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Impact of some Plant Growth Promoting microorganisms and Humic Acid on Phosphorus Solubilization for increasing Murcott tangerine Production

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ABSTRACT

Egyptian lands generally suffer from a deficiency of phosphorus because a large part of the phosphorus nutrient is fixed in the soil in the form of tricalcium phosphate (TCP) due to high temperatures and lack of organic matter with an increase in pH. Two field experiments were conducted at in a private orchard (Pico Farm) located in Giza governorate, Egypt during two seasons (2021-2022) to study the influence of plant growth promoting microorganisms (PGPM) and humic acid on phosphate solubilization of Murcott tangerine trees *(Citrus sinensis (L.) Osbeck x Citrus reticulata Blanco)* with winter management and/or after fruit set compared to control a randomized complete block design. All tested microorganisms were apparently able to trigger PGPM properties. However, *Trichoderma asperellum* appeared to be superior in IAA, P-solubilization *Bacillus megatherium* recorded the best production of exopolysaccarides. As for the nature of siderophores, two strains exhibited hydroxymate nature whereas, carboxylate nature was showen by *Trichoderma*. on the contrary of catecholate nature which was not produced by either strains. The obtained results showed a significant rise in all measured parameters for yield efficiency, fruit set percentage, leafy inflorescence's percentage, fruit quality attributes, bio-chemical contents and mineral content of leaves as compared with the control. Optimum results were obtained from the T_7 (Humic acid with winter management + after fruit set) and T_4 (PGPM with winter management + after fruit set) treatments.

Keywords: Murcott tangerine trees, PGPM and solubilization phosphorus.

1. Introduction

Citrus (*Citrus spp*.) is a widely grown and commercially popular fruit crop (Wu *et al.,* 2011). Citrus is the most popular in Egypt, their fruitful acreage reached over 451530 Fed., yielding approximately 4708427 tons/Fed .(Source: Ministry of Agriculture and Land Reclamation/Annual Report, 2022).

The Murcott originated from the efforts of citrus pioneer Walter Tennyson Swingle to create innovative citrus hybrids. Although the trees grow straight, their branches frequently break or bend at the ends from heavy fruiting. Fruit should be shielded from the wind and other environmental elements while it ripens since it usually grows at the edge of the tree. Although the Murcott's true ancestry is uncertain, it is most likely a tangor a hybrid of a sweet orange and tangerine. The Murcott fruit has a characteristic tangerine form and is medium in size, with an average diameter of 2.5–3 inches. The fruit is perfect for the fresh fruit market and reaches maturity in March. Certain factors, such as climate, growing circumstances, and tree age, might affect when fruit ripens exactly. The application of substances such as humic acid and bio fertilizers can greatly enhance the productivity of Murcott tangerine by hamessing their positive impact on soil quality and plant characteristics. Al-Kraawi *et al.* (2020) on Lemon citrus (*Citrus limon* [L.] Burm); Ihsan *et al.* (2019) on Volkameriana seedlings; Hameed *et al.* (2018) on kinnow mandarin (*Citrus reticulata*); Maidan and Maree (2018) on orange (*Citrus sinensis* L.); Al-Hayani (2016) on lemon; Abobatta (2015) on Valencia orange (*Citrus sinensis* L.).

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One of the most widely distributed metallic elements in the earth's crust, is phosphorus it can be found in both organic and inorganic forms in soil. (Gyaneshwar *et al.,* 2002). Plants utilized phosphorus as inorganic form (orthophosphate) (Hinsinger, 2001), and severely lacking due to phosphorus sorption (fixation) capacity. Phosphorous is essential for several metabolic processes, including signal transmission, photosynthesis, nitrogen fixation in legumes, and crop quality. (Khan *et al*, 2014). According to Ozanne (1980), phosphorus appears to be a structural phospholipid coenzyme component and is a component of living things' DNA genetic memory, which is involved in development and reproduction. Phosphorus plays a specific role in the metabolism of carbon and the production of membranes during photosynthesis (Wu, 2005), Elongation and proliferation are components of root architecture (Borch *et al.,* 1999; Williamson *et al.,* 2001). One of the various mechanisms that Plant Growth Promoting Rhizobacteria (PGPR) possess is the solubilization of phosphorus through the use of organic acids, which function as chelating phosphor ions and release the P straight into solution (He *et al.,* 2002).

Phosphate Solubilizing Bacteria (PSB): Since the 1950s, the use of PSB as a bio-fertilizer has increased the availability of plant phosphorous (Khan *et al.,* 2009). Microbial inoculants can be used as an alternative source, which is not only cost-effective but also environmentally friendly. Continued investigation of the natural diversity of the soil microorganisms, and optimization of the microbial interactions within the soil rhizosphere, is a pre-requisite for the development of more effective microbial inoculant with the ability to solubilize phosphorus. A soil's sustainable productivity is largely dependent on its ability to provide essential nutrients to growing plants. Micronutrient deficiencies have become a major obstacle to soil productivity, stability, and sustainability (Bell and Dell 2008). The naturally occurring rhizospheric phosphorus solubilizing microorganism (PMS) has been around since 1903. The number of microbial species play a key role in the process of P solubilization. Fungi in soil are able to travel long distances more efficiently than bacteria and may therefore play a more important role in the formation of soil phosphorus (Venkateswarlu *et al.,* 1984).

The main ways through which soil microorganisms solubilize nutrient include the release of complex compounds like organic acid anions and siderophores, the release of protons and hydroxyl ions, the release of CO_2, the secretion of extracellular enzymes (also known as biochemical Phosphorus mineralization), and the release of phosphorus during substrate degradation. Soil microorganisms play a crucial role in the soil phosphorus cycle, which involves processes such as precipitation, sorption, desorption, and mineralization. In such conditions, it is recommended to use biofertilizers. According to Bolan and Duraisami (2003), PSB greatly enhanced phosphorus nutrition.

Trichoderma, a type of filamentous fungus commonly utilized in agriculture, is crucial in promoting sustainable agriculture and soil productivity. It achieves this by enhancing soil physical and chemical properties and maintaining beneficial soil microbial communities (Zin and Badaluddin 2020). One of its key contributions is the production of organic acids, which effectively reduce soil pH and enhance soil nutrient availability (Poveda, 2021). *Trichoderma* also aids in the transformation of soil enzymes and nutrients (Fu *et al*., 2019). The fungal cellular wall is primarily composed of polysaccharides, specifically glycogen, glucan, galactan, mannan, galactomannans, xylomannans, polygalactosamsinde, chitin, chitosans, cellulose, and polyuronides (Ohno, 2007). These polysaccharides play significant roles in the rheology, growth, pathogenicity, cell protection, resistance, chemical signal transfer, and stress related pathways of fungi (Jaroszuk *et al.,* 2020).

