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Fertilizing Potentiality of Fungal-Treated Olive Mill Solid Waste to improve Some Growth and Physiological Parameters of *Vicia faba* L.

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THE MAIN objective of the present work is to biodegrade the phenols content of olive mill solid waste by a locally isolated fungus under optimized culture conditions and study the effect of the crude olive mill solid waste (OMSW), fermented olive mill solid waste (FOMSW), as well as the residue of water extracted olive mill solid waste (ROMSW) on some growth and physiological biomarkers of *Vicia faba. Aspergillus tamarii* was the most efficient fungus of ten isolated fungi, where it completely (100%) degraded the phenolic contents of OMSW under the optimized culture conditions. Data showed that applying FOMSW and ROMSW resulted in a significant alleviation of OMSW adverse effects on plant shoots and roots, chlorophylls content, chlorophyll stability index, quantum yield of PSII (Fv/Fm), carbohydrates and protein content as well as the macro-elements K, P, Mg and Ca content. This improvement was increased with increasing concentrations of ROMSW, while it increased up to 4% of FOMSW. Even as the crude OMSW negatively affects the growth and physiological attributes of the tested plant, all treatments stimulated them. It seems that detoxification of OMSW either by *A. tamarii* or water resulted in a marked reduction of phenolics and rising in pH that might improve the soil characteristics and allows a secure and efficient dumping of OMSW.

Keywords: Biofertilizer, Biodegradation, Faba bean, Fungi, Phenolics, Photosynthesis.

Introduction

Olive production is considered as an important national income source for most Mediterranean countries (Nogueira et al., 2015). Oil extraction from olives produces large quantities of waste by-products that can destructively affect water resources, plants as well as soil microbes (Ahmed et al., 2019). Waste recycling is being applied to produce valuable fertilizers or amendments to improve the structure of the soil. The recycling of olive mill waste as organic compost can enhance soil fertility as well as reduce CO_2 emissions. (Regni et al., 2017). The possibilities of these biomass waste sources as organic fertilizers have been evaluated by studying its effective management. The biomass waste contains valuable nutrients, which can be put to good use if managed properly (Chew et al., 2019).

Olive mill solid waste (OMSW) elicits a certain amount of toxicity owing to its phenolic constituents. OMSW has been considered as a potential source of pollution either in land or in water and great efforts have been applied to find lucrative solutions for its management (Marra et al., 2018). Several investigations have been carried out using microorganisms capable of growing aerobically on diluted OMSW with the objective of reducing its initial organic load and its phenolic content (Aggelis et al., 2003; Nogueira et al., 2015). Different chemical and biological treatment technologies have been employed for the reduction of phenol content in industrial wastes. The biological management has become helpful because of simplicity of the process, cost effectiveness, and its potentiality to bend toxic resources into secure products (Bramhachari et al., 2016; Youssef et al., 2019).

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Different microbial strains can use the phenolic compounds present in OMSW as a carbon source for the production of different organic compounds useful in agricultural processes. Filamentous fungi (*Aspergillus* spp., *Fusarium* spp, etc.) have been used to degrade the phenolic compounds of industrial wastes such as OMW (Karatay & Donmez, 2014). The fermented OMSW can be used as an organic biofertilizer instead of the traditional chemical fertilizers, also, it improves soil quality. Organic biofertilizers are not only a good source for plant nutrition but also they improve soil microbial activity and promote the activities of critical soil enzymes and plant growth hormones. Accordingly, they play a fundamental role in the ecosystem (Omer, 2012). Rhizosphere microorganisms have the impending to induce plant growth and help in managing plant diseases and abiotic stresses in the soil throughout the production of phytohormones and significant root morphological changes, which improve plant-water relations, nutritional status and stimulate the defensive mechanisms of plants (Goswami & Deka, 2020).

Faba bean (*Vicia faba* L.) is considered as one of the most important legumes in Egypt. It has become one of the strategic crops due to its income to the farmers. Furthermore, faba bean is a popular legume food and used worldwide as an important source of protein for human and animal nutrition (Cazzato et al., 2012).

The main intent of the current work was to isolate and identify one local fungus efficient for degrading phenolic compounds dominant in OMSW. Furthermore, to study the effect of raw OMSW; fermentation residues (FOMSW) as well as residues of water extracted (ROMSW) on some growth biomarkers, physiological and biochemical analysis on *Vicia faba* L. to appraise its potentiality as a biofertilizer.

Materials and Methods

Microbial preparation

The tested fungi were isolated from OMSW and soil, purified, and identified according to Watanabe (2002) and CBS (2006). The medium that contains the best ingredients of the culture medium that favored the formation of enzyme system responsible for hydrolysis of OMSW phenols was (g L^{-1}): OMSW, 80; K_2HPO_4, 3.40; KH_2PO_4, 4.30; $MgSO_4.7H_2O$, 0.30; $(NH_4)_2SO_4$, 1.0; yeast extract, 0.05; 10ml of trace elements solution (mg L^{-1}) of $MnCl.4H_2O$, 1.0; $FeSO_4.7H_2O$, 0.6; $CaCl_2.H_2O$, 2.6; $Na_2MoO_4.2H_2O$, 6 (Santos & Linardi, 2004). For optimization of medium composition and cultural conditions, two-phase optimization approach was carried out, the first deals with evaluating the significance of the different fermentation medium constituents and culture conditions using the Plackett-Burman design (Plackett & Burman, 1946). Response surface methodology (Box & Bhenken, 1960) was applied to determine the optimal level of each key independent variables and their interaction.

Olive mill solid waste

The OMSW was collected from three olive mills located at Dabaa area–western coastal region of Egypt. Recently produced OMSW was moved shortly to the laboratory, before use it was dried and passed through sieve (2mm). Three groups were prepared: crude olive mill solid waste (OMSW), residue of water extracted olive mill solid (ROMSW) and fermented olive mill solid waste (FOMSW).

