Egyptian Journal of Biological Pest Control, 25(2), 2015, 367-377

Management of tomato damping-off disease caused by *Fusarium oxysporum* and *Rhizoctonia solani* using chemical and biological degradable olive mill waste water

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(Received: July 1, 2015 and Accepted: September 3, 2015)

ABSTRACT

Damping-off of tomato seedlings caused by *Rhyizoctonia solani* and *Fusarium oxysporum* is a common fungal disease causing severe seedlings death in the nursery. Cultural and biological control are the only tools in organic crops to manage this disease. Possibility of using of olive mill waste waters (OMW) against the disease as eco-friendly method depends on its polyphenol compounds. Biological treatment using OMW as organic agro-wastes aganist some of the indigenous fungal strains of *Aspergillus wentii* and *A. niger* and with *Pleurotus ostreatus* as reference strain showed ability to degradable polyphenolic compounds. Also, chemical treatment of OMW with H_2O_2 solution (30% w/v at 0.55 *M* pH 9, under UV radiation /150 min) showed similar results. Cell less OMW tested as antagonistic against both pathogens, showed a potent effect with increasing concentration, spatially *R. solani* was more sensitive than *F. oxysporum*. However, adding of chemical and biological treated OMW as will as crud OMW on tomato seeds (Castle rock and Global CVs), germination were unclear. In this study, the relative tomato seeds germination of both genotype recorded highest values at OMW (10% for crude OMW and 5% chemically treated OMW). All the treatments significantly reduced percent damping-off of tomato seedling over untreated control, in terms of suppressing the disease incidence with increasing plant survival. Use of OMW, either crude or biologically treated with *P. ostreatus,* significantly increased dehydrogenase activity in rhizosphere region of infested tomato plants after 45 days of seedling than chemical treatment of OMW. Obtained results slightly showed that there was a variation in numbers and types of compounds present after each biological and chemical treatment of OMW and large amounts of degraded compounds in OMW chemically treated. Also, degradation process of polyphenolic compounds continued to give very small non-toxic compounds such as CO_2 and H_2O as revealed by GC-MS analysis of OMW.

Key words: Tomato, Damping-off, Olive Mill Waste Water, Seed germination, *Fusarium oxysporum*, *Rhizoctonia solani*.

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is one of the most popular and commonly grown vegetables in the world and can be grown either in the field or under greenhouse conditions. Tomato production has a major role in global horticulture, ranking second in importance to potato in many countries. Damping-off is one of the worst diseases of tomato occurring in the nursery and can kill both germinating seeds and young seedlings (Agrios, 2005). Several fungi that are widely distributed in soils can cause this disease, including *Rhizoctonia solani*, *Pythium* spp., *Phytophthora* spp., *Sclerotinia* spp. and *Fusarium* spp. (Stephens *et al.*, 1982). Damping-off of tomato seedlings is the most common disease caused by *Rhizoctonia solani* (Moussa, 2002) and *Fusarium oxysporum* Snyder and Hansen (Smith, 2007) as soil-borne fungal pathogens. *R. solani* develops in both cultured and non-cultured soils and lives in the soil in the form of sclerotia and does not generate asexual spores (Huang *et al.* 2011). Chemical fungicides are often used when losses from *R. solani* are substantial. Intensive use of chemical fungicides has not only created problems of fungicide resistance and increased contamination of the soil, but may also have adverse high toxicity on microbial communities. In addition, chemical control is not completely effective, and *Rhizoctonia* disease remains a persistent problem (Huang *et al.* 2011). *F. oxysporum* was first described over 100 years ago in the UK caused *Fusarium* wilt, at present, losses from *Fusarium* wilt can be very high given susceptible host virulent pathogen combinations; yield losses of *Fusarium* wilt up to 45% were recently reported in India (Ramyabharathi *et al.*, 2012). Moreover, losses from *Fusarium* crown rot in greenhouse tomato have been estimated at up to 90 and 95% in Tunisia and Canada, respectively and the disease incidence was estimated as 100% in the field in the USA (Hibar *et al.*, 2007).

Strategies to control soil-borne diseases are limited because cultivars with complete resistance are not available. Also, control of the soil-borne pathogens is difficult because of their ecological behavior, their extremely broad host range and the high survival rate of resistant forms such as chlamydospores and sclerotia under different environmental conditions. Many research studies have shown that biological control offers an environmentally friendly alternative to protect plants against soil-borne pathogens (Weller *et al.*, 2002). Although the number of bio-control products is increasing, these products still represent a very small

proportion of fungicides (Fravel, 2005). Therefore, other alternatives to control damping-off and root rot are currently of great importance. Use of organic amendments plays an important role in the outcome of the plant–pathogen interactions (Abawi and Widmer, 2000). Biological control strategies based on the application of organic amendments and microorganisms have been explored intensively over the past decade. Among the different soil amendments that have been evaluated for their suppressive effect against plant pathogens are compost preparations made from olive mill wastes (Alfano *et al.*, 2011).

Olive mill waste water (OMW) is a major environmental problem owing to its high organic load and antimicrobial properties, particularly for Mediterranean countries. The production rate of olive oil is about 1.4-1.8 million tons per year in the Mediterranean resulting in 30 million tons of by-products (Barbera *et al.*, 2013). Many studies have reported the efficacy of OMW composts on suppressing a number of soil-borne plant pathogens including *R. solani* on cucumber seedlings (Trillas *et al.*, 2006), *Phytophtora nicotianae*, *Fusarium oxysporum* f. sp. *lycopersici*, *F. oxysporum* and *Pythium ultimum* causing damping-off on tomato plants (*Lycopersicon esculentum* Mill.) (Alfano *et al.*, 2011).

