

Effect of rock phosphate and bacteria solubilizing nutrients soil application on yield and fruit quality of olive trees cv. "Picual" under the arid zones

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Abstract. This study was carried out throughout two seasons and Located in a private olive grove on Cairo-Ismailia Desert Road in Egypt. The aim of this experiment was to use different amounts of rock phosphate (1, 2 or 3 kg/tree) with different time of additions (2 or 3 times) for phosphorus and sulfur release bacteria to form 6 treatments beside the control (untreated trees) on the mineral contents of the leaves, the vegetative growth, the yield, and the quality of the fruit of Picual olive trees planted in sandy soil and watered with drip irrigation for two seasons. The outcomes demonstrated that application of 2 or 3 kg rock phosphate per tree for two or three times of phosphorus and sulfur release bacteria gave the highest yield and improves fruit quality. Generally, the treatment of 2 kg rock phosphate for three times of P and S release bacteria is recommended due to its clear effect among all treatments on the abovementioned parameters.

1 Introduction

One of the world's most significant economic fruit crops is olives (*Olea europaea* L.) and particularly in the Mediterranean region. Olive trees tolerate drought, salinity and climatic changes more than many other fruit crops. Furthermore, increasing yield and fruit quality of olive trees under the arid zones depend on proper agricultural, technical and economic management due to the scarcity of natural resources. Olive trees areas increased quickly in Egypt and reached about 89942 hectares with total production of fruits about 1080091 tons [1].

Phosphorus is the second most essential nutrients and is taken up in largest amount after nitrogen [2]. Since it is essential for most crops' fruit setting and root growth, an appropriate supply is necessary for several physiological processes, including seed production, water-use efficiency, root and seedling growth, uniform maturity, and early heading [3]. Briefly, phosphorus deficiency causes a significant loss in productivity of crops. Using rock phosphate as an alternative to phosphate fertilizers leads to significant environmental,

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agricultural and economic benefits. Utilizing natural element compounds has been shown in numerous studies to enhance the physical and chemical properties of soil as well as increase soil nutrient availability and water uptake [4]. Natural elements compounds such as rock phosphate are used as a source of some nutrients, this management is considered organic or clean agriculture, and these compounds improve soil structure.

Bio-fertilization and soil microbes particularly phosphorus solubilizing bacteria play an effective and active role in dynamics of soil phosphorus. These microorganisms either release phosphorus in the soils through release of organic acids or prevent precipitation of Mg^{+2} and Ca^{+2} to cover phosphorus deficiency in alkaline / calcareous soils [5]. The use of phosphorous-dissolving bacteria is an effective method that will provide new means to improve the efficiency of phosphorous use [6]. Therefore, bio-fertilizers may contribute to the development models of sustainable agriculture [7].

The current study set out to find out how rock phosphate in combination with bacteria that release sulfur and phosphorus affected the olive trees (Picual cv.) vegetative growth, leaf mineral content, fruit yield (kg per tree), and fruit quality traits.

2 Materials and methods

This experiment was conducted in a private olive orchard on Cairo-Ismailia Desert Road, Ismailia Governorate, Egypt, over the course of two seasons (2022 and 2023). Eight-year-old Picual cv. olive trees that were planted at a 4 x 6 meter, grown in sandy soil, and drip-irrigated were used in the experiment. An analysis of the orchard's soil and water can be found in Tables (1) and (2). In addition, the chosen trees received standard horticultural procedures.

Table 1. Analysis the water from the orchard well.

pH	EC(dSm ⁻¹)	Soluble cations (meq/l)				Soluble anions (meq/l)			
		Ca ⁺⁺	Mg ⁺⁺	Na ⁺	K ⁺	CO3 ⁼	HCO3 ⁻	Cl ⁻	SO4 ⁼
7.84	6.27	15.0	10.5	42.6	0.20	-	1.9	43.5	22.9

Table 2. Analysis of the orchard soil's chemical and physical characteristics.

Depth parameter s of simple (cm)	pH	EC(dSm ⁻¹)	Soluble cations (meq/l)				Soluble anions (meq/l)				N (ppm)	P (ppm)
			Ca ⁺	Mg ⁺	Na ⁺	K ⁺	CO3 ⁼	HCO3 ⁻	Cl ⁻	SO ⁴⁻		
Superficial sample	8.19	1.23	2.7	1.3	7.5	1.0	-	1.0	10.0	1.5	53.3	22.4
30 cm	8.58	0.97	4.5	1.5	3.6	0.7	-	0.9	2.8	6.6	36.18	19.5
60 cm	8.62	0.45	1.8	0.6	2.0	0.4	-	0.6	2.6	1.6	19.04	0.12

2.1 Experimental design

The experiment was designed with fully randomized sectors. Apart from the control treatment, there were six treatments in the experiment. There were three replicates for every treatment, and each replicate comprised a single tree. The treatments included the addition of phosphate rock as a source of phosphorus beside the nutrients solubilizing bacteria in the form of a simple experiment.

2.2 Experimental material

The amounts of rock phosphate (1, 2 or 3 kg per tree / year) and organic fertilizer (2 kg compost (2.00:3.00% N) plus 0.5 kg agricultural sulfur per tree) was applied for all treatments. During the final week of December, all applications were made superficially and with a shovel.

The agricultural and biological institute's microbiology department generously provided liquid cultures of two bacteria, *Bacillus megaterium* var. *phosphaticum* and *Acidithia bacillus thiooxidans*, for use as biofertilizer at the National Research Center. To produce a cell suspension, each organism was grown independently in batch culture until it reached the late exponential phase. Each tree received 25 milliliters of the mixture that was created on the spot by combining cultures of *Bacillus megaterium* var. *phosphaticum* and *Acidithia bacillus thiooxidans*. This treatment was applied twice (in January and March) or three times (in January, March, and May) throughout the season.

2.3 Treatments

This experiment was designed to study the effect of rock phosphate (1, 2 or 3 kg/tree) with different time of additions (two or three times) for phosphorous and sulfur release bacteria to form six treatments beside the control (21 trees for all treatments) during two studied seasons as follow:

- (T0) untreated trees (control)
- (T1) 1kg rock phosphate plus bacteria at two times
- (T2) 1kg rock phosphate plus bacteria at three times
- (T3) 2kg rock phosphate plus bacteria at two times
- (T4) 2kg rock phosphate plus bacteria at three times
- (T5) 3kg rock phosphate plus bacteria at two times
- (T6) 3kg rock phosphate plus bacteria at three times.

Three replicates (one tree per replicate) for each treatment were selected at random in the first part of October for each season.

2.4 Measurements

Each growing season's shoot length (measured in centimeters) and the number of leaves per one-year-old shoot was recorded during the first week of July.

In July, mature samples of leaves were taken from each tree's four directions. The samples were then dried in a drying oven at 70°C until they reached a constant weight. The following estimate was made for the elemental concentration in the leaves:

- 1- N% - By following [8] instructions and using the modified micro-Kjeldahl method.
- 2- P% - Was calculated using the method [9] described.
- 3- Ca% and K% were determined using flame photometry in accordance with (10) methodology.
- 4- As explained by [11], atomic absorption (Model, spectronic 21 D) was used to spectrophotometrically determine the percentage of magnesium and the concentrations of Fe, Mn, Zn, and Cu.

Yield: At the maturity stage, ripe fruit samples were collected in mid-October, and each tree's yield weight (Kg) was determined.

Fruit quality: To measure the fruit quality, thirty fruits were randomly chosen from each tree.

1. The average fruit's weight (gm) and volume (cm³)
2. Fruit shape (L/D ratio), fruit length L (cm), and fruit diameter D (cm).

3. Fruit weight in grams, seed weight in grams, and pulp to seed ratio were computed.

2.5 Statistical analysis

According to [12], an analysis of variance (ANOVA) was performed using the Costat program for the data collected from the two seasons of the experiment 2022 and 2023. The least significant domains (LSR) were used with a probability of 5%, per [13].

3 Results and Discussion

3.1 Shoot length and No. of leaves per shoot

The results displayed in Table (3) demonstrated how the treatments that were administered had an impact on shoot length. The highest values in this regard were obtained from T4 (3 kg rock phosphate + bacteria at the three times), as they were 55.6 and 52.43 cm during both study seasons. Results in the same table indicated that all treatments had a significant impact on the number of leaves per shoot during both study seasons. Thus, in the first season, the trees treated with 2 kg of rock phosphate plus bacteria three times (T4) and 3 kg of rock phosphate plus bacteria three times (T6) showed the highest number of leaves per shoot (63 and 65, respectively). Additionally, the highest value ever recorded for T4 and T6 was in the second season (60 and 62, respectively).

Table 3. Effect of rock phosphate soil application and nutrients solubilizing bacteria on shoot length and no. of leaves / shoot of Picual olive trees in 2022 and 2023 seasons.

Treat.	Shoot length (cm)		No. of leaves / shoot	
	2022	2023	2022	2023
T0	32.5 f	30.51 f	43 e	41 f
T1	41.2 e	39.09 e	51 d	48 e
T2	41.9 e	39.40 e	53 c	50 d
T3	49.5 c	44.81 d	58 b	53 c
T4	54.6 b	51.48 b	63 a	60 a
T5	48.8 d	45.76 c	58 b	55 b
T6	55.6 a	52.43 a	65 a	62 a

Mean in each column with similar letter(s) are not significantly different at 5 % level.

3.2 Leaf contents of Macro elements

The percentages of nitrogen, phosphorus, potassium, calcium, and magnesium in leaves are displayed in Table (4) along with the various rates at which rock phosphate combined with bacteria that release phosphorus and sulfur are affected. For each treatment, the nitrogen percentage increased significantly. Applying 2 kg of rock phosphate + bacteria three times (T4) produced the highest percentages in the first and second seasons, 2.87% and 2.74%, respectively. Phosphorus percentages in the same table showed no discernible variation over the course of the two seasons between any treatment and control. The tree treated three times with bacteria plus three kg of rock phosphate (T6) showed the highest values in this regard, with multiple instances of 0.24% and 0.22% in the first and second seasons.

Table 4. Effect of rock phosphate soil application and nutrients solubilizing bacteria on macro elements of Picual olive leaves in 2022 and 2023 seasons.

Treat.	N%		P%		K%		Ca%		Mg%	
	2022	2023	2022	2023	2022	2023	2022	2023	2022	2023
T0	1.88 f	1.79 f	0.21 a	0.19 a	0.79 e	0.76 e	2.08 d	1.98 f	1.53 b	1.46 e
T1	2.50 c	2.38 b	0.18 a	0.17 a	0.98 b	0.93 b	2.20 c	2.09 d	1.65 a	1.57 b
T2	2.18 e	2.08 e	0.21 a	0.20 a	0.89 d	0.85 d	2.28 b	2.17 c	1.58 b	1.51 c
T3	2.40 d	2.27 d	0.21 a	0.20 a	0.98 b	0.92 b	2.30 b	2.15 c	1.55 b	1.46 e
T4	2.87 a	2.74 a	0.19 a	0.18 a	0.92 c	0.88 c	2.38 a	2.26 a	1.58 b	1.51 c
T5	2.60 b	2.48 b	0.18 a	0.17 a	1.08 a	1.03 a	2.31 b	2.19 b	1.55 b	1.48 d
T6	1.83 f	1.75 g	0.24 a	0.22 a	0.67 f	0.64 f	2.18 c	2.07 e	1.68 a	1.60 a

Mean in each column with similar letter(s) are not significantly different at 5 % level.

In terms of the percentage of potassium found in leaves, the trees (T5) treated with 3 kg of rock phosphate plus bacteria twice produced the highest values 1.08% and 1.03%, respectively, in the first and second seasons. Regarding the percentage of calcium in the leaves, various treatments had an impact during both seasons. The plants treated with T4 had the highest percentages of calcium in their leaves (2.38% and 2.26%) in the first and second seasons, respectively. The percentage of magnesium in leaves was impacted by the treatments that were used. Applying 1 kg of rock phosphate plus bacteria twice (T1) and 3 kg of rock phosphate plus bacteria three times (T6) to the soil produced the highest magnesium leaf percentages (1.65 and 1.68%, respectively) during the first season. Furthermore, the highest value ever recorded with T6 during the second season was (1.60%).

3.3 Leaf contents of Micro elements

The data in Table (5) showed the treatments did not lead to a significant increase in iron concentration in the leaves compared to the control. According to data from the same study, every treatment increased the amount of Mn in the leaves when compared to the control treatment (table 5). The trees treated with 1 kg of rock phosphate plus bacteria three times (T2) showed the highest values, 48.51 and 46.25 ppm in the two seasons under study. The zinc content of the leaves in the same table was not significantly impacted by the various treatments. According to the results shown in the same table, the additions made during the two seasons had no discernible effect on the leaf content of Copper. Applying 3 kg of rock phosphate plus bacteria to the soil three times produced the highest values (7.42 and 7.08 ppm) in both study seasons.

Table 5. Effect of rock phosphate soil application and nutrients solubilizing bacteria on micro elements of Picual olive leaves in 2022 and 2023 seasons.

Treat.	Fe ppm		Mn ppm		Zn ppm		Cu ppm	
	2022	2023	2022	2023	2022	2023	2022	2023
T0	133.65 a	127.41 a	38.61 c	36.81 cd	21.00 a	20.01 a	5.94 a	5.66 a
T1	105.30 d	100.39 c	46.00 a	43.85 ab	17.60 a	16.78 a	4.50 a	4.29 a
T2	120.29 b	114.67 b	48.51 a	46.25 a	18.71 a	17.84 a	4.46 a	4.20 a
T3	121.50 b	113.67 b	37.00 c	34.92 d	20.30 a	19.16 a	4.50 a	4.19 a
T4	122.50 b	116.27 b	39.61 bc	36.85 cd	19.71 a	18.37 a	4.41 a	4.15 a
T5	104.25 d	99.38 d	43.00 ab	40.99 b	21.60 a	20.59 a	6.00 a	5.72 a
T6	116.10 c	110.68 c	45.54 a	43.41 ab	20.10 a	19.01 a	7.42 a	7.08 a

Mean in each column with similar letter(s) are not significantly different at 5 % level.

3.4 Yield and fruit quality

In regards to yield as kg/tree, Table (6)'s data made it clear that, during the study seasons, the treatments greatly increased yield in comparison to the untreated trees. Accordingly, the trees that were treated with 2 kg of rock phosphate plus bacteria three times (T4) yielded the most in the first season (kg/tree) at 38.67 kg/tree. Additionally, in the second one, since there were (49 and 48 kg/tree, respectively), the maximum values were given with T4 and T6. Conversely, in both study seasons, the control trees gave the minimum yield values in this regard (17.33 and 27.50 kg/tree).

Table 6. Effect of rock phosphate soil application and nutrients solubilizing bacteria on yield, fruit weight and fruit volume of Picual olive trees in 2022 and 2023 seasons.

Treat.	Yield Kg/tree		Fruit weight (g)		Fruit volume (cm ³)	
	2022	2023	2022	2023	2022	2023
T0	17.33 d	27.50 d	7.34 c	7.00 d	7.50 abc	7.25 bc
T1	22.67 c	34.00 c	7.47 bc	7.12 c	7.25 bc	7.00 c
T2	30.00 b	41.50 b	6.95 d	6.63 e	7.00 bc	7.25 bc
T3	32.67 b	42.50 b	7.91 a	7.35 a	8.00 a	7.50 b
T4	38.67 a	49.00 a	7.67 ab	7.31 b	7.75 ab	7.75 a
T5	22.67 c	35.50 c	7.36 c	7.02 d	7.50 abc	7.75 a
T6	37.33 a	48.00 a	6.76 d	6.45 f	7.00 c	6.50 d

Mean in each column with similar letter(s) are not significantly different at 5 % level.