NEMATOPHAGAL ABILITY OF JORDANIAN ISOLATES OF *PAECILOMYCES VARIOTII* ON THE ROOT-KNOT NEMATODE *MELOIDOGYNE JAVANICA*

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Summary. The distribution and nematophagal ability of local isolates of *Paecilomyces variotii* against the root-knot nematode (RKN), *Meloidogyne javanica*, was investigated under laboratory conditions. Eighty RKN-infected root samples from fig trees, tomato, aubergine and cucumber were collected from three geographical areas of Jordan (Safi, Central Jordan Valley and Jerash). *Paecilomyces variotii* occurred in 10% of the samples and was found in both females and egg masses of *M. javanica*. The local isolates of *P. variotii*, as nematode antagonists, resulted in egg parasitism of about 61.4% compared to 68.5% for *P. lilacinus*. Moreover, both species were able to parasitize females and freed eggs and to reduce hatch of second stage juveniles. Under laboratory conditions, *P. lilacinus* parasitized females on agar plates significantly more than local isolates of *P. variotii*. Isolates of *Paecilomyces lilacinus* and *P. variotii* parasitized heat-killed eggs to similar extents, but at rates higher than those of live eggs inside egg masses, suggesting that both species possess high saprophytic ability.

Keywords: Antagonistic fungi, biological control, Jordan, *Paecilomyces lilacinus*.

The root-knot nematodes (RKN), *Meloidogyne* spp., are considered among the most damaging and widely distributed nematode pests. These sedentary endo-parasites attack a wide range of crops (Barker *et al.*, 1985) and reduce world crop production by 5% (Sasser and Carter, 1985). In Jordan, RKN have been reported since 1962 (Abu-Gharbieh, 1992) and *M. javanica* (Treub) Chitw., *M. incognita* (Kofoid *et* White) Chitw., and *M. arenaria* (Neal) Chitw. have been found in many crops under irrigation, *M. javanica* being the most widely distributed species (Abu-Gharbieh *et al.*, 2005). The cultivated area in Jordan exceeds 215,000 hectares, concentrated in two regions: the Ghor Rift Valley (250-450 m below sea level) and the Eastern uplands (more than 350 m above sea level).

Several measures have been employed worldwide to control RKN, namely: legislative, cultural, physical, chemical and biological (Abu-Gharbieh, 1992). Interest in the use of biological control agents has increased significantly during the past 30 years. According to Kerry (1992), the withdrawing of several nematicides from the market, for health and environmental reasons, has lead to the use of nematophagous fungal and bacterial bio-agents in a number of soils and subsequently to the development of a number of commercial products based on nematophagous fungi and bacteria.

In this context, the efficacies of several nematophagous fungi have been cited as making them promising bio-agents and/or antagonists. Of these, nematode trapping fungi (*Arthrobotrys conoides, A. dactyloides*) (Hazmi *et al.*, 1982), fungal parasites of eggs and females (*Paecilomyces lilacinus* (Thom) Samson, *Dactylella oviparasitica* Stirling *et* Mankau, *Pochonia chlamydosporia* Zare, Gams *et* Evans, *Hirsutella rhossiliensis* Minter *et* Brady (Stirling and Mankau, 1979; Jatala, 1986; Jaffee *et al.*, 1994), and several other fungi (*Paecilomyces variotii* Bainier, *Fusarium equiseti* (Corda) Sacc. produce metabolites toxic to nematodes (Mankau, 1980; Sayer, 1980). In a recent investigation on fungi associated with RKN, *P. variotii* was isolated from three locations in Jordan (Hijaz, 2003), wherein the proportion of eggs parasitized by one isolate of *P. variotii* reached 88.8%.

However, little information is available on whether *P. variotii* isolated from some RKN females and eggs in Jordan is parasitic, antagonistic or a saprophytic colonizer. This study aimed to investigate the geographical distribution and nematophagal ability of local isolates of *P. variotii* against *M. javanica*, under laboratory conditions.