OMW is an acidic base has a high concentration of organic material and is resistant to biodegradation due to toxic effects of some organic compounds, mainly polyphenols (Khatib *et al.*, 2009). Many studies established that these wastes have a high fertilizer value when applied to the soil because of the high organic matter content and some mineral nutrient content (Paredes *et al.*, 1999). However, despite the potential agronomic value, soil amendment with OMW is also known for its antimicrobial activity (Kistner *et al.*, 2004). El Hadrami *et al.* (2004) observed negative effects of OMW on germination of chickpea, durum wheat, tomato and maize. They compared the toxicity of OMW with the corresponding doses of their polyphenolic extracts and concluded that phenols contained in the OMW are the main compounds implicated in suppression of seeds germination. Yangui *et al.* (2008) pointed out the importance of hydroxytyrosol, a low molecular weight phenolic substance present in the olive mill waste waters in reducing *Verticillium* wilt symptoms development on tomato plants.

This research aimed to study the effect of chemical and biological treated and untreated OMW on soil-borne plant pathogenic fungi *R. solani* and *F. oxysporum* *in-vitro* and *in-vivo* to verify their suppressive effect against these two soil-borne pathogenic fungi and also analyzed the direct effect of OMW on tomato seeds germination.

MATERIALS AND METHODS

Materials

The fungus *Pleurotus ostreatus* (NRRL–2366) was provided by Northern Regional Research Laboratory, Illinois, Peoria, USA and was used as standard strain in the present study. Rose-bengal chloramphenicol agar medium was used for isolation of fungi from OMW; Potato Dextrose Agar (PDA) medium (Martin, 1950) was used for maintenance of the isolated fungi and Czapeks Dox Agar medium was used for identification of isolated fungi. All previous media were obtained from Difco Company (England). Tomato seedlings of two genotypes Castle rock and Global seedlings by age 15 days were obtained from Agriculture Ministry Greenhouse, Dokki, Giza, Egypt. Hydrogen peroxide was obtained from El-Nasr Co. (Egypt).

Methods

Isolation and identification of pathogenic fungi

R. solani and *F. oxysporum* were originally isolated from tomato plants rhizosphere exhibiting symptoms of damping-off which were grown in pots inside experimental greenhouse at Department of Vegetables Pathology, Plant Pathology Institute (PPI), Agricultural Research Center (ARC), Giza, Egypt. These dominant rhizosphere fungi were isolated by plate count dilution method as described by Xinyu *et al.* (2007) using PDA medium. All isolates were purified, using single spore and hyphal tip techniques according to method described by Goh (1999). The obtained isolates were stored at 4°C in tubes containing PDA. *F. oxysporum* isolates were identified morphologically based on characteristics of the macro conidia (shape and size), phialids, micro conidia, chlamydospores, and colony growth traits (Leslie and Summerel, 2006). Meanwhile, *R. solani* isolates were identified morphologically based on cultural characteristics, hyphal anastomosis, and number of nuclei per hyphal cell (Blazier1 and Conway, 2004). Pathogenicity test of selected isolates were done against tomato seedling grown in soil artificially inoculated with *F. oxysporum* or *R. solani* isolates previously grown on barley grain medium. This experiment was carried out in pots at the greenhouse of ARC.

Chemical composition of OMW

Olive mill wastewater (OMW) was obtained from Horticulture Research Institute, ARC,

Table (1): Chemical composition of OMW

Component		Amount in 100 ml
Moisture		86.68%
Total solids		13.32 g
Ash		0.86 g
Total nitrogen (without centrifugation)		4.24 g
Total nitrogen (after centrifugation)		2.61 g
Total carbohydrates.		4.20 g
Total lipids		0.82 g
Phenolic compounds		0.56 g
COD		9.00 g
BOD_5		4.10 g
Minerals	Potassium	75.0 mg
	Calcium	2.7 mg
	Sodium	30.6 mg
	Zinc	18.0 mg
	Magnesium	38.0 mg
	Iron	19.0 mg
	Manganese	4.0 mg
	Phosphorus	0.19 mg
pH		4.1
EC		10.6 dS/m

dS/m: deci-siemens/meter

Giza, Egypt and was kept at - 20°C until use. Chemical composition of OMW was determined according to listed references in the table (1); phenolic compounds were extracted according to the method of Elena *et al.* (2006) as follows: 10.0 ml of OMW was mixed with 15 ml of hexane; the mixture was dynamically shaken and centrifuged for 5 min at 3000 rpm. The non polar phase was separated to remove lipids. Extraction of phenolic compounds was then carried out with 10 ml of ethyl acetate after acidification by HCl to pH 2. The aqueous phase was separated. The ethyl acetate was evaporated under vacuum and the dry residue wás dissolved in 5 ml of methanol and then total phenols were determined using spectrophotometer as described by Swain and Hillis (1959).