Bacillus megaterium var. phsphaticum, also known as phosphobacterium, is a large rod shaped bacterium that is classified as Gram's positive (Menkina, 1963). The ability of *Bacillus megatherium* var. phsphaticum to enhane solubilization of mineral phosphorus (P) is widely acknowledged (Vazquez *et al.,* 2000). Various mechanisms have been suggested to explain this P solubilization, which involve the secretion of both organic and inorganic acids as well as the release of phosphatase enzymes that break down organic P compounds (Stevenson, 1986).

Humic acid is a crucial component of the soil organic matter which is highly efficient in enhancing the fertility of soil and plant production (Pettit, 2004). Humic acid has a substantial impact on plant health in agriculture as it enhances chemical, physical, and biological characteristics of soil. Its hydrophilic groups (carboxyl, hydroxyl, amide) effectively function as chelating agents, improving soil P^H, cation exchange, water holding capacity, and soil texture (Karakurt *et al.,* 2009).

The current study tended to explore the effects of plant growth promoting and humic acid on flowering, fruit set, fruit quality and yield of Murcott tangerine trees.

2. Materials and Methods

This research was carried out in two consecutive seasons, years, 2021 and 2022, focusing on Murcott tangerine trees (*Citrus sinensis (L.) Osbeck x Citrus reticulata Blanco)*. Ten-year-old Murcott tangerine trees grafted on Volkamer lemon (*Citrusvolkameriana)*rootstock planted 5 × 5 m apart in sandy soil at Pico Farm, a private orchard in Giza governorate, Egypt. The trees were cultivated under a drip irrigation system to study the impact of soil application of two phosphate solubilization microorganisms (PSM) ($5.2X10^6$ CFU/ml of *Trichoderma asperellum* and $6.3X10^8$ CFU/ml of *Bacillus megatherium*) or humic acid (670 mg/L) provided by the Bio-fertilizer Production Unit, Agric. Microbiology. Dept., Soils, Water and Environ. Res. Inst. (SWERI), Agric. Res. Center (ARC), Giza, Egypt. The applications were made 50 cm away from the tree trunk and 30-50 cm deep from the soil surface in either late December (during winter management) or late May (after fruit set) or both times. Five L/tree/time was applied to enhance phosphorus dissolution in the soil in a form accessible to plants, promoting growth, flowering, fruit set, ripening standardization, increased total soluble solids percentage, yield, fruit quality, and subsequent production increase. Table (1) show data on certain physical and chemical characteristics of the soil as determined using the techniques outlined by Page *et al.* (1982).

Table 1: Physical and chemical properties of the soil Property

Property	**2020/2021**	**2021/2022**
Sand %	50.28	50.35
Silt %	22.23	21.81
Clay %	24.77	26.72
Texture grade	Sandy Loam	
pH (1:2.5)	7.60	6.98
EC (dS m^{-1})	1.83	1.54
OM (%)	0.41	0.39
Total-N (%)	0.030	0.031
Available-P (mg kg^{-1})	3.81	4.11
Available-K (mg kg^{-1})	100.38	98.17

Twenty-one healthy trees with consistent growth and fruit production were selected for the study, and they were randomly divided into groups. An experiment was conducted using a full randomized block design, with three replicates assigned to each treatment. The study included seven different treatments:

T_1 = Control
T_2 = Plant Growth Promoting Microorganisms (PGPM) with winter management
T_3 = Plant Growth Promoting Microorganisms (PGPM) after fruit set
T_4 = Plant Growth Promoting Microorganisms (PGPM) with winter management plus after fruit set
T_5 = Humic acid with winter management
T_6 = Humic acid after fruit set
T_7 = Humic acid with winter management plus after fruit set

2.1. Microbiological and Horticultural studies.

2.1.1. Microbiological studies

Humic acid and microorganisms that solubilize phosphate were generously provided by the Biofertilizer Production Unit at the Agriculture Department. Microbiol., Dept., SWERI, ARC, Giza, Egypt. Humic compounds from composted rice straw (Table 2) are obtained by squeezing and diluting the humic compounds present in the compost.

Table 2: Chemical characteristics of composted rice straw

Physico- chemical and biological compost properties	
pH (1 : 10 extract)	6.8
E.C (dS/m)	4.30
Organic matter (%)	46.50
Total-N (%)	1.17
Total-P (%)	0.50
Total-K (%)	1.32
C/N ratio	17.89
Total soluble-N (mg/kg)	963.2
Available-P (mg/kg)	302.8
Available-K (mg/kg)	513.2

In this study, we have selected two kinds of plant growth promoting rhizobacteria, specifically phosphate solubilizing microorganisms (PSM) (*Bacillus megatherium* and *Trichoderma asperellum* OR23476). Prior to their application, we were intending to evaluate the effectiveness of these microorganisms as plant growth promoting (PGP) in the following way:

The quantification of indole acetic acid production was performed using Gordon and Weber's (1951) method. Activity of phosphate solubilization: Gupta *et al.* (1994) performed a screening procedure to identify the technique for phosphate solubilization activity. The clear area surrounding the microbial colony suggests that phosphorus has been dissolved (Noori and Saud, 2012). The solubilization index (SI) was computed following the method outlined by Edi-Premono *et al.* (1996). The quantitative evaluation of inorganic phosphate solubilization was carried out using the method outlined by Pikovskaya (1948). Watanabe and Olson (1965) subsequently measured the phosphorus levels in each sample. Evaluation of siderophore production. The method used for assessing siderophore activity was conducted by Alexander and Zuberer (1991) using Chrome azurol Sulfonate (CAS) media. Ghosh *et al.* (2017) identified the hydroxymate nature of siderophores, while Farokh *et al.,* (2011) determined the catecholate nature of siderophores. Ghosh *et al.* (2017) and Vogel (1992) identified the carboxylate nature of siderophores. Extracelluer polysaccharides (EPS) isolation and purification as described by Breedveld *et al.* (1990) and Aranda-Selverio *et al.* (2010).

2.2. Horticultural studies

2.2.1. Bio-chemical contents

2.2.1.1. Leaf pigments contents

Chlorophyll (a), (b) and total chlorophylls as well as carotenoids were measured according to Nornai, (1982) and expressed as (mg/100g F.W.).

2.2.1.2. Leaf indole and phenol contents

Phenol was calculated as mg/g dry weight as reported by Daniel and George (1972), and indole was calculated as mg/g dry weight in accordance with Larsen *et al.* (1962), amended by Selim *et al.* (1978).

2.2.1.3. Flowering and fruit set parameters.

For every tree, the proportion of fruit set and woody inflorescence at the flower balloon stage was calculated for each of the four main branches.

- Percentage of fruit set= (Total number of fruit sets / Total number of flowers) × 100.
- Leafy inflorescence percentage = (Number of leafy inflorescences overall / Total number of florescence's) ×100.

2.3. Yield efficiency.

Tree size, measured in canopy volume, and tree height were computed using the following formula: 0.5236 x height x diameter square (Turrell, 1946). Every year, the amount of fruit produced is documented. Yield efficiency was measured as yield in relation to tree volume (Yield efficiency = kg of fruits / m^3 canopy of tree).

