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Effect of olive mill wastewater (OMW) on growth of tomato and germination of certain vegetable seeds

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Abstract

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One of the serious environmental problems that face most Mediterranean countries especially Jordan, is Olive mill wastewater (OMW). Fortunately, scientists were able to conquer this problem by many solutions including OMW biological treatment and re-use in agriculture. This research was conducted to study the effect of OMW on tomato plant cultivation and seed germination of selected cultivars. The experiments were conducted at The Hashemite University which is located in an arid region, with three treatments of OMW including water (control), 50% diluted OMW, and 100% treated (concentrated OMW) in field and in greenhouse. While seed germination experiment was conducted under normal light conditions and 25°C. For tomato plants, the total yield, dry weight of root, aboveground biomass and accumulative number of flower. The results showed significant difference between those treated with 50% diluted OMW and those treated with 100% OMW or control and these differences included all variables measured except for the root dry weight. Concentrated OMW (100%) and 75% diluted OMW were highly toxic to the germinating of all seeds studied including chickpea, melon, cucumber, tomato and squash. As for the Microbial community analysis in soil, OMW treatments lead to a significant increase in fungal and bacterial counts throughout irrigation at elevated concentrations of OMW. However, OMW at a 50% concentration can be used in irrigation of crops like tomato plants.

Keywords: Olive mill waste water; tomato; seed germination; microbial community; vegetable crops

Introduction

Olive oil has a high nutritional value, reasonable prices and higher demand which encourage farmers to cultivate more olive trees to cover the increased global demand (Dermeche et al., 2013). Worldwide, 10 million hectares were cultivated with olive trees of which, Mediterranean countries occupied 90% of cultivated areas (FAOSTAT, 2018). Jordan ranked 8th regarding olive oil producing among nations with about 15 million cultivated olive trees (Al-Mefleh et al., 2020). As a results of olive oil extraction processes, 300 000 m^3 of olive mill wastewater (OMW) were discharged annually (Fraij & Massadeh, 2015) which causes many environmental problems if not used correctly (Perez et al., 1998). In Jordan, OMW should be collected and moved to the nearest evaporation ponds for final disposal. Hence, it is essential to find effective ways to cope with these massive volumes of OMW. Chemical analysis showed that although OMW is acidic, phytotoxic, have high organic load and low biodegradability which prevents its direct discharge into wastewater treatment systems (Sobhi et al., 2007; Jaradat et al., 2018), but it has high organic matter (N, P, K and mg) and anti-bacterial effects due to its high polyphenolic content (El-Hassani et al., 2009; Jaradat et al., 2018).

Jordanian soil is poor and exposed to erosion (Al-Eisawi, 1985). However, addition of amendments rich in organic material such as OMW could be useful solution for these problems, as they might increase soil fertility and may improve soil water retention, hydraulic conductivity, and porosity (Mekki et al., 2006). Jordan is also a well known country for its dry nature, poor soil and low water resources (Al-Eisawi, 1996; Al-Ghzawi et al., 2018). Thus, the objectives of the present study were to investigate the influence of OMW on the growth and productivity of tomato plants as a major vegetable crop in Jordan and other countries and also to investigate the impact of OMW on seed germination of other important vegetable crops.

Materials and Methods

OMW sampling and physiochemical properties

OMW was collected from a three-phase mill located around The Hashemite University campus (Zarqa/Jordan) in small containers of 20 L and stored in cold room at 4°C in the dark until use. The main characteristics of OMW are listed in Table 1.

Table 1. Physical and chemical characteristics of OMW (Massadeh & Modallal, 2008)

Parameter	Unit	Minimum Reading	Maximum Reading
TDS	mg/L	16 984	80 355
TSS	mg/L	14 207	46 188
COD	mg/L	78 536	160 096
BOD	mg/L	23 248	63 271
T-P	mg/L	158	403
NH_4-N	mg/L	22	68
TKj-N	mg/L	398	1036
Na	mg/L	130	384
Ca	mg/L	276	757
Mg	mg/L	38	63
K	mg/L	2053	5492
Cl	mg/L	486	1111
Phenolics	mg/L	7739	10 432