MATERIALS AND METHODS

Fungal isolates. Eighty *M. javanica*-infected root samples were taken from fig trees, tomato, aubergine and cucumber plantations. Samples were collected during Spring of 2007 from three geographically different agricultural areas of Jordan, as follows: 35 samples from Safi (southern Ghors, 450 m below sea level), 30 samples from the Central Jordan Valley (central Ghors, 250 m below sea level), and fifteen samples from Jerash (500 m above sea level). The areas sampled are among the major vegetable production areas in Jordan, where RKN-infested open field plantations are under low chemical control measures. Twenty-five samples were

taken from farms of the FAO Regional Project for IPM (Farmer Field Schools) distributed in the Safi area and the Central Jordan Valley. Ten egg masses and ten females were hand-picked from each sample using a stereomicroscope, surface sterilized with 70% alcohol for about two minutes, and washed with sterile distilled water (SDW). Egg masses and females were placed in 90 mm diameter Petri dishes containing approximately 15 ml of Potato Dextrose Agar (PDA) medium (39 g PDA and 1 g streptomycin sulphate in 1,000 ml Distilled Water (DW) (Morgan-Jones *et al.*, 1981)) and incubated for 10 days at 25 ºC. Fungal colonies, isolated from females and egg masses, were purified and cultured on PDA at 25 ºC. Four isolates were sent for identification to the Plant Clinic Diagnostic and Advisory Service (CABI biosciences, Bakeham Lane, UK). A standard isolate of *P. lilacinus* (strain CBS 432.87 from Peru) was obtained from Centraalbureau voor Schimmelcultures (CBS, Netherlands) and used for comparison.

RKN isolation and rearing. An isolate of RKN was obtained, from a field of aubergine in the Central Jordan Valley and was identified as *Meloidogyne javanica*, according to the morphology of the perineal patterns of females (Hirschmann, 1985). Root-knot nematode eggs were extracted from a single egg mass and surface-sterilized using 0.5% sodium hypochlorite solution (Barker *et al.*, 1985). Newly emerged second stage juveniles were reared on roots of aubergine grown in methyl bromide sterilized soil (453 g/10 m^3 soil). The RKN isolate was then increased on aubergine plants in pots in a greenhouse at 25-30 °C.

Fungal parasitism of M. javanica *eggs inside egg masses*. Galled aubergine roots were dissected under a stereomicroscope and RKN egg masses were hand-picked (Barker *et al.*, 1985). Ten egg masses per fungal isolate were used for each replicate. Egg masses were washed with DW to remove soil debris, surface-sterilized with 70% alcohol for about two minutes and washed again three times with SDW (Morgan-Jones *et al.*, 1981), before they were transferred to 5-cm-diameter plates containing 1% agar medium: 10 g agar and 1 g streptomycin sulphate in 1,000 ml DW. Petri dishes were previously inoculated centrally with a 5 mm disc taken from the edge of 7-day-old colonies of *P. variotii* or *P. lilacinus* growing on PDA, using a heat sterilized cork borer, and then ten egg masses were placed about 2-3 mm away from the fungus (Freire and Bridge, 1985).

The saprophytic ability of eight local *P. variotii* isolates was assessed on heat-killed egg masses obtained by heating at 60 ºC for 5 minutes (Stirling and Mankau, 1978). Control treatments for all experiments were obtained following the same procedures as for the fungus-inoculated treatments, except for using PDA discs that were not inoculated with fungus. Dishes of all treatments were randomly kept at 25 ºC under continuous white illumination for 15 days and each treatment was replicated five times.

The numbers of parasitized eggs were assessed after extracting the eggs from the infected egg masses. Egg masses were shaken in 1% sodium hypochlorite solution for 4 minutes (Barker *et al.*, 1985) and freed eggs were collected by sieving through a 20 µm mesh sieve, washed several times with SDW and re-suspended in 10 ml SDW. Two drops of 0.1% lactophenol cotton blue were added to help in differentiation between healthy and infected eggs. Half a millilitre of suspension was examined under a microscope at 100× magnification and three counts were taken for each isolate. Emerging juveniles were counted directly in the agar plates under a stereomicroscope at 45× magnification. Another 0.5 ml egg suspension was placed on PDA plates and kept under the same conditions for 15 days to re-isolate and confirm the identity of the fungus in the parasitized eggs.

