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Study of the Phytotoxicity of Olive Mill Wastewater on Germination and Vegetative Growth – Case of Tomato *(Solanum lycopersicum* L.)

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ABSTRACT

Olive mill wastewater (effluents from olive oil extraction during harvest) is a source of environmental pollution due to its high acidity and heavy loads of polyphenols and organics. Our aim is to study the phytotoxicity of olive mill wastewater (OMW) at different concentrations on the germination and growth of tomatoes (*Solanum lycopersicum* L.). To assess this, we measured the percentage of germination (GP), the germination inhibition rate (GIR), the length of the main stem, the number of leaves, and the biomass of the stem, leaves, and roots. The germination test was carried out by placing 25 tomato seeds on filter paper in Petri dishes and then irrigating with different concentrations of OMW (0%, 1%, 2%, 5%, 7%, 10%). The boxes were placed in an incubator at a temperature of 25 °C for 10 days. The results obtained show that, after 10 days of germination, the GP is maximal (more than 98%) at concentrations of less than 7% ($r = -0.98$; $p<0.000$) and with an "inhibitory" effect at more than 7% ($r = +0.98$; $p<0.000$). However, the growth in length (8.514±2.612 cm) and the number of leaves (4.667±0.866 leaves) were maximum, respectively, at 2% and 1%, after a period of 30 days. However, the dry weight is maximum (0.235±0.049 g) at 5% OMW, however, there is no significant difference in the fresh and dry weights of the stems and leaves for different concentrations. In light of these results, the OMW of crushing units using the continuous three-phase process could be used as an irrigation source at concentrations below 7%, for better tomato yield.

Keywords: OMW, tomato, germination percentage, germination inhibition rate, valorization.

INTRODUCTION

Morocco is the fifth largest producer of olive oil in the world, with 140,000 tons, or 5% of the world production [Tarik, 2021]. However, the olive industry generates liquid waste (olive mill wastewater) and solid waste (pomace). These olive mill wastewaters (OMW) are effluents generated during the olive oil harvest season during the olive seasons (3 to 4 month) [Lakhtar and al., 2010] and consist mainly of oil emulsion, pulp, crushed seeds and large amounts of water [Shabir and al., 2022]. Due to their high content of phenolic compounds, OMW have a black color, high acidity, high organic matter content and high resistance to biodegradation [Alaoui and al., 2022; Domingues and al., 2021; Enaime and al., 2020]. The annual production of OMW in the Mediterranean is more than 30 million m^3, and in Morocco about 685,000 m^3 per year [Massadeh and al., 2022].

In Mediterranean countries, OMW is considered a dangerous effluent due to its high concentration of high levels of organic loads and polyphenolic compounds [Hamimed and al., 2022]. However, discharge of untreated OMW into receiving environments, including rivers, can lead to discoloration, degradation, and disturbance of ecosystems such as Oued Oussefrou [Zghari and al., 2018] and Oued Oum He Rbia River [EL ALAMI and FATTAH, 2020]. The phytotoxicity of OMWs is mainly attributed to their phenolic content as well as the presence of other organic compounds such as aldehydes and short-chain fatty acids [Shabir and al., 2022], so they must require treatment before discharge into the receiving environment. Several studies have focused on the treatment processes of OMW, such as biological (aerobic, anaerobic) [Enaime and al., 2020; M. Rusan and al., 2016], physico-chemical (coagulation/ floculation, membrane filtration,...) [El Herradi and al., 2016, 2017; Hanafi and al., 2010, 2009], thermal (incineration, evaporation...) and distillation neutralization [Ouabou and al., 2014; Rusan and Malkawi, 2016]. However, the richness of OMW in organic matter and soil fertilizing elements, namely, nitrogen (N), phosphorus (P), potassium (K), and magnesium (Mg) [Chaari and al., 2015; Dakhli and Maalej, 2017], allows these effluents to be recycled as eco-fertilizers at a lower cost for soils, as well as a source of irrigation water in the Mediterranean countries suffering from water scarcity and soil degradation [Ahmali and al., 2020; Belaqziz and al., 2016; de los Santos and al., 2019; Mekki and al., 2013, 2006; Piotrowska and al., 2011]. Using OMW as a soil amendment will increase soil fertility and improve plant performance, but it needs to be streamlined. [Di Bene and al., 2013; Mechri and al., 2007], in Jordan [Ayoub and al., 2014], in Tunisia [Chaari and al., 2015]. According to Sahraoui and al, (2012), the direct application of OMW to the soil can be a process of valorization of these effluents, potentially increasing their fertility, especially in phosphorus, nitrogen, and potassium [Bargaoui and al., 2020; Chaari and al., 2015; Gargouri and al., 2022; Sahraoui and al., 2012]. In this framework, our research work is focused on OMW study of the phytotoxicity of OMW on the germination and vegetative growth of tomato seeds to determine the optimal concentrations to have the best yield and determine the phytotoxic concentration.

