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RESEARCH ARTICLE

Influence of Plant Growth-Promoting Rhizobacteria and vermicompost tea on a pomegranate tree

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Abstract

This research studied the effects of Plant Growth-Promoting Rhizobacteria (PGPR), and vermicompost tea on the physical and chemical properties of pomegranate (Manfalouty cultivar) fruits during two consecutive seasons, 2020 and 2021. Trees were sprayed with PGPR (3 ml/liter of water), and vermicompost tea (one liter/20 liters of water), and also vermicompost was added (one liter / 5 liters of water) to the soil at a distance of 50 cm from the trunk of the tree, were used in separate or in combined forms, three times a year, the first one in the middle of July (after fruit set), the second after a month of the first application, and the last one after a month of the second. The results showed that all treatments increased and improved all parameters when compared to the control. The physical and chemical properties of the fruits improved to some extent in the second season compared to the first one, which was due to the cumulative effect of vermicompost in the soil. In comparison to the other treatments in the two seasons, the treatment that included three treatments as a mix of foliar application and soil addition had the best effectiveness.

Keywords: Organic Agriculture; Pomegranate; Plant Growth Promoting Rhizobacteria; Vermicompost tea.

1. Introduction

The pomegranate (*Punica granatum*) is an important horticulture crop grown all over the world and is considered one of the most popular fruits in tropical and subtropical areas around the world. The skin, seeds, and pulp of the fruit have several therapeutic characteristics, and it has an important place in the pharmaceutical and food industries due to its nutritional value, chemical compounds, and antioxidant compounds (Damar *et al.,* 2014). There are nearly 153 phytochemicals in the fruit, including catechin, ellagic acid, and procyanidins, as well as triglycerides and, fatty acids, terpenoids, sterols, flavonols, and other phytochemicals. Tannins, anthocyanin, polyphenols, and antioxidants A, E, and C are found in fruit juice and have an important function in the health of cardiac blood vessels and normal blood circulation. In addition, pomegranate is abundant in bioflavonoids and organic acids such as anthocyanins, ascorbic acid, ellagic acid, gallic acid, caffeine acid, catechin, and minerals, amino acids, quercetin, and rutin (Jurenka, 2008). Furthermore, pomegranate fruit contains carbohydrates, sugar, fiber, fat, protein, phenolic compounds, and small amounts of iron, phosphorous, Zinc, Potassium, Calcium, and Manganese (Lansky and Newman, 2007). Fertilizers are necessary for modern agriculture because they supply vital plant nutrients. Chemical fertilizers (such as calcium nitrate,

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urea, diammonium phosphate, ammonium phosphate, and others) have a significant impact on global food production because they act as rapid nourishment for plants, causing them to develop more quickly and efficiently. Excessive and unbalanced use of these chemical inputs is causing increasing harmful effects and a significant deterioration of soil health. It also upsets soil microbes, lowers pH, causes P_2O_5 and K_2O build-up, reduces soil fertility, pollutes groundwater, and has negative health consequences (Macit *et al.,* 2007; Meenakshi Suhag, 2016).

The importance of sixteen essential plant nutrients in the correct amounts to achieve the best crop production is well recognized (for example, macronutrients as N, P, K, Ca, Mg, and S, and micronutrients as Fe, Zn, Cu, Mo, Mn, B, and Cl). Plants require N, P, and K to improve their stress resistance to pests, diseases, drought, and cold (Tasi *et al.,* 2007).

Using various organic manures and fertilizers to preserve the soil's Physico-chemical and biological qualities is a good idea. Organic matter will not only supply vital nutrients, such as micronutrients, but it will also enhance the physical state of the soil, promote aeration, and provide improved root growth and production opportunities. It is a good method for long-term horticulture with minimal chemical input highly accessible at a low price. To preserve the soil productivity and fertility, integrated nutrient management is a production method that favors the utilization of organic material to the greatest extent possible is encouraged, whereas synthetically made agro-inputs are reduced as possible. Vermicompost use in fruit crops has been shown to improve nutrient availability soil physical conditions, and enzymatic activity (Maheswarappa *et al.,* 1999; Sudhakar *et al.,* 2002).

Earthworm excreta are high in humus, macronutrients, and micronutrients, and are known as vermicompost. It has the potential to increase soil health, agricultural output, and the physical qualities of the soil. Physical qualities of soil, such as structure, texture, and tilth, are important in determining a land's agronomic potential (Azarmi *et al.,* 2008). Root penetrability, potential rooting volume, nutrient uptake, and mobility, soil aeration, and water availability are all influenced by these qualities (Roy *et al.,* 2006). Soil texture also has an impact on soil moisture content as well as chemical qualities like cation exchange capacity (CEC), or the soil's ability to store positively charged ions (Delgado and Gomez, 2016).

For instance, a region with a high proportion of sand is easy to cultivate, but it has poor moisture retention and low CEC, allowing plant nutrients and water to rapidly leak out of the rooting zone. As a result, adding composts like vermicompost to the soil (especially sandy soil) is helpful because it helps raise the soil organic matter (SOM) composition, which helps improve soil aeration, maintain excellent soil aggregation, prevent soil erosion, and boost nutrient availability (Roy *et al.,* 2006). In addition, vermicompost contains a variety of plant nutrients like N, P, K, Ca, Mg, S, Fe, Mn, Zn, Cu, and B, all of which help to increase the nutrient content of different plant components like roots, shoots, and fruits (Theunissen *et al.,* 2010).

Because of its humus content, vermicompost can keep nutrients for a long time and has a higher water-holding capacity and porosity than traditional compost (Rajiv *et al.,* 2010) Vermicompost is currently widely utilized in agriculture, and its potential to produce high crop yields is well documented, as evidenced by reports for the wheat (Yousefi and Sadeghi, 2014), and tomatoes (Zucco *et al.,* 2015) and the use of vermicompost had the same effect as the application of inorganic fertilizers indicating that vermicompost is a viable alternative to chemical fertilizer application (Singh *et al.,* 2008).

Farmers, on the other hand, frequently get low-quality fruits and yields due to a lack of effective nutrition management. Improved management practices, such as the use of plant growth-promoting rhizobacteria, have been found to increase plant growth, yield, and quality by producing plant growth regulators (auxin, gibberellins, cytokinins, etc.), mineralizing complex nutrients, fixing atmospheric nitrogen, and facilitating nutrient uptake, resulting in a resurgence of interest in sustainable and organic cultural practices. (Esitken *et al.,* 2005, Tripathi *et al.,* 2014).

