

Short Paper

Pathogenicity of *Paecilomyces marquandii* on eggs of *Meloidogyne incognita*

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Abstract: Among fungi, species of the genus *Paecilomyces* are considered as promising biological control agents with high potential to reduce root-knot nematode (RKN) and other nematode populations. In this research we investigated a soil hyphomycetes, *Paecilomyces marquandii* and its pathogenicity on eggs of RKN *in vitro*. In greenhouse test, root weight, plant length, fresh weight and dry weight of plants, gall index and proportion of infected eggs were determined. *P. marquandii*, effectively promoted growth of plants inoculated with *M. incognita* by suppressing its pathogenesis as root galling and reducing egg mass production. At 25 °C, a great number of nematode eggs were parasitized by *P. marquandii*, inhibiting juvenile development.

Keywords: Biological control, Nematophagous fungi, *Paecilomyces marquandii*, *Meloidogyne incognita*, Iran

Introduction

Root-knot nematodes (*Meloidogyne* spp.) cause high economic losses to agricultural crops worldwide mainly in tropical and sub-tropical regions (Sikora and Fernandez, 2005). They infect more than 2000 plant species, and reduce world crop production by about 5%, however losses in individual fields may become much higher (Agrios, 2005). For several decades the use of chemical nematicides was one of the primary means of controlling root-knot nematodes. However, their negative impact on environment and ineffectiveness after prolonged use have led to a total ban or restricted use of most nematicides and an urgent need for safe and more effective options (Zuckerman and Esnard, 1994). Biological control is a safe approach to manage plant pests

and diseases and many studies have been focused on fungal agents to find an effective and ecologically-friendly means to reduce the population of plant-parasitic nematodes (Sorribas *et al.*, 2003). Some of the opportunistic bio control agents like soil hyphomycetes have shown great promise (Hooper and Evans, 1993; Jatal, 1985; Kerry and Crump, 1980). *Paecilomyces* is a hyphomycetous fungus with more than 30 recognized species (Bainier, 1907). *Paecilomyces* has different habitats comprised of soil, decaying plant residues, food products and insects. Some *Paecilomyces* species mainly parasitize sedentary nematodes such as *Meloidogyne* spp. and cyst nematodes. Among these, *P. marquandii*, an egg parasite of cyst and root-knot nematodes, has been studied extensively as a potential biocontrol agent (Chen *et al.*, 1999; Marban-Mendoza *et al.*, 1992; Soares *et al.*, 2011). Carris and Glawe in 1989 reported *P. marquandii* from eggs of *Heterodera glycines* (Carris and Glawe, 1989). The potential of this fungus as a nematode biocontrol agent has not been studied in Iran. This study was performed to

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evaluate the biocontrol potential of *P. marquandii* on eggs of *Meloidogyne incognita* in Iran.

Materials and Methods

Isolation of *P. marquandii*

Paecilomyces marquandii isolates were obtained from soil and identified based on molecular characters (Accession numbers: JQ013001 to JQ013003). As all isolates have similar molecular and morphological characteristics, in this study the isolate JQ013001 was used *in vitro* and in pot experiments. Moreover, all isolates had similar effects on eggs in preliminary experiment.

Production of nematode egg masses for *in vitro* pathogenicity test

Galled roots were collected from tomato seedlings growing in soil infested with the nematode. Egg masses were handpicked using a stereomicroscope and transferred aseptically to 9-cm diameter Petri dishes containing potato dextrose agar (PDA, 20 g/l, Difco, USA) kept for 48 h at 25 °C, and healthy uninfected individuals that did not develop fungal or bacterial infection were used for pathogenicity test.

In vitro Pathogenicity tests

For *in vitro* pathogenicity tests, 1% water agar supplemented with antibiotics (penicillin + streptomycin sulphate) was prepared. Sterile egg masses (500 eggs / petri dishes) were placed on growing fungal colonies. After 3 weeks, number of infected eggs and intact eggs (immature and mature) were counted using a microscope at 40 × and 100 × magnifications. Percentage egg infection was calculated by dividing number of infected eggs by total eggs multiplied by 100 (Wang *et al.*, 2005).

