



Inhibitory Potential of Olive Leaf Extract on Plant Pathogenic Fungus *Verticillium dahliae* Kleb.

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Introduction

Olive is one of the most important Mediterranean fruit and oil crops but its sustained cultivation is endangered with plant pathogenic bacteria and fungi. Fungus *Verticillium dahliae* is the most important fungal disease of olive with increasing incidence in Europe. Olive leaves, an important byproduct of olive cultivation and also recently recognized as a source of important natural compounds with antibiotic and antimycotic properties, are used for production of olive leaf extract. The aim of this study was to screen potential of water solutions of commercially available olive extract (Magdis, Zagreb, Croatia) to inhibit *in vitro* growth of two dangerous non-defoliating *V. dahliae* isolates, obtained from Spain and Croatia.

Materials and methods

Preparation of plant material

In order to test the antifungal effect of olive leaf extract on the growth and development of the pathogen *Verticillium dahliae*, a mass of 2,5 g of olive leaf powder (Magdis, Zagreb, Croatia) was immersed in 50 mL of sterile distilled water according to the modified method of Varo et al. (2017).

Water extraction

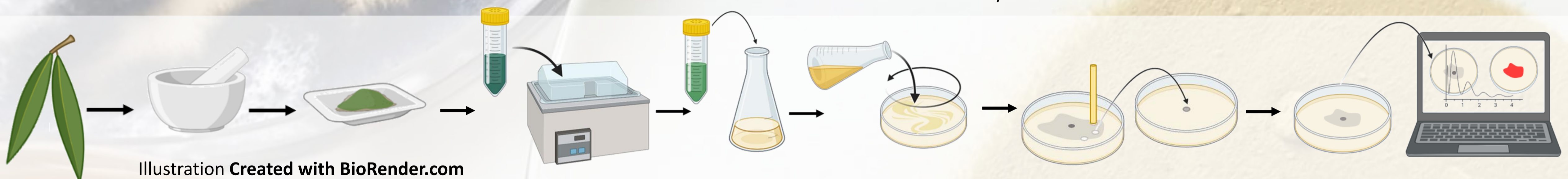
The conventional water extraction was carried out according to the modified method of Šain et al. (2020) in a termomixer at 45 °C for 5 hours with mixing (500 rpm). After dissolving, the solution was centrifuged at 10,000 rpm/5 minutes, and the extract was filtered using a 0,22 µm diameter filter disc and stored at 4 °C until use.

Antifungal activity

According to the poisoned food method (Qadoos et al., 2016), test petri dishes contained water extract added to melted PDA medium (45 °C) in four different concentrations (5, 50, 500, 5000 mg/L according to Varo et al., 2017), respectively. Control petri dishes contained only PDA medium. Test and control Petri dishes were inoculated with 5 mm diameter micellar disc taken from the edge of 6-day-old cultures of *V. dahliae* (Croatian and Spanish isolate) and incubated in a climate chamber for 6 days at 25 °C in the dark.

Fungal growth measurement

Results were obtained on 6th day after incubation using the *ImageJ* computer program (Schneider et al., 2012). According to Guzmán et al. (2014) the growth area of fungal colonies of both isolates was measured. The obtained inhibition percentages were calculated using the mean value of the pathogen growth area.



Research results

Growth of pathogen *V. dahliae* was inhibited by 2, 2, 12, 45 % (Spanish isolate) and 9, 36, 70, 81 % (Croatian isolate) at olive leaf extract concentrations of 5, 50, 500, 5000 mg/L, respectively. Results suggest variable susceptibility of isolates of *V. dahliae* to extract with high susceptibility of Croatian isolate to even low concentrations of extract. In addition to growth rate, extract reduced fungal colony density and microsclerotia formation.

Graph 1. Antifungal effect of olive leaf extract on the growth and development of the Spanish isolate of *V. dahliae*.

