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POTENTIAL USE OF OLIVE OIL MILL WASTEWATER TO CONTROL 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, filtered and non-sterilized olive OMW was tested *in vitro* a) on mycelium growth of *Pythium* spp., *V. dahliae, S. sclerotiorum* and *F. oxysporum* f.sp. *lycopersici* on PDA medium, b) on sporulation of *Penicillium* species and *Botrytis cinerea* on infected with the pathogen fruits (mandarin and red pepper) and c) on tomato plants (seedlings) infected with the fungus *V. dahliae* or *S. sclerotiorum* . The *in vitro* results shows that the filter sterilized olive OMW inhibits the growth of all tested fungi mycelium. Olive OMW decreased fungi spores (conidia) number on infected fruits and acted positively on tomato plant growth.

1.Introduction

During olive oil extraction a large amount of solid and aqueous residues as olive oil mills wastewaters (olive OMWs) produced annually worldwide where the majority of it 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 and flavonoids (Gonzales *et al*. 1999; Hamdi, 1992). Some of these phenols are responsible for several biological effects, including antibiosis (Rodrvguez *et al.* 1988) and phytotoxicity (Capasso *et al*. 1992). They also appear to be involved in the defense of plants against invading pathogens, including bacteria, fungi and viruses (Marsilio *et al.* 2001). 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 plant post-harvest diseases.

2.Material and methods

2.1.Effect of olive OMW on the mycelium growth of fungus

The antifungal effect of olive OMW against plant pathogens (*Fusarium oxysporum* f.sp. *lycopersici, Pythium* spp., *Sclerotinia sclerotiorum* and *Verticillium dahliae*) were tested *in vitro*. Tested 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 25ml of olive OMW were added into 1l agar and further sterilized by autoclaving (120 ^{o}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 21ºC for six days and fungi mycelium growth was recorded.

2.2.Antimicrobial activity of olive OMW on fruits treated with pathogens

Two common species of *Penicillium* (*P. italicum and P. digitatum*), isolated from mandarin fruits and *Botrytis cinerea* isolated from red horn (sweet) peppers 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 at 10^6 spores/ml. A 50ml of olive OMW were added in each flask. Mandarin fruits were surface sterilized and soaked for 3 min in 1l beakers contained 500 ml of the above spore and olive OMW solution. After that time fruits removed from the flasks, dried for 10 min in a laminar flow unit and incubated at 21ºC for 12 days. Olive OMW was passed through Whatman filter paper No 2 before added to each beaker. The same procedure was followed for red horn peppers inoculated with *B. cinerea.* After the incubation time, the spore number of each mandarin fruits or peppers were count by scraped each treated fruit surface into 1l beaker contained 500ml distill 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 mandarin fruits and infected with spores and olive OMW red peppers. Equal numbers of mandarin fruits and red peppers soaked only in olive OMW and only in fungus spore suspension were used as control.

2.3.Effect of olive OMW on tomato plants infected with Fusarium oxysporum f.sp. lycopersici

In this experiment, two tomato varieties were used, cv. Roma and cv. Marmande. Plant pathogens used were *V. dahliae* and *S. sclerotiorum,* both isolated from tomato plants. For each variety, 42 tomato seedlings were incubated with spores (10^6 conidia/ml) of *V. dahliae* and 42 seedlings with *S. sclerotiorum* with mycelial suspension collected from 10 PDA petri dishes. In *V. dahliae* treatment from the 42 plants 21 of them were incubated in *V. dahliae* conidia suspension for 10 min and 21 were incubated in the conidial suspension treated with olive OMW (5 ml/100 ml in total solution). In *S. sclerotiorum* treatment, 21 tomato plants were incubated in *S. sclerotiorum* mycelial suspension for 30 min and 21 were incubated in mycelial 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 250ml pots and kept in a glasshouse. Plants were harvested 45 days after planting and stem height were measured. Tomato plants (21plants/variety) were used as control.

2.4.Statistical analysis

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

3.Results and discussion

3.1.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 inhibits the growth of all tested fungi mycelium (Fig. 1). Sterilized olive OMW had similar effect on the mycelia growth of *Pythium* spp., *V. dahliae, S. sclerotiorum* and *F. oxysporum* f.sp. *lycopersici* with the untreated control. However, sterilised olive OMW seems to have some positive effect on the mycelium growth of all tested fungi (Fig 1).