Greenhouse pathogenicity tests

Pots filled with sterilized soil were arranged in a completely randomized design in the greenhouse. The desired concentrations of *P. marquandii* were prepared and placed in separate vials (Potato dextrose medium was used for culturing in order to obtain mycelium for blending to make mycelial suspension for inoculations). The treatments were

as follows: (T1) Tomato plants without any inoculation, (T2) Tomato plants with nematode inoculum only, (T3) Tomato plant with *P. marquandii* only, (T4) Tomato plant inoculated with both *P. marquandii* and nematode inocula. The level of inoculum of *M. incognita* was 2000 freshly hatched J2s in each pot and/or one gram mycelium/pot. All treatments were replicated four times, and pots were kept in a greenhouse (25-27 °C) allowing plants to grow for two months.

Parameters evaluation

After 8 weeks of growth in greenhouse, the soil was washed off the roots of plants from each treatment. The following plant parameters and nematode indexes were considered; plant length, plant fresh weight, plant dry weight, gall index, egg mass index and percentage of eggs infected with *P. marquandii*. Plant length and fresh and dry weights were determined by standard methods, and mean values were then calculated. Gall index (GI) and egg mass index (EMI) were rated on a scale of 0 - 5, where 0 = completely healthy root system and 5 = more than 100 galls or egg masses in root system (Hartman and Sasser, 1985). To determine the percentage of eggs infected with *P. marquandii*, randomly egg masses from the roots of plants in T2 and T4 were stained with cotton blue in lacto phenol. The number of eggs infected with *P. marquandii* was counted under a compound microscope and the percentage of infected eggs was calculated.

Statistical analyses

A completely randomized design was used. Stat.10 for Windows (SPSS Inc., 2000) was used for statistical analysis. The means of treatments were compared with the Least Significant Differences (LSD) test at $P = 0.05$.

Results

Mycelium of *P. marquandii* isolate tested in our experiments penetrated the eggs of *M. incognita* on water agar (Fig. 1, A) and parasitized eggs between 60 to 80% *in vitro*. Infected eggs contained mycelium inside as well as on their egg shell (Fig. 1, B). Tomato plants inoculated

with *M. incognita* showed significant reduction in their growth (Table 1). The isolate of *P. marquandii*, increased the top weight and decrease the root weight compared with the nematode control. The weight of roots was significantly higher in plants treated with *M. incognita* alone which was attributed to the presence of larger galls in the roots (Table 1). When tomato plants were inoculated with *P. marquandii*, there was no significant difference in fresh and dry weights of the plants in comparison to tomato plants without any inoculation (control). In tomato plant inoculated

with *P. marquandii* and nematode inoculums, fresh and dry weights of plants were significantly greater than plants inoculated with *M. incognita* alone and the weights differed significantly from the control (Table 1). Root gall and egg mass were significantly reduced in plants treated with *P. marquandii* when compared with those treated with *M. incognita* alone. The values were comparable with those treated with uninoculated control plants. In all experiments, fewer eggs hatched in treatments containing *P. marquandii* than in treatments with *M. incognita* only.



Figure 1 Infected eggs of *Meloidogyne incognita* colonized by *Paecilomyces marquandii* hyphae (A), *P. marquandii* conidiophores formed on an infected egg (B). Bars = 25 μ m.

Table 1 Effect of *Paecilomyces marquandii* on fresh and dry weight, gall and egg mass production and infection of eggs on tomato plants 2 months after inoculation with *Meloidogyne incognita*.

Treatments	Length (cm)	Fresh weight (g)	Dry weight (g)	Root weight (g)	GI	EMI	Infected eggs (%)
T1	65.0 (\pm 0.9) a	39.8 (\pm 1.3) a	5.7 (\pm 1.2) a	8.3 (\pm 0.5) a	-	-	0
T2	42.0 (\pm 1.5) c	23.0 (\pm 2.1) c	2.2 (\pm 1.4) c	13.2 (\pm 0.8) b	5	5	0
T3	59.2 (\pm 1) a	36.3 (\pm 1.6) a	4.9 (\pm 1.2) a	7.9 (\pm 0.4) a	-	-	0
T4	55.1 (\pm 0.8) b	32.3 (\pm 1.5) b	3.9 (\pm 0.8) b	5.4 (\pm 0.3) c	2	1	70

T1 = Tomato plants without any inoculation, T2 = nematode inoculum only, T3 = *P. marquandii* only, T4 = *P. marquandii* and nematode inoculum.