Notes: TDS = total dissolved solids; TSS = total suspended solids; COD = chemical oxygen demand; BOD5 = biological oxygen demand (after incubation for 5 days at 30°C); T-P = total phosphorus; NH_4-N = ammoniacal nitrogen; TKj-N = total nitrogen

Study site

This study was conducted at the campus of The Hashemite University, Zarqa (32°04′N, 36°12′E; altitude: 550-650 m) that lies 40 km east of Amman. The mean annual precipitation is approximately 115 mm, falling mainly between October and April. The recorded temperature ranges between 0.0°C in December and/or January to around 40°C recorded in July and/or August. The prevailing wind is west north with a mean monthly wind speed ranged between 2.0 to 7.8 knots. According to bioclimatic classification, the area is specifically classified as arid Mediterranean while for vegetation type it is typically Irano-Turanian (steppe) region (Al-Eisawi, 1985). The area is mainly of rocky limestone hills, flat plains and broad wadis.

Effect of OMW on tomato

Greenhouse experiment

Seeds of tomato were sown, on potting trays (45 × 25 × 8 cm) that have been filled with a mixture of 2/3 pasteurized black soil and 1/3 pro-mix (Premier Promix, Premier Horticulture Ltee, Riviere-du-Loup, QC, Canada). Two weeks old seedlings with uniform vigour (13 cm length with 4-6 leaves) were individually transplanted into separate pots (20 cm diameter) containing 1/1 pasteurized red soil and peat moss. Potted seedlings were arranged randomly on a greenhouse bench and maintained under greenhouse conditions (24 ± 2°C with 15 h of light/day at photon flux density minimum of 350 ± 50 µmol m^{-2} s^{-1}). The experiment was one factor and five replicates in a completely randomized design. Potted seedlings were left for one week to be established and any non-healthy plant was replaced by the already prepared spare potted plants. One week later, three levels of irrigation accomplished as follows: (1) 0% OMW (control = tap water) (2) 100% OMW (the collected stock solution) and (3) 50% OMW (prepared by dilution (1:1) of the stock solution with tap water). At the beginning of each week, the plants had received 100 ml of the assigned irrigation treatment. However and whenever needed all plants were irrigated by similar quantities of tap water. During flowering, the number of flowers formed by each plant were counted. Mature fruits were collected and weighed separately for every plant. Three months after treatments application, all tomato plants were carefully removed from the soil, the roots were thoroughly washed and the plant was cut to separate above ground and below ground biomass. Treated and control plant materials (leaves or roots) from each pot were separately placed in paper bags, oven dried at 80°C for 72 h, and then weighed.

Field experiment

A piece of land 10×10 m was prepared and bordered using wooden sticks and robes. 15 (50 × 50 cm) plots were prepared with 2 m alleys between any of the two plots. Five of two-week old tomato seedlings were transplanted into each plot with uniform spaces between individual plants. The five plants were dealt with as one experimental unit. Seedlings were left for 2-weeks for establishment, then the three levels of irrigation were accomplished the same as in the greenhouse experiment. The experiment was one factor and four replications (a total of 20 plants for each irrigation treatment) in a completely randomized design. Data were collected exactly the same as was in the greenhouse experiment.

Effect of OMW on seed germination of certain vegetable crops

Seeds of chickpea *(Cicer arietinum)*, melon (*Cucurbita ceae*), cucumber (*Cucumis sativus*), tomato (*Solanum lycopersicum*), and squash (*Cucurbita maxima*) were studied for their germination after being subjected to OMW.

Four Whatman #1 filter papers were placed into 15cm diameter Petri plates, papers were saturated with distilled water, and excess water was decanted. Four OMW concentrations were prepared by diluting the stock solution to 0%, 10%, 25% and 50%. OMW treatments were applied on five replicates of 10 seeds for each species. The 10 seeds per plate were placed on the moistened filter paper and the plates were sealed with parafilm. Plates were incubated at 26 ± 2C under two 34 W cool white fluorescent lights on a 16 h day 8 h night cycle. Germinated seeds were counted and removed every day for a period of 10 days. Filter papers were kept moist through periodic spraying with the assigned OMW solution.