2.4. Fruit quality attributes.

At harvest time, in the 1st week of March, a sample of ten fruits per replicate was chosen in order to assess the fruit quality as below:

2.5. Fruit physical attributes.

Fruit peel thickness (mm), fruit shape index (length/width), average fruit weight (g),size (ml) and juice weight percentage (w/w).

2.6. Fruit chemical attributes. Total soluble solids percentage, total acidity percentage and vitamin C (mg/ 100 g. juice) were calculated as well according to (AOAC, 2000).

2.7. Mineral content of leaves

In September, mature leaves from non-bearing spring flushes were gathered for the two seasons to determine leaf mineral content.

a. Nitrogen using the semi-micro Kjeldahl technique, as ascribed by Pregl (1945).
b. Phosphor spectrophotometer at 882 volts using the Murphy and Riely (1962) technique.
c. According to Brown and Lilleland (1946) a flame photometer was used to assess potassium.
d. Assessed the nitrogen and the hydrolysis of carbohydrates in mature leaves (Dubois *et al.* 1956). Then the C/N ratio was calculated as total carbohydrates to nitrogen ratio.

2.8. Statistical analysis.

A full randomized complete block design was set up with seven treatments and three replicates for each treatment. Each season, the collected data underwent one-way analysis of variance (ANOVA) as outlined by Snedecor and Cochran (1980) utilizing the M-STAT software. The different treatments were compared using the Duncan's (1958) range tested at a significance level of 0.05 to determine the mean values.

3. Results and Discussion

3.1. Evaluation of plant growth promoting activities of PGPM *in vitro*

Table (3) illustrates the quantitative analysis of indole acetic acid as a measure of PGP-related characteristics of microorganisms in *vitro*. In respect to IAA production, *B. megatherium* was followed by *T. asperellum* in the order of production. The relative levels were 81.00μgmL^{-1} and 109.11μgmL^{-1}. This finding is corroborated by studies by Mara *et al.* (2014) and El-Sayed *et al.* (2016), which found that *Trichoderma* isolates are potent IAA-producing microorganisms and that using L-tryptophan precursor as an inducer had a beneficial influence on this phytohormone's synthesis. Furthermore, a significant percentage of microorganisms have been shown to be able to create plant growth hormone (IAA), which stimulates the growth of roots as well as greater branching and surface area. This information was reported by Badawi *et al.* (2011).

The qualitative assessment of microorganism's solubilization of insoluble tricalcium phosphate is displayed in Table (3). Two microbial strains were able to solubilize insoluble tricalcium phosphate in contrast to the control. Compared to *Bacillus megatherium*, which recorded 107.13 and 83.50 ppm, respectively, *Trichoderma* fungus solubilized more phosphorus. Due to their larger colonies and higher biomass than bacteria, fungi solubilized more P (Illmer and Schinner, 1995). Table (3) displays the soluble index (SI) for each phosphate-solubilizing microorganism depending on colony diameter and the halo-zone surrounding it. As colony diameter increased, so did the halo-zone. Findings indicated that *Trichoderma* with SI = 1.85 cm, the results indicated that *Trichoderma* was the most effective phosphate solubilizer. This is because it's possible that organic acids are the primary cause of P solubilization. The solubilization index and the amount of organic acids generated showed the strongest positive association (Alam *et al.,* 2002).

Many microorganisms produce extracellular polymeric substances, which are widely used in the food and beverage industries as well as the adhesives industry. The results shown in Table (3) indicated that *B. megatherium* produced more EPS than *T. asperellum*, with respective values of 39.12 and 23.16

gL^{-1}. High molecular weight substances known as exopolysaccharides influence soil P solubilization indirectly (Yi *et al.,* 2007). Diverse exopolysaccharides have been observed to exhibit distinct binding affinities with various metals, as well as varying binding strengths among the metals themselves (Ochoa-Loza *et al.,* 2001). When PSB simultaneously produced a significant amount of effective organic acids, EPS from phosphate-solubilizing bacteria indirectly contributed to the solubilization of TCP (Yanmei *et al.,* 2008).

Microorganisms suffering from iron deficiency produce siderophores, which are complexing agents. The data obtained in Table (3) declared that *B. megatherium* produced 59.18% of siderophores, while *T. asperellum* produced 45.72%. Regarding the various types of siderophores, *T. asperellum* alone produced carboxylate siderophores, while *B. megatherium* and *T. asperellum* both produced hydroxymate siderophores. On the other hand, it can be produced by *T. asperellum* and *B. megatherium's* catecholate siderophores. In soils, insoluble Pi was limited by the cations Ca, Fe, and Al (Luyckx *et al.,* 2020). The physicochemical stabilization of organic P compounds and the environmental regulation of phosphate imopilization levels in soil are affected differently by the geochemical precipitation of P (Dodd And Sharpley 2015). A chemically diverse class of secondary metabolites known as siderophores has a strong affinity for iron (Gu *et al.,* 2020). There are three distinct types of siderophores based on their functional group: hydroxamates, catecholates, and carboxylates (Verma *et al.,* 2012; Neilands, 1995).

Table 3: In vitro some plant growth promoting activities properties of *Bacillus megatherium* and *Trichoderma asperellum* PGP-related properties of the tested microorganisms *in vitro*.

Microbial strains	Amount of IAA (ppm)	Dissolved phosphorus (ppm)	SI	EPS gL^{-1}	Siderophores %
T. asperellum	109.11	107.13	1.85	23.16	45.72
B. megatherum	81.00	83.50	0.98	39.1	59.18

	Siderophores nature		
	Hydroxymate	**Catecholate**	**Carboxylate**
T. asperellum	++	-	+
B. megatherum	++	-	-

Enzymes such as dehydrogenase and phosphomonoestrase are used to demonstrate the biological activity of the rhizosphere of Murcott tangerine trees. When compared to other treatments, the data in Table 4 demonstrated the significant increase in dehydrogenase activity. In the first and second seasons, application of (T_7) resulted in maximum dehydrogenase activity values of 230.0 and 246.4µg TPF/g/24 h, respectively. Dehydrogenase activity was strongly influenced by PGPR, which also behaved similarly to other treatments. Application of T_7 increased alkaline phosphates (88.05 – 85.30) and acid phosphates (87.26 – 85.42) percent during two growing seasons, respectively, following the same trend. Additionally, data in Table (4) did not demonstrate a statistically significant increase in available phosphorus as a result of various treatments during the first season; instead, T_7 demonstrated the most effective treatment during the second season. Application of rhizospheric humic acid boosted the number of microorganisms in the root zone (Deepa, 2001). Therefore, the combination of PSM and humic acid results in an enhancement of rhizosphere soil enzymes, promoting efficient utilization of nutrients. The concurrent elevation of both enzyme classes as a result of humic acid addition may suggest the influence of organic matter on phosphorus dissolvent, enabling the proliferation of other microbial communities. Kucey (1983) demonstrated that a carbon deficiency could lead to an absence of a significant correlation between phosphate solubilizers and available-P, as they solubilize P constitutively rather than being inducible or that microbial numbers reflect native-P status rather than present P status. Additionally, imbalanced phosphorus levels in the soil caused shifts in soil microbial communities (Ducousso-Detrez *et al.,* 2022).

