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Short Communication

Biocontrol potential of *Myrothcium roridum* Tode ex Fr. (Hypocreales: Incertae sedis) against root-knot nematode *Meloidogyne incognita* (Kofoid & White) Chitwood (Tylenchida: Heteroderidae)

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ABSTRACT

Biocontrol efficacy of *Myrothecium roridum* against the southern root-knot nematode, *Meloidogyne incognita*, was evaluated in pot experiments on tomato. When treated with the culture filtrate of *M. roridum*, egg hatching was significantly reduced. Inhibition rate of egg hatching was dose dependent, which increased with the increment of concentration of culture filtrate. Three days after treatment the highest rate of 78.9% was observed with 10 mL of original culture filtrate of *M. roridum*. The number of root galls was reduced with the treatment of the culture filtrate. Significant difference was observed between treatment and non-treatment. However, biocontrol efficacy was not dose-dependent, ranging from 67.5% to 74.3%. *M. roridum* caused inhibition of mobility of second-stage juveniles of *M. incognita* at all concentrations tested. The rate showed positive correlation with concentrations of the culture filtrate, showing the highest rate of 82.6% in 4% of the culture filtrate.

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Introduction

Root-knot nematodes belonging to the genus *Meloidogyne* are serious pests which infect roots of various plant species, such as tomato, cucumber, and watermelon (Affokpon et al., 2011; Zhang et al., 2015; Davis, 2007). Root-knot nematodes are easily found in tropical and subtropical area, but now get distributed in temperate climates as well. Their second-stage juveniles (J2s) penetrate plant roots and construct specialized site which is called giant cell. The nematodes produce galls around the giant cells that negatively affect the uptake of nutrients and water in host plants, resulting in yield loss (Milligan et al., 1998).

Four major *Meloidogyne* species, including the southern root-knot nematode *M. incognita* (Kofoid & White) Chitwood (Tylenchida: Heteroderidae), peanut root-knot nematode *M. arenaria* Chitwood (Tylenchida: Heteroderidae), tropical root-knot nematode *M. javanica* (Treub) Chitwood (Tylenchida: Heteroderidae), and the northern root-knot nematode *M. hapla* Chitwood (Tylenchida: Heteroderidae), are the most serious pests of a wide range of vegetable species (Sasser and Carter, 1985). Annual economic loss caused by the root-knot nematodes was estimated at $100 billion USD worldwide (Sikora and Fernàndez, 2005). These species have seriously damaged vegetables in greenhouse in Korea, as well (Choo et al., 1987). Damage has increased due to repeated cultivation with the same crop every year. It's fairly difficult to prevent these nematodes in time since the disease symptoms are recognized on the foliage of plant hosts after extensive damages have already done to the root system. The problematic attributes, *e.g.*, a variety of host range, high reproductive rate, and endoparasitic nature, make it worse to control these nematode species (Manzanilla-Lopez et al., 2004; Trudgill and Blok, 2001).

Chemical nematicides and fumigants were preferentially used as a controlling method, but they have been phased out due to both their toxicity and ineffectiveness. Alternative biological control using antagonistic fungi such as *Paecilomyces lilacinus* (Thom) Samson (Eurotiales: Trichocomaceae), *Pochonia chlamydosporia* (Goddard) Zare & W. Gams (Hypocreales: Clavicipitaceae), and *Trichoderma longibrachiatum* Rifai (Hypocreales: Hypocreaceae) has been tried to control *Meloidogyne* spp. (Anastasiadis et al., 2008; Upadhyay et al., 2000; Zhang et al., 2015).

Myrothecium species is one of the antagonistic microorganisms and has been received increasing research interest. In this study, biological potential of unexplored *Myrothecium* species was evaluated by investigating inhibition rate of egg hatching and mobility of J2s of *M. incognita*. In addition, suppression of root galling formation was investigated.

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Materials and methods

Preparation of biological agent

Myrothecium roridum Tode ex Fr. (Hypocreales: Incertae sedis) was cultured on potato dextrose agar (PDA; BD, USA) plate for 7 days at 30 °C. Spores were collected by adding 0.9% saline solution to the plate and spore suspension was prepared at a concentration of 1×10^3 spores per mL, using a haemocytometer. For seed cultures, spore suspension of *M. roridum* was incubated in 500 mL Erlenmeyer flasks containing 100 mL of seed medium. The medium consisted of 5% (*w*/*v*) glucose (Duksan, Korea), 2% (*w*/*v*) bacto peptone (BD, USA), 0.8% (*w*/*v*) KH_2PO_4 (Duksan, Korea), 0.2% (*w*/*v*) CH_3COOH (Duksan, Korea), 0.1% (*w*/*v*) NaCl (Duksan, Korea), and 0.05% (*w*/*v*) $MgSO_4 \cdot 7H_2O$ (Duksan, Korea) in 1 L of distilled water. Then, the flasks were incubated for 72 h on a rotary shaker (IS-971RF, Jeio Tech, Korea) at 25 °C and 180 rpm. For main culture, 150 mL of seed broth was transferred to 5 L jars (Bio CnS, Korea) containing 3 L of potato dextrose broth (PDB; BD, USA) and cultured at 30 °C and 500 rpm with an aeration rate of 1.0 vvm. After 120 h culture, culture broth was autoclaved and centrifuged at 7000 rpm for 10 min to remove *Myrothecium* cells. Resulting filtrate was collected for the following experiments.