The use of bio-fertilizers containing helpful microorganisms rather than synthetic chemicals has been shown to increase plant development by increasing nutrient delivery. This could aid in the preservation of environmental health and soil fertility (Egamberdieva, 2009). Azotobacter, Bacillus, Erwinia, Arthrobacter, Rhizobium, Beijerinckia, Serratia, Enterobacter, Flavobacterium, Acinetobacter, Alcaligenes, Azospirillium, and Burkholderia are among the bacterial genera that contain plant growth-promoting rhizobacteria (PGPR) (Rodriguez and Fraga, 1999). The mechanisms of PGPR are unknown, but it performs the following functions, (a) solubilization of inorganic phosphate and/or mineralization of organic phosphate and/or other nutrients (Jeon *et al.,* 2003)(b) the ability to produce plant hormones like gibberellins (Gutierrez-Manero *et al.,* 2001); auxins (Jeon *et al.,* 2003), and cytokinins (Garcia de Salamone *et al.,* 2001),(c) antagonism against phytopathogenic microorganisms through (Dey *et al.,* 2004), and (d)symbiotic N_2 fixation (Sahin *et al.,* 2004).

Numerous researches have indicated that seed or root inoculation with microorganisms capable of generating plant growth regulators improves plant growth and development. Nevertheless, less research has been done on the impact of PGPR capable of generating plant growth regulators in fruit trees being introduced via floral or foliar inoculation on yield and plant growth (Zahir *et al.,* 2004). In previous studies, it was found that PGPR could stimulate growth and increase yield in apple, sweet cherry, citrus, raspberry, high bush blueberry, mulberry, and apricot (De Silva *et al.,* 2000; Sudhakar *et al.,* 2000; Esitken *et al.,* 2003; 2006; Aslantas *et al.,* 2007).

The demand for organically cultivated pomegranate fruits is expanding in the Middle East, America, and Europe as people become more health-conscious. With these considerations in mind, the current study was conducted to determine the effect of organic manures on the development and yield of pomegranate cv. Manfalouty.

2. Materials and methods

The experimental study was conducted over two growing seasons (2020 and 2021) to study how vermicompost and Plant Growth Promoting Rhizobacteria (PGPR) affected pomegranate trees of the Manfalouti cultivar, which were grown at the experimental orchard of the Pomology Department, Faculty of Agriculture, Assiut University. 40 pomegranate trees were chosen that were 15 years old, as uniform as possible, spaced 5x5 meters apart, and grown in clay soil. The trees that were studied were healthy, uniform in vigor, and divided for this investigation. Irrigation, pest management, and other traditional agricultural practices were used on all of the trees, and in December, fertilizer (FYM @ 20 kg/tree) was administered to all of the trees (including the control). Three times a year, the trees were treated with the following applications on the same tree: The first is in the middle of July (after the fruit has been set), the second is after the first application month, and the third is after the second application month.

1-Control (sprayed with distilled water and without any mineral fertilization).

2-Sprayed with PGPR (3ml/ liter of water/ tree).

3-Sprayed with a combination between PGPR (3ml/ liter of water/ tree) and vermicompost tea (one liter of vermicompost/20 liters of water/tree).

4-Sprayed with PGPR (3ml/ liter of water/ tree) and Soil supplement of vermicompost (one liter of vermicompost for every 5 liters of water/tree).

5 -Sprayed with vermicompost tea (one liter of vermicompost/20 liters of water/tree).

6 -Sprayed with vermicompost tea (one liter of vermicompost/20 liters of water/tree) and Soil supplement of vermicompost (one liter of vermicompost for every 5 liters of water/tree).

7 -Sprayed with a combination between PGPR (3ml/ liter of water/tree) and vermicompost tea (one liter of vermicompost/20 liters of water/tree) and Soil supplement of vermicompost (one liter of vermicompost for every 5 liters of water/tree).

8 -Soil supplement of vermicompost (one liter of vermicompost for every 5 liters of water/tree).

Each treatment sprayed five trees, and only three replicates of each treatment were taken, with two fruits gathered from each tree.

2.1. How to prepare vermicompost tea

For each kilogram and a half of vermicompost, 10 liters of water were added and placed in a water-permeable cotton bag. To obtain pH, combine 25 grams of molasses, 5 grams of magnesium sulfate, 1 gram of monopotassium phosphate, and citric acids. 6-10 grams humic acid - ventilate for 48 hours before removing it from the solution and using it.

2.2. Preparation of PGPR

This study used four strains of PGPR: Bacillus circulans (Potassium-solubilizing), Paenibacillus polymyxa (Nitrogen fixation), and Bacillus megaterium (Phosphate-solubilizing bacteria). These strains of Bacteria were grown in King's medium B until reached 10^9 cells /ml^{-1} (Atlas, 1995). And Pseudomonas fluorescens (inhibiting harmful microbes, generating growth-stimulating plant hormones, and creating higher disease resistance in plants) was grown on specialist media for it and it can use as powder or liquid. Taken 2 ml of each strain to make a combination of them, and then took 3 ml of it and put it in a liter of water.

2.2.1. Leaf constituents

At harvest date, leaves were randomly collected from the middle part of non-fruiting shoots of each replicate tree in both seasons to determine N.P.K content and total chlorophyll.

2.2.2. N.P.K content

Leaf samples were cleaned with tap water firstly, and then with distilled water, and non-ionic detergent before being dried at 70°C in an air oven and manually pulverized with a mortar and pestle. In a muffle oven, one gram of powder was burned for 25 minutes at 550°C. The resultant white ash was then dissolved in 10 mL of 2 N HCl and 100 mL of distilled water for macro-and micronutrient analyses. (Chapman and Pratt, 1961). A flame photometer was used to determine potassium content, and a spectrophotometer was used to determine phosphorous content. The Kjeldahl technique was used to determine total nitrogen content (Olsen et al.,1954; Jackson, 1973) .

2.2.3. Total chlorophyll content

was determined with a SPAD-502-meter (Minolta Camera Co., Osaka, Japan).

2.2.4. Yield weight

When fruits reached the ripening stage in each season (approximately in the middle of October), they were harvested separately from each tree, and their weight was calculated by kilogram per tree.

2.2.5. Fruit cracking %, fruit sunburn%, and marketable fruit %

The number of fruits cracked (FCP), sunburned (FBP), and marketable fruit (MFP) was determined for each treatment at the time of harvest as follows- :

$$FCP = \frac{NCF}{TNF} \times 100$$

$$FBP = \frac{NBF}{TNF} \times 100$$

$$MFP = \frac{[TNF - NCF - NBF]}{TNF} \times 100$$

**NBF is the number of sunburned fruits .*

**TNF is the total number of fruits .*

**NCF is the number of cracked fruits .*

2.3. Fruits properties

Two fruits were taken randomly from the yield of each tree (3 replicates) and made as a composite sample for both chemical and physical determinations.

2.4. Physical characteristics

The weight of each fruit, arils and peel of fruit were determined in grams by using an analytical balance.

2.5. Chemical properties

2.5.1 Total soluble solids (TSS): According to A.O.A.C. (1990), the soluble solids content in fruit juice was determined using a hand refractometer.

