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EFFECTS OF FIVE FUNGAL ISOLATES ON HATCHING AND PARASITISM OF ROOT-KNOT NEMATODE EGGS, JUVENILES AND FEMALES[1]

by
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Summary. *Paecilomyces lilacinus, Phoma herbarum* and three isolates of *Fusarium oxysporum* differed significantly in their ability to parasitize eggs and females of *Meloidogyne javanica. P. lilacinus* and *F. oxysporum-1* significantly ($P<0.05$) parasitized more than 70% of eggs and females while *F. oxysporum-3* parasitized less than 20%. Also, *P. lilacinus* and *F. oxysporum-1* had the greatest suppressive effect on hatching. In general, control Petri-dishes and those treated with *F. oxysporum-3* had the highest proportions of hatched eggs, but exhibited the least levels of egg parasitism. The fungus *P. lilacinus* significantly ($P<0.05$) parasitized eggs of *M. javanica, M. incognita* and *M. arenaria* but no significant differences were detected in the levels of parasitism.

Root-knot nematodes are serious pests in Kenya and efforts have been directed towards the screening and development of fungal antagonists for use in nematode control (Makhatsa *et al*, 1993; Oduor-Owino *et al*, 1993). A wide range of fungi have been isolated (Oduor-Owino and Waudo, 1995) though only *Paecilomyces lilacinus* (Thom) Samson and *Fusarium* spp. had a significant effect on nematode eggs (Oduor-Owino *et al* 1993; Oduor-Owino *et al*, 1996); some of the isolates have been deposited at the International Mycological Institute with reference numbers, 357758 to 357762 (Oduor-Owino and Waudo, 1995).

This paper describes *in-vitro* tests conducted to evaluate the effects of five different fungal isolates on hatching and parasitism of *Meloidogyne javanica* (Treub) Chitw, juveniles, eggs and females. Results on fungal parasitism of *M. javanica, M. incognita* (Kofoid et White) Chitw. and *M. arenaria* (Neal) Chitw. eggs by *P. lilacinus*, the most virulent egg pathogen isolated, are also presented.

Materials and methods

Five different fungal isolates were obtained from root-knot nematode eggs and used in six tests. Egg masses of *M. javanica* were collected from tomato (*Lycopersicon esculentum Mill.*) cv Moneymaker grown in fungi/nematode infested field soil collected from Kakamega district in Kenya. The egg masses were dispersed in distilled water and the suspension passed through 150, 75 and 38 mm aperture sieves. Eggs collected on the 38 mm aperture sieve were washed five times with distilled water and resuspended in 20 ml of distilled water. One ml of the suspension containing c. 1000 eggs was poured onto a Petri-plate containing 1.5% water agar (WA) medium supplemented with 0.1%

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chloramphenicol solution (Sikora *et al*, 1990). All plates were randomly arranged on a greenhouse bench and left for two days.

After the incubation period, individual eggs were aseptically transferred onto new WA plates and incubated for four days to allow fungal growth. To obtain pure fungal cultures, each fungal colony was aseptically transferred to new WA plates using a sterile platinum loop and again incubated for four days. Single fungal colonies were transferred and cultured on potato dextrose agar medium (PDA) in 9 cm-diameter Petri-dishes for ten days at 20 °C. The fungi were sent to Prof. R.A. Samson of the Central Bureau Voor Schimel cultures, The Netherlands, for identification. *F. oxysporum, P. lilacinus* and *Phoma herbarum* were frequently isolated and were therefore used in the six tests described below.

In test 1, the effect of isolates of *F. oxysporum, P. lilacinus* or *P. herbarum* on the parasitism of *M. javanica* eggs was determined on WA. Fungi free eggs were obtained from tomato cv Moneymaker grown in sterile sand. PDA plugs (7 mm diameter) were cut from the periphery of ten-day-old fungal colonies with a cork borer and placed at the centre of 1.5% WA in 9-cm-diameter Petri-dishes. Each plate was inoculated with a single plug and incubated at 20 °C until the whole agar surface was covered with the mycelium of a particular fungus. One ml of sterile distilled water containing c. 1000 eggs was then spread over each fungal-covered WA surface. Control plates had one ml of distilled water and a non-colonized PDA plug. The plates were arranged in a randomized design with ten replicates and incubated for two weeks at 25 °C (Freire and Bridge, 1985). After the incubation period, 100 eggs per plate were examined at random using a microscope at x 100 mg, and the proportion parasitized was determined (Kerry and Crump, 1977).

