

# Olive-mill wastewater spreading in Southern Tunisia: effects on a barley crop (*Hordeum Vulgare* L.)

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**Abstract:** This study was conducted to assess the impact of different concentrations of Olive mill waste water (OMWW) on the phenological behavior of a local barley variety (Arthaoui.) during three consecutive crop seasons. A complete randomized block design was used with four amounts of OMWW equivalent to 0 m<sup>3</sup>/ha (T0), 15 m<sup>3</sup>/ha (T1), 30 m<sup>3</sup>/ha (T2) and 45 m<sup>3</sup>/ha (T3) and three replications. The results showed a highly significant reduction as well as of the tiller and ears number compared to the control according to the increase of OMWW concentrations especially for the highest amounts either T2 and T3 (30 and 45 m<sup>3</sup>/ha). This reduction was although observed but it was less accentuated for the treatment with an amount of 15 m<sup>3</sup>/ha compared to the other rates. In addition, barley yield components were negatively affected by “OMWW” in particular yields plots that received higher doses as 30 and 45 m<sup>3</sup>/ha. Obviously, the straw and seed yield are significantly affected with relatively different degrees depending on the applied dose but also on the cumulative effect of successive applications during the three years of study. Indeed, richness of these effluents on salts especially in sodium and chlorides, on polyphenols and other compounds with variable toxicity is causing physiological disturbances that are negatively reflected at different phenological stages of barley as tillering, stem elongation and heading giving the poor results in terms of seed yield.

**Keywords:** OMWW, Barley, tiller number, ear number.

## Introduction

The economic and social importance of olive cultivation in Tunisia and in the Mediterranean countries is well known. However, the olive industry is confronted

with the issue of the disposal of its waste water, mainly due to “OMWW”, waste resulting from the trituration of olives leading in particular to surface water pollution and ecological toxicity environment. These environmental problems are attributed above all to the very high concentrations of organic compounds resulting from the relatively high biological oxygen demand of margins at the order of 200 g / L.

This high organic load, mainly composed of polyphenols as well as short and long chain fatty acids, is believed to be responsible for the phytotoxic and antimicrobial effect of these effluents.

These compounds can inhibit the growth of microorganisms, especially bacteria Capasso *et al.* (2002a, 2002b), thus affecting the mineralization process in the soil.

These considerations have led several researchers, nationally and internationally, to choose the path of treatment and exploitation of OMWW to limit their pollution, Gharsallah (1999), Leger *et al.* (2000), Kissi *et al.* (2001), Garcia *et al.* (2002), Garrido *et al.* (2002), Pozo *et al.* (2002) and Fenice *et al.* (2003).

However, the processes developed up to now remain very limited and their cost is very high Hamdi (1993a, 1993b). Consequently, treatment by natural evaporation basins remains the most used technique despite its impact on the natural environment, particularly the groundwater.

On the other hand, numerous authors recorded a fertilizing effect of margins attributed to their very high concentration of potassium and organic matter Ros de Ursinos (1981), Briccoli Bati & Lombardo (1990), Ammar & Ben Rouina (1999) and Ben Rouina & Ammar (1999). These authors consider that OMWW may be a source of potential fertilization in particular for sandy soils. This is an asset for a possible valorization of these effluents in agronomy.

The main objective of this study was to verify, in a semi-arid environment such as southern Tunisia, the possibility of directly applying margins without prior treatment to an annual crop such as barley. We also sought to analyze the effects of three successive applications of these effluents on the same experimental site on the dynamics of phenological plant growth.

The choice of the dose range applied in the present work was based on the results of a previous study conducted by Dakhli *et al.* (2009) on an experimental plot in the southern Tunisian field. From this research, many useful conclusions have been drawn. Indeed, the application of 4 doses of OMWW equivalent to 50, 100 and 200 m<sup>3</sup>/ha was tested in the presence of an irrigated Barley crop in order to evaluate the impact of these effluents on the chemical characteristics and on the phenological behavior and the yield of the plant.