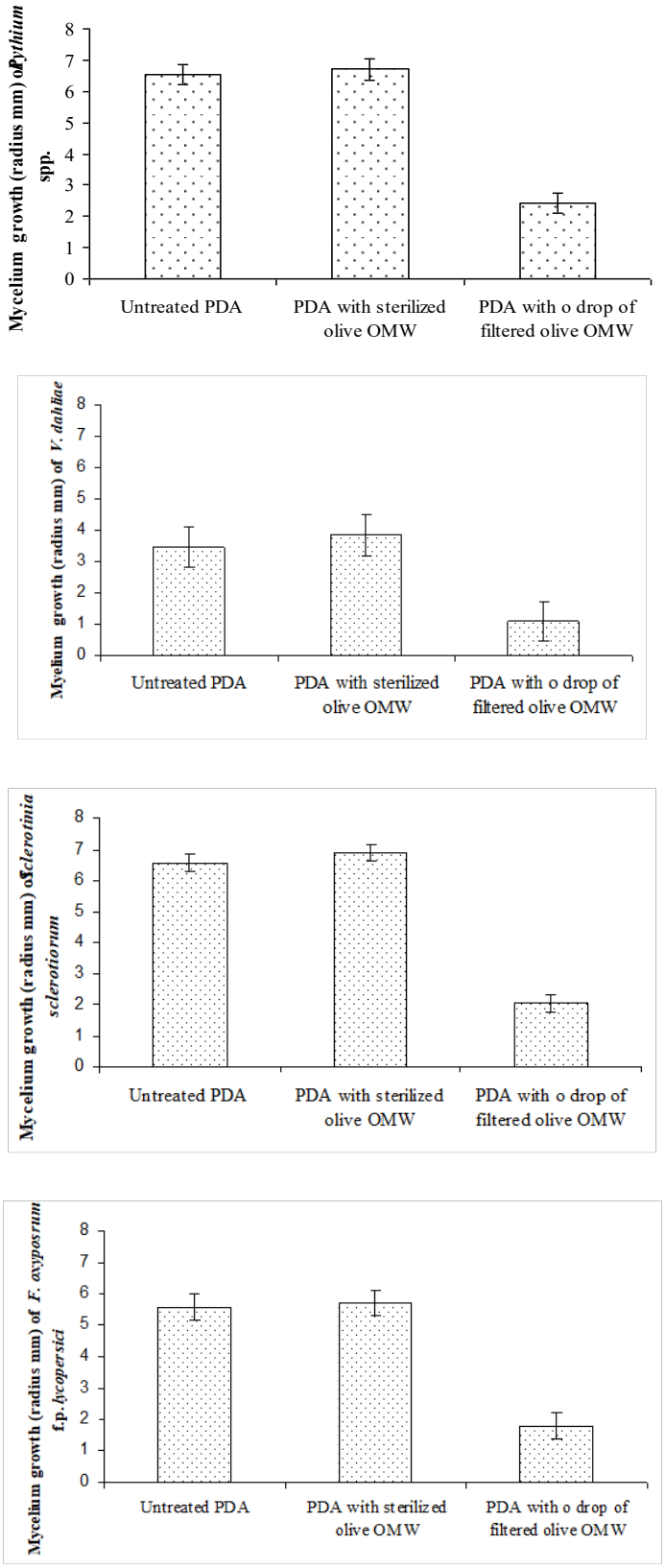

Figure 1. Effect of sterilized and filtered olive oil mill wastewater (olive OMW) on the mycelium growth of *Pythium* spp., *V. dahliae, S. sclerotiorum* and *F. oxysporum* f.sp. *lycopersici*.

3.2.Antimicrobial activity of olive OMW on fruits treated with pathogens

The olive OMW reduced the number of *Penicillium* spp. (P<0.001) and *B. cinerea* (*P*<0.001) spores from mandarin fruits and red peppers respectively. On mandarin fruits the average spore's number was $4,7x10^6$ for mandarin fruits infected only with *Penicillium* species and $0,9x10^2$ for mandarin fruits infected with *Penicillium* species and treated with olive OMW (Fig. 2). On red horn (sweet) peppers the average spore's number was $4.6x10^5$ for peppers infected only with *B. cinerea* and $2,2x10^2$ peppers infected with *B. cinerea* and treated with olive OMW.

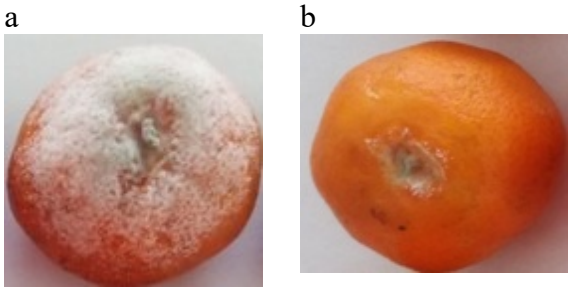

Figure 2. Mandarin fruits infected with *Penicillium* species only (a), with *Penicillium* species and treated with olive OMW (b).