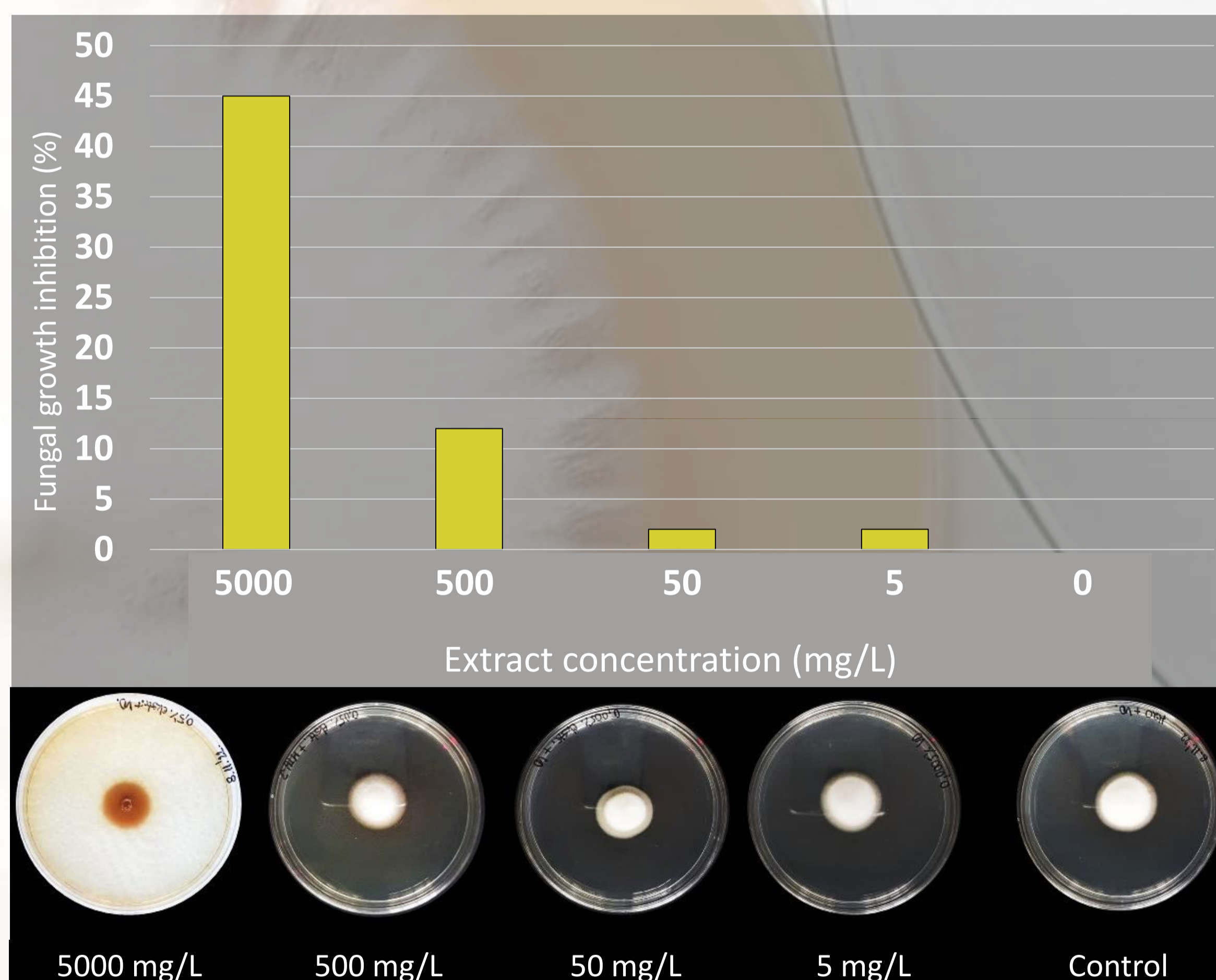


Figure 1. Antifungal effect on colony growth and development of the Spanish isolate of *V. dahliae* at different concentrations of olive leaf extract under *in vitro* conditions.

Graph 2. Antifungal effect of olive leaf extract on the growth and development of the Croatian isolate of *V. dahliae*.

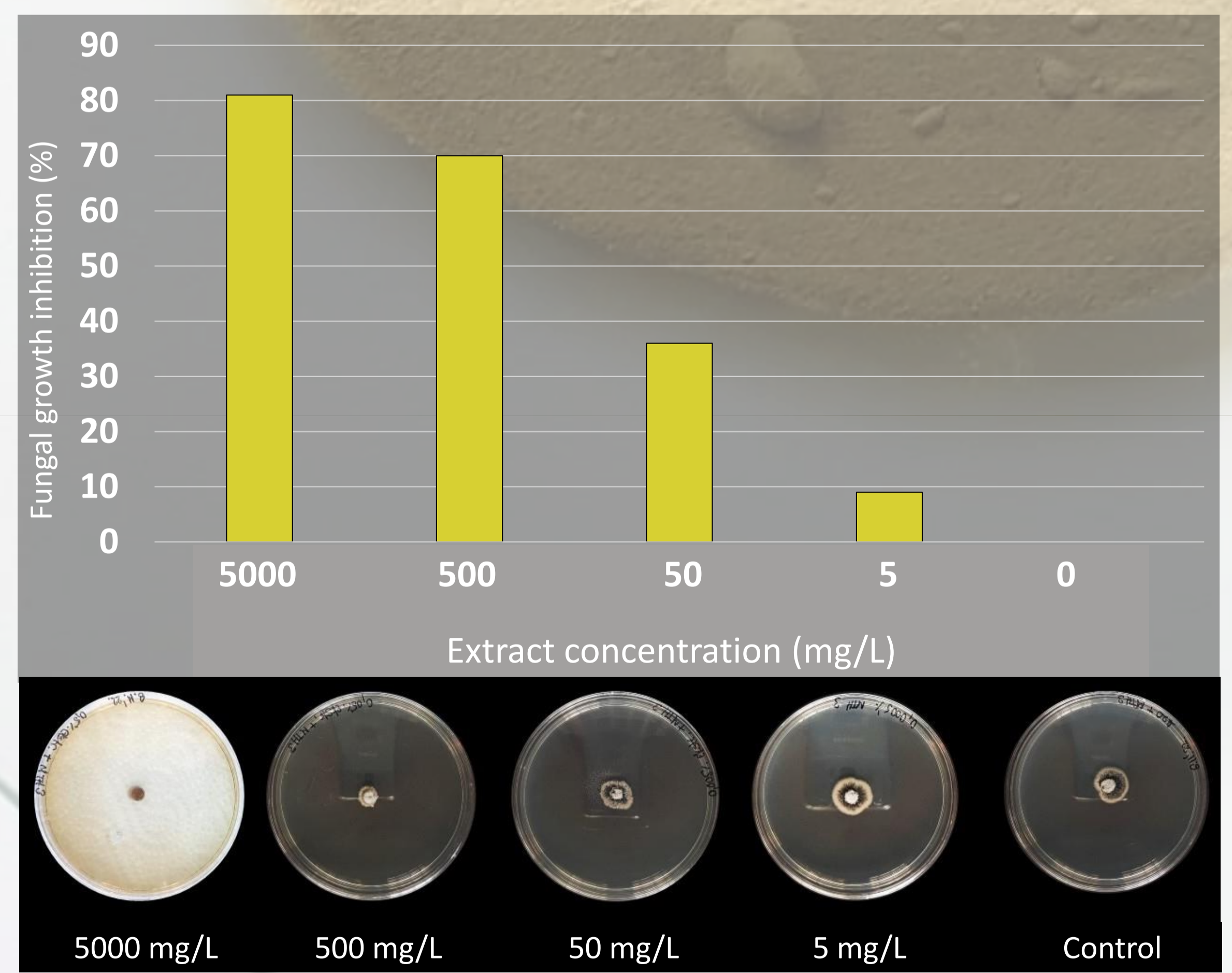


Figure 2. Antifungal effect on colony growth and development of the Croatian isolate of *V. dahliae* at different concentrations of olive leaf extract under *in vitro* conditions.

Conclusions

1. Non-defoliating isolates of fungus *V. dahliae* from Spain and Croatia are susceptible to inhibitory effects of olive leaf extract on mycelial growth, with Croatian isolate being susceptible even at low concentrations.
2. Olive leaf extract reduced density of aerial mycelium and formation of microsclerotia of Croatian and Spanish isolates of *V. dahliae*
3. Water solutions of olive leaf extract could have future applications in novel methods of biological control of *Verticillium* wilt of olive

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