Bio-fertilizers issued from anaerobic digestion for growing tomatoes under irrigation by treated wastewater: Targeting circular economy concept

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Dear Dr Amir,

I am pleased to tell you that your work has now been accepted for publication in International Journal of Environmental Science and Technology.

Thank you for submitting your work to this journal

With kind regards
M. Abbaspour
Editor-in-Chief
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Abstract

Tomatoes (*Solanum lycopersicum*) plant were provided with bio-fertilizers issued from anaerobic digestion of olive mill wastewater without and with 1%, 5% of phosphate residues in mesophilic conditions for 25 days. 1% of raw substrates (OMW raw; OMW+1%PR raw; Olive mill wastewater+5%Phosphate residues raw; and phosphate residues) and digestates (Olive mill wastewater digestate, Olive mill wastewater+1%Phosphate residues digestate and olive mill wastewater+5%Phosphate residues digestate) was provided fortnightly to the plants. Reclaimed water from a wastewater treatment plant located in the study site was used for automatically controlled irrigation. It contained a low level of chemical fertilizers to compare tomato plant growth, leaf analysis, steam water potential, production yield, and fruit quality results to plants fed with bio-fertilizers. Generally, parameters and results were progressively increased during the growing and harvesting stage, which refer to the essential elements that cover the plant's needs. Plants fed with biofertilizers showed the most extended plant height (Olive mill wastewater+5% Phosphate residues raw), and the best accumulation of essential elements in leaves (Olive mill wastewater+1% Phosphate residues digestate and Olive mill wastewater+5%Phosphate residues digestate). The maximum average fruit weight per treatment (35.5 g) was obtained when applying the digestates mixture of Olive mill wastewater raw and Olive mill wastewater+5% Phosphate residues. The maximum yield production per plant was obtained when applying phosphates residues. Biofertilizers (digestates) showed good performances, high fruit quality and perfect tomato yield production compared to the control plants. Results obtained during this study are considered promising regarding environmental framework. However, this study was done in a lab-scale and needs to be applied in a large-scale to provide more data on the effectiveness of the digestates application. It is also recommended to apply these biofertilizers on different crops and various soils for a better evaluation.

Keywords: Olive mill wastewater, phosphate residues, reclaimed water, wastewater, biological treatment.

1. Introduction

Environmental governance faces new challenges such as climate change, water scarcity, waste management, energy, and natural resource depletion. These challenges are threatening the ecosystem and human safety, but water scarcity seems to be the most dangerous problem because of the increasing population in the world and, consequently, raise demand on freshwater, while water resources are minimal (Wiek and Larson, 2012; Arenas-sánchez et al., 2016). It has been reported in the UN World Water Development Report (UNESCO) that 340 million people suffered from water scarcity (Almer et al., 2017). Water scarcity has increased in many regions since 1970 and will continue in the future due to rapid population growth, accelerated economy and agricultural water consumption (Arenas-sánchez et al., 2016). The Middle East and Mediterranean regions are the most countries suffering from water scarcity. At the same time, they are considered under high or extremely high water stress, where 56 % of the available water is used in irrigation (Creel and Souza, 2002; Carlo et al., 2018). Generally, it has been reported that more than 70% of fresh water is used only in agriculture, and the quantity is still increasing as a result of economic and population growth (Voulvoulis, 2018). Therefore, treated wastewater use in agriculture is one of the best alternative strategies that have numerous benefits, such as reducing pressure on freshwater, reducing chemical fertilizers, protecting of natural resources instead of dumping wastewater into rivers and agricultural lands (Aziz and Farissi, 2014; Carlo et al., 2018; Voulvoulis, 2018).

On the other hand, organic waste quantity reached alarming values due to human activities. For example, the World Bank estimated 2,2 billion tons of municipal organic waste is produced each year all over the world. The Mediterranean region is considered the highest olive oil producer by 95 % of the global amount (Türkekul et al., 2010; Zafeiriou et al., 2012; Killi and Kavd, 2013). Consequently, this industry results in tremendous waste called olive mill wastewater (OMW), which is considered the most phytotoxic organic waste (Dermeche et al., 2013). In Morocco, OMW is dumped in large quantities without any treatment, and therefore has a negative impact on natural resources and the ecosystem. Besides, Morocco has the largest phosphate reserve and is considered the second largest phosphate producer after the United States and the first exporter of phosphate and its derivatives (Aroussy et al., 2016; Benbrik et al., 2020; Haddaji et al., 2021). This production is accompanied by large quantities of phosphate waste (waste rock, phosphate sludge and tailings) that present a serious threat to the environment. Air pollution, agricultural land deterioration and large quantities of stocks that modify an area's landscape are the major environmental damages of phosphate waste (Hakkou et al., 2016). Thus, waste management and new strategies are essential to reduce all kinds of waste's negative impact.