Fungal parasitism of freed M. javanica *eggs*. In order to assess the role of the gelatinous matrix in the parasitism process, freed *M. javanica* eggs were collected on a sieve of 20 µm aperture by surface-sterilizing twenty egg masses with 1% sodium hypochlorite solution for 2 minutes. Eggs were then washed several times with SDW and re-suspended in 25 ml SDW. Half a millilitre of freed egg suspension was plated onto 5 cm diameter Petri dishes containing 1% agar previously inoculated with 5 mm PDA plugs containing the fungus. After 15 days, 50 eggs were examined under a microscope at 100× magnification and the numbers of parasitized and non-parasitized eggs were recorded.

Parasitism of M. javanica *females*. Galled aubergine roots were dissected under a stereomicroscope and females embedded in root tissues were hand-picked, surface sterilized with 70% alcohol and washed three times with SDW. Ten females were placed on 5-cm 1% agar plates previously inoculated with 5 mm discs taken from 7-day-old colonies of *P. variotii* or *P. lilacinus*. After 10 days, the numbers of parasitized females were recorded and parasitism was confirmed by examining females directly under a microscope at 400× magnification.

RESULTS

Identification and occurrence of P. variotii *isolates*. Eight out of the eighty (10%) RKN-infected root samples showed fungal colonies with morphological characteristics resembling those of the genus *Paecilomyces*. Positive samples were distributed in agricultural areas where very little chemical management was undertaken (Table I). Five *P. variotii* were isolated from Farmers Field Schools (FFS, Integrated Pest Management project in the Near East, FAO, Jordan).

Four isolates, namely Safi I, Safi II, CJVII and JII, were identified by CABI biosciences, UK, as *P. variotii* and deposited under IMI number 395999. The colonies grown on PDA had almost the same sandy-brown colour and irregular edges with conidiogenous cells pro-

Table I. Distribution and occurrence of *Paecilomyces variotii* associated with *Meloidogyne javanica* in Jordan.

Area/Location	*P. variotii* isolates	Isolated from	Host Plant	Samples associated with *P. variotii*/Total samples (% occurrence)
Safi/Southern Ghors	*Safi I*	Egg mass	Tomato	3/35 (8.6%)
	Safi II	Female	Tomato	
	Safi III	Egg mass	Tomato	
Kraimeh/Central Jordan Valley	*CJV I1*	Female	Aubergine	3/30 (10%)
	CJV I2	Female	Aubergine	
	CJV II	Female	Cucumber	
Jerash	*J I*	Egg mass	Fig	2/15 (13.3%)
	J II	Egg mass	Fig	
Total samples associated with *P. variotii*/Total number of samples (Average % occurrence)				8/80 (10%)

ducing chains of ellipsoidal conidia, typical for *P. variotii* (Fig. 1), which is known to be a thermo-tolerant fungus producing the toxic metabolites patulin and viriditoxin (CABI identification report).

Parasitism of M. javanica *eggs*. All *P. variotii* local isolates revealed significantly (P = 0.05) greater ability in parasitizing eggs of *M. javanica* in egg masses on agar plates (average 58.5%), compared to the control treatment, but no significant differences among them were found. However, these values of parasitism were significantly lower than that of the isolate of *P. lilacinus* from Peru (average 68.5%) (Table II). On the other hand, the hatching percentages were significantly decreased in all isolates (average 24.0%), but with no significant differences among the isolates, compared to the control in

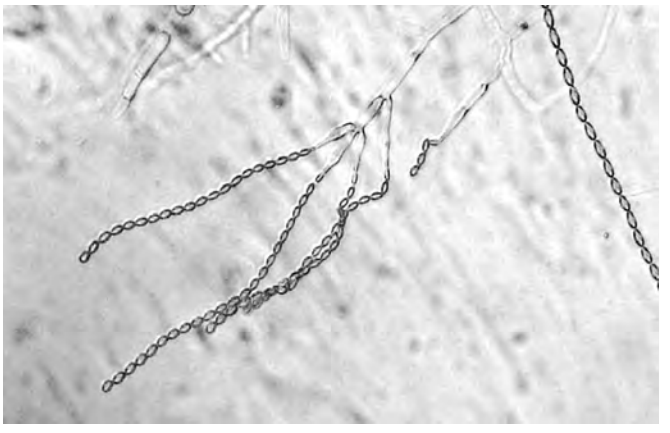

Fig. 1. *Paecilomyces variotii* conidiogenous cells producing chains of ellipsoidal conidia (400×).