MATERIALS AND METHODS

Sampling

OMW was collected from the storage basin of a three-phase centrifugal olive mills located in Tifelt, Khémisset province in the Rabat-Salé-Kénitra region of Morocco, during the olive seasons. The effluents were collected and transported in sterile containers.

Physicochemical characterisation of OMW

The physico-chemical parameters measured are: The pH using a pH meter (ORION STAR A111). Electrical conductivity (ms/cm) using a conductivit metre (CON 700). The dry matter is determined after evaporation of a 20ml volume of OMW in a porcelain capsule previously dried and tared, at 105°C until constant weight. The sample is then weighed after cooling to determine its dry weight (g/l). The dry matter is calcined in a muffle furnace at 550°C. After being cooled, the incineration products constituting the mineral matter are weighed. The organic matter is determined by the difference between the dry weight and the weight after calcinations [Rodier and al., 2009]. The chemical oxygen demand COD (mgO$_2$/l) is determined by measuring the excess of potassium dichromate in acid milieu in the presence of sulfate silver. Biochemical oxygen demand BOD$_5$ (mgO$_2$/l) is determined by the respirométrique method [Rodier and al., 2009].The total phenolic compounds content has been determined according to a standard method [Box, 1983] using the Folin-Ciocalteu reagent and Gallic acid as standard.

Germination test

The germination test was carried out on tomato seeds (*Solanum lycopersicum L*) of Moroccan origin "Campbell 33" commercial genotypes. The seeds were physically examined, as damaged or unhealthy seeds were excluded. Then, 25 seeds the seeds were placed on filter paper in 9 cm diameter Petri dishes (25 seeds/ dish). They were irrigated with OMW of concentrations 0%, 1%, 2%, 5%, 7% and 10%. The dishes were placed in an incubator at a temperature of 25°C for 10 days. During this period, the Petri dishes were irrigated once every two days. A seed is considered germinated when the radical reaches 2 mm (ISTA, 2018). The measured parameters were the following: germination percentage (GP), germination inhibition rate (GIR). Using the following formulas:

Germination percentage: [Shabir and al., 2022]

$$GP = (S_G/S_T) \times 100 \tag{1}$$

where: S_G – the number of seeds germinated,
S_T – the total number of seeds.

Germination inhibition rate (GIR):

$$GIR\ (\%) = ((GP_C - GP_S)/ GP_C) \times 100 \quad (2)$$

where: GP_C – percentage of the control;
GP_S – percentage of the sample.

Growth test

After the germination test, tomato growth was monitored for a 30-day period in plastic pots at room temperature and natural light in the laboratory. Five seeds were moved into pots filled with sandy soil from the High School of Technology in Salé (0–20 cm deep). The pots were periodically irrigated with different concentrations of OMW (0%, 1%, 2%, 5%, 7%, and 10% (2–3 times per week). The length of the main stem and the number of leaves were the monitoring parameters. After 30 days of monitoring, the aerial parts were separated from the roots to measure the fresh weight of stems, leaves, and roots. Dry biomass was determined after oven drying at 70 °C to a constant mass and weighing with a precision balance.

