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ORIGINAL ARTICLE

Olive mill wastewater effects on durum wheat crop attributes and soil microbial activities: A pilot study in Syria

Jehan Khalil[1] · Hasan Habib[1] · Michael Alabboud[2] · Safwan Mohammed[3]

[1] Faculty of Agricultural, Department of Soil Science, Damascus University, Damascus, Syria
[2] College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran
[3] Faculty of Agricultural and Food Sciences and Environmental Management, Institute of Land Use, Technology and Regional Development, University of Debrecen, Debrecen, Hungary

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Abstract Olive mill wastewater is one of the environmental problems in semiarid regions. The main goals of this study were to investigate the impacts of different olive mill wastewater levels on durum wheat (*Triticum aestivum* var. Douma1) production and soil microbial activities (i.e., bacteria and fungi). A pot experiment was conducted during the growing seasons 2015/2017 to evaluate the effect of three levels of olive mill wastewater on both growth and productivity attributes of wheat. *Vertisol* soil samples were collected from southern Syria. Two months before wheat cultivation, three levels of olive mill wastewater: T5 (5 L/m ^{2}), T10 (10 L/m^2) and T15 (15 L/m ^{2}) were added to pots filled with the collected soil samples. Also, a control (T0) free of olive mill wastewater was considered as a reference. Results showed a significant increase ($p < 0.05$) in germination rate (%), plant height (cm), ear length (cm), kernels number, kernels weight per ear (g) and grain yield (g/m^2) compared to control. However, T5 treatment did not induce a significant increase in terms of ear length, kernels weight per ear or yield (in the second season). On the other hand, T10 treatment had recorded the best results compared with the other two treatments (T5, T15). Similarly, the results showed a significant increase in the number of bacterial and fungi cells by increasing olive mill wastewater concentration. This research provides promising results toward using olive mill wastewater in an eco-friendly way under Syrian conditions.

Keywords *Vertisol*; olive wastewater · *Triticum durum* · Semiarid regions; food security · Mediterranean region

✉ Safwan Mohammed
safwan@agr.unideb.hu

1 Introduction

The disposal of olive mill wastewater (OMWW) is considered one of the recurrent environmental problems related to the olive oil industry in the Mediterranean region such as Tunisia (S'habou et al. 2009; Sahraoui et al. 2014; Mekki et al. 2006a, b), Greece (Kavvadias et al. 2014; Gikas et al. 2018), Spain (Paredes et al. 1999; Lozano-García et al. 2011) and Italy (Sciarria et al. 2013). One ton of olives usually produces one/two tons of OMWW according to oil-extracting technology (traditional and continuous systems), since one kg of olive requires an additional 2L of water in the continuous extraction systems (Lanciotti et al. 2005). Thus, serious environmental problems such as surface and groundwater body contamination, besides severe soil pollution, have been continuously noticed near production areas, which are usually agricultural lands (Zema et al. 2019; Paraskeva and Diamadopoulos 2006; Chaari et al. 2015).

A growing body of the literature has highlighted the possibilities of using OMWW in Mediterranean countries. One way is to spread it over agricultural lands as a secondary source for crop fertilization and soil amendment. For instance, after nine years of using three OMWW doses (50, 100 and 200 m^3· ha^{-1}) in Tunisia, Chaari et al. (2015) recommended using OMWW in organic production although of its high phenolic compounds content. In Southern Italy, Zema et al. (2019) noticed a sudden decrease in soil infiltration after irrigation by OMWW, in contrast to a marked increase after three weeks of treatment. Other soil parameters such as aggregate stability and nutrient contents (i.e., organic matter, phosphorous, potassium) increased. On the contrary, Barbera et al. (2014) reported that OMWW had a negative impact on plant germination and plant root system. Thus, extra caution is required when applying OMWW due to its adverse side effects on soil–plant system such as toxicity toward plants and soil biota (Barbera et al. 2014; Zema et al. 2019). Other reports of OMWW possible implications and effects in the agricultural sector can be found in Table 1.