The results of the study showed that at a dose of 50 m<sup>3</sup>/ha, OMWW did not pose risks to salinity, high phenolic concentrations, potassium content and pH. On the contrary, they induce an improvement of certain chemical properties of the soil (organic matter content and potassium) without improving the productivity of barley.

However, application rates of OMWW greater than 50 m<sup>3</sup>/ha result in a highly significant decrease in barley yield followed by disturbances in phenological stages due to the accumulation of phenolic substances and the excessive increase in sodium concentrations, chlorides and sulphates and consequently higher levels of soil salinity in the short and long term.

The results showed also that OMWW has a negative impact on the germination rate of barley once sowing is carried out directly after the application of these effluents. Indeed, for the highest doses (T2: 100 m<sup>3</sup>/ha and T3: 200 m<sup>3</sup>/ha), the germination rate was severely penalized during the first weeks. This inhibition persists until the fourth week for T3 treatment. However, the low dose T1 (50 m<sup>3</sup> / ha) did not induce a major effect on the inhibition of germination, whatever the interval between the sowing date and the date of application. The date of application to the soil of a dose T1 appears independent of the date of sowing.

In conclusion, the results showed that the dose of 50 m<sup>3</sup>/ha can be adopted as a sustainable rate for barley fertilizer irrigation with possibilities to optimize this quantity according to the cultivated variety, the cultivation techniques adopted and the irrigation water quality. Studies have also reported that the rate of 50 m<sup>3</sup> /ha-year (Mekki *et al.*, 2006) significantly reduces the risk of groundwater contamination by avoiding the accumulation of high BOD load in the soil.

We suggest, based on our previous results, that the following guidelines should be respected and taken into consideration when applying OMWW for this research work:

- Evenly distribute OMWW at doses lower than 50 m<sup>3</sup>/ha;
- The application of OMWW must adequately precede sowing to be beneficial. (Barley seeding should be carried out 2 weeks after OMWW spreading date).

## Material and Methods

### *Experimental Site and OMWW application*

Experimental work was carried out in field experiment located at the IRA (Institute of Arid Regions) in southern Tunisia (governorate of Medenine). North latitude: 33° 16' 21", East longitude: 10° 19' 30". Climatic conditions were typically Mediterranean: with an average annual rainfall of 150 mm occurring mostly in autumn and spring and a mean annual air temperature ranging from 18 to 20 °C.

The soil chosen was isohumic with the following characteristics: sandy texture (90% sand, 3% loam, and 6% clay), pH (7.11), EC (2.32 dS.m<sup>-1</sup>), % CaCO<sub>3</sub> (5.55) and % OM (0.98).

The fresh OMWW was taken from a three-phase continuous extraction factory located in Saadane- southern Tunisia. The physicochemical characteristics of crude OMWW are summarized in Table 1 and correspond to the mean values of 3 analyzes.

Table 1 - Physicochemical characteristics of olive mill waste water used in ferti-irrigation (values after represent  $\pm$  standard deviation).

CHARACTERISTICS	DATA
pH	4.8 $\pm$ 0.2
Electrical conductivity (Ds/m)	10.0 $\pm$ 0.52
COD (g/L)	98.0 $\pm$ 2.1
BOD (g/L)	66.0 $\pm$ 2.4
Total organic carbon (g/L)	26.0 $\pm$ 2.4
Total nitrogen Kjeldahl (g/L)	1.6 $\pm$ 0.1
Carbon/Nitrogen	16.25 $\pm$ 0.48
Phenolic compounds (g/L)	8.8 $\pm$ 0.3
Potassium (g/L)	6.1 $\pm$ 0.2
Calcium (g/L)	1.1 $\pm$ 0.1
Phosphorus-Olsen (g/L)	0.35 $\pm$ 0.02
Magnesium (g/L)	0.42 $\pm$ 0.01
Sodium (g/L)	1.57 $\pm$ 0.01
Chlorides (mg/L)	0.65 $\pm$ 0.4

The experimental design consisted of thirty six plots according to a complete randomized block design with three replicates per treatment. Plots with 2 $\times$ 3 m<sup>2</sup> size, were delimited, mechanically ploughed and leveled to subsequently amend them with OMWW (olive mill waste water).