Statistical analyses

Data were analysed by one-way ANOVA, where the factor was OMW concentration, and the influencing factors were germination percentage, germination inhibition rate, stem size, leaf number, and biomass. If the statistical difference was significant, multiple means were compared using Tukey's test. For all comparisons, differences were considered significant at the 5% probability level.

RESULTS

Characterization of olive mill wastewater

Physicochemical analysis revealed that OMW have an acidic pH (pH=5.32), due to the presence of organic acids such as phenolic and fatty acids [Achak and al., 2009; Chaari and al., 2015; Elabdouni and al., 2020; Shabir and al., 2023]. A fairly high electrical conductivity (EC =15.33 ms/cm) compared to 28.23 ms/cm found by [El Ghadraoui and al., 2021], this high value can be explained by the salting added to preserve the olives until crushing [Achak and al., 2008; Chaari and al., 2015; Elabdouni and al., 2020; Shabir and al., 2023]. This value belongs to the conductivity range (between 10 and 50 ms/cm) [Tsioulpas and al., 2002]. However, the polyphenol content (0.321 g/L) remains relatively low but is within the range of the literature (0.1 and 17.5 g/L) [Benaddi and al., 2022]. The OMW are rich in organic matter as shown by the values of COD (10560 mgO$_2$/l) and BOD$_5$ (410 mgO$_2$/l), these are relatively low to the amounts cited by other researchers [Bargougui and al., 2019] (Table 1). The total phenol content is about 0.321 g/l, it depends on the olive variety, its olive oil storage time of OMW and its extraction process [Sassi and al., 2006; Yaakoubi and Aghanchich, 2021; Zaier and al., 2017], this value is within the literature range of the literature (0.1 and 17.5 g/L) [Benaddi and al., 2022].

Germination test

Germination percentage of tomato seeds

The daily evolution of germination percentage of tomato seeds (*Solanum lycopersicum L*) treated with different concentrations of OMW as a function of time showed that germination starts from the first day of incubation, at 1 and 2%, including controls.

The germination percentage reached its maximum on the 3rd day (more than 80%) and then remained almost constant beyond this day. Regarding the 5% and 7% concentrations, we note a delay in the germination process, which starts only after the second day to be maintained to a maximum beyond the third day, with a fluctuation interval of 60% to 100% for 5%, whereas, beyond the seventh day, the fluctuation interval is included between 60% and 80%, for the 7% concentration. However, seed germination at 10% started only on the 3rd day as seen in the graph (Figure.1).

Table 1. Physicochemical characteristics of olive mill wastewater

Parametres	OMW raw
pH	5.32
Electrical conductivity (ms/cm)	15.33
Mineral matter (g/l)	47.91
Organic matter (g/l)	112.41
COD (mg O$_2$/l)	10560
BOD$_5$ (mg O$_2$/l)	410
Polyphenols (g/l)	0.321