Inhibitory effect of OMW against mycelial growth of *R. solani* and *F. oxysporum*

To study the activity of OMW against mycelial growth of *R. solani* and *F. oxysporum*, different concentrations were prepared (0.5, 1, 2, 3, 4, and 5% v:v) by using cell-less OMW filtered through 0.45-mm filters under aseptic conditions. Different volumes of OMW filtrate were added to a sterilized PDA before solidifying to obtain the proposed concentrations and then rotated gently to ensure equal distribution of the added filtrate. After mixing, the amended PDA was dispensed into 9-cm-diameter Petri dishes and allowed to cool. Five-millimetre-diameter plugs of agar from young pure cultures of *R. solani* or *F. oxysporum* were placed at the center of each plate with the surface mycelium facing down on the test PDA medium. The plates were incubated at 28±2°C, and the radial growth of mycelium was measured at 24-h intervals for a 7 days. Controls were run with inoculated PDA without OMW addition. Three replicates of each concentration were used plus one temporary plate. Plates showed contamination or abnormal growth diameter was discarded. The mycelial radial growth (cm) of tested pathogen in treated and control plates were recorded after 7 days of incubation, Growth (%) and Growth Reduction (%) of the causal pathogens was calculated according to Hmouni *et al.* (1996) using following formula:

$$\text{Growth Reduction (\%)} = \frac{[A_c - A_t]}{Ac} \times 100$$

- Where; A_c: diameter of each colony in control; A_t: diameter of each colony in treatment.

Degradation of phenolic compounds in OMW

To get maximum degradable phenolic compounds of OMW crude, some chemical and biological treatments were carried out according the methods described by Afify *et el.*, (2009) as follows:

Chemical treatments

This treatment was performed on OMW solution, the optimum conditions of phenolic compounds decomposition was done by oxidation using H_2O_2 solutions (30 % w/v) at 0.55 *M* at pH 9, then solution was subjected to UV radiation by using UV lamp (Camag Co. Ltd., Switzerland) at 254 nm for 150 min.

Biological treatment

The fungi *Aspergillus wentii* and *A. niger* as well *Pleurotus ostreatus* were isolated from OMW. The isolated fungi were purified according to method described by Goh (1999) and identified followed description by Samson *et al.* (2007). The microbial strain was sub-cultured every 30 days and maintained at 4°C. All isolates were grown mainly in 250 ml conical flask containing 50 ml of sterilized 20% OMW (121°C for 20 min). The flasks were incubated at 25°C on rotary shaker (150 rpm) for one week. The resulting biomass was collected by centrifugation and filtrate was used for determination of total phenols according to Sayadi and Ellouz (1993), and biologically oxygen demands according to Lenore and Wpcf (1992) and then used for farther bio-experiments.

Phytotoxicity of chemical and biological treated OMW

OMW biologically pretreated with *P. ostreatus, A. niger* and *A. wentii* or chemically pretreated with 0.55 *M* H_2O_2/UV for 150 min were evaluated for toxicity toward seeds germination and sprouts growing of Castle rock and Global tomato genotypes.

Seed germination test

The effect of crude and treated OMW on seed germination was carried out using germination test according to the methods described by Piotrowska *et al.* (2006). Tomato seeds of Castle rock and Global CVs were germinated on chemically treated OMW: water by 5 and 10% (v:v) and biologically treated by 10 and 20% (v:v) using *A. wentii*, *A. niger* and *P. ostreatus* and untreated OMW by 10 and 20% (v:v). Before the beginning the test, seeds were surface sterilized by soaking in 2.7% sodium hypochlorite solution for 3.0 min, then rinsed thoroughly with sterilized water. The sterilized seeds (25 seeds of both tomato cultivars) were transferred on a surface sterilized filter paper (Whatman No.1) placed in sterilized 10 cm Petri dish. The filter paper was initially moistened with 5 ml of the prepared solutions and was replenished as needed. Four plates for each concentration and three replicates for each treatment were arranged in complete randomized distribution. The Petri dishes were wetted daily with 2.0 ml of tested OMW treatments, while irrigation water (tap water) was considered as a control treatment using sterilized syringes. Dishes with seeds were hermetically sealed with Parafilm in Lab Guard Class II Type A/B3 Laminar Flow - Biological Safety Cabinet to prevent evaporation and then incubated in the dark, with alternating temperatures of 20 and 30°C for 16 and 8 h, respectively. Seeds were considered germinated when the radical and hypocotyls together appeared then germination percentage was calculated. A primary root ≥ 2 mm was considered as the end germination point.

$$\text{Relative Germination \% (R.G.)} = [100 \times (G_t/G_c)]$$

- Where: G_t and G_c are the numbers of roots germinated in the treated and control samples, respectively.

Sprouts growing (plant tolerant)

A pot experiment was done to evaluate treated OMW on sprouts growing of tomato plants (Castle rock and Global CVs) and it's tolerant against phytotoxicity by OMW. Experiments were conducted under green house conditions at Microbiology Department, Soils, Water and Environment Research Institute, Ministry of Agriculture and Land Reclamation (MoA), Giza, Egypt during autumn season (2013). Sandy loam soil (native Ismailia soil) was used. The physico-chemical and microbiological properties of the used soil were determined after sterilizing by autoclave as described by Jakson (1958) (Table 2). The soil was moistened with distilled water up to 60% of its water-holding capacity and autoclaved for 1 h at 121°C twice, for 2 successive days. The soil was maintained at 60% of its water-holding capacity for a week. After that, the soil was again autoclaved twice for 20 min at 121°C, then placed in plastic pots (30 cm diameter) each containing 10 kg of autoclaved soil.

