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# SEED GERMINATION AND MORPHO-PHYSIOLOGICAL CHARACTERIZATION OF THREE TOMATO (*LYCOPERSICON ESCULENTUM*) VARIETIES IRRIGATED WITH TREATED WASTEWATER

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**ABSTRACT**. The ability to reuse treated wastewater (TWW) would be of significant benefit to agriculture whilst at the same time providing a valuable water resource. This study concerned the effects of three various levels of treated wastewater (0%, 50% and 100% TWW) on seed germination and plant development of three different varieties of tomato (*Lycopersicon esculentum*) (*viz.* Toufan, Heinz and Bouzina). Irrigation with TWW persisted 15 days for the germination stage and 120 days for the growth and development stage. A control plot irrigated with a pure water (groundwater) was also set up in order to compare the seeds and the plants response to different concentrations of irrigation water. The final germination was expressed as a percentage of the total number of seeds in each treatment. This study has demonstrated that treated wastewater improves the germination in Heinz variety and had no effect on both Toufan and Bouzina varieties whether it is pure or diluted by half. For the growth stage, the statistical analysis showed that the Toufan variety has tolerated successfully the TWW irrigation with its high and moderate concentrations, and this by analysing all the morpho-physiological parameters studied in this work (leaves numbers, stem and root length and dry biomass, relative water content and rate water loss).

**Keywords:** wastewater reuse; water quality; experimental crops; plant growth; salinity; agriculture.

## INTRODUCTION

Sustainable water management is one of the main focuses of sustainable development, as water must satisfy

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current and future generations (Loucks and van Beek, 2005). In addition, agriculture is the largest consumer of water resources. Given the scarcity of conventional water resources, we cannot neglect the idea of reusing the enormous quantities of wastewater discharged into the wild or into the sea (Jones, 2014). With more concerns due to recent droughts, the use of this source of water for a useful purpose, such as agricultural and other plant production, is sensible, since most of them contain organic matter, as well as N, P and K compounds, nutrients that need to be added as fertilizers to achieve adequate yields providing these ingredients (Muyen *et al*., 2011). Farmers, particularly those in the continental regions, are interested in the use of wastewater in the measure where it has to pass a good treatment (Meneses *et al*., 2010). According to FAO (2010), treated wastewater is spread in over 20 million hectares of land worldwide. At the same time, in line with wastewater utilization practices, several advanced technology treatments have been built for agricultural and landscape irrigation, particularly in arid and semi-arid regions (Sato *et al*., 2013).

The recovery of treated wastewater (TWW) is considered as an essential component in the integrated water resources management policy (Larsen *et al.,* 2016). In fact, the richness of treated wastewater with nutrients will contribute to the recycling of these elements and the reduction of the excessive use of fertilizers (Mouhani *et al*., 2012). Currently, Algeria has 134 treatment stations, WWTP (waste water treatment plants) and lagoons, in operation with an installed capacity estimated at 12 million EQH (equivalent population) or 800 $hm^3$/year. In Algeria, wastewater will represent a very significant volume of almost two billion $m^3$, if the demand for water is completely satisfied at this time. With this volume, once purified, for ecological considerations or protection of water resources, will be highly appreciated as to its use by agriculture or industry (De Fraiture and Wichelns, 2010). The reuse of treated wastewater, especially for agricultural purposes, has become one of the main focuses of the strategy of the water resources sector in Algeria (Kellis *et al*., 2013).

Many research studies and projects have been accomplished on the practice and effects of wastewater as irrigation source on different crops (Pedrero *et al*., 2010; Malchi *et al*., 2014), by increasing the amount of minerals in the foliage of the plants (Bedbabis *et al*., 2010; Carbonell *et al*., 2011; Bañón *et al*., 2011).

The reuse of wastewater requires close coordination between the different structures involved in reuse operations at all levels. Tomato is included in the category of industrial crops allowed for this irrigation practice (Gatta *et al*., 2015). Here, we present the results of a diagnostic greenhouse study in which we investigated the effects of treated wastewater application on the germination and growth of three tomato (*Lycorpersicon esculentum*) varieties, commonly grown crops in Algeria.

The main aim of the study was to develop a rapid assessment methodology for evaluating treated wastewater effect, by investigating the toxicity of the wastewater to both seed germination and vegetative plant growth.

