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Biocontrol of melon wilt caused by *Fusarium oxysporum* Schlect f. sp. *melonis* using seed treatment with *Trichoderma* spp. and liquid compost

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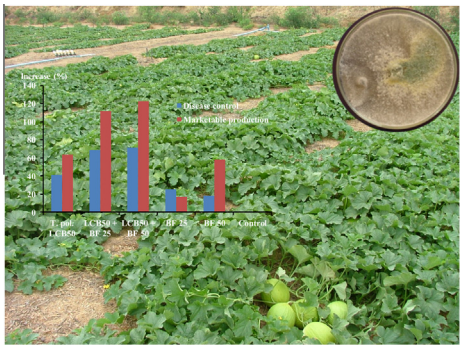
HIGHLIGHTS

- *T. polysporum* controlled efficiently melon wilt in a naturally infested crop field.
- *T. polysporum* and fertigation with liquid compost had a synergist effect on control.
- Synergism between *T. polysporum* and liquid compost doubled fruit production.

GRAPHICAL ABSTRACT



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ABSTRACT

The control of melon wilt caused *Fusarium oxysporum* f. sp. *melonis* (*Fom*) has become a complex problem for melon (*Cucumis melo* L.) growers worldwide. In this study, we evaluated the ability of *Trichoderma* spp. to control melon wilt under field conditions, and the application of liquid compost as a food-based strategy to improve the biocontrol efficiency of the selected strain. In a first experiment, we evaluated the use of *Trichoderma harzianum* LCB47, *Trichoderma viride* LCB48, *Trichoderma koningii* LCB49, and *Trichoderma polysporum* LCB50 to control melon wilt in a naturally infested soil. The treatment with *T. polysporum* LCB50 (*Tp*) showed the highest efficiency to control melon wilt (44.85%), increasing the fruit yield in 43%. In the second experiment, *Tp* was applied as seed treatment, and repeated once at 15 days after transplanting. Two doses of liquid compost: 25 (LC25) and 50 mL pL^{-1} (LC50), were applied by fertigation on a weekly basis along the crop development. In this experiment, *T. polysporum* LCB50 applied alone resulted in a significant ($P < 0.05$) control of wilt (32.2%), and 27% increase in fruit production. Single application of both doses of LC did not significantly reduced disease incidence. However, a strong synergistic effect was observed applying *Tp* and LC25 and LC50, resulting in a highly significant wilt control (68 and 72%, respectively) and an increase in productivity. The use of *Tp* + LC50 treatment increased in 100% the production of commercial fruits. From the results, a strategy based on the use of *T. polysporum* LCB50 and an organic matter source is proposed for the integrated management for melon wilt.

1. Introduction

Soil-borne plant pathogens have become a serious problem for melon crops in the main melon-producing areas of the world

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(Soriano-Martin et al., 2006; Zhao et al., 2011). Farming practices, such as the cultivation of susceptible genotypes, the damaging of seedling at transplant, the inappropriate management of irrigation, and the lack of an appropriate crop rotation may have been responsible for the increase in the severity of soil-borne diseases (Ghorbani et al., 2008; Sidique et al., 2012). *Fusarium* wilt of melon is caused by *Fusarium oxysporum* f. sp. *melonis* (*Fom*), that is a persistent soil inhabitant fungus. *Fom* colonizes soil organic matter and crop residues in the field, causing damages to subsequent crops of susceptible melon cultivars (Freeman et al., 2002).

Fungicide-based strategies usually adopted to control soil-borne pathogens have not achieved reliable results due to adsorption by soil colloids, and microbial degradation (Cook and Baker, 1983; Cadkova et al., 2013). Moreover, increasing concern about food and environmental contamination stimulated the search for environmentally friendly alternatives to pesticides for the management of plant diseases. Therefore, the use of antagonistic microorganisms for the biological control of melon wilt may become a substitute to an integrated management approach.

Some works have been reporting the selection of potentials antagonist to *Fom* in the last years. Bafti et al. (2005), reported that a strain of *Streptomyces olivaceous* showed anti-*Fusarium* activity in culture media. Tziros et al. (2007) showed that some pseudomonads applied to seeds reduced disease severity at early stages of wilt development and, although it did not inhibit watermelon wilt, they kept it at lower levels. Fungal BCAs have been detected among different genera, as *Penicillium oxalicum*, for example, that suppressed *Fom* wilt on melon and watermelon both in growth chamber and field experiments (De Cal et al., 2009). *Aspergillus* strains isolated from compost piles also shown potential as BCAs of melon wilt (Suárez-Estrella et al., 2007). Freeman et al. (2002) reported that a mutant non-pathogenic strain of *Fom* significantly reduced mortality of muskmelon seedlings in cross-protection experiments. The authors also showed that the mutant strain efficiently colonized seedling roots and lower stem tissue.