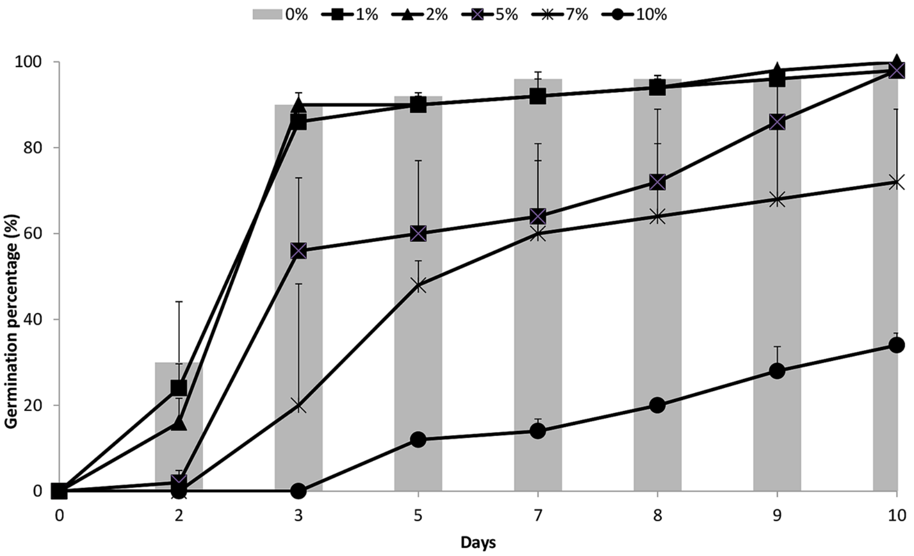

Figure 1. Cumulative germination percentage of tomato seeds as a function of time

Germination inhibition rate

The final germination percentage (FGP) of tomato seed after 10 days at concentrations of 0%, 1%, 2% and 5% ranges from 98% to 100%. However, this percentage reached 72% at a concentration of 7% and a marked decrease in germination at 10%, with only 34% of the seeds germinating. Fisher's test did not show significant differences between control GP concentrations of 1% ($p<1$), 2% ($p<1$), 5% ($p<1$), and 7% ($p<0.055$), this difference appeared significant between control and the concentration of 10% ($p<0.001$). The FGP decreases with increasing OMW concentrations ($r=-0.81$; $p<0.000$) while the average of inhibition rate (GIR) increases with increasing concentrations ($r=+0.81$; $p<0.000$), from 5%, where ($t=4.944$; $p<0.01$). On the other hand, a germination inhibition rate of 66% is observed at 10% concentration of OMW (Figure.2).

Plant growth

Effect of OMW on the length of the main stem

The length of the tomato plant shows growth over time, for all concentrations, but with a difference in growth rate. These speeds are maximum at concentrations 0% (81%), 1% (98%), and 2% (98%) then they decrease at concentrations 5% with a growth speed of 77%, the low growth speed (53%) is observed for concentration 10%. The ANOVA shows a very significant difference between the mean stem lengths

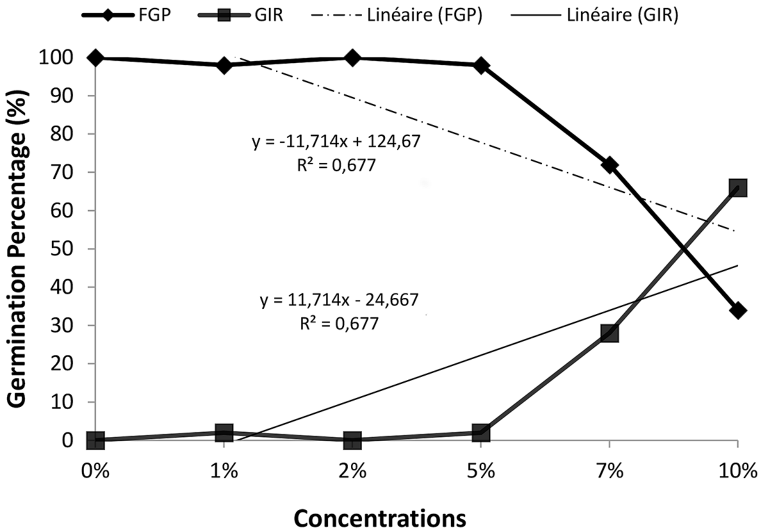

Figure 2. Final germination percentage (FGP) and germination inhibition rate (GIR) under OMW treatment

at the different concentrations (Fisher=17.484; $p<0.000$). However, the comparison of means using Tukey test shows that the 2% and 1% stimulate more growth in the length of the main stem compared to the control, with maximum lengths varying between 8.514±2.612cm at 2% and 7.522±1.660 cm at 1% (Table 2).