Metabolizable compost compounds are present, serving as energy sources for microorganisms and potentially leading to increased dehydrogenase enzyme activity (Bueis *et al.,* 2018). *B. megatherium*, a phosphate solubilizing bacteria, can solubilize di and tri calcium phosphate by releasing organic acids with carboxylic and hydroxide groups (Rodriguez and Fraga, 1999). Phosphatase and phytase enzymes from phosphate solubilizing bacteria can convert organic phosphatic compounds into mineral phosphorus (Richardson *et al.,* 2009). *T. harzianum* has the ability to enhance soil phosphorus supply through phosphatase activity and expedite the conversion of organic phosphorus compounds into mineral phosphorus in the soil (Zhu *et al.,* 2022).

Table 4: Available P and some enzymatic activities in the rhizosphere of Murcott tangerine trees were observed due to the application of different treatments.

Treatment	Available phosphorus (ppm)	Dehydrogenase (µg TPF/g/24 h)	Acid Phosphatase (mg PNP/g DW soil/hr)	Alkaline Phosphatase (mg PNP/g DW soil/hr)
		1st season		
T_1	4.98a	122.8g	7.540f	11.64e
T^2	5.16a	143.8f	8.830ef	13.68d
T_3	5.32a	158.8e	9.750df	15.76c
T_4	5.56a	176.2d	10.76cd	16.77c
T_5	6.18a	198.2c	12.17bc	18.86b
T_6	6.62a	215.8b	13.25ab	20.54ab
T_7	6.82a	230.0a	14.12a	21.89a
		2nd season		
T_1	5.18b	133.0g	8.160d	12.66e
T^2	5.23ab	145.8f	8.950d	13.87de
T_3	5.42ab	162.3e	9.970cd	15.45cd
T_4	5.68ab	179.2d	11.00bc	17.05c
T_5	6.52ab	200.0c	12.28b	19.04b
T_6	6.80ab	238.1b	14.62a	22.66a
T_7	7.15a	246.4a	15.13a	23.46a

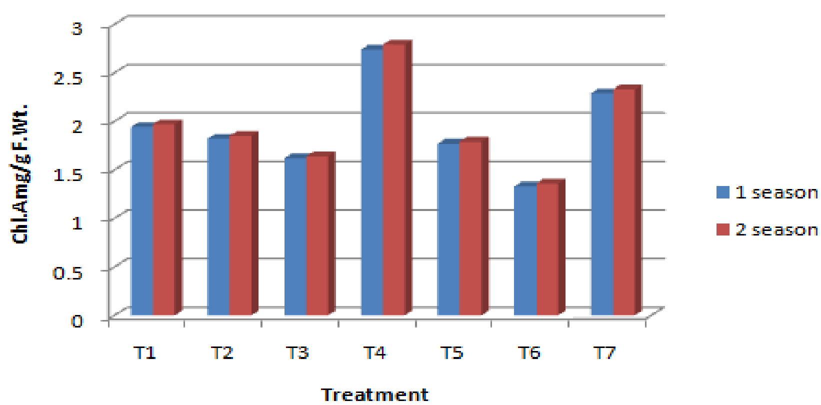
T_1 = Control
T_2 = PGPM with winter management
T_3 = PGPM after fruit set
T_4 = PGPM(with winter management + after fruit set)
T_5 = Humic acid with winter management
T_6 = Humic acid after fruit set
T_7 = Humic acid (with winter management + after fruit set)

As for available phosphorus, data in Table (4) indicated that different treatments did not have a significant impact on available P in the first season. However, in the second season, the performance of different treatments varied, with T_7 showing the highest available P (7.15 PPm), while the other treatments had similar effects to the control. According to Al-Bahrani, (2015), Bacillus and Pseudomonas, either alone or in combination, increase phosphorus availability by producing organic acids that enhance phosphorus availability. Additionally, humic acid enhances the release of phosphorus into the soil solution and prolongs the slow dissolution and persistence of phosphorus minerals in the soil when added (Abdel–Razzak and El–Sharkawy, 2013).

This could be a result of bacteria impacting soil pH through organic acid production or phosphatase production, aiding in phosphorus release, and humic acids promoting microbial growth in soil (Bano and Musarrat, 2003; Burkowska and Donderski, 2007).

3.2. Leaf determinations
3.2.1. Bio-chemical contents
3.2.1.1. Leaf pigments contents

Figures (1, 2, 3, and 4) were used to analyze the photosynthesis of Murcott tangerine trees based on the levels of chlorophyll a, b, total, and carotenoids pigment. The trees showed significant changes in chlorophyll levels when treated with PGPM during winter management and after fruit set (T_4). Chlorophyll a and total chlorophyll increased by approximately 41.23 - 41.62% and 19.87 – 19.17% in the 1st and 2nd seasons, respectively. However, chlorophyll b and total carotenoids were more influenced by treatment (T_7), with increases of 27.38 - 26.43% and 78.60 - 79.80% in the 1st and 2nd seasons. According to Kumar *et al.* (2019), the combined use of inorganic fertilizers, organic manure, and bio-fertilizer greatly increased the chlorophyll levels in acid lime fruits. The minor amount of phosphorus in the soil indicates a reduction in ATP and NADPH synthesis and the activation of genes associated with photosynthesis. Therefore, these reductions are mirrored in the chlorophyll index as it serves as a representation of photosynthetic pigments. Hence, applying phosphate close to *Trichoderma* may have influenced the plant's ATP production and the activation of genes related to photosynthesis due to higher chlorophyll levels. Komal *et al.* (2022) reported that most plants exhibit higher levels of chlorophyll in their mature leaves compared to their young leaves, highlighting the positive effect of PGPM on leaf chlorophyll levels. The study found that combining different PGPM had a more significant influence on plant chlorophyll content than administering them individually. Variations in chlorophyll levels can be caused by various factors that impact leaf characteristics like size, form, structure, and chloroplast distribution, such as water, soil, and temperature conditions. According to Cano-Castro *et al.* (2024), PGPRs are necessary when nutrients are not easily accessible in the soil or near the rhizosphere. Some of the mechanisms that contribute to the stimulation of leaf pigment contents include producing hormones that boost chlorophyll a, b, total, and carotenoids in the leaves, as well as engaging in other activities that enhance plant growth. In recent years, numerous PGPR strains have been discovered, and their impacts on leaf pigment levels, plant growth, disease and insect resilience, and the stimulation of systemic plant defenses have been thoroughly researched. Some varieties have demonstrated to be crucial for boosting agricultural yield.