Preparation of aqueous extract

Stock aqueous extract (OMSWAE) and subsequent dilutions were obtained by the following methods: 1000ml of distilled water were mixed with 100g dried OMSW, conducted in dark for 24hrs at 25°C, centrifuged for 15min at 5000xg. The supernatant was adjusted to pH 6.8; this would be (100%) full strength concentration. Sequences of dilutions (0, 5, 10, 20 and 40%) were equipped from the stock solution (El-Darier et al., 2015).

Germination bioassay

Petri-dish experiment was applied to investigate the biological activity of OMSWAE on faba bean plumule (PL) and radicle lengths (RL), fresh weight (FW) and dry weight (DW). Prior to germination, seeds were drenched 2min in 2% chlorex for sterilization, after washed by distilled water numerous times. Finally, the seeds were soaked in aerated distilled water for 24hrs, ten seeds were regularly arranged in Petri-dishes (15cm diameter) lined by two discs of Whatman No.1 filter paper under normal laboratory conditions. Ten ml of the OMSWAE (0, 5, 10, 20 and 40%) were added daily. Inhibition percentage (IP) was calculated according to Zhen et al. (2008):

$$IP= [1 – (Treatment/Control)] \times 100.$$

Growth bioassay

To test the potential effects of OMSW, ROMSW and FOMSW on some growth and physiological attributes of faba bean, pot experiment was achieved for two weeks. At the end of the experiment, homologous faba bean individuals were harvested, washed with distilled water and finally separated into roots and shoots.

The three groups of waste were mixed with a sandy clay loam soil at different concentrations (2, 4, 8 and 16%). One treatment containing untreated soil was used as a control. Soils in the plastic pots (20cm diameter, 2lcm length) were compacted to about field density. Ten sterilized faba bean seeds were planted in each pot. The pots were kept under normal laboratory conditions, and watered every two days (at 70% field capacity) with tap water.

Physiological and biochemical analysis

Determination of photosynthetic pigments

Leaf chlorophyll extraction was prepared using N,N-dimethyl formamide (4ml) for 24hrs at 4°C. Chlorophylls content was quantitatively determined using a spectrophotometer-double beam (T80 UV–Vis) at wave lengths of 646.8 and 663.8nm (Porra, 2002). Total carotenoids content was calculated according to Moran (1982). Chlorophyll stability index (CSI %) was measured using the formula noted by Sivasubramaniawn (1992):

(Total Chl. in treated leaves/ Total Chl. in control leaves) × 100.

Measurement of quantum yield of PSII (Fv/Fm)

Chlorophyll fluorescence measurements were performed with chlorophyll fluorimeter OS-30P pulse modulated (Opti-sciences, Hudson, and USA) following the procedure described by (Branquinho et al., 1997). Leaves were dark-adapted for 30 min with leaf-clips before each measurement.

Extraction and estimation of total available carbohydrates (TAC)

The powdered plant material (100mg) was introduced into a boiling tube, ten ml of 0.7N HCl were then added and the tube was placed in a boiling water bath for 30min. The hydrolysate was neutralized and adjusted to known volume. TAC was estimated following the method described by Murata et al. (1968).

Extraction and estimation of total protein (TP)

Total protein was extracted by adding 10ml of 0.5N NaOH to about 100mg of the oven-dry plant material which was left over night. After centrifugation the extract was completed to volume with distilled water (Rausch, 1981). Estimation of total protein was done as the method explained by Hartree (1972).

Extraction and estimation of total phenolics

Total phenolics content was estimated according the method of Demiray et al. (2009). Samples and standard absorbance was measured using spectrophotometer-double beam (T80 UV–Vis) at 765nm.

Energy dispersive spectroscopy (EDS) for elements analysis

Elemental analysis was carried out using an Energy Dispersive X-ray Fluorescence (EDS) spectrometer at the special unit of Electron Microscope, Faculty of Science, Alexandria University. Link ISIS analyzer programmed attached with Scanning Electron Microscope (JSM-5300) was employed for the data collection. Elements values correspond to the average of three replicate spectra attained from each tissue and each treatment (Chen et al., 2014).

Statistical analysis

Results of statistical analyses were carried out according to Duncan's multiple range tests using SPSS-20. Following the method described by Sokal & Rohlf (1995), data were subjected to one-way ANOVA. Differences between treatment-means were considered statistically significant at $P \leq 0.05$.

Results

Microbial bioremediation of OMSW

It was carried out to get rid and/or degrade phenolic compounds and other toxic materials that render its use as a biofertilizer and the fungal cell contents may add beneficial substances to the plant growth. This goal of work was started by isolation and identification of some fungi from OMSW and soil. *Aspergillus tamarii* was the most efficient in degradation of phenols (64.07%) from the initial content 8.46g kg^{-1}. While the other tested fungi showed different activities of phenols degradation. In an attempt to maximize phenol degradation by the experimental fungus (*A. tamarii),* different six culture media with varied ingredients (qualitatively and quantitatively) were tested. The best medium was used as basal medium for phenol degradation. The time course study of phenols of OMSW degradation by *A. tamarii* indicated that 7 days of incubation represent the best growth period for *A. tamarii* to produce phenols hydrolyzing enzymes, at that period 84.6% of phenols were degraded.

In order to optimize degradation of phenols content of OMSW by the tested fungus, statistical experimental designs was applied. Two-phase optimization approach was carried out, the first deals with evaluating the significance of the different fermentation medium constituents and culture conditions using the Plackett-Burman design of the tested 14 trails, the medium consists of (g L^{-1}): OMSW, 80; K_2HPO_4, 3.40; KH_2PO_4, 4.30; $MgSO_4.7H_2O$, 0.30; $(NH_4)_2SO_4$, 1.0; yeast extract, 0.05; $MnCl.4H_2O$, 10.0 (µg) ; $FeSO_4.7H_2O$, 0.6(µg); $CaCl_2.H_2O$, 26 (µg) ; $Na_2MoO_4.2H_2O$, 100 (µg); inoculum size 3ml ($6x10^6$ spores ml^{-1}) and 25ml medium/250 ml Erlenmeyer flasks, shacked at 180rpm for 7 days at 30±2˚C and initial pH 6 appeared to be optimum for phenols degradation (96.28%) that represent about 14% increase as compared to the best tested medium for phenols degradation. Thereafter, response surface methodology was applied to determine the optimal level of each key independent variable and their interaction. The most significant variables included, medium volume/250 ml Erlenmeyer flask $Na_2MoO_4.2H_2O$ and $(NH_4)_2SO_4$.

