# Study of the phytotoxic potential of olive mill wastewaters on a leguminous plant '*Vicia faba* L.'

Jawaher Sdiri Ghidaoui, Lobna Bargougui, Mohamed Chaieb and Ali Mekki

## ABSTRACT

We investigated the study of the phytotoxic potential of olive mill wastewaters (OMW) on the germination and growth responses of the faba bean '*Vicia faba* L.' leguminous plant cultivated under an arid Mediterranean climate. The results showed that the raw OMW blocked the germination of the seeds tested, while OMW treated soil extracts stimulate the seeds' germination rate. The monitoring of the faba bean growth parameters in the different OMW treated soils showed that the optimal growth of the faba bean plants has been recorded for the soil amended with 25 $m^3$ $ha^{-1}$ of OMW. Although the 50 $m^3$ $ha^{-1}$ dose is the most suitable for the soil studied, our results showed that the 25 $m^3$ $ha^{-1}$ dose is the most suitable for the vegetative development, as well as for the productivity of the plant tested '*Vicia faba L.*'.

**Key words** | germination, growth, leguminous plant, soil amendments

**Jawaher Sdiri Ghidaoui**
**Lobna Bargougui**
**Ali Mekki** (corresponding author)
Laboratory of Sustainability of Olive Growing and Arboriculture in Semi-arid and Arid Regions,
Olive Tree Institute,
Sfax,
Tunisia
E-mail: *a_mekki_cbs@yahoo.fr*

**Lobna Bargougui**
**Mohamed Chaieb**
Laboratory of Plant Biodiversity and Dynamics of Ecosystems in Arid Environment,
Faculty of Sciences of Sfax,
Tunisia

**Ali Mekki**
Laboratory of Environmental Bioprocesses,
Center of Biotechnology of Sfax,
AUF (PER-LBP), BP: 1177,
3018 Sfax,
Tunisia

## INTRODUCTION

The world's olive groves cover nearly 11 million hectares with more than 900 million olive trees, of which almost 95% are in the Mediterranean basin (IOC 2017). Olive growing plays a major role in the economies of the Mediterranean countries, particularly in Spain, Italy, Greece, Tunisia and Turkey (Bargougui *et al.* 2018). Tunisia has become the excellent Northern African olive grove with olive farms of over than 1.8 million hectares and nearly 88 million olive trees, corresponding to 79% of the total arboreal area (Meftah *et al.* 2018). Nevertheless, olive oil extraction industry produces a large amount of olive by-products such as olive mill wastewaters (OMW) (Magdich *et al.* 2016). Similarly, the production of a high amount of OMW in a short period, its pollution load with high chemical oxygen demand (COD between 50 and 150 g $L^{-1}$) and its phytotoxic properties constitute serious problems for OMW disposal (Mahmoud *et al.* 2010; Mekki *et al.* 2012). The OMW phytotoxic and antimicrobial properties have been extensively investigated and are associated with the presence of phenolic compounds and free fatty acids (Obied *et al.* 2005; Saadi *et al.* 2007; Mekki *et al.* 2013). Several investigators have reported on the inhibition of plant and microbial growth by low-molecular-weight phenols present in olive mill wastes (Fiorentino *et al.* 2003; Allouche *et al.* 2004; Isidori *et al.* 2005; Mekki *et al.* 2007).

Despite their polluting nature, OMW have a great potential as fertilizer and soil improver given their high water content (86–95%) and their high levels of organic matter (OM), essentially potassium mineral elements (Mekki *et al.* 2013; Magdich *et al.* 2015; Chatzistathis & Koutsos 2017). Thus, the use of OMW as an organic soil amendment constitutes a promising alternative for improving soil fertility and textural and structural balance (Yaakoubi *et al.* 2009; Abichou *et al.* 2014; Gargouri *et al.* 2014; Chatzistathis & Koutsos 2017; Caruso *et al.* 2018).

The main objective of this study is the ecological valorization as soil and crops biofertilizer of OMW. Specifically, we are interested in the study of the following

(i) If OMW exerts phytotoxic effects on the seeds and what concentrations affect the plant.
(ii) The effects of OMW on the functioning of rhizobial symbiosis in the rhizosphere.

doi: 10.2166/wst.2019.373

(iii) The level of tolerance of the *Vicia faba* L. species to OMW high concentrations.

## MATERIALS AND METHODS

### Olive mill wastewaters sampling and characterization

OMW used in this work comes from a continuous extraction system located in the Gremda region (Sfax, Tunisia). The pH and the EC have been measured using the method of Sierra *et al.* (2007). Suspended matter (SM) has been determined by the filtration technique. Dry matter (DM) has been evaluated by drying at 105 °C. OM and mineral matter (MM) have been expected after calcinations at 550 °C for 4 h. Kjeldahl total nitrogen (TN) has been assessed using a standard method (Kandeler 1995). The chemical oxygen demand (COD) has been determined according to Knechtel (1978). Five-day biochemical oxygen demand ($BOD_5$) has been estimated according to Aloui *et al.* (2007). The mineral elements (K, Ca and Na) have been carried out by flame photometer referred to Mathieu & Pieltain (2003). The OMW residual oil content (ROC) has been determined by using the method of Ayadi *et al.* (2001). The total phenolic compounds content has been determined according to a standard method (Box 1983). The main physico-chemical and microbiological parameters of these effluents are summarized in Table 1.