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T$_5$ = Humic acid with winter management
T$_6$ = Humic acid after fruit set
T$_7$ = Humic acid (with winter management + after fruit set)

Fig. 1: Impact of some plant growth promoting microorganisms (PGPM) and humic acid on Murcott tangerine trees Chl.A content (mg/g F.Wt.) in leaves during the two successive seasons.

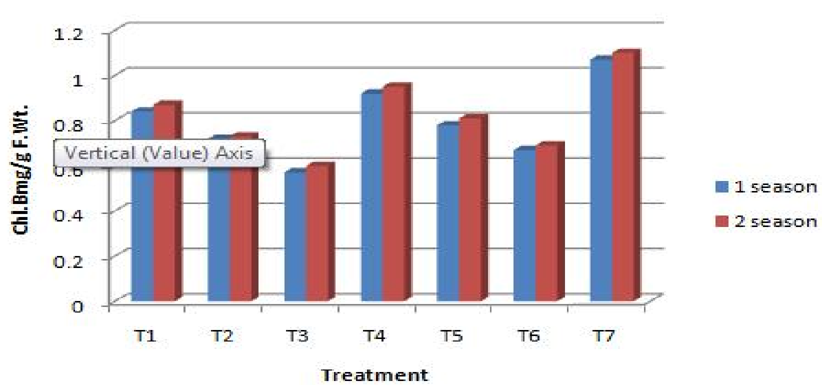

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Fig. 2: Impact of some plant growth promoting microorganisms (PGPM) and humic acid on Murcott tangerine trees Chl.B content (mg/gF.Wt.) in leaves during the two successive seasons.

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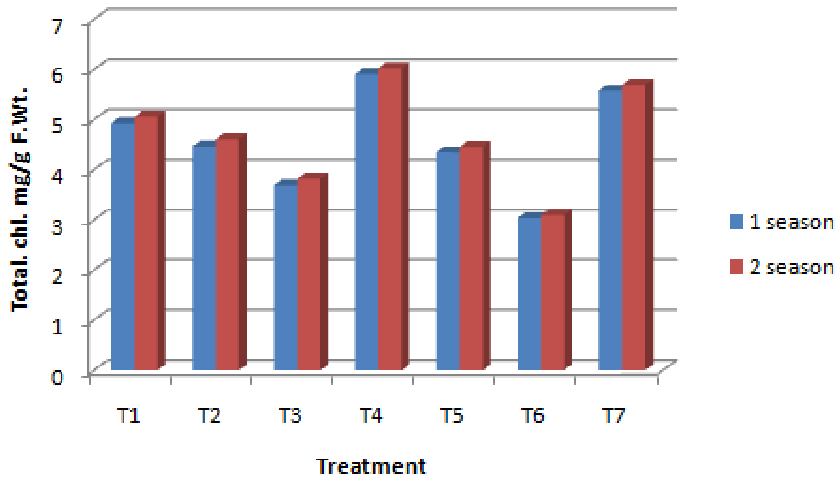

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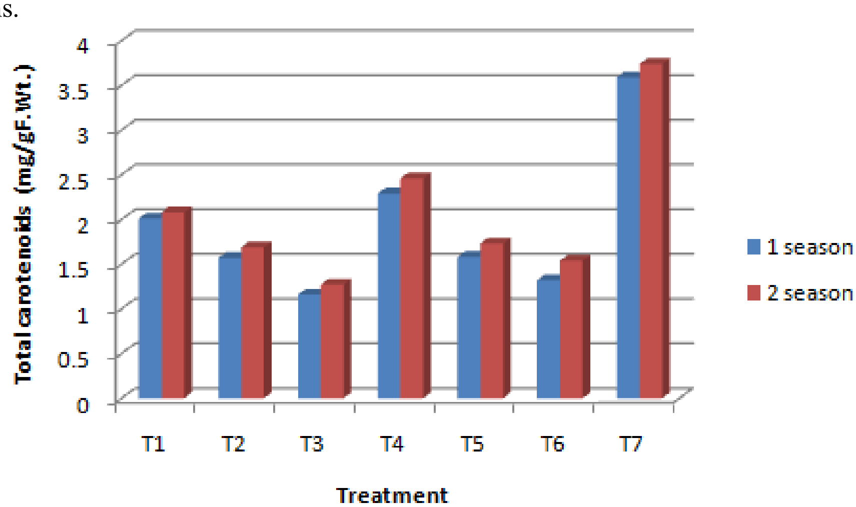
Fig. 3: Impact of some plant growth promoting microorganisms (PGPM) and humic acid on Murcott tangerine trees total chlorophyllscontent (mg/gF.Wt.) in leaves during the two successive seasons.



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T_6 = Humic acid after fruit set
T_7 = Humic acid (with winter management + after fruit set)

Fig. 4: Impact of some plant growth promoting microorganisms (PGPM) and humic acid on Murcott tangerine trees total carotenoids content (mg/gF.Wt.) in leaves during the two successive seasons.

3.2.1.2. Leaf indole and phenol contents

Table (5) displays how various treatments impact the chemical composition of Murcott tangerine leaves over two seasons. Bigatton *et al.* (2024) noted that the use of eco-friendly farming methods has gained popularity in recent times, with T_7 showing the lowest phenols production at 0.73 mg/100g and 0.71 mg/100g for T_6 compared to the control (1.25 and 1.23 mg/100 d. wt). Additionally, indole leaf content increased significantly due to PGPM during winter management and after fruit set (T_4) by 77.03 – 78.67% in the two seasons. Many research papers have demonstrated that PGPM are able to act as bio-fertilizers, directly stimulating plant development by improving the absorption of certain nutrients through different mechanisms such as producing siderophores, fixing nitrogen, solubilizing minerals, absorbing phosphate and potassium, and sequestering iron, among others. Furthermore, PGPM has the ability to improve bacteria-synthesized exopolysaccharides (EPS), amino acids, 1-aminocyclopropane-1-carboxylate (ACC) deaminase, volatile organic compounds (VOCs), sugars (which prevent deterioration), and phytohormones such as cytokinin (CK), auxin or indole-3-acetic acid (IAA), ethylene (ET), salicylic acid (SA).

Table 5: Impact of some plant growth promoting microorganism (PGPM) and humic acid on Murcott tangerine trees leaf growth promoters and inhibitors during the two successive seasons.

	Phenol (mg/100g D.Wt)	**Indole (mg/100g D.Wt)**	**Phenol (mg/100g D.Wt)**	**Indole (mg/100g D.Wt)**
Treat.	**1st Season**		**2nd Season**	
T_1	1.25a	2.09c	1.23a	2.11c
T_2	1.11b	1.85d	1.09b	1.93d
T_3	1.17ab	1.31f	0.91c	1.33f
T_4	0.93c	3.70a	1.17ab	3.77a
T_5	0.85d	1.62e	0.83d	1.67e
T_6	0.67f	1.13g	071e	1.17g
T_7	0.73e	2.85b	0.64f	2.94b

At the 5% level, there is no significant difference between the mean and the same letter.