Table (2): Physical and chemical properties of the sterilized experimental soil

Mechanical properties	
Sand	70.7 %
Silt	30.3 %
Clay	27.0 %
Soil texture	Sandy
Chemical properties	
Organic matter	0.247 %
Organic carbon	0.143 %
Total nitrogen	0.020 %
Total phosphorus	0.200 ppm
Available phosphorus	0.025 ppm
Water holding capacity	25.00 %
pH	7.75
EC	1.150 ds/m
Anion and cations properties	
Carbonate	Trace
Bicarbonate	1.130 Meq/l
Chloride	0.200 Meq/l
Sulphate	0.070 Meq/l
Calcium	0.640 Meq/l
Magnesium	0.110 Meq/l
Sodium	0.290 Meq/l
Potassium	1.130 Meq/l
Microbiological properties	
Total fungal count	0.77×10^6/g soil cfu
Total bacteria count	1.7×10^6/g soil cfu
Total actinomycetes count	1.3×10^6/g soil cfu

- Meq/l: mill equivalent/l, ds/m: deci-siemens/metre and Cfu: colony formed unit

Five seedlings of Castle rock or Global tomato genotype were planted into each pot. Six replicates of each treatment were done. Concentration 20% of chemically and biologically treated OMW as well as crude OMW was added twice with irrigation water before plantation once every week. After plantation by 15 days, OMWs were added three times with one week up to 3 weeks interval. The pots were completely arranged randomized. Numbers of dead plants as affected by phytotoxicity in comparison with untreated control were calculated two times, the first after 15 days and the second after 45 days of plantation, according to the methods described by Yangui *et al.* (2008).

Effect of different chemical and biological OMW treatments on suppressing tomato damping-off disease

A pot experiment was done to evaluate treated OMW on suppressing damping-off disease of tomato plants under greenhouse conditions as follow:

Inoculums preparation of pathogenic fungi

Pure culture of *R. solani* and *F. oxysporum* isolates were sub-cultured on PDA in Petri-dishes and then incubate at 28 ± 2°C/10 days, then kept at 4°C till use in the following experiment. Artificial soil infestation by both pathogens grown on barley grain medium was carried out. The barley grain were soaked in 5% sucrose solution for 12 h then placed in conical flasks and autoclaved at 15 kg/m^2 pressure and 121°C for 30 min. The flasks were inoculated by liquid culture of the tested fungi (1 ml/flask) and shaken manually to dispense the inoculum on barley grain. The flasks were incubated under dark and static conditions in an incubator at 25±2°C for 7 days. However, the flasks were shaken manually for a few minutes daily to promote uniform colonization of the fungi on barley grain.

Soil infestation

Soil infestation was done by adding inoculums of both tested pathogens at the rate of 5g/kg soil before sowing. Incubated pots were covered with plastic film for one week at room temperature to promote pathogens growth. Then the dose of fresh 10% chemical and biological treated OMW as well as crude OMW were added by 100 ml/for each pot (inoculated soil and non-inoculated soil), this procedures were done twice by irrigation water before plantation once every week. Thirty tomato seedlings (Castle rock or Global CVs) at the stage of two true leaves were sterilized by immersion in ethanol 75% for 5 min, followed by extensive rinsing in sterile distilled water then transplanted into 6 pots (five plants/pot) for each dose. There were three replicates for each tested dose. Pots were placed under ambient conditions and monitored for a growing period of 3 weeks. After plantation by 7 days, OMWs were added three times once a week up to 3 weeks. Plants showing symptoms of damping-off were noted and recorded twice after 15 and 30 days from transplanting date. There were three controls, one for non-infested soil without amendment with OMW, other infested soil by pathogenic tested fungi without amendment with OMW or treated OMW and another for infested soil with amendment of fungicide Carbendazim 50% W.P., by rate 60ml / 20 liter distilled water.

Percentage of infection and healthy survivals in each treatment was determined after 30 days of sprouts transplanted using the following formula:

$$(\%)\ \textbf{Infection} = \frac{\text{Total number of infected seedling}}{\text{Total number of sown seedling}} x\ 100$$

$$(\%)\ \textbf{Survived seedling} = \frac{\text{Total number of healthy seedling}}{\text{Total number of sown seedling}} x\ 100$$

Determination of dehydrogenase activity

Dehydrogenase activity was determined after 45 days of tomato planting in soil to study the correlation with microbial activity in the rhizosphere region. The dehydrogenase activity was determined according to the method described by Casida *et al.* (1964). Enzyme activity (based on dry soil) was calculated in µg of tri phenyl formazan (TPF) / dry soil/day.

Identification of phenolic compounds in all OMW treatments using GC/MS

Polyphenols in OMW were monitored by using gas chromatography coupled with mass spectroscopy (GC/MS) analysis. Analyses were carried out in "Center Laboratory of Biotechnology, PPI, ARC, MoA, Giza, Egypt" as described by Knupp *et al.* (1996).

Extraction: 10 ml of treated OMW and untreated OMW were defatted by washing three times with 20 ml of hexane. The pH was adjusted to pH 4.5 with hydrochloric acid solution then phenolic compounds were extracted twice with 20 ml of ethylacetate. The organic phase was evaporated to dryness at 40°C by using rotary evaporator.

Derivatization: To the phenolic compound residue, 50 µl of pyridine was added and the tube was retained for 10 min at 60°C. The tubes were retained for 60 min at 60°C after addition of 200 µl tri-methyl chlorosilane (TMCS) and 500 µl of BSA (N,O-bis-tri-methyl silyl acetamide).

GC/MS analysis: The derivative sample was analyzed by Agilent 6890 gas chromatography instrument coupled with an Agilent MS-5975 inert XL mass selective detector and an Agilent auto-sampler 7683-B (Agilent Technologies, Little Fall, NY, and USA). A capillary column HP-5MS (5% phenyl methyl siloxane) with dimensions of 30 m × 0.25 mm i.d., 0.25 µm film thickness was used for the separation of polyphenol compound. The initial oven temperature was 100°C, held for 2 min, ramped at 5°C min^{-1} to 290°C and held for 20 min. The volume injected was 1.0 µl. The solvent delay was 6 min and total run time was 60 min. MS transfer line temperature was at 280°C. The MS was operated in full scan in electron ionization mode with an electron multiplier voltage of 2200 V. The mass-spectra of the unknown compounds were compared with Chem. Station 6890 Scale Mode software with two libraries (NIST and Wily) which provide best information about the identification of active compound.

