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Mycorrhizal Fungi and Rock Phosphate Application to Mitigate Soil Salt Stress and Its Effect on Productivity of 'Picual' Olives and 'Wonderful' Pomegranates

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ABSTRACT

Field experiments were carried out to determine the effect of times and rates of mycorrhizal fungi and bacteria mixture applications for rock phosphate inoculation to mitigate the impacts of soil and water salt stress for enhance the productivity of 'Picual' olive trees in a private orchard at Cairo-Ismailia desert road, Ismailia governorate and 'Wonderful' pomegranate trees in a private orchard at Cairo-Alexandria desert road, Alexandria governorate, Egypt. Experiments included applications of rock phosphate (RP) at 1 kg / tree and 25 cm^3 of bacteria mixture were applied once at the end of January and inoculated with mycorrhiza fungi (MF) at 100 g/ tree divided at different rates and times as the follow; (T1) application of mycorrhiza fungi at 100 g/ tree on the end January, (T2) application of mycorrhiza fungi at 50 g/ tree on the end January and 50 g/ tree on mid-March, (T3) application of mycorrhiza fungi at 50 g/ tree on the end January and 25g/ tree on mid-March and 25 g/ tree on mid-May and (T4) control trees (untreated with mycorrhiza fungi). The results showed that the inoculation of rock phosphate with microbial stimulant (mycorrhizal fungi + bacteria mixture) had an obvious effect in enhancing the studied parameters, leaf mineral content, yield and fruit quality of 'Picual' olive and 'Wonderful' pomegranate trees and water productivities as compared with control. As a result, it can be recommended to inoculate the rock phosphate with bacteria mixture and mycorrhizal fungi at rate of 1 kg of rock phosphate +25 cm^3 of bacteria mixture + 50 g of mycorrhizal fungi / tree on the end of January and 25 g of mycorrhizal fungi each in mid-March and mid-May for 'Picual' olive trees. However, application of mycorrhiza fungi was 50g of mycorrhizal fungi / tree on the end January and 50 g/tree on mid-March for 'Wonderful' pomegranate trees to mitigate the impacts of soil and water salt stress on the trees under the same conditions, thus enhancing productivity.

Keywords: Soil and water salinity, olive, pomegranate, mycorrhizal fungi, bacteria mixture, rock phosphate, water productivity.

1. Introduction

While olive trees (*Olea europaea* L.) are grown all over the world, they are most commonly farmed in Mediterranean nations, where they are thought to be among the species most suited to a climate similar to that of the Mediterranean (Fraga *et al.*, 2021; Hegazi *et al.*, 2021). Olive trees have been farmed for their highly nutritious fruits and oil, which can lower the risk of heart disease and even prevent cancer (Wong *et al.,* 2021). In Egypt, olive area reached about 112.851 hectare, producing 1.137.075 tons, according to FAO, (2022). The Mediterranean region may see a rise in the irrigation of olive trees with saline water as a result of the detrimental effects of population growth and climate change on the availability and quality of fresh water (Chartzoulakis, 2011). Pomegranate (*Punica*

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granatum L.) in Egypt is currently considered one of the most promising export fruit crops, especially 'Wonderful' cultivar, as it is a late variety with high productivity, large fruit, rich red arils, high juice, and good taste. Egypt is the fifth-largest exporter in the world with a total planted area of approximately 31,987 hectares, an overall production of 382,587 tons, and an export of nearly 82,866 tons in 2020, according to Ministry of Agriculture and Land Reclamation (Ministry of Agriculture and Land Reclamation, 2020). Also, it is known for its health benefits that may help prevent heart disease, and cancer. This is due to it containing high levels of polyphenol compounds (Mahmoud *et al.,* 2019).

Microbial stimulants, having plant growth-promoting bacteria and mycorrhizal fungi (MF), are enhancing plant growth, nutrient uptake, soil quality, and plant resistance to abiotic stress (Herawati *et al.,* 2021). A novel class of biofertilizers called mycorrhizal fungus is being employed to boost plant growth by enhancing plant nutrition and biodefense. However, large-scale replication of MF propagules is difficult due to the requirement of the plant host (Ben Ahmed *et al.,* 2012). Because of positive effects of arbuscular mycorrhizal fungi on soil structure, nutrient availability, and carbon sequestration, Arbuscular mycorrhizal fungi maintain soil environment and increase plant adaptation, growth and vegetation adaptability to abiotic stressors, such as a lack of water, salinity, and ion toxicity. Arbuscular mycorrhizal fungi can promote plant health and productivity and help agricultural sustainability, ecology management, and climate change alleviation (Wahid *et al.,* 2020).

After nitrogen, phosphorus is the macronutrient that is most readily absorbed, ranking second in importance (Reddy *et al*., 2004; Shen *et al*., 2011). Most crops depend on it for fruit setting and root growth, therefore having an adequate supply is crucial for several physiological processes, including uniform maturity, early root and seedling creation, and efficient use of water (Cordell *et al*., 2009). It is essential for energy storage and transfer, respiration, cell division, and photosynthesis (Reddy *et al*., 2004). In a nutshell, a shortage of it results in a major loss in crop output. One of the biggest obstacles to production in alkaline soils is phosphorus shortage. In many places, the high cost of phosphorus fertilizers may be an impassable barrier, making the only long-term, low-cost solution to be the exploitation their own rock phosphate (RP) (Orozco-Patiño and Medina-Sierra, 2013). In developing countries, soluble phosphate fertilizers are usually too expensive for agriculture. Rock phosphate (RP) is considered the cheapest type of phosphate fertilizer, and its source may be sedimentary or igneous, and its locations are at Abu Tartur (Western Desert), Safaga (Red Sea), and Sebaiya (Nile Valley) in Egypt (Hellal *et al.,* 2019). RP, although relatively insoluble, is easily minable which makes it economical to use as a natural phosphate fertilizer when compared to industrial phosphate fertilizers (Zhan *et al., 2*021).

In terms of sustainability, the biological dissolution of phosphate rock is more environmentally friendly. The dynamics of soil phosphate are greatly influenced by soil microbes, particularly phosphorus-solubilizing bacteria (PSB), which release organic acids to dissolve phosphorus in the soil (Afzal and Bano, 2008). Thus, these bacteria may assist to the creation of models for sustainable agriculture (Arif *et al.,* 2018). Phosphorus-solubilizing bacteria (PSB) are a useful strategy that could offer fresh ways to boost the efficiency of phosphorus use (Estrada-Bonilla *et al.,* 2017). The phosphorus will be available for utilization by plants due to its biological solubility, which will minimize environmental pollution.