The antagonistic potential of the five fungal isolates on *M. javanica* juveniles was assessed in test 2. Freshly hatched juveniles obtained from egg masses were rinsed with sterile distilled water and then placed on a 38 µm sieve resting above water in a shallow dish. The egg masses were then left for one week to hatch at room temperature. After the incubation period, hatched juveniles were washed three times with sterile distilled water. A 1 ml suspension of c. 1000 juveniles in water was transferred onto each WA plate covered with the mycelium of the particular fungal isolate. The control treatment had the same quantity of juveniles added to WA plates inoculated with fungal-free (uncolonized) PDA plugs. All plates were incubated for 14 days at 25 °C in a randomized design with ten replicates. The proportion of juveniles parasitized, if any, was determined by randomly counting 100 juveniles per plate using a Hawksley's slide and a stereomicroscope at x 400 magnification.

The ability of the five fungal isolates to parasitize eggs within egg masses was investigated in test 3. Fungal-free egg masses of *M. javanica* were picked from galled roots of tomato cv Moneymaker maintained in a glasshouse. They were washed five times with distilled water and put in Petri-dishes containing 1.5% WA medium supplemented with 0.1% chloramphenicol solution (Sikora *et al.*, 1990). Ten plates each received four egg masses per plate. The plates had been centrally inoculated with 7 mm diameter PDA plugs of the respective fungal isolates and incubated at 25 °C for 11-days when the whole agar surface was covered with mycelium of each fungus. Control treatments were represented by plates containing four egg masses per plate but centrally inoculated with a fungal-free-7 mm diameter PDA plug. All plates were incubated for 14 days at 25 °C in a randomized design with ten replicates. After the incubation period, the amount of egg parasitism was determined by crushing the egg masses in a tissue homogenizer, spreading one ml of egg suspension containing c. 1000 eggs on WA and counting the proportion of eggs parasitized (i.e. those showing fungal growth) (Kerry and Crump, 1977).

The affect of fungal isolates on hatching was assessed in test 4. Four fungal-free egg masses from glasshouse cultures were crushed and the released juveniles (J_O) and intact eggs suspended in 10 ml of distilled water and then counted. The juvenile/egg suspension was then spread onto 1.5% WA which had been centrally inoculated with PDA plugs of the respective fungal isolates and incubated. Control plates had no fungus.

After 14 days, the percentage of eggs hatched (% hatch) was determined at x 400 magnification using the formula of Kaplan *et al.* (1992):

$$\% \text{ Hatch} = \frac{J_{14}\text{-}J_O \times 100}{Eggs_O}$$

Where J_{14} = the number of juveniles observed at day 14

J_O = the number of juveniles observed at day zero

$Eggs_O$ = the number of intact eggs counted at day zero

Mature fungal-free *M. javanica* females from a glass house culture were used in test 5 to assess their parasitism on WA. The females were dipped in 0.5% sodium hypochlorite solution (NaOCL) for one minute, washed three times with sterile distilled water and once in 0.1% chloramphenicol (antibiotic) solution. One hundred surface sterilized females were then put on WA plates that had been centrally inoculated with a 7 mm-diameter PDA plug of the respective fungal isolate. Control plates had females treated as above and placed on WA plates inoculated with PDA medium without any fungus. All plates were replicated ten times and kept in a randomized design for ten days at 25 °C. The number of females parasitized (those exhibiting fungal growth) was counted.

The relative susceptibility of *M. javanica, M. incognita* and *M. arenaria* eggs to *P. lilacinus* was determined in test 6 to evaluate the host range of this isolate. The fungus was chosen because it had the highest levels of egg parasitism in all the tests and had consistent parasitic effects on *M. javanica* eggs in previous studies (Makhatsa *et al*, 1993). The egg masses of *M. incognita* and *M. arenaria* were provided by Prof. De Guiran of INRA, France. *M. javanica* eggs were isolated from farmer's fields in Nairobi Kenya. The eggs from Kenya and France were used to inoculate (separately) tomato cv Moneymaker (grown in the glass house). Fungal-free egg masses for each nematode species were picked off from galled roots after three subsequent nematode generations and were homogenized in 10 ml of sterile distilled water. The released eggs were suspended in 20 ml of distilled water before dispensing 1 ml of the egg-suspension containing c. 1000 eggs onto Petri-dishes containing 1.5% WA medium supplemented with chloramphenicol solution. The WA surfaces were covered with the mycelium of *P. lilacinus* that had been transferred onto the plates 11 to 18 days before egg-plating. Control plates had one PDA plug each but no fungus. All plates were arranged on the laboratory bench for 14 days with six replicates per nematode species. After the incubation period, egg parasitism was determined as described by Kerry and Crump (1977).

ANOVA was used for data analysis.