Soil microbial counts

Three replicates of 10 g soil samples were collected from pots of the greenhouse experiment at different time (2, 6 and 10 weeks) post treatments of OMW. Using a spatula soil samples were collected from 5 cm depth and then transferred to labelled, sterile Petri plates. The soil of each sample was mixed thoroughly and then 1 g of each sample was transferred to 9 ml sterile distilled water. Serial dilutions of 10^{-2} up to 10^{-5} were prepared from each soil sample. 1 ml of 10^{-3} (in the case of fungi) and 10^{-5} (in the case of bacteria) dilution of each soil sample was transferred to blank Petri plates followed by about 15 ml of molten media (around 40°C) of either nutrient agar (for bacterial count) or Sabaroud dextrose agar (for fungal count). The plates were gently moved to mix the contents, left to solidify under room temperature and then incubated at 25-30°C for 24 h in the case of nutrient agar and for 5 days in the case of Sabaroud agar. Microbial count was estimated by calculating the colony forming unit (CFU) per 1 ml.

Statistical and data analysis

One Way ANOVA was used to test the significant differences among treatment levels and Tukey's test was used to separate the means of different treatments at $P = 0.05$.

Results

Effect of OMW on the vegetative and reproductive parameters of tomato grown under field conditions

Under 50% OMW treatment, all standard vegetation and reproductive parameters were significantly affected compared to untreated control (0%). Dry weight of aboveground biomass and root biomass of tomato plants were significantly greater when treated with 50% OMW compared to untreated control. On the other hand, under 100% OMW treatment, the above ground biomass was significantly lower and there was no significant difference in root biomass occurred on comparison to 50% OMW (Table 2).

Table 2. Effect of different OMW concentrations (0%, 50% and 100%) on growth and yield of tomato plants

	OMW concentration		
	0%	50%	100%
Greenhouse experiments			
Total yield of tomato plant, g	15.65±6.02b	36.3±36.3a	10.17±1.83b
Root dry weight, g	1.06±0.37b	3.47±0.44a	1.77±0.46b
Above ground dry weight, g	6.2±1.42b	15.73±1.41a	7.57±1.82b
Number of flowers	1.33±0.33b	2.67±0.33a	1.33±0.33b
Field experiments			
Total yield of tomato plant, g	73.03±19.92b	193.5±28.82a	95.14±16.53b
Root dry weight, g	11.89±5.93b	17.77±2.69a	15.73±3.91ab
Above ground dry weight, g	56.38±15.11b	92.66±20.44a	46.06±8.401b
Number of flowers	2.08±0.6b	5.08±0.6a	2.67±0.31b

Notes: Values refer to means ± SEM (average of 4 and 5 replicates in field and greenhouse experiments, respectively), Within rows means with a common letter are not significantly different according to Tukeys' test at $P = 0.05$

The total number of flowers produced per plant as well as the accumulative yield of tomato fruits per plant were also significantly increased at 50% OMW treated compared to untreated control plants. No such increase occurred under 100% OMW compared to untreated treatment (Table 2).

Effect of OMW on the vegetative and reproductive parameters of tomato grown in greenhouse environment

Under greenhouse environment, 50% OMW treatment induced a significant increase in all vegetative and reproductive parameters compared to both 0% (untreated) and 100% treatments (Table 2). The increase in biomass of vegetative growth as well as in the yield was 2-folds or more in the case of 50% OMW treatment compared to other treatments.

Effect of OMW on seed germination of certain crop seeds

Different responses in germination potential were obtained from different crop seeds (Figure 1). 10% OMW reduced significantly the germination potential of melon and tomato seeds but no such reduction obtained for chickpeas, squash and cucumber. In the meanwhile, 25% reduced significantly the germination of all seeds except squash seeds. However, 50% OMW reduced significantly the germination of all studied crop seeds with 0% germination for squash, melon and tomato (Figure 1).