Effect of OMW on the number of leaves

The number of leaves after 30 days increased progressively with the difference in the speed of progression, where this speed was maximum (98%) at 2%. However, the ANOVA shows a very highly significant difference between the average number of leaves (Fisher=6.798; $p<0.000$) after 30 Days. After 30 days, the number of leaves gradually increased with the speed of progress, which reached a maximum of 2% (98%). However, ANOVA showed a very significant difference between the average number of leaves (Fisher = 6.798; $p<0.000$) after 30 days. Furthermore, the maximum number of leaves is (4.667±0.866 leaves) recorded at a concentration of 1%, and the minimum number is (2.8±0.789 leaves) at a concentration of 10% (Table 3).

Evaluation of the effect of OMW at different concentrations on the vegetative growth of tomato is expressed by the length of the main stem and the number of leaves. The Pearson test applied to the results obtained, namely the size of the main stem and the number of leaves of tomato plants, showed the presence of a highly significant positive correlation ($p<0.01$) with a correlation coefficient $r = 0.4999$.

Effects of OMW on tomato biomass

The evolution of the fresh and dry weight of the concentration of the stem as a function of OMW is decreasing (r=-0.85) with a minimum weight of 0.125 g at 10% and a maximum of 0.375 g at 5%. In fact, the ANOVA shows a significant difference between the average fresh weight (F=3.617; $p<0.05$). The maximum dry weight is 0.07 g at 5% and the minimal 0.025 g at 10% with (F=3.294; $p<0.089$) (Figure 3a). For leaf fresh weight, the trend was decreasing (r=-0.94) with a maximal fresh weight of 0.26 g at 2% and a minimum weight of 0.09 g at 10%. ANOVA did not show significant differences between mean fresh weight (F=1.424; $p<0.336$). The maximum dry weight is 0.07 g at 5% and the minimum is 0.025 g at 10% with (F=1.267; $p<0.385$) (Figure 3B). The evolution of the fresh and dry weight of the root is increasing between concentrations of 0% and 5% with r=-0.91, however, this evolution decreases from 5% with r=0.96, reaching a maximum fresh weight of 0.475 g at 5% and a minimum weight of 0.125 g. ANOVA showed a significant difference between average fresh weight (F=4.021; $p<0.05$). The maximum dry weight is 0.235 g at 5% and minimal 0.055 g at 10% with (F=16.341; $p<0.002$) (Figure 3C).

DISCUSSION

The valorization of OMW to improve the vegetative aspect showed quite variable germination percentage from one species to another in terms of the concentration used. Thus, in tomatoes, the

Table 2. One-way ANOVA "concentration effect" on tomato stem length as a function of days

Concentration	Days								R^2 (Speed of regression)
	0	9	11	13	20	22	26	30	
0%	0±0.00	1.26±0.859 (ab)	1.9±1.4 (bc)	2.56±1.111 b	3.588±0.770 b	4.187±1.193 b	4.5±1.291 abc	6.333±1.041 cd	0.976 (81%)
1%	0±0.00	1.7±0.837 (b)	2.85±0.224 (c)	4.12±1.215 c	5.4±1.542 c	6.222±1.380 c	6.566±1.640 cd	7.522±1.660 d	0.972 (98%)
2%	0±0.00	3.26±1.479 (c)	4.5±1.539 (d)	5.79±1.698 d	6.55±1.986 c	7.03±2.177 c	7.437±2.516 c	8.514±2.612 d	0.894 (98%)
5%	0±0.00	1±0.00 (ab)	2±0.00 (bc)	3.21±0.491 bc	3.74±0.837 b	4.39±1.265 b	5.085±1.033 bc	5.285±1.113 bc	0.969 (77%)
7%	0±0.00	0.2±0.2 (ab)	0.7±0.274 (ab)	1.1±0.95 ab	2.5±0.471 ab	3.17±0.472 ab	3.857±0.627 ab	4.028±0.663 ab	0.963 (66%)
10%	0±0.00	0±0.00 (a)	0±0.00 (a)	0±0.00 (a)	1.95±0.762 a	2.42±0.911 a	2.75±0.422 a	3.03±0.177 a	0.858 (53%)
F (*p* value)		11.298 (p<0.000)	18.463 (p<0.000)	38.552 (p<0.000)	21.326 (p<0.000)	16.941 (p<0.000)	12.525 (p<0.000)	17.484 (p<0.000)	

Note: values with the same letters do not have significant difference at $p<0.05$; *** – very highly significant difference, R^2 – determination coefficient.