## MATERIAL AND METHODS

### Plant material, growth condition and treatment

Experiments were conducted in greenhouse of the Regional Enterprise of Rural Engineering (RERE Aures, Batna, Algeria) (Lat: 35°33'45.40"N; Long: 6°10'38.07"E). Treated wastewater (TWW) of the National Sanitation Office Maintenance Management Directorate Batna Unit WWTP-Timgad (Algeria) (Lat: 35°29'58.99"N; 6°28'20.19"E) was used in 2018 as irrigation water. Water quality parameters are provided in *Table 1*.

According to this table, the determination of nutrients shows relatively low concentrations of nitrates, nitrites and orthophosphate (the maxima in $mg.L^{-1}$ are, respectively, 3.4, 0.1 and 4.84), whereas the ammonium level was 2.75 $mg.L^{-1}$.

The TWW pollutants has demonstrated that oxygen demands (COD and BOD) reach low values (COD = 51 mg $O_2.L^{-1}$, BOD = 3.25 $mg.L^{-1}$). It is also the case for temperature, electrical conductivity (EC), salinity and pH. Heavy metal concentrations are under the detection limit.

Effluent waters were stored in 20-liter tanks. The TWW was used undiluted (100% TWW), and at concentrations of 50% TWW and 0% TWW (control: groundwater).

Dilutions were performed using groundwater. Irrigations have been performed in frequent intervals to create water stress on plants. Excess water was drained from the pot under free drainage conditions. The seeds of three tomato (*Lycopersicon esculentum* L.) varieties (*viz*. Toufan, Heinz and Bouzina) have been used and compared. The 1000-seed weight was between 25 and 28 g.

**Table 1 - Wastewater Treatment Annual Report for 2018 (Wastewater treatment plants WWTP-Timgad, Batna)**

| Parameters | Values |
|---|---|
| SM ($mg.L^{-1}$) | 1.65 ± 0.94 |
| BOD5 ($mg.L^{-1}$) | 3.25 ± 0.54 |
| COD ($O_2.L^{-1}$) | 51.0 ± 18.34 |
| N-$NH_4$ ($mg.L^{-1}$) | 2.75 ± 2.33 |
| N-$NO_2$ ($mg.L^{-1}$) | 0.10 ± 0.05 |
| N-$NO_3$ ($mg.L^{-1}$) | 3.40 ± 2.52 |
| $PO_4^{3-}$ ($mg.L^{-1}$) | 4.84 ± 3.30 |
| Dissolved $O_2$ | 4.83 ± 1.10 |
| Salinity ($\mu S.cm^{-1}$) | 0.43 ± 0.04 |
| Conductivity ($\mu S.cm^{-1}$) | 1133.5 ± 72.1 |
| Temperature (°C) | 15.7 ± 4.23 |
| pH | 7.56 ± 0.16 |

Suspended matter (MES), Biological oxygen demand (BOD5), Chemical oxygen demand (COD), Ammonium ($NH_4^+$), Nitrite ($NO_2^-$), Nitrate ($NO_3^-$), Orthophosphates ($PO_4^{3-}$).

### Seed germination parameter

The experiment was conducted at the Laboratory of Ecology and Environment Department, University of Batna 2, Algeria (Lat: 35°38'10.32"N; Long: 6°16'31.52"E).The sowing (four replicates of 25 seeds × three treatments × three varieties) was realized in Petri dishes of 10 cm diameter, papered with two layers of Whatman filter paper and soaked with 20 ml with the appropriate solution (50% and 100% of TWW) or groundwater for the control (0% TWW). Seeds were incubated under continuous dark at the laboratory temperature 25°C (± 2 °C).

The papers were changed with the same treatment each three days to prevent waste accumulation. The seeds were moistened with the appropriate solutions of TWW and kept wet throughout the 15 days of the experiment. The germination criterion was taken into account when radicle had pierced the tegument with 2 mm in length (Egley and Chandler, 1978).

The Petri dishes were arranged every two days, according to a randomized design to eliminate any effect of the position in the seed culture room.

