ARTICLE DOI: 10.2478/V10133-009-0017-3 A&EB

BIOLOGICAL CONTROL OF *BOTRYTIS* FRUIT ROT (*GRAY MOLD*) ON STRAWBERRY AND RED PEPPER FRUITS BY OLIVE OIL MILL WASTEWATER

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ABSTRACT

The antifungal activity of olive oil mill wastewater (olive OMW) on Botrytis cinerea Pers was investigated. The effect of sterilized, filtered and non sterilized olive OMW was tested in vitro: a) on mycelium growth of B. cinerea on PDA agar plates and b) on strawberry and pepper fruits infected with the pathogen. The results show that the filter sterilized olive OMW inhibits the growth of B. cinerea mycelium in vitro probably due to the activity of the phenolic compounds contained on olive OMW. Furthermore, olive OMW significantly decreased fungus mold formation on the tested fruits.

Keywords: biocontrol, olive oil mill wastewater, antifungal activity, *Botrytis cinerea*

Biotechnol. & Biotechnol. Eq. 2009, **23**(4), 1489-1491

Introduction

Botrytis fruit rot (gray mold) is caused by the fungus *Botrytis cinerea* and is the most important disease of strawberry worldwide. The fungus can cause gray mold rot on many fruits and vegetables as pepper. Gray mold appears as tan or brown watersoaked lesion that becomes grayish or dried-out on fruits. On pepper, the infection appears during storage but it starts already in the field. The infected fruits are covered with fine white-gray or tan mold. On strawberry this disease causes severe pre-harvest losses, primary due to infection that has started by spores produced on dead strawberry foliage within the field. Epidemics of *Botrytis* fruit rot are initiated by conidia produced in dead strawberry leaves. Conidia are dispersed by air, water or harvesting and ultimately infect different floral parts including stamens and petals. After infecting the flower, the fungus eventually invades maturing fruit and causes rot. The fungus can also spread to adjacent fruit by direct contact. As the epidemic progresses, the pathogen sporulates on diseased flowers and fruit, and these become important sources of inoculum (6).

Control of *Botrytis* fruit rot requires a combination of chemical, cultural and biological control methods. Main recommendations for reduction of the infection with *Botrytis* are the usage of highly active botryticides. The removal and destruction of dead or infected plant parts as well the removal of diseased fruit from within the plant canopy will reduce the amount of inoculum capable of producing new infections.

Regarding biocontrol many fungi, yeasts and bacteria have been found to be effective in controlling *Botrytis* diseases due to enzymes and antibiotics they produced. Other products with anti-botrytis activity could be the residues of olive OMW. The residues of olive OMW appeared to have effective antifungal activity against soil born plant pathogens (12). The use of olive OMW for fruit and vegetable protection against post-harvest diseases could be a promising solution for preventing losses of fruits and vegetables as mentioned above. The aim of this work was to examine the antifungal (anti- *Botrytis* activity of olive OMW against post-harvest diseases caused by the fungus *B. cinerea*.

Materials and Methods

Effect of olive OMW on the mycelium growth of fungus

The antifungal effect of olive OMW against mycelia plugs of *B. cinerea* was tested *in vitro.* Treatments were conducted on PDA plates (Potato Dextrose Agar; DIFCO) with a) olive OMW added into the medium and autoclaved and b) a drop (4 μm) of filter sterilized olive OMW (using a syringe Millipore filter 0.2 μm) added onto the agar surface. These are fully described in Vagelas et al. (12). Fifteen agar plates per treatment were inoculated with a mycelium plug (5 mm in diameter) of *B. cinerea* and the same number of plates was used as control. Plates were incubated at 21^{0}C for six days and fungi mycelium radius growth was recorded.

Antimicrobial activity of olive OMW on fruits inoculated with *B. cinerea*

Botrytis cinerea isolated from strawberry fruits was used for this experiment. Spores suspension was prepared by collecting spores of above fungus from 12 days old cultures. Three agar plates per fungus culture were used to collect spores. Spores were collected in 1l Erlenmeyer flask according to the method described by Vagelas et al. (12). In each flask spores suspension was adjusted at 10^6 spores/ml. Red horn peppers and strawberry fruits were surface sterilized and soaked for 3 min in 1l beakers that contained 500 ml of the above fungus spore suspension solution. After that time fruits were removed from the beakers, dried for 10 min in a laminar flow unit, treated with olive OMW

mixed with talc powder 1:6 v:v and incubated at 21^{0}C for 16 days. After the incubation time, the mycelium (molt) formation of each fruit of pepper or strawberry was recorded and mold formation was sorted in five classes (0 to 4), where 0 is equal to healthy fruits; 1=slightly mold fruits and 4=heavy mold fruits.