Table II. Effects of *Paecilomyces variotii* and *P. lilacinus* isolates on live eggs and heat-killed eggs in egg masses, freed eggs, egg hatching and females of *Meloidogyne javanica* under laboratory conditions*

Treatment	Egg parasitism (%)	Egg hatching (%)	Heat-killed egg parasitism (%)	Freed egg parasitism (%)	Female parasitism (%)
P. lilacinus Peru	68.5 a	21.8 b	90.7 a	71.7 a	96.0 a
P. variotii Safi I	61.4 b	23.4 b	90.3 a	74.8 a	83.3 b
P. variotii Safi II	59.5 b	23.0 b	87.6 a	74.0 a	75.3 b
P. variotii Safi III	59.9 b	22.8 b	87.8 a	73.2 a	72.7 b
P. variotii CJV I1	58.3 b	26.2 b	84.7 a	71.6 a	75.3 b
P. variotii CJV I2	57.4b	24.4 b	89.0 a	72.1 a	83.3 b
P. variotii CJV II	58.8 b	24.5 b	87.6 a	70.0 a	86.0 b
P. variotii J I	55.9 b	24.8 b	85.7 a	72.3 a	69.3 c
P. variotii J II	56.5 b	23.2 b	89.6 a	72.4 a	76.7cb
Control	0.0 c	47.8 a	0.0 b	0.0 b	0.0 d

*Averages with the same letter in columns are not significantly different according to Duncan's multiple range test (P = 0.05).

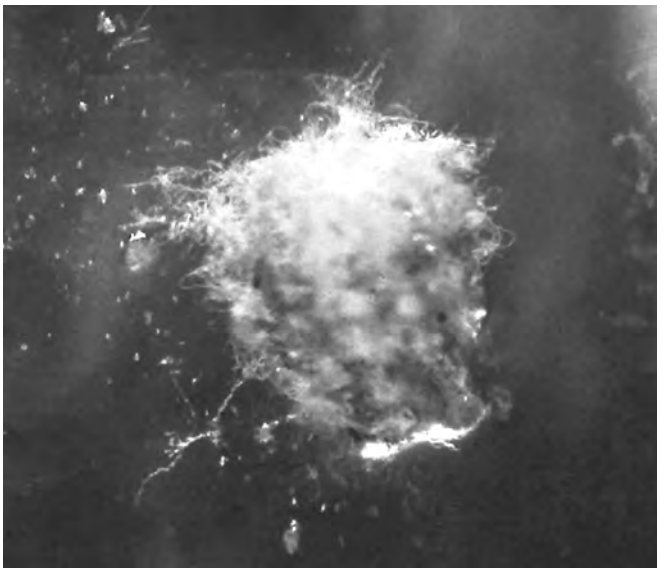

Fig. 2. *Meloidogyne javanica* egg mass parasitized by *P. variotii*, showing white mycelium growth protruding from the gelatinous matrix (45×).

which 47.8% of the eggs hatched on agar plates (Fig. 2, Table II).

The parasitism of heat-killed eggs inside egg masses by the isolates of *P. variotii* and *P. lilacinus* on agar plates was quite high (89.6%) and significantly different from the control, but with no significant differences among the fungus-inoculated treatments (Table II). Freed eggs on agar plates were also parasitized by *P. variotii* isolates (72.5%) and the *P. lilacinus* isolate (71.7%). Both fungi were significantly different from the control (Table II).

Parasitized egg masses exhibited white fungal hyphal growth protruding from the gelatinous matrix (Fig. 2). The fungus was successfully re-isolated from infected eggs (Fig. 3) and the identity was confirmed for both *P. variotii* and *P. lilacinus*.

