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Assessment of entomopathogenic fungi and their extracts against a soil-dwelling pest and soil-borne pathogens of olive

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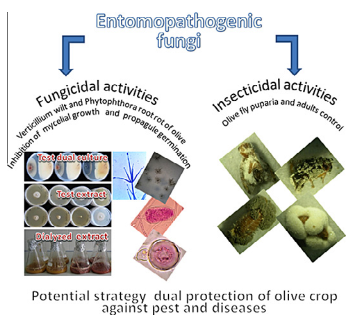
HIGHLIGHTS

- *Beauveria* and *Metarhizium* have shown potential in soil treatments against preimaginals and adults.
- *Beauveria* and *Metarhizium* have been inspected for antifungal activity against soil-borne pathogens.
- *Beauveria* and *Metarhizium* caused 40–62% inhibition of *Phytophthora* and *Verticilium* growth rates (GR).
- Crude extracts and dialyzed of *Beauveria* and *Metarhizium* caused 100% inhibition of the pathogens GR.
- These dialyzed fractions also caused inhibition of reproductive propagules of the pathogens.

GRAPHICAL ABSTRACT



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ABSTRACT

The olive fruit fly *Bactrocera oleae* (Gmelin) and the olive pathogens *Verticillium dahliae*, *Phytophthora megasperma* and *Phytophthora inundata* are major worldwide limiting factors for olive tree production. Entomopathogenic fungi, such as *Metarhizium* spp. and *Beauveria* spp., are suitable for olive fly control by either targeting adults or pupariating larvae and puparia in the soil beneath the tree canopy. Such soil treatments could have an additional impact on other communities of microorganisms present in the soil, such as soil-borne olive pathogens. The present work explores the possible dual biocontrol of both olive pests and diseases with entomopathogenic fungi. Twelve indigenous isolates of *Beauveria bassiana*, *Beauveria pseudobassiana*, *Beauveria varroae* and two isolates of *Metarhizium brunneum* from olive crops (soil and phylloplane) were evaluated in soil treatments against medfly (*Ceratitis capitata* Wied.) pre-imaginals. There were significant differences among the isolates in the total percentage of non-viable puparia and the total the puparia and adults showing fungal outgrowth, with percentages ranging from 27.5% to 82.5%, which highlights the potential of soil treatments with entomopathogenic fungi not only to reduce the target pre-imaginals but also the adults emerging from them, therefore contributing to the overall reduction of the next adult generation. The strains EAMa 01/58-Su of *M. brunneum* and EABb 09/16-Su of *B. bassiana* were evaluated for antifungal activity against two strains of *V. dahliae* (V117, V004), one of *P. megasperma* (PO20), and one of *P. inundata* (PO47) with one strain of *Trichoderma atroviride* used as a reference in the antagonism assays. In the dual plate culture assays, *T. atroviride* caused a 64–79% inhibition of *Phytophthora* spp. and *V. dahliae* mycelial growth. Meanwhile, the mycelial growth inhibition rates of *M. brunneum* and *B. bassiana* were 42–62% for *Phytophthora* and 40–57% for *V. dahliae*, with both entomopathogens causing inhibition halos, supporting the mechanism of antibiosis. Such a mechanism is also supported by the inhibitory activity of the crude extracts of these two *M. brunneum* and *B. bassiana*

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strains against these olive pathogens. Also, after the dialysis of the crude extracts against water in a molecular porous membrane with a cut-off of 3500 Da, the antifungal activity was mostly retained (100% inhibition rates) in the dialyzed fractions, indicating that the active compounds were secondary metabolites. Likewise, these dialyzed fractions inhibited the germination of conidia (96–100%) and microesclerotia (58–96%) of *V. dahliae* and propagules of *P. megasperma* (38–100%). These results indicate that entomopathogenic fungi have the potential for dual biocontrol of olive soil pests and diseases.

1. Introduction

The olive tree *Olea europaea* L. (Oleaceae) is an important crop in many countries and has acquired a large socio-economic importance within the Mediterranean region (IOOC, 2012). In fact, the olive tree is planted in all regions of the globe between 30° and 45° latitude of both hemispheres. Spain, with over 320 million olive trees, is the number one producer of olive oil, at 35–40% of worldwide production, with 75% of its production coming from Andalusia (FAO, http://faostat.fao.org/).

The most important insect pest of the olive tree worldwide, with an emphasis in Mediterranean countries, is the olive fruit fly *Bactrocera oleae* (Gmelin) (Diptera: Tephritidae). The fly lays its eggs in the olive fruit and the larvae feed and grow in the mesocarp. The infestation leads to direct (table olives are unsuitable for consumption) or indirect (reduction in oil quality) damage (Tazanakakis, 2006). *B. oleae* (Gmelin) is largely responsible for approximately 60% of losses due to insects (Mazomenos et al., 1997) and 15% of the total crop losses, estimated at approximately 800 million euro per year (Quesada-Moraga et al., 2009).