The objective of this work was to study the effect of times and rates of mycorrhizal fungi application for rock phosphate inoculation to mitigate the impacts of soil and water salt stress on productivity of 'Picual' olive and 'Wonderful' pomegranate trees.

2. Materials and Methods

Two distinct field experiments on two distinct fruit crops olives and pomegranates during the course of two consecutive seasons, 2021 and 2022, are included in the study. Nine-year-old "Picual" olive trees, cultivated at 4x6 meters in sandy soil with drip irrigation in a private orchard near Cairo-Ismailia desert route, Ismailia governorate, Egypt, were used in the initial field trial. Table 1 lists the mechanical and chemical characteristics of soil. According to the US agronomic classification of soil salinity, the soil salinity of the soil paste for the studied loamy sand soil (ECe) = 9.1 dS-1 equals (EC1:5) = 1.23 dS-1 for the 1:5 soil/water extract, and the irrigation water analysis was displayed in Table (2), where it was categorized as acute problem water (Ayers and Westcot, 1985). In the second field trial, ten-year-old "Wonderful" pomegranate trees were grown at space of 3 × 5 meters between the trees in sandy soil with drip irrigation on a private "Hegazi" farm situated 57 kilometers from Cairo on the road to Alexandria, Egypt. Table (3) displays the mechanical and chemical characteristics of the soil, whereas Table (4) displays the irrigation water analysis.

Table 1: Soil physical and chemical properties of the olive farm before applying studied treatments

Particle size distribution (%)						
Sand		**Silt**		**Clay**	**Texture**	
82.3		8.6		9.1	loamy sand	
Chemical soil properties						
pH (1:2.5)	**EC dS^{-1} (1:5)**	**N (ppm)**		**P (ppm)**	**Organic matter %**	
8.19	1.23	53.3		22.4	0.60	
Soluble cations (me/l)				**Soluble anions (me/l)**		
Ca^{++}	**Mg^{++}**	**Na^{+}**	**K^{+}**	**$CO_3^{--}+HCO_3^{-}$**	**Cl^{-}**	**$SO4^{--}$**
2.7	1.3	7.5	1.0	1.0	10.0	1.5
Hydro-physical properties						
Bulk density g cm^{-3}	**Total porosity %**	**Saturation %**	**Field Capacity %**	**Wilting percentage %**	**Available water %**	**Hydraulic conductivity m day^{-1}**
1.58	40.27	21.8	14.5	5.4	9.1	4.08

Table 2: The irrigation water analysis of the olive farm

Properties	**pH**	**EC dSm^{-1}**	**SAR**	**Soluble cations (me/l)**				**Soluble anions (me/l)**			
				Ca^{+2}	**Mg^{+2}**	**Na+**	**K^{+}**	**CO_3^{-2}**	**HCO^{-3}**	**Cl^{-}**	**$SO4^{-2}$**
Value	7.84	6.27	11	15.0	10.5	42.6	0.20	-	1.9	43.5	22.9

Table 3: Soil physical and chemical properties of the pomegranate farm before applying studied treatments

Particle size distribution (%)										
Sand			**Silt**			**Clay**		**Texture**		
94.64			2.73			2.63		sandy		
Properties	**pH (1:2.5)**	**ECdS^{-1} (1:5)**	**Soluble cations (me/l)**				**Soluble anions (me/l)**			
			Ca^{+2}	**Mg^{+2}**	**Na+**	**K^{+}**	**$CO3^{-2}$**	**HCO^{-3}**	**Cl^{-}**	**$SO4^{-2}$**
Value	7.68	2.65	3.73	1.1	20.88	0.88	-	0.86	22.03	3.7

Table 4: The irrigation water analysis of the pomegranate farm

Properties	**pH**	**EC dSm^{-1}**	**Soluble cations (me/l)**				**Soluble anions (me/l)**			
			Ca^{+2}	**Mg^{+2}**	**Na+**	**K^{+}**	**$CO3^{-2}$**	**HCO^{-3}**	**Cl^{-}**	**$SO4^{-2}$**
Value	8.4	1.19	1.5	1.12	8.45	0.89	-	1.4	6.46	4.1

2.1. Soil preparation, Experimental design and Treatments

Studied treatments were conducted in saline sandy soil irrigated with saline ground water. All trees were received the same horticultural practices. Farmyard manure (F) was added to the soil at 15 kg/tree for all treatments in December. The experiment had four treatments included application of rock phosphate (RP) at 1 kg / tree with 25 cm^3 of bacterial mixture (BM). Each treatment was applied once at the end of January and inoculated with mycorrhiza fungi (MF) at 100 g/ tree divided at different rates and times as shown in Table (5), except control trees untreated with MF. Olive and pomegranate trees were fertilized with the doses recommended by the Egyptian Ministry of Agriculture for modern sandy soil. Organic fertilizer was added during the month of December, and then the superphosphate fertilizer doses were reduced by 50% and replaced with phosphate rock. Three replicates of each treatment, each represented by a single tree, were organized in a completely randomized block design.

Table 5: Inoculations of mycorrhiza fungi (MF) and bacterial mixture (BM) as microbial stimulants with rock phosphate (RP) in the experiments

Treatments	T1	T2	T3	T4 (Control)
Times	Rates			
January	100 g of MF +25 cm^3 of BM + 1 kg of RP/ tree	50 g of MF +25 cm^3 of BM + 1 kg of RP/ tree	50 g of MF +25 cm^3 of BM + 1 kg of RP/ tree	25 cm^3 of BM + 1 kg of RP/ tree
March	-	50 g of MF/ tree	25 g of MF/ tree	-
May	-	-	25 g of MF/ tree	-

2.2. Microbial stimulants preparation

a) Mycorrhizal fungi (MF)

Mixed spores of mycorrhizal genera: *Via Glomus* spp., *Giaspora* spp. and *Acaulospora* spp. were prepared after propagation and mixed with sand and then added 100g to each treated tree divided into rates at different times, and each gram included 500 spore /g inoculum.

b) Bacterial mixture (BM)

The two bacteria that made up the bacterial mixture were *Bacillus megaterium* var. phosphaticum and *Acidithia bacillus thiooxidanse*, which were prepared by the National Research Center's Agricultural and Biological Institute's microbiology department. Cultures were mixed by ratio (2 *Bacillus megaterium*: 1 *Acidithia bacillus*) on site then all trees of pomegranate and olive received 25 cm^3 of the mix.