One of the best methods to treat organic waste is anaerobic digestion (AD), a biological process consisting of biodegradation of organic matter by different microbial communities under anaerobic conditions (Siddique and Wahid, 2018; Tallou et al., 2020). This technique is very effective because it results in valuable by-products in terms of energy and agricultural profitability. AD generates biogaz that can produce heat and electricity, while the solid and liquid residues called digestate are considered rich in micro and macro-nutrients and can be used as bio-fertilizer in agriculture (Siddique and Wahid, 2018; Carfagno et al., 2019). Bio-fertilizers cannot bring all the essential elements that plants need to grow and produce fruits. Therefore, bio-fertilizers from organic waste can be coupled with chemical fertilizers to reduce the cost and the negative effects of synthetic fertilizers on soil and human health.

Furthermore, bio-fertilizers are eco-friendly, cheap, sustainable and have numerous advantages comparing to chemical fertilizers. They improve soil fertility and enhance plant growth due to their biological properties and nutrient contents (Garcia-gonzalez and Sommerfeld, 2016; Zaffar et al., 2020). In contrast, chemical fertilizers negatively affected the environment such as eutrophication, soil contamination and human health problems (Koh. Rae-Hyun and Song, 2007; Comer et al., 2019). Contrary to the chemical fertilizers, biofertilizers could synchronize the release of nutrients with plant needs due to the progressive mineralization of organic matter. They are promoted to harvest the naturally available biological system of nutrient mobilization. (Akhtar et al., 2020; Riaz et al., 2020) There are few research studies on bio-fertilizers application in agriculture. Therefore, this thematic needs to be systematically and deeply studied (Lili et al., 2016).

Several authors examined the agronomic potential of digestates issued from anaerobic digestion process. For example, (Cristina et al., 2020) used two liquid and two solid anaerobic digestates from treated sewage sludge. These digestates were applied on tomato plants (*Solanum lycopersicum*) to evaluate the fertilizing quality during three months. Results showed an enhancement of growth parameters and no phytotoxic impact was observed. In addition, soil properties and tomato leaves contents showed an enrichment in essential elements. Other research papers (Sierra et al., 2007), presented beneficial results of olive mill wastewater application on soils where the soil fertility increased due to phosphorous, nitrogen, and other essential elements. However, nitrogen immobilization, increase in phenolic compounds and salinity concentration have been observed. In their study (El-Bassi et al., 2021) converted olive mill wastewater into biochar using pyrolysis and then used as an organic amendment for soil. It was highlighted that biochar from olive mill wastewater supplied tomato plants during the crop season with all important nutrients, and therefore, the biochar produced has ideal potential as biofertilizer. Generally, biofertilizers application can be of great benefits for poor and eroded soils. For instance, in recent study (Slepetiene et al., 2020), the agronomic value of digestate formulated from agricultural waste was evaluated in Lithuania, and applied on eroded soils. Authors found that phosphorous and potassium provided from the digestates are essential, especially in the first 0-40 cm soil layer. The soil fertility and amount of mobile humic acids were enhanced respectively 5 times and 1,6 times compared to soil without digestate. It has been proved from the literature that anaerobic digestion results in high-value and stable biofertilizer product that showed high performance when applied as an alternative instead of chemical fertilizers in agriculture (Opatokun et al., 2017; Elalami et al., 2020; Cesaro, 2021). Basically, phosphate residues or phosphate rocks are mixed with different organic waste to formulate phospho-compost that presented positive results when applied to the soils (El Maaloum et al., 2020; Haouas et al., 2020; Oyeyiola and Omueti, 2019; Selvaraju, 1999). However, to the best of our knowledge this is the first time where phosphate residues are mixed with olive mill wastewater under anaerobic digestion process for additional minerals up-taking. In addition, wastewaters resulting from different sources (municipal wastewater, industrial wastewater, olive mill wastewater, etc.) can be treated with different techniques and processes. For example, the constructive wetland is a practical, low-cost technology used for treating domestic, urban and industrial wastewaters. This method showed positive results in terms of reducing toxic pollutants (Sehar et al., 2016; Sehar and Nasser, 2019; El Ghadraoui et al., 2020).

This paper aims to evaluate the potential use of digestates issued from anaerobic co-digestion of olive mill wastewater with phosphate residues on tomato plant during the crop season. Moreover, irrigation of tomatoes was assessed using reclaimed water from wastewater treatment plant in Murcia, Spain. To the best of our knowledge, OMW was co-digested for the first time with PR and combined with reclaimed water for growing tomatoes. The study was carried out during the crop season from April until September 2019 in the CEBAS-CSIC center in Murcia, Spain.