Syria is a Mediterranean country with more than 79,000 hectares of olive trees. This fact renders olive trees the third largest cultivated crop in terms of area and production after cereals and cotton (Mohammed et al. 2019). Notably, exporting olive oil contributes 1.5–3.5% of the national gross domestic product (GDP), with Syria ranking the sixth largest country in olive production worldwide (Mohammed et al. 2019). In recent years, the olive sector has witnessed significant development in terms of expanding cultivation area, number of trees and production, which has led to an increase in the amount of OMWW produced from the oil industry. In 2012, the total OMWW was estimated to be 1 million cubic meters, and this amount is expected to increase in the next few years as a result of the continued expansion of olive cultivation and the introduction of 2–3 million new trees yearly into the production cycle (CBS, 2012).

OMWW is a major environmental problem in Syria due to many reasons such as: (1) the immediate and random dumping of OMWW without a proper treatment, which leads to soil pollution and the degradation of its physical, chemical and biological characteristics; (2) OMWW usually reaches lakes, rivers and groundwater, rendering these water resources undrinkable and posing a significant threat to aquatic life, especially in the coastal part of Syria; and (3) since chlorine is used to purify water in Syria, it might interact with the high percentage of polyphenolic compounds in OMWW polluted water causing the toxic chlorophenol to be formulated. Such material is even more dangerous to human health than phenol (Takriti et al. 2009). In this sense, The Syrian Ministry of Agriculture and Agrarian Reform (MoAAR) passed the law No. 190/T of 2007, which aims to regulate the use of OMWW in agricultural lands between 50 and 80 m^3/ha according to extraction method (traditional, centrifugal) (Mahmoud et al. 2012).

Few researches were carried out to investigate the impact of local OMWW on some soil properties and plant production. Mahmoud et al. (2012) noticed that long-term (15 years) irrigation with OMWW in south-western Syria affected the topsoil layer making it more fragmented and prone to preferential solute transport. Similarly, Hayfa et al. (2016) concluded that adding OMWW had significantly enhanced some soil properties (i.e., electrical conductivity; organic matter content, soil porosity, total Cu and total Mn); and the quality of tomato fruit (*Solanum lycopersicum*) (i.e., dry matter; total dissolved solids).

The *vertisol* covers roughly 9% of the Syrian land, while it dominates in some areas located to the northeast of the country near the Turkish–Iraqi borders in addition to Houran plain, the central region and the northwestern regions (Mohammed et al. 2020). Since this soil is the dominant type in the Syrian agricultural areas, the main goal of this research was to investigate the possible use of

Table 1 Implication of OMWW in the agricultural sector in different regions around the world

Region	Reference	Impact
Southern Spain	Paredes et al. (1999)	OMWW and the sludges contain substantial quantities of nutrients compared with compost and manures. It is essential to be characterized before usage for agricultural purposes
Southern Spain	Ramos-Cormenzana et al. (1996)	*Bacillus pumilus* reduced the phenol content of OMWW
Tunisia	Zouari & Ellouz (1996)	Reduction of COD by 50% by using bacterial mixtures obtained from local area
Tunisia	Hachicha et al. (2008)	A mixture natural fertilizer between olive oil production process, OMWW and poultry manure positively affected potato production
South-western Syria	Mahmoud et al. (2010)	Direct implementation of OMWW, without any treatments, for 15-year reduced soil hydraulic conductivity, drainable porosity ($\Phi < 30$ μm), and increased the susceptibility of groundwater pollution
Italy	Di Serio et al. (2008)	Direct increase in soil respiration activity due to 16 l/mg of OMWW, which was highly correlated with decomposition of OM
–	Barbera et al. (2013)	Adding OMWW to soil increase C/N ratio and inhabited mineralization due to limited N sources in the soil
Tunisia	Mekki et al. (2009)	Recommended OMWW amendment before using for agricultural purpose or adding to soil
Italy	Ferri et al. (2002)	Spreading OMWW on *Triticum durum* field decreased plant number and badly affected the leaf area index and reduced the total dry matter. Also, leaf was subjected to necrotic spots (20–30%)
Morocco	Hanifi & El Hadrami (2008)	For *Zea mais L*, OMWW inhabited coleoptile elongation between 32 and 74% and radicle growth by 84%

OMWW in *Vertisol* for wheat production as a proposed solution to minimize the negative environmental impact of OMWW. The detailed goals were to (1) highlight the dynamic interaction between wheat production and three levels of OMWW in *Vertisol* and (2) investigate the impact of different levels of OMWW on soil microbiological activities.

2 Materials and methods

2.1 Olive wastewater (OMWW) and soil samples

Olive mill wastewater was collected from a local centrifugation-based system press located in Isim village in the city of Qatana (southern Syria; 25Km from Damascus). The collected OMWW samples were placed in 20-L plastic containers and stored at a low temperature to avoid chemical and biological transformations during the study period (2015–2017). Different physical and chemical properties were measured as follows: pH (Conyrrs 1988); EC (Jones, 2001); ash percentage (%); water content (%); organic matter content (Jone 2001); total nitrogen (Bremner 1996); total P, Ca, Mg, Na and K (Jackson 1958); microelements and heavy metals (Jones, 2001); phenols (Lee et al., 2003); oil content (NAM 2010); COD and BOD (Muthuvel & Udaasoorian, 1999) (Table 2).

Vertisol soil samples were collected from Al-Thahalla village, Suwayda governorate (southern Syria) and prepared for wheat plantation. It is worth mentioning that soil samples were analyzed before and after treatments (data not shown).

2.2 Experimental design

A completely randomized experiment was carried out in triplicates during two consecutive agricultural seasons of 2015–2016 and 2016–2017 (Fig. 1). For that, 4 kg of homogeneous soil samples (*vertisol*) was distributed in plastic containers with an 18 cm diameter. OMWW was then added to the pots at three rates: 5 l/m^2 (T5), 10 l/m^2 (T10) and 15 l/m^2 (T15). Taking into consideration the bulk density of *vertisol* samples (1.13 g/cm^3), a surface unit of 1m^3 and 0.3 m in depth would weigh 339 kg. Thus, 4 kg of *vertisol* (each pot) will require 59, 118 and 117 ml of OMWW for T5, T10 and T15 treatments, respectively. After addition, OMWW was thoroughly mixed the pots were left for two months prior to cultivation with regular watering (80% of field capacity) during this period, according to the recommendations of Ortiz-Garcia et al. (1997). By cultivation time, wheat kernels were added to the pots at a rate of 10 grains per pot and then were irrigated regularly to keep soil moisture around 80% of field capacity.

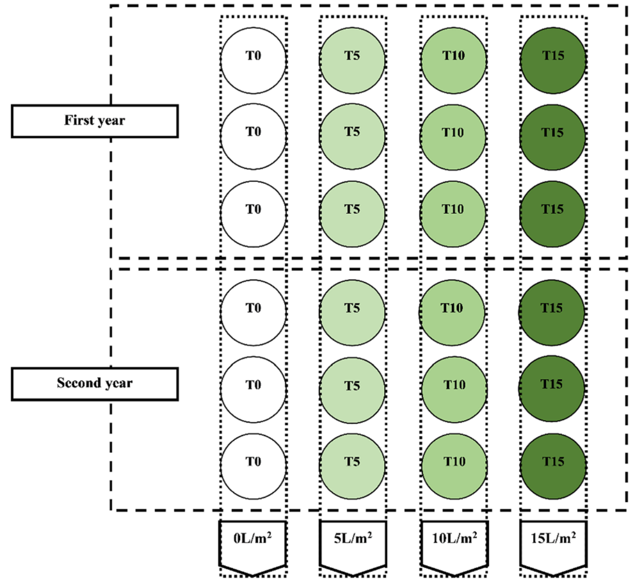

Fig. 1 Experimental design, first season (cultivation 4/12/2015, harvesting 16/6/2016) and second season (cultivation 9/12/2016, harvesting 20/6/2017)

Table 2 Physicochemical properties of OMWW

OMWW properties	Value	OMWW properties	Value
pH	4.63 ± 0.0648	Fe (ppm)	18.30 ± 1.5725
EC (dS/m)	8.82 ± 0.0566	Cu (ppm)	2.92 ± 0.5617
Ash (%)	0.80 ± 0.0163	Mn (ppm)	3.46 ± 0.4114
Water (%)	92.40 ± 0.2007	Zn (ppm)	2.83 ± 0.0455
Organic matter (g/L)	68.00 ± 1.6330	Cd (ppm)	0.12 ± 0.0082
N (ppm)	812.63 ± 4.2845	Pb (ppm)	0.56 ± 0.0432
P (ppm)	284.36 ± 2.6426	Total phenols (g/L)	8.12 ± 0.5146
Ca (ppm)	151.32 ± 2.6618	Oil (g/L)	3.16 ± 0.2406
Mg (ppm)	78.41 ± 1.6330	COD (g/L)	142.26 ± 2.9334
Na (ppm)	54.60 ± 0.5666	BOD (g/L)	58.37 ± 2.3088
K (ppm)	2231.00 ± 21.6969	COD/BOD	2.43 ± 0.0478

2.3 Wheat morphological and yield traits

Various morphological and yield-related traits of wheat plants were monitored. The germination percentage, plant height (at harvest), ear length (at harvest), the weight of grains per ear (post-harvest), the number of grains per ear (post-harvest) and yield per pot (post-harvest) were calculated as an average for the three replicates.

2.4 Microbiological analysis

Bacterial and fungal numbers in soil samples were evaluated using the volumetric dilution method until 10^{-5} for bacterial cultures and 10^{-4} for fungal cultures. Then, the dilutions were cultured in Petri dishes with three replicates for each dilution.

For bacterial culture, the soil extract environment was used (1 kg of soil + 1 L of distilled water) sterilized by autoclave and then filtered. The culture was supplemented with agar (at 1.5%), and the pH degree was regulated to 7 using 0.1 M HCl, since the original extract was alkaline. Three Petri dishes were cultivated from 10^{-5} dilution, where 1 ml of this dilution was spread in each dish. Then, the culture environment was poured over it, stirred gently and then left to harden. After that, the dishes were placed inverted in the incubator and incubated at a temperature ranging between 30 and 35 °C.

As for fungi culture, Potato Dextrose Agar (PDA) environment was used. The pH was set to 6. Three Petri dishes were cultivated from 10^{-4} dilution. One milliliter of this dilution was placed in each Petri dish, and the culture environment (PDA) was poured over it and left to harden. The dishes were incubated in an inverted position at a temperature of 25 °C (Tepper et al. 1987).

Bacterial and fungal numbers were then calculated using the following formula, as suggested by Tepper et al. (1987):

$$\mathrm{CFU} = \mathrm{ACN}/\mathrm{DF} \tag{1}$$

where CFU is the bacterial/fungal cells number, CAN is the average cells count in the diluted culture, and DF is the dilution factor.

2.5 Statistical analysis

The experiment was conducted in a complete randomized design, with three replications per treatment. The normal distribution of the experimental data was evaluated by applying Shapiro–Wilk test. The treatment's means were compared using one-way ANOVA and Tukey's HSD ($p < 0.05$). Additionally, the correlation matrices between variables (i.e., germination (Ger.) in %, plant height (Hig.) in cm, ear length (Ear) in cm, kernels number (Ker.) per ear, kernels weight (Ker.Wie) in g, yield in g/m^2) were plotted.

Statistical analysis was carried out using GenStat v-12 (Payne et al. 1987), SPSS software (v24; IBM, Chicago, IL, USA) (SPSS Inc. 1988), EViews software package (v10; New York, USA) (McCullough, 1999) and GraphPad Prism (v6; GraphPad Software, Inc).

3 Results

3.1 OMWW and wheat growth

3.1.1 Germination percentage

A significant increase in germination rates was observed under all treatments in comparison with control treatment. Although OMWW increased germination rates in the second season, this increase was only significant under T10 treatment, where the highest germination percentage was observed (Fig. 2a).

3.1.2 Plant height

The results obtained from both seasons showed that OMWW application had significantly increased plant height compared to the control treatment. The most significant effect in terms of plant height was observed in T10 treatment. Furthermore, it was noticed that plant height was significantly increased by increasing OMWW treatment from T5 to T 10 and decreased again under T15, with this decrease being significant in the second season (Fig. 2c).

3.1.3 Ear length

Both T10 and T15 treatments induced a significant increase in ear length compared to the control and T5 treatments in both seasons. In contrast, no significant differences were found between the control and T5 treatment. Additionally, T10 resulted in the highest values of this attribute with 7.86 cm and 6.86 cm in both seasons, respectively, and significantly higher than those of T15 treatment (Fig. 2b).

3.1.4 Kernels number and weight per ear

Kernels' number per ear increased significantly under OMWW application when compared to the control treatment. Furthermore, significant differences were found between the three levels. The highest value was noticed under T10 treatment in the second season with 51.28 kernels per ear (Fig. 2d).

OMWW treatments showed a significant increase in kernels' weight per ear compared to the control sample.

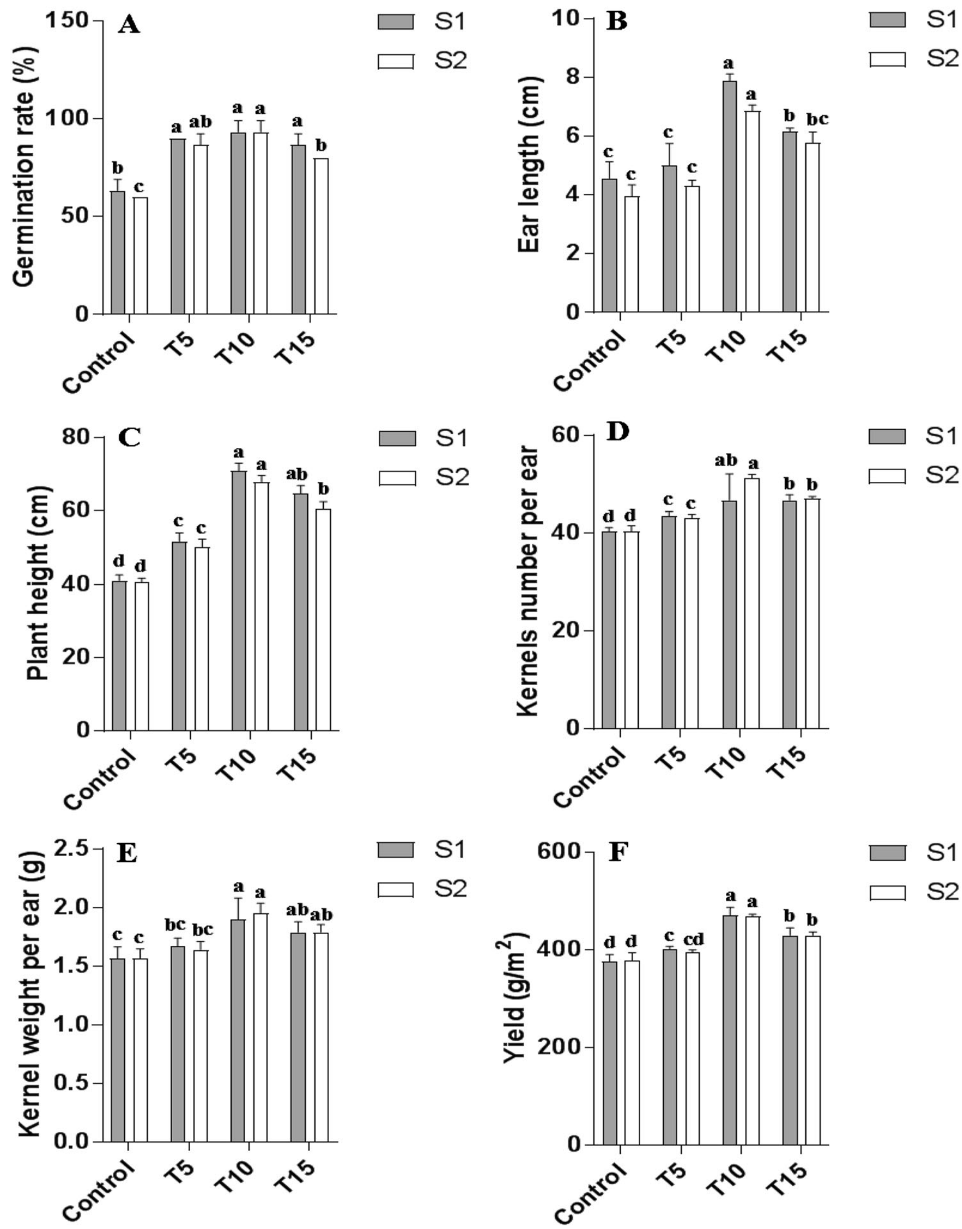

Fig. 2 Impact of OMWW treatments on studied parameters: **a** Ger. %, **b** Hig. in cm, **c** Ear in cm, **d** Ker.per ear, **e** Ker.Wie in g, **f** yield in g/m^2). Columns with similar litters have no significant differences based on Tukey's HSD test ($p < 0.05$)

The highest recorded observations were under T10 treatment with 1.90 g/ear and 1.95 g/ear in the first and second seasons, respectively. Similar to ear length results, T5 treatment did not induce a significant increase in kernels' weight per ear when compared to the control (Fig. 2e).

3.1.5 Yield

The yield was calculated and expressed based on m^2 basis. The results showed a significant increase in total yield compared to the control for T15, T10 and the first season under T5 treatment. The highest increase was recorded under T10 treatment with 24.94 and 23.84% yield increase in the first and second seasons, respectively. Simultaneously, the lowest increase was recorded under T5 treatment with only 6.37 and a 4.26% increase in the first and second seasons, respectively (Fig. 1f).

In this context, Fig. 3 depicts the correlation matrix between studied variables of wheat. Interestingly, a significant ($p < 0.001$) highly positive correlation was observed between all the studied variables, which revealed a positive impact of different treatments in wheat production. The highest correlations were $r_{\text{Hig. Vs. Yield}}$ (0.94, $p < 0.001$) and $r_{\text{Ear. Vs. Yield}}$ (0.90, $p < 0.001$), while the lowest correlations were $r_{\text{Ker. Vs. Ker.Wie.}}$ (0.62, $p < 0.001$) and $r_{\text{Ger. Vs. Ker.Wie}}$ (0.64, $p < 0.001$). Nonetheless, more

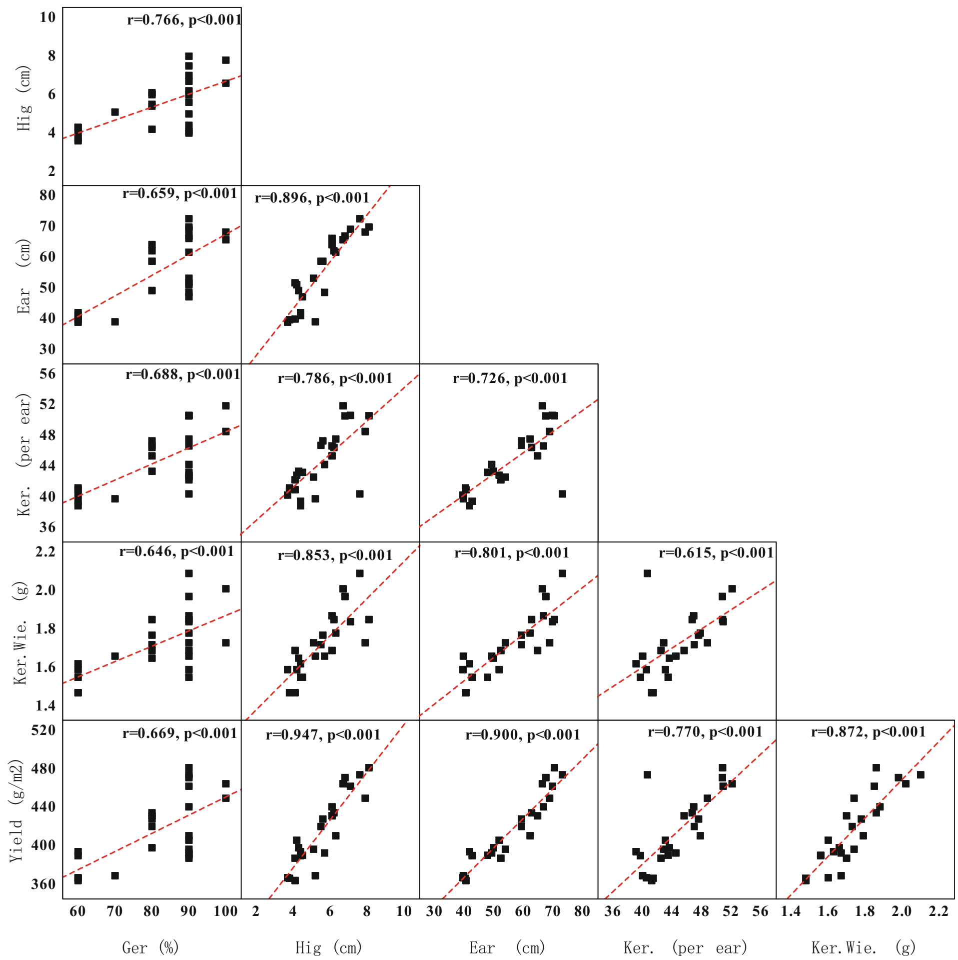

Fig. 3 Correlation matrix between wheat studied variables

experiments should be conducted to verify the output of the correlation matrix.

3.2 OMWW and microbiological activities

3.2.1 Bacterial count

The highest bacterial count was recorded under T10 treatment in both seasons with 43.18 and 40.91 CFU/ 10^{-5} g soil. However, no significant differences were recorded between T10 and T15 treatments in the first season. The lowest observations were recorded in control in both seasons, which lead to the conclusion that olive wastewater treatment had increased the bacterial count in general, and this increase was significant starting from T5 treatment (Fig. 4a).

3.2.2 Fungal count

The statistical analysis results in both seasons indicated a significant increase in the number of fungi by each increase in the concentration of added olive wastewater treatments. T15 treatment recorded the highest counts in both seasons with 11.58 and 11.21 CFU /10^{-4} g soil, respectively (Fig. 4b).

4 Discussion

The current results showed that OMWW treatment might induce an increase in kernel germination at any concentration (T5–T15). It was reported that the direct cultivation post-OMWW treatment without an induced bacteriological decomposition decreased the germination percentage in wheat (Casa et al. 2003) or inhibited germination even if diluted (Mekki et al. 2006a, b). Additionally, a decrease in tomato seed germination rate was observed after OMWW addition at rates of 40 and 80 m^3/ha (Piotrowska et al. 2006). This decrease was attributed to the increased phytotoxicity after OMWW addition, especially under higher rates (80 m^3/ha). However, it was apparent that 45 days of incubation were almost enough to restore normal germination rates under lower rates. The current study results of increased germination percentage under OMWW might be

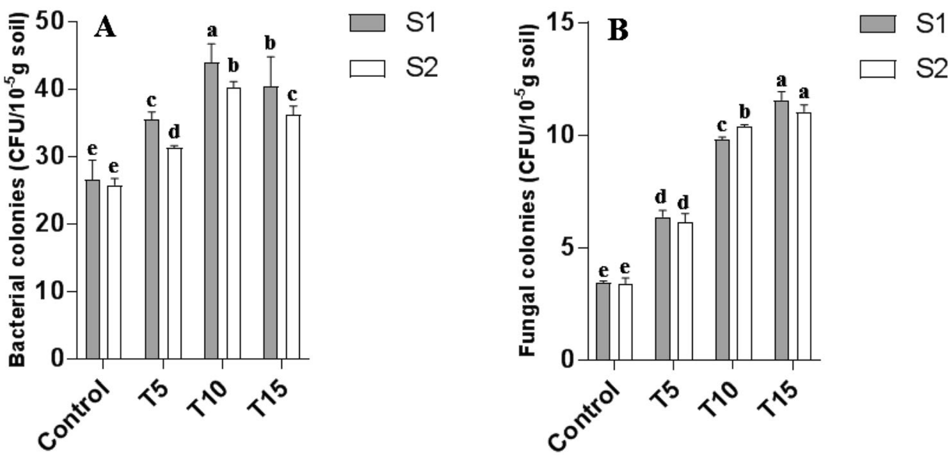

Fig. 4 Impact of OMWW treatments on microbiological activities: **a** bacterial count, **b** fungal count. Columns with similar litters have no significant differences based on Tukey's HSD test ($p < 0.05$)

attributed to the decrease in the overall phytotoxicity after an incubation period of two months with an increase in germination assistant substances such as humic acids. Although humic acid content was not measured in the current work, other studies showed that OMWW has good composting potential (Madejón et al. 1998; Cigarra et al. 1999; Tomati et al. 1995). Consequently, humic acids resulted from OMWW application might stimulate enzyme systems in the kernel and, thus, provides the energy needed for the embryo during the transformation from the non-photosynthetic phase to the autotrophic phase (Mekki et al. 2013). Therefore, a bacteriological decomposition is crucial after OMWW application.

The observed increase in plant height due to OMWW treatments was previously noted by (Alibrahim et al. 2008), who reported significant increases in wheat plant height under olive wastewater treatment of 5, 10 and 20 m^3/1000 m^2. Pomace treatment, which is another by-product of olive oil extraction, induced an increase in kernels weight and kernels number in the area unit (Brunetti et al. 2005), which is similar to the current observations.

The current increase in yield under OMWW treatments was expected due to the increase in both kernels number per ear and average kernel weight per ear under olive wastewater treatments. These results are similar and exceed those of Mekki et al. (2006a, 2006b), who reported a 5% yield increase in wheat under olive wastewater treatment compared to the control sample as a result of the increase in both kernel weight and ears per plant.

Mechri et al. (2008); Saadi et al. (2013); and Mohawesh et al. (2019) reported that phenolic content was decreased after a period of adding OMWW, which might be the case in the current study due to the two-month waiting period. Therefore, the observed increase in the bacterial cell's numbers in all OMWW-treated soils was due to the decreased inhibitory effect of phenolic compounds accompanied by a high organic matter content, which plays an essential role in supplying the bacterial cells with the nutrients and energy needed for their activity. This observation corresponds with Piotrowska et al. (2006) and Badran (2011) reports of total bacterial numbers increase after a period of adding wastewater. On the other hand, the slight decrease in CFU under T15 treatment compared to T10 might be due to phenols exceeding a certain inhibitory threshold. Similarly, Naeb (2011) previously found that adding wastewater to the soil in large quantities can negatively affect the microbial activity and the processes of decomposition of organic matter in the soil due to the high percentage of phenolic substances in it, while the small quantities positively affect the activity of microorganisms in the soil.

As for fungal cell numbers after OMWW treatments. Naeb (2011) found a decrease in the number of fungi after adding wastewater to the soil at a rate of (20 l/ m^2) compared to adding it at a rate of 10 l/m^2. This observation might be due to the wastewater phenolic compounds, sugars, fats and proteins, in addition to some aromatic compounds that inhibit the growth of these microorganisms (Tushan et al. 2010). However, the consistent increase in the current study might be due to the degradation of toxicity-inducing substances after the 2-month waiting period, similar to Zenjari et al. (2006).

5 Conclusion

In this research, *vertisol* was treated with three different levels of OMWW T5 (5 L / m ^{2}), T10 (10 L/ m^2) and T15 (15 L / m ^{2}) and then planted with wheat for two successive seasons. Soil treatments of different levels of OMWW showed a significant effect on wheat growth and production. Both kernel germination and plant height increased with OMWW application compared with the control sample. Similarly, ear length, kernels number per ear and average kernel weight increased significantly. These results were a direct consequence of enhancing the physico-chemical properties of soil. Notably, our results reveal that T10 (10 L/ m^2) was superior among other treatments. Thus, this amount of OMWW (10 L/ m^2) could be recommended for agricultural application.

The current results on wheat production promising and could render the reported treatment as a sustainable solution for OMWW problem in Syria. However, further investigation should be conducted under different agro-climatic zones, various pre-application period or bacterial degradation treatments to ensure the positive impact of OMWW under Syrian conditions, especially in *vertisol*.

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Declarations

Conflict of interest The author declares that they have no conflict of interest.

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