GI = gall index; EMI = egg mass index.

Values are means \pm SD of four replicated plots per treatment. Means followed by the same letters in a column are not significantly different (LSD test, $P = 0.05$).

Discussion

M. incognita severely infected tomato, and reduced the fresh and dry weight of the plants. Our results corroborate the findings of Chen *et al.* (1999) who observed that *P. marquandii*, lowered root-galling severity, reduced reproduction of *M. hapla*, and increased lettuce yield in organic soil (Chen *et al.*, 1999). It is clear that, fungal hyphae of *P. marquandii* penetrate egg of *M. incognita* with enzymes and pressure following the formation of a simple appressorium (Alamgir Khan *et al.*, 1997). The entire contents of the egg are then used as food resource by the fungus, completely destroying the embryo/larva in the process. Egg containing embryos or larvae get infected by the fungus (Alamgir Khan *et al.*, 1997). The nematophagous fungus *P. marquandii* produces proteases in solid-state fermentation and liquid medium (Soares *et al.*, 2011). Moreover, Khan *et al.* (2004) demonstrated that a serine protease secreted by fungus of genus *Paecilomyces* was effective in reducing the hatching of eggs and the destruction of hatched *M. javanica* juveniles (Khan *et al.*, 2004). In a study with potted banana plants, *Streptomyces costaricanus*, *Bacillus thuringiensis*, and *P. marquandii* in combinations were generally not as efficacious for nematode management as when each of the organisms was applied individually against *Radopholus similis* and *Helicotylenchus multincinctus* (Esnard *et al.*, 1998). *P. marquandii* has been reported from eggs of *Heterodera glycines*, but the potential of this fungus as a nematode biocontrol agent of *H. glycines* has not been studied (Carris and Glawe, 1989). Marban-Mendoza *et al.* (1992) indicated that *P. marquandii* is one of the natural soil organisms that contributed to nematode suppression in the chinampa agricultural soils (Marban-Mendoza *et al.*, 1992). The present study concludes that *P. marquandii* is a potential biocontrol agent causing reduction in the number of root knot nematode *M. incognita* and thereby improving plant growth parameters. Pathogenicity of *P. marquandii* on *M. incognita* is a new report from Iran.

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بررسی بیماری‌زایی *Paecilomyces marquandii* روی تخم‌های نماتود ریشه‌گرهی *Meloidogyne incognita*

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چکیده: در میان قارچ‌ها، گونه‌های جنس *Paecilomyces* از نظر مهار زیستی اهمیت بسزایی دارند ضمن این‌که پتانسیل بالقوه‌ای در مهار زیستی نماتودها و کاهش جمعیت نماتود ریشه‌گرهی دارند. در این بررسی از قارچ هیفومیست *Paecilomyces marquandii* برای بررسی بیماری‌زایی روی تخم‌های نماتود ریشه‌گرهی در شرایط آزمایشگاه استفاده شد. در آزمایشات گلخانه‌ای وزن ریشه، طول گیاه، وزن تر و خشک گیاهان، شاخص گال و نسبت تخم‌های آلوده محاسبه شد. در این بررسی مشخص شد که *P. marquandii* به‌طور مؤثری رشد گیاهان مایه‌زنی شده با نماتود ریشه‌گرهی را با کاهش ایجاد گال و توده تخم نماتود افزایش داد. هم‌چنین در دمای ۲۵ درجه سانتی‌گراد تعداد زیادی از تخم‌های نماتود توسط *P. marquandii* پارازیته شد.

واژگان کلیدی: مهار زیستی، قارچ‌های نماتود خوار، *Meloidogyne incognita*، *Paecilomyces marquandii*، ایران