**Table 1** | Olive mill wastewaters characteristics (average values of three replications ± SD)

| Parameters | Values ± SD |
|---|---|
| pH (25 °C) | 3.8 ± 0.02 |
| EC (mS $cm^{-1}$) | 9.39 ± 0.05 |
| Water content (%) | 94.8 ± 1 |
| Suspended matter (g $L^{-1}$) | 21.35 ± 1 |
| Dry matter (g $L^{-1}$) | 52 ± 1 |
| Organic matter (g $L^{-1}$) | 30.8 ± 1 |
| Mineral matter (g $L^{-1}$) | 21.2 ± 0.5 |
| TOC (g $L^{-1}$) | 17.9 ± 0.5 |
| COD (g $L^{-1}$) | 74 ± 1 |
| $BOD_5$ (g $L^{-1}$) | 21 ± 0.5 |
| $BOD_5$/COD | 0.28 ± 0.01 |
| Nitrogen (g $L^{-1}$) | 0.48 ± 0.02 |
| Carbon/Nitrogen | 37.29 ± 0.5 |
| K (g $L^{-1}$) | 7.1 ± 0.1 |
| P (g $L^{-1}$) | 0.54 ± 0.01 |
| Na (g $L^{-1}$) | 1.22 ± 0.02 |
| Cl (g $L^{-1}$) | 0.78 ± 0.02 |
| Ca (g $L^{-1}$) | 0.72 ± 0.02 |
| Mg (g $L^{-1}$) | 0.63 ± 0.02 |
| ROC (%) | 1.21 ± 0.05 |
| Phenolic compounds (g $L^{-1}$) | 1.94 ± 0.1 |
| Total microflora ($10^3$ CFU. $ml^{-1}$) | 245 ± 5 |
| Fungi ($10^2$ CFU. $ml^{-1}$) | 112 ± 2 |

SD: Standard deviation ($P \leq 0.05$).

### Soil origin, sampling and characterization

The soil has been collected in February 2018, from a non-cultivated area of the experimental station 'Taous' of the Olive Tree Institute of Sfax (Southern Tunisia). Soil texture has been determined using the pipette method (Thomas *et al.* 2012). Soil water retention capacity (SWRC) has been evaluated gravimetrically by saturating the soil overnight. The pH and EC have been measured using a pH meter and an EC meter, respectively. Dry matters (DM), organic matters (OM) and minerals matters (MM) have been determined according to Sierra *et al.* (2007). The various soils nitrogen forms as total nitrogen (TN), ammoniacal nitrogen ($NH_4$-N), and nitrate nitrogen ($NO_3$-N) have been expected according to the Kjeldahl method (Kandeler 1995).

### Faba bean *Vicia faba* L. seeds origin

The faba bean *Vicia faba* L. seeds have been obtained in the form of marketed seeds (agronomical information: 1,000 seeds weight (1.4 kg); green grain at the harvest stage) and treated with preservatives (in water at 20 °C, to soften and weaken the skin, allowing germination easier and faster) for use in germination tests and sowing in soils treated by OMW and in control soil.

### Experimental set-up

The soil collected from an uncultivated plot of the experimental station 'Taous' has been well homogenized and then distributed in plastic pots. The pots used have a diameter of 30 cm, a depth of 30 cm and a volume capacity of the order of 25 kg of soil. Each pot has been seeded with five faba bean seeds. After germination, only three seedlings have been taken per pot. The experiment has been carried out in five repetitions for each OMW dose (the OMW characteristics as N, P and K contents are summarized in Table 1) and in comparison with a control pot irrigated with tap water (i.e. 15 seedlings per batch of pots). Plant irrigation has been done manually using a watering can every week at 25% of the SWRC (a water quantity equal to 25% of SWRC of the 25 kg of soil per pot).

### Olive mill wastewaters application

Four OMW doses have been tested, respectively; 25, 50, 75 and 100 m³ $ha^{-1}$. The OMWs amounts are calculated on the basis of the pots surface and not on the pots volume since we talked about the doses in m³ $ha^{-1}$. The OMWs added quantities corresponds 175 mL $pot^{-1}$, 350 mL $pot^{-1}$, 525 mL $pot^{-1}$ and 700 mL $pot^{-1}$, respectively, for the doses mentioned above. The OMW has been sufficiently homogenized and then added to the soil already prepared in the pots. The OMW addition has been carried out on February 26, 2018. The study has been conducted in a plastic greenhouse at the Olive Tree Institute (Sfax, Tunisia) and ambient conditions.

### Phytotoxicity evaluation

The phytotoxicity of OMW has been determined by measuring the germination index (GI %) of faba bean seeds in raw OMW and treated soils extracts in comparison with distilled water as control medium (Zucconi *et al.* 1981).

### Study of the olive mill wastewaters effects on plants growth

In order to follow the effects of the different doses of OMW on the growth of the faba bean plants, three plants have been kept in each pot (i.e. 15 plants per dose of OMW and row of pots). The monitoring of the different morphological and biochemical parameters has been realized from the germination of the seeds until the cultivation of the plants over about 150 days.

### Morphological parameters monitoring

The effects of OMW on some morphological parameters of the faba bean plants (as the plants heights, the stems thicknesses, the leaves number and areas and the flowers number) have been determined for all plants cultivated and throughout the growing period.

The height of the plants has been followed by measuring the length of the stems for the different plants. The measurement has been made twice a week during the study period. The circumference of the plant stems has been measured twice a week during the study period. Counting of the number of leaves has been carried out twice a week in the same time with the parameters relating to the growth of the stems. The leaf area measurement has been determined according the method established by Tattini *et al.* (1995). The counting of the flowers has been done during the flowering period and this for all plants. The pods numbers and lengths have been determined for each plant as they appeared.

### Biochemical parameters monitoring

The effects of OMW on some biochemical's parameters of the faba bean plants (as the pods number and length, the seeds number per pods, the seeds weights, the root part/shoot part (R/S) ratio, the chlorophyll pigments content, the soluble sugars and starch content and the proteins content) have been assessed for all plants and in relation with different OMW doses applied.