2.3. Measurements

The effects of treatments in the experiments were studied by evaluating influence on the following parameters:

a. Vegetative growth parameters

Length of shoots and number of leaves per shoots: Four branches, evenly spaced along the directions of the tree, were tagged at the beginning of April. When growth ceased in the middle of July, the length of the shoot was measured and the number of leaves per shoots were counted.

b. Leaf mineral contents

In late july, twenty leaves were taken from the central section of the tagged shoots on each replicate tree. The leaves were then dried in an electric oven at 70°C for 48 hours, or until their weight remained consistent, then grounded and digested by sulfuric acid and perchloric acid. Leaf mineral content of N, P, K, Ca, Mg, Zn, Cu, Fe and Mn were determined on dry weight basis according to the method described by AOAC, (1995).

c. Yield

At the end of September and October, pomegranate and olive fruits were harvested respectively from each replicate tree, then weighted and yield as Kg/tree was estimated.

2.4. Fruit quality

a. Olive fruits

To calculate the fruit weight (g), volume (cm3), length (cm), diameter (cm), shape index (calculated as L/D ratio), seed and pulp weight (g), and pulp/seed ratio, samples of 20 fruits from each replication tree, or 60 fruits from each of the applied treatments, were chosen at random during harvest.

Fruit oil content was calculated from the dry fruit using the method described by AOAC, (1995). Fruit oil was extracted using a soxthelt extraction apparatus and petroleum ether with a boiling point of 40/60ºC to extract oil from dried fruit flesh.

b. Pomegranate fruits

Samples of three fruits from each replicate tree i.e. 9 fruits from each of treatment were picked randomly at harvest to determine fruit weight (g), weight of 50 arils (g), juice volume of 50 arils (cm3), TSS of juice (%) was measured by using a digital refractometer, acidity of juice (%) was determined as Citric acid by titration with a solution of NaOH (0.1 N), using phenolphthalein as an indicator according to AOAC, (1995) and then TSS/Acidity ratio calculated.

2.4. Water Productivity

Water productivity (WP) points out effectiveness use of irrigation water on crop production. WP was calculated as fruit yield per water unit. These increases due according to Pereira *et al.* (2012) in the following:-

$$WP = Yield/\ Irrigation\ water\ amount\ (kg\ m^{-3})$$

2.5. Statistical analysis

The collected data were analyzed using variance according to the method described by Gomez and Gomez, (1984). Means were compared by the Tokay Kramer test at 5% level of probability.

3. Results

3.1. Times and Rates of Mycorrhizal Fungi Applications for rock phosphate inoculation on 'Picual' olives under saline soil and water stress

3.1.1. Effect on vegetative growth

Results presented in the Table (6) indicated that, shoot length and number of leaves / shoot were affected by times and rates of mycorrhizal fungi applications on rock phosphate compared to untreated trees during the both seasons of study. Application by mycorrhiza fungi at the rate of 50g/tree on the end of January and 25g each of mid-March and mid-May (T3) gave the highest values (47.33 & 49.66 cm and 52 &56 leaves / shoot) in both study seasons, respectively. On the other hand, the lowest one recorded in control trees (32 & 34.17 cm and 32 & 33 leaves / shoot) in first and second seasons, respectively.

Table 6: Effect of times and rates of mycorrhizal fungi using for rock phosphate inoculation on shoot length and no. of leaves / shoot of 'Picual' olive trees in 2021 and 2022 seasons

Treatments	Shoot length (cm)		No. leaves / shoot	
	2021	2022	2021	2022
T1*	38.67 b	39.00 c	46 b	50 b
T2	44.67 ab	44.80 b	49 ab	50 b
T3	47.33 a	49.66 a	52 a	56 a
T4	32.00 c	34.17 d	32 c	33 c

* Applications of mycorrhiza inoculum were at 100 g/ tree on the end January (T1), 50 g/ tree on the end January with 50 g/ tree on mid-March (T2), and 50 g/ tree on the end January and 25 g/ tree on mid-March with 25 g/ tree on mid-May (T3). T4 acts a control (untreated with mycorrhiza inoculum).

3.2. Effect on leaf mineral content

3.2.1. Macro elements

It is obvious from Table (7) that macro elements in leaves of 'Picual' olive trees were influenced as a result of rock phosphate inoculation with mycorrhizal fungi compared to untreated trees during both seasons of the study. The differences between the dates and rates of applying mycorrhiza fungi were significant in both seasons.

The application of mycorrhiza fungi at the rate of 50g/tree in the end of January and 50g in mid-March (T2) gave the highest nitrogen leaf content (2.40 %) in the first season, while mycorrhiza fungi application at the rate of 50g/tree in the end of January and 25g each of mid-March and mid-May (T3) gave the highest nitrogen leaf content (2.47%) in the second season. On the other hand, the lowest attained in the control (1.47 and 1.95 %) during 2020 and 2021 seasons respectively. Concerning of phosphorus and potassium leaf content, mycorrhiza fungi application at the rate of 50g/tree on the end of January and 25g each of mid-March and mid-May (T3) gave the highest values (0.53 & 0.58 % and

0.65 & 0.92 %) in first and second seasons, respectively. On the other side, the lowest values (0.35 & 0.38 % and 0.53 & 0.67 %) recorded in the control during the first and second seasons, respectively.

Table 7: Effect of times and rates of mycorrhizal fungi using for rock phosphate inoculation on macro elements of 'Picual' olive trees in 2021 and 2022 seasons.

Treatments	N (%)	P (%)	K (%)	Mg (%)	Ca (%)
			2021		
T1*	2.17 c	0.41 b	0.64 a	0.25 a	1.60 a
T2	2.40 a	0.47 b	0.58 b	0.22 ab	1.59 a
T3	2.30 b	0.53 a	0.65 a	0.23 a	1.40 b
T4	1.74 d	0.35 c	0.53 c	0.18 b	1.30c
			2022		
T1	2.31 b	0.44 b	0.80 bc	0.28 a	1.83 a
T2	2.45ab	0.47 b	0.84 b	0.21 ab	1.60 c
T3	2. 47a	0.58 a	0.92 a	0.26 a	1.67 b
T4	1.95 c	0.38 c	0.67 c	0.20 ab	1.35 d

* Applications of mycorrhiza inoculum with 25 cm^3 of bacteria mixture + 1 kg of rock phosphate/ tree were at 100 g/ tree on the end January (T1), 50 g/ tree on the end January with 50 g/ tree on mid-March (T2), and 50 g/ tree on the end January and 25 g/ tree on mid-March with 25 g/ tree on mid-May (T3). T4 acts a control (untreated with mycorrhiza inoculum).