2. Materials and Methods

2.1. Anaerobic digestion process and samples characterization

in Beni Mellal region located in the center of Morocco, OMW was collected (60L) during the olive oil production season, which is from November until March. PR is abundant in the Khouribga region (Morocco), where phosphates' extraction is in large quantities. Three batch reactors of 10L total volume and 8L working capacity were filled with an initial substrate for 25 days of hydraulic retention time (HRT), at a temperature of 37°C, mechanical agitation of 60 rpm and initial pH range [6.5-7.5]. Bioreactors were covered with tissues formed each one from 3 cm thick polyester opaque material with a thin layer of aluminum to avoid light from entering and consequently affecting the AD process. Each one is equipped with a mechanical agitator and output from where the biogaz can be measured and collected. Bioreactors initial composition was: i) Batch reactor 1: OMW. ii) Batch reactor 2: OMW + 1% PR. iii) Batch reactor 3: OMW + 5% PR.

After 25 days of AD treatment, raw samples (substrates) and final samples (digestates) were stored to be applied as bio-fertilizers. Cations and anions of the initial substrate and final digestate were characterized using mass spectrometry for cation (Ion Chromatography (chromato-graph (Metrohm, Switzerland)), and by Inductively Coupled Plasma Spectrometer (ICP-ICAP 6500 DUO Thermo, England).

2.2. Wastewater treatment process and steps

Reclaimed water used in irrigation of tomato plants in this study was obtained from the Balsicas, Roldan and Lo Ferro municipal wastewater treatment plant (WWTP) in Murcia region, after a treatment that consisted of pre-treatment (coarse screen, fine screen, sieving, degritter and degreaser), double-stage activated sludge with prolonged aeration, and secondary clarifier. This reclaimed water was employed in this study as a model of water with low microbiological quality.

2.3. Tomato plants preparation and plantation

Tomato is known to be the second most important vegetable in the world after potato. In Europe, Spain is the second largest producer of tomato after Italy. Murcia region (Place where located the greenhouse where the experiment was done) in the south of Spain is one of the first five producers of tomato. Tomato short ripening duration, high productivity rate, and adequate climate in Spain are the major reasons for using tomato as convenable crop for this study (Chiurciu *et al.*, 2020). Tomato (*Solanum lycopersicum)* was grown during crop season in a greenhouse located on a research platform from CEBAS-CSIC in the Balsicas, Roldan and Lo Ferro municipal (WWTP) in Murcia region (south-eastern Spain). The greenhouse characteristics, average temperature, surface area, relative air humidity, and transpiration were 15°C, 680 m^2, 67%, and 0.5 Lm^{-2}. Organic soil used in this experiment was coconut fiber due to its poverty in essential elements. The pH value of coconut fiber was (5.5-6.5), E.C was (<1 mS/cm), and the capacity of water retention was (8-9). Pots of 10 Litres of working volume, which corresponds to 7 Kg of Coconut fiber were filled and positioned randomly in two lines of 20 pots each line. The length of each line was 13.5 m on a working surface of 42 m^2. The total number of drippers used in this work was 80, which refers to 1.85 dropper/m^2.

After two weeks in the nursery (Baby Plant, SL, Santomera, Murcia), two tomato plants were transplanted in each pot and connected with a two-irrigation dripper. A head-unit programmer and electro-hydraulic valves automatically controlled the irrigation during the four months of the experiment. All pots received precisely the same quantity of chemical fertilizers (N-P_2O_5-K_2O-CaO), but less than the required amount for growing tomato to evaluate the effect of bio-fertilizers. This reduction was highlighted in the introduction section, where many authors prefer the combination of bio-fertilizers and chemical fertilizers. Finally, for each treatment, there are five pots and ten-tomato plants to have representative results. Bio-fertilizers were mixed with irrigation water and applied on the surface fortnightly in a proportion of 1% of the coconut fiber weight (7 Kg). The total irrigation water amount of 2995,15 m^3/ha was applied during the 4 months with a flow of 2,2 L/h per dropper. The number of irrigations per day and duration was regulated regarding plant growth. During the first month, 10 minutes of irrigation repeated from 3 to 9 times every day. Afterward, and when the plant starts to grow, 3 to 5 minutes of irrigation repeated 11 to 18 times per day. This difference in number of irrigation and duration is due to the plant status and sometimes to the irrigation system problems. Those parameters were chosen according to the reference and international instructions.

Plant growth was measured every week during the four months of the experiment and secondary steams were amputated every week in order to ensure plant growth and fruit quality. When plants started to grow, they were connected with agricultural rope and tightened into the wire above to keep straight the plants and avoid overcrowding.

2.4. Leaf mineral analysis

Leaves were cleaned using alconox 0.1% detergent, washed with tap water, then with a diluted solution of 0.005% Chloridric acid (HCl) and finally washed again using distilled water. After that, leaves were positioned on a filter paper and left to drain before being dried for a minimum of 2 days at 65°C in an oven. Dried leaves were ground and digested using nitric-perchloric acid (2:1). Aqua Regia acid HCl/ Nitric acid (HNO_3) was used to digest the repetitions of (0,25g). Metals, macronutrients (Potassium (K), Calcium (Ca), Magnesium (Mg), Nitrate (NO_3^-), Phosphate PO_4^{3-}), micronutrients (Copper (Cu), Manganese (Mn), Iron (Fe), Zinc (Zn)) and phytotoxic elements (Sodium (Na^+), Boron (B^{3+}), Chloride (Cl^-)) of leaves were determined using inductively coupled plasma (ICP-ICAP 6500 DUO Thermo, England) and ion chromatography with a Chromatograph Metrohm (Switzerland) (Pedrero et al., 2013; Nicolás et al., 2016).