Two experiments were conducted in order to identify the effect of olive OMW on fruits (strawberries or pepper) infected by *B. cinera*. Each experiment had two treatments, fruits infected with B. cinerea conidia and treated with olive OMW in form of talc powder and fruits infected with *B. cinerea* conidia and treated with talc powder only; four replicates per treatment with twelve fruits (strawberries or pepper) per replicate were used. Equal numbers of healthy fruits (red peppers or strawberries), treated with olive OMW in form of talc powder or fungus conidia suspension were used as control. All fruits were kept in closed plastic boxes.

Statistical analysis

Data of all treatment were analyzed using the Minitab statistical package. Analysis of variance was followed by comparison of means for significant effect using LSD. Differences were considered to be significant at P≤0.05.

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 filter sterilized olive OMW inhibited the growth of the tested *B. cinerea* mycelium (**Fig. 1**). Sterilized (autoclaved) olive OMW had similar effect on the mycelia growth of *B. cinerea* with the untreated control (**Fig. 1**).

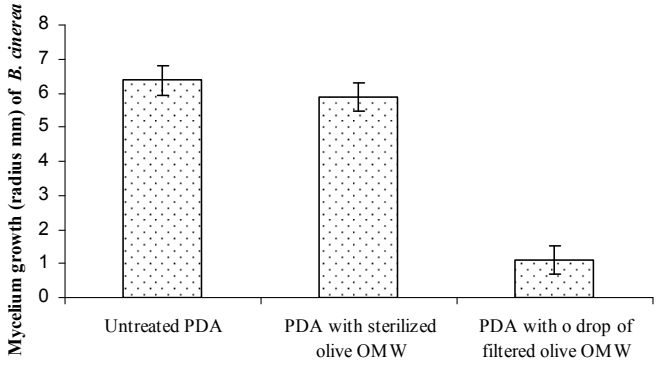

Fig. 1. Effect of sterilized (autoclaved) and filtered (filter sterilized) olive oil mill wastewater (olive OMW) on the mycelium (radius) growth of *B. cinerea*

Antimicrobial activity of olive OMW on fruits treated with *B. cinerea*

Pepper and strawberry fruits treated with olive OMW in form of talc powder significantly reduced *B. cinerea* mold formation (**Table 1**). A high mold formation was recorded only in treatments with fruits (strawberry or pepper) treated by a) fungus conidia suspension and b) infected with *B. cinerea* conidia and treated only with talc powder (**Table 1**, **Fig. 2** and **Fig. 3***)*. Moreover, in those treatments the mold produced a strong alcohol odor.

TABLE 1

Effect of olive OMW on mold formation of *B. cinerea* after 16 days incubation at 21^{0}C

	Treatments*				
Tested fruit:	**A**	**B**	**C**	**D**	**E**
Pepper	1	4	0	0	4
Strawberry	1	3	0	0	4

***A**: infected with *B. cinerea* conidia and treated with olive OMW in form of talc powder; **B**: infected with *B. cinerea* conidia and treated only with talc powder; **C**: untreated fruits, **D**: treated only in olive OMW with talc based powder and **E**: treaded only with *B. cinerea* conidia suspension

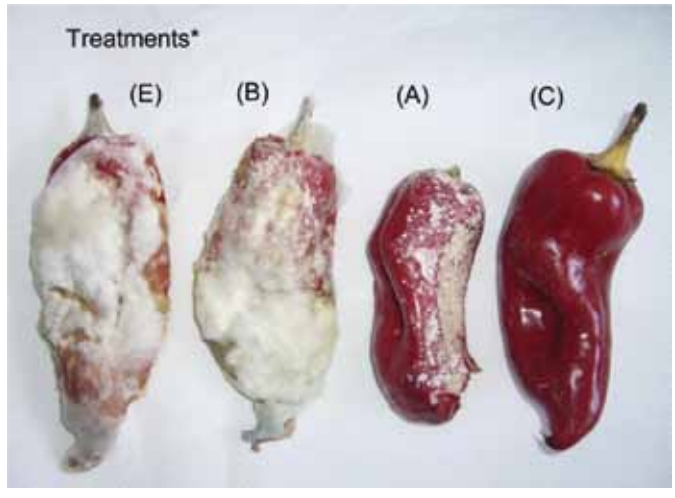

Fig. 2. Effect of olive OMW on mold formation of *Botrytis cinerea* on red horn pepper. Where **E**: pepper fruit treaded only with *B. cinerea* conidia suspension; **B**: pepper fruit infected with *B. cinerea* conidia and treated only with talc powder; **A***:* pepper infected with *B. cinerea* conidia and treated with olive OMW in form of talc powder; and **C**: untreated fruits (absolute control)