As for leaf percentage of magnesium and calcium, inoculation of rock phosphate with mycorrhizal fungi at 100g/ tree on the end January (T1) exhibited the maximum values (0.25 & 0.28 % and 1.60 & 1.83 %) in both seasons of study, respectively. Meanwhile, control trees (rock phosphate without inoculation by mycorrhiza fungi) gave the minimum percentages (0.18 & 0.20 % and 1.30 & 1.35 %) in 2021 and 2022 seasons, respectively.

3.2.2. Micro elements

The effect of using mycorrhizal fungi at various rates and times to inoculate the rock phosphate on micro elements of 'Picual' leaves during 2021 and 2022 seasons are illustrated in (Table 8). The highest increment of leaf content from iron in both seasons was recorded by using mycorrhiza fungi application at the rate of 50 g/tree on the end of January and 50g in mid-March (T2). Mycorrhiza fungi application at the rate of 50g/tree on the end of January and 25g each of mid-March and mid-May (T3) gave the highest leaf content of manganese (43 and 45.17 ppm) in first and second seasons, respectively. Leaf content of zinc was no significant affected by different treatments in both studied seasons.

Table 8: Effect of times and rates of mycorrhizal fungi using for rock phosphate inoculation on micro elements of 'Picual' olive trees in 2021 and 2022 seasons

Treatments	Fe (ppm)	Mn (ppm)	Zn (ppm)	Cu (ppm)
			2021	
T1*	131.50 b	39.00 c	19.16 a	6.00 a
T2	138.00 a	32.00 d	20.30 a	6.32 a
T3	125.30 c	43.00 a	20.70 a	7.12 a
T4	109.38 d	23.41 e	19.13 a	5.18 a
			2022	
T1	135.85 ab	40.11 ab	20.09 a	7.00 ab
T2	138.17 a	43.02 ab	21.08 a	7.08 ab
T3	135.76 ab	45.17 a	21.17 a	7.50 a
T4	125.00 b	29.00 b	20.02 a	6.67 b

* Applications of mycorrhiza inoculum with 25 cm^3 of bacteria mixture + 1 kg of rock phosphate/ tree were at 100 g/ tree on the end January (T1), 50 g/ tree on the end January with 50 g/ tree on mid-March (T2), and 50 g/ tree on the end January and 25 g/ tree on mid-March with 25 g/ tree on mid-May (T3). T4 acts a control (untreated with mycorrhiza inoculum).

Also leaf content of copper was no significant affected by different treatments in the first season, while in the second season the application of mycorrhiza fungi at the rate of 50g/tree on the end of January and 25g each of mid-March and mid-May (T3) gave the highest value compared with the other treatments. Generally, the lowest values of leaf content from iron, manganese, zinc and copper were for control trees that received phosphate rock as single applications without mycorrhizal fungi during the two seasons of study.

3.2.3. Effect on olive yield

Results in Figure 1 indicate that inoculation of rock phosphate with mycorrhizal fungi show significant increase in yield of tree comparing with rock phosphate as single applications (control trees) during both seasons. Application of mycorrhiza fungi application at the rate of 50g/tree on the end of January and 25g each of mid-March and mid-May (T3) was affective in enhancing the yield of tree, recording (41 and 48.67 Kg/tree), compared to the control trees (27.5 and 29 Kg/tree) in both study seasons, respectively.

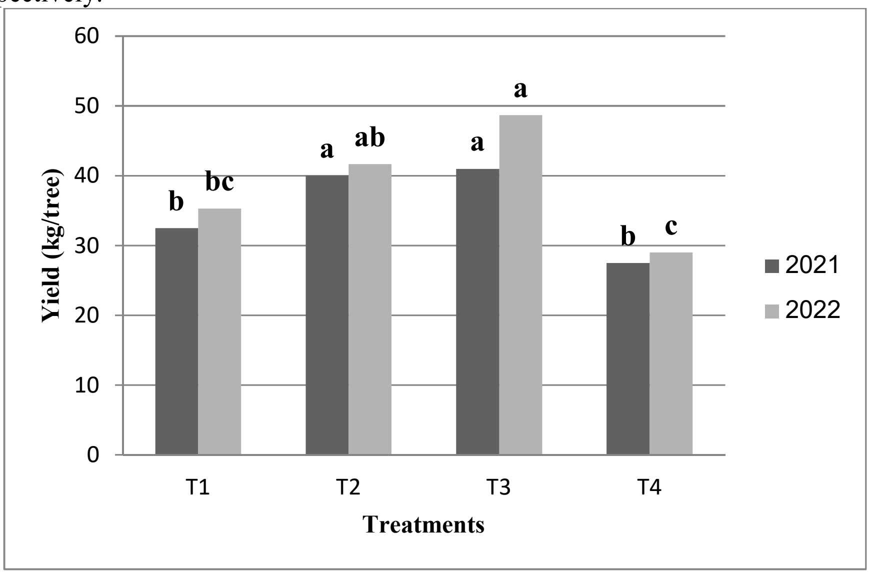

Fig. 1: Effect of times and rates of mycorrhizal fungi using for rock phosphate inoculation on yield of 'Picual' olive fruits in 2021 and 2022 seasons.

Applications of mycorrhiza inoculum with 25 cm^3 of bacteria mixture + 1 kg of rock phosphate/ tree were at 100 g/ tree on the end January (T1), 50 g/ tree on the end January with 50 g/ tree on mid-March (T2), and 50 g/ tree on the end January and 25 g/ tree on mid-March with 25 g/ tree on mid-May (T3). T4 acts a control (untreated with mycorrhiza inoculum).

3.2.4. Effect on fruit quality

The obtained results in Table (9) indicated that the highest fruit volume (8.50 & 8.53 cm^3), fruit length (2.73 cm), fruit diameter (2.17 & 2.20 cm) and fruit shape index (1.2304 &1.2409) were obtained by application of mycorrhiza fungi application at the rate of 50g/tree on the end of January and 25g each of mid-March and mid-May (T3) in both seasons of the study compared to other treatments during 2021 and 2022 seasons respectively.