Only the final germination percentage (FGP) for each tomato varieties and irrigation-treatment were calculated using the following formula:

$$FGP\ (\%) = \frac{\Sigma ni}{N} \times 100$$

where, *FGP* is final germination percentage, ni is the number of germinated seeds at final day of test, and *N* is the total number of incubated seeds per test (Côme, 1970).

**Morpho-physiological parameters**

Seeds were germinated in plastic pot (Top diameter: 28 cm; Bottom diameter: 20 cm; Height: 24 cm) containing 4.5 kg of mixed substrate (two volumes of sand mixed with one volume of compost) (EC = 1200 $Ohm.cm^{-1}$; pH = 5.0-6.5; NPK = 1.2 $kg.m^{-3}$; N = 30 $g.m^{-3}$; $P_2O_5$ = 25 $g.m^{-3}$; $K_2O$ = 50 $g.m^{-3}$) and arranged according to the method of complete randomized blocks with (four replicates × three treatments × three varieties) under greenhouse conditions. Sand was sieved at 2 mm to eliminate wastes and coarser material.

After 120 days of treatment, leaf, stem, and root samples were harvested from control and TWW-treated plants for estimation of various parameters.

Stem length (SL), root length (RL) and leaves number per plant (LNP) of four plants (n=4) from each treatment were recorded after 120 days of treatment. For measurement of dry weights, leaves, stems and roots were excised from control and wastewater-treated plants and the fresh weight was noted immediately. Later, they were wrapped in pre-weighed aluminium foils and kept in an incubator at 80°C for 48 h before the dry weight was recorded.

Leaf fresh weight (LFW) was immediately noted after sampling and subsequently immersed into distilled water for 8 h at room temperature.

Leaves were then blotted dry and leaf turgid weight (LTW) was taken prior to incubating at 80°C for 48 h. After incubation period, leaf dry weight (LDW) was also noted.

The leaf relative water content (RWC) was calculated using following formula (Barrs and Weatherley, 1962):

$$RWC\ (\%) = \frac{LFW - LDW}{LTW - LDW} \times 100$$

The rate water loss (RWL) was calculated using (Clarke *et al.*, 1989) formula:

$$RWL\ (mg/cm2.min) = \frac{(FW - FW2h)/DW}{LA \times 120}$$

where, *FW* = leaf fresh weight determined immediately after leaf harvesting; *FW2h* = leaf fresh weight measured after 120 min., under laboratory conditions; *DW* = leaves dry weight measured after drying in an oven at 50°C, for 2 h; LA = leaf area ($cm^2$), estimated by the method of Paul *et al.* (1979).

**Statistical analysis**

All the experiments were conducted with four replicates (n=4) and the results were expressed as mean ± standard deviation (SD). All the data were subjected to one-way (treatment) and two-way (treatment, varieties) analysis of variance (ANOVA) and Duncan's multiple-range test ($p < 0.05$) using SAS Version 9.0 (Statistical Analysis System) (2002) software.

## RESULTS AND DISCUSSION

**Seed germination**

*Fig. 1* showed the variation in seed germination rate of three tomato varieties (Heinz, Toufan and Bouzina) subjected to different treatments (three types of treated wastewater irrigation at different concentrations: 0%, 50% and 100%). This figure showed that the FGP was not negatively affected by TWW concentration. Indeed, the increase in TWW concentration improved the FGP of the variety Heinz, decreased slightly the FGP of Toufan at 50% TWW and had no effect on Bouzina seeds. We clearly observed the positive effect of irrigation with treated wastewater, compared to groundwater. The delay in germination differed between species and is likely due to variation in seed size, seed coat permeability, differential uptake of nutrients and toxins and metabolism.

The maximum germination rate was recorded at the 50% treatment level for both Toufan and Bouzina varieties, registering respective values of 97% and 95%. On the other hand, for the Heinz variety, the highest germination rate was recorded under 100% TWW irrigation with a value of 75% (*Fig. 1*). The analysis of two-factor variance (*Table 2*) showed a significant difference between the three treatments, for the germination rate parameter in both Heinz and Toufan varieties. However, Bouzina variety showed a similar way in the three treatments, justifying the non-significance of the result by recording germination rates fairly close.