2.5. Fruit quality and tomato yield

Total fruit production, total weight (g), average fruit weight per treatment (g) and production per plant (g) were measured during the harvesting season starting from the second week of August until the end of September. Five tomato fruit from each treatment were sampled and stored at -17°C to evaluate fruit quality. Color values on tomato fruit surfaces were measured using a Minolta color chromameter (CR-300, Minolta, Ramsey, NJ) tristimulus color analyser, calibrated to a white porcelain reference plate. The colour space coordinates *L*, a*, b** and chroma (*(a*2 + b*2)1/2)* was measured for each tomato fruit from (five repetition for each treatment) three different positions around the equatorial zone to obtain the Hue° index following this equation (Andrés and Perla, 2014; Saad et al., 2016): *[Hº = arctg (a*/ b*)]*. Tomato fruit hardness was measured using a compression test with Lloyd instrument (model LR10K, Fareham Hants, U.K.) and equipped with two flat plates of 12×18 cm. This test consists of the maximum force required to deform 1% of the tomato fruit at an average speed of 25 mm/min. In order to assess titratable acidity (TA) and total soluble solids (TSS), a sample of 50 mL per tomato was used. TA was determined by titration of 10mL of juice using 0.1 $mol.L^{-1}$ of a diluted solution of Sodium Hydroxide (NaOH) to pH 8.1 and the TSS was measured using a handheld refractometer (Atago N1, Tokyo, Japan) (Pedrero et al., 2013; Nicolás et al., 2016; Bertin and Génard, 2018; Carlo et al., 2018).

2.6. Statistical analysis

Data related to tomato yield production, biofertilizers application, length, average stress water potential and fruit quality parameters were processed statistically through analysis of variance (ANOVA). Means where separated for all data by Tukey's post hoc test, where $p<0.05$ was considered statistically significant. Different lowercase letters (a-d) represent significant differences between different biofertilizer's application. All statistical analyses were performed with R software, version 3.3.3 (The R Foundation for Statistical Computing, Vienna, Austria).

3. Results and Discussion

3.1. Substrates and digestates characterization

The results presented in *Table 1* showed the richness of raw substrates and digestates in terms of essential elements (Macro and Micronutrients) with a slight difference in some elements. All the results are compared to each other and to the Spanish Legislation ('Spanish Regulations for Water Reuse Royal Decree 1620 / 2007 of 7 December', 2011). In general, AD was effective in reducing the phytotoxicity of the raw substrates (OMW, OMW+1%PR and OMW+5% PR). The majority of elements quantity was decreased after the AD process. Some elements as well as Ca, Mn, Cl and Zn reached removal of 50%, 50%, 70% and 80% respectively. In our case, Ca presence in both raw substrates and digestates is a positive value for plants and soil. The soil used in this work is deficient in terms of macro and micronutrients. Therefore, the role of calcium is soil is to decrease the pH value and detoxify some heavy metals (Aluminium (Al), Mn, Cadmium (Cd), etc.) that could be toxic for plants (Fageria and Moreira, 2011). Other elements (Fe, K, Mg and P) quantity was slightly reduced. Moreover, the main positive outcome of the AD process was the increase in NO^{3-} and Sulphate (SO_4^{2-}) availability where the quantity of NO^{3-} was undetected in raw substrates and reached an average of 15 mg/L, which is below the limit value as reported (Mohidin *et al.*, 2015). For SO_4^{2-} only a slight increase was observed after AD process. In addition, other heavy metals (Cd, Cu, (Lithium) Li, Cobalt (Co) etc.) quantities (results not showed) were very low and under limits (Roy *et al.*, 2006). All macro and micronutrients present in PR are in low quantities and under limits except for Ca (20 mg/L). B^{3+} is present in all the raw substrates and digestates at an average amount of 6 mg/L for the other samples. This quantity of B^{3+} is slightly above the optimal range (2.0-4.0 mg/L) and this is probably due to different chemicals used in the olive oil industry (cleaning agents) (Pedrero et al., 2013; Deng et al., 2019). For the Ca element, the quantity in digestates was reduced by about 50% after AD where it varies generally from 0.4 to 2 g/L in raw substrates, and from 0.4 to 1 g/L in digestates. Fe^{2+} average quantity present in all samples is 40 mg/L which is considered high. K^+ average quantity existing in raw substrates and digestates is 4 g/L which present high-level value but can be used to avoid Na^+ and Cl^- toxicity. Na^+ and Cl^- quantity illustrated is very high, and this is due to the overuse of Sodium Chloride (NaCl) in olive oil industries in Morocco to improve olive oil extraction. Even though, it was reported that the presence of NaCl increase fruit quality, but also increase in salinity was noticed (Dorais and Paradopoulos, 2001). High level of Na^+ results from using NaOH solution to adjust pH in bioreactors before anaerobic digestion (Cruz et al., 2007; Bolzonella et al., 2017). Contrary to the Cl^- which is reduced by more than 50% after AD process. Even plants absorbed a large amount of Cl^-, but still in the plant tolerability range, and does not present any risk to the plant, according to literature (Roy *et al.*, 2006; Fageria and Moreira, 2011). PO_4^{3-} was also slightly reduced after AD but still above the limits (1.0-2.0 g/L) and exceeded the plant needs. The availability of Nitrogen (N), Phosphorous (P) and K elements were reflected and observed during the growing and harvesting stages where plants showed good growth (length, steam potential, flowering and fruit quality) (Worsfold et al., 2016).