The control of *B. oleae* is based on chemical insecticides, particularly organophosphates, which are used as bait (insecticides mixed with an attractant) or cover sprays. However, concern has arisen regarding the negative impact that such chemicals have on the olive tree ecosystem and the discovery of insecticidal resistance in some olive fly populations frequently treated with these chemicals (Daane and Johnson, 2010). Likewise, globally changing plant protection policies aim to reduce pesticide risks on the environment and human health by reducing dependence on chemical control. Thus, research on old and new control options has been reinforced (Daane and Johnson, 2010). As a result of these studies, microbial control with entomopathogenic fungi has been shown to have potential as an alternative approach to olive fly management and can complement and partially replace current chemically based olive fly adult management practices (Quesada-Moraga et al., 2009).

The olive fly is a multivoltine species, completing from 2 to 5 generations per year, as a function of the region and local conditions, mainly temperature and relative humidity (Santiago-Alvarez and Quesada-Moraga, 2007). During development, third instar larvae drop from fruits to the ground, burrow into the soil, and form a puparium, overwintering as pupa several centimeters below the soil surface (Tremblay, 1994; Alfaro-Moreno, 2005). Compared with other entomopathogenic microorganisms, entomopathogenic fungi have the advantage of contact action, which allows for the direct penetration of the host cuticle and makes them suitable for olive fly control targeting either adults or pupariating larvae and puparia in the soil beneath the tree canopy (Ekesi et al., 2007).

The entomopathogenic mitosporic ascomycetes *Beauveria* spp. and *Metarhizium* spp. are frequently isolated from the soils of olive orchards in Spain with several isolates of both species having the potential for olive fly adult and puparia control (Quesada-Moraga et al., 2006, 2007, 2008). To optimize the efficacy of soil treatments, the effects of temperature, moisture (Garrido-Jurado et al., 2011c) and soil physicochemical properties (Garrido-Jurado et al., 2011b) on the availability, movement, and virulence of conidia against tephritid puparia, and even the effects of such soil treatments on the soil-dwelling non-target arthropods, have been studied (Garrido-Jurado et al., 2011a). Understanding the relationship among the insect pest, the fungi, and the environment appears to be important in their role as biological control agents.

To this end, recent evidence indicates that many of these entomopathogenic fungi play additional roles in nature, such as plant endophytes associating with the rhizosphere, plant growth-promoting agents and even antagonists of plant pathogens, which could allow for the exploitation of their full crop protection potential (Vega et al., 2009). The application of entomopathogenic fungi in the soil beneath the tree canopy for the control of pupariating larvae of *B. oleae* could have an impact on other communities of microorganisms present in the soil, such as soil-borne plant pathogens. While it has been reported that *Beauveria bassiana* (Ownley et al., 2004, 2008a,b) and *Lecanicillium* spp. (Askary et al., 1998; Benhamou and Brodeur, 2000, 2001; Kim et al., 2007, 2008) are antagonistic to plant pathogens, the potential antagonistic effect of entomopathogenic fungi against soil-borne olive pathogens remains unknown.

The soil-borne fungus *Verticillium dahliae* Kleb. and the oomycete *Phytophthora* spp. are two major constraints on olive cultivation in the Mediterranean Basin, causing *Verticillium* wilt and root rot diseases, respectively (Sánchez-Hernández et al., 2001; López-Escudero and Mercado-Blanco, 2011; Jiménez-Díaz et al., 2012). The goal of the present work is to explore the potential antagonistic activity of several strains of the entomopathogenic fungi *Beauveria* spp. and *Metarhizium* spp., selected for their virulence toward tephritid puparia against the olive soil-borne pathogens *V. dahliae* and *Phytophthora* spp. and to elucidate their possible mechanisms of antagonism.

2. Materials and methods

2.1. Insects

The olive fruit fly *B. oleae* is very hard, if impossible, to rear and manipulate under laboratory conditions (Fletcher, 1987); therefore, *Ceratitis capitata* Wied. is used worldwide as a model. Pupariating third instar *C. capitata* larvae were reared under the insectary conditions of a 16:8 h light:dark photoperiod with 50–60% relative humidity (RH) and 26 ± 2 °C. The adult flies were provided water and a standard artificial diet of yeast autolysate + sucrose (1 + 4 by weight). The larvae were reared on wheat bran + sucrose + beer yeast + nipagin + nipasol + water (20 + 5 + 1 + 0.5 + 0.5 + 10 by weight).

2.2. Fungal isolates

Seven indigenous strains of *B. bassiana*, three strains of *B. pseudobassiana*, two strains of *B. varroae*, and one strain of *Metarhizium brunneum* (Table 1) from the culture collection at the Department of Agricultural and Forestry Sciences and Resources (AFSR) of the University of Cordoba (Spain) were selected for screening. These strains originated from the soil, olive leaves and weeds of an organic olive orchard at Bobadilla in the Malaga province in