Leaves sampling has been done after 90 days of germination. Foliar analysis (fresh weight, dry weight, water content and mineral content) has been performed for the different plants. As soon as the plants are mature, two pods from each plant have been taken (i.e. 10 pods per row and per dose of OMW). The fresh weight of the pods, the number of seeds and the fresh weight of the seeds have been determined.

At the stage of maturity of the faba bean plants (90 days after germination), five plants from each row of pots have been sampled. The plants have been rinsed with tap water, the aerial and root parts have been separated and the following parameters have been determined: (i) the root part/aerial part ratio; (ii) the DM yield and (iii) the fresh weights (FW).

The faba bean plants productivities have been determined by weighing pods and mature seeds after harvesting. For chlorophyll pigments, chlorophyll *a* (chl *a*), *b* (chl *b*) and carotenoid (carrot) have been evaluated according to Hartmut & Lichtenthaler (1987) method. Soluble sugars and starch contents of leaves and seeds have been assessed by the method of McCready *et al.* (1995). The organic nitrogen and protein contents in the aerial parts, as well as the root parts, have been determined according to Bargougui *et al.* (2018).

### Statistical analyses

All the parameters studied have been carried out using SPSS software (Statistical Package for the Social Sciences, version 20).

Results have been expressed in mean standard deviation, using analysis of variance (ANOVA) ($p = 0.05$). The different letters (a, b, c, d and e) for the same parameter indicate a significant difference between the different treatments. Homogeneous averages (no significant difference) represent the same subgroup (with the same letter of significance).

## RESULTS AND DISCUSSION

### Olive mill wastewaters characterization

The raw OMW used in this work comes from a three phase's continuous extraction system. The main physico-chemical and microbiological parameters of these effluents are summarized in Table 1. As shown in this table, OMW have an acidic pH (pH = 3.8), mainly due to their richness in organic acids (Chaari *et al.* 2015). In addition, OMW show a high value of electrical conductivity (EC = 9.4 mS $cm^{-1}$). This parameter reflects the significant concentration of salts in these effluents (Mekki *et al.* 2013; Magdich *et al.* 2015). The OMW studied are rich in organic compounds, which can be explained by their high COD (74 g $L^{-1}$) and $BOD_5$ (21 g $L^{-1}$). Alternatively, these effluents illustrate a high level of potassium (7.1 g $L^{-1}$) and relatively high concentrations of sodium, calcium and phosphorus. Indeed, the content of MM varies according to the conditions of conservation of the olives, the extraction system, the degree of ripening, as well as the variety of olives (Proietti *et al.* 2015).

The contents of DM and OM are of the order of 52 g $L^{-1}$ and 30.8 g $L^{-1}$, respectively. Such results are in agreement with studies done on Tunisian OMW (Ben Rouina *et al.* 2014). Besides, the OMW contains large amounts of SM (21.35 g $L^{-1}$) and high levels of phenolic compounds, which give them antimicrobial potency (Magdich *et al.* 2012). The rate of residual oils content (ROC = 1.21%) in the OMW studied is relatively elevated (Ayadi *et al.* 2001).

Microbiological analyzes show that only a few microorganisms are able to grow in OMW (Table 1). They are essentially fungi (yeasts and molds), which, thanks to their acidophilic and halophilic characteristics, can adapt to the high acidity and salinity of OMW (Mekki *et al.* 2006).

### Characterization of the control soil

Table 2 summarizes all the granulometric and physicochemical characteristics of the control soil studied. The analysis of the results shows that the control soil is sandy (sand = 86.2%), with an alkaline pH (8.85). The OM content is very low (OM = 0.92% DM), which confirms the previous works proving that Tunisian soils are very poor in OM (Mekki *et al.* 2018). The electrical conductivity (EC) is of the order of 316 µS $cm^{-1}$, which means a deficiency of mineral elements. The TN content is 0.22 g $kg^{-1}$, so the soil analyzed can be considered as very poor in nitrogen content. Then again, the soil studied shows a weak cation exchange capacity (CEC = 3.25 meq 100 $g^{-1}$ DM) which can be explained by the low soil humus content (Magdich *et al.* 2012).

**Table 2** | Soil control granulometric and physicochemical characteristics (average values of three replications ± SD)

| Soil characteristics | $S_C$ ± SD |
|---|---|
| Sand (%) | 86.2 ± 1 |
| Clay (%) | 11.6 ± 0.5 |
| Silt (%) | 2.2 ± 0.1 |
| pH (25 °C) | 8.85 ± 0.2 |
| EC (µS $cm^{-1}$) | 316 ± 2 |
| SWRC (%) | 30.28 ± 0.5 |
| CEC (meq% DM) | 3.25 ± 0.2 |
| OM (g $Kg^{-1}$ DM) | 3.2 ± 0.05 |
| TOC (g $Kg^{-1}$ DM) | 1.8 ± 0.02 |
| TN (g $Kg^{-1}$ DM) | 0.22 ± 0.01 |
| Organic Nitrogen | 0.18 ± 0.01 |
| Mineral Nitrogen | 0.03 ± 0.001 |
| C/N ratio | 8.18 ± 2 |
| P (g $Kg^{-1}$ DM) | 0.038 ± 0.01 |
| K (g $Kg^{-1}$ DM) | 0.69 ± 0.05 |
| Na (g $Kg^{-1}$ DM) | 0.36 ± 0.02 |
| Ca (g $Kg^{-1}$ DM) | 0.19 ± 0.02 |

SD: Standard deviation ($P \leq 0.05$).