The decision of applying 1% of organic fertilizers fortnightly was taken considering the results of samples characterization (*Table 1*) to avoid phytotoxicity of soil and plants. Therefore, during four months of growing, harvesting and fruit quality testing, only four out of eighty plants fed with initial substrates (OMW raw) could not survive. Plants were replaced with good ones and showed good growth which means that the four plants accidentally died (Plants died have no root which probably could be an error of implantation). It was also highlighted (Annobill, 2007; Fageria and Moreira, 2011; Alcalde-Sanz et al., 2017) that the combination between organic and chemical fertilizers is a good decision regarding the SDGs of organic waste and soil management. It has been noticed also during the growing stage that plants fed with substrates and digestates have more roots comparing to reference. This is certainly due to N, P and K's amount in substrates and digestates that support root growth (root hair, lateral root, root length, depth and density) (Fageria and Moreira, 2011; Mohidin et al., 2015). From the results above, AD was effective in terms of reducing phytotoxicity and providing essential elements (Macro and micronutrients) for tomato. The pH and electric conductivity (EC) of substrates were acidic (average pH=4; average EC=12 mS/cm) and digestates becomes neutral (average pH=6.5; average EC=20 mS/cm) which confirm the AD effectiveness and the reduction of different toxic elements as shown in *Table 1* (Mengistu et al., 2015; Siddique and Wahid, 2018).

3.2. Plants growth

Plants growth was measured every week during the crop season. Every 10 days secondary steams were cut-off to keep the principal steam, leaves and flowers to ensure the perfect growth. Therefore, the plants grew in good health and conditions. This was confirmed by the growing rate of 10 to 20 cm per week, which indicates the regular nutrition and controlled conditions of tomato growth. All plants reached good height (152 cm on average) during the 4 months except plants fed with PR where they have the lowest height (116,58 cm). This is certainly due to PR's clay effect where it was observed that when the application of samples (raw substrates and digestates), PR stifles plants because it conserves irrigation water for a long time and plant breath becomes difficult. It was also observed the presence of green algae in soils of plants fed with PR, which is resulted from high humidity level, which interfere with tomatoes transpiration (Bertin and Génard, 2018). When it is difficult to breathe, it will influence plants' biochemical reactions and therefore decrease in absorbing essential elements for normal growth. Application of 1% of PR, which refers to 70g every 15 days, negatively influenced plant growth where 20% of plants died because of clay effect and not all plants reached the expected growth.

In contrast, plants fed with PR have more flowers compared to other applications (field observation). Clay effect has not been observed in other applications. Therefore, PR should be applied in very low quantities (<1%) or should be applied once at the beginning or before the plantation stage because of the low rate of dissolution. The right use of PR is mixing it in low quantities with other organic waste as in our study to provide phosphorus for plants (Annobill, 2007). This was observed during the experiment, where there was no difference between reference and other treatments in the first two months. This is because of the time-release of different essential elements from the substrates and digestates (Annobill, 2007; Kupper et al., 2014; Xu et al., 2017). Another difference is illustrated in *Figure 1* where the plants fed with digestates are higher than plants provided with substrates except for OMW+5%PR raw. This is undoubtedly due to a reduction in the quantity of some toxic elements after the AD process (Mohidin et al., 2015; Xu et al., 2017). In general, biofertilizers fed the plants slowly and need time for mineralization. Contrary to chemical fertilizers, nutrients are available immediately but unsustainable. This difference in sustainability can result in nutrient loss with drainage for chemical fertilizers (Xu et al., 2017).