2.5.2 Titratable acidity: was estimated as ml of citric acid per 100 ml of juice through titration with NaOH at 0.1N using phenolphthalein as an indicator as outlined in the A.O.A.C. (1990).

$$\text{Titratable acidity (\%)} = \frac{\mathrm{N}x\ \mathrm{BS}\ x\ 0.06404}{\mathrm{V\ (ml)}} x100$$

N = Standard solution
BS= NaOH was adjusted by using a known volume of oxalic acid 0.1M
V= Total juice volume (ml)

2.5.3 Total anthocyanins

The determination of the total anthocyanin was realized by the method proposed by Di Stefano et al.,1989. The absorbance was measured at 540 nm after the samples were diluted with a solution of 70/30/1 (v/v/v) ethanol/water/HCl (concentrated). The overall anthocyanin contents were represented as malvidin-3-glucoside equivalents due to the lack of a malvidin-3-glucoside standard.

TA540 nm (mg/L) = A540 nm16.7d

*Where A540 nm is the absorbance at 540 nm and d is the dilution.

2.6. Statistical analysis

The experiment was set up as a three-replication randomized complete blocks design. SPSS software was used to analyze variance (ANOVA). The LSD test was used to determine to mean differences. (Least significant difference) at P < 5% based on Gomez and Gomez (1984) in Statistics 8.1 (Analytical Software, 2008).

Table 1. Analysis of the tested soil before starting the study

Characters		Character	
Sand (%)	15.43	Total N (%)	0.16
Silt (%)	33.22	Available P (mg/kg)	21.61
Clay (%)	51.35	Available K (mg/kg)	401.33
Texture	Clay	DTPA-extractable (mg/kg)	
pH (1:1 suspension)	8.10	Fe	13.19
E.C (dS/m^{-1})	2.69	Mn	15.16
Organic matter (%)	1.32	Zn	2.35
$CaCo_3$ (%)	3.66	Cu	2.11

Table 2. Analysis of the vermicompost tea used in experimental

Characters (These contents per liter)	
natural material & element	35%
Natural plant extracts	30%
N	3%
P	1.5%
K	20%
Mg	0.12%
Si	5%
Fe	350 ppm
Zn	335 ppm
Mn	160 ppm

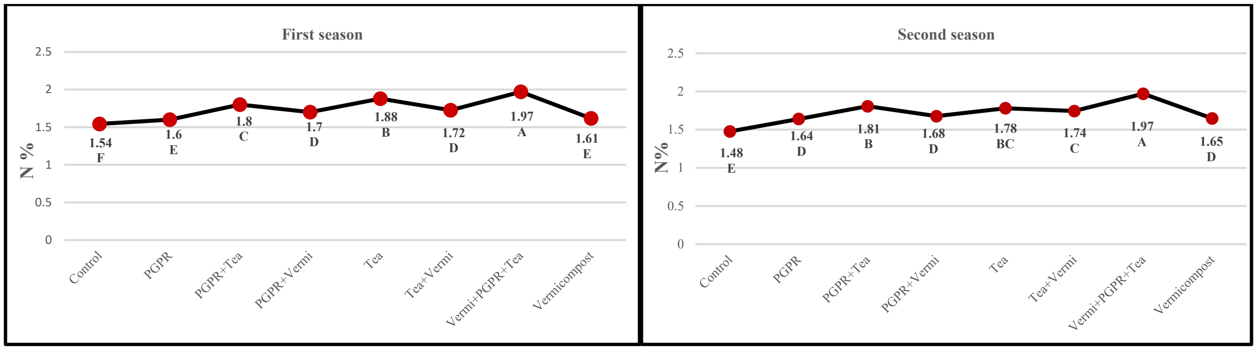
3. Result

3.1. The parameters of leaves included N, P, K percentage, and chlorophyll

3.1.1. N%

Data in Fig.1 illustrated that all treatments gave a good impact on the percentage of nitrogen in leaves in both successive seasons. In the first season, the biggest rate was found in $\mathbf{T_7}$ (1.97%), while $\mathbf{T_5}$ (1.88%) came in second followed by other treatments with significant differences, and the smallest rate was found in the control (1.54%). In the second season, T_7 had the biggest percentage (1.97%), followed by other treatments with a significant difference, and the smallest percentage was found in the control (1.48%).

Fig. 1. The influence of PGPR and vermicompost tea on N % of pomegranate leaves (Manfalouty cultivar) during 2020 and 2021 seasons

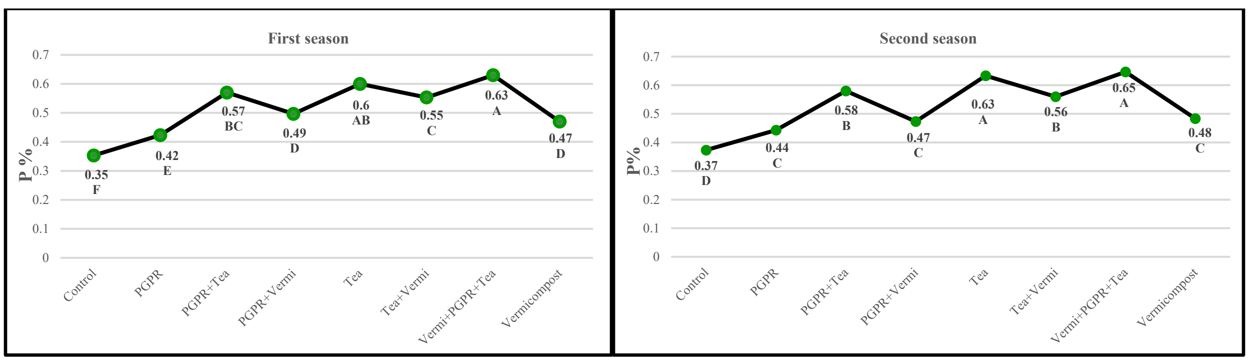


**Means separation by LSD tests at P ≤ 0.05. The same letters of data indicate that are not significantly different. Ascending order starts from (A) means the highest value until reaches the letter which has the lowest value.*

3.1.2. P%

Data in Fig. 2 proved that all treatments had an affirmative effect on the phosphor percentage of leaves during two conductive seasons. The highest rate was found in $\mathbf{T_5}$ (0.6, 0.63%), and $\mathbf{T_7}$ (0.63, 0.65%), respectively, and the lowest rate was found in the control (0.35, 0.37%), in two successive seasons, respectively, with significant differences between them. And other treatments were located among them with significant differences.