The wastewater individually increased the biomass and NPK resulting in taller and leafier plants as compared to tap water (Farooq *et al.*, 2010). The plants survival in a given environment depends in part on their reaction at the germinal stage (Mansouri and Kheloufi, 2017) and becomes the most important factor for the continuity of the life cycle of the plant (Bewley *et al.*, 2012). Bedouh and Bekhouche (2014) reported that TWW treatment has a positive effect on onion (*Allium cepa*) seedlings emergence. These results indicated a tolerance of these seeds to the unfavorable conditions of the medium. According to Sharma (2012), the mechanisms of high catalase and peroxidase capacities in cotyledons contribute to the detoxification and restoration of thermodynamic equilibrium with the medium.

The results of Marzougui *et al.* (2018) showed that treated urban wastewater had a better contribution to the germination rate of hina (*Lawsonia inermis*). The causes of the variability of the germinative properties are numerous and depend mainly on the genetic heritage. Environmental factors may alter the expression of these genetic properties (Côme, 1970).

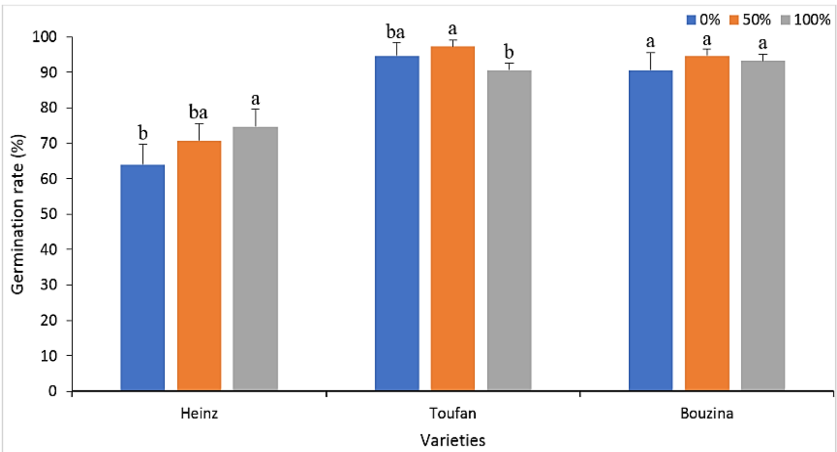


**Figure 1 - Effect of treated wastewater irrigation on final germination rate of three tomato varieties after 15 days of various levels of treatments. Means, in each box, with similar letters are not significantly different at the 5% probability level using Duncan's test.**

**Morphological traits**

According to *Fig. 2*, the leaves number and the root length for the three tomato varieties was not statistically influenced by the irrigation level. Indeed, the values are fairly close together where the lowest number of leaves was recorded in the Bouzina variety.

The SL was influenced by the type of treatment in the three varieties of tomato, where the most remarkable effect was noted in Bouzina and Heinz varieties *(Fig. 2)*. However, the change in stem length in Toufan variety was not influenced by the level of irrigation by recording fairly close average values (*Fig. 2*).

Overall, the number of leaves, stem length and root length are higher in the 50% and 100% TWW-irrigated Toufan variety (*Table 2, Fig. 2*). The first plant part interacts with soil and irrigation solution is the roots and it is almost inevitable that the crops are affected by salt or other mineral concentration (Kheloufi *et al.*, 2016; Kheloufi *et al.*, 2018). The reason that the root and shoot length are affected negatively by TWW is due to the inhibition of cytokinesis and cell expansion (Han *et al.*, 2011). The increase in osmotic pressure around the roots because of rhizosphere environment can also prevent water uptake by root and results with short root (Özkara *et al*., 2011).

**Plant dry biomass**

According to *Fig. 3*, no significant effect was observed on the dry biomass of the leaves, stems and roots of Toufan variety plants at the three treatment levels. In addition, this variety showed the highest values for dry biomass for the three organs studied.