3.3. Leaf mineral analysis

Approximately 80% of essential elements absorbed by tomato plants (roots) are transported to the shoots. The abundance of nutrients in the soil makes easy transport to the shoots. Accordingly, plants accumulate nutrients in large quantities even above the needs to use them, especially in producing fruits or nutrients deficiency (Fageria and Moreira, 2011). Limit concentrations for an element depend on the stage of plant growth. From the *Table 2* below, it can be noticed the presence of all essential elements for tomato growth. In addition, other micronutrients are not shown but they still present in adequate quantity and below limits. All tomato plants fed with low chemical fertilizers (control) or bio-fertilizers (raw substrates and digestates) showed a normal leaves composition and limit toxicity values. Even the high level of Na^+ in bio-fertilizers, but the plants absorbed minimal quantity. This is due to the presence of K^+, which reduce the plant absorption, and transport of high Sodium quantities to the leaves (Sainju et al., 2014; Hochmuth et al., 2018). In general, tomato plants did not show any essential element deficiency symptoms during the growing stage (field observation). These results can confirm the good physiological and biochemical processes during the crop season except PR's clay effect for some plants (20%). Total Carbone and Total N average values for all treatments are 41.85 g/100g and 4.23 g/100g respectively, according to commercial tomatoes instructions (Rattin, Andriolo and Witter, 2002).

3.4. Tomato yield

In general, yield production (Total fruit number, total production per plant) of tomato fruits doubled in plants fed with substrates and digestates compared to the control *(Table 3)*. Control and plants fed with OMW+5%PR digestate have the lowest number of fruits produced (89 and 79 tomato fruits respectively). Total fruit number, total production, and average production per plant in case of plants fed with substrates were high regarding plants fed with digestates, but most of these results did not present significant difference according to the ANOVA test. In contrast, average fruit weight was enhanced in all plants fed with digestates where the maximum average fruit weight recorded for plants fed with OMW+5%PR digestate (53.5 g ±0,9 d), and the results presented significant difference according to the Tukey’s HSD test between average fruit weight of plants fed with raw substrates, digestates and control. Even plants fed with PR showed the lowest height, but they recorded the highest total weight (7091.8 g ±314 c) and average production per plant (709.2 g/plant ±31,4 d). It was also confirmed that they present the highest flowering rate (field observation). Obviously, the presence of bio-fertilizers boosted the yield production due to the available nutritional elements confirmed by the ANOVA test at $p<0.05$ (Mekki and Ahmed, 2005; Abdel et al., 2013; Oyeyiola, 2018).

3.5. Fruit quality

Tomato fruit quality for consumption can be determined from different properties (color, size, TA, hardness, Brix°, taste index and maturity index). As reported in the literature, Malic acid and citric acid are the most important tomato fruit acids (Dorais and Paradopoulos, 2001; Ili et al., 2017). It is reported in *Table 5* that TA of tomatoes fed with substrates and digestates is higher than reference. In addition, plants fed with digestates (OMW digestate, OMW+1%PR digestate and OMW+5%PR digestate) are more acidic than tomatoes provided with substrates. Thus, digestates resulted from the AD process improved photosynthesis of plants and consequently a higher carbohydrate accumulation in fruits. These results are similar to those presented in the previous study (Ili et al., 2017; Rodríguez-ortega et al., 2019).

The TSS content in tomatoes is generally composed of reducing sugars. The TSS content obtained in this work ranged between 4.76-5.76 Brix° and differences are not significant in this case. All plants (references, substrates and digestates) are considered rich in organic acids *(Table 4)* and these results are in accordance with the literature (Zoran et al., 2014; Ili *et al.*, 2017). As it has been highlighted, the results of soluble solids (TSS) confirm that plants do not suffer any pathogenic disease or physiological problems (Rodríguez-ortega *et al.*, 2019). The taste index was calculated and showed promising results for all plants fed with substrates and digestates (<0.7) except for reference which the value was under limit of sweetness (0.61). It is also noticed that the taste index for tomatoes fed with digestates is slightly higher than in the case of substrates supply (Zoran et al., 2014). Hue° and Hardness results presented a slight difference for all treatments (Reference, substrates and digestates) but are considered in accordance with the commercial tomato properties and previous studies on red tomato (Andrés and Perla, 2014; Saad et al., 2016). The red color in tomato fruit is due to Lycopene pigment where about it constitutes 80 % of tomato color and it appears in the pericarp and locular tissues (Dorais and Paradopoulos, 2001; Rodriguez et al., 2006; Rodríguez-ortega et al., 2019). Many parameters can affect fruit quality, light intensity, irrigation rate, temperature, mineral nutrition, and salinity. However, in our experiment, fruit quality seems to be adequate for commercial use, where no pathogenic, physiological disorder was noticed during the growing and harvesting stage.

4. Conclusion

New challenges that threaten our planet today need more awareness, consciousness, and knowledge to reach a circular economy system that produces goods, ensures the economy and protects the environment. Therefore, the experiment conducted in this work evaluates the impact of using bio-fertilizers instead of chemical fertilizers and reclaimed water in irrigation instead of consuming the available quantity of fresh water in agriculture. This work will contribute to providing data and results to this area where there is a lack of scientific research because of the difficulty and the interfering of different factors (physical-chemical parameters, different organic waste, microbiological and pathogenic threats, etc.). Results obtained during the crop season of tomato under greenhouse using bio-fertilizers and treated wastewater for irrigation are considered encouraging regarding environmental framework but at the same time need more confirmation and analysis in the long term to be commercialized. Plants fed with biofertilizers showed the highest plant height, and good accumulation of macro and micronutrients in tomato leaves especially when applying (OMW+1%PR digestate) and (OMW+5%PR digestate). The maximum average fruit weight per treatment was obtained when applying (OMW+5%PR digestate), which was in accordance with commercial tomatoes size. Biofertilizers (digestates) showed good performances, high fruit quality and perfect tomato yield production.