Fig. 2. The influence of PGPR and vermicompost tea on P % of pomegranate leaves (Manfalouty cultivar) during 2020 and 2021 seasons

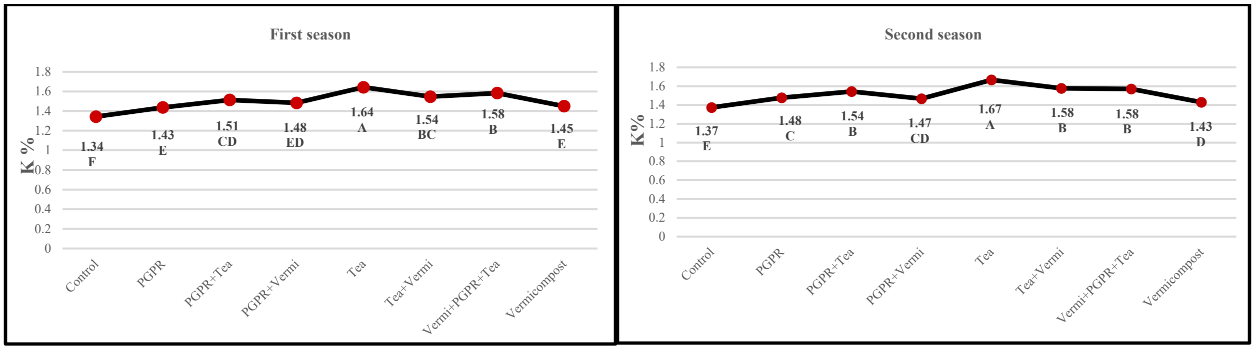


**Means separation by LSD tests at P ≤ 0.05. The same letters of data indicate that are not significantly different. Ascending order starts from (A) means the highest value until reaches the letter which has the lowest value.*

3.1.3. K%

Data in Fig. 3 described the effect of treatments on the amount of potassium in leaves. In two successive seasons, all treatments gave a positive effect on the rate of potassium compared with the control. The highest rate was found in $\mathbf{T_5}$ (1.64, 1.67%), and the lowest rate was found in the control (1.34, 1.37%), respectively during the two-season. In the first season, $\mathbf{T_7}$ (1.58%) came after $\mathbf{T_5}$, with a significant difference, and then the other treatments followed them. In the second season, $\mathbf{T_3}$(1.54%), $\mathbf{T_6}$, and $\mathbf{T_7}$ (1.58,1.58%) had insignificant differences between them and came after $\mathbf{T_5}$ with significant differences, and other treatments came after them.

Fig. 3. The influence of PGPR and vermicompost tea on K % of pomegranate leaves (Manfalouty cultivar) during 2020 and 2021 seasons



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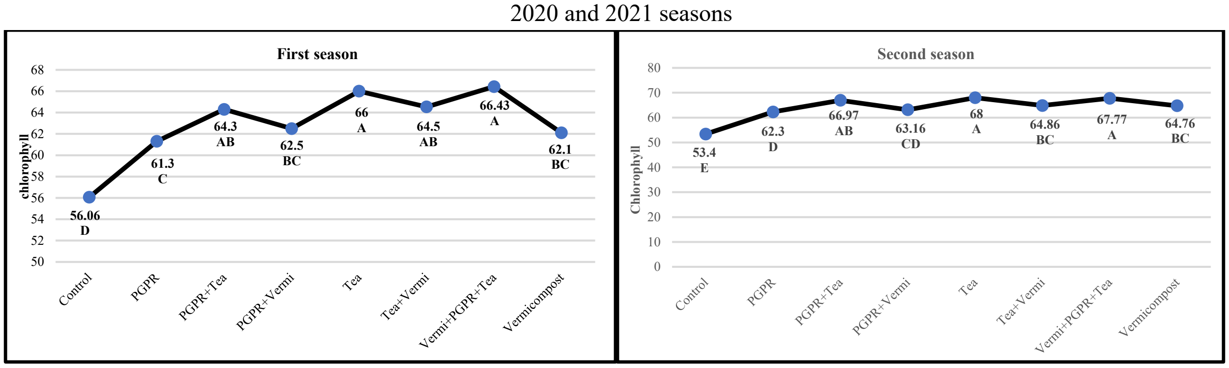
3.1.4. Total chlorophyll

Data in Fig.4 clarified that all treatments had an effective influence on the amount of chlorophyll in leaves. There were significant differences between the treatments compared with the control. In the first season, the best effect was found in **T_5** (66) and **T_7** (66.43) followed by **T_6** (64.5) with trivial differences, and other treatments came second, and the least effect was found in the control (56.06). In the second season, the best effect was found in both **T_5** (68) and **T_7** (67.77), followed by **T_3** (66.97) with insignificant differences, whereas other treatments followed them with significant differences, and the least effect was found in the control (53.4).

Fig. 4. The influence of PGPR and vermicompost tea on total chlorophyll of pomegranate leaves (Manfalouty cultivar) during 2020 and 2021 seasons



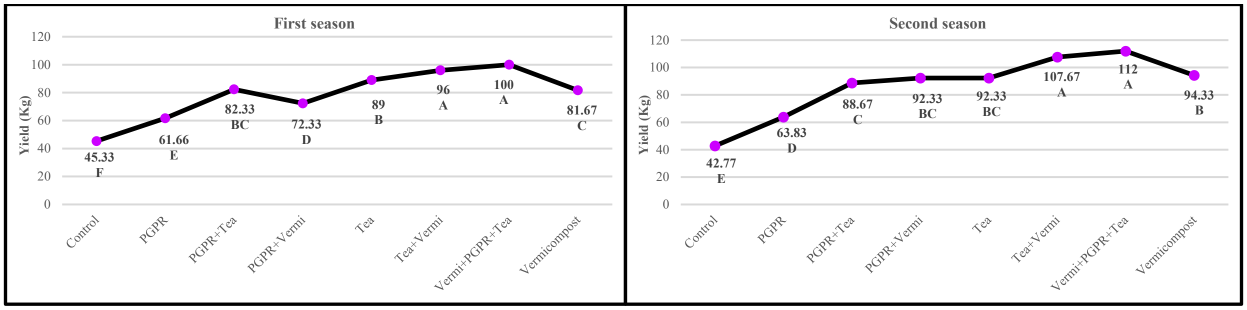
**Means separation by LSD tests at P ≤ 0.05. The same letters of data indicate that are not significantly different. Ascending order starts from (A) means the highest value until reaches the letter which has the lowest value.*

3.2. Physical parameters of fruit

3.2.1. Yield (kg)

The results in Fig.5 proved that all treatments had an impact clearly on yield during successive seasons. In two seasons, the highest value was found in **T_6** (96,107.67kg), and **T_7** (100, 122 kg) respectively, and other treatments came in second and the lowest value was found in the control (45.33, 42.77 kg), respectively with significant differences. In addition, the data illustrated the interaction between the two seasons and proved that there was a significant difference between the two seasons and the second season was the best compared with the first one.