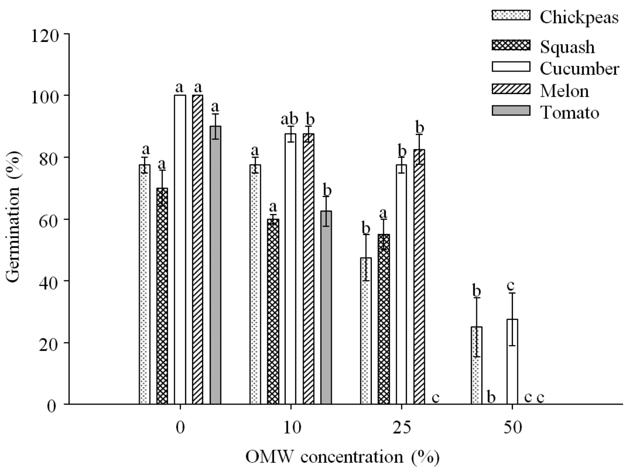

Fig. 1. Effect of OMW concentrations on germination of selected vegetable crop seeds

Notes: Means are average of 5 replicates. Error bars represent the standard errors of the means. Within each crop; means with a common letter are not significantly different according to Tukeys' test and at P = 0.05

Effect of OMW treatments on soil microbiology under field conditions

Results indicated that both parameters, the total number of bacterial and fungal (Figure 2) colony forming units (CFU) were significantly increased under 50% and 100% OMW treatments compared to untreated (0% OMW) soil. Moreover, 100% treatment resulted in more microbial colony forming units than 50% OMW treatment.

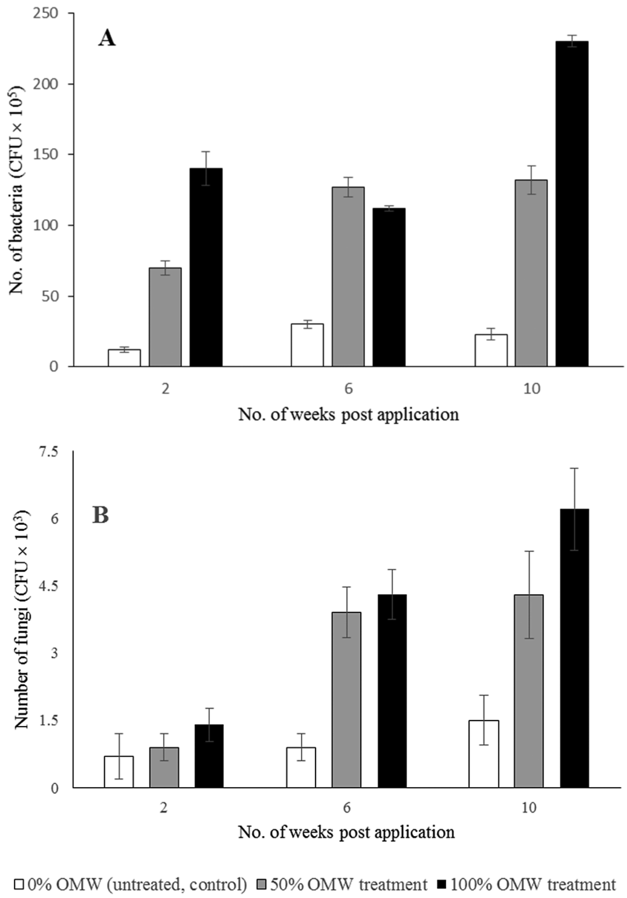

Fig. 2. A – Bacterial and B – Fungal counts (CFU) in soil post to OMW application under greenhouse conditions

Notes: Means are average of 5 soil samples. Error bars represent the standard errors of the means

Discussion

In the current study, 50% and 100% and control (0%OMW) dilutions of OMW were used as a pre-treatment technique with tomato Plant. Dilution is considered as the principal pretreatment technique that positively affect OMW phytotoxicity compared to all other pretreatment techniques (Komilis et al., 2005). Current results showed that tomato plants grown in the field and in greenhouse showed improvement in performance in terms of yield, number of flowers and biomasses of roots and aboveground parts, this happened only under 50% OMW concentration. Although the 100% OMW concentration never induced such improvement but still didn't reduce growth parameters of tomato plants compared to untreated control plants. This was consistent with the findings of Tattini et al. (1991) who claimed that OMW could serve as an important fertilizer to save and improve the root/shoot ratio of both peach and olive plants. Furthermore, El-Hassani et al. (2009) studied the effect of OMW on Date Palm trees and found that the palm growth was efficiently raised by OMW spreading.