Results and discussion

The five fungal isolates significantly differed ($P < 0.05$) in the extent to which they parasitized *M. javanica* eggs and females. The highest percentage of eggs parasitized was obtained with *P. lilacinus* (71%) and *F. oxysporum*-1 (Fo-1) (68%). These values were significantly higher than those obtained with *P. herbarum* (57%), *F. oxysporum-2* (Fo-2) (24%) and *F. oxysporum-3* (Fo-3) (18%). The above five fungi parasitized 92, 82, 29, 26 and 9% of eggs within egg masses, respectively. *P. lilacinus* (PL) signif-

icantly parasitized more females (87%) than all the other isolates (Fig. 1). The least parasitism (19%) was associated with *F. oxysporum-3* (Fo-3) (Fig. 1). *M. javanica* juveniles were not parasitized. In general, *P. lilacinus* parasitized eggs of *M. javanica, M. incognita* and *M. arenaria* equally.

The fungi *P. lilacinus, F. oxysporum-1* (Fo-1) and *P. herbarum* which parasitized most eggs *in vitro* had the greatest inhibitory effect on hatching, though their effects on hatching were similar (Fig. 2). Control plates had the greatest ($P < 0.05$) proportions of eggs hatched (Fig. 2).

Although high levels of females and egg were parasitized on WA, this may not reflect waht could be achieved in the natural soil ecosystem where rhizophere competence is high; up to 30.9% of *M. javanica* eggs were parasitized in sterile soil (Oduor-Owino *et al.*, 1996). However, the high proportion of females parasitized reveals that *M. javanica* females were a better substrate for *P. lilacinus* and *F. oxysporum-1* (Fo-1) than the eggs or juveniles. Since female root-knot nematodes play a vital role in enhancing nematode populations, studies on the interaction between fungal antagonists and female root-knot nematodes should be intensified in order to gain more information that could be utilized in the management of these organisms in the soil ecosystem.

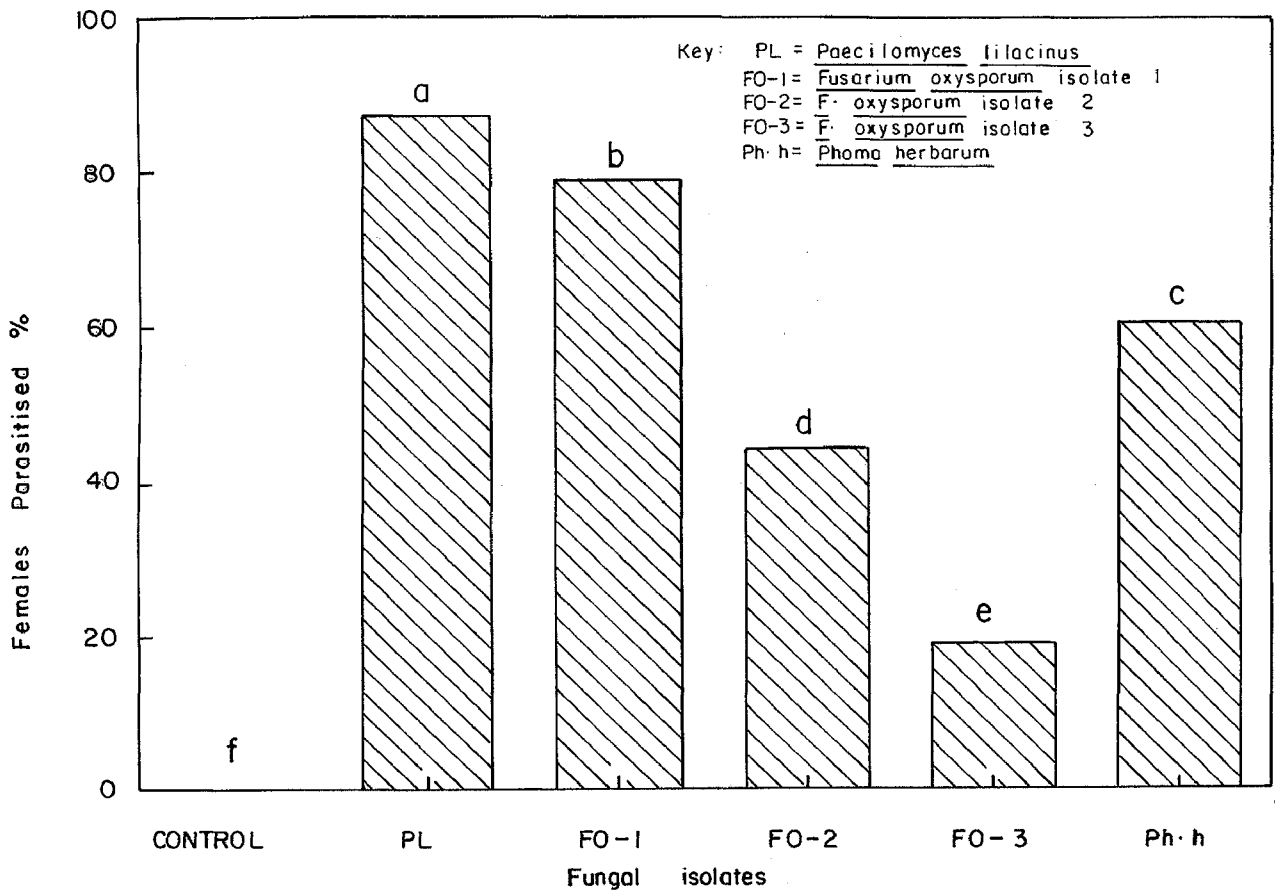

Fig. 1 - Parasitism (%) of *Meloidogyne javanica* females on water agar by five fungal isolates. Bars with different letters are significantly ($P = 0.05$) different according to Duncan's Multiple Range Test.

