

Effect of treated wastewater irrigation on physiological and agronomic properties of beans *Vicia faba*

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Abstract—The current study investigated the effect of two doses (50%, and 100 %) of treated wastewater (TWW) on biometric and physiologic parameters of *Vicia faba* beans after 40 days of exposure.

Our data showed a decrease in shoots and roots length and weight in plants amended with TWW. Moreover, a significant decrease in Chlorophyll 'a', 'b' and carotene content was observed in plants irrigated with 100% of TWW.

These findings provided new insights on TWW reuse which can cause different types of stress as it may affect the development of cultivated crops.

Keywords—Treated wastewater, *Vicia faba*, growth, chlorophyll, carotene.

I. INTRODUCTION

Tunisia is a country where the agricultural sector is a priority representing its most important natural resource. However, the annual rainfall is very irregular both in space and time and almost this country belongs to arid and semi-arid climate [1]. In arid and semi-arid regions, variations in rainfall accompanied by successive periods of drought generate undesirable impacts on water availability [2].

In Tunisia, the reuse of treated wastewater (TWW) is part of freshwater resources mobilization strategy and sustainable development of water resources [3]. Nevertheless, once properly treated, wastewater could replace freshwater and decrease this pressure on natural resources to be conserved for other purposes. However, from a quantitative point of view, wastewater is a source

of water always available. Indeed, TWW can ensure the balance of the natural water cycle and preserve resources by reducing harmful discharges into the natural environment [4]. On the other hand, and as has been reported by numerous study the TWW reuse could cause harmful effects on soils and even on living beings such as plant, invertebrates and microorganisms [5,6,7,8,9].

In the end of the past century the use of microbial, animal and human cell culture for toxicity evaluation [10,11,12] have been replaced using animal and vegetable bioindicators. Numerous international studies supported by United Nations Environment Program (UNEP), World Health Organization (WHO) and US Environmental Protection Agency (US-EPA) have validated plant-based bioassays for toxicity monitoring [13,14,15].

Vicia faba is commonly used as a model for cytological, physiological and toxicological studies [16,17,18,19] for many reasons such as its availability around the year, easy to cultivate and does not require sterile conditions. This plant could be used for toxicity assessment of various contaminants in soils i.e. heavy metals, aromatic compounds, pesticides etc.

Vicia faba is one of the oldest domesticated food legumes. Its importance in terms of food and agriculture is reflected by the occupied area worldwide (3.6 million hectares) in more than 50 countries and gives a total production of 4 million tons per year.

Many studies have investigated the effect of TWW on agronomic and physiological aspect of numerous vegetables and improve that those effluent constitute a

reliable source of nutrients i.e. (nitrogen, phosphorus, potassium) and organic matter that enhance soil fertility and productivity [20,21,22,23,24]. However, the chemical composition of TWW could influence vegetable growth, uneven fruit maturity and quality and quantity of yields due to the potential presence of heavy metals, surfactants and pharmaceuticals [25]. The metallic and salt stress have gained an increasing attention, and this was reported by several studies [26,27,28,29,30] which founded that growth factors and physiological properties of many plants are affected when exposed to wide varieties of contaminants that can potentially exist in TWW.

In this context, the current study was, therefore, carried out to evaluate the effect of municipal TWW irrigation on agronomic (growth dynamic properties) and physiological properties (chlorophyll and carotenoid contents) of *Vicia faba* plants.

II. MATERIALS AND METHODS

2.1. Soil sampling:

The soils used for this research were collected from an organic farming plot in the region of Chott-Mariem. The soils were sampled from the depth of 0-15 cm. Before use, samples were air-dried and crushed to pass a (<2 mm) screen.

2.2. Water sampling:

Secondary TWW have been collected in glass bottles from wastewater treatment plant of Northern Sousse, Tunisia, managed by the National Office of Sanitation (ONAS).

2.3. Experimental design:

Dry Certified seeds of beans (*V. faba* *Aguadulce*) obtained from local production were germinated on moistened filter paper at 22°C, when the primary roots were about 2–3-cm long, the seedlings were transplanted in the containers containing 1 kg of soils. Before transplantation soils were moistened with deionized water (Control), diluted TWW (50%) and TWW (100%) brought to 70 % of its holding capacity and this was maintained during the experimentation, five replicates per condition were used.

