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Preliminary selection and evaluation of fungicides and natural compounds to control olive anthracnose caused by *Colletotrichum* species

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ABSTRACT

The commercial control of olive anthracnose is mainly based on the use of copper-based fungicides, although alternatives to reduce the quantity of copper in orchards are required. A total of 45 products, including antifungal compounds (commercial and experimental products), inorganic salts, and natural compounds (organic products and plant extracts), were evaluated against *Colletotrichum godetiae* or *C. nymphaeae* by *in-vitro* sensitivity tests or by bioassays using detached fruits. Moreover, copper oxychloride was evaluated for plants bearing fruits. Systemic fungicides and the protectant folpet were the most effective in inhibiting mycelial growth, while copper sulfate and trifloxystrobin were the most effective in inhibiting conidial germination. Fruit bioassays showed that tebuconazole or trifloxystrobin were the most effective against pathogen infection. Plant extracts were ineffective in controlling the pathogen. For potted plants, treatments with copper oxychloride delayed the onset of fruit rot and branch dieback.

1. Introduction

Anthracnose of olive (*Olea europaea* subsp. *europaea* L.) is the most important fruit disease of this crop globally (Cacciola et al., 2012; Moral et al., 2014; Talhinhas et al., 2005). Spain is the leading olive-producing country, with 25% of the world acreage and nearly 45% of the global production (Moral et al., 2014). In this country, olive anthracnose causes over $100 million in economic losses each year due to fruit drop and its negative impact on the oil quality obtained from affected fruits (Moral et al., 2009, 2014). Economic losses are even proportionally higher than in Spain in humid areas where susceptible cultivars to the disease are dominant such as Portugal, where the pathogen often affects 50% of the fruits (Talhinhas et al., 2018).

Olive anthracnose is caused by at least 13 *Colletotrichum* species that belong to the complexes *C. acutatum* J.H. Simmonds, *C. gloeosporioides* (Penz.) Penz. & Sacc., and *C. boninense* Moriwaki, Toy. Sato & Tsukib. (Cacciola et al., 2012; Moral et al., 2014). For example, *C. godetiae* Neerg. is dominant in Greece, Italy, Montenegro, and Spain (Cacciola et al., 2012; Moral et al., 2014); *C. nymphaeae* (Pass.) Aa is the prevalent species in Portugal (Talhinhas et al., 2005); and *C. acutatum* is the most common species in Tunisia (Chattaoui et al., 2016).

The pathogen mainly infects fruits at maturity stage, causing rot (Fig. 1a). Subsequently, the affected fruits mummify, and most of them fall to the soil (Cacciola et al., 2012; Moral et al., 2009, 2014). The olive oil from infected fruits shows undesirable physicochemical and organoleptic parameters (Leoni et al., 2018; Moral et al., 2014). Affected olive trees also show defoliation and dieback of shoots and branches as a consequence of the toxins (aspergillomarasmines) produced by the pathogen in mummified fruits remaining in the tree canopy (Cacciola et al., 2012; Moral et al., 2009, Fig. 1b).

The disease cycle starts with the infection of developing fruits by water-splashed conidia during spring rains (Moral et al., 2009). Under Mediterranean conditions, the infection remains latent until the onset of the fruit ripening (fall-winter). Then, the epidemic development is highly driven by the rainfall, the ripening of the fruit (since unripening fruits are frequently resistant), and the cultivar resistance (Moral et al., 2008; Moral and Trapero, 2012). Because of the exponentially growing anthracnose epidemic during fall-winter and the very restricted use of

Abbreviations: BCO, Biological Control Organisms; PDA, Potato Dextrose Agar; SHAM, Salicylhydroxamic Acid; RGI, Relative Growth Inhibition; RGeI, Relative Germination Inhibition; RApI, Relative Appressorium Formation Inhibition; EC_{50}, Effective Concentration (μg ml^{-1}) Inhibiting 50%; CI_{95}, Confidence Intervals at 95%; DSI, Disease Severity Index; RAUDPC, Relative Area Under the Disease Progress Curve; ANOVA, Analysis of Variance; HSD, Honest Significant Difference Test