Fig. 5. The influence of PGPR and vermicompost tea on yield (kg) of pomegranate fruits (Manfalouty cultivar) during 2020 and 2021 seasons

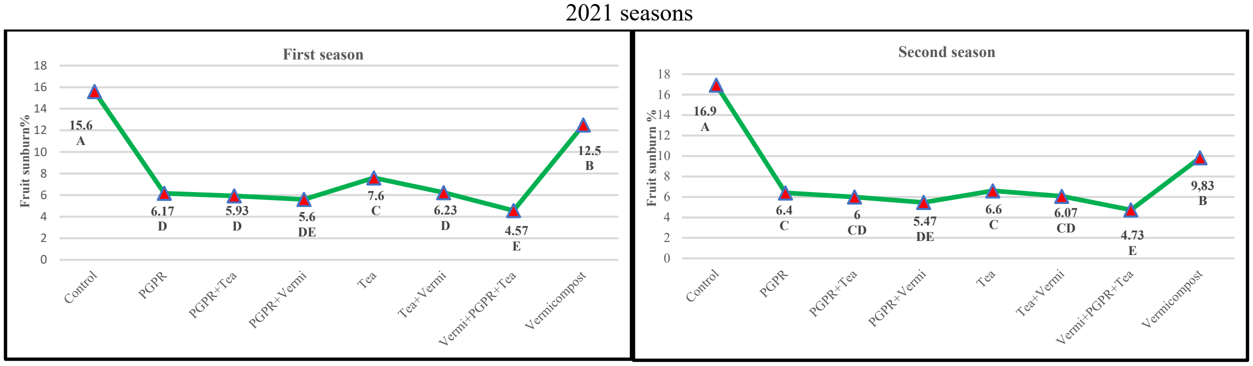


**Means separation by LSD tests at P ≤ 0.05. The same letters of data indicate that are not significantly different. Ascending order starts from (A) means the highest value until reaches the letter which has the lowest value.*

3.2.2. Fruit sunburn %

The result in Fig. 6 demonstrated that all treatments decreased the sunburn of fruits in comparison to the control with significant differences. The highest percentage was in the control (15.6, 16.9 %), followed by $\mathbf{T_8}$ (12.5, 9.83%), and the lowest percentage was in $\mathbf{T_7}$ (4.57, 4.73%), in two successive seasons, respectively, and the other treatments came second with trivial differences

Fig. 6. The influence of PGPR and vermicompost tea on fruit sunburn % of Pomegranate (Manfalouty cultivar) during 2020 and 2021 seasons

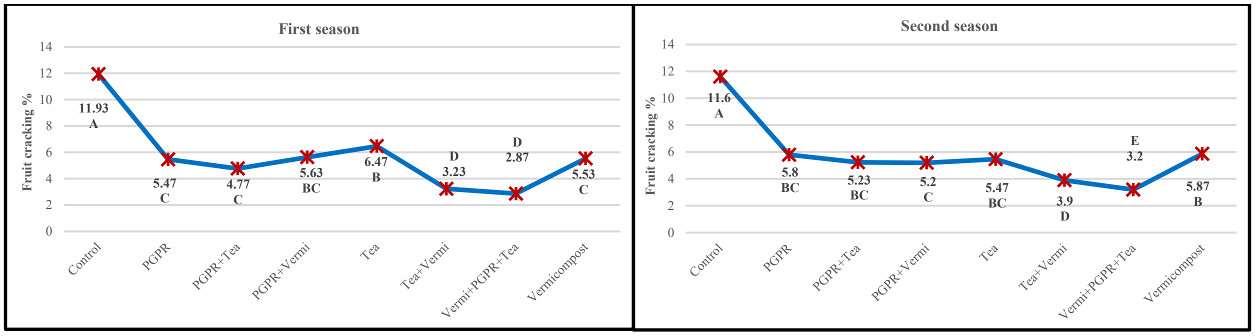


**Means separation by LSD tests at P ≤ 0.05. The same letters of data indicate that are not significantly different. Ascending order starts from (A) means the highest value until reaches the letter which has the lowest value.*

3.2.3. Fruit cracking %

The result in Fig. 7 clarified the effect of treatments on the percentage of fruit cracking and proved that all treatments gave a good impact on decreasing the percentage of fruit cracking. There were significant differences between all treatments compared with the control. The highest rate was found in the control (11.93, 11.6%), in both seasons respectively, and the lowest rate was found in both $\mathbf{T_6}$ and $\mathbf{T_7}$ (3.23, 2.87%) respectively, in the first season and in$\mathbf{T_7}$ (3.2%) only in the second season, wherever, other treatments came second with smallest differences.

Fig. 7. The influence of PGPR and vermicompost tea on fruit cracking % of pomegranate (Manfalouty cultivar) during 2020 and 2021 seasons

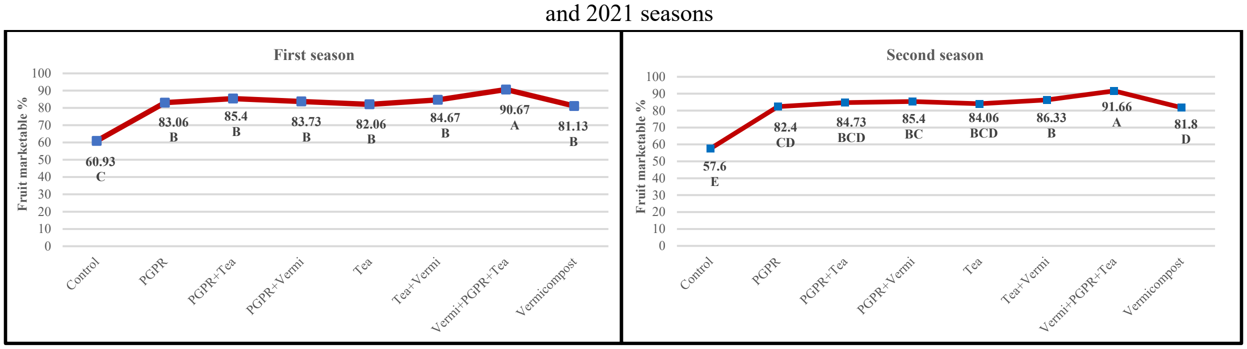


**Means separation by LSD tests at P ≤ 0.05. The same letters of data indicate that are not significantly different. Ascending order starts from (A) means the highest value until reaches the letter which has the lowest value.*

3.2.4. Fruit marketable%

Data in Fig.8 illustrated that all treatments had a positive influence on fruit marketable percentage, and improved the characteristics of fruit, thus increasing their marketability in a good way as possible. In both consecutive seasons, there were significant differences between the treatments and the control. In the first season, the highest percentage was found in $\mathbf{T_7}$ (90.67%) and the lowest percentage was found in the control (60.93%), while the other treatments came second with significant differences, but between them, they were nonsignificant differences. In the second season, the biggest effect was found in $\mathbf{T_7}$ (91.66%) and the least effect was found in the control (57.6 %), and the other treatments came second with a significant difference, while between them there were trivial differences.