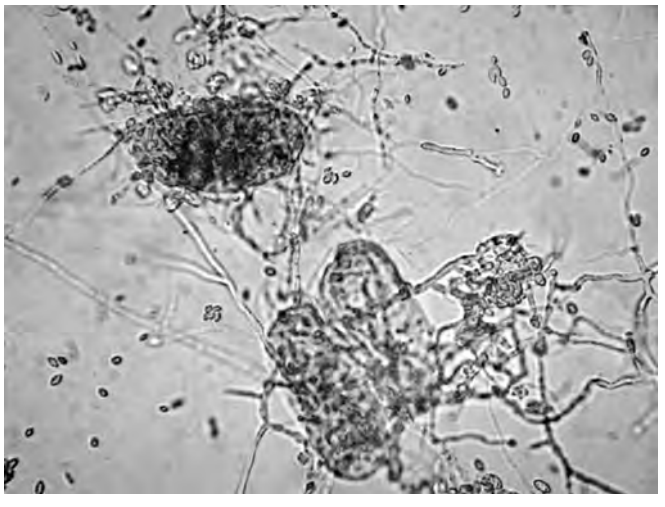

Fig. 3. *M. javanica* eggs parasitized by *P. variotii* as seen under microscope at 100× magnification.

Parasitism of M. javanica *females*. Nearly 96% of *M. javanica* females were parasitized by *P. lilacinus*, compared to 72.7-86% of the different isolates of *P. variotii* (Table II). Parasitism was observed 3 days after inoculation in females placed near the fungal disc. At this time, white fungal mycelium protruding from the parasitized female bodies was observed (Fig. 4). However, females which were inoculated further from the inoculated disc were still not parasitized after this time. The fungus identity was confirmed by examining diseased females under a microscope at 400×.

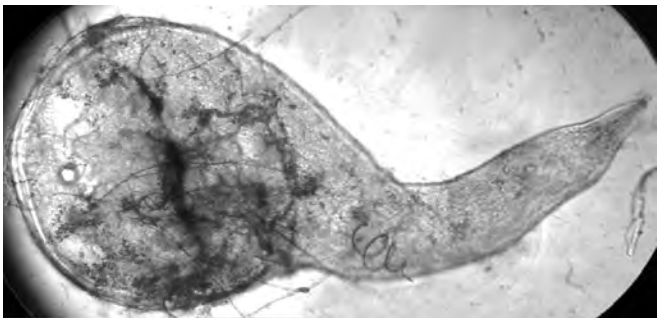

Fig. 4. *M. javanica* female parasitized by *P. variotii* observed three days after inoculation (100×).

DISCUSSION

As reported earlier by Hijaz (2003), our data confirmed that Jordanian soils harbour a good percentage of *P. variotii*. More than 60% of the isolates of this fungus (five out of eight isolates) were obtained from farms that apply integrated pest management according to a regional project (Integrated Pest Management Project in the Near East, FAO), suggesting that application of integrated soil management measures supports soil bioactivity whereas chemical dependent farms may not have this characteristic.

All isolates of *P. variotii* colonies grown on PDA were sandy-brown in colour and had irregular edges with conidiogenous cells producing chains of ellipsoidal conidia, while colonies of *P. lilacinus* were typically purple in colour. Although parasitism of eggs inside egg masses and females seems to be slightly lower in *P. variotii* than in *P. lilacinus*, the percentage of females parasitized by *P. variotii* isolates was greater than 70% for all isolates and both species gave acceptable parasitism percentages in all laboratory tests. This may suggest that local isolates of *P. variotii* act in nature as a nematode bio-agent, as they were isolated from different geographical areas with great diversity in climatic conditions and from different RKN- infected crops (Table I).

Paecilomyces lilacinus and local isolates of *P. variotii* were able to parasitize the heat-killed eggs in nearly equal rates, and by 30% more than the corresponding live eggs (non-heat-treated). This may suggest that both fungi possess saprophytic ability in addition to the abili-

ty to parasitize live eggs inside egg masses. We expect that such saprophytic ability will provide the local isolates of the fungus with an advantage for sustainability in the soil and may enable population build-up of these isolates. Such a phenomenon may be of particular importance in reducing the nematode population before the cropping season begins, particularly if the fungus is added right at the beginning of the growing season.

The local isolates of *P. variotii* are thought to be adapted to the local harsh soil conditions since they were first isolated from areas in which soil temperatures might exceed 40 ºC, a temperature to which such isolates are well adapted. It is expected that these thermotolerant isolates will prove to have long-term viability and exhibit good management efficiency compared with other, imported bio-agents, such as *P. lilacinus*. Nevertheless, to assess the potential of Jordanian *P. variotii* isolates as bio-control agents, further experiments under field conditions are required.

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