 and 7.65) in bioassay 1, 2, 3, 4, 5 and 6, respectively. In each column, means followed by the same letter (superscript) are not significantly different at *P*=0.05 according to Tukey's studentized range test. nt, not tested. n=4.

Table 9: Percentage paralysis and mortality of mixed stages of *Radopholus similis* in different culture filtrate concentrations of two endophytic *Fusarium oxysporum* isolates after 6 hrs exposure.

Isolate		% paralyzed *R. similis*		% mortality of *R. similis*	
V5W2	CF concentration (%)	Bioassay 1	Bioassay 2	Bioassay 1	Bioassay 2
	100	75.1 ± 9.3^{a}	81.4 ± 0.9^{a}	95.7 ± 0.1^{a}	95.8 ±2.1^{a}
	50	63.9 ± 2.5^{a}	67.1 ± 4.2^{b}	68.0 ± 4.4^{b}	52.3 ±3.1^{b}
	25	57.9 ± 4.5^{a}	61.8 ± 2.1^{b}	19.3 ± 4.2^{c}	20.9 ±1.4^{c}
	10	46.8 ± 10.0^{a}	49.4 ± 1.6^{c}	13.1 ± 3.8^{c}	23.9 ±2.5^{c}
	5	52.6 ± 5.4^{a}	41.8 ± 1.1^{c}	22.6 ± 5.6^{c}	21.1 ±2.5^{c}
	2.5	51.5 ± 9.3^{a}	39.4 ± 1.1^{c}	22.9 ± 3.3^{c}	23.2 ±1.4^{c}
	P-value	0.1682	<0.0001	<0.0001	<0.0001
	LC50	-	-	21.9 ± 2.5	20.4 ± 6.4
Eny1.31i	CF concentration (%)	Bioassay 1	Bioassay 2	Bioassay 1	Bioassay 2
	100	89.7 ± 0.7^{a}	95.2 ± 1.4^{a}	96.8 ± 1.1^{a}	98.5 ± 1.4^{a}
	50	53.4 ± 4.5^{b}	80.7 ± 2.8^{b}	75.7 ± 5.7^{b}	77.9 ± 4.2^{b}
	25	57.4 ± 6.3^{b}	70.2 ± 1.5^{b}	27.4 ± 3.4^{c}	33.2 ± 1.2^{c}
	10	43.5 ± 1.5^{b}	55.2 ± 2.3^{c}	17.7 ± 1.1^{c}	37.7 ± 1.9^{c}
	5	42.9 ± 6.4^{b}	50.3 ± 2.1^{c}	13.6 ± 2.7^{c}	34.7 ± 4.9^{c}
	2.5	43.4 ± 6.3^{b}	51.6 ± 3.1^{c}	23.1 ± 3.3^{c}	36.0 ± 3.9^{c}
	P-value	<0.0001	<0.0001	<0.0001	<0.0001
	LC50	-	-	18.4 ± 5.2	9.6 ± 1.7
Control 1	-	49.5 ± 6.1^{b}	46.2 ± 1.1^{c}	19.5 ± 3.6^{c}	36.8 ±1.3^{c}
Control 2	-	40.3 ± 3.1^{b}	20.8 ± 2.9^{c}	22.1 ± 4.9^{c}	64.4 ±3.9^{b}

CF= culture filtrate. In control 1, the pH was unadjusted to 5.29 and 5.22, and in control 2, the pH was adjusted to the average pH of the test fungal filtrates (8.21 and 8.68) in bioassay 1 and 2, respectively. Means followed by the same letter (superscript) for each isolate in each bioassay are not significantly different at P=0.05 according to Tukey's studentized range test. LC50 values were calculated using the Calcusyn 1.1 software for dose-effect relationships. n=4.

Figure 1: Percentage hatch of *Radopholus similis* eggs in culture filtrates of nine endophytic Fusarium oxysporum isolates after 3, 5, 9, 12 and 15 days incubation, sdw = sterile distilled water control, Control CF=Un-inoculated broth. A, B and C are repeat bioassays. Errors bars represent standard errors of the mean, n=3.