2.4. Growth measurements:

Five replicates were taken for each treatment were used to calculate the mean of each measurement. Plants were collected after 40 days of exposure to three conditions including control one. The measurements taken were the following:

- Length of the root and shoot system.
- Fresh weights of the root and shoot system
- Number of nodes.

2.5. Chemical content: Photosynthetic pigments

For this purpose, 1g of fresh leaves, was extracted by grinding in a mortar using 20 ml 80% acetone, with small amount of pure (Silica Quartz), and 0.5 g calcium

carbonate to equalize the cellular sap acidity. The extract was filtered and collected in Eppendorf's tubes.

The optical density (DO) of the extract was measured at wave lengths 663, 645, and 440.5 nm [31] to estimate chlorophyll 'a' and 'b', and carotenes respectively, using a Spectrophotometer (VWR-UV-3100-PC) and a vitreous cell (thickness of photo route 1 cm). Three replicates were used for each treatment, and the amount of pigment present in each sample was calculated according to the following equations:

- mg Chlorophyll "a" / g-tissue
= $12.7 \cdot DO_{663} - 2.69 \cdot DO_{663} \cdot v / (w \cdot 1000)$

- mg Chlorophyll "b" / g-tissue
= $29.9 \cdot DO_{663} - 4.68 \cdot DO_{663} \cdot v / (w \cdot 1000)$

- mg Carotenoids / g-tissue
= $46.95 \cdot DO_{440.5} \cdot (DO_{440.5} - 0.268 \cdot \text{Chlorophyll "a"} + \text{"b"})$

Whereas, W, the fresh weight by grams for extracted tissue; V, the final size of the extract in 80% acetone; DO, optical density at specific wave length.

2.6. Statistical analysis:

The non-parametric Mann–Whitney U-test was used to compare the data from plants exposed to 50% and 100% of TWW with data from the control soil (irrigated with deionized water).

III. RESULTS

The difference of shoots and roots weight of *V. faba* beans after 40 days of exposure to 50% and 100% of TWW is given in **Figure 1 a and b**. Results showed a significant variation between treatments. Indeed, shoots weight (**Fig.1a**) decreased significantly comparing to control plants irrigated with fresh water with a value of $17,93 \pm 0,75$ g.

However the root weight (**Fig.1b**) increased significantly in plants exposed to soils irrigated with diluted TWW ($8,16 \pm 0,23$ g). In contrast, a slight decrease was recorded in root's weight of plants exposed to 100% which reaches $7,6 \pm 0,2$ g.

On the other hand, **Figure 2 a and b** illustrate the length variation of faba bean's plant shoot and root after 40 days of exposure to 50% and 100% of TWW.

Accurately to weight variation, the length of shoots (**Fig.2a**) decrease progressively with TWW dose to reach $51,33 \pm 4,6$ mm in plants exposed to 100 % of TWW.

Thus, roots length (**Fig.2b**) decreased significantly in plants exposed to 50 % of TWW and reach $15,66 \pm 1,15$ mm which represents approximately 50% of control mean ($28 \pm 3,46$ mm).

Finally, the number of nodes in *V.faba* plants after exposure to TWW are shown in **Figure 3**, whatever, the number of nodes didn't show any significant changes between treatments, it's almost the same in all the experiments.

Figure 4 a and **b** reported the effect of 40 days of irrigation with two doses (50% and 100%) of TWW on chlorophyll "a" and "b". It was noticed that chlorophyll "a" content decrease significantly in the plants exposed to 50% of TWW to reach $0,6 \pm 0,21$ mg/g-fresh tissue compared to control plant where the content was $1,37 \pm 0,04$ mg/g-fresh tissue.

However, the concentration of chlorophyll "b" (**Fig.4b**) decreased by the increasing of TWW dose reaching its lowest $0,61 \pm 0,09$ and $0,77 \pm 0,03$ mg/g-fresh tissue when exposed respectively to 50% and 100% of TWW.

By following carotenoid content after exposure of bean plants to TWW it appears from results illustrated in **Fig.5** that TWW irrigation inhibits the carotenes formation and this was clear in plants exposed to 100% of TWW where means reach its minimum at $0,253 \pm 0,001$ mg/g-fresh tissue.

IV. DISCUSSION

In our study, TWW application on *Vicia faba* beans for 40 days was assessed through the measurement of biometric parameters which were modified after exposure to 50% and 100% of TWW.