T_1 = Control
T_2 = PGPM with winter management
T_3 = PGPM after fruit set
T_4 = PGPM(with winter management + after fruit set)
T_5 = Humic acid with winter management
T_6 = Humic acid after fruit set
T_7 = Humic acid (with winter management + after fruit set)

3.3. Flowering and fruit set parameters include.

The performance of various treatments on leafy inflorescences and fruit set in the two seasons was summarized in Table (6). During both seasons, PGPM with winter management and after fruit set (T_4) led to a notable increase in all parameters by 28.15 - 29.18 and 26.29 - 26.11 % for leafy inflorescence and fruit set, respectively. Moreover, there was a notable increase in leaf dry matter percentage with the application of humic acid during winter and after fruit set (T_7). The findings align with previous research indicating that fertilization methods incorporating organic and bio-fertilizers improve the percentage of orange trees bearing fruit (EL-Aidy *et al.,* 2018). The effectiveness of humic acid is comparable to that of auxins, which are known to delay abscission according to various studies.Therefore, it can be seen as causing an increase in fruit production (Nikbakht *et al.,* 2008). Simultaneously, auxin's direct influence on fruitlet drop could lead to an increase in fruit set or a postponement in fruitlet abscission. This impact is only visible when ethylene production is minimal or nonexistent. PGPM strains, known for producing plant hormones such as auxins and cytokinins, have the ability to modify the function of the bacterial enzyme ACC deaminase. This enzyme helps halt the production of the growth-inhibiting hormone ethylene, while also promoting either plant cell elongation or division (Halil *et al.,* 2011).

Phytohormones regulate the flowering process, influencing both the flowering patterns and yield. Growth regulators like auxins (e.g., Inodol-3-acetic acid; IAA) impact the differentiation of floral primordia and the initiation of flowering. Moreover, cytokinins like Zeatin are involved in the regulation of floral meristem function, specifically in cell differentiation and fruit development. Gibberellins (GA1, GA3, GA4, and GA7) mainly affect the start of flower development. Similar to Jasmonic Acid (JA), some substances play a role in the ripening of flowers and fruits. It is important to mention that every process is controlled by a dominant hormone, but these processes rely on the interactions and hormonal equilibrium. Farmers have the ability to manipulate soil microbial communities to enhance flowering by influencing the production and balance of phytohormones. These tiny organisms, called Plant-growth-promoting rhizobacteria (PGPR), have the potential to enhance the process of flowering (Bigatton *et al.,* 2024).

Table 6: Impact of some plant growth promoting microorganisms (PGPM) and humic acid on flowering and fruit set of Murcott tangerine trees during the two successive seasons.

Treat.	Leafy inflorescence percentage	Fruit set percentage	Leafy inflorescence percentage	Fruit set percentage
	1st Season		2nd Season	
T_1	64.61bc	3.077abc	65.42bc	3.143ab
T_2	75.07ab	3.357abc	77.66ab	3.440ab
T_3	56.64c	2.290bc	56.94c	2.350ab
T_4	82.80a	3.897ab	84.51a	3.963ab
T_5	73.94ab	3.670ab	74.29ab	3.737ab
T_6	55.75c	1.973c	55.83c	2.177b
T_7	78.67a	4.023a	79.69ab	4.130a

At the 5% level, there is no significant difference between the mean and the same letter.

T_1 = Control
T_2 = PGPM with winter management
T_3 = PGPM after fruit set
T_4 = PGPM(with winter management + after fruit set)
T_5 = Humic acid with winter management
T_6 = Humic acid after fruit set
T_7 = Humic acid (with winter management + after fruit set)

3.4. Yield efficiency.

The yield efficiency of Murcott tangerine trees was evaluated based on the yield Parameter, as shown in Table (7) Peak productivity response to humic acid occurred both during the winter dormant period and after fruit set in the two seasons (T_7). Bio-fertilizers create different phytohormones that help citrus grow by absorbing nutrients, improving photosynthesis, controlling cell division and size, and increasing proline. Various studies support the idea that inoculating with bio-fertilizers releases phytohormones like cytokinins, gibberellins, indole acetic acid, auxins, and ethylene. Thus, bio-fertilizers play a crucial part in enhancing citrus growth, boosting yield, and improving fruit quality.

Humic acid can enhance the respiration intensity of fruit trees' root systems and provide energy for their daily functions due to its redox properties. In addition, humic acid contains numerous active genes which can boost plant physiological functions, enhance catalase and polyphenol oxidase activity in fruit trees, and promote rapid root growth in young trees, resulting in a flourishing root system and robust stems, branches, and leaves. Strong, vibrant photosynthesis accelerates the uptake and utilization of nutrients, resulting in an increase in flower production and the rate of flower bud development in plants. This ultimately leads to a higher average in yield and fruit weight per plant, as evidenced by Arancon *et al.,* (2003).

The use of organic and bio fertilizers in citrus farming brings many advantages such as enhancing fruit quality, boosting nutrient levels in the soil, minimizing pollution, and maintaining ecological harmony. Therefore, incorporating organic fertilizers in citrus groves will protect the soil and improve production quality, leading to financially viable returns. Organic fertilizers are an integral component of initiatives aimed at boosting citrus production and preserving the environment (Martinez-Alcantara *et al.,* 2016).

Table 7: Impact of some plant growth promoting microorganism (PGPM) and humic acid on Murcott tangerine trees yield efficiency during the two successive seasons.

Treat.	Yield efficiency(kg/m^3 canopy)	
	1st Season	2nd Season
T_1	2.310c	2.370c
T_2	1.943d	1.950d
T_3	1.533e	1.590e
T_4	2.813b	2.843b
T_5	1.853d	1.890d
T_6	1.460e	1.490f
T_7	3.317a	3.393a

At the 5% level, there is no significant difference between the mean and the same letter.

T_1 = Control
T_2 = PGPM with winter management
T_3 = PGPM after fruit set
T_4 = PGPM(with winter management + after fruit set)
T_5 = Humic acid with winter management
T_6 = Humic acid after fruit set
T_7 = Humic acid (with winter management + after fruit set)

3.5. Fruit quality attributes

3.5.1. Fruit physical attributes

The Murcott tangerine trees were evaluated for fruit quality aspects including peel thickness, shape, weight, size, and juice percentage in Table (8). The trees injected with winter management and after fruit set showed the highest values for weight, size, and juice percentage (T_4), while the control group (T_1) had the lowest values in both seasons. The study found that there were no notable variations in fruit shape among treatments when humic acid or two different phosphate solubilization microorganism were applied to the soil. In relation to the effect of the microorganisms strains tested in the soil injection of this study, the results from Figure (7) indicated that the control group (T_1) substantially raised the peel thickness of Murcott tangerine trees in both study seasons. However, there were distinct variations in the peel thickness (mm) following the introduction of rhizobacteria that enhance plant growth into the soil (T_4). The results provided aligned with the research conducted by Bigatton *et al.* (2024), revealing that the bacterial strains tested in the soil injection of this study also enhanced fruit physical characteristics such as fruit weight, size, and juice percentage. Consequently, using the bacterial strains can result in higher-quality fruit. The bacterial treatments also greatly changed the chemical composition of the fruit. Due to the influence of bacterial-produced phytohormones on fruit setting, fruit characteristics, and plant growth, bacterial treatments could indirectly affect fruit quality, leading to observed correlations among several factors analyzed.