As for weight of fruit, seed and pulp of 'Picual' olives Table (10) the statistical analysis indicated that there was a slight significant difference between the treatments during the two seasons of the study. With that record mycorrhizal fungi application at 50g/tree on the end of January and 25g each of mid-March and mid-May (T3) the highest values of fruit weight and pulp/ seed ratio in both seasons of the study compared to other treatments during 2021 and 2022 seasons respectively.

3.2.5. Effect on fruit oil content

It is clear from Figure (2) that oil content of fruit was impacted significantly as a result of rock phosphate inoculation with mycorrhizal fungi compared to control trees during both seasons of the study. Oil content of fruits significantly increased due to mycorrhiza fungi application. The highest oil content was obtained from the rate of 50g/tree on the end of January and 25g each of mid-March and mid-May (T3) (19.34 and 20.67%) during 2021 and 2022 seasons respectively followed by (T2) and (T1) without a significant difference, whereas the lowest oil content attained in the control trees (16.55 and 17%) during 2021 and 2022 seasons respectively.

Table 9: Effect of times and rates of mycorrhizal fungi using for rock phosphate inoculation on fruit volume, length, diameter and shape of 'Picual' olives in 2021 and 2022 seasons

Treatments	Fruit volume (cm^3)		Fruit length (cm)		Fruit diameter (cm)		Fruit shape (L/D ratio)	
	2021	2022	2021	2022	2021	2022	2021	2022
T1*	7.50 ab	7.00 a	2.63 a	2.63 a	2.11 b	2.16c	1.21b	1.23ab
T2	7.50 ab	7.20 a	2.64 a	2.64 a	2.15 ab	2.18 b	1.23 ab	1.21ab
T3	8.50 a	8.53 a	2.73 a	2.73 a	2.17 a	2.20 a	1.23 a	1.24 a
T4	6.83 b	7.56 a	2.54 a	2.54 a	2.11 b	2.12 d	1.17 c	1.19 b

* Applications of mycorrhiza inoculum with 25 cm^3 of bacteria mixture + 1 kg of rock phosphate/ tree were at 100 g/ tree on the end January (T1), 50 g/ tree on the end January with 50 g/ tree on mid-March (T2), and 50 g/ tree on the end January and 25 g/ tree on mid-March with 25 g/ tree on mid-May (T3). T4 acts a control (untreated with mycorrhiza inoculum).

Table 10: Effect of times and rates of mycorrhizal fungi using for rock phosphate inoculation on fruit physical characteristics of 'Picual' olives in 2021 and 2022 seasons

Treatments	Fruit weight (g)		Seed weight (g)		Pulp weight (g)		Pulp/seed ratio	
	2021	2022	2021	2022	2021	2022	2021	2022
T1*	7.26 b	7.54 ab	1.02 a	1.05 a	7.48 a	8.17 a	7.33 b	7.79 a
T2	7.24 b	8.00 ab	0.83 b	0.95 b	6.31 b	7.21 ab	7.59 ab	7.61 a
T3	8.29 a	9.17 a	0.83 b	0.91 b	6.40 b	6.85 ab	7.70 a	7.56 a
T4	7.00 b	7.18 b	0.86 b	0.85 b	6.40 b	6.33 b	7.44 ab	7.56 a

*Applications of mycorrhiza inoculum with 25 cm^3 of bacteria mixture + 1 kg of rock phosphate/ tree were at 100 g/ tree on the end January (T1), 50 g/ tree on the end January with 50 g/ tree on mid-March (T2), and 50 g/ tree on the end January and 25 g/ tree on mid-March with 25 g/ tree on mid-May (T3). T4 acts a control (untreated with mycorrhiza inoculum).

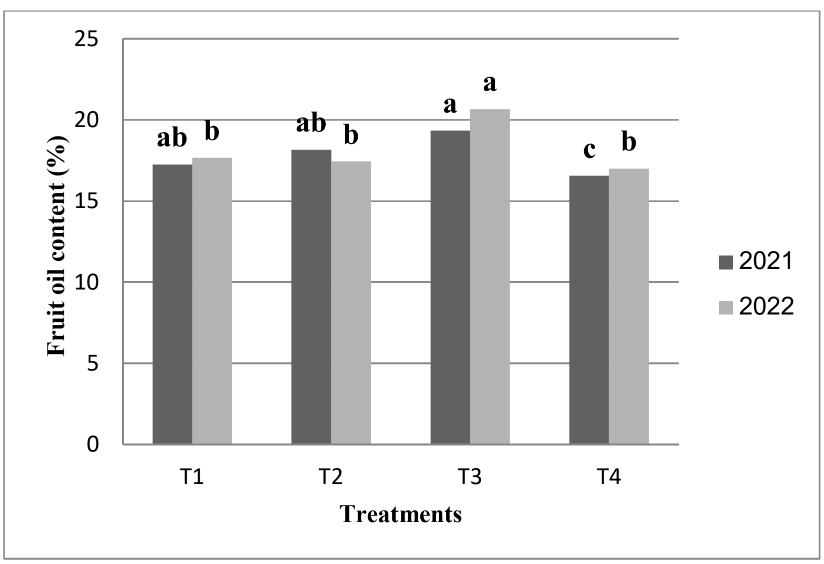

Fig. 2: Effect of times and rates of mycorrhizal fungi using for rock phosphate inoculation on oil content of 'Picual' olive fruits in 2021 and 2022 seasons.

Applications of mycorrhiza inoculum with 25 cm^3 of bacteria mixture + 1 kg of rock phosphate/ tree were at 100 g/ tree on the end January (T1), 50 g/ tree on the end January with 50 g/ tree on mid-

March (T2), and 50 g/ tree on the end January and 25 g/ tree on mid-March with 25 g/ tree on mid-May (T3). T4 acts a control (untreated with mycorrhiza inoculum).

3.3. Times and Rates of Mycorrhizal Fungi Applications for rock phosphate inoculation on 'Wonderful' pomegranates under saline soil and water stress

3.3.1. Effect on leaf mineral content

It is obvious from Table (11) that macro elements in leaves of 'Wonderful' pomegranate trees were influenced as a result of rock phosphate inoculation with mycorrhizal fungi compared to untreated trees during both seasons of the study. The differences between the dates and rates of applying mycorrhiza fungi were significant in both seasons.

The application of mycorrhiza fungi at the rate of 100g/ tree mycorrhiza fungi on the end January (T1) gave the highest nitrogen leaf content followed by application of mycorrhiza fungi application at the rate of 50g/tree in the end of January and 25g each of mid-March and mid-May (T3) in the first and second season. On the other hand, the lowest attained in the control during 2021 and 2022 seasons.