Under exposure to numerous pollutants that can reach soils through TWW reuse in irrigation [32,33,34,35], plants can be subject to different types of stress mainly metallic and salt stress.

Globally, a decrease of the length and weight of the shoots and roots was recorded by increasing TWW dose. This decrease could be related to the high amount of salts present in TWW as reported by [36,37,38,39,40,41] who assessed the effect of salts on different plants and they found that it could cause several changes through negative effects on photosynthesis process, changes in enzymatic activity, decrease on the carbohydrates level and growth hormones.

Otherwise, metal content in TWW can exert an inhibitory effect on growth parameters, then they are strongly poisonous to the metabolic activities. However, an exceeded dose of heavy metals such as (Cd, Zn, Pb, Cr...) could cause phytotoxicity and this was proved by many authors [42,43,44,45].

Interestingly, chlorophyll is a clue element for plant's life which contributes to ATP production from the sun's light energy, it is indeed a good biomarker to assess plant's state under stress or exposure to toxics.

Our results regarding a decrease in chlorophyll 'a', 'b' are in concordance with several studies [46,41] which reported that salinity lead to the decrease of chlorophyll rates in barely and beans plant. The second factor that may influence photosynthetic process in *V.faba* beans is the trace elements uptake and this was by inhibiting

chlorophyll biosynthesis and reducing the activity of enzymes involved in CO₂ fixation [47,48,45].

Indeed, in stressed plants the carbon metabolism seems to be inhibited, then, in general the amount of amino acids was lower than in normal plants as proved by Gadallah, 1999 and this was due to the inhibition of amino acids incorporation into proteins under salt/metal stress.

As a part of national strategy to face water scarcity and to save freshwater resources, TWW constitute a sustainable way to manage water resources in the Mediterranean arid and semi-arid regions. But, like every strategy with all its positive effects (i.e. natural fertilizer, source of nutrients, availability), it presents many undesirable effects mainly (heavy metals, high amount of NA⁺ and Cl⁻ cations, pathogens...).

However, this can obviously affect normal development of plants and functional properties of soils receiving this non-conventional water. Moreover, many studies have been assessed the effect of TWW reuse on soils, plants and soil organisms [50,51,52,53,54,55] and in most cases they found that those effluent modify the physicochemical properties of soils, and physiological properties of plants and other organisms.

V. CONCLUSION

Our study showed that TWW reuse affect strongly growth and physiological parameters of *Vicia faba* beans, and this by decreasing shoots and roots length and weight than chlorophyll 'a' and 'b' content. Results also highlighted the effect of TWW on carotene content which decreased after 40 days of exposure.

Overall, TWW reuse as an alternative to save freshwater resources, it could be a good way ensuring the transfer of nutrients, organic matter and minerals in soils.

But, if these effluents are not subject to a periodic control, they can be a source of pathogens and potentially hazardous chemical substances (salts, heavy metals and surfactants), accumulated in soils, then as a result unfavorable effects on crop quality and productivity.

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An acknowledgement section may be presented after the conclusion, if desired.

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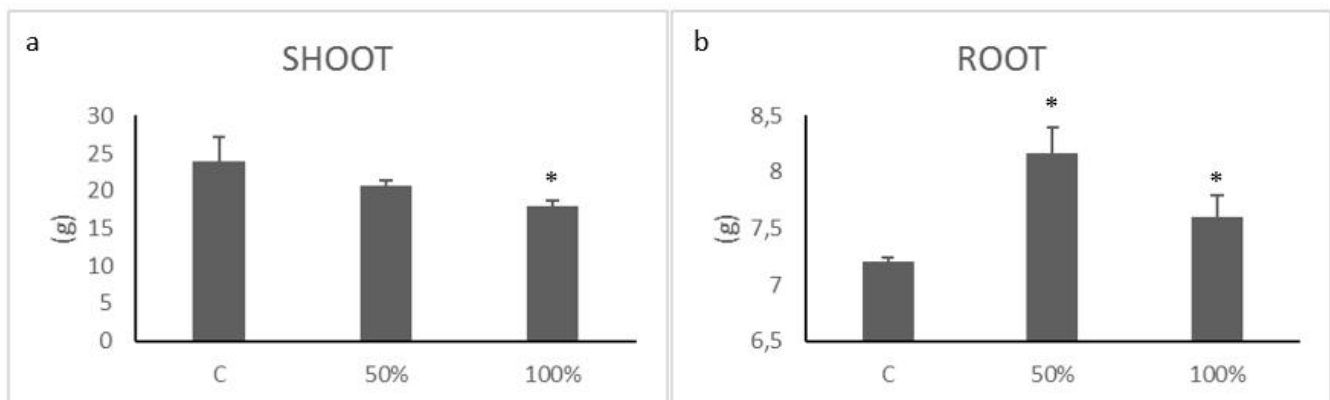