Ten trails were made and the results analyzed mathematically revealed an optimum response of the following concentrations: $(NH_4)_2SO_4$,1.8g L^{-1}; $Na_2MoO_4.2H_2O$, 60µg L^{-1} and medium volume 17ml /flask with phenol degradation of 100% after 138hrs (5.75 days) instead of 168hrs (7 days). So under the optimized conditions, phenol content (8.46g kg^{-1}) of OMSW was completely degraded by *A. tamarii* after 138hrs of incubation. The results revealed that phenols content 8.46g kg^{-1} was completely degraded, total carbohydrates recorded decrease and total lipids were significantly reduced. On the other hand, the total protein content has a significant increases and pH becomes neutral (Under publication). These results indicated that it is safe to use the fermented OMSW (FOMSW) as a biofertilizer.

Germination bioassay

The effect of different concentrations of olive mill solid waste aqueous extract (OMSWAE) on faba bean seeds was evaluated (Fig. 1). PL and RL lengths, as well as their FW and DW, were notably decreased with the increase in OMSWAE concentration.

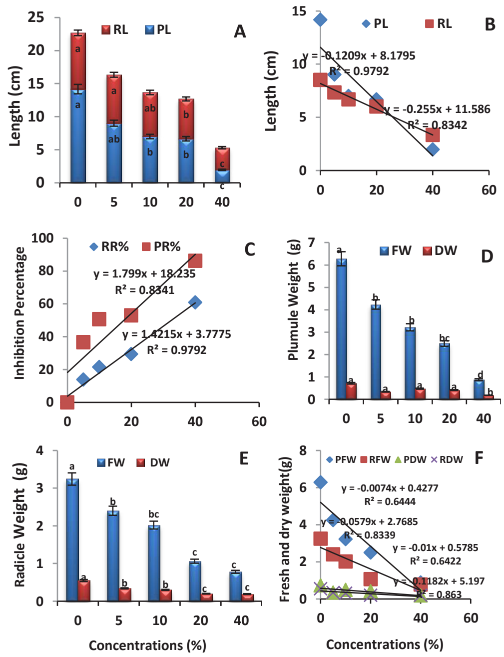

Fig. 1. Effect of different concentrations of OMSWAE on (A) radicle length (RL) and plumule length (PL), (B) regression analysis for radial and plumule lengths,(C) reduction percentages of radicle and plumule lengths, (D) plumule fresh and dry weights, (E) radicle fresh and dry weights and (F) regression analysis of plumule and radicle fresh and dry weights of *Vicia faba* L. at 9 day-old seedlings [Values are means ± SD based on triplicate independent determinations, and different letters mean significant difference as evaluated by Duncan´s multiple comparison test].

Growth bioassay

A pot experiment was carried out by application of three treatments (OMSW, ROMSW, and FOMSW) of olive mill solid waste with different concentrations (2, 4, 8, and 16%) mixed with sandy clay loam soil.

Root and shoot lengths

Generally, a 2% concentration level of OMSW showed a slight increase in root and shoot lengths of faba bean compared to control

(Table 1). Conversely, the lengths of the two organs significantly decreased to values of 5.3 and 8.3, respectively, relative to the control (6.25 and 16.5) in response to higher concentration (16%). Detoxification by water extraction (ROMSW) significantly enhanced shoot and root lengths in all concentrations. At 16% of ROMSW concentration, the increase in both shoot and root lengths attained nearly 2-fold more than the control. The root and shoot lengths of faba bean plants grown in soil mixed with OMSW fermented by *Aspergillus tamarii* showed a significant increase at low concentrations (2 and 4%) compared to the control, on the other hand, high concentration showed a marked inhibition. Furthermore, the application of OMSW, ROMSW, and FOMSW at different concentrations significantly increased root: shoot (R/S) ratio in faba bean plants.

Fresh (FW) and dry (DW) weights

Data presented in Table 1 showed that mixing soil with different concentrations of raw OMSW resulted in a significant reduction of FW and DW. Mixing soil with the ROMSW showed an increase in the fresh and dry weights of shoots, while the two estimates for roots were reduced nearly by 33 and 17%, respectively, at 16% concerning the control. It is clearly demonstrated that there was a gradual increase to 8% in the FW and DW of shoots and roots in soil containing *A. tamarii*-FOMSW. Treatment with 4% FOSMW resulted in a significant increase in shoots and roots, the increase values for FW reached about 1.6- and 1.9-fold, respectively, and the corresponding values for DW were 1.6- and 2.3-fold, respectively.

Photosynthetic pigments

Data in Fig. 2 revealed that the total chlorophylls content was significantly decreased by increasing concentrations of OMSW comparing to untreated plants. The total chlorophylls contents estimated at 16% were reduced by 52% and chlorophyll stability index (CSI) reached 48% compared to control plants, whereas the corresponding value was 113% in ROMSW-treated plants (Fig. 2). The increase in Chl. a and Chl. b was significantly noticed in accordance with the increase in ROMSW concentrations reached about 1.13-fold and 1.06, respectively, at 16%. Application of FOMSW enhanced the synthesis of photosynthetic pigments particularly at the concentration of 4% and the CSI value was 117%. The trend of carotenoids content was significantly increased with increasing concentrations for all treatments.

TABLE 1. Effect of different concentrations of olive mill solid waste (OMSW), residue of water extracted olive mill solid waste (ROMSW), and fermented olive mill solid waste with *Aspergillus tamarii* (FOMSW) on shoots and roots fresh and dry weights (g 5ind^{-1}), lengths and root/ shoot ratio (R/S) of *Vicia faba* L. at 15 day-old plants.