Fig. 8. The influence of PGPR and vermicompost tea on fruit marketable% of pomegranate (Manfalouty cultivar) during 2020 and 2021 seasons



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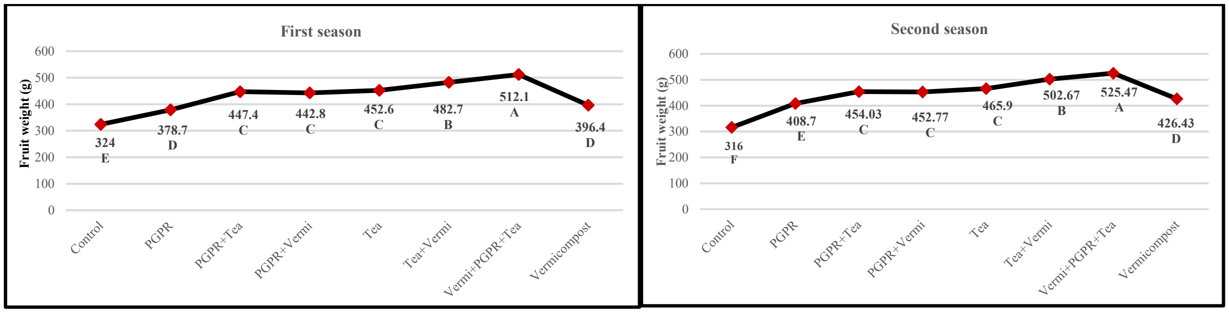
3.2.5. Fruit weight

Data in Fig. 9 illustrated that all treatments had a positive effect on fruit weight in two successive seasons compared with the control. The treatment that had a combination of vermicompost tea application, PGPR, and vermicompost tea spraying gave the best value (512.1, 525.47 g) followed by $\mathbf{T_6}$ (482.7, 502.67 g) compared to the other treatments, and the control gave the lowest value (324, 316 g) in both seasons, respectively.

Fig. 9. The influence of PGPR and vermicompost tea on fruit weight (g) of pomegranate (Manfalouty cultivar) during 2020 and 2021 seasons



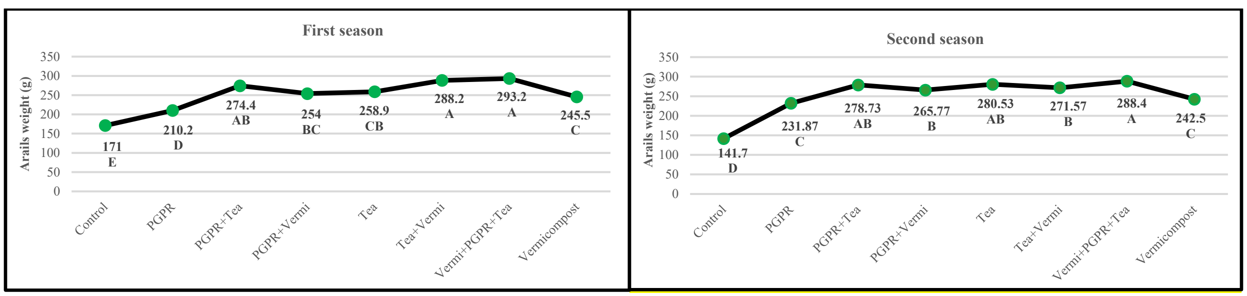
**Means separation by LSD tests at P ≤ 0.05. The same letters of data indicate that are not significantly different. Ascending order starts from (A) means the highest value until reaches the letter which has the lowest value.*

3.2.6. Arails weight (g)

Data in Fig. 10 showed that all treatments gave an acceptable effect on arails weight compared with the control. In the first season, $\mathbf{T_6}$ and $\mathbf{T_7}$ had the highest values (288.2, 293.2 g), respectively followed by $\mathbf{T_3}$ (274.4 g), and there were insignificant differences between them. The minimum value relatively for treatment, was found in $\mathbf{T_2}$ (210.2 g), and the lowest value was found in the control (171 g).

In the second season, the best amount was found in $\mathbf{T_7}$ (288.4 g) then, $\mathbf{T_3}$ and $\mathbf{T_5}$ (278.73, 280.53 g), respectively without a significant difference between them, followed by $\mathbf{T_4}$ and $\mathbf{T_6}$ (265.77, 271.75 g), respectively, and the minimum amounts relative to treatments were found in $\mathbf{T_2}$ and $\mathbf{T_8}$ (231.87, 242.5 g), respectively, and the lowest amount was found in the control (141.7 g).

Fig. 10. The influence of PGPR and vermicompost tea on arails weight (g) of pomegranate fruits (Manfalouty cultivar) during 2020 and 2021 seasons

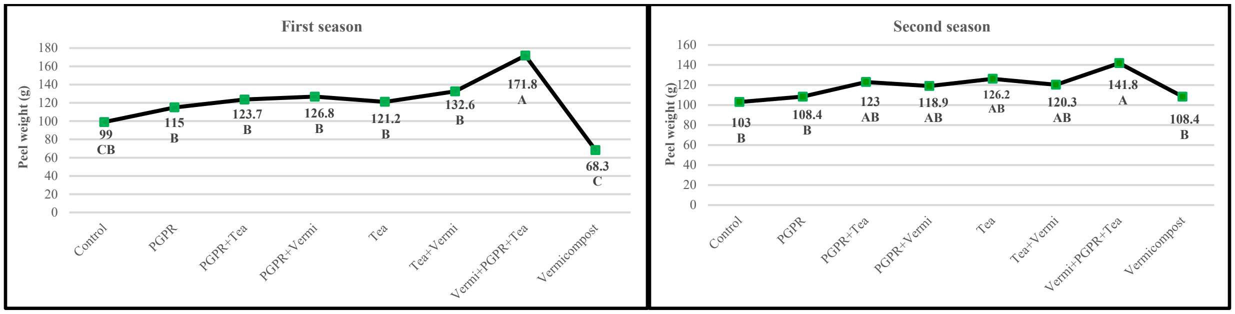


**Means separation by LSD tests at P ≤ 0.05. The same letters of data indicate that are not significantly different. Ascending order starts from (A) means the highest value until reaches the letter which has the lowest value.*

3.2.7. Peel weight

Data in Fig. 11 demonstrated that in the first season, the biggest value was found in $\mathbf{T_7}$ (171.8 g), followed by the other treatments with significant differences, and the lowest value was found in $\mathbf{T_8}$ (68.3 g). In the second season, there were trivial differences between some treatments and control, but the biggest value was found in $\mathbf{T_7}$(141.8 g) and the lowest value was found in the control (103 g).

Fig. 11. The influence of PGPR and vermicompost tea on peel weight (g) of pomegranate fruits (Manfalouty cultivar) during 2020 and 2021 seasons



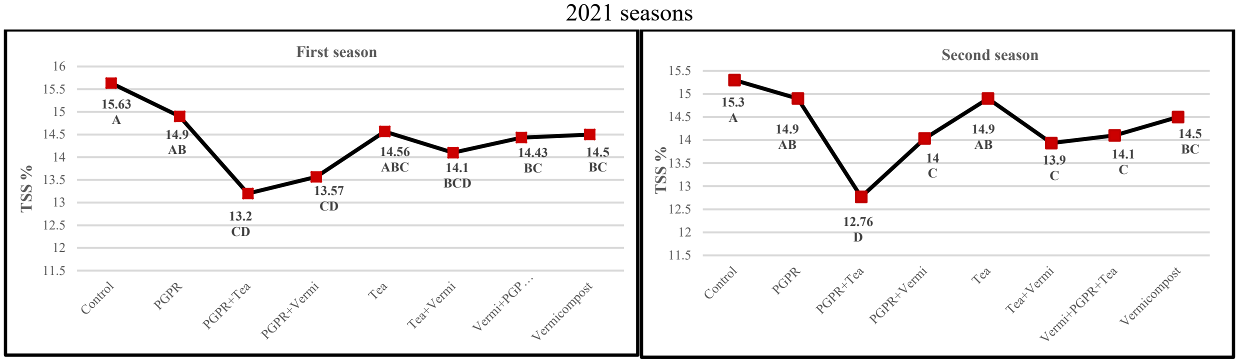
**Means separation by LSD tests at P ≤ 0.05. The same letters of data indicate that are not significantly different. Ascending order starts from (A) means the highest value until reaches the letter which has the lowest value.*