There were significant negative correlations between fruit weight and fruit fructose concentration. The use of bacteria decreased all pomological features when compared to the control group. However, various treatments led to the highest increases in the levels of overall soluble solids, as well as fructose and glucose. The reason for this is that fruit elongation increases as fruit sugar concentration decreases. Strong positive connections were observed between total soluble solids, fructose, glucose concentrations, and plant vegetative growth. Enhanced vegetative growth leads to larger and better quality fruit due to an increase in photosynthesis and the production of soluble materials like sugars.

Table 8: Impact of some plant growth promoting microorganisms (PGPM) and humic acid on Murcott tangerine trees of fruit physical attributes during the two successive seasons.

Attributes / Treatments	Fruit weight (g)	Fruit size (ml.)	Peel thickness (mm)	Fruit shape	Juice percentage
			1stseason		
T_1	137.45c	145.50d	2.55a	0.86a	36.50b
T_2	153.00bc	161.25cd	1.90ab	0.82a	35.70b
T_3	139.50c	144.00d	1.95ab	0.84a	28.70c
T_4	179.00a	188.50a	1.85b	0.79b	41.60a
T_5	160.50b	167.25c	1.93ab	0.81a	34.90b
T_6	142.75c	144.50d	2.10a	0.84a	26.40c
T_7	173.50ab	179.00b	1.81b	0.80a	40.20a
			2nd season		
T_1	135.25d	141.75de	2.58a	0.88a	37.70b
T_2	155.25c	158.50c	1.87bc	0.83a	35.90b
T_3	143.00cd	146.50d	1.91bc	0.83a	29.40c
T_4	183.50a	192.25a	1.73c	0.80a	41.90a
T_5	158.00c	162.50c	1.89bc	0.83a	35.20b
T_6	145.50cd	148.25d	2.00b	0.82a	26.90c
T_7	174.00b	181.25b	1.70c	0.81a	40.90a

At the 5% level, there is no significant difference between the mean and the same letter.

T_1 = Control
T_2 = PGPM with winter management
T_3 = PGPM after fruit set
T_4 = PGPM(with winter management + after fruit set)
T_5 = Humic acid with winter management
T_6 = Humic acid after fruit set
T_7 = Humic acid (with winter management + after fruit set)

3.5.2. Fruit chemical attributes

Chemical properties of certain fruits were assessed in Murcott tangerine trees to examine the impact of various treatments over the two seasons. The results shown in Table (9) indicate a lower impact of vitamin C content in the first season, while the increase was highest in the second season with treatments T_4 and T_7. A rise of 35.41% and 29.16% above the control was documented. Applying humic acid post-

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fruit set (T_6) increased acidity levels, while using humic acid during winter and post-fruit set (T_7) resulted in a notable rise in total soluble sugar levels over two growing seasons.

Zahghoul *et al.* (2015) explored how PGPR affect the productivity and fruit quality of oranges, showing a significant boost in orange tree productivity, particularly in fruit set, fruit number, and fruit yield. In contrast, chlorophyll, firmness, vitamin C content, and SSC% all showed an increase in two seasons, while the SSC/acid ratio decreased. These parameters are crucial for assessing fruit maturity and quality. Nour El-Din *et al.* (2012) found that sustained organic farming of citrus crops resulted in enhanced soil organic carbon levels in Mediterranean citrus systems, as reported by Novara *et al.* (2019).

Scotton *et al.* (2018) found that a four-year timeframe was enough for transitioning away from chemical fertilizers to organic nutrient management, promoting fungal flora and their activity. Duarte *et al.,* (2010) disclosed that the levels of organic acid in organic citrus juice were elevated. By giving proper focus and spreading knowledge about organic farming techniques. The highest sugar levels achieved when using organic fertilizers along with biodynamic elements may be due to the movement of nutrients from photosynthesis and the buildup of more food reserves in the plant, especially in the fruits (Crane, 1969). The decline in acidity levels may be linked to a rise in phosphorous levels (Rajput and Haribabu, 1985).

Table 9: Impact of some plant growth promoting microorganism (PGPM) and humic acid on Murcott tangerine trees of chemical properties the fruit during the two successive seasons.

Treat.	Juice T.S.S. %	Juice Tot. Ac. %	Vitamin C (mg/100 ml juice)	Juice T.S.S. %	Juice Tot. Ac. %	Vitamin C (mg/100 ml juice)
	1st Season			2nd Season		
T_1	8.62ab	1.037b	24.47a	9.64b	1.420abc	24.00c
T_2	8.83ab	0.860b	24.97a	9.67ab	0.920abc	27.00bc
T_3	8.54ab	1.097ab	20.23b	9.41b	1.670ab	18.80d
T_4	9.49a	0.950b	26.50a	10.34a	0.640c	32.50a
T_5	8.83ab	1.037b	25.50a	9.13b	1.210abc	29.00ab
T_6	7.89b	1.323a	20.87b	9.97b	1.810a	19.50d
T_7	9.25a	0.950b	26.10a	10.65a	0.730bc	31.00ab

At the 5% level, there is no significant difference between the mean and the same letter.

T_1 = Control
T_2 = PGPM with winter management
T_3 = PGPM after fruit set
T_4 = PGPM(with winter management + after fruit set)
T_5 = Humic acid with winter management
T_6 = Humic acid after fruit set
T_7 = Humic acid (with winter management + after fruit set)

Humic acid was shown to be a successful bio-fertilizer that enhanced plant nutrient uptake, leading to positive effects on plant growth and fruit quality parameters. In this particular situation, it was discovered by El-Khayate and Abdel Rehiem (2013) that applying dissolving phosphate as bio-fertilizer to lemon trees resulted in higher acidity and vitamin C levels in the fruits. The use of compost can improve soil fertility by introducing nutrients, enhancing soil physical, chemical, and biological characteristics, boosting soil organic matter levels, and aiding in the growth and yield of various plants (Adugna, 2016). PGPM inoculants can be effectively utilized to sustainably produce crops while maintaining soil fertility and supplying plants with phytohormones.