Concerning of phosphorus leaf content, mycorrhiza fungi application at the rate of 50g/tree on the end of January and 25g each of mid-March and mid-May (T3) gave the highest values in first and second seasons, respectively, while no significant between (T1) and (T2) in both seasons. On the other side, the lowest values recorded in the control during the first and second seasons.

Likewise, application of mycorrhiza fungi application at the rate of 50g/tree in the end of January and 25g each of mid-March and mid-May (T3) recorded the maximum values of potassium leaf content in the first seasons. While in the second season, there was no significant difference between the other treatments. Meanwhile, control trees (rock phosphate without inoculation by mycorrhiza fungi) gave the minimum percentages of potassium leaf content in 2021 and 2022 seasons.

As for leaf percentage of magnesium, inoculation of rock phosphate with mycorrhiza fungi at the rate of 50g/tree in the end of January and 25g each of mid-March and mid-May (T3) exhibited the maximum values (0.31 %) in the first season of study. At the same time, there is no significant between (T1) and (T2). In the second season, there was no significant difference between the (T1), (T2) and (T3) in leaf percentage of magnesium. Meanwhile, control trees (rock phosphate without inoculation by mycorrhiza fungi) gave the minimum percentages (0.19 and 0.22 %) in 2021 and 2022 seasons, respectively.

The application of mycorrhiza fungi at 50g/ tree on the end January and 50g/ tree on mid-March (T2) gave the highest significant calcium leaf content followed by application of mycorrhiza fungi at the rate of 100g/ tree mycorrhiza fungi on the end January (T1) in the first and second seasons. On the other hand, the lowest attained in the control during 2021 and 2022 seasons.

Table 11: Effect of times and rates of mycorrhizal fungi using for rock phosphate inoculation on leaf macro elements of 'Wonderful' pomegranate trees in 2021 and 2022 seasons.

Treatments	N (%)		P (%)		K (%)		Mg (%)		Ca (%)	
	2021	2022	2021	2022	2021	2022	2021	2022	2021	2022
T1*	2.40 a	2.59 a	0.28 b	0.32 b	1.64 b	1.83 a	0.22 b	0.25 a	1.60 ab	1.61 b
T2	2.17 b	2.34 b	0.29 b	0.30 b	1.57 c	1.82 a	0.24 b	0.24 a	1.77 a	1.70 a
T3	2.23 ab	2.47 a	0.35 a	0.38 a	1.66 a	1.83 a	0.31 a	0.25 a	1.40 b	1.55 b
T4	1.73 c	1.90 c	0.22 c	0.27 c	1.54 d	1.53 b	0.19 c	0.22 b	1.34 b	1.43 c

* Applications of mycorrhiza inoculum with 25 cm^3 of bacteria mixture + 1 kg of rock phosphate/ tree were at 100 g/ tree on the end January (T1), 50 g/ tree on the end January with 50 g/ tree on mid-March (T2), and 50 g/ tree on the end January and 25 g/ tree on mid-March with 25 g/ tree on mid-May (T3). T4 acts a control (untreated with mycorrhiza inoculum).

3.3.2. Micro elements

The effect of using mycorrhizal fungi at various rates and times to inoculate the rock phosphate on micro elements of 'Wonderful' pomegranate leaves during 2021 and 2022 seasons are illustrated in (Table 12).

The highest increment of leaf content from iron in both seasons was recorded by using mycorrhiza fungi application at the rate of 50g/tree on the end of January and 50g in mid-March (T2). Control trees untreated with mycorrhiza fungi (T4) gave the highest leaf content of manganese (58.28 and 59.02 ppm) in first and second seasons, respectively, while

Table 12: Effect of times and rates of mycorrhizal fungi using for rock phosphate inoculation on leaf micro elements of 'Wonderful' pomegranate trees in 2021 and 2022 seasons

Treatments	Fe (ppm)		Mn (ppm)		Zn (ppm)		Cu (ppm)	
	2021	2022	2021	2022	2021	2022	2021	2022
T1*	122.17 b	105.00 b	40.00 c	49.45 b	17.1 c	17.60 d	6.33 a	6.48 b
T2	138.83 a	130.00 a	32.83 d	39.35 c	22.6 b	20.40 b	8.02 a	7.50 a
T3	102.03 c	98.60 c	50.67 b	51.25 b	30.9 a	24.80 a	4.17 b	5.02 c
T4	82.33 d	91.40 d	58.28 a	59.02 a	14.43c	16.20 c	2.90 b	3.98 d

* Applications of mycorrhiza inoculum with 25 cm^3 of bacteria mixture + 1 kg of rock phosphate/ tree were at 100 g/ tree on the end January (T1), 50 g/ tree on the end January with 50 g/ tree on mid-March (T2), and 50 g/ tree on the end January and 25 g/ tree on mid-March with 25 g/ tree on mid-May (T3). T4 acts a control (untreated with mycorrhiza inoculum).

Table 13: Effect of times and rates of mycorrhizal fungi using for rock phosphate inoculation on yield of 'Wonderful' pomegranate trees in 2021 and 2022 seasons

Treatments	No. Fruit/tree		Fruit weight (g)		Yield (Kg/tree)	
	2021	2022	2021	2022	2021	2022
T1*	58 c	54 c	463.33 b	486.11a	26.87c	26.25 c
T2	71 a	76 a	545.33 a	467.5 a	38.72 a	35.53 a
T3	64 b	63 b	539.17 a	467.5 a	34.51b	29.45 b
T4	29 d	42 d	520.00 a	426.67 b	15.08d	17.92 d

* Applications of mycorrhiza inoculum with 25 cm^3 of bacteria mixture + 1 kg of rock phosphate/ tree were at 100 g/ tree on the end January (T1), 50 g/ tree on the end January with 50 g/ tree on mid-March (T2), and 50 g/ tree on the end January and 25 g/ tree on mid-March with 25 g/ tree on mid-May (T3). T4 acts a control (untreated with mycorrhiza inoculum).

Application of mycorrhiza fungi at the rate of 50g/tree on the end of January and 50g in mid-March (T2) gave lowest values of manganese leaf content (32.83 and 39.35 ppm) in 2021 and 2022 seasons, respectively. The application of mycorrhiza fungi at the rate of 50g/tree on the end of January and 25g each of mid-March and mid-May (T3) gave the highest value of zinc leaf content in both studied seasons. Application of mycorrhiza fungi at 50g/ tree on the end January and 50g/ tree on mid-March (T2) gave the highest value of copper leaf content in the first and second season. Generally, the lowest values of leaf content from iron, zinc and copper were for control trees that received phosphate rock as single applications without mycorrhizal fungi during the two seasons of study.