Trichoderma species have shown highly promising results against *Fom* under different experimental conditions. In the studies of Suárez-Estrella et al. (2007), *Trichoderma harzianum* 2413 reduced the incidence of melon wilt in a greenhouse experiment. In previous research, we showed that selected isolates of *Trichoderma* spp. were efficient to control soil-borne pathogens of melon in field conditions (Gava and Menezes, 2012). *Trichoderma* spp. are the most widely studied biological control agents (BCAs) for root and shoot pathogens, applied even in post-harvest (Woo et al., 2014). *Trichoderma* species are soil inhabitants, competitive saprophytes, and facultative mycoparasites that can colonize the soil and rhizosphere (Harman et al., 2004). Their mechanisms of action include the lysis of fungal hyphae with enzymes such as chitinase, proteases, and glucanases (Shahid et al., 2014), the induction of phytoalexin accumulation by the host (Yedidia et al., 2003), the production of antibiotics (El-Hasan et al., 2006; Reino et al., 2008) and modulation of plant hormones (Martínez-Medina et al., 2010).

Several microorganisms have been reported as plant pathogen antagonists, but only a small number were applied on a commercial scale. Most of this are due to a lack of consistency of the results from field trials (Fravel, 2005). Biotic and abiotic interactions interfere with the colonization of soil and rhizosphere, affecting BCA efficiency. Soil organic matter content and quality are a constraint to soil colonization by saprophytic fungi as *Trichoderma* (Hoitink and Boehm, 1999). In addition to BCAs, perhaps the application of organic residues or fertilizers could create a food-based strategy to improve their population and efficacy. Some studies have already shown promising results in controlling soil-borne plant diseases by applying compost to the soil (Egwanatum and Lane, 2009; Suárez-Estrella et al., 2007). Organic compost itself has also served as a source of BCA microorganisms isolation (Zhao et al., 2011).

Seed treatment is an attractive method for applying antagonistic microorganisms, as other methods involve the application of larger amounts of propagules. However, the survival of the BCAs in the soil after the application is essential for the efficient control of the pathogen. According to Hoitink and Boehm (1999), the selection of BCAs able to colonize the host rhizosphere or the introduction of a food base allows the preferential colonization of the soil and rhizosphere by the BCAs. In previous studies, Gava and Menezes (2012) demonstrated that *Trichoderma* spp. isolates used in the present work were able to colonize the rhizosphere of melon plants actively, controlling soil-borne plant pathogens in field conditions.

Melon growers intensively employ drip irrigation systems. So, applying organic matter to extensive areas become easier when it is in liquid form. Liquid composts are produced by the submerged composting of organic wastes for 15 to 60 days, under aerobic or anaerobic conditions. Liquid composts (LC) are rich in dissolved organic matter and support a large and diverse microorganism population (Ghorbani et al., 2005). Applying LCs directly to the soil or shoots has resulted in the control of plant diseases in different pathosystems (Lorito et al., 2010). Therefore, application of BCA and liquid composts by irrigation are likely a good strategy for soil-borne pathogen control. Considering that, the objectives of this study were (a) to select effective *Trichoderma* spp. isolates for the biological control of melon wilt and (b) to evaluate the effectiveness of the combined application of *Trichoderma* spp. and liquid compost for the biocontrol of melon wilt in naturally infested field conditions.

2. Materials and methods

2.1. Inoculum production and formulation

The fungi used in this study were *Trichoderma harzianum* LCB47, *Trichoderma viride* LCB48, *Trichoderma koningii* LCB49, and *Trichoderma polysporum* LCB50 has been maintained in the Emprapa Tropical Semi-Arid microorganism collection. Conidial production was performed as described by Cavalcante et al. (2008). Briefly, 100 μL of a suspension containing 10^5 conidia mL^{-1} were added to plastic bags filled with 200 g of autoclaved parboiled rice. The bags were kept in an incubation chamber (28 ± 2 °C) for 12 days to reach maximum sporulation. Subsequently, the biomass was partially dehydrated in a drying chamber (30 ± 2 °C) for three days. The spores were extracted using a spore extractor Mycoharvester M5 (ACIS R&D, Devon, UK).

A technical grade auto-adhesive preparation was made by adding the spores to a solution containing appropriate amounts of soluble starch, pectin, and carboxymethylcellulose to achieve a final concentration of 10^9 conidia mL^{-1}. The viability of the spores after processing was determined by transferring 100 μL of a conidial suspension (10^5 conidia mL^{-1}) into 4.5 cm Petri dishes containing potato dextrose agar (PDA) medium (Difco). Twelve hours after sowing, the rate of spore germination was determined by counting the number of germinated spores within five microscopic fields at 400× magnification. At least 300 spores were counted for each sample.

2.2. Seed and seedling production

Fungicide-free AF682 hybrid melon seeds (Sakata Seeds Sudamerica Ltd) were used for all experiments. The seeds were treated with 20 mL of the technical preparation and manually agitated for approximately 30 s. The seeds of the control treatment were treated with the same preparation without the addition of conidia. After treatment, seeds were air-dried overnight and