Statistical analyses

Least significant differences (LSD) were calculated by COSTAT software on the basis of

ANOVA statistics for the completely randomized means and calculated through Duncan's Multiple Range test at significant level of 5% for one factor each time (one way analysis).

RESULTS AND DISCUSSION

Inhibitory effect of OMW against mycelial growth of *R. solani* and *F. oxysporum*

The incorporation of OMW in the culture medium showed an effective antifungal activity against *R. solani* and a high inhibition of mycelial growth was observed for all tested doses (Table 3). However, for *F. oxysporum*, the highest growth reduction was observed at 5% of tested concentrations. These results indicated that *R. solani* was probably more sensitive to the OMW than *F. oxysporum*. Also, reduction rate (%) of both pathogens was increased by increasing OMW concentration in the growth media. These results are in-agreement with those of Yanguia *et al.* (2008) who stated that *F. oxysporum* was found to be more resistant to polyphenols than *R. solani*. This resistance is expressed by the very small inhibition zone recorded around the well and by the intensive formation of chlamydospores.

Biological evaluation of treated OMW

Effect of treated OMW on germination of tomato seeds

Results in table (4) revealed highly significant differences in the germination percentages values of tomato seeds for Castle rock and Global genotypes. The relative tomato seeds germination of both genotypes recorded highest values when seeds irrigated with tap water supplemented by low concentration of OMW (10% for crude OMW and 5% chemically treated OMW) than that supplemented by high concentration of OMW (20% for crude OMW and 10% chemically treated OMW).

Concerning biologically treated OMW, data revealed the same trend, *i.e.* low OMW concentrations were more effective than the high ones. In addition, OMW treated with *A. wentii* achieved best results on tomato seed germination, followed by OMW treated with *A. niger* then OMW treated with *P. ostreatus* at last. In general, Casa *et al.* (2003) mentioned that after treating with liquid culture of *Lentinus edodes* in OMW, a highly significant increase in germinability was observed in comparison to control, indicating that phytotoxicity had been considerably reduced. So, the use of the whole fungus had a much stronger detoxifying effect on OMW.

2. Effect of treated OMWs on growing tomato seedlings

Data presented in table (5) and figure (1) revealed that 13.33% of plants of both Castle rock and Global CVs were found dead after 15 up to 45 days when irrigated with crude OMW or chemically treated OMW. In regard to plants irrigated with biologically treated OMW, the obtained results were contrasted where the percentages of dead plants increased. Extreme effect (dead plants %) was recorded with plants irrigated by *A. wentii* treated OMW, where the percentage of dead plants reached 40.0 and 93.33% after 15 and 45 days, respectively, for Castle rock genotype and 86.33% for Global genotype either after 15 or 45 days. Biologically treated OMW with *A. niger* had also high effect on plants but less than that seen with plants irrigated with *A. wentii* treated OMW. The least effect was seen at plants irrigated by *P. ostreatus* treated OMW where dead plants % reached 40.0% either after 15 or 45 days for both genotypes. On contrast, plant tolerance showed reversed results where the maximum plant tolerance percentage was seen at plants irrigated by chemically treated OMW (86.67%), followed by *P. ostreatus* treated OMW (60.00%) then other biologically treated OMWs, for Castle rock tomato genotype. Plant tolerance percentage, for Global genotype, reached the maximum value with plants irrigated with *P. ostreatus* treated OMW (60.00%), as seen with Castle rock genotype, followed by plants irrigated with *A. niger* treated OMW (20.00%) then those irrigated with *A. wentii* treated OMW.

In concern to the biological treatments, OMW treated with *P. ostreatus* gave the best result in comparison to that treated with *A. niger* or *A. wentii*, This result may be due to the ability of *P. ostreatus* to adapt and degrade the phenolic compounds to beneficial substances such as carbon and nitrogen sources. In *P. ostreatus*, laccase was considered of being responsible for the oxidation of phenolic compounds and aromatic amines, by reducing molecular oxygen to water (Tsioulpas *et al.*, 2002).

Effect of different chemical and biological OMW treatments on suppressing tomato damping-off disease

Data presented in table (6) revealed that soil infested with only *F. oxysporum* and *R. solani* had significantly increased damping-off of tomato seedlings and severely reduced survival healthy plants than untreated control. Percentage of tomato plants showing symptoms of damping-off was significantly reduced by all treated OMW and the suppressive effect was observed against both *R. solani* and *F. oxysporum* infection. However, highest dose (20%) of OMW significantly reduced the percentage of infected tomato seedlings compared with the chemical fungicide. This could be related to the high toxicity of phenolic compounds in OMW against the tested pathogenic fungi. This finding was confirmed by results of seedling germination of

Table (3): Effect of different concentration of OMW on *in-vitro* mycelial growth of *R. solani* and *F. oxysporum*