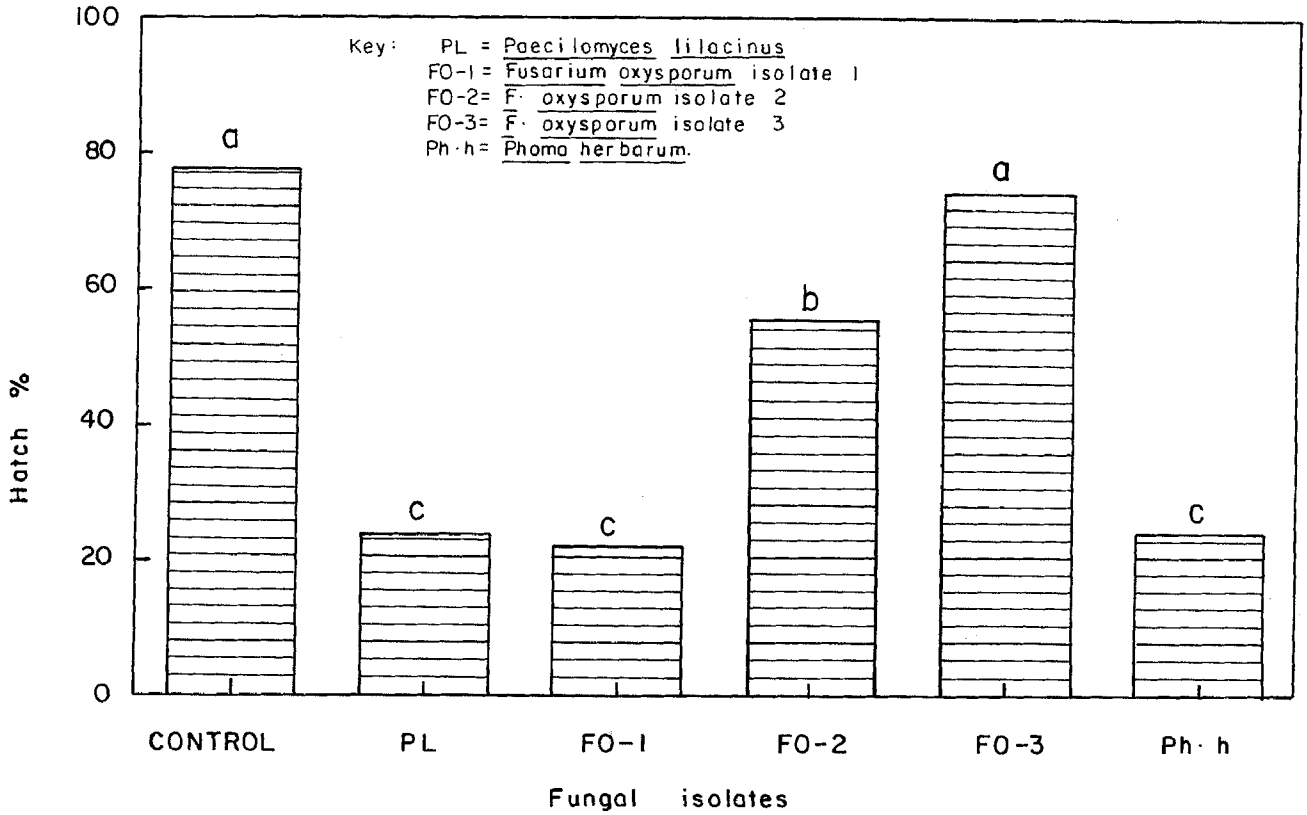

Fig. 2 - Mean percentages (%) of hatched *M. javanica* juveniles as influenced by five fungal isolates. Bars with different letters are significantly (P = 0.05) different according to Duncan's Multiple Range Test.

In general, the ability of fungal isolates to parasitize eggs and inhibit hatching as observed with *P. lilacinus* and *F. oxysporum-1* (Fo-1) is an important factor in the biological control of phytonematodes. Such isolates would help to reduce the population of infective juveniles below the injurious threshold. Therefore, more work should be conducted along this line in order to develop and acquire isolates with adequate antagonistic potential against root-knot nematodes.

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