### The faba bean: criteria of choice and agronomic characteristics

The bean is the best rotation crop for wheat. It breaks the evolutionary cycles of cereal pest fungi (foot rot, Fusarium, etc.) and improves the soil nitrogen content with an average intake of 30 kg $ha^{-1}$ (Jensen *et al.* 2010). Like all legumes, the bean seed is rich in protein, with average values between 20% and 40% of the total DM (Cepona *et al.* 2010). In the Mediterranean basin, the bean cultivation accounts for almost 25% of the total cultivated area and the world production of beans (FAO 2015). In Tunisia, beans and faba beans are the most widely grown legume species, with areas of up to 38% of the cultivated farmland.

## Effects of olive mill wastewaters on the faba bean germination

In order to study the OMW phytotoxic potential, faba bean seeds germination has been determined in raw OMW, in OMW treated soils extracts, in comparison with a control medium (pure water), as well as the pure water irrigated soil extract (Figure 1).

As shown in the Figure 1, raw OMW totally blocks the faba bean seeds' germination. This confirms previous studies postulating the OMW phytotoxicity (Ben Rouina *et al.* 2014; Magdich *et al.* 2015). The addition of OMW to soils considerably reduces their phytotoxic potential, which explains the values of the GIs (%) summarized in the same figure (Figure 1). Compared with pure water irrigated soil extract (Spw), we notice that the GI (%) increases to reach its maximum for the OMW treated soil extract at the dose of 25 $m^3\ ha^{-1}$ ($S_{25}$) with a GI about 129%, and then it decreases with the increase of the OMW doses as 50, 75 and 100 $m^3\ ha^{-1}$. This decrease of the GI is especially perceptible for the $S_{75}$ and $S_{100}$ extracts and can be deciphered by the high salinity and phenolic compounds content at such applied doses (Mekki *et al.* 2007; Saadi *et al.* 2007).

## Effects of olive mill wastewaters on the morphological parameters of the faba bean plants

Results showed that plants cultivated in OMW treated soils demonstrate an increase in shoot elongation, with a maximum extension of 62.9 cm observed for $S_{50}$ soil after 80 days of germination (Figure 2).

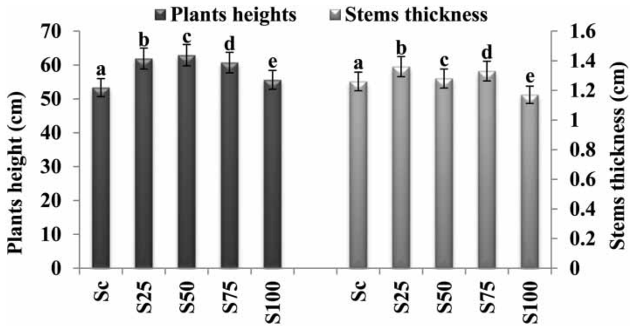


**Figure 2** | Evolution of the plants heights and the stems thicknesses in olive mill wastewaters irrigated soils extracts in comparison with soil control (Sc) extract after 80 incubation days.

Monitoring the evolution of stems' thicknesses, results showed that the plants that grow in the soil $S_{25}$ have the thickest stems, followed by those cultivated in the control soil (Sc) and in the $S_{50}$. The plants grown in soil treated by 100 $m^3\ ha^{-1}$ ($S_{100}$) showed the lowest elongation and thickening values compared to others grown in $S_{25}$, $S_{50}$, $S_{75}$ and in the control soil (Figure 2).

Concerning the effects on leaves growth (number and circumferences), the application of OMW does not lead to any significant increase in leaves number for the plants grown in amended soils, compared with those grown in control soil (Figure 3). In fact, leaves number for plants of the $S_{25}$, $S_{50}$ and $S_{75}$ remains practically close to that of control plants, whereas plants cultivated in $S_{100}$ showed the least leaves number. Conversely, the evolution of leaves areas showed a clear increase for plants grown in $S_{25}$ and $S_{50}$ and illustrate a gradual decrease for plants cultivated at higher OMW doses ($S_{75}$ and $S_{100}$) in comparison with control plants (Sc) (Figure 3). Likewise, the flowers number has

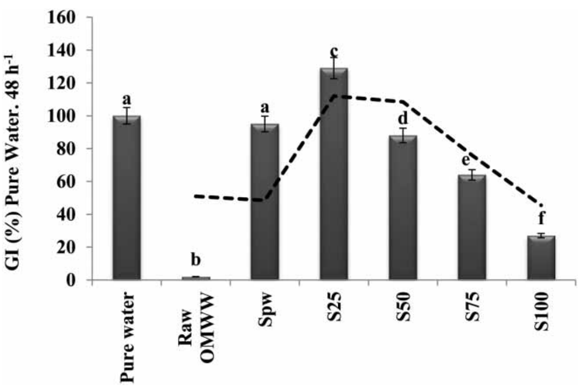


**Figure 1** | *Vicia faba* L. seeds germination indexes (GI (%)) in raw olive mill wastewaters (OMW) and OMW irrigated soils extracts in comparison with soil pure water extract (Spw) and pure water.

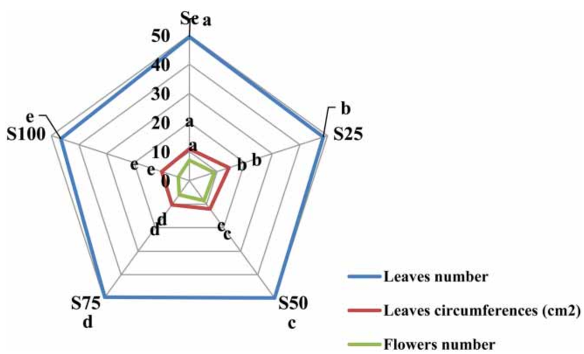


**Figure 3** | Evolution of the leaves number, leaves circumferences and the flowers number in olive mill wastewaters irrigated soils extracts in comparison with soil control (Sc) extract after 80 incubation days.

been influenced by the OMW added quantities. In fact, Figure 3 shows that the maximum number of flowers is recorded in plants grown in $S_{25}$ and $S_{50}$ soils compared to control soil and $S_{75}$ and $S_{100}$. As for the other morphological parameters, the plants grown in the $S_{100}$ showed the lowest flowers number value, which confirms the toxic effect of the 100 $m^3$ $ha^{-1}$ dose for the faba bean plants (Fiorentino *et al.* 2003; El Hajjouji *et al.* 2007).