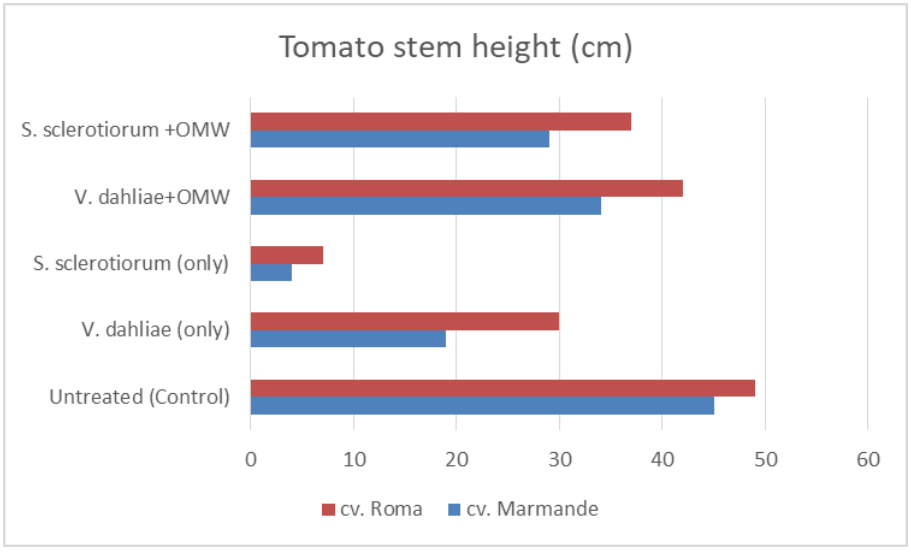

Figure 3. Effect of olive OMW on tomato stem height infected with *V. dahliae* spores or with *S. sclerotiorum* mycelia suspension.

3.3.Effect of olive OMW on tomato plants infected with *Fusarium oxysporum* f.sp. *lycopersici*

There was a statistically significant difference (*P*<0.001) between untreated (Control) and treated plants with *V. dahliae* spores or *S. sclerotiorum* mycelia suspension on the height of tomato stem (Fig. 3). Olive OMW produced more plant biomass (shoot length) than those infected only with *V. dahliae* spores or with *S. sclerotiorum* mycelia suspension (Fig. 3).

4.Conclusions

Olive oil mill wastewater (olive OMW) contains phytotoxic components capable of inhibiting the growth of microorganisms (Ramos-Cormenzana *et al.* 1995) and plants (Martin et al., 2002). Olive OMW contains phenolic compounds (Ramos-Comenzana *et al.* 1995) polysaccharides, lipids, proteins, and a number of monocyclic and polymeric aromatic molecules (Ethaliotis *et al.* 1999) which might exhibit inhibition effects towards some specific microorganism populations. In the current study filtered sterilised olive OMW significantly reduced the growth of *Pythium* spp., *V. dahliae, S. sclerotiorum* and *F. oxysporum* f.sp.

lycopersici. According to D'Annibale *et al.,* (2004) phenolic compounds are the main determinants of the phytotoxic effect of olive residues. Thus, the phenolics of olive OMW used in this experiment had negative effect on all tested fungi *in vitro*. The used for olive OMW sterilization probably removed or destroyed the phenolic compounds from olive OMW solution resulted a same or a better growth media for all tested fungi *in vitro*. Furthermore, the production of the two species of *Penicillium* (*P. italicum and P. digitatum*) and *B. cinerea* spores on fruits inhibited by olive OMW. We assume that the presence of phenolic compounds on olive OMW suppresses fungi reproduction and possible could offer a protection on fruits from post-harvest diseases. Tomato plants infected with *V. dahliae* spores or *S. sclerotiorum* mycelia suspension and treated with olive OMW produced well developed plants compared with the plants infected only with *V. dahliae* spores or *S. sclerotiorum* mycelia suspension. Same results have been reported (Bonanomi *et al.* 2006, Vagelas *et al.* 2009) for olive mill residues affect saprophytic growth and disease incidence of foliar and soilborne plant fungal pathogens same as *Penicillium* spp., *B. cinerea*, *V. dahliae* and *S. sclerotiorum* presented in this research. Overall, we believe that the olive OMW due to phenolics have antifungal activity and could possibly use against soil borne fungal pathogens and fruit parasites such as *Penicillium* spp., causing plant or post-harvest diseases, respectively.

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