Fig.1: Effect of TWW irrigation (C: control, Diluted TWW: 50% and TWW: 100%) on *Vicia faba* (a) shoots and (b) roots weight after 40 days of exposure. Results represent the Mean \pm SD of at least 5 replicates. (*) Statistically significant differences ($p < 0.05$) comparing to control.

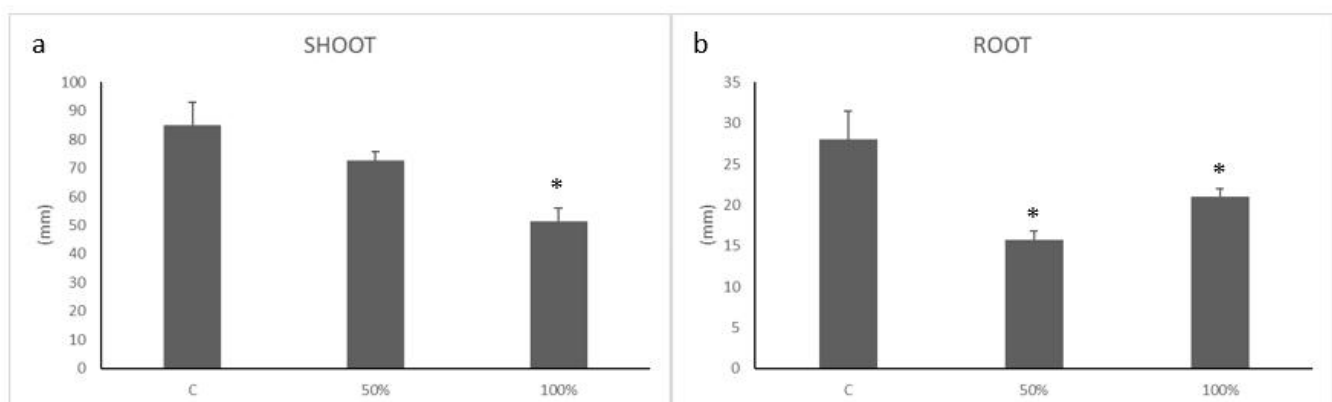


Fig.2: Effect of TWW irrigation (C: control, Diluted TWW: 50% and TWW: 100%) on *Vicia faba* (a) shoots and (b) roots length after 40 days of exposure. Results represent the Mean \pm SD of at least 5 replicates. (*) Statistically significant differences ($p < 0.05$) comparing to control.