## Effects of olive mill wastewaters on the faba bean biochemical parameters and productivities

The effects of OMW on the pods production and quality are shown in Figure 4. The $S_{25}$ dose gives the highest yield in terms of pods number/per plant, followed by that of the control soil. These findings are confirmed by the analysis of pods elongation which clearly showed that plants grown in soil $S_{25}$ have the best yields compared to plants grown in control soil Sc and in soils $S_{50}$, $S_{75}$ and $S_{100}$ (Figure 4). The seed quality is closely related to the physiological state of the plant. It also depends on the pod condition. The analysis of seeds number/pod, seeds FW and seeds dry weights (DW) of the different plants clearly shows that plants grown in $S_{25}$ soil have the best seeds quality compared to plants grown in others amended soils and control plants (Figure 4). However, plants grown on $S_{50}$ and $S_{75}$ showed seeds with similar qualities, while plants grown on soil $S_{100}$ have the lowest yields in terms of FW and DW. These results confirm the phytotoxicity exerted by the 100 $m^3$ $ha^{-1}$ dose on the faba bean plants (Hachicha *et al.* 2006; Abid *et al.* 2007).

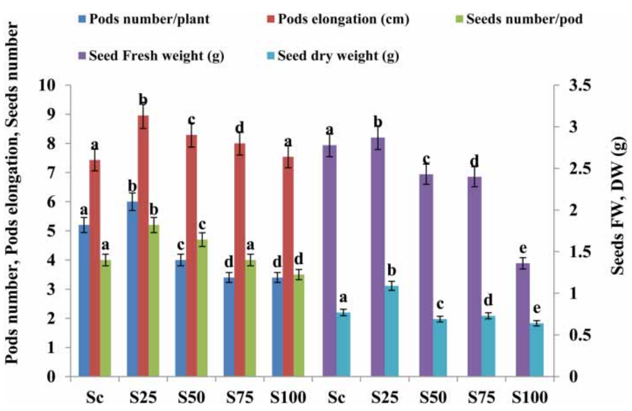


**Figure 4** | Evolution of the faba bean plants biochemical's parameters (pods number, pods elongation, seeds number and seeds weights) in olive mill wastewaters irrigated soils extracts in comparison with soil control extract (Sc) after 120 incubation days.

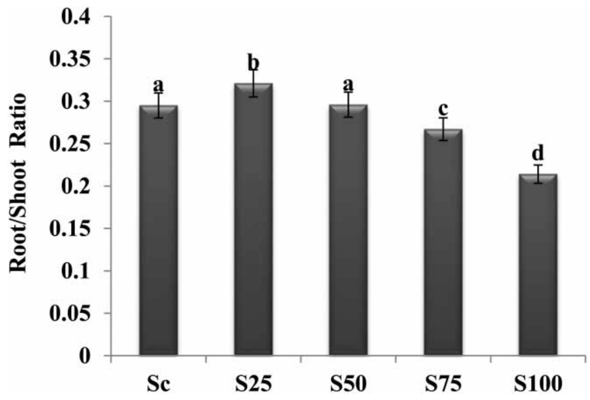


**Figure 5** | Evolution of the faba bean plants shoot/root ratios in olive mill wastewaters irrigated soils extracts in comparison with soil control extract (Sc) after 120 incubation days.

The root part/shoot part ratio (R/S) reflects the ability of the plant to develop its rhizosphere in relation to its aerial part. This development is closely dependent on the environment surrounding the root system and the availability of nutrients (Ipsilantis *et al.* 2009; Mekki *et al.* 2018). Figure 5 illustrates that plants grown in soil $S_{25}$ have the highest R/S ratio. Plants growing in $S_{50}$ and $S_{75}$ soils show R/S ratios comparable to those of control plants while the lowest ratios are recorded in plants grown in the $S_{100}$. Thus, the large amount of phenolic OM and the high salt content resulting from the 100 $m^3$ $ha^{-1}$ dose inhibit the root development of the faba bean plants grown in $S_{100}$ soil (Figure 5) (Assaf *et al.* 2009).

Chlorophyll is the main assimilating pigment of photosynthetic plants. This pigment, located in the chloroplasts of plant cells, intervenes in photosynthesis to intercept light energy, the first step in the conversion of this energy into chemical energy (Ipsilantis *et al.* 2009). Figure 6

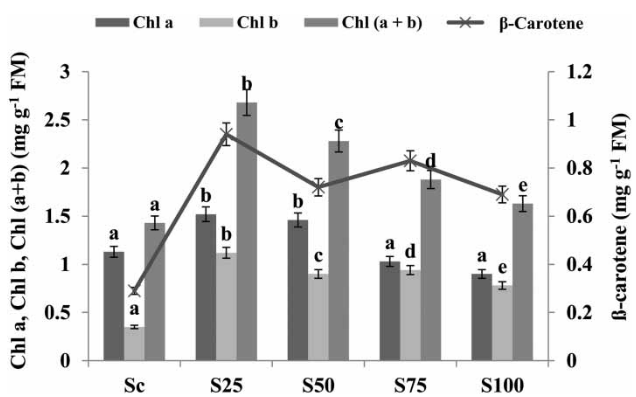


**Figure 6** | Evolution of the faba bean plants chlorophyll pigments (chl *a*, chl *b*, chl ($a+b$), *β*-carotenoid) in olive mill wastewaters irrigated soils extracts in comparison with soil control (Sc) extract after 150 incubation days.

