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Management of the root-knot nematode *Meloidogyne incognita* on tomato with combinations of different biocontrol organisms

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ABSTRACT

The nematicidal effect of *Pseudomonas fluorescens*, *Paecilomyces lilacinus*, *Pichia guilliermondii* and *Calothrix parietina* singly or in combination was tested against root-knot nematode, *Meloidogyne incognita*. Treatments with *P. fluorescens* and *P. lilacinus* caused mortality of *M. incognita* as 45% and 30% of juveniles after 48 h of exposures, respectively compared to water control *in vitro*. Under greenhouse conditions, all treatments reduced the disease severity and enhanced plant growth compared to untreated control. Application of *P. fluorescens*, *P. lilacinus* and *P. guilliermondii* Moh 10 was more effective compared to *C. parietina*. There was a negative interaction between *C. parietina* and either *P. lilacinus* or *P. guilliermondii*. Fresh and dry weight of shoots and roots of plants were significantly reduced as a result of infection with *M. incognita*, however application of biocontrol agents singly or in mix recovered this reduction. Moreover, they enhanced the growth parameters compared with the control. Our results proved that application of different biocontrol agents (*P. fluorescens*, *P. lilacinus* and *P. guilliermondii*) not only has a lethal effect on nematode, but also enhances the plant growth, supplying many nutritional elements and induction the systemic resistance in plants. Presence of *C. parietina* as a soil inhabitant cyanobacterium could antagonize biocontrol agents leading to the reduction of their practical efficiency in soil.

1. Introduction

The root-knot nematodes (*Meloidogyne* spp.) are sedentary endoparasites and are among the most damaging agricultural pests attacking a wide range of crops (Sahebani and Hadavi, 2008), in particular vegetables, causing dramatic yield losses mainly in tropical and sub-tropical agriculture (Kiewnick and Sikora, 2006). In Egypt, root-knot disease of tomato caused by *Meloidogyne incognita* is an important disease (El-Sherif et al., 1999). The infection starts with root penetration of second-stage juveniles (J2) hatched in soil from eggs encapsulated in egg masses laid by females on the infected roots.

Environmental side effects associated with chemical control and the recent loss of methyl bromide as a multipurpose soil fumigant have spurred research into nematode control alternatives (Nico et al., 2004; Kiewnick and Sikora, 2006).

Many attempts have been made to use antagonistic bacteria and fungi to control root-knot nematodes (Khan et al., 2008). The damage caused by root-knot nematodes could be managed by application of microorganisms antagonistic to *Meloidogyne* spp., or compounds produced by these microbes (Ashraf and Khan, 2010). Different fungal strains isolated from nematodes, soil and plants were shown to produce substances that inhibit nematode egg hatch or kill nematodes (Khan and Saxena, 1997; Nitao et al., 1999). Khan et al. (2003) showed that the fungus *Paecilomyces lilacinus* penetrates nematode eggs and cuticles through the production of the lytic enzymes serine protease and chitinase.

Antagonistic bacteria have been repeatedly shown to be promising microorganisms for the biological control of plant-parasitic nematodes (Giannakou et al., 2004). *Pseudomonas aeruginosa* (Siddiqui et al., 2000) and other *Pseudomonas* spp. (Ali et al., 2002) have shown good results for the control of *Meloidogyne* spp. Many species of *Pseudomonas* were reported as plant growth-promoting rhizobacteria (PGPR) producing iron-chelating siderophores, antibiotics or hydrogen cyanide, and these compounds have been implicated in the reduction of deleterious and pathogenic rhizosphere microorganisms, creating an environment more favorable for root growth (Siddiqui, 2006).

Recently, attention has been paid to application of antagonistic yeasts for the inhibition of different plant disease (Tian et al., 2002; El-Ghaouth et al., 2003). However, a little information is available

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about application of yeasts as biocontrol of nematodes. Youssef and Soliman (1997) found that an Egyptian isolate of *Saccharomyces cerevisiae* reduced population of *M. incognita* on Egyptian henbane, *Hyoscyamus muticus* and improved its growth. Hashem et al. (2008) screened 22 yeast strains for their efficacy in suppression of *M. incognita* on grapevines and found that the highest percentage of nematode mortality was achieved by application of *Pichia guilliermondii*, *Pachytrichospora transvaalensis*, *Candida albicans* and *Geotrichum terrestre*. Utilization of antagonistic yeasts as an alternative appears to be a promising technology (Fan et al., 2002).

However there is a little information about using of cyanobacteria to inhibit the nematode population (Kumar et al., 1993; Dhanam et al., 1994; Khan et al., 2005, 2007), Flores and Wolk (1986) mentioned that some filamentous cyanobacteria secrete antibiotics that kill other strains of cyanobacteria. A planktonic filamentous cyanobacterium, *Oscillatoria* spp., inhibited growth of co-cultured cyanobacteria and eukaryotic algae, owing to the excretion of secondary metabolites (Bagchi et al., 1990). Issa (1999) reported the production of antibiotics by *Oscillatoria angustissima* and *Calothrix parietina* that inhibited the growth of some microorganisms.

Certain biochemical change occurring after application of biocontrol agents can act as markers for induced systemic resistance (Schönbeck et al., 1981). These include accumulation of certain enzymes, such as peroxidase (PO) (He et al., 2002). The PO enzyme has been implicated in the hypersensitive response, the formation of papillae, and the polymerization of lignin from monomeric lignols (Bestwick et al., 1998).

