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BIOACTIVITY OF OLIVE OIL MILL WASTEWATER AGAINST POST-HARVEST DISEASES

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KEY WORDS*: Olive oil mill wastewater, antifungal activity, post-harvest diseases*

ABSTRACT

The antifungal activity of olive oil mill wastewater (olive OMW) was investigated. The effect of sterilized, filtered sterilized and non sterilized olive OMW was tested a) on mycelium growth of Botrytis cinerea in vitro and b) on strawberries fruits infected with the fungus Botrytis cinerea in vivo. The results show that the filtered sterilized olive OMW inhibits the growth of Botrytis cinerea mycelium in vivo confirming the antifungal activity of the phenolic compounds which contained on olive OMW in vitro.

INTRODUCTION

During olive oil extraction a large amount of solid and aqueous residues known as olive oil mill wastewaters (olive OMWs) are produced annually worldwide where the majority of it is produced in the Mediterranean basin. The uncontrolled disposal of olive OMW is becoming a serious environmental problem due to its high content in 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 fruits protection against microorganism could be a solution for residues management and nature protection. The main objective on this study was to examine the post-harvest biological control of grey mould (*Botrytis cinerea* Pers.: Fr.) on fresh-market strawberries with olive OMW.

MATERIAL AND METHODS

Effect of olive OMW on biological control of grey mould (Botrytis cinerea Pers.: Fr.) on fresh-market strawberries

Botrytis cinerea isolated from market strawberries was used for this experiment. Spores suspension was prepared by isolating spores of above *Botrytis* species, from 7 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

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surface with 3ml distilled water and filter the produced solution through sterilized muslin. In each flask spores suspension was adjusted at 10^6 spores/ml. A 50ml of olive OMW were added in each flask.

Fresh-market strawberries 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 21^{o}C for 12 days. Olive OMW was passed through Whatman filter paper No 2 before added to each beaker. After the incubation time, the spores number of each strawberry fruits was counted by scraping fruits surface into 200ml beaker which contained 50ml distill water.

The spore number per treatment and per beaker was counted by optical microscope using a hematocytometer.

Also, after the incubation period, the mycelium (molt) formation of each strawberry fruit was recorded and mold formation was sorted in six classes (0-5, as reported by Vagelas *et al.* 2009), where 0 is equal to healthy fruits, 1=slightly mold fruits and 5=heavy mold fruits. The experiment had fourteen replicates and four treatments; strawberry fruits infected with spores and olive OMW and strawberries infected only with spores, treated only in olive OMW and treated only with sterilized water were used as control.

In vitro assessment of antimicrobial activity of olive OMW on Botrytis cinerea mycelia

The antifungal effect of olive OMW against *Botrytis cinerea mycelia* was tested *in vitro*. Tests were made on PDA (Potato Dextrose Agar; DIFCO) in 9 cm Petri dishes. 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 (121 ^{0}C for 20 min). In the second treatment a drop (50 μl) of filter sterilized 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 fungus which was 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 drop. Equal plate numbers were used as control (without olive OMW). Plates were incubated at 21^{o}C for six days and fungus mycelium growth was recorded.

Statistical analysis

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

RESULTS

*Effect of olive OMW on biological control of grey mould (*Botrytis cinerea *Pers.: Fr.) on fresh-market strawberries*

The olive OMW significantly reduced the number of *B. cinerea* (P<0.001) spores. The average spore's number was 3.4x10^6 for strawberry fruit infected only with *B. cinerea* and 1,6x10^2 conidia/strawberry fruit infected with *B. cinerea* and treated with olive OMW. Further, a high mold formation was recorded only in treatments with strawberry fruits treated with fungus conidia suspension (Figure 1).

In vitro assessment of antimicrobial activity of olive OMW on Botrytis cinerea mycelia

There was a statistical significant difference between filtered sterilized olive OMW and control (untreated PDA and sterilized with olive OMW PDA), (P<0.001). The total phenols content and antioxidants of filtered sterilized olive OMW could be an explanation of olive OMW antimicrobial activity in vitro confirming the biological control of grey mould by olive OMW as presented with the *in vivo* studies.

The filter sterilized olive OMW significantly inhibited the growth of *Botrytis cinerea* mycelia and showed only fungistatic activity against grey mould *in vitro* probably due to phenols content (Figure 2).

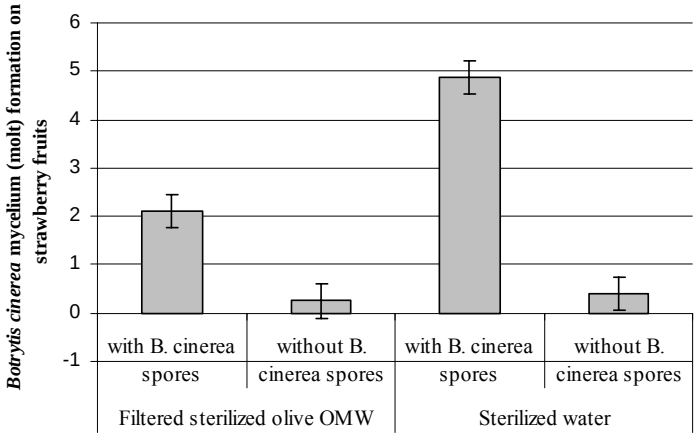

Figure 1. Effect of sterilized and filter sterilized olive oil mill wastewater (olive OMW) on the mycelium mold formation of *Botrytis cinerea* on strawberry fruits.

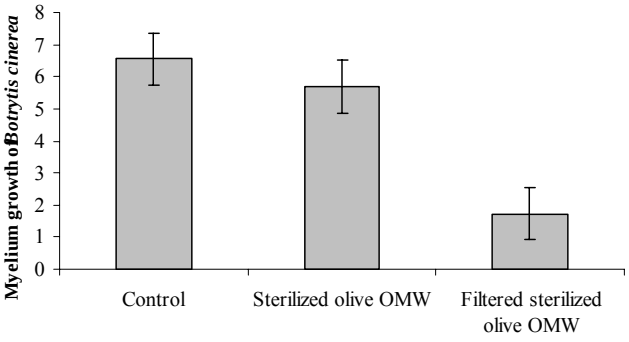

Figure 2. Effect of sterilized and filter sterilized olive oil mill wastewater (olive OMW) on the mycelium growth of *Botrytis cinerea*.

DISCUSSION

Olive oil mill wastewater (olive OMW) contains phytotoxic components capable of inhibiting the growth of microorganisms (Ramos-Cormenzana *et al.*, 1996) 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 filter sterilised olive OMW significantly reduced the growth of *Botrytis cinerea*. 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 *Botrytis cinerea* mycelia *in vitro*. The used for olive OMW sterilization at 121 ^{0}C for 20 min 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 *B. cinerea* spores on fruits inhibited by olive OMW. We assume that the presence of phenolic compounds on olive OMW suppresses fungus reproduction and possible could offer a protection on strawberry fruits from post-harvest diseases. Overall we believe that the olive OMW due to phenolics have antifungal activity and could possible used against fruit fungal pathogens for preventing post harvest diseases.

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