**Table 2 - Mean comparison and analysis of variance effects of treated wastewater irrigation on FGP (final germination percentage), LN (leaves number), LDW (leaves dry weight), SL (stem length), SDW (stem dry weight), RL (root length), RDW (root dry weight), RWC (relative water content) and RWL (rate water loss)**

| Parameters | Sources of variation | Df | F | *p* | Duncan's multirange test |
|---|---|---|---|---|---|
| FGP | TRT | 2 | 4.16 | 0.0267 | Heinz$^{B}$ Toufan$^{A}$ Bouzina$^{A}$ |
| | VAR | 2 | 151.00 | <0.0001 | |
| | TRT × VAR | 4 | 3.84 | 0.0135 | |
| LN | TRT | 2 | 5.30 | 0.0115 | Heinz$^{A}$ Toufan$^{A}$ Bouzina$^{A}$ |
| | VAR | 2 | 1.46 | 0.2505 | |
| | TRT × VAR | 4 | 1.45 | 0.2461 | |
| LDW | TRT | 2 | 1.84 | 0.1785 | Heinz$^{B}$ Toufan$^{A}$ Bouzina$^{B}$ |
| | VAR | 2 | 5.02 | 0.0140 | |
| | TRT × VAR | 4 | 2.61 | 0.0577 | |
| SL | TRT | 2 | 7.01 | 0.0035 | Heinz$^{B}$ Toufan$^{A}$ Bouzina$^{C}$ |
| | VAR | 2 | 22.61 | <0.0001 | |
| | TRT × VAR | 4 | 3.65 | 0.0167 | |
| SDW | TRT | 2 | 3.60 | 0.0412 | Heinz$^{B}$ Toufan$^{A}$ Bouzina$^{B}$ |
| | VAR | 2 | 10.21 | 0.0005 | |
| | TRT × VAR | 4 | 3.85 | 0.0133 | |
| RL | TRT | 2 | 4.00 | 0.0301 | Heinz$^{A}$ Toufan$^{A}$ Bouzina$^{A}$ |
| | VAR | 2 | 0.53 | 0.5929 | |
| | TRT × VAR | 4 | 2.67 | 0.0534 | |
| RDW | TRT | 2 | 2.62 | 0.0913 | Heinz$^{A}$ Toufan$^{A}$ Bouzina$^{A}$ |
| | VAR | 2 | 0.81 | 0.4566 | |
| | TRT × VAR | 4 | 1.44 | 0.2490 | |
| RWC | TRT | 2 | 1.01 | 0.3779 | Heinz$^{B}$ Toufan$^{A}$ Bouzina$^{B}$ |
| | VAR | 2 | 21.80 | <0.0001 | |
| | TRT × VAR | 4 | 3.69 | 0.0161 | |
| RWL | TRT | 2 | 0.30 | 0.7456 | Heinz$^{A}$ Toufan$^{A}$ Bouzina$^{A}$ |
| | VAR | 2 | 1.47 | 0.2480 | |
| | TRT × VAR | 4 | 1.02 | 0.4128 | |

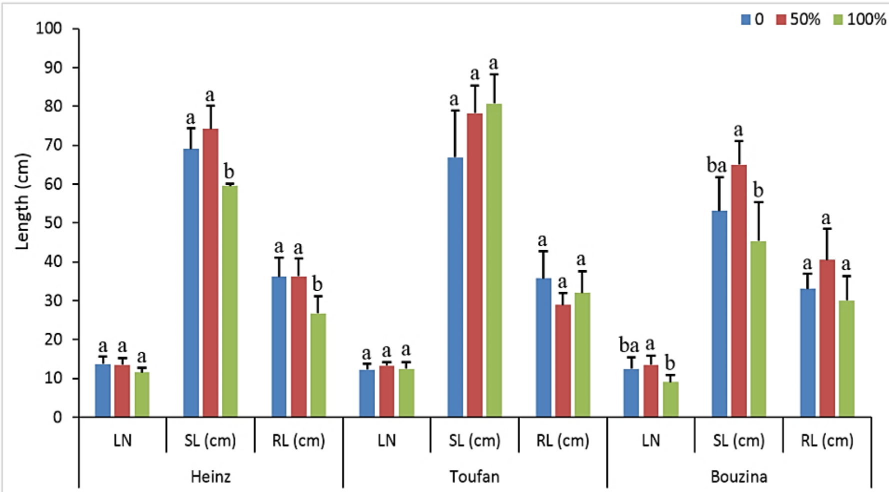


**Figure 2 - Effect of treated wastewater irrigation on leaves number (LN), stem length (SL) and root length (RL) of three tomato varieties after 120 days of various levels of treatments. Means, in each box, with similar letters are not significantly different at the 5% probability level using Duncan's test.**

According to Bedouh (2012), the production of biomass is greater in plants watered by TWW than those of the controls. According to *Fig. 3*, TWW irrigation seems to significantly affect the dry biomass of Bouzina variety plants. In fact, 100% TWW irrigation reduced this biomass by 80% in the leaves, 72% in the stems and 72% in the roots, compared to control. However, no effect was observed by 50% TWW irrigation. For Heinz variety, the only effect of TWW irrigation was observed on SDW (*Fig. 3*). The increase in TWW concentration decreased the dry biomass by 65% at 100% TWW.