Treatment (%)	Fresh weight (FW) (g)		Dry weight (DW) (g)		Length (cm plant^{-1})		R/S
	Shoot	Root	Shoot	Root	Shoot	Root	
0	8.780±1.02^{a}	4.240±0.49^{a}	0.800±0.08^{a}	0.307±0.03^{a}	16.5±1.92^{a}	6.25±0.64^{a}	0.379^{a}
OMSW							
2	6.000±0.63^{b}	3.580±0.38^{b}	0.524±0.06^{b}	0.270±0.03^{ab}	18.3±1.93^{b}	8.0±0.93^{b}	0.437^{a}
4	5.980±0.59^{b}	2.700±0.26^{b}	0.520±0.05^{b}	0.225±0.02^{b}	16.7±1.64^{a}	7.7±0.75^{b}	0.461^{b}
8	3.500±0.36^{c}	1.820±0.19^{c}	0.340±0.03^{c}	0.198±0.02^{b}	11.3±1.15^{c}	5.7±0.54^{a}	0.504^{bc}
16	3.960±0.46^{c}	1.400±0.16^{c}	0.370±0.04^{c}	0.193±0.02^{b}	8.3±0.97^{d}	5.3±0.62^{a}	0.639^{c}
ROMSW							
2	6.840±0.66^{b}	3.360±0.33^{b}	0.637±0.07^{b}	0.347±0.04^{a}	24±2.33^{e}	8.3±0.87^{b}	0.346^{a}
4	7.800±0.74^{a}	3.120±0.30^{b}	0.710±0.09^{ab}	0.313±0.04^{a}	26.7±2.54^{e}	10.7±1.29^{c}	0.401^{a}
8	8.800±0.80^{a}	3.620±0.33^{b}	0.726±0.09^{ab}	0.283±0.03^{a}	27±2.45^{ef}	12.0±1.48^{c}	0.444^{b}
16	10.160±1.18^{d}	2.840±0.33^{b}	0.837±0.08^{a}	0.256±0.02^{a}	31±3.60^{f}	13.7±1.25^{c}	0.442^{ab}
FOMSW							
2	9.520±0.97^{ad}	8.320±0.85^{d}	0.890±0.10^{a}	0.652±0.08^{c}	17.63±1.80^{a}	6.25±0.73^{a}	0.355^{a}
4	13.730±1.65^{d}	8.100±0.98^{d}	1.270±0.13^{d}	0.698±0.07^{c}	20.33±2.45^{b}	7.13±0.73^{ab}	0.351^{a}
8	12.350±1.52^{d}	7.600±0.94^{d}	1.250±0.15^{d}	0.674±0.08^{c}	16.5±2.04^{a}	6.67±0.80^{a}	0.404^{a}
16	7.000±0.75^{a}	5.700±0.61^{a}	0.755±0.107^{a}	0.527±0.06^{c}	8.48±0.91^{d}	5.95±0.64^{a}	0.702^{c}

Values are means ± SD based on triplicate independent determinations, and different letters mean significant difference as evaluated by Duncan´s multiple comparison test.

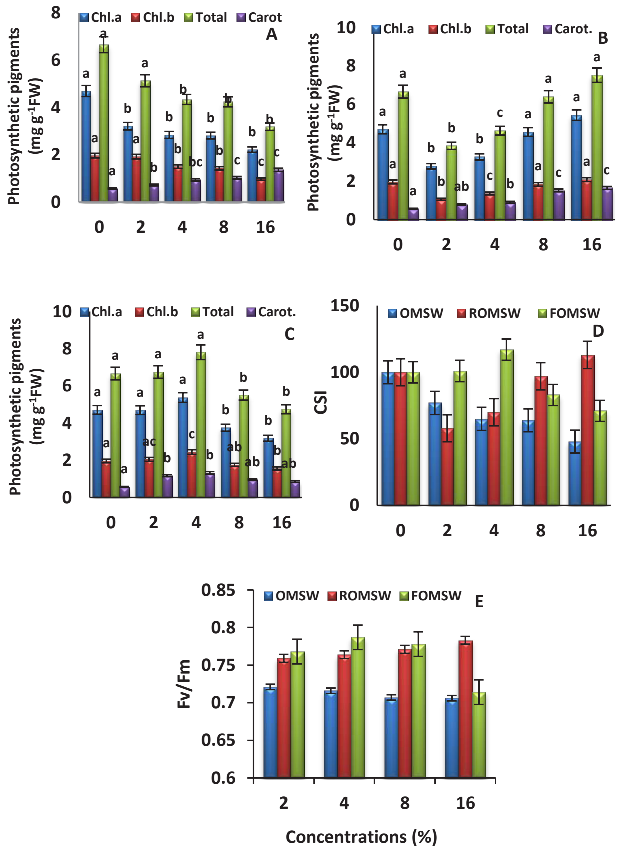

Fig. 2. Effect of different concentrations of (A) olive mill solid waste (OMSW), (B) residue of water extracted olive mill solid waste (ROMSW), and (C) fermented olive mill solid waste with *Aspergillus tamari* (FOMSW) on photosynthetic pigments content, chlorophyll stability index (D) and (E) quantum yield of PSII (Fv/Fm) of *Vicia faba* L. at 15 day-old plants [Values are means ± SD based on triplicate independent determinations, and different letters mean significant difference as evaluated by Duncan´s multiple comparison test].

Measurement of quantum yield of PSII (Fv/Fm)

The maximal photochemical efficiency of PSII (Fv/Fm) decreased by increasing concentrations of OMSW, the reduction percentage was 10% compared to the control in leaves treated with 16% OMSW (Fig. 2E). Applications of ROMSW resulted in a slight reduction with increasing concentrations, attained a similar value of control at 16%, otherwise leaves treated with FOMSW showed a higher value at 4%.

Total available carbohydrates and total protein

A significant reduction in TAC content was detected in OMSW- treated shoots and roots. The decrease in TAC was relatively prominent at high concentrations of crude OMSW-treated plants. The reduction percentage at 16% achieved 33 and 38% for shoots and roots, respectively, compared to the control. On contrary, ROMSW treated plants at 16% showed a significant increase in TAC with increasing concentrations the values were 2-fold and 1.6-fold higher for shoots and roots respectively, concerning untreated plants. At 4% of FOMSW, the increase in shoots and roots was 1.3-fold and 1.1-fold, respectively, (Fig. 3E). The total protein content was significantly affected by applying the different concentrations of OMSW, commonly; there was a general trend of decrease in the content of TP with increasing waste concentrations. The reduction values recorded for the TP at the highest concentration were about 11% and 17% for shoots and roots, respectively, compared with the control. Application of ROMSW showed a notable increase in TP content with increasing waste concentrations in both shoots and roots. At 16%, for example, the increase values were about 1.6- and 1.8-fold in shoots and roots, respectively. Likewise, the application of FOMSW on faba bean plants resulted in a significant increase in TP reaching the highest value at 4% in shoots (1.7-fold) as presented in Fig. 3F.