OMW concentration (%)	Mycelial radial growth (cm)			
	R. solani		*F. oxysporum*	
	Mean ± SD	Growth Reduction %	Mean ± SD	Growth Reduction %
0.50%	0.90 ± 0.04 [a]	89.89	9.50 ± 0.42 [ab]	1.04
1.00%	0.50 ± 0.00 [a]	94.38	9.20 ± 0.31 [ab]	4.17
2.00%	0.50 ± 0.00 [a]	94.38	9.00 ± 0.49 [b]	6.25
3.00%	0.50 ± 0.00 [a]	94.38	4.20 ± 0.21 [c]	56.25
4.00%	0.50 ± 0.00 [a]	94.38	2.60 ± 0.09 [d]	72.92
5.00%	0.50 ± 0.00 [a]	94.38	1.00 ± 0.07 [e]	89.58
Control	8.90 ± 0.60 [b]		9.60 ± 0.30 [a]	
L.S.D. (0.05)	*** 0.40		*** 0.54	

- Each value represents the mean ± S.D (Standard Division) and mean of three replicates.
- Values in the same column with the same letter are not significantly at ($p \leq 0.05$); *** = High significant values.

Table (4): Effect of crude and treated OMWs on germination (%) of tomato (Castle rock and Global CVs) seeds

Treatment	Tomato cultivars			
	Castle rock		Global	
	Germination	Relative germination (%)	Germination	Relative germination (%)
Control	98.00 ± 2.00 [a]	100.00	56.00 ± 4.00 [b]	100.00
OMW (10%)	60.00 ± 4.00 [e]	61.22	21.00 ± 1.00 [f]	37.50
OMW (20%)	21.00 ± 2.00 [f]	21.43	0.00 ± 0.00 [g]	0.00
COMW (5%)	79.00 ± 6.00 [cd]	80.61	66.00 ± 5.00 [a]	117.86
COMW (10%)	75.00 ± 5.00 [d]	76.53	62.00 ± 4.00 [a]	110.71
BOMW (*A. wentii*, 10%)	96.00 ± 7.00 [a]	97.96	45.00 ± 4.00 [c]	80.36
BOMW (*A. wentii*, 20%)	85.00 ± 6.00 [bc]	86.73	40.00 ± 2.00 [cd]	71.43
BOMW (*A. niger*, 10%)	94.00 ± 8.00 [ab]	95.92	51.00 ± 5.00 [b]	91.07
BOMW (*A. niger*, 20%)	71.00 ± 5.00 [d]	72.45	45.00 ± 3.00 [c]	80.36
BOMW (*P. ostreatus*, 10%)	75.00 ± 7.00 [d]	76.53	36.00 ± 2.00 [d]	64.29
BOMW (*P. ostreatus*, 20%)	58.00 ± 4.00 [e]	30.00	30.00 ± 1.00 [e]	30.00
LSD (0.05)	*** 9.19		*** 5.52	

- COMW: Chemically treated OMW and BOMW: Biologically treated OMW
- Each value represents the mean ± S.D (Standard Division) and mean of three replicates.
- Values in the same column with the same letter are not significantly at ($p \leq 0.05$);
- *** = High significant values.

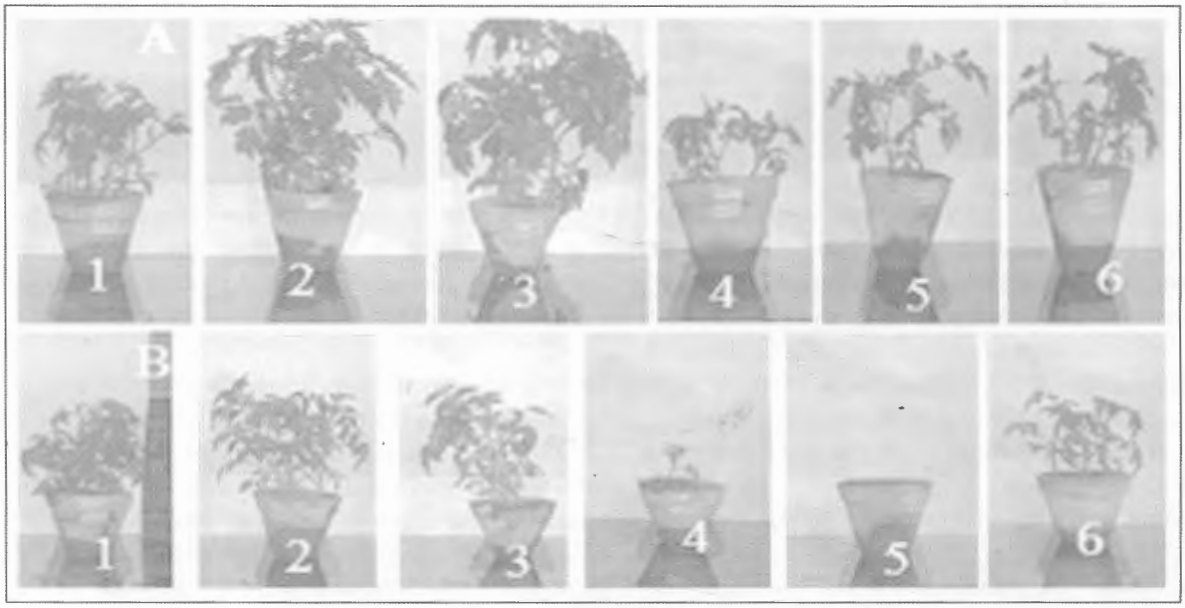

Figure (1): Effect of crude and treated OMWs on growth of tomato seedlings.
A: Castle rock tomato genotype, B: Global tomato genotype,
1: Control, 2: OMW, 3: COMW, 4: BOMW (*A. wentii*), 5: BOMW (*A. niger*), 6: BOMW (*P. ostreatus*).