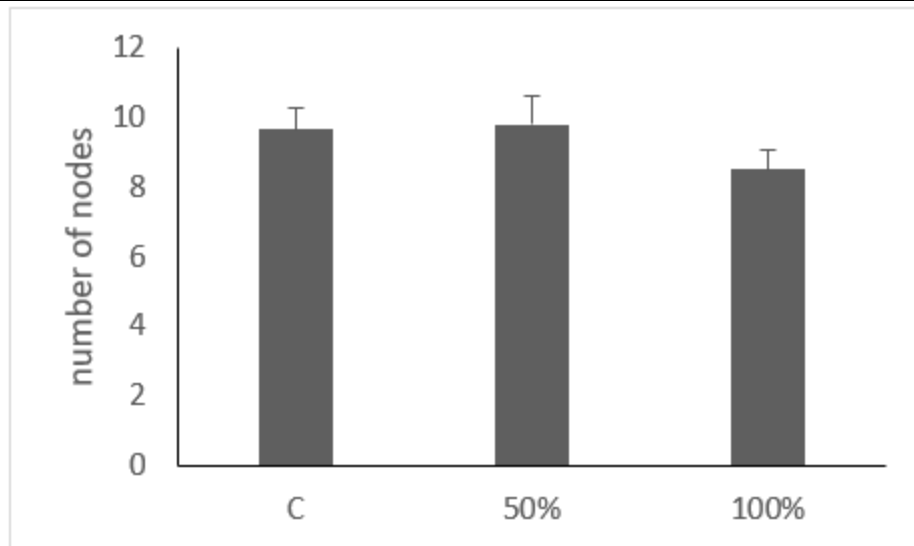


Fig.3: Effect of TWW irrigation (C: control, Diluted TWW: 50% and TWW: 100%) on the number of nodes of *Vicia faba* after 40 days of exposure. Results represent the Mean \pm SD of at least 5 replicates. (*) Statistically significant differences ($p < 0.05$) comparing to control.

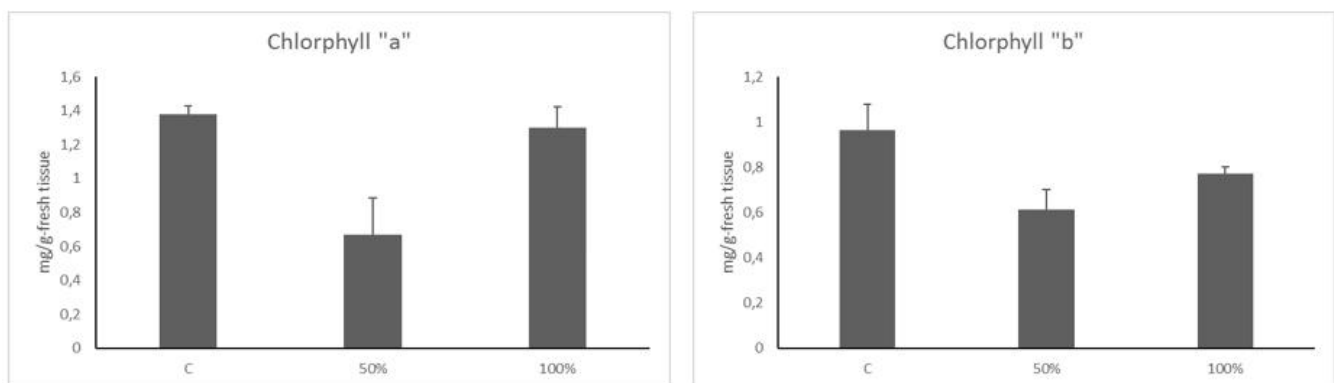


Fig.4: Effect of TWW irrigation (C: control, Diluted TWW: 50% and TWW: 100%) on (a) chlorophyll 'a' and (b) chlorophyll 'b' content of *Vicia faba* after 40 days of exposure. Results represent the Mean \pm SD of at least 5 replicates. (*) Statistically significant differences ($p < 0.05$) comparing to control.

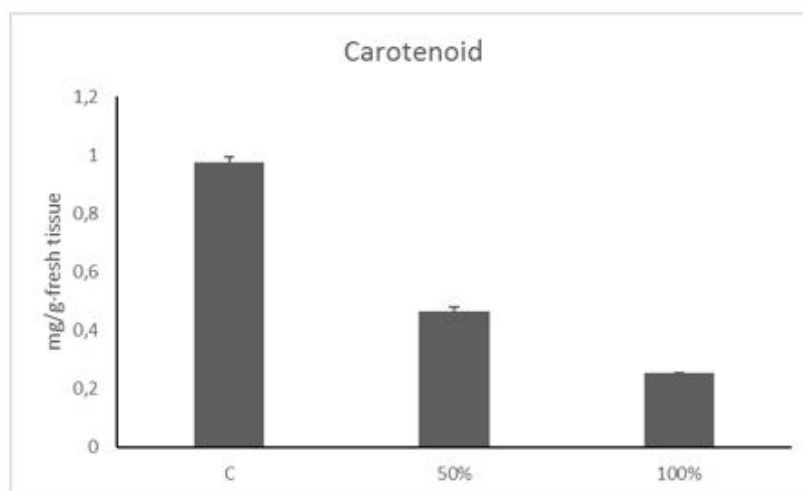


Fig.5: Effect of TWW irrigation (C: control, Diluted TWW: 50% and TWW: 100%) on carotene content of *Vicia faba* after 40 days of exposure. Results represent the Mean \pm SD of at least 5 replicates. (*) Statistically significant differences ($p < 0.05$) comparing to control.