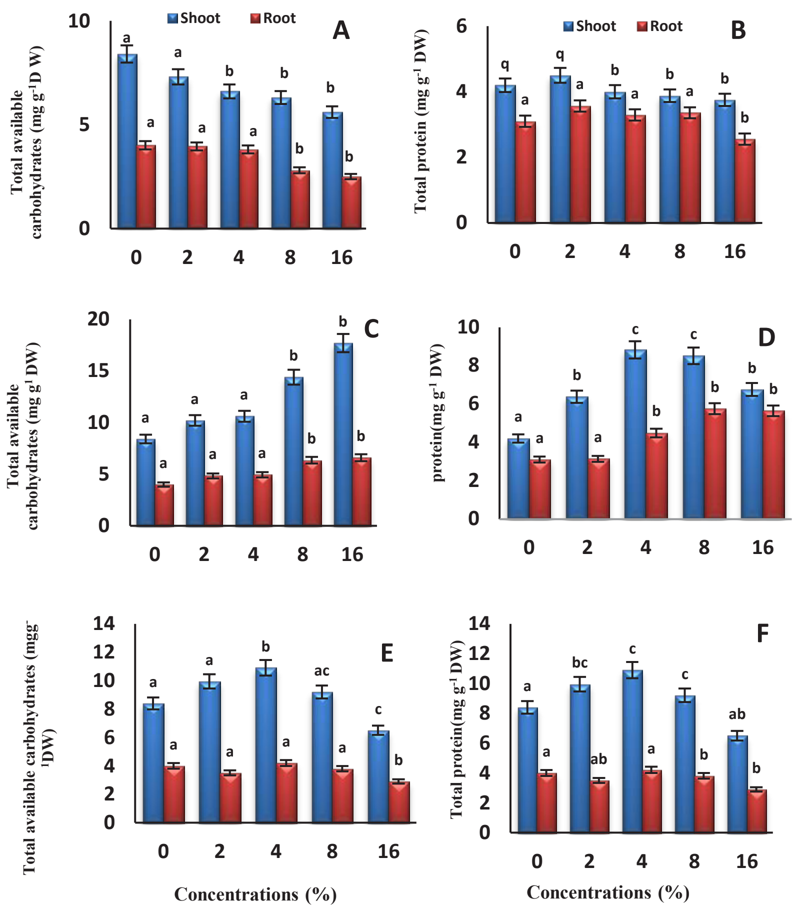

Fig. 3. Effect of different concentrations of (A & B) olive mill solid waste (OMSW), (C & D) residue of water extracted olive mill solid waste (ROMSW), and (E & F) fermented olive mill solid waste with *Aspergillus tamari* (FOMSW) on total available carbohydrates and total protein content (mg g^{-1} DW) of *Vicia faba* L. at 15 day-old shoots and roots [Values are means ±SD based on triplicate independent determinations, and different letters means significant difference as evaluated by Duncan´s multiple comparison test].

Total phenolics

The variation in total phenolics content of control and treated samples showed significantly increased values in shoots of faba bean grown in the three treatments compared to that of control (Fig. 4A). Total phenolic content of control samples was 3.96mg g^{-1} DW and that of treated shoots was 6.6, 8.57 and 10.67mg g^{-1} DW for the concentration of 16% mixed waste with the soil of OMSW, ROMSW, and FOMSW, respectively. Faba bean plants grown in soil mixed with ROMSW or FOMSW exhibited a significant accumulation of total phenolic compounds in the shoots; the accumulation increased in accordance with waste concentration. Application of regression analysis resulted in a value of coefficient (R^2) of determination of about 0.89, 0.75 and 0.78, for OMSW, ROMSW, and FOMSW, respectively (Fig. 4B).

Energy dispersive spectroscopy (EDS) for elements analysis

The results of elemental analysis such as Na, Mg, P, K, Ca, Fe, Cu and Zn showed that the application of OMSW, ROMSW, and FOMSW caused variations in element percentages compared to the control (Fig. 5 and Table 2). A marked increase in the percentage of K, P, Ca, and Mg was recorded in plants treated with ROMSW and FOMSW, the increasing values of K were nearly about 1.1- and 1.2 - fold, respectively compared to the control. Conversely, a great reduction of K percentage in plants treated with OMSW was detected; the percent inhibition was 23%. There was a noticeable increase in microelements Cu and Zn in shoots of faba bean- treated with OMSW, the increase values were 2.5 and1.7- fold, respectively compared to the control.

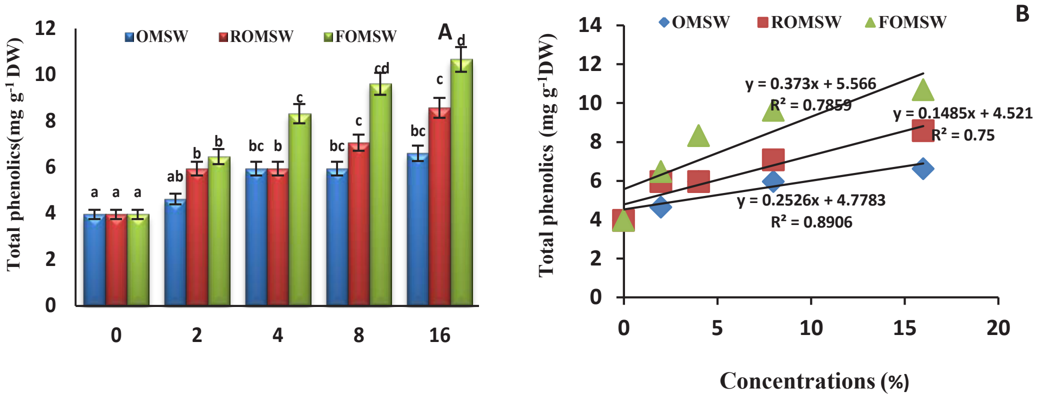

Fig. 4. Effect of different concentrations of olive mill solid waste (OMSW), residue of water extracted olive mill solid waste (ROMSW), and fermented olive mill solid waste with *Aspergillus tamarii* (FOMSW) on (A) total phenolics and (B) regression analysis of *Vicia faba* L. at 15 day-old shoots [Values are means ± SD based on triplicate independent determinations, and different letters mean significant difference as evaluated by Duncan´s multiple comparison test].

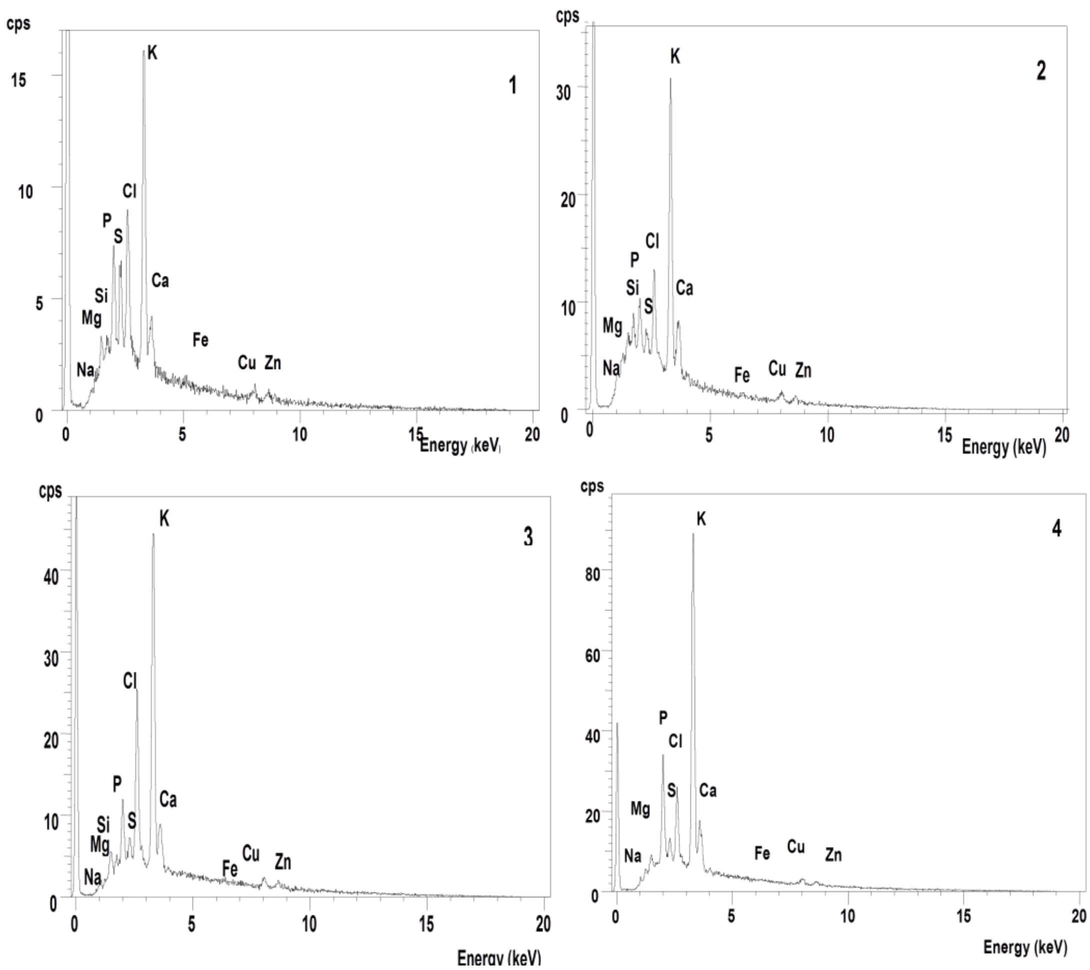

Fig. 5. The energy dispersive spectroscopic (EDS) analysis of elements in 15 day-old *Vicia faba* shoots of untreated (1) and treated with 8% (2) olive mill solid waste (OMSW), (3) residue of water extracted olive mill solid waste (ROMSW), and (4) fermented olive mill solid waste with *Aspergillus tamari* (FOMSW).

TABLE 2. The percentages of elements in 15 days-old *Vicia faba* shoots of untreated (1) and treated with 8% (2) olive mill solid waste (OMSW), (3) residue of water extracted olive mill solid waste (ROMSW), and (4) fermented olive mill solid waste with *Aspergillus tamarii* (FOMSW).

Treatment elements	Control	OMSW	ROMSW	FOMSW
	Element concentration in %			
Na	0.8	2.1	0.9	0.7
Mg	2.6	1.9	4.9	4.3
Si	6.2	11.4	4.3	2.9
P	9.5	7..9	10.9	11.5
S	4.8	4.8	2.2	3.0
Cl	16.5	18.9	11.7	11.7
K	43.4	33.5	48.8	50.7
Ca	9.0	5.7	11.5	10.4
Fe	1.3	3.0	1.6	1.1
Cu	1.5	3.7	1.8	1.0
Zn	1.8	3.0	1.4	0.7

Discussion

Much consciousness towards sustainability and technological advances for solid waste management has been implemented to reduce the generation of unnecessary waste, particularly in developing countries. The recycling of this waste is being applied to produce valuable organic matter, which can be used as fertilizers or amendments to improve the soil structure (Chew et al., 2019). OMSW is very rich in organic matter (73.24%) comprising of a relatively large amount of lignin, cellulose, hemicelluloses, lipids, and carbohydrates as well as phenolics (8.46g kg^{-1}). The electrical conductivity is about 6 dSm^{-1} and the acidic pH value is about 5.1. OMSW possesses considerable amounts of mineral nutrients such as nitrogen, phosphorous, potassium, sodium, calcium, magnesium, iron, copper, manganese and zinc (El-Darier et al., 2015)

In the Mediterranean basin, the most serious environmental problem associated with the agro-industrial sector is the disposal of OMSW. Numerous organic and inorganic compounds were detected in OMSW in high concentrations. The phenolic compounds seem to be responsible for OMSW phytotoxicity and microbial inhibition (Ammar et al., 2005). These render its use as soil fertilizer where it is toxic to plants, animal cells and microorganisms (Mekki et al., 2006). Crude OMSW has insufficiently stable matter, and when applied to soil, it may induce a number of negative effects on the soil properties and plant growth (Komilis et al., 2005). OMSW before its application to the soil might be, consequently, subjected to suitable treatments for stabilization of its organic amendments, and diminshing its environmental hazard (Brunetti et al., 2007). Several treatments have been projected to reduce the toxicity of OMSW phenolic content of which the most promising is the use of microorganisms capable of degrading its phenol content (Hamdi et al., 1991; Mechri et al., 2011). In this respect, the bioremediation of OMSW using specific strains of fungi has been used. Additionally, the ability of fungi to degrade different phenolic compounds was reported (Ghanem et al., 2009; 2013).

Data of the current study revealed that the proximate chemical analysis of the fermented OMSW as compared to crude OMSW indicated that the pH value of the fermented waste tends to be neutral (pH 7) which is due to the different metabolic activities of *A. tamarii*. The acidity of crude OMSW may be due to its content of organic acids and other compounds (Garcia et al., 2000). Paredes et al. (2000) found that the pH of OMSW was 5.17. The neutral pH value of the FOMSW favors its use as a fertilizer than the acidic pH value of crude waste. The total carbohydrates of crude OMSW were noticeably reduced (3.5 fold decrease) after fermentation; this indicates that *A. tamarii* assimilated most of (71.4%) total carbohydrates during its fermentation process. However, the total proteins showed about a 27.7% increase, due to the fungal content of proteins. As for the total lipids (oils), it was markedly decreased from 16.5g kg^{-1} to 1.0g kg^{-1}, this indicates that *A.*

tamarii assimilated the crude OMSW oils, and so, it has lipases. In accordance, Dayanandan et al. (2012) reported that that *A. tamarii* has lipases. The chemical analysis of OMSW for lipids, polyphenols and carbohydrates content was concomitant with that recorded by Filidei et al. (2003).

The affiliated aim in the current laboratory-scale study was to assess the effect of OMSW, FOMSW, and ROMSW on some physiological parameters to determine the most appropriate waste to increase soil fertility.

In the present study, the application of olive-mill waste aqueous extract to the germinating *Vicia faba* L. seeds exhibited a severe reduction to PL and RL lengths with increasing the extract concentrations, which may be credited to the continuation of the phenolic compounds. It is known that OMSW suppresses seeds germination of different plant species (Niaounakis & Hakvadakis, 2004). Massoudinejad et al. (2014) reported that the phytotoxic effects of the phenolic compounds contained within the OMSW had a bad impact on germination. Some plant extracts or residues may inhibit germination, emergence and subsequent growth of other plants by exuding toxic substances. These substances are called allelochemicals (Madany & Khalil, 2017; Hassanein et al., 2019). When these allelochemicals are taken up by germinating seeds of the same or of other plant species, there may be some degrees of germination and emergence inhibition or growth injury (Hassanpouraghdam et al., 2010). Bora et al. (1999) found that the elongation of radicle and epicotyl was reduced in all treatments of *Acacia auriculiformis* extract proportional to the concentration levels, they suggested that the inhibitory effect was related to the presence of allelochemicals including tannins, wax, flavonoids and phenolic acids.

The use of OMSW has been seen to have a detrimental effect on faba bean growth. Increasing of OMSW concentrations (2, 4, 8, and 16%) resulted in a marked reduction of length, fresh and dry weight of shoots and roots. This was accompanied by a notable decline of chlorophylls content, chlorophyll stability index, the efficiency of PSII (Fv/Fm), total available carbohydrates, total proteins, and the macro-elements K, P, Mg and Ca contents. These observations could be related to the phytotoxic effects of absorbed phenolic compounds and the inhibitory effect of accumulation of Cu and Zn in faba bean shoots and roots. In agreement with these observations, Ouzounidou et al. (2008) reported that application of olive mill waste for tomato cultivation caused a significant limitation of absorption and translocation of K, Na, Fe, Ca, and Mg, which caused deficiencies of those elements. Asfi et al. (2012) assessed that OMSW stress influences photosynthetic pigments and damages the photosynthetic apparatus. Additionally, Rocchetta & Kupper (2009) reported that Cu and Zn stress resulted in the generation of reactive oxygen species (ROS) causing oxidative stress and inhibition of the photosynthetic machinery and, also, may have a phytotoxic effect limiting crop yield and quality. Under prevailing experimental conditions, total available carbohydrates and protein contents in the shoots and roots of OMSW-treated faba bean plants were significantly decreased with increasing the crude waste concentrations (Fig. 3), indicating the suppression of plant growth.

Ouzounidou et al. (2010) concluded that the inhibitory effects of olive mill waste on plant growth might be related to its high mineral salt content, low pH and the occurrence of phytotoxic compounds, particularly polyphenols. Similarly, the current study showed that OMSW was characterized by high mineral salts, low pH and high phenolics content (Rahmanian et al., 2014). Thus, the decrease in the growth of faba bean under prevailing experimental conditions might be attributed to the decrease in soil acidity and the increase of the phytotoxic effect of phenolics on plasma membrane integrity, as well as a disturbance in metabolic processes (Zhang et al., 2010).

Application of the ROMSW and FOMSW to the soil, improved the growth, chlorophyll contents, CSI, chlorophyll a fluorescence (Fv/Fm), TAC, TP and macronutrient concentrations. This improvement was increased with increasing concentrations of ROMSW, while it increased up to 4% of FOMSW then gradually declined. These observations might be due to the increase in K, P, Mg and Ca contents and the decrease in Cu and Zn concentrations resulting in increasing the biosynthesis of photosynthetic pigments (Fig. 2) and hence improving the photosynthetic machinery, as well as shifting off the oxidative stress caused by Cu and Zn. Efthimiadou et al. (2010) appraised that nutrients like P, K, and Mg improve the root growth, increase the intake of water which helps in stomatal regulation, also the photosynthetic rate and stomatal conductance of the corn plants. Under the prevailing experimental conditions, there was a marked increase of carotenoids and total phenolics contents with increasing the waste level in the soil. These findings could be attributed to the removal of generated ROS under waste treatment. Kalaji et al. (2012) confirmed that carotenoids are essential for the photoprotection of the photosynthetic apparatus.

It is shown that washing and *A. tamarii*-treatment waste resulted in an increase in the pH from 5.1 to 6.8 and 7, respectively, and decrease total phenolics from 8.46g kg^{-1} to 2.5g kg^{-1} and 0.0g kg^{-1} waste, respectively. Consequently, the improvement of soil characteristics, due to washing or *A. tamarii*-treatment of waste, by increasing pH could be result in protecting the plasma membrane integrity and, hence, the growth. It is clearly demonstrated that treatment with FOMSW resulted in complete degradation of phenolics and raising the pH. Accordingly, the application of FOMSW to the soil might improve the soil characteristics, in which raising pH and decreasing external phenolics. Kistner et al. (2004) reported that the addition of bio-OMWW resulted in significantly higher shoot length and the number of leaves, fresh weight of leaves stems and roots. Moreover, Ouzounidou et al. (2012) findings were that the bio-treated OMW alleviated the significant decrease in photosynthetic activity observed in raw OMW applications. Moreover, Salman et al. (2014) pointed out that bioremediation may produce valuable products including excellent fertilizer. Additionally, Anwar et al. (2015) and El-Darier et al. (2015) recorded that bio-degradable OMSW can successfully be co-composted with different types of organic wastes to get a higher quality composted product. Recently, Regni et al. (2017) findings were that the recycling of the OMSW as a soil amendment is a win-win strategy to transform a potential environmental threat associated with its disposal into an important resource and to improve the soil quality.

Although, there was a marked increase of faba bean growth in *A. tamarii*-fermented waste, the growth decreased in high concentrations and this was accompanied by a significant increase of phenolics content in faba bean shoots. These findings could be related to the presence of some fungal metabolite (s) produced during the fermentation acting as toxic substances. Whereas, the increase of accumulated phenolics could be related to removing these toxic responsibilities as phenolic compounds are considered as a class of antioxidant agents and their antioxidative properties is due to their ability to chelate transition metal ion, the inhibition of superoxide-driven Fenton reaction (Luo et al., 2004).

Conclusion

The toxicity of phenolics and acidity of OMSW-contaminated soil might be considered important parameters as most polluting agents for inhibition of faba bean growth. According to this study OMSW detoxification (water or microbiologically) might result in a marked reduction of phenolics and rising in pH which allows safe and economical disposal of OMSW. Using *A. tamarii* in the current study, as a bio-degradable for OMSW resulted in the complete absence of the phytotoxic action of phenols leading to positive effects of detoxified OMSW, may be attributed to its effect on the increase in macronutrients K, P, Mg and Ca availability at the soil that act as bio-organic fertilizers that improve the plant growth.

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استخدام المخلف الصلب لعصر الزيتون كسماد لنبات الفول بعد ازالة سميته بالمعالجة بالفطريات

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يعتبر المخلف الصلب لعصر الزيتون أحد أهم الملوثات البيئية لاحتواءه علي العديد من المركبات السامة والتي تؤثر سلبا علي إمكانية استخدامه كسماد عضوي للتربة. لذا هدفت الدراسة الحالية إلى التخلص الحيوي من أكبر ملوثاته وهى المواد الفينولية وذلك باستخدام أحد الفطريات المعزولة محليا تحت الظروف المزرعية المثلي لنمو الفطر. ودراسة تأثير مخلف العصر الخام والمخلف بعد عملية التخمر وكذلك بعد الاستخلاص بالماء بالاضافة لتأثير الماء المستخدم في عملية الاستخلاص علي الانبات وبعض علامات النمو والدلالات الفسيولوجية لنبات الفول.أظهرت الدراسة أن فطر الأسبرجيللس تماري كان الأكفأ من ضمن العشر فطريات المختبرة في التخلص الحيوي كلية من المركبات الفينولية. أظهرت النتائج أن إضافة المخلفات الصلبة بعد الاستخلاص المائي والمتخمر بفطر الأسبرجيللس تماري إلى التربة أدت إلى تحسن ملحوظ لعلامات النمو ومحتوى الكلوروفيل ومؤشر الاستقرار الكلوروفيلي وكفاءة الطيف الوميضي للنظام الضوئى الثانى (Fv/Fm) والمحتوى الكلي للكربوهيدرات والبروتين ومحتوى بعض المغذيات الكبرى مثل البوتاسيوم والفسفور والكالسيوم ،هذا التحسن ازداد بزيادة تركيزات المخلف المعالج بالماء بينما ازداد حتى تركيز4 % مع المخلف المعالج بالتخمر الفطري. على عكس المخلف الخام الذى كان له الأثر السلبى على علامات النمو والصفات الفسيولوجية للنبات قيد الدراسة فقد نتج عن استخدام المخلفات الصلبة بعد الاستخلاص المائي وتلك المتخمرة بفطر الأسبرجيللس تماري انخفاض ملحوظ فى محتوى الفينول وارتفاع فى الرقم الهيدروجينى والتى من شأنها تحسين خصائص التربة. أكدت الدراسة أن المخلف الصلب لعملية عصر الزيتون عند معالجته (بالماء أو الفطر) يتم نزع سميته نتيجة الاختزال الواضح للمحتوى الفينولي وميل الأس الهيدروجيني للتعادل، مما يسمح بالتخلص الاقتصادي الآمن من المخلف بعد تلاشي مسبب السمية ولهذا يمكن بصورة مقبولة استخدامه كسماد عضوي حيوي لتحسين خواص التربة وبالتالي نمو النبات.