3.3 Chemical parameters of fruit

3.3.1. TSS%

Data in Fig. 12 showed the effect of treatments on the total soluble solids of fruits. In the first season, the biggest value was found in the control (15.63%) and the lowest values were found in **T$_3$**, and **T$_4$** (13.2, 13.57%), respectively, with insignificant differences, while there were trivial differences between the control and **T$_2$** and **T$_5$** (14.9, 14.56%). Also, there were small differences among other treatments. In the second season, the control had the biggest value (15.3%) but **T$_3$** (12.76%) gave the smallest value with a significant difference compared with the other treatments, while there were no significant differences between the control and **T$_2$** and **T$_5$**, both of them had the same rate (14.9%).

Fig. 12. The influence of PGPR and vermicompost tea on TSS% of pomegranate fruits (Manfalouty cultivar) during 2020 and 2021 seasons

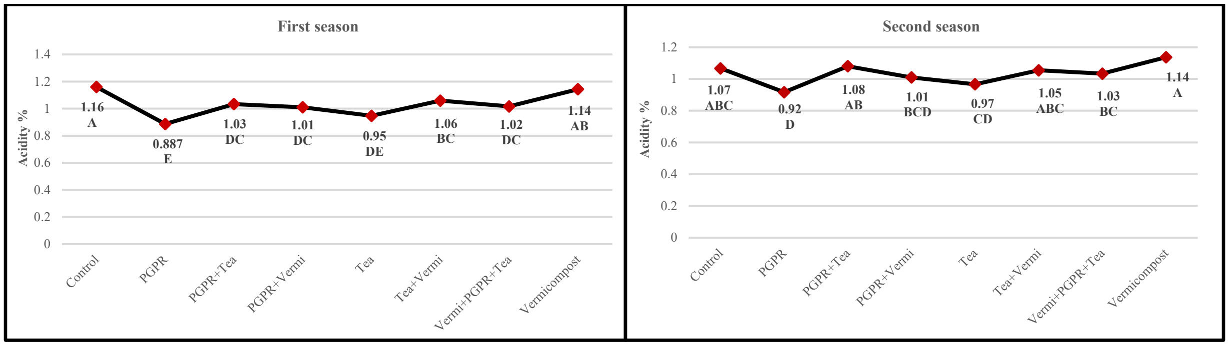


**Means separation by LSD tests at P ≤ 0.05. The same letters of data indicate that are not significantly different. Ascending order starts from (A) means the highest value until reaches the letter which has the lowest value.*

3.3.2. Acidity%

The results in Fig. 13 demonstrate the effect of treatments on the percentage content of acidity in fruits. In the first season, the highest rate was found in the control (1.16%) and the lowest rate was found in **T$_2$** (0.88%), with a significant difference, and there was no significant difference between the control and **T$_8$** (1.14%), followed by **T$_6$** (1.06%), and there were small differences between other treatments. In the second season, the highest rate was found in **T$_8$** (1.14%) and the lowest rate was found in **T$_2$** (0.92%), with a significant difference, and there was no significant difference between **T$_3$**, **T$_6$**, and **T$_8$** (1.08, 1.05, and 1.14%), respectively, and the control (1.07 %). Also, there were small differences between other treatments.

Fig. 13. The influence of PGPR and vermicompost tea on acidity % of pomegranate fruits (Manfalouty cultivar) during 2020 and 2021 seasons



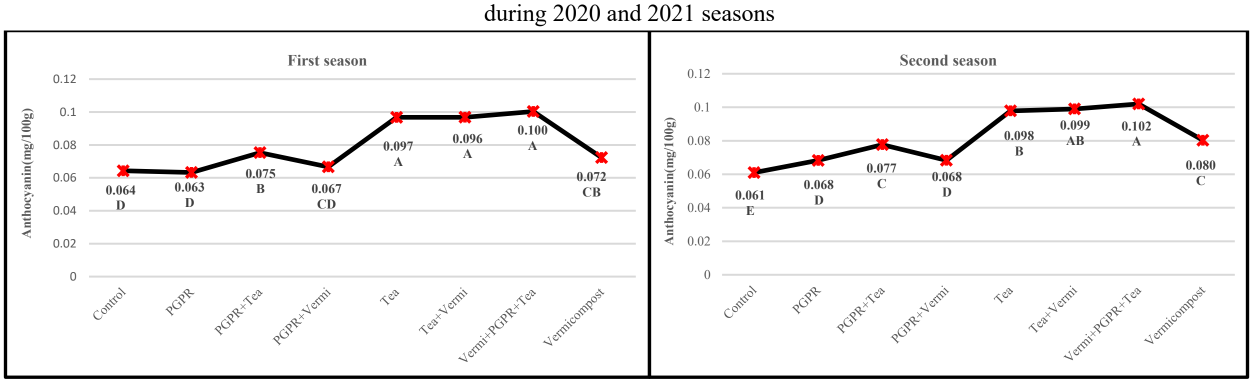
**Means separation by LSD tests at P ≤ 0.05. The same letters of data indicate that are not significantly different. Ascending order starts from (A) means the highest value until reaches the letter which has the lowest value.*

3.3.3. Anthocyanin

Data in Fig.14 explain the effect of treatments on anthocyanin in juice. In the first season, the highest value was found in both **T_5**, **T_6**, and **T_7** (0.097, 0.096, and 0.100 mg/100gm) respectively, and the lowest percentage was found in **T_2** and the control (0.063, 0.064 mg/100gm) respectively, and other treatments came between them with a trivial difference. In the second season, all treatments had a positive impact, better than in the first season. There were significant differences between all treatments compared with the control. The best effect was in **T_7** (0.102 mg/100gm), and then **T_6** (0.099 mg/100gm) with a trivial difference and other treatments came second with significant differences, and the least effect was found in the control (0.061 mg/100gm).

In addition to the above, the data clarified that the interaction between the two seasons proved that the second season had a positive effect better than the first season.

Fig. 14. The influence of PGPR and vermicompost tea on anthocyanin (mg/100gm) of pomegranate fruits (Manfalouty cultivar) during 2020 and 2021 seasons



**Means separation by LSD tests at P ≤ 0.05. The same letters of data indicate that are not significantly different. Ascending order starts from (A) means the highest value until reaches the letter which has the lowest value.*

4. Discussion

This study illustrated that the use of vermicompost and PGPR in a separate form, or a combined form, as an alternative chemical fertilizer application, achieved a positive effect on the physical and chemical properties, and yield production, of pomegranate fruits, compared with untreated fruits.