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Tables

Table 1: Essential elements concentration (mg/L) in raw substrates and digestates samples before agronomic use.

Essential Elements	OMW raw	OMW digestate	OMW+PW 1 % raw	OMW+PW 1 % digestate	OMW+PW 5 % raw	OMW+PW 5 % digestate	PR
B^{3+}	6,39	7,37	6,32	6,56	6,41	6,48	0,11
Ca^{2+}	483,70	402,30	1028	653	2356	1036	20,62
Fe^{2+}	56,91	55,07	52,38	39,44	39,12	31,77	0,04
K^{+}	5476	4745	4888	4268	4822	4200	3,55
Mg^{2+}	308,40	291,80	350	278,50	365,20	283,60	5,12
Mn^{2+}	5,41	3,04	5,42	2,95	5,16	2,41	0,03
Na^{+}	1626	7943	1574	6556	1735	5211	9,96
P^{3-}	714,60	464,50	688	471,60	613,70	294	0,15
Zn^{2+}	4,44	0,68	4,41	1,81	3,92	0,69	0,01
Cl^{-}	2446,03	868,45	2429,84	931,51	2495,71	920	4,09
NO_3^{-}	<1,0	13,21	9,75	17,76	<1,0	20,18	1,72
PO_4^{3-}	1956	1927	2117	1610	1723	1164	<1,0
SO_4^{2-}	803,90	882,27	1107,73	1239,66	1036,80	1310,48	8,34

Table 2: Essential elements concentration (g/100g) in tomato leaves after harvesting stage

elements	Control	OMW raw	OMW digestate	OMW+ 1%PR raw	OMW+1 %PR digestate	OMW+5 %PR raw	OMW+5% PR digestate	PR
B^{3+}	0,84	1,47	1,29	0,66	0,68	0,82	0,90	0,68
Ca^{2+}	1,98	3,93	3,56	1,15	1,16	1,43	2,16	0,76
Cd^{2+}	0,10	0,31	0,17	0,11	0,03	0,25	2,32	<0,01
Cu^{2+}	0,99	1,53	2,18	0,38	0,26	0,33	0,80	0,22
Fe^{2+}	1,01	1,67	1,71	1,53	0,78	2,03	0,98	1,11
K^{+}	2,85	2,67	2,63	2,31	2,31	1,88	2,32	2,36
Mg^{2+}	0,54	0,69	0,63	0,38	0,37	0,40	0,47	0,33
Mn^{2+}	1,18	1,57	1,76	0,82	0,83	0,97	0,72	0,56
Na^{+}	0,45	0,55	0,55	0,56	0,30	0,26	0,23	0,46
P^{3-}	0,78	0,88	0,86	0,66	0,52	0,54	0,62	0,68
Zn^{2+}	0,47	0,58	0,56	0,82	0,35	0,72	0,38	0,39

Table 3: Tomato yield production after harvesting stage; Total fruit number per treatment, Total production (g), Average fruit weight (g) and Average production/plant (g).

Treatment	Total fruit number	Total production (g)	Av. Fruit Weight (g)	Av. production/Plant(g)
Control	89 ±6,40 a	3946.5 ±388,57 a	36,5 ±0,6 a	325,3 ±38,3 a
OMW raw	184 ±7,66 b	6866.4 ±353,88 b	37,3 ±0,8 a	686,64 ±35,4 b
OMW digestate	141 ±6,08 b	6019.1 ±252,08 b	42,7 ±0,6 b	601,9 ±31,8 ab
OMW+1%PR raw	167 ±7,24 b	6310 ±344,86 c	37,8 ±0,7 a	631 ±34,5 ab
OMW+1%PR digestate	134 ±8,49 b	5685 ±259,71 b	42,4 ±0,8 b	568,5 ±37,7 c
OMW+5%PR raw	172 ±4,02 ab	6770.8 ±181,24 ab	39,4 ±0,4 ab	677,1 ±18,1 b
OMW+5%PR digestate	79 ±9,12 a	4228.9 ±416,05 b	53,5 ±0,9 d	422,9 ±52,5 c
PR	181 ±5,40 b	7091.8 ±314 c	39,2 ±0,5 a	709,2 ±31,4 d

Different letters (a,b and c) next to the values indicate significant differences according to Tukey's HSD test between total fruit number, total production, average fruit weight and average production per plant at $p<0.05$. Values are the mean of 10 tomato plants for eight different biofertilizers application compared the control.