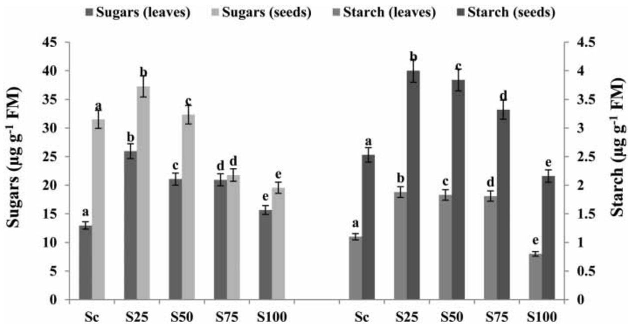


**Figure 7** | Evolution of the faba bean plants carbohydrates contents (sugars, starch) in olive mill wastewaters irrigated soils extracts in comparison with soil control (Sc) extract after 150 incubation days.

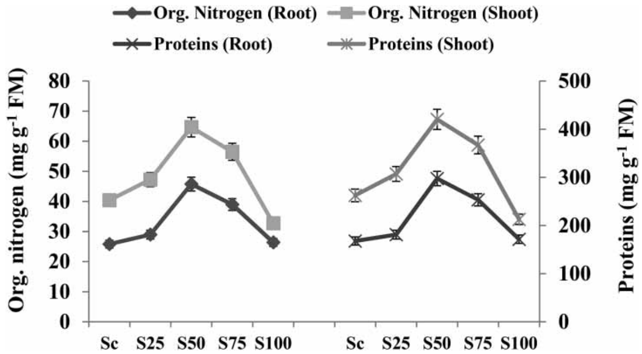


**Figure 8** | Evolution of the faba bean plants organics matters (organic nitrogen, proteins) in in olive mill wastewaters irrigated soils extracts in comparison with soil control (Sc) extract after 150 incubation days.

illustrates the impact of the OMW addition on the content of bean leaves in these photosynthetic pigments. The obtained results showed that the levels of these different pigments (chl $a$, chl $b$, chl $(a+b)$, $\beta$-carotenoid) increase proportionally with the increase of OMW dose up to the dose 50 $m^3$ $ha^{-1}$, then they decrease for the doses 75 and 100 $m^3$ $ha^{-1}$. Compared with control plants, optimal chlorophylls pigments amounts are recorded for $S_{25}$ and $S_{50}$ soils. In this context, Magdich *et al.* (2016) demonstrated the improvement in the content of chlorophyll pigments in olive leaves irrigated by OMW.

The organic plant material is constituted by proteins, lipids, phenolic monomers and polymers and mainly by polysaccharides (such as cellulose, hemicellulose, starch, etc.) (Kistner *et al.* 2004). The contents of the various bean leaves of reducing sugars and starch are summarized in Figure 7. As shown in the figure, plants grown in amended soils have significantly higher values for these mentioned compounds compared to those grown in control soil. Indeed, the wealth of OMW in organic compounds, essentially carbohydrates, enriched the soil in these compounds which has a positive effect on the composition of the plants' leaves. In the same context, the analysis of seeds' content in these organic compounds shows a concordance of the contents of sugars and starch with those of the leaves, as well as in relation to the OMW doses applied (Figure 7). Indeed, the dose 25 $m^3$ $ha^{-1}$ showed the elevated content in these compounds of the plants studied.

The control soil is initially very poor in nitrogen content. However, the organic nitrogen content increases with the OMW doses applied, whose maximum values are recorded for the soil amended with 50 $m^3$ $ha^{-1}$ with 65 mg $g^{-1}$ for the aerial part and 45 mg $g^{-1}$ for the root part (Figure 8). For $S_{75}$ and $S_{100}$ soils, the decrease in this content can be deciphered by the excess of nutrients exceeding the threshold tolerable by plants (Zenjari & Nejmeddine 2001). In addition, knowing that the organic nitrogen (Org.N) reveals the protein content ([proteins] (mg $g^{-1}$) = 6.5 × [Org.N]), our results clearly show the improvement in the protein content of the plants grown in the $S_{25}$ and $S_{50}$ soils. While for plants grown in $S_{75}$ and $S_{100}$ soils, we recorded a remarkable decrease in protein content (data not shown). In this context, Zenjari & Nejmeddine (2001) have shown that OMW bring a surplus of nitrogen to the soil which stimulates the nitrogen assimilation of plants. Besides, El Hassani *et al.* (2009) reported that protein levels in OMW treated spearmint plants are higher than in untreated plants.

## CONCLUSION

The results obtained in this research work showed that the application of raw OMW totally blocked the germination of the faba bean seeds. However, treated soil extracts stimulate the germination rate compared to the control soil extract. The growth of the faba bean plants has been differently influenced by the OMW in relation to the quantities applied. Indeed, the optimal growth has been recorded for the plants grown in the soil amended with 25 $m^3$ $ha^{-1}$. Results demonstrate also that OMW addition stimulate the biological fixation of the atmospheric nitrogen by the faba bean plants roots in symbiosis with the rhizobia, which represents a key asset for the 'green enrichment' of the soil in plants assimilated nitrogen. Moreover, in addition to their benefits to the soil, OMW influences the protein content of this leguminous species.

## ACKNOWLEDGEMENTS

This work has been carried out in the Olive Tree Institute of Sfax, Tunisia. The facilities and services of the Direction of the Institute and the staff of the experimental station are gratefully acknowledged.