Annually, since 2009, OMWW spreading application was done in one application on the same plot for three consecutive years: 2009 (First year), 2010 (Second year) and 2011 (Third year). The three contributions were made on the same site and with the same experimental design.

Amendments were applied annually in December (from 2009 to 2012), spreading the waste on the soil surface, followed by arable- level homogenization. After a period of 15 days of rest deemed necessary for drying the soil, the incorporation of OMWW was subsequently carried out by manual tillage.

Each year, OMWW application was done at rates equivalent to 15 m<sup>3</sup>/ha, 30 m<sup>3</sup>/ha and 45 m<sup>3</sup>/ha. Also T0 plots were not amended and served as control: soil where OMWW was never spreading. All the treatments were done in triplicate, distributing the plots alternatively.

The choice of the different doses was adopted following a series of optimizations made following our previous research work, the last one in 2009 in the framework

of a master's work, which showed that doses above 50 m<sup>3</sup> / ha of OMWW negatively affect the behavior of barley (*Hordeum vulgare* L.). T1 = 15 m<sup>3</sup>/ ha, T2 = 30 m<sup>3</sup>/ ha and T3 = 45 m<sup>3</sup>/ ha were used for this purpose.

A local barley variety: Ardhaoui was adopted in irrigated areas. Sowing was carried out two weeks after each OMWW application with a seeding rate of 136 kg/ha.

In order to investigate the influence of waste application on crop growth dynamics, we studied the evolution of tillering and heading stage resulted in a weekly follow-up of 10 plants per plate chosen at random according to a diagonal arrangement.

At harvest, grain and straw yield, and seed weight were recorded. The statistical analysis was performed either for each year or globally for the 3-year period.

## Results

### *Effect of OMWW on Barley growth*

#### *Tillering stage*

For the three companions, OMWW induced a very significant reduction in the number of tillers compared to the controls particularly for T2 (30 m<sup>3</sup>/ha) and T3 (45 m<sup>3</sup>/ha) doses.

This reduction proportional to the increase of the allocated dose tends to increase from one companion to another (Figure 1).

Indeed, in the first crop year, we recorded an average of 10 and 8 tillers for respectively for plots receiving the highest doses of OMWW such as T2 (30 m<sup>3</sup>/ha) and T3 (45 m<sup>3</sup>/ha) with a highly significant difference compared to the control where we recorded 17 tillers.

However, the T1 dose (15 m<sup>3</sup>/ha), produced the lightest reduction with 13 tillers (Table 2).

During the second season and independently of the dose used, OMWW have always negatively affected the tillering stage (Figure 3). Indeed, the number of tillers registered a significant reduction compared to the control for all the doses incorporated. Plots receiving T1 (15 m<sup>3</sup>/ha), as in the first season, recorded the lowest damage with 13 tillers compared with T2 (30 m<sup>3</sup>/ha) and T3 (45 m<sup>3</sup>/ha) where an average of 5 and 6 tillers was recorded respectively (Table 2). In this second companion, the inhibitory effect of OMWW was significantly more pronounced in comparison with the previous year especially for T2 and T3 doses. The cumulative effect of successive application of these effluents at doses greater than 15 m<sup>3</sup>/ha may be at the origin of this decrease (Figure 1).

Moreover, in addition to the number of tillers, we recorded a delay in the date of the start of tillering, in comparison with the control. This delay was one week for

the T1 dose (15 m<sup>3</sup>/ha). Beyond this dose, this depressive effect becomes prejudicial. Indeed, the application of the highest doses T2 (30 m<sup>3</sup>/ha) and T3 (45 m<sup>3</sup>/ha) induced an inhibition of the tillering stage resulting from a blockage of the process which lasted three weeks (Figure 1).

In the third companion, the effect of OMWW on the number of tillers is more pronounced. The same findings were made during the third companion, except that the degree of damage was further magnified in comparison with previous companions. Indeed, the application of OMWW has led to a considerable reduction in the number of tillers irrespective of the dose applied. This decrease is highly significant compared to the control for the different doses adopted. Indeed, this reduction is by 45% for T1 (15 m<sup>3</sup>/ha). On the other hand, T2 (30 m<sup>3</sup>/ha) and T3 (45 m<sup>3</sup>/ha) doses recorded a highly significant reduction by 80% and 90% respectively (Table 2); which can be described as catastrophic in comparison with the control.

In addition, a delayed initiation of tillering process of one-month was recorded especially for the highest doses of OMWW, namely T2 (30 m<sup>3</sup>/ha) and T3 (45 m<sup>3</sup>/ha). These doses affected the speed of the tillering process. This braking also lasted a month. However, this delay is less pronounced for the T1 dose (15 m<sup>3</sup>/ha), it lasted only two weeks (Figure 1).

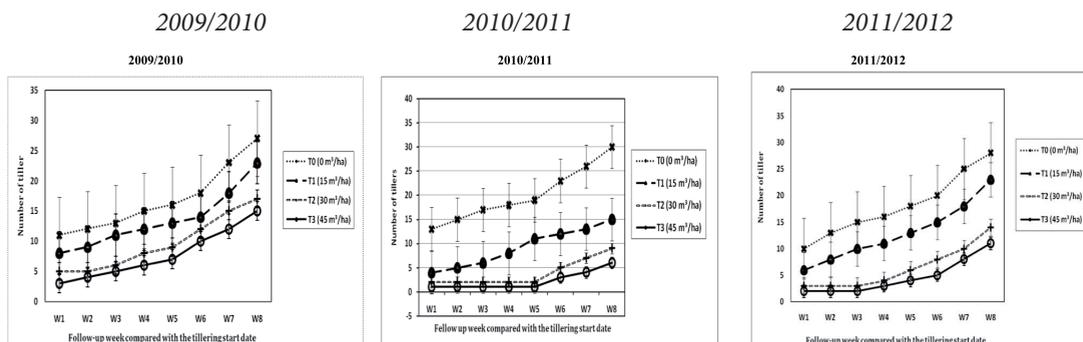


Figure 1 - Evolution of tiller number after OMWW spreading since three successive years.

In addition to the decrease in the number and growth rate of the tillers, effect of OMWW on the plants was of moderate phytotoxicity with some necrosis of younger leaves and symptoms of yellowing, wilting and premature senescence of oldest leaves in the run-up stage. In response, the crop did not produce new leaves.

These manifestations can be the consequences of an excessive concentration of salts in the soil relative to the doses of OMWW brought in, in particular the very high levels of sodium and chlorides. This situation is aggravated from one companion to another because of the accumulated amount of salts brought by these effluents.

Indeed, previous work carried out by Dakhli *et al.* (2009 and 2013) showed a delay in initiation of tillering by one week compared with the control, induced by doses of 100 m<sup>3</sup>/ha and 200 m<sup>3</sup>/ha. These doses induced an inhibition resulting on a blockage of the tillering process which lasted one month and a shortening of the heading stage.

Table 2 - The average number of tillers recorded during three successive farming companions

Rate of OMWW	Mean*
First year: 2009-2010	
T0 (0m <sup>3</sup> /ha)	16,87 ± 3,65 A
T1 (15m <sup>3</sup> /ha)	13,5 ± 1,89 AB
T2 (30m <sup>3</sup> /ha)	9,62 ± 0,84 BC
T3 (45m <sup>3</sup> /ha)	7,75 ± 0,41 C
Second year: 2010-2011	
T0 (0m <sup>3</sup> /ha)	18,12 ± 5,13 A
T1 (15m <sup>3</sup> /ha)	13 ± 1,81 B
T2 (30m <sup>3</sup> /ha)	6,37 ± 0,67 C
T3 (45m <sup>3</sup> /ha)	4,62 ± 0,43 C
Third year 3: 2011-2012	
T0 (0m <sup>3</sup> /ha)	20,12 ± 6,89 A
T1 (15m <sup>3</sup> /ha)	9,25 ± 0,83 B
T2 (30m <sup>3</sup> /ha)	3,87 ± 0,23 C
T3 (45m <sup>3</sup> /ha)	2,25 ± 0,14 C

\* Averages with the same letter are not statistically significant at the 5%

### Heading stage

For the three years of experiment, OMWW spreading to soil at 30 m<sup>3</sup>/ha and 45 m<sup>3</sup>/ha allowed a highly significant reduction in the number of spikes compared to the controls. The highest doses recorded the highest reductions.

This reduction, although observed in the first year, was accentuated during the second and third companion for the same doses (Figure 2).

Indeed, in the first companion, OMWW negatively affected the heading stage of barley irrespective of the dose applied. However, the degree of impact depends on the amount used. The highest doses T2 (30 m<sup>3</sup>/ha) and T3 (45 m<sup>3</sup>/ha) recorded highly significant reductions in the number of spikes with an average of 9 and 8, respectively, compared to the control without OMWW where we observed an average

of 15 spikes. This reduction is around 50%. However, for the plots that received the T1 dose (15 m<sup>3</sup>/ha), we recorded an average of 12 spikes, a reduction of about 20% with a significant difference compared to the control and to T2 (30 m<sup>3</sup>/ha) and T3 (45 m<sup>3</sup>/ha) (Table 3, Figure 2).

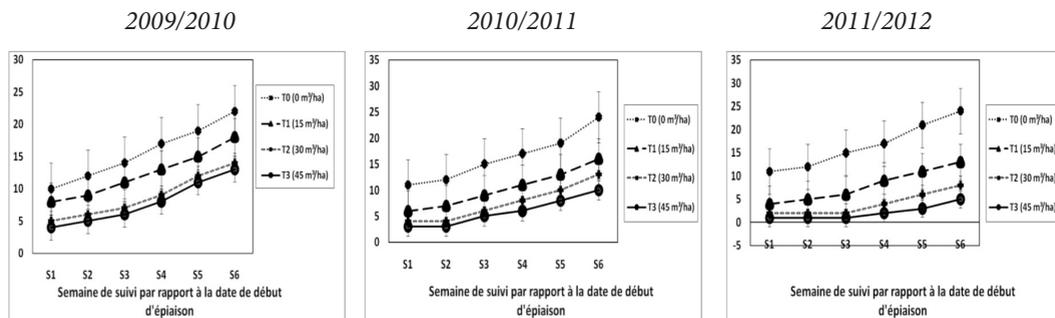


Figure 2 - Evolution of spike number after OMWW spreading during three successive years.

Table 3 - The average number of spikes recorded during three successive farming companions.

OMWW doses	Mean*
First year: 2009-2010	
T0 (0m <sup>3</sup> /ha)	15,66 ± 3,32 A
T1 (15m <sup>3</sup> /ha)	12,33 ± 1,67 AB
T2 (30m <sup>3</sup> /ha)	8,83 ± 0,79 B
T3 (45m <sup>3</sup> /ha)	7,83 ± 0,46 B
Second year: 2010-2011	
T0 (0m <sup>3</sup> /ha)	11,33 ± 1,54 A
T1 (15m <sup>3</sup> /ha)	8 ± 0,82 B
T2 (30m <sup>3</sup> /ha)	2,33 ± 0,15 C
T3 (45m <sup>3</sup> /ha)	1,66 ± 0,11 C
Third year: 2011-2012	
T0 (0m <sup>3</sup> /ha)	9,66 ± 0,87 A
T1 (15m <sup>3</sup> /ha)	7,66 ± 0,44 A
T2 (30m <sup>3</sup> /ha)	2,16 ± 0,18 B
T3 (45m <sup>3</sup> /ha)	1,16 ± 0,13 B

\* Means with the same letter are not statistically significant at the 5% threshold.

The second year showed a more pronounced impact. Indeed, with an average of 8 spikes, the T1 dose (15 m<sup>3</sup>/ha) recorded a significant reduction of the number of spikes by 25% compared to the control. This reduction is catastrophic for the highest doses, namely T2 (30 m<sup>3</sup>/ha) and T3 (45 m<sup>3</sup>/ha). It is by 80% with a highly significant difference compared to the control (Table 3, Figure 2). In this second year, the negative effect of cumulative successive doses of OMWW begins to appear.

The penalization of the number of spikes recorded during two previous cropping years is more pronounced during the third season.

This could be explained by a cumulative effect of chemical substances in the soil after 3 years of application of OMWW. The results of the third cropping year showed the catastrophic impact of successive doses of OMWW greater than 15m<sup>3</sup>/ha on the heading stage (Table 2, Figure 3).

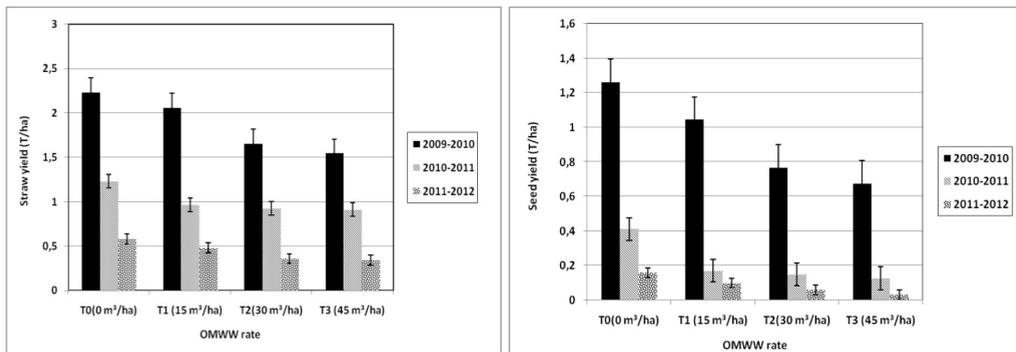


Figure 3 - Effect of OMWW spreading in Barley straw and seed yield.

### Effect of OMWW spreading in Barley straw and seed yield

For the three companions, straw yield was decreased to varying degrees of OMWW treatments as compared to control.. This reduction is correlated with the assigned dose.

The reduction induced by the T1 dose (15 m<sup>3</sup>/ha) is significant. However, it is highly significant for T2 doses (30 m<sup>3</sup>/ha) and T3 (45 m<sup>3</sup>/ha) compared to the control.

Indeed, we recorded in the first companion a yield of 2.058 T / ha for the dose T1 (15 m<sup>3</sup>/ha) or a reduction by 8%. The difference is not significant compared to the control for which a yield of 2.23 T / ha was recorded.

For the highest doses of OMWW the straw yield, showed a highly significant reduction by 27% compared to the control. A yield of 1,653 and 1,545 T/ha was recorded for T2 (30 m<sup>3</sup>/ha) and T3 (45 m<sup>3</sup>/ha), respectively.

The cumulative effect of successive annual applications of OMWW on the straw yield begins to appear in the second companion to increase during the third companion.

In the second year of experiment, this reduction is by 17% for the three doses adopted, namely T1 (15 m<sup>3</sup>/ha), T2 (30 m<sup>3</sup>/ha) and T3 (45 m<sup>3</sup>/ha). The difference is statistically significant compared to the control. Although it worsened much more at the end of the third companion, reaching rates by 62% and 58% respectively for T2 (30 m<sup>3</sup>/ha) and T3 (45 m<sup>3</sup>/ha).

The Barley grain yield over the 3-year period decreased from the first to the last years.

For the first year of trial, a reduction by 18% compared with the control was recorded for the T1 (15 m<sup>3</sup>/ha). However, this decline is highly significant for T2 (30 m<sup>3</sup>/ha) and T3 (45 m<sup>3</sup>/ha) by 61% and 54% respectively.

For the second crop year, this reduction is much more important. Indeed, it is of the order of 59% relative to the control respectively for the doses T1 and T2 and by 69% for the dose T3.

Concerning the third year of experiment, the dose T1 (15 m<sup>3</sup>/ha) induced a significant reduction in the grains yield by 37% compared to the control. However, T2 (30 m<sup>3</sup>/ha) and T3 (45 m<sup>3</sup>/ha) doses were distinguished by catastrophic damage with a yield reduction by 62% and 81%, respectively. It is likely that the reproductive organs were damaged in year 3, causing low spike fertility and the lowest yield in the 3-year period. These results confirmed those obtained by (Bonari and Ceccarini.,1993 ; Rhinali et al., 2003) for wheat and barley crop.

## Discussions

Barley yields have been negatively affected by OMWW, in particular the yields of the plots receiving the highest doses 30 m<sup>3</sup>/ha and 45 m<sup>3</sup>/ha. The straw yield and of course the grains yield are very affected with relatively variable degrees depending on the dose applied but also on the cumulative effect of the successive omww inputs during three years of study.

This effect, although significant for plots receiving the 15 m<sup>3</sup>/ha dose, is less aggressive. The reduction in yield increases from one year to another whatever the quantity of OMWW applied.

In this context, Dakhli *et al.* (2009; 2013a, b) have shown that 100 m<sup>3</sup>/ha of OMWW induce a significant reduction in dry matter and seed yield. This reduction is highly significant at a dose of 200 m<sup>3</sup>/ha.

The yield is necessarily dependent on the variety of barley used, but mainly on the availability of adequate mineral nutrition. These negative results can only be attributed to an exogenous nutritional stress caused by OMWW application.

The concordance observed between the degree of reduction of the various yield components and the dose of the margins applied during the three cropping years shows suspicion against chemical characteristics specific to these effluents.

Indeed, the richness of these effluents in salts, polyphenols and other more or less toxic compounds is at the origin of the physiological disturbances which have translated negatively at the level of the various phenological stages of barley such as germination, emergence, tillering and heading, thus induced consequencely the catastrophic results obtained in terms of grains yield and productivity.

Salinity inhibits especially the absorption and the transport of the major elements, which limits the supply of the plant in essential elements for its growth (Soltani *et al.*, 1993).

Adverse effect of salinity on the above parameters might be due to fewer uptakes of water and nutrients from the growing media due to higher concentration of salts present in the root zone, which may causes imbalances in osmotic pressure. Reduced growth under salt stress might be due to reduced transport of essential nutrient to the shoot (Tarmatt and Munns, 1986; Dageret.*al.*, 2004).

Salinity may directly or indirectly inhibit cell division, cell enlargement (Calu, 2006), which results in reduction of shoot length, number of leaves, dry matter accumulation, leaf size, mobilization of food material from source to sink and increased root shoot ratio. Singh and Singh (1991) reported that yield and yield attributes decreased markedly with increasing levels of sodicity.

Indeed, other research has shown that in the presence of high concentrations of NaCl, reduction in barley growth is accompanied with an increase in the accumulation of Na<sup>+</sup> and Cl<sup>-</sup> in the tissues and a decrease in K<sup>+</sup> and Ca<sup>2+</sup>.

(Ashraf, 2004; Saqib *et al.*, 2005) demonstrated that Na<sup>+</sup> is the primary cause of ion specific damage, resulting due to a range of disorders in enzyme activation and protein synthesis (Tester & Davenport, 2003).

On the other hand, in the isolated walls of barley roots, Na<sup>+</sup> and Ca<sup>2+</sup> compete for the same adsorption sites, while K<sup>+</sup> is fixed at other sites (Stassart *et al.*, 1981). This leads to an alteration in K<sup>+</sup>/Na<sup>+</sup> selectivity and a loss of K<sup>+</sup> tissue (Hajji & Grignon., 1985 and Kent & Lauchli., 1985). K<sup>+</sup> and Ca<sup>2+</sup> become limiting factors for growth when the medium is enriched with NaCl. (Greenway., 1962a,b; Munns., 1993; Mansour., 2000 and Bounaquba *et al.*, 1996).

These results are consistent with those of Soltani *et al.* (1990) which showed that NaCl exerts its depressive effect on growth by limiting the supply of the plant to Ca<sup>2+</sup>.

The decrease of plant growth and biomass accumulation under salinity stress have been shown by other authors (Magio *et al.*, 2007; Neocleous and Vasilakakis, 2007; Singh and Prasad, 2009). Salinity limits plant growth and productivity (Ghazi and Al-Karaki, 2006; Ashraf and Foolad, 2007; Rewald *et al.*, 2012). Decrease of root weight of soybean was parallel with enhancement of NaCl concentration in nutrition solution. Salinity stress diminished plant growth and decreased total dry weight (Dolatabadia *et al.*, 2011). The reduction in growth under salinity stress could be due to the low water potential, reduction of photosynthesis (Ziska *et al.*, 1990 and Jeschke

*et al.*, 1992; Wang *et al.*, 2012; Abou-Leila *et al.*, 2012; Boughalleb *et al.*, 2012), ion toxicity (Munns *et al.*, 2006) and disturbance in mineral uptake (Kingsbury *et al.*, 1984; Kyparassis *et al.*, 1995 and Wang *et al.*, 1997; Abou-Leila *et al.*, 2012).

In general, as with all plants, the response to stress results in a precipitation of the phenological stages and a shortening of the vegetative cycle in order to arrive as quickly as possible in the seeds production and to ensure the perennality of the species despite the productivity and quality of the seeds.

## Conclusion

The results of three years of study led to the conclusion that the application of OMWW induces a highly significant reduction in the number of tillers and spikes. This reduction, although observed in the first year, has become increasingly pronounced thereafter under the cumulative effect of the succession applications of OMWW.

On the other hand, all yield components of barley were negatively affected by OMWW. The straw yield and of course the grain yield are very affected with relatively varying degrees depending on the applied dose but also according to the cumulative effect of the successive intakes during the three years of study.

Although this effect was significant for the 15 m<sup>3</sup>/ha plots, it was still less aggressive.

The yield is necessarily dependent on the barley variety used and the availability of adequate mineral nutrition. These negative results can only be attributed to an exogenous nutritional stress caused by OMWW application.

The concordance observed between the degree of reduction of the various yield components and the dose of OMWW applied during the three crop years demonstrates suspicion of the chemical characteristics specific to these effluents.

Notably, their very high levels of salts (sodium and chlorides), excess of phenols and other toxic organic compounds in the soil due to cumulative successive inputs reduced growth straw yield and barley productivity.

This can be at the origin of the ionic perturbations limiting the supply of the plants with major mineral elements necessary for their growth but also with a prior nitrogen immobilization.

Indeed, the cumulative effects of the successive inputs of OMWW had a negative effect on the mineralization process (microbial activity) due to the excessive accumulation of salts and concentrations and phenolic compounds. This is the cause of the harmful physiological repercussions due to disturbances of hydro-mineral soil balance and, above all, nitrogen immobilization, which have negatively affected the different phenological stages of barley, including tillering and heading thus giving very low results in terms of grain yield.

The use of OMWW as fertilizer has not yielded encouraging results regarding

straw and grain yields of barley. On the contrary, it constituted a factor of stress and metabolic disturbance.

In sum, the contribution of OMWW as an organic amendment proved to be of no agronomic interest. On the contrary, it can be a source of trouble especially for annual crops with a relatively short root system such as Barley. The same effect can also be true for other cultivated and even spontaneous species, hence the weeding power of these effluents evoked by other previous works.

For perennial species (trees and shrubs), this effect may not be immediately noticeable, especially at relatively moderate doses. However, the successive contribution of OMWW due to their weed control can lead to a degradation of the spontaneous vegetation, source of endogenous organic matter, and the regression of the microflora of the soil especially in the arboreal areas in this case the olive groves.

Especially since the majority of olive groves in central and southern Tunisia receive almost no organic exogenous amendment. It is therefore necessary to be very vigilant to any attempt at this level.

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