3.4. Effect on pomegranate yield

Results in Table (13) & Figure (3) indicate that inoculation of rock phosphate with mycorrhizal fungi show significant increase in yield of 'Wonderful' pomegranate trees comparing with rock phosphate as single applications (control trees) during both seasons. Application of mycorrhiza fungi at 50 g/ tree on the end January and 50 g/ tree on mid-March (T2) was affective in enhancing number of fruit / tree and fruit weight, which was reflected in an increase in the yield of tree (Kg), recording (38.72 and 35.53 Kg/tree), compared to the control trees (15.08 and 17.92 Kg/tree) in both study seasons, respectively.

Applications of mycorrhiza inoculum with 25 cm^3 of bacteria mixture + 1 kg of rock phosphate/ tree were at 100 g/ tree on the end January (T1), 50 g/ tree on the end January with 50 g/ tree on mid-March (T2), and 50 g/ tree on the end January and 25 g/ tree on mid-March with 25 g/ tree on mid-May (T3). T4 acts a control (untreated with mycorrhiza inoculum).

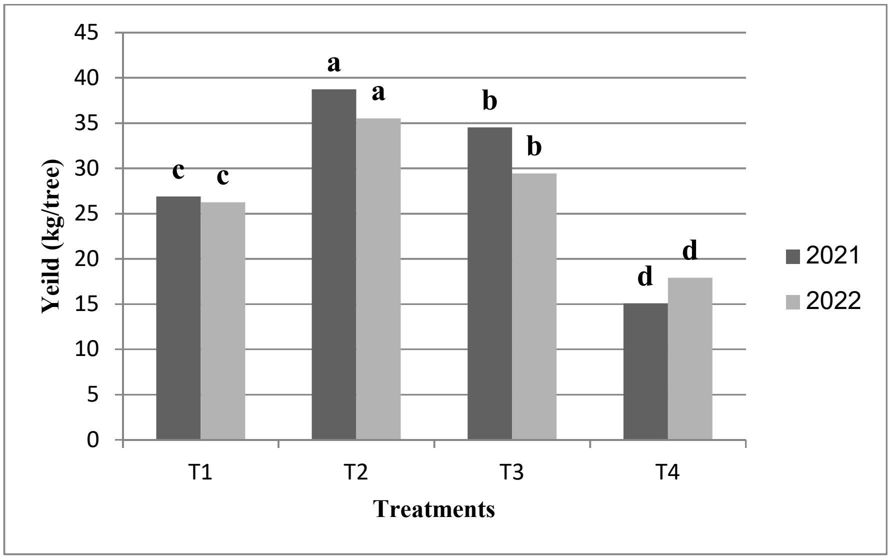

Fig. 3: Effect of times and rates of mycorrhizal fungi using for rock phosphate inoculation on yield of 'Wonderful' pomegranate fruits in 2021 and 2022 seasons.

3.5. Effect on fruit quality

The obtained results in Table (14) indicated that the quality of 'Wonderful' pomegranate fruits were influenced as a result of rock phosphate inoculation with mycorrhizal fungi compared to untreated trees during both seasons of the study.

Highest weight of arils and juice volume were obtained by application of mycorrhiza fungi at 100g/ tree on the end January (T1) in both seasons of the study. Highest value of TSS % of juice was obtained in (T2) during 2021 season, while in 2022 season was recorded with (T1). Lowest value of acidity % of juice was recorded by the application of mycorrhiza fungi at 50g/ tree on the end January and 50g/ tree on mid-March (T2) during 2021 and 2022 seasons. As for TSS/Acidity ratio, the statistical analysis indicated that the mycorrhiza fungi application at 50g/ tree on the end January and 50g/ tree on mid-March (T2) recorded the highest values in both seasons of the study compared to other treatments during 2021 and 2022 seasons respectively.

Table 14: Effect of times and rates of mycorrhizal fungi using for rock phosphate inoculation on fruit quality of 'Wonderful' pomegranate in 2021 and 2022 seasons

Treatments	Weight of 50 arils (g)		Juice volume of 50 arils (cm^3)		TSS (%)		Acidity (%)		TSS/Acidity ratio	
	2021	2022	2021	2022	2021	2022	2021	2022	2021	2022
T1*	21.12 a	23.92 a	15.87a	16.95 a	15.17 b	16.2 a	3.78ab	3.55b	4.01b	4.56ab
T2	20.80 a	22.31b	15.00 a	15.85b	16.50 a	15.3 b	3.07 b	3.07 c	5.37a	4.98a
T3	20.92 a	20.13 c	14.90 a	13.65c	14.87 b	15.3 b	3.78ab	3.75 b	3.93b	4.08b
T4	21.03 a	19.80 c	14.4 a	13.6 c	13.73 c	14.1c	4.61a	4.90 a	2.98c	2.88c

* Applications of mycorrhiza inoculum with 25 cm^3 of bacteria mixture + 1 kg of rock phosphate/ tree were at 100 g/ tree on the end January (T1), 50 g/ tree on the end January with 50 g/ tree on mid-March (T2), and 50 g/ tree on the end January and 25 g/ tree on mid-March with 25 g/ tree on mid-May (T3). T4 acts a control (untreated with mycorrhiza inoculum).

3.6. Olive and pomegranate water productivity

Applications of mycorrhiza inoculum at 100 g/ tree with 25 cm^3 of bacteria mixture + 1 kg of rock phosphate/ tree on the end January (T1) recorded the highest water productivities of olive fruits. While, 50 g/ tree on the end January and 25 g/ tree on mid-March with 25 g/ tree of mycorrhiza inoculum on mid-May (T3) attained the highest water productivities of pomegranate fruits (Table 15).

Table 15: Effect of times and rates of mycorrhizal fungi using for rock phosphate inoculation on water productivities of olive and pomegranate trees

Treatments	Olive (WP)	SD	Pomegranate (WP)	SD
T1*	2.83 a	0.10	1.28 b	0.11
T2	2.14 b	0.13	2.67 a	0.07
T3	2.57 a	0.10	2.93 a	0.12
T4	1.78 b	0.19	2.76 a	0.15

*Applications of mycorrhiza inoculum with 25 cm^3 of bacteria mixture + 1 kg of rock phosphate/ tree were at 100 g/ tree on the end January (T1), 50 g/ tree on the end January with 50 g/ tree on mid-March (T2), and 50 g/ tree on the end January and 25 g/ tree on mid-March with 25 g/ tree on mid-May (T3). T4 acts a control (untreated with mycorrhiza inoculum). SD: standard deviation.