Generally, the aim of using fertilization, whatever the kind, whether chemical or organic, is to increase tree vegetative, root growth, and so the yield. Continuous cropping without using appropriate manure and overuse of mineral fertilizers is the main factor contributing to lower crop yields (Ndayisaba, 2013).

In addition to, the negative effects of chemical fertilizers on the environment, human, and plant health, many countries around the world have reduced the use of chemical fertilizers and replaced them with organic fertilizer substitutes and bio-fertilizer. When organic fertilization was applied, it caused less damage to plant tissue and increased plant growth (Neri *et al.,* 1998). Also, bio-fertilizers were used to improve the plant growth, yield, and fruit quality, as in pomegranate (El-Salhy *et al.,* 2015). The bio-fertilizer helps to preserve soil and water resources for future generations (Abd al-Hadi and Abd al-Adeem, 2005). Previous research has found that applying PGPR to several plants, such as sugar beet, barley (Cakmakci *et al.,* 2001), raspberry (Orhan *et al.,* 2006), apple (Esitken *et al.,* 2009), and, can increase production and quality indices (Aslantas *et al.,* 2007). They were also shown to be capable of generating IAA, cytokinin, and antimicrobial substance production in plant shoots, as well as having N_2-fixing and phosphate-solubilizing abilities (Aslantas *et al.,* 2007; Esitken *et al.,* 2009). Foliar applications of PGPR have considerable influences on fruit set, pomological properties, color amounts, chemical structures, and characteristics of vegetative growth of sour cherry (Karakurt *et al.,* 2011).

A direct influence of auxin on fruitlet abscission could result in a delay in fruitlet abscission and/or an increase in the number of fruitlets in a set. Only when ethylene production is low or non-existent is this effect visible (Guardiola, 2008). It is well known that Plant hormone-producing PGPR strains, such as auxins and cytokinins, can stimulate plant cell elongation or cell division, and/or alter bacterial 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity, which prevents the production of the plant growth-inhibiting hormone ethylene (Patten and Glick, 2002). (Penrose *et al.,* 2001).

Furthermore, it was demonstrated that foliar applications of the bacterial strains boosted vegetative growth indices as leaf area and shoot development. As a result, trees treated with a foliar application of these bacterial strains may produce higher-quality fruit. The bacterial treatments also changed the chemical content of the fruit. Bacterial treatments may have an indirect effect on fruit quality because phytohormones produced by bacteria alter fruit set, pomological properties, and vegetative growth. This can be explained by the fact that when fruit sugar content and color hues decrease, fruit enlargement increases (Aslantas and Karakurt, 2007). Color hues become darker as the brightness, redness, and yellowness level values increase (Orhan *et al.,* 2006). The levels of glucose, fructose, and total soluble solids had substantial positive relationships with plant vegetative development. Higher vegetative growth means more photosynthesis and soluble matter syntheses, such as sugars, which affects fruit quality parameters including size, composition, and color (Karakurt *et al.,* 2011).

There was a study that illustrated that PGPR could increment strawberry yield, and other previous studies with the PGPR tested on tomatoes, corn, sugar beet, and barley indicated comparable results, validating our findings in this study (Pırlak and Kose, 2009). The PGPR strain enhanced yield and quality metrics in apple (Aslantas *et al.,* 2007), tomatoes (Turan *et al.,* 2004), sugar beet, and barley (Cakmakci *et al.,* 2001). And also, floral and foliar spray with Bacillus strain incremented growth and yield in apricot (Esitken *et al.,* 2002; 2003). Likewise, de Silva et al. (2000) illustrated that Pseudomonas fluorescens Pf5 was used to improve the leaf area and stem diameter of high bush blueberries. It was also demonstrated in a study by Aseri *et al.* (2008), who found that after 4 months, dual inoculation Punica granatum plants with G. mosseae and A. brasilense had the maximum total chlorophyll. In this regard, the use of PGPR is considered a sustainable agricultural method for increasing the growth and yield of crops (Sturz *et al.,* 2000; Shoebitz *et al.,* 2009), despite the mechanism of PGPR-induced promotion of growth and yield of several crops is not even completely figured out (Dey *et al.,* 2004).

Because it has a higher nutritional content than standard composts, vermicompost is excellent organic manure for improving plant growth and output. This is owing to the action of earthworms has enhanced the pace of mineralization and increased the degree of humification, porosity, aeration, drainage, and water-holding capacity, all of this found in vermicompost. The presence of microbiota, notably fungi, bacteria, and actinomycetes, in vermicompost makes it appropriate for plant growth. Vermicompost contains plant-available nutrients such as nitrates, phosphates, exchangeable calcium, and soluble potassium. Plant growth regulators and other plant growth influencing compounds generated by microorganisms are also present. Organic wastes produced by earthworms were found and had cytokinins and auxins. Certain metabolites, such as vitamin B, vitamin D, and related compounds, are released into the soil by earthworms. In addition to improved N availability, C, P, K, Ca, and Mg availability on the form of a facilitator for plant uptake, all these were included in vermicompost (Joshi *et al.,* 2015).

Plant nutrients such as N, P, K, Ca, Mg, S, Fe, Mn, Zn, Cu, and B are found in vermicompost, and their uptake increases plant nutrition, photosynthesis, and the chlorophyll content of the leaves, as well as the nutrient content of the various plant components (roots, shoots, and fruits). The high percentage of humic acids in vermicompost benefits plant health by promoting the synthesis of phenolic compounds like anthocyanins and flavonoids, which may increase plant quality and function as a pest and disease deterrent (Theunissen *et al.,* 2010). Vermicompost exhibited the same effect as inorganic fertilizer application, demonstrating that vermicompost is a viable alternative to chemical fertilizer application (Singh *et al.,* 2008; Rajiv *et al.,* 2010).

This study proved that vermicompost alone increased the nutritional content, fruit quality, and yield as is the case in Flame Seedless grapes (*Vitis vinifera* L.) and Zaghloul dates (*Phoenix dactylifera* L.), according to Kassem & Marzouk (2002), and Marzouk & Kassem (2011), respectively.

5. Conclusion

The results proved that all treatments increased and improved all physical and chemical parameters of leaves and fruits when compared to the control, and those properties were improved to some extent in the second season compared to the first, which was due to the cumulative effect of vermicompost in the soil. In comparison between all treatments in the two successive seasons, the recommended treatment included a mix of PGPR, vermicompost tea spraying, and soil application of vermicompost tea for the best effectiveness.

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Data Availability Statement

Data presented in this study are available on fair request from the respective author.

Ethics Approval and Consent to Participate

This work carried out at Soils and Water Department and Pomology Department, Faculty of Agriculture, Assiut University and Microbial Department, Soils, Water and Environ Research Institute, Agricultural Research Center and followed all the departments instructions.

Consent for Publication

Not applicable.

Conflicts of Interest

Declare no conflict of interest.

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