It is not yet known which components of the wastewater are likely to be responsible for the chronic phytotoxicity observed (Michael-Kordatou *et al.*, 2015). However, there is some evidence suggesting that sodium (Netzer *et al.*, 2014), ethanol (Ge *et al.*, 2012) and polyphenols (Barbera *et al.*, 2013) are all potentially phytotoxic constituents. The detailed analysis of inorganic components in the root and shoot tissue provided some insights (Tangahu *et al.*, 2013). Research further revealed that salinity plays an important role in the quality of wastewater. Indeed, salinity not only affects seed germination (Mansouri and Kheloufi, 2017), but also significantly reduced the subsequent growth of seedlings (Shelef *et al.*, 2013). This reduction in seedling growth is either due to accumulation of mineral ions in their cell sap or because of the failure of sub-cellular organelles to adjust high osmotic potential of cell sap (Lisar *et al.*, 2012).

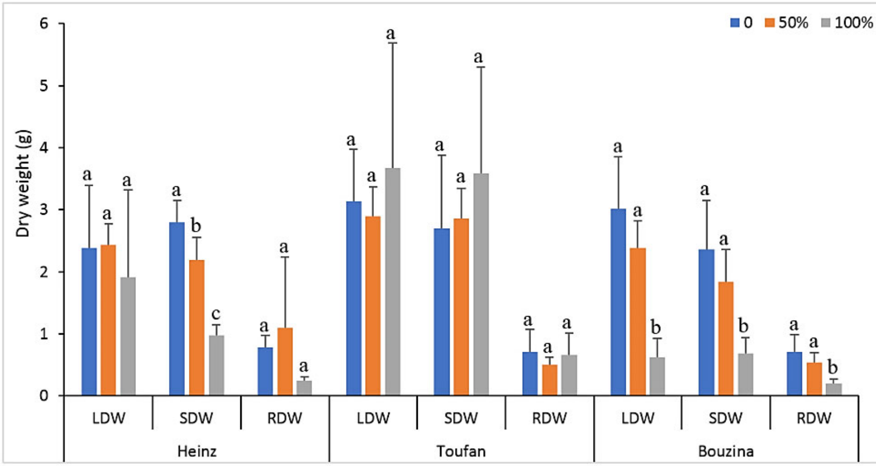


**Figure 3 - Effect of wastewater irrigation on leaves dry weight (LDW), stem dry weight (SDW) and root dry weight (RDW) of three tomato varieties after 120 days of various levels of treatments. Means, in each box, with similar letters are not significantly different at the 5% probability level using Duncan's test.**

**Physiological traits**

According to *Fig. 4*, no significant effect was observed on the RWC of the leaves of Toufan variety plants at the three treatment levels. In addition, this variety showed the highest values of RWC, compared to Heinz and Bouzina varieties. Indeed, TWW irrigation did not produce a notable change in the water content of the plants leaves, indicating a relatively high resistance as well to dehydration, which will certainly contribute to some degree of TWW tolerance.

For Bouzina variety, the RWC decreased slightly with increase in TWW at 50% (*Fig. 4*). However, the RWC increases under 100% TWW with a mean value similar to the control. The opposite phenomenon was observed in the Heinz variety where RWC decreases only under 100% TWW recording a loss of 11%, compared to control (*Fig. 4*).

According to *Fig. 5*, no significant effect was observed on the RWL of the leaves of both Heinz and Toufan varieties at the three treatment levels. Indeed, TWW irrigation did not produce a notable change in the water loss of the plants leaves, which will certainly contribute to some degree of TWW tolerance.

This improvement could be due to stomatal closure, it will typically induce the limitation of gas exchange and alter the rate of photosynthesis and metabolism (Wang and Nii, 2000).