## REFERENCES

Abichou, M., Gargouri, K., Rhouma, A. & Khatteli, H. 2014 Long term effect of olive mill waste water spreading on microbial population and the natural floristic composition of a sandy soil. *Agriculture Science Engineering Technology Research* **1**, 16–25.

Abid, N., Chamkha, M., Godon, J. J. & Sayadi, S. 2007 Involvement of microbial populations during the composting of olive mill wastewater sludge. *Environmental Technology* **28**, 751–600.

Al-Assaf, S., Sakata, M., McKenna, C., Aoki, H. & Phillips, G. O. 2009 Molecular associations in acacia gums. *Structural Chemistry* **20**, 325–336.

Allouche, N., Fki, I. & Sayadi, S. 2004 Toward a high yield recovery of antioxidants and purified hydroxytyrosol from olive mill wastewaters. *Journal of Agriculture & Food Chemistry* **52**, 267–273.

Aloui, F., Abid, N., Roussos, S. & Sayadi, S. 2007 Decolorization of semisolid olive residues of 'alperujo' during the solid state fermentation by *Phanerochaete chrysosporium*, *Trametes versicolor*, *Pycnoporus cinnabarinus* and *Aspergillus niger*. *Biochemical Engineering Journal* **35**, 120–125.

Ayadi, M., Khlif, M., Rekik, B., Arous, N. & Rekik, H. 2001 Olive oil extraction: effect of different steps of centrifugal process paste on some olive oil qualitative characteristics. In: *International Congress Center Berlin, Germany*, September, 16–20.

Bargougui, L., Guergueb, Z., Chaieb, M., Braham, M. & Mekki, A. 2018 Agro-physiological and biochemical responses of Sorghum bicolor in soil amended by olive mill wastewater. *Agricultural Water Management* **212**, 60–67.

Ben Rouina, B., Bedbabis, S., Boukhris, M. & Ferrara, G. 2014 Effect of irrigation with treated wastewater on soil chemical properties and infiltration rate. *Journal of Environmental Management* **133**, 45–50.

Box, J. D. 1983 Investigation of the Folin–Ciocalteau phenol reagent for the determination of polyphenolic substances in natural waters. *Water Research* **17**, 511–522.

Caruso, C., Maucieri, C., Cavallaro, V., Borin, M. & Barbera, A. C. 2018 Olive mill wastewater spreading and AMF inoculation effects in a low-input semi-arid Mediterranean crop succession. *Archives of Agronomy and Soil Science* **64** (14), 2060–2074.

Cepona, K., Marget, P., Peyronnet, C., Carrouea, B., Arese, P. & Duc, G. 2010 Nutritional value of faba bean (*Vicia faba* L.) seeds for feed and food. *Crop Research* **115**, 329–339.

Chaari, L., Elloumi, N., Mmseddi, S., Gargougri, K., Ben rouina, B., Mechichi, T. & Kallel, M. 2015 Changes in soil macronutrients after a long-term application of olive mill wastewater. *Journal of Agriculture Chemistry and Environment* **4**, 1–13.

Chatzistathis, T. & Koutsos, T. 2017 Olive mill wastewater as a source of organic matter, water and nutrients for restoration of degraded soils and for crops managed with sustainable systems. *Agricultural Water Management* **190**, 55–64.

El Hajjouji, H., Fakhareddine, N., Baddi, G. A., Winterton, P., Bailly, J. R., Revel, J. C. & Hafidi, M. 2007 Treatment of olive mill wastewater by aerobic biodegradation: an analytical study using gel permeation chromatography, ultraviolet-visible and Fourier transform infrared spectroscopy. *Bioresource Technology* **98**, 3513–3520.

El Hassani, F. Z., Zinedine, A., Mdaghri Alaoui, S., Merzouki, M. & Benlemlih, M. 2009 Use of olive mill wastewater as an organic amendment for Mentha spicata L. *Industrial Crops Productions* **32**, 343–348.

FAO 2015 Food and Agriculture Organisation of the United Nations 2015 Tunisie: *Analyses de la filière oléicole (In French)*.

Fiorentino, A., Gentili, A., Isidori, M., Monaco, P., Nardelli, A., Parallela, A. & Temussi, F. 2003 Environmental effects caused by olive mill wastewater: toxicity comparison of low-molecular-weight phenol components. *Journal of Agriculture& Food Chemistry* **51**, 1005–1009.

Gargouri, K., Masmoudi, M. & Rhouma, A. 2014 Influence of olive mill wastewater (OMW) spread on carbon and nitrogen dynamics and biology of an arid sandy soil. *Communications Soil Sciences and Plant Analyses* **45**, 1–14.

Hachicha, S., Chtourou, M., Medhioub, K. & Ammar, E. 2006 Compost of poultry manure and olive mill wastes as an alternative fertilizer. *Agronomy for Sustainable Development* **26**, 135–142.

Hartmut, K. & Lichtenthaler, N. 1987 Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. *Methods in Enzymology* **148**, 350–382.

IOC 2017 International Olive Council, *Olivae.* N° 124.

Ipsilantis, I., Dimitrios, G., Karpouzas, H., Kalliope, K., Papadopoulou, R. & Ehaliotis, C. 2009 Effects of soil application of olive mill wastewaters on the structure and function of the community of arbuscular mycorrhizal fungi. *Soil Biology & Biochemistry* **41**, 2466–2476.

Isidori, M., Lavogna, M., Nardelli, A. & Parella, A. 2005 Model study on the effect of 15 phenolic olive mill wastewater constituents on seed germination and *Vibrio fischeri* metabolism. *Journal of Agricultural and Food Chemistry* **53**, 8414–8417.

Jensen, E., Steen, P., Mark, B. & Hauggaard, N. H. 2010 Faba bean in cropping systems. *Field Crops Research* **115** (3) 203–216.