Table (5): Effect of crude and treated OMWs on growth of tomato (Castle rock and Global genotypes) plants

Treatment	Castle rock			Global		
	Dead plants (%) after		Plant tolerance	Dead plants (%) after		Plant tolerance
	15 days	45 days	(%)	15 days	45 days	(%)
Control	0.00 [b]	0.00 [d]	0.00	0.00 [d]	0.00 [d]	0.00
OMW	13.33 [b]	13.33 [d]	86.67	13.33 [d]	13.33 [c]	86.67
COMW	13.33 [b]	13.33 [d]	86.67	13.33 [d]	13.33 [c]	86.67
A. wentii	40.00 [a]	93.33 [a]	6.67	86.33 [a]	86.33 [a]	16.67
A. niger	53.33 [a]	77.33 [b]	26.67	53.33 [b]	80.00 [a]	20.00
P. ostreatus	40.00 [a]	40.00 [c]	60.00	40.00 [c]	40.00 [b]	60.00
LSD $_{(0.05)}$	***13.62	***12.05		***12.80	***11.79	

- COMW: Chemically treated OMW and BOMW: Biologically treated OMW
- Each value represents the mean ± S.D (Standard Division) and mean of six replicates.
- Values in the same column with the same letter are not significantly at ($p \leq 0.05$); *** = High significant values.

Table (6): Effect of different treated olive mile waste water on disease % and survival % as well as affected by *F. oxysporum* and *Rhizoctonia solani*

Treatment				OMW	COMW	BOMW (*A. wentii*)	BOMW (*A. niger*)	BOMW (*P.ostreatus*)	Control (-)	Control (+) with F.	Control (+) without
After 15 days of plantation	Castle Rock	*Fusarium oxysporium*	Mean	1.67	3	3	3.33	3	0	ND	ND
			Infection %	33.33	60	60	66.67	60			
		Rhizoctoni Solani	Mean	1.33	1	4	3.33	1			
			Infection %	26.67	20	80	66.67	20			
	Global	*Fusarium oxysporium*	Mean	1.67	3	3.67	4	3.67	0		
			Infection %	33.33	60	73.33	80	73.33			
		Rhizoctoni solani	Mean	1.33	1.33	4	2.33	1.67			
			Infection %	26.67	26.67	80	46.67	33.33			
After 45 days of plantation	Castle Rock	*Fusarium oxysporium*	Mean	1.33	2	3.33	3.33	4	0	1.33	1.33
			Infection %	26.67	40	66.67	66.67	80		26.67	26.67
		Rhizoctoni Solani	Mean	1.67	1.33	4	4	1		0.33	1
			Infection %	33.33	26.67	80	80	20		6.67	20
	Global	*Fusarium oxysporium*	Mean	1.67	3	3.67	4	4.33	1.33	0	1
			Infection %	33.33	60	73.33	80	86.67		0	20
		Rhizoctoni Solani	Mean	1.33	2	4.33	2.33	3		2	4.33
			Infection %	26.67	40	86.67	46.67	60		40	86.67
Survival (%)	Castle Rock	*Fusarium oxysporium*		73.4	60	33.4	33.4	20	100	73.33	73.33
		Rhizoctoni solani		66.6	73.4	20	20	80		93.33	80
	Global	*Fusarium oxysporium*		66.6	40	26.6	20	13.4	98.67	100	80
		Rhizoctoni solani		73.4	60	13.4	53.4	40		60	13.33

- COMW: Chemically treated OMW and BOMW: Biologically treated OMW
- Control (-): healthy tomato sprouts without any treatment;
- Control (+) with F: infested tomato sprouts treated with chemical fungicide (Carbendazim 50% W.P.);
- Control (+) without: infested tomato sprouts without any treatments.
- Each value represents the mean ± S.D (Standard Division) and mean of three replicates.
- ND: Non detectable

tomato in soil free from soil-borne pathogens but containing chemical and biological treated OMW (Table. 6). The significant reduction of damping-off incidence on tomato plants using the OMW amendment was attributed to the effect of polyphenols and other chemical compounds. Several researchers have demonstrated that only a few microorganisms are able to survive in this by-product, because it contains various simple and complex phenolic compounds characterized by high antimicrobial activity (Kistner *et al.*, 2004).

Also, these suppression effects in case of biological treated OMW may have different mechanisms of action including interference with spore germination or germ tube elongation inhibition through abnormal hyphal swelling (Jung *et al.*, 2003). They can also be responsible for lyses and complete degradation of the fungal hyphae and suppression by competition for nutrients could occur (Bailey and Lazarovits, 2003).

Dehydrogenase activity in rhizospheric area

Data in table (7) revealed that, in general the use of OMW either crude or biologically treated with *P. ostreatus* significantly increased dehydrogenase activity in rhizosphere region of tomato plants after 45 days of seedling. Results showed that crude OMW increased dehydrogenase activity whereas recorded 45.40 µg TPF/g dry soil/day. Biologically treated OMW with *P. ostreatus* increased dehydrogenase activity reachimg 55.90 µg TPF/g dry soil/day.