All microorganisms that have been applied as biocontrol agents against nematodes were tested singly, and there is no available information about the efficacy of mixing the bioagents. We suggest that a combination of biocontrol agents could be more likely to have a greater variety of traits responsible for suppression of root-knot nematode over a wide range of environmental conditions. Therefore, the objective of this study was to evaluate the efficacy of application of different biocontrol agents: *P. fluorescens*, *P. lilacinus*, *P. guilliermondii* and *C. parietina*, singly or in combination, as soil amendment for the control of the root-knot nematode *M. incognita* on tomato plants.

2. Materials and methods

2.1. Nematode inoculum

The inoculum of root-knot nematode, *M. incognita* isolated from naturally infected tomato was obtained from pure culture raised by single egg mass and maintained on roots of tomato plants (*Solanum lycopersicum* L., nematode susceptible variety Super Marmande) in greenhouse. The *Meloidogyne* was identified on the basis of the perineal pattern system of mature females according to Taylon et al. (1956) and Seinhorst (1966). Infected plants were uprooted from soil and entire root system was dipped in water and washed gently to remove adhering soil. Egg masses of *M. incognita* were picked using forceps. Egg masses were rinsed with sterile water, placed in 0.5% sodium hypochlorite, to dissolve the gelatinous matrix, agitated for 4 min and rinsed with sterile water on a sieve having 26 μm pores. The eggs were incubated for five days using modified Baermann funnel method to obtain second-stage juveniles (J2). The nematode inoculum was used for *in vitro* and pot experiments.

2.2. Preparation of biocontrol agents

2.2.1. Bacterial strain

P. fluorescens (PF7) used in this study was isolated from tomato rhizosphere. A 48 h-old-culture in King's B medium (KBM) was centrifuged at 10,000 rpm for 10 min. The pellets (bacterial cells) were washed thrice with sterilized distilled water by centrifugation. The optical density (OD) of the solution was adjusted to obtain 10^8 CFU/ml with the help of a UV–visible spectrophotometer (A610 nm).

2.2.2. Yeast strain

The yeast strain *P. guilliermondii* Moh 10, used in this study was previously isolated and identified in a previous study (Hashem, 2005) and showed nematicidal effect (Hashem et al., 2008). Yeast culture was prepared by growing this strain in yeast extract malt extract broth (YMB) and incubated at 25 ± 1 °C with shaking (150 rpm) for 48–72 h. The obtained yeast cells were palletized via centrifugation (10,000 rpm) for 10 min and resuspended in sterilized tap water to desired concentration (~10^8 CFU/ml).

2.2.3. Fungal strain

P. lilacinus AUSB3026 was obtained from the culture collection of the Department of Botany, Faculty of Science, Assiut University, Egypt. It was grown on potato dextrose agar (PDA) for seven days at 28 ± 1 °C and a spore suspension was prepared by adding sterile water to the culture and the spore concentration (1.7×10^5 spores per ml) was estimated using a hemocytometer.

2.2.4. Cyanobacterium strain

C. parietina ASGC50 strain was obtained from the culture collection of the Department of Botany, Faculty of Science, Assiut University, Assiut, Egypt. It was isolated from soil (Issa, 1999). The culture of cyanobacterium was grown in BG-11 medium ($NaNO_3$, 1500 mg l^{-1}; K_2HPO_4, 400 mg l^{-1}; $MgSO_4.7H_2O$, 75 mg l^{-1}; $CaCl_2.H_2O$, 36 mg l^{-1}; citric acid, 6 mg l^{-1}; ferric ammonium citrate, 6 mg l^{-1}; EDTA, 1 mg l^{-1}; Na_2CO_3, 20 mg l^{-1} and 1 ml l^{-1} of micronutrient solution) (Rippka and Herdman, 1993). The culture was grown at 25 °C under continuous illumination of 92-μmole m^{-2} s^{-1} with cool fluorescent lamps and suspension was bubbled with 5% CO_2-enriched air. The Cyanobacterial cells were homogenized and the concentration was adjusted to 10^5 CFU/ml.

2.3. Effect of different biocontrol agents on mortality of nematode in vitro

Nematicidal effect of *P. fluoresces*, *P. guilliermondii*, *P. lilacinus* and *C. parietina* was evaluated against *M. incognita* under laboratory conditions. Three hundreds of *M. incognita* juveniles were transferred to the suspensions of microorganisms (10^8 cell ml^{-1} of *P. fluorescens*, 10^5 CFU ml^{-1} of *P. lilacinus*, 10^8 cell ml^{-1} of *P. guilliermondii* and 10^5 cell ml^{-1} of *C. parietina*) separately in sterilized Petri dishes, and sterilized distilled water served as a control. Each treatment had five replications. Separate sets of Petri dishes were maintained for each period of observation (48 h). Then, mortality of nematodes was assessed by checking the motility and confirmed by touching the juvenile with fine needle.

2.4. Effect of different biocontrol agents on the seedling growth of tomato

To ensure that the biocontrol agents have no harmful effect on seeds and seedlings, the following experiment was carried out. Tomato seeds were immersed in the biocontrol agent suspensions, which contained separately 10^8 cell ml^{-1} of *P. fluorescens*, 10^5 CFU ml^{-1} of *P. lilacinus*, 10^8 CFU ml^{-1} of *P. guilliermondii* and 10^5 cell ml^{-1} of *C. parietina*. Sterile distilled water (DW) was used as control. Seeds were gently shaken for 2 h, blot dried, plated on wet blotters and the germination was tested using the filter paper method. The treated seeds were incubated at 28 °C for one week. Periodically, the water contents were readjusted and samples were taken for assaying germination. The viability of seedlings was