Kandeler, E. 1995 Total nitrogen. In: *Methods in Soil Biology* (F. Shinner, R. Ohlinger, E. Kandeler & R. Margesin, eds). Springer, Berlin, Germany, pp. 406–408.

Kistner, T., Nitz, G. & Schnitzler, W. H. 2004 Phytotoxic effects of some compounds of olive mill wastewater (OMW). *Fresenius Environmental Bulletin* **13**, 1360–1361.

Knechtel, R. J. 1978 A more economical method for the determination of chemical oxygen demand. *Water Pollution Control* **116**, 25–29.

Magdich, S., Jarboui, R., Ben, B., Boukhris, M. & Ammar, E. 2012 Science of the total environment a yearly spraying of olive mill wastewater on agricultural soil over six successive years. *Science of the Total Environment* **430**, 209–216.

Magdich, S., Ben Ahmed, C., Boukhris, M., Ben Rouina, B. & Ammar, E. 2015 Olive mill wastewater spreading effects on productivity and oil quality of adult chemlali olive (*Olea europaea* L.) in the South of Tunisia. *International Journal of Agronomy and Agricultural Research* **6**, 56–67.

Magdich, S., Abid, W., Boukhris, M., Ben Rouina, B. & Ammar, E. 2016 Effects of long-term olive mill wastewater spreading on the physiological and biochemical responses of adult Chemlali olive trees (*Olea europaea L.*). *Ecology Engineering* **97**, 122–129.

Mahmoud, M., Janssen, M., Haboub, N., Nassour, A. & Lennartz, B. 2010 The impact of olive mill wastewater application on flow and transport properties in soils. *Soil and Tillage Research* **107**, 36–41.

Mathieu, C. & Pieltain, F. 2003 Soil chemical analysis, selected methods 'Analyse chimique des sols, méthodes choisies' Lavoisier. *Techniques et Documents (In French), Paris* 388–575.

McCready, R. M., Guggolz, J., Silviera, V. & Owens, H. S. 1995 Determination of starch and amylase in vegetables. Application to peas. *Analytical Chemistry* **22**, 1156–1158.

Meftah, O., Guergueb, Z., Braham, M., Sayadi, S. & Mekki, A. 2018 Long term effects of olive mill wastewaters application on soil properties and phenolic compounds migration under arid climate. *Agricultural Water Management* **212**, 119–125.

Mekki, A., Dhouib, A. & Sayadi, S. 2006 Changes in microbial and soil properties following amendment with treated and untreated olive mill wastewater. *Microbiological Research* **161**, 93–101.

Mekki, A., Dhouib, A. & Sayadi, S. 2007 Polyphenols dynamics and phytotoxicity in a soil amended by olive mill wastewaters. *Journal of Environmental Management* **84**, 134–140.

Mekki, A., Aloui, F., Dhouib, A. & Sayadi, S. 2012 Effects of Phanerochaete chrysosporium on biologic activity of soil amended with olive mill wastewaters. *Journal of Soil Science and Environmental Management* **3**, 1–8.

Mekki, A., Dhouib, A., Feki, F. & Sayadi, S. 2013 Review: effects of olive mill wastewater application on soil properties and plants growth. *International Journal of Recycling of Organic Waste in Agriculture* **2**, 15.

Mekki, A., Aloui, A., Guergueb, Z. & Braham, M. 2018 Agronomic valorization of olive mill wastewaters: effects on *Medicago sativa* growth and soil characteristics. *Clean, Soil, Air, Water* **100**, 1–9.

Obied, H. K., Allen, M. S., Bedgood, D. R., Prenzler, P. D., Robards, K. & Stockmann, R. 2005 Bioactivity and analysis of biophenols recovered from olive mill waste. *Journal of Agriculture & Food Chemistry* **53**, 823–830.

Proietti, P., Federici, E., Fidati, L., Scargetta, S., Massaccesi, L., Nasini, L., Regni, L., Ricci, A., Cenci, G. & Gigliotti, G. 2015 Effects of amendment with oil mill waste and its derived-compost on soil chemical and microbiological characteristics and olive (*Olea europaea L.*) productivity. *Agriculture, Ecosystems and Environment* **207**, 51–60.

Saadi, I., Laor, Y., Raviv, M. & Medina, S. 2007 Land spreading of olive mill wastewater: effects on soil microbial activity and potential phytotoxicity. *Chemosphere* **66**, 75–83.

Sierra, J., Marti, E., Garau, M. A. & Cruanas, R. 2007 Effects of the agronomic use of olive oil mill wastewater: field experiment. *Science of The Total Environment* **378**, 90–94.

Tattini, M., Gucci, R., Coradeschi, M. A., Ponzio, C. & Everard, J. D. 1995 Growth, gas exchange and ion content in Olea europea plants during salinity stress and subsequent relief. *Physiologia Plantrium* **95**, 203–210.

Thomas, E. Y., Omueti, J. A. I. & Ogundayomi, O. 2012 The effect of phosphate fertilizer on heavy metal in soils and *Amaranthus caudatus*. *Agriculture Biology Journal North America* **4**, 145–149.

Yaakoubi, A., Chahlaoui, A., Rahmani, M., Elyachioui, M. & Oulhote, Y. 2009 Effect of spreading olive wastewaters on soil microflora. *Agro-solutions* **20**, 36–42.

Zenjari, B. & Nejmeddine, A. 2001 Impact of spreading olive mill wastewater on soil characteristics: laboratory experiments. *Agronomy* **21**, 749–755.

Zucconi, F., Forte, M., Monaco, M. & De Bertoldi, M. 1981 Biological evaluation of compost maturity. *Biocycle* **22**, 27–29.

First received 10 May 2019; accepted in revised form 31 October 2019. Available online 11 November 2019