4. Discussion

Salinity of soil is one of the most dangerous environmental stresses that limits yield and growth of horticultural crops, all over the world. Nearly 20 % cultivated and half of the irrigated lands in the world are affected by salinity. Salinity is still expanding, which poses a greater threat to sustainable development of agriculture (Mastrogiannidou *et al.,* 2016).

High salinity levels lead to a decrease in all growth parameters and nutrient contents (Mansy *et al.,* 2018). It is clear from Tables (1 and 3) that the soil in the areas under this study is characterized by poor soil structure, low water field capacity, deficiency of organic matter and nutrient insufficiency. Moreover, as is in, olives and pomegranates were irrigated with saline water with acute problem (Tables 2 and 4). In light of this, the aim of the study was to use some applications of microbial stimulants inoculation at different times and rates using mycorrhizal fungi for rock phosphate inoculation to mitigate the effects of soil and saline water stress on the productivity of olive trees "Picual cv." and pomegranate trees "Wonderful cv.".

Soil amendments play an effective role in increasing the nitrogen content in leaves, shoots and yield of trees irrigated with saline water (Chehab *et al.,* 2020). Rock phosphate fertilizer is a soil amendment, which has been reported to decompose to replace some of the available calcium lost or reduced from the saline soil ecosystem. Available calcium can replace sodium in the soil solution and in the soil exchange complex (Indriani *et al.,* 2016). Microbial biostimulants are also a biological tool to increase the quality standards of various horticultural crops, as well as reduce soil degradation. Efforts to improve the quality of sandy soil include the use of mycorrhizal fungi and soil conditioners (Wahid *et al.,* 2020).

The results of the study showed that the soil application of mycorrhizal to olive and pomegranate trees increased the yield and improved the quality of fruits, as well as the oil content of olive. It has a high ability to dissolve nutrients and convert them into an easily absorbed form, especially the element phosphorus, which is transformed by mycorrhizal from the complex form to the simple form that is easy to absorb. Compared to the leaves of untreated trees, this led to an increase in vegetative growth, all of which eventually lead to an increase in the amount of yield and an improvement in the quality fruits of 'Picual' olive and 'Wonderful' pomegranate. Mycorrhizae also secrete organic acids that increase the acidity of the soil and reduce its alkalinity, which allows for an increase in the availability of nutrients in the soil and an increase in the ability of the roots of trees to absorb it, especially the element phosphorus, and thus the amount of traditional fertilizers added to the soil decreases and the symptoms of nutrient deficiency on the plant decrease.

The results are in line with those reported by Mikiciuk *et al.* (2019) who cleared that the addition of mycorrhizal to the soil during plant cultivation or during winter service increases the yield and quality of fruits and improves plant growth. Due to the intense growth of mycorrhizal filaments, this reduces the use of traditional phosphate fertilizers. Similar results were obtained by Jamiołkowska and Michałek (2019) who showed that the use of mycorrhizal increases the resistance of the root system of plants to pathogens, improves plant resistance, and increases crop yield. It is also possible to explain the increase in tree productivity through the effect of mycorrhizal on increasing the trees resistance to environmental and biotic stress, and this was confirmed by (Pešakovi´c *et al.,* 2013; Sowik *et al.,* 2016). Similar results were obtained by Zhu *et al.* (2012) who observed that the mycorrhizal fungus, when growing around the roots of plants, synthesizes plant hormones that stimulate growth, such as indole acetic acid, which increases the growth and elongation of roots, and this explains the increased root density of trees treated with mycorrhizal compared to other trees. In a similar study, Wu *et al.,* (2022) cleared that the ground cover of mycorrhizal to olive trees increased the uptake of phosphorus as well as the nitrogen element, and the uptake of potassium was not affected, where the study found that the addition of mycorrhizal to the soil enhanced the gene expression of genes that transport the element phosphorus in the roots of olive trees. This highlights the important role of mycorrhizal in increasing the uptake of the element phosphorus. In another study, Iula *et al.* (2021) found that tomato plants treated with mycorrhizal increased their fresh and dry weight compared to untreated plants, and thus the yield increased. In addition, Faisal *et al.* (2010) cleared that the addition of mycorrhizal to pepper plant led to a significant increase in the number of fruits and the total yield, compared to the untreated plants.

Elmajdoub and Marschner (2015) studied the influence of mycorrhizae and soil conditioner on chemical characteristics and biological activity of soil, showed that organic amendments can help soil microbial community adapt to salinity. The high carbon availability from organic amendments provides microbes with the energy needed to synthesize of organic osmolytes. Mycorrhizal fungi are growth promoters, nutrient-enriching and phytoremediative bio-factors that provide protection to plants from diseases and resistance to drought, salinity, and heavy metal toxicity. Biostimulants such as arbuscular mycorrhizae fungi promote soil enzymatic and microbial activities and increase the nutrients solubility and mobility, which enhance the soil fertility, increase plant metabolism and improve crop quality (Wahid *et al.,* 2020). Rock phosphate with inoculation if mycorrhizal fungi in the soil enhanced crop yield since the phosphorus mineralization and uptake increased (Harikumar, 2017).

Moreover, (Osorio and Habte, 2001) mentioned that there are collaboration between P solubilizing microorganisms and mycorrhizal fungi, which was more obvious in the P uptake than in the growth. Mycorrhiza and azotobacter significantly increased growth and yield. This supports the results of our study, where rock phosphate-fertilized with inoculation by microbial stimulants both olive and pomegranate trees had significantly higher yield and fruit quality under the soil and water salt stress.

4. Conclusion

From the above results, it can be concluded that applications of mycorrhiza inoculum with 25 cm^3 of bacteria mixture + 1 kg of rock phosphate/ tree were at 100 g/ tree on the end January (T1) insignificantly increased olive yield and decreased pomegranate yield. It could be detected that applications of mycorrhiza inoculum with 25 cm^3 of bacteria mixture + 1 kg of rock phosphate/ tree were at 50 g/ tree on the end January and 25 g/ tree on mid-March with 25 g/ tree on mid-May (T3) is the best management under this study for both of olive and pomegranate trees to mitigate the impacts of soil and water salt stress as well as enhancing the productivity of trees.

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