Table (7): Effect of crude and treated OMWs on dehydrogenase activity in rhizospheric area of tomato plants after 45 days

Treatment	Dehedrogenase activity*
Control	31.90 ± 0.45
OMW	45.40 ± 0.43
COMW	47.43 ± 0.43
BOMWP	55.90 ± 0.43
LSD	1.88

*µg TFP/g dry soil/day, COMW: Chemically treated OMW and BOMWP: Biologically treated OMW with *P. ostreatus*

Table (8): GC/MS analysis of ethylacetate extracts of crude, chemically and biologically treated OMWs

Serial	Compound name	Crude OMW	Peak area percentage			
			Treatment type			
			Chemical	*A. wentii*	*A. niger*	*P. ostreatus*
1	4-Hydroxy-phenylethanol	0.4	-	-	-	-
2	*N*-Acetyltyramine	13.1	12.8	-	-	-
3	4-Aminothio-phenol	11.6	29.8	-	-	-
4	4- Hydroxy-Benzenemethanol	0.4	0.2	-	-	-
5	Benzaldehyde-3-amino-oxime	14.5	-	-	-	-
6	2-Butyl-5-ethyl-4-methyloxazol	17.3	18.3	-	-	-
7	3-Hydroxy-1,2-dimethyl-9H-carbazol	4.6	-	-	-	-
8	*p*-Hydroxymethyl-diphenylmethane	2.1	-	-	-	16.5
9	2-Ethylphenol	-	1.0	-	-	-
10	Diethylphthalate	-	0.7	-	-	-
11	2-Aminobenzenethiol	-	0.9	-	-	-
12	4-Methoxy-benzenamine	-	-	-	-	0.6
13	4-Methyl-2,6-di-tert-butylphenol	-	-	-	-	0.8
14	2.3.4-Trimethoxy-benzyl alcohol	-	-	-	-	2.3
15	9,12-Octadeca-dienoic acid methyl ester	-	-	-	-	0.4
16	15-Octadecenoic acid methyl ester	-	-	-	-	0.6
17	Octadecanoic acid methyl ester	-	-	-	-	0.5
18	9-Octadecenoic acid	-	-	-	-	11.1
19	Dibutylphthalate	-	-	4.1	-	-
20	Palmitic acid	0.6	1.9	2.1	11.0	5.8
21	Oleic acid	2.0	5.2	0.8	25.3	3.4
22	Stearic acid	0.4	-	-	10.3	-
23	Di-(2-ethylhexyl) phthalate	0.6	0.5	26.0	10.2	7.5
24 -44	Total other	32.4	28.7	67.0	43.2	68.3

Dehydrogenase activity recorded with biologically treated OMW was higher than that recorded in control and chemically treated OMW. In general, increases of dehydrogenase activity in response to OMW applications may be due to that the chemically and biologically treated OMW added value of easily degradable C and N substrates, resulted in high levels of extractable C and N, leding to a rapid increase of soil microorganisms respiration and increase of microbial biomass of OMW amended to soil.

GC/MS analysis for ethylacetate extracts of chemical and biological treated OMW

As shown in table (8), there were 38 compounds extracted from crude OMW, only 12 of them were identified. The most abundant compounds were 2-butyl-5-ethyl-4-methyloxazol (17.3%), benzaldehyde-3-amino-oxime (14.5%), *N*-acetyl-tyramine (13.1%) and 4-aminothiophenol (11.6%). In chemically treated OMW, there were 44 compounds and 10 of which were identified. The most abundant compounds were 4-aminothiophenol (29.8%), 2-butyl-5-ethyl-4-methyloxazol (18.3%) and *N*-acetyltyrammine (12.8%). There were several compounds identified in crude OMW and didn't appear in chemically treated OMW, *e.g.* benzaldhyde-3-amino-oxime and others. In contrast, some compounds appeared in chemically treated OMW only and were identified, *e.g*; 2-ethylphenol, 2-aminobenzenethiol and diethylphthalate. This may be due to the decomposition of phenolic compounds by H_2O_2 and UV in the chemical treatment. In other words, chemical treatment of OMW resulted in the degradation of several compounds. In biologically treated OMW, data showed that most of the compounds were decomposed by microorganisms, except OMW treated with *P. ostreatus.* Regarding this treatment, 39 compounds appeared, only 11 compounds of them were identified, *e.g*; *p*-hydroxymethyl diphenylmethane (the major, 16.5%), 9-octadecanoic acid (11.1%), and others. In biologically treated OMW with *A. wentii*, there were 19 compounds, only 4 of them were identified, *e.g*: Dibutylphthalate (4.1%). In OMW treated with *A. niger* there were 25 compounds, only 4 compounds were identified. Three fatty acids were identified in all OMWs in variable amounts, except for stearic acid that wasn't identified in chemically treated and biologically OMW treated with *P. ostreatus* and *A. wentii*. This means that stearic acid was degraded by H_2O_2/UV and these fungi. The most abundant compounds identified in OMW treated with *A. niger* were fatty acids (46.6%) and di-(2-ethylhexyl) phthalate (10.2%). The major compound identified in OMW treated with *A. niger* was oleic acid (25.3%) and in OMW treated with *A. wentii* was di-(-2-ethylhexyl) phthalate (26.0%).

From the obtained results, it was markedly observed that there was a variation in numbers and types of compounds present after each treatment. Appearance of large amounts of degraded compounds in OMW chemically treated is due to the ability to degrade the compounds easily and in a short time and also the degradation process continued to give very small non-toxic compounds such as CO_2 and H_2O. GC-MS analysis of OMW showed that 50% were monomeric, with a predominance of hydroxytyrosol, tyrosol, caticol, benzaldhyde, caffeic acid and others. In accordance to Chiou *et al.* (2008), the time factor was important in degradation process, as they found that phenolic compounds required more time to be completely mineralized into CO_2 and water by photo-catalysis.

Generally, use of OMW as its or degraded biologically or chemically for controlling such soil-borne plant pathogen practically under greenhouse or filed conditions is recommended.

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