Olive oil mill wastewater (olive OMW) contains a number of biologically active substances capable of inhibiting the growth of microorganisms (11) and even plants (9). Many phenolic, free fatty acids and aromatic compounds have been detected (7, 11) in olive oil mill residues associated with phytotoxic and antimicrobial properties of these residues (10). Several investigators reported that the inhibition of microbial growth and the toxic activity of olive OMW caused by different chemical compounds of olive residues (1, 2, 7). Low molecular-weight phenolic compounds seem to be the main determinants of the anti-microbial effect of olive residues (5, 8) while high molecular-mass polyphenols, organic acid, lipids, oligosaccharides and glycoproteins can contribute to the phytotoxic potential of the waste (4). Thus, several methods have been developed to degrade phenols in olive oil residues.

In the current study filtered sterilised olive OMW significantly reduced the growth of *B. cinerea* mycelia and probably the phenolics of olive OMW used in this experiment had negative effect on *B. cinerea* mycelia *in vitro*. The used of olive OMW sterilization probably removed or destroyed

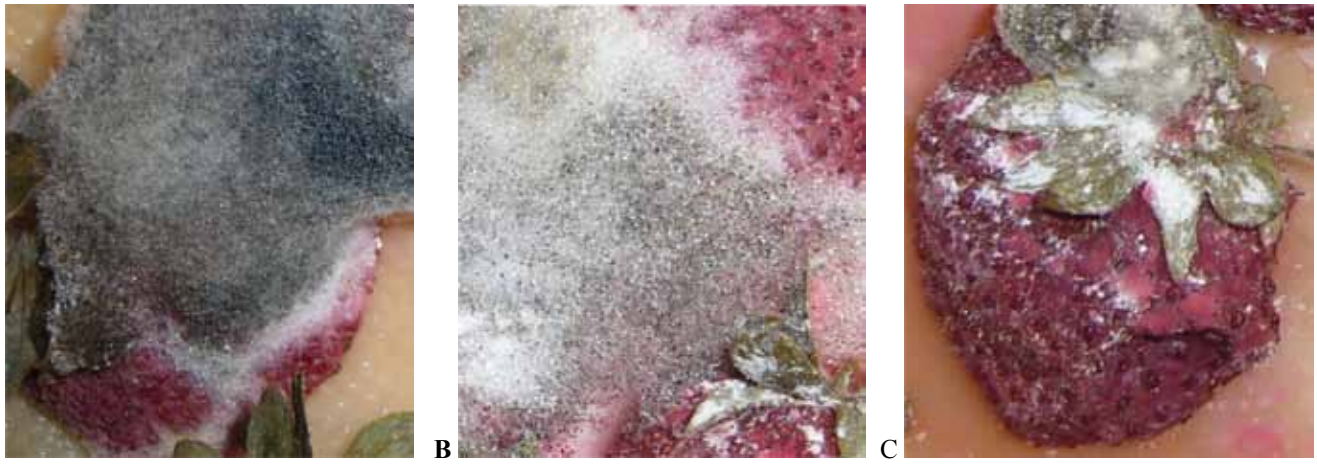

Fig. 3. Effect of olive OMW on mold formation of *Botrytis cinerea* on strawberry fruits. Where **A**: strawberry fruit treaded only with *B. cinerea* conidia suspension; **B**: strawberry fruit infected with *B. cinerea* conidia and treated only with talc powder and **C**: strawberry infected with *B. cinerea* conidia and treated with olive OMW in form of talc powder

phenolics in olive OMW liquid resulting in a same growth media for the tested fungus. Furthermore, the production of *B. cinerea* mold formation on tested fruits was significantly inhibited by olive OMW. We assume that the presence of phenolic compounds on olive OMW suppresses pathogen mycelium and possibly it could offer a protection on fruits from post-harvest diseases such as *Botrytis* fruit rot (*gray mold*). Similar results have been reported (3, 12) for olive mill residues to affect saprophytic growth and disease incidence of foliar, soilborne and post harvest plant pathogens same as *B. cineria* that was presented in this study.

Conclusions

The results of this work demonstrated the high potential of olive OMW as a fungicide against fruit parasites causing post harvest diseases. The results presented here only showed the anti-fungus activity of phenolics that remained in olive residues after filter sterilization. In future studies, it would be interesting to study the antifungal activity of this phenol content(s) and to evaluate it on further *in vitro* and *in vivo* studies for potential use as a bio-fungicide.

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