Present results showed that OMW affected seed germination, for instance, in our research the results showed that tomato plant was more sensitive to OMW, it's germination occurred until 10%, in contrast with squash which showed high germination that reached 50%, whereas the rest of the studied plants, the germination of melon, cucumber and chickpea seeds occurred till 25%. Our results indicated that pure OMW (100%) and 75% concentration were highly toxic to the germination of all plants' seeds used in the study. Our results agreed with findings of El Hadrami et al. (2004) and Aqeel & Hameed (2007) who studied the effect of OMW on the germination of cress seeds. The reduction in seed germination might be caused by the phenolic compounds in OMW. These effects might also be due to the lipophility of phenolic compounds, which could modify the accessibility of elements inside the biological membranes (Aqeel & Hameed, 2007).

Researchers observed that the controlled spreading of OMW will not produce phytotoxic effects on trees and crops species (Aqeel & Hameed, 2007). It is important to know that there are several methods that can be used to minimize the phytotoxic effect of OMW and make it better in working as a fertilizer. According to Aqeel & Hameed (2007), when OMW was applied prior to planting; 100% of the trees survived which could be due to the presence of high organic and inorganic compounds in OMW as indicated in Table 1. However, there are many other reasons that may explain these improvements, such as the fact that the acidity of OMW is neutralized by soil carbonates alkalinity. Belaqziz et al. (2008) confirmed this possibility by suggesting that the soil carbonate is converted to bicarbonate which in turn moves and accumulates in deeper soil horizon. On the other hand, Broja et al. (2006) and Aqeel & Hameed (2007) suggested another explanation for these improvements as they showed that OMW storage throughout the experiment plays an important role in pre-treatment effluents of OMW which develop and inherit detoxification properties. In fact, OMW indigenous microorganisms exert important phenolic degradation capabilities. This kind of information was confirmed by Belaqziz et al. (2008) who illustrated that soil treated with OMW showed noticeable relevant stimulation paying attention to leaf area, shoot height, root length, spike and stem when studied the effect of OMW on maize crop (*Zea mays*) in Morocco. Furthermore, Ouzounidou et al. (2008) showed, while studying the influence of OMW on tomato growth, that increasing OMW concentration decrease plant performance and also confirmed the results which indicate that plants treated with high OMW concentrations produced lesser but bigger tomato fruits compared to plants treated with lower OMW concentrations.

OMW also can affect the soil microflora, and the impact that OMW leaves on it can be measured with two basic methods: (1) Temporary enrichment of the soil with readily available C source and (2) The addition of wastewater which contains inhibiting components to some microorganisms (Fraij & Massadeh, 2015). In the present study, the bacterial and fungal counts were significantly increased when OMW concentration was increased, and this might be due to the process of enriching the soil with easily decomposable carbon sources and inorganic nutrients as well as the increased amounts of recalcitrant polymerized phenols and concurrent immobilization of Nitrogen (N). The above dramatic change in the soil nutritional status may favor certain fungal and bacterial communities while hampering the growth of others which in turn help plants (Al-Eitan et al., 2021).

In conclusion, OMW was a suitable supplement for cultivating tomato but after diluting it. the total yield, dry weight of root, aboveground biomass and accumulative number of flower were all enhanced when irrigating tomato plants with 50% diluted OMW. Concentrated OMW (100%) and 75% diluted OMW were highly toxic to the germinating of all seeds studied including tomato. As for the Microbial community analysis of soil, OMW treatments lead to a significant increase in fungal and bacterial counts throughout irrigation at elevated concentrations of OMW. However, OMW at a 50% concentration can be used in irrigation of crops like tomato plants which in turn could be useful in eliminating its phytotoxic activity in nature, reuse as a rich source of nutrients, and in saving the economic penalty upon the olive mills.

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