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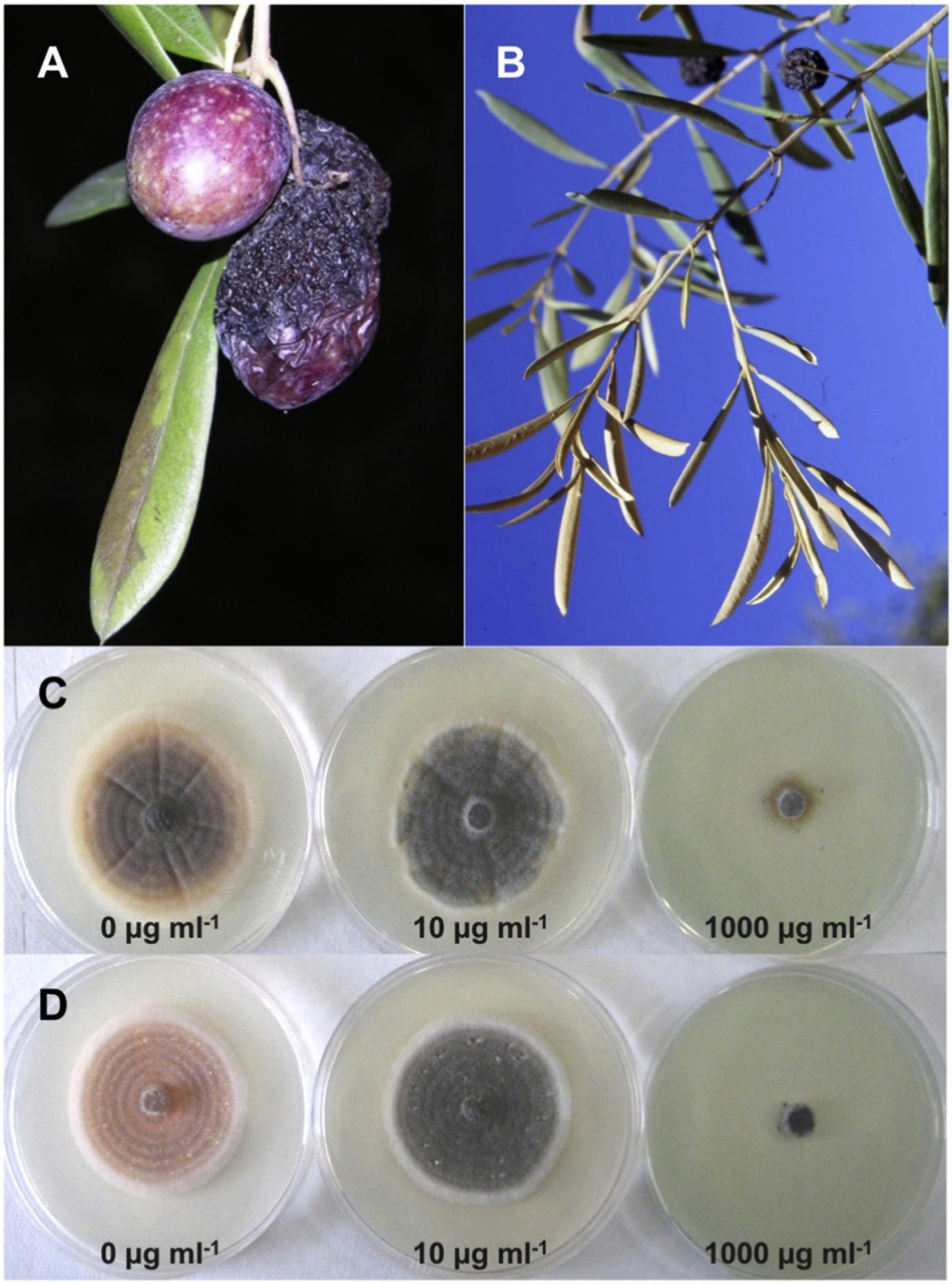

Fig. 1. Symptoms of *Colletotrichum* species affecting olive [(a) Fruit rot and (b) dieback of shoots]. Colonies of (c) *C. godetiae* isolate Col-104 and (d) *C. nymphaeae* isolate Col-116 growing on potato dextrose agar amended with copper sulfate at doses ranging between 0 and 1000 µg ml^{-1} of metallic copper.

curative fungicides, most of them soluble in the fruit oil, the most useful control strategy is the application of nonfat-soluble protective fungicides at the beginning of fall such as copper-based fungicides (Cacciola et al., 2012; Moral et al., 2014). After that, copper-based fungicides must be repeated in wet fall since they can wash-off, although these re-applications can be delayed if the rains persist due to the difficulty in performing treatments (Moral et al., 2014).

Some of the advantages of copper-based fungicides include i) their high efficiency against several olive pathogens (i.e., *Spilocaea oleagina*, *Pseudocercospora cladosporioides*, and *Pseudomonas savastanoi*); ii) their reduced cost; iii) their low toxicity for olive trees and long persistence on the trees; iv) the low potential for the appearance of resistant isolates due to the multisite mode of action of the Cu^{2+} ion; and iv) their use is authorized, even after flowering, in both organic and conventional olive farming (Cacciola et al., 2012; Moral et al., 2014; Roca et al., 2007).

Unfortunately, long-term use of copper-based fungicides may lead to the accumulation of copper in soils, with potential phytotoxic and adverse environmental effects (Komárek et al., 2010). To avoid soil contamination by copper, different types of management strategies could be used, such as i) the use of other commercial fungicides with a higher efficacy against the pathogen (Roca et al., 2007); ii) the use of plant extracts with fungicidal activity (Flors et al., 2004), iii) the use of biological control organisms (BCO), iv) the optimization of the fungicide application methods and times (Moral et al., 2014), v) the selection of copper-based compounds with high efficacy against the pathogen (Roca et al., 2007), and vi) the use of new copper formulations (i.e., micron or submicron particles) that allow an important reduction in the use of metallic Cu^{2+} per ha (Civardi et al., 2015). Even so, the number