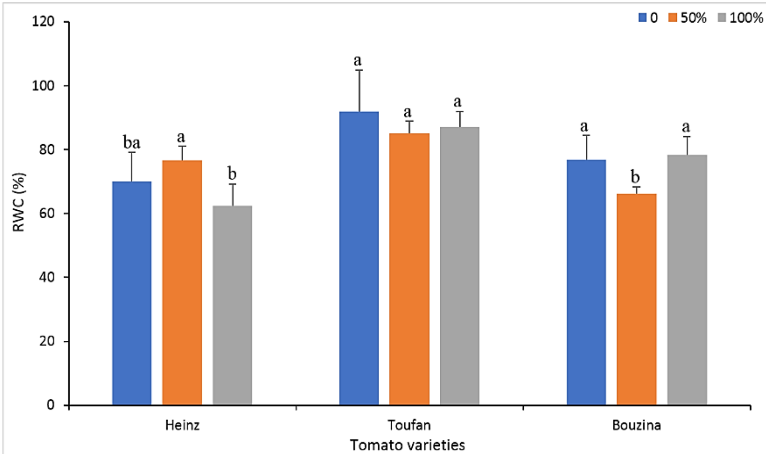


**Figure 4 - Effect of treated wastewater irrigation on relative water content (RWC) from the leaves of three tomato varieties after 120 days of various levels of treatments. Means, in each box, with similar letters are not significantly different at the 5% probability level using Duncan's test.**

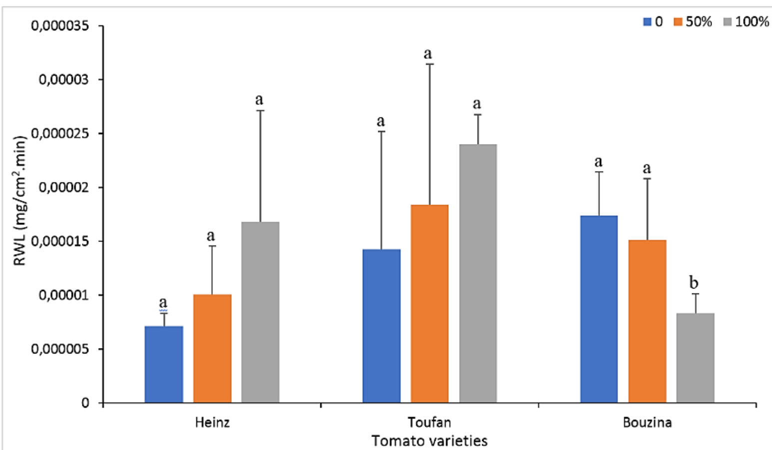


**Figure 5 - Effect of treated wastewater irrigation on the rate water loss (RWL) from the leaves of three tomato varieties after 120 days of various levels of treatments. Means, in each box, with similar letters are not significantly different at the 5% probability level using Duncan's test.**

RWL and RWC have been suggested as screening techniques to identify genotypes under drought stress (Gunes *et al.*, 2008). Indeed, these traits are direct measurements of plant water deficit and good criterions for the selection of drought tolerant plants (Farshadfar *et al.*, 2001). This tolerance is also depending on the plant capacity to accumulate many minerals (NaCl or heavy metals) in the vacuole, to avoid reaching toxic concentrations in the cytoplasm, a mechanism that is especially efficient in some succulent, highly tolerant dicotyledonous halophytes (Ruiz and Blumwald, 2002). On the other hand, the only tomato variety affected by TWW irrigation was Bouzina variety whose leaves recorded a reduced RWL to 53%, compared to the control (*Fig. 5*).

## CONCLUSIONS

This study has demonstrated that treated wastewater improves the germination in Heinz variety and had no effect on both Toufan and Bouzina varieties whether it is pure or diluted by half. The statistical analysis showed that the Toufan variety has tolerated successfully the TWW irrigation with its high and moderate concentrations, and this by analysing all the morpho-physiological parameters studied in this work. The use of TWW with dilution is therefore practically limited based on freshwater availability and cost, in conjunction with overall volumes of treated wastewater production. Using the TWW as irrigation water would be a great help and we will economize groundwater even if we have to use a diluted TWW at a low concentration.

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