Table 4: Effects of bio-fertilizers on tomato fruit quality parameters: TA, TSS, hardness and Hue°.

Biofertilizers application	Titratable Acidity (TA) %	Total Soluble Solids (%)	Hardness (%)	Hue°
Control	0,492 ±0,07 a	4,98 ±0,42 a	55,60 ±5,22 a	42,60 ±1,12 a
OMW raw	0,558 ±0,10 ab	5,32 ±0,90 b	54,60 ±5,59 a	42,25 ±2,89 a
OMW digestate	0,734 ±0,27 c	4,92 ±0,50 a	58,20 ±6,14 a	46,36 ±1,49 b
OMW+1%PR raw	0,566 ±0,08 b	5,08 ±0,73 b	49,8 ±5,85 b	44,17 ±1,28 ab
OMW+1%PR digestate	0,626 ±0,17 c	4,76 ±0,74 b	55,86 ±6,46 b	45,21 ±1,89 b
OMW+5%PR raw	0,726 ±0,40 c	5,04 ±0,74 a	52,60 ±5,55 a	42,62 ±3,09 a
OMW+5%PR digestate	0,762 ±0,32 d	4,98 ±0,37 c	56,40 ±3,78a	44,95 ±1,39 ab
PR	0,644 ±0,17 d	5,78 ±0,61 c	54,40 ±3,05 a	44,03 ±1,25 ab

Different letters (a,b,c and d) next to the values indicate significant differences between Titratable acidity, Total Soluble Solids, Hardness and Hue° at $p<0.05$. Values are the mean of 10 tomato plants for eight different biofertilizers application compared the control.

Figures

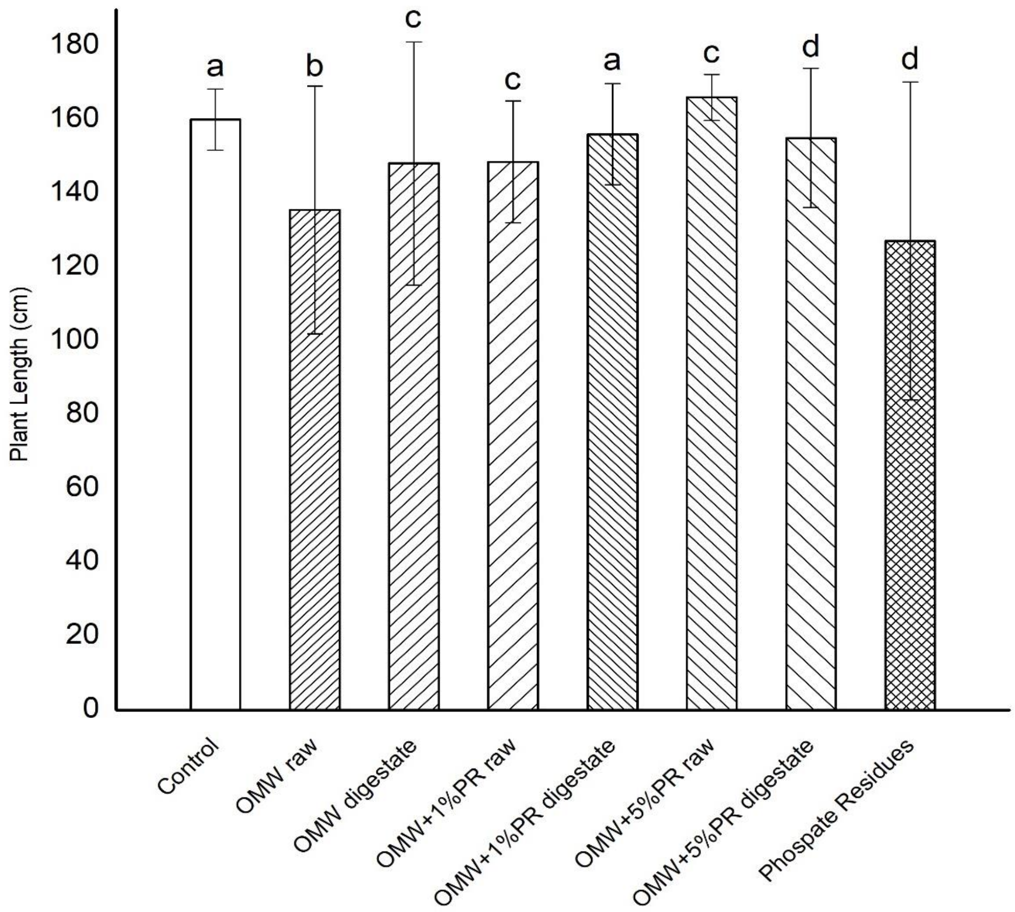

Figure 1: Average maximum length of tomato plant for each treatment during growing and harvesting stage (Different letters above the error bars indicate significant differences between Average Plant Length of each application at $p<0.05$)