Combining organic manure and bio-fertilizers with chemical fertilizers enhances soil's physicochemical properties, organic carbon, nitrogen, and phosphorus content, leading to higher fruit yield in citrus such as mandarin orange (Lalrinfela and Varte, 2021). According to Darmawan (2017), humic acid can enhance plant growth by boosting plant cell energy, leading to increased ion exchange activity that speeds up root system growth, making roots longer for quicker nutrient absorption. Soil acidity or alkalinity affects how easily plants can take in nutrients (Handini *et al.* 2020).

The use of *Bacillus circulans* biofertilizer along with varying nitrogen levels and 120 units of potassium resulted in improved growth, yield, and quality of Valencia orange. It led to the highest values of quality indicators such as juice percentage, TSS, titratable acidity, ascorbic acid, and total sugar. (El-Khawaga and Maklad, 2013).

In an experiment, Kumar *et al.* (2019) discovered that combining inorganic fertilizers with organic manure and biofertilizer significantly improved the biochemical characteristics of acid lime fruits. The application of 50% RDF + 75% FYM + 75% vermi compost + Biofertilizers (Azotobacter + 25g PSB + 10g VAM) resulted in the highest levels of TSS (7.99 Brix), acidity (6.09%), TSS: acidity ratio (1.42), reducing sugar (1.08%), ascorbic acid content (35.41 mg/100 ml juice), and chlorophyll. Adding compost to soil can improve its fertility by providing nutrients, enhancing soil properties, increasing organic matter, and promoting plant growth and yield. Abo-Ogiala and Khalafallah (2019) conducted a study examining how gypsum and compost impact the growth and yield of Washington navel oranges in saline-sodic soils. They found that using 10 tons/fed of gypsum along with 15 tons/fed of compost was the most effective method for improving the growth, yield, and quality of the oranges.The use of liquid organic fertilizers (derived from animals and plants) in drip irrigated citrus may serve as a substitute for conventional mineral fertilizers (Martinez-Alcantara *et al.* 2016).

3.6. Mineral content of leaves.

Leaf nutrient concentration was measured using nitrogen (N), phosphorus (P), and potassium (K), with the results displayed in Table (10). Humic acid application after fruit set (T_6) led to a notable 8.09% and 8.63% increase in N levels, while PSM treatment during winter and after fruit set (T_4) significantly raised leaf P levels by 63.00% and 38.21%, respectively. The use of PGPR after fruit set resulted in a 7.82% and 6.77% increase in leaf K levels compared to the control in the 1st and 2nd seasons. An evident increase in the leaf levels of N, P, and K occurred due to the application of PGPM and humic acid, since they are recognized as beneficial substances for enhancing root structure in order to absorb nutrients from the soil solution. The positive effect of using the mixed inoculum in this study on leaf mineral concentration (NPK) could be attributed to various factors such as the presence of nitrogen-fixing bacteria, as well as phosphate and potassium solubilizing bacteria in the inoculum being analyzed.

Table 10: Impact of some plant growth promoting microorganisms (PGPM) and humic acid on Murcott tangerine trees of fruit physical attributes during the two successive seasons.

Attributes / Treatments	Nitrogen (%)	Phosphorous (%)	Potassium (%)	C/N ratio
		1st season		
T_1	2.310ab	0.100cd	1.153b	20.76c
T_2	2.08ab3	0.1200abc	0.983c	24.89b
T_3	2.397a	0.0500d	1.240a	15.20d
T_4	1.917b	0.1633a	0.820d	27.37a
T_5	2.120ab	0.1000bcd	1.000c	22.50c
T_6	2.497a	0.0500d	1.207ab	11.70e
T_7	1.937b	0.1400ab	0.880d	24.39b
		2nd season		
T_1	2.317b	0.123a	1.180a	20.78c
T_2	2.097d	0.133a	1.020b	25.18b
T_3	2.437a	0.050c	1.260a	15.27d
T_4	1.970e	0.170a	0.850c	28.26a
T_5	2.193c	0.110ab	1.030b	22.83c
T_6	2.517a	0.060bc	1.233a	12.17e
T_7	1.993e	0.153a	0.913c	25.08b

At the 5% level, there is no significant difference between the mean and the same letter.

T_1 = Control
T_2 = PGPM with winter management
T_3 = PGPM after fruit set
T_4 = PGPM(with winter management + after fruit set)
T_5 = Humic acid with winter management
T_6 = Humic acid after fruit set
T_7 = Humic acid (with winter management + after fruit set)

Gaber and Nour El-Din (2005) indicated comparable outcomes from the inoculation of apple trees with an inoculum containing *Azospirillum sp.* and *B. megatherium*. This resulted in increased plant root surface area, improving the acquisition of macro- and micro-elements from the soil. Additionally, the inoculation led to the release of plant hormones such as cytokinins and auxins, promoting root cell division and size (Jaha and Saraf, 2015), and the release of polysaccharides and organic acids in the soil, ultimately lowering the soil pH (Steenhodt and Vanderleyden, 2006). This could be due to the combined impact of inoculation and bio-stimulants spray, which improved nutrient absorption and plant growth. Additionally, they also improve the absorption and movement of various nutrients, leading to higher levels of carbohydrate and protein production. They promote cell division and tissue growth, boost plant resistance to root diseases, and enhance the production of hormones like IAA, GA3, and cytokinins. These resultsled to in improved root development, increased plant growth, and ultimately better fruit quality and productivity (Kannaiyan, 2002).

The application of organic and biofertilizers enhances soil physical and chemical properties, with the degree of improvement varying based on the rate of application (Hamed *et al.,* 2014 and Muhammad and Sharif, 2019). In addition, using organic and bio-fertilizers can enhance the presence of NPK and organic matter in the soil while reducing its pH, according to Yang *et al.* (2019). The overall effectiveness of various treatments on the mineral content of leaves (C/N ratio) was summarized in table (10) for two seasons. During two seasons, PGPR with winter management and after fruit set significantly increased mineral content of leaves by 28.15 – 29.18% for C/N ratio respectively.

4. Conclusion

In conclusion, this study suggests that all plant growth promoting microorganisms (PGPM) and humic acid increased the yield efficiency, flowering, fruit set, fruit quality, bio-chemical contents, and mineral content of leaves of Murcott tangerine trees as compared to the untreated control, thus, it could be indicated that plant growth promoting microorganisms (PGPM) strains have a great potential to synthesize plant growth regulators, such as IAA and cytokinin, and can fix nitrogen and solubilized phosphorus, they may be effective means for biological control of bacterial and fungal plant diseases and increase photosynthesis, transpiration, leaves chlorophyll content. These treatments are safe, effective, and easily adopted by growers. Therefore, they should be considered as bio-fertilizer for fruit production in sustainable and ecological agricultural systems. Our finding recommended that humic acid and plant growth promoting microorganisms (PGPM) when used (with winter management + after fruit set) are the potential tools for sustainable agriculture. For this reason, there is an urgent need for researchers to clear definition of what PGPR and humic traits are useful and necessary for different environmental conditions and plants.

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