Nematode preparation

Root samples infected by *M. incognita*, were collected from tomato greenhouse at the National Institute of Horticultural & Herbal Science, Suwon, Korea. Egg masses of *M. incognita* were picked off infected tomato roots. They were placed in a solution of 1% sodium hypochlorite and agitated at 3000 rpm for 5 min (Affokpon et al., 2011). Eggs were collected through 60, 100 and 400 mesh sieves placed in series, rinsed with distilled water, and used in the experiment. Eggs were incubated for 3 days, and freshly hatched J2s were harvested using a Baermann funnel (Park et al., 2012).

Effect of M. roridum on egg hatching

Pots (7.0 cm in diameter and 7.0 cm in height) were filled with 100 g of sterile sand and inoculated with 10 mL of egg solution consisting of 2000 eggs of *M. incognita*. Three concentrations of *M. roridum*, *e.g*., 120 h-cultured filtrate, 10×, and 100×, were prepared for the treatment. Ten mL of prepared solution of *M. roridum* was poured into a pot while 20 mL of distilled water was used as a control. Pots were shaken to mix them up for 1 min and placed at 25 °C for 72 h. Hatched J2s were harvested using a Baermann funnel and the number of nematodes was counted using a stereo zoom microscope (SZ61, Olympus, Japan). Experiments were replicated five times. Inhibition rate of egg hatching (IR) was calculated as follows:

$$IR = [(N_a - N_b)/N_a] \times 100(\%)$$

where N_a = nematode numbers in control, N_b = nematode numbers in the treatment.

Effect of M. roridum on root galling formation

Pot medium (Baroker, Seoul Agricultural Supply Company, Seoul, Korea), which consisted of 85% organic matter, 8% vermiculite, 5% zeolite, and 2% fertilizers with pH 5.5–6.0 was autoclaved at 121°C for 25 min before experiments. Pots were filled with 100 g of sterile pot medium and inoculated with 10 mL of nematode solution consisting of 2000 J2s. The autoclaved culture filtrate was formulated as follows: 10 mL of culture filtrate + 20 mL of tap water, 20 mL of culture filtrate + 10 mL of tap water, and 30 mL of culture filtrate. Thirty milliliters of tap water was used as a control. One week-old seedlings of tomato were transplanted in each pot 2 days after nematode inoculation. Each treatment was replicated five times. The number of root galls was counted 4 weeks after seedling. Biocontrol efficacy (BE) was calculated as follows:

$$BE = [(N_a - N_b)/N_a] \times 100(\%)$$

where N_a = the number of root galls in control, N_b = the number of root galls in the treatment.

Effect of M. roridum on inhibition of juvenile motility of M. incognita

Suspension of J2s of *M. incognita* in antibiotic solution (penicillin at 500 U/mL and streptomycin at 500 ppm) was prepared to evaluate inhibition effect of *M. roridum* on motility of J2s. Four concentrations of autoclaved culture filtrate of *M. roridum*, *i.e*., 1%, 2%, 4%, and 8%, were prepared, and 500 μL of each solution was poured into a well in 12-well plate (SPL Life Science Cat. No. 30012). The same amount of nematode suspension containing 60 J2s was released into each well. The plate was placed at 25°C. Inhibition rate was determined at 24 h, checking motility of J2s. Juveniles were considered dead when touched with fine needle. The same amount of antibiotic solution and 0.9% saline solution was used as a control. Experiments were replicated five times. Biocontrol efficacy (BE) was calculated as follows:

$$BE = [(N_a - N_b)/N_a] \times 100(\%)$$

where N_a = the number of nematodes motile in control, N_b = the number of nematodes motile in the treatment.

Statistical analysis

Data were analyzed using PASW software (Version 17, SPSS Inc., CA, USA). Analysis of variance (ANOVA) and Tukey's honestly significant difference (HSD) tests were used to determine significant differences between treatments.

Results

Effect of M. roridum on egg hatching

As shown in Fig. 1., significant difference in egg hatching rate was observed between concentrations ($F = 15.8$; df = 2,12; $P < 0.001$). Treatment of autoclaved culture filtrate of *M. roridum* significantly reduced egg hatching at all concentrations. Inhibition rate of egg hatching of *M. incognita* decreased with the increment of dilution rate of the

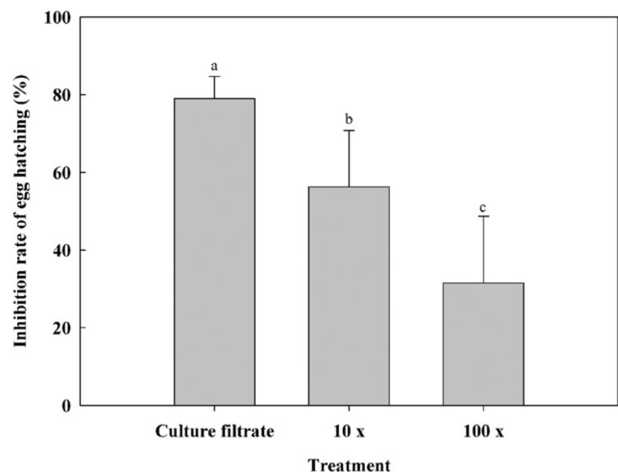

Fig. 1. Effect of *Myrothecium roridum* on inhibition rate of egg hatching. Different letters on the column indicate values are significantly different at P $_amp_$lt; 0.05 (Tukey's HSD).

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