Table 3. One-way ANOVA “concentration effect” on the number of leaves according to the days

Concentration	Jours							R^2 (speed of regression)
	0	11	13	20	22	26	30	
0%	0±0.00	0±0.00	1.111±1.054 c	2±0.866 ab	3.285±1.113 bc	3.8±0.837 b	4±0.000 abc	0.932 (68%)
1%	0±0.00	0±0.00	2.1±0.316 d	3.1±0.994 c	3.778±0.441 c	4±0.000 b	4.667±0.866 c	0.962 (77%)
2%	0±0.00	0±0.00	2±0.000 d	2.4±0.843 bc	3.8±0.422 c	3.875±0.354 b	4.142±0.378 bc	0.716 (92%)
5%	0±0.00	0±0.00	0.8±1.033 bc	2.2±0.632 bc	2.6±0.843 ab	3.428±0.787 b	3.428±0.787 abc	0.922 (60%)
7%	0±0.00	0±0.00	0.2±0.632 ab	1±1.054 a	2.2±1.135 a	3.143±1.069 ab	3.286±0.951 ab	0.874 (56%)
10%	0±0.00	0±0.00 a	0±0.00 a	1.8±0.632 ab	1.8±0.632 a	2.2±1.033 a	2.8±0.789 a	0.857 (45%)
F (*p* value)			17.956 (p<0.000)	6.629 (p<0.000)	10.546 (p<0.000)	6.907 (p<0.000)	6.798 (p<0.000)	

Note: values with the same letters do not have significant difference at $p<0.05$; *** – very highly significant difference, R^2 – coefficient of determination.

percentage of germination is therefore highest after 10 days (98%) at concentrations of 1%, 2% and 5%, with a delay for germination at concentrations greater than 5%. These GP are still higher than those achieved (69%), with dilution below 1/32 and close to a dilution of 1/64 and 1/128 in the same species (Tomato), with a delay in the germination process of 2 to 3 days in the germination process [El Herradi and al., 2017]. However, in *Vicia faba* beans, GP is 70% at concentrations of 1%, 5%, and 10%, with a delay of 2 days [Yaakoubi and Aghanchich, 2021]. Likewise, the GP in species such as *Triticum durum, Cichorium intybus, Hedysarum coronarium* and *Vicia faba* exceeds 80% with 10% concentration [Muscolo and al., 2010].

The delay in the germination process encountered in several species at concentrations is often attributed to either embryonic dormancy or impermeability of the seed coat to water [El Herradi and al., 2017; Yaakoubi and Aghanchich, 2021]. In this work, a dilution of OMWs greater than 7% inhibits tomato seed germination, the delay in the germination process encountered in several species at concentrations is often attributed to either embryonic dormancy or impermeability of the seed coat to water [El Herradi and al., 2017; Yaakoubi and Aghanchich, 2021]. In this work, a dilution of OMWs greater than 7% inhibits tomato seed germination, This is consistent with the results of Komilis and al. (2005) agreed that a 1/10 dilution of OMW completely abolished the phytotoxicity of tomato seeds and almost completely abolished the phytotoxicity of chicory seeds, in fact, dilution allows the reduction of phenols and organic acids and is accompanied by a reduction of nutrient salts, particulate matter, and organic matter content, minimizing the negative effects on plants and soil and preventing the clogging of the irrigation system [Komilis and al., 2005; Muscolo and al., 2010]. Similarly, a dilution of 1/8 (12.5%) reduced the inhibitory effect for radish, and cucumber, and phytotoxic for lettuce [Andreozzi and al., 2008]. Thus, the use of OMW with a dilution of 1/6 inhibited the germination of wheat seeds by 69.9% [Shabir and al., 2022], while, a 100% inhibition was observed in tomatoes at a concentration of 1/16 [El Herradi and al., 2017]. Many studies have attributed the inhibitory effect of OMWs at high concentrations on germination as a result of the toxic action of salinity, organic acids, and polyphenols [Komilis and al., 2005; Yaakoubi and Aghanchich, 2021; Zohaib and al., 2014]. Moreover, this variation in germination rates could be due, not only to the dilution rate, but probably to other factors such

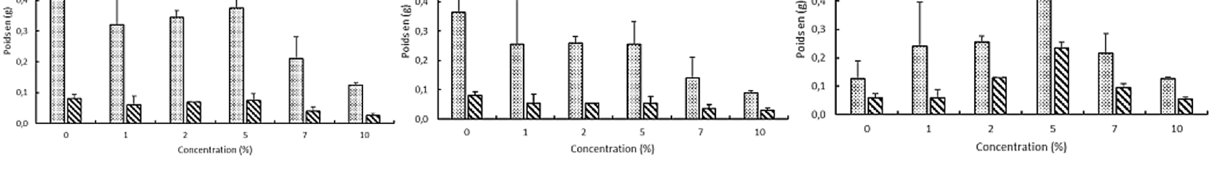

Figure 3. Effects of OMW on fresh and dry weight as a function of concentration, (a) stem, (b) leaf, (c) root

as COD, BOD, phenolic compounds content and electrical conductivity, and genotype [Andreozzi and al., 2008]. However, the work of Dakhli and al. (2021) in the Faba bean shows a reducing effect of growth according to concentrations. Indeed, the high salinity level could lead to a reduction of the plant's water uptake capacity by reducing the growth rate, the number of leaves, and roots, with time [Hasanuzzaman and al., 2013]. According to Houshia and al, (2019), the high level of acidity will be able to lead to reduced growth in tomatoes [Houshia and al., 2019]. Recent studies have shown that the irrigation of tomato plants by OMW did not cause toxicity symptoms in fruits and plants [de los Santos and al., 2021; El-Bassi and al., 2021]. On the other hand, our results show a significant effect of OMW on the vegetative growth of tomato plants. These results are comparable to those found by Khalil and al. (2021), where OMW positively affects, at doses of T5 (5 L/m^2) and T10 (10 L/m^2), the height of wheat plants. This could be attributed to the organic and nutritional loading of OMW, as well as the reduction in levels of phenols and other phytotoxic compounds as a result of the different dilutions [Fausto Cereti and al., 2004; Khalil and al., 2021; Rusan and Malkawi, 2016; Sidari and al., 2010]. This positive vegetative growth is attributed to the high amounts of protein, polysaccharides, humic acids, and macro and micro mineral elements present in these effluents [Faraloni and al., 2023; Sawalha and al., 2014].

CONCLUSIONS

OMW is a major environmental problem due to the uncontrolled discharge of residues from the olive industry into the environment. With these constraints, laws on waste management (28.00), the law on water (10–95), and the new strategy "Generation Green 2020–2030" encourage the protection of the environment as well as the prohibition of pollution.

The law also says that effluents cannot be discharged or spread to pollute the receiving environment. To take advantage of this, several techniques of recovery of these wastes are put in place especially in the field of agriculture namely the use of composting, fertilizer, and as irrigation water after dilution. From this perspective, this study showed that the use of OMW with a concentration of 5% stimulates the vegetative growth of tomatoes and the production of fresh biomass compared to the control. This can be considered as a solution to the OMW problems. Despite the efforts undertaken by the authorities of the field, it remains to multiply the efforts to establish effective and less expensive techniques of treatments and valorization, while insisting on the hard application of the regulations of environmental protection.

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