BIOACTIVITY OF OLIVE OIL MILL WASTEWATER AGAINST PLANT PATHOGENS AND POST-HARVEST DISEASES

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ABSTRACT

The antifungal activity of olive oil mill wastewater (olive OMW) was investigated. The effect of sterilized and filter sterilized olive OMW was tested in vitro: a) on mycelium growth of Fusarium oxysporum f.sp. lycopersici, Pythium spp., Sclerotinia sclerotiorum and Verticillium dahliae on PDA agar plates; b) on sporulation of Penicillium italicum and P. digitatum and Botrytis cinerea on infested with the pathogens fruit and-vegetables (oranges and red horn peppers) and c) on planta, tomato plants infested with the fungus F. oxysporum f.sp. lycopersici. The results show that the filter sterilized olive OMW inhibits the mycelium growth of all tested fungi in vitro, probably due to phenolic compounds which are contained in olive OMW. Furthermore, olive OMW decreased fungi spores' number on infested fruits-vegetables and acted positively on tomato plants fresh weights, infested with the soil borne pathogen F. oxysporum f.sp. lycopersici.

Keywords: olive oil mill wastewater, antifungal activity, plant and post-harvest diseases

Introduction

During olive oil extraction a large amount of solid and aqueous residues as olive oil mill wastewaters (olive OMWs) are produced annually worldwide where the majority of it is being produced in the Mediterranean basin. The uncontrolled disposal of olive OMW is becoming a serious environmental problem due to its high content of phenolic compounds: tannins (7) and flavonoids (8). Some of these phenols are responsible for several biological effects, including antibiosis (12), antimicrobial (2) and phytotoxic properties (3). They also appear to be involved in the defense of plants against invading pathogens, including bacteria, fungi and viruses (9). The use of olive OMW for plant and harvested fruit protection against microorganism could be a solution for residues management and nature protection. The main objective on this study was to examine the antifungal activity of olive OMW against plant pathogens and post-harvest diseases.

Materials and Methods

Effect of olive OMW on the mycelium growth of fungus

The antifungal effect of olive OMW against soil borne plant pathogens (*Fusarium oxysporum* f.sp. *lycopersici, Pythium* spp., *Sclerotinia sclerotiorum* and *Verticillium dahliae*) were tested *in vitro*. Tests were made on PDA (Potato Dextrose Agar; DIFCO). Treatments were PDA plates with a) olive OMW added into the medium and autoclaved and b) a drop of filter sterilized olive OMW (using a syringe filler 0.2 μm) added onto the agar surface. In the first treatment a 35ml of olive OMW were added into 1l agar and further sterilized by autoclaving (120°C for 20 min). In the second treatment a drop (50 μl) of sterilized filtered olive OMW was added onto the centre of each plate. Fifteen agar plates per treatment, were inoculated with a mycelium plug (5 mm in diameter) of the above fungi (depended of the treatment) taken from the periphery of 7 days old fungal colonies. Mycelia plugs were placed onto the centre of each plate or next to the olive OMW drops. Equal plate numbers per fungus treatment were used as control (without olive OMW). Plates were incubated at 21°C for six days and fungi mycelium growth was recorded.

Antimicrobial activity of olive OMW on fruits treated with pathogens

Two common species of *Penicillium* (*P. italicum and P. digitatum*), isolated from oranges and *Botrytis cinerea* isolated from red horn (sweet) pepper were used for this experiment. Spores suspension was prepared by collecting spores of above *Penicillium* species, from 8 days old cultures. Three agar plates per fungus culture were used to collect spores. Spores were collected in 1l Erlenmeyer flask which contained distilled water, by washing the agar surface with 3ml distilled water and filtered that solution throw sterilized muslin. In each flask spores suspension was adjusted to 10^6 spores/ml. A 35ml of olive OMW were added in each flask. Oranges were surface sterilized and soaked for 3 min in 1l beakers that contained 500 ml of the above spore and olive OMW solution. After that time fruits were removed from the flasks, dried for 10 min in a laminar flow unit and incubated at 21°C for 12 days. Olive OMW was passed through Whatman filter paper No. 2 before being added to each beaker. The same procedure was followed for red horn peppers incubated with B. cinerea. After the incubation time, the spore number of each fruit of oranges or peppers was counted as each treated fruit surface was scraped into 200ml beaker, containing 50 ml distilled water. The spore number per treatment and per beaker was counted in optical

microscope using a hematocytometer. The experiment had fourteen replicates per treatment and two treatments; infected with spores and olive OMW oranges and infected with spores and olive OMW red peppers. Equal numbers of fruits (oranges) and vegetables (red peppers) soaked only in olive OMW and only in fungus spore suspension were used as controls.

Effect of olive OMW on tomato infested with *Fusarium oxysporum* f.sp. *lycopersici*

In this experiment 42 tomato seedlings were incubated with spores (10^6 conidia/ml) of F. oxysporum f.sp. lycopersici (Fol). Twenty one of them (tomato seedlings) were incubated in Fol conidia suspension for 10 min and 21 were incubated in the conidial suspension treated with olive OMW (5 ml/100 ml in total solution). Olive OMW was passed through Whatman filter paper No. 2. All treated plants were planed into 200 ml pots and kept in a glasshouse. Plants were harvested 40 days after planting. The height and fresh weight of tomato plants were measured.

Statistical analysis

Data were analyzed using the Minitab statistical package. Analysis of variance was used to assess treatments effect.

Results and Discussion

Effect of olive OMW on the mycelium growth of fungus

There was a statistically significant difference between filtered olive OMW and control (untreated PDA and sterilized with olive OMW PDA), (P<0.001). The filtered olive OMW inhibited the growth of all tested fungi mycelium **(Fig. 1)**. Sterilized olive OMW had similar effect on the mycelia growth of *F. oxysporum* f.sp. *lycopersici, Pythium* spp., *S. sclerotiorum* and *V. dahliae* with the untreated control. However, sterilized olive OMW seamed to have some positive effect on the mycelium growth of all tested fungi **(Fig 1)**.

Antimicrobial activity of olive OMW on fruits treated with pathogens

The olive OMW reduced the number of *Penicillium* species (P=0.009) and *B. cinearia* (P<0.001) spores from oranges and red peppers respectively. On oranges the average spore's number was $3.8x10^5$ for oranges infected with *Penicillium* species and $2.1x10^2$ for oranges infected with *Penicillium* species and treated and olive OMW. On red horn (sweet) peppers the average spore's number was $5.3x10^5$ for red horn peppers infected only with *B. cinearia* and $1.8x10^2$ peppers infected with *B. cinearia* and treated with olive OMW.

Effect of olive OMW on tomato infested with *Fusarium oxysporum* f.sp. *lycopersici*

There was a statistically significant difference between untreated, treated with *Fusarium oxysporum* f.sp. *lycopersici* (Fol) and olive OMW and treated plants only with Fol, on the height (P<0.001) and fresh weight (P<0.001) of tomato stems (**Fig. 2**). Olive OMW + Fol treatment produced more plant biomass (shoot length and fresh weight) than those infested only with *F. oxysporum* f.sp. *lycopersici* (**Fig. 2**).

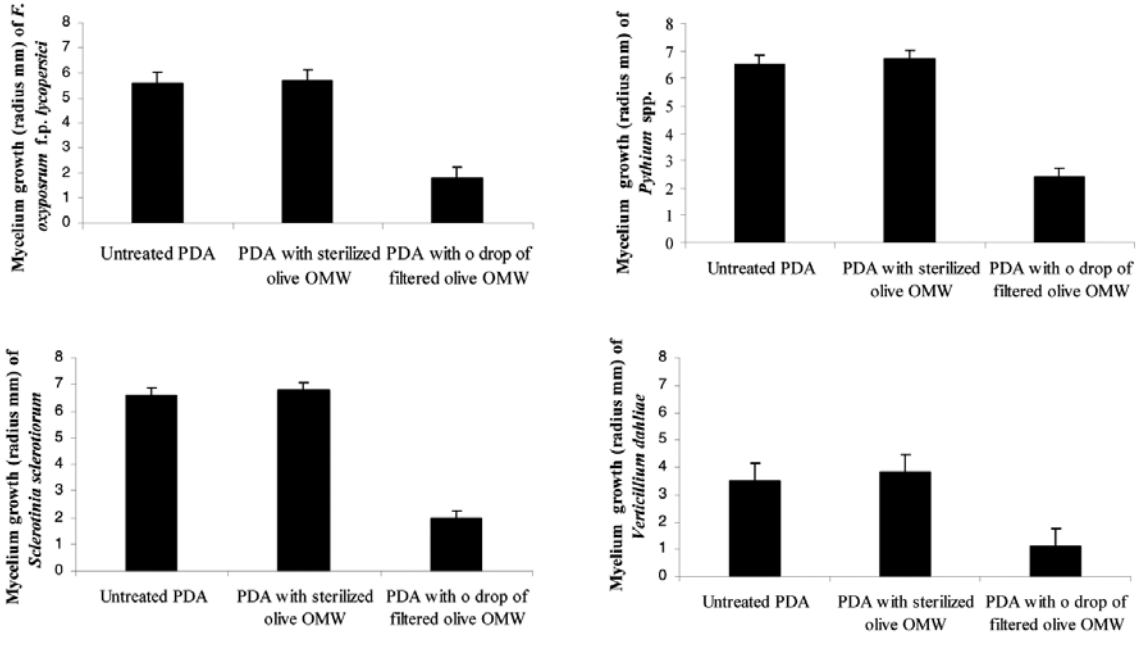

Fig. 1. Effect of sterilized and filtered olive oil mill wastewater (olive OMW) on the mycelium growth of *F. oxysporum* f.sp. *lycopersici, Pythium* spp., *S. sclerotiorum* and *V. dahliae*

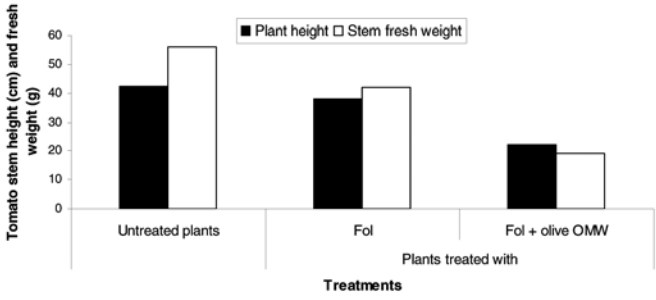

Fig. 2. Effect of olive OMW on tomato height and stem fresh weight infected with the fungus *Fusarium oxysporum* f.sp. *lycopersici* (FoI)

Olive oil mill wastewater (olive OMW) contains components capable of inhibiting the growth of microorganisms (10). Olive OMW contains phenolic compounds (11), polysaccharides, lipids, proteins and a number of monocyclic and polymeric aromatic molecules (5), which might exhibit inhibition effects towards some specific microorganism populations. In the current study filter sterilized olive OMW significantly reduced the growth of important soil borne plant pathogens as *F. oxysporum* f.sp. *lycopersici, Pythium* spp., *S. sclerotiorum* and *V. dahliae*. According to D'Annibale et al. (4) phenolic compounds are the main determinants of the antimicrobial effect of the olive residues. Thus, probably the phenolics (6) of olive OMW used in this experiment had a significant inhibitive effect on all tested fungi *in vitro* in agreement with D'Annibale et al. (4) and Bonanomi et al. (1). The use of olive OMW sterilization, probably removed or destroyed the phenolic compounds from olive OMW solution resulting in the same or a better growth media as the untreated (control) for all tested fungi *in vitro*. Furthermore, the production of the two species of *Penicillium* (*P. italicum and P. digitatum*) and *B. cineria* spores on fruits-vegetables was significantly inhibited by olive OMW. We assume that the presence of phenolic compounds on olive OMW suppresses the fungal reproduction and could possibly offer a protection on fruits and vegetables from post-harvest diseases. Finally, tomato plants infested with *F. oxysporum* f.sp. *lycopersici* and olive OMW, produced better developed plants compared with the plants infested only with *F. oxysporum* f.sp. *lycopersici.*

Conclusions

Overall we believe that the olive OMW due to the phenolics have antifungal activity and could possibly be used against soil borne plant pathogens, fruit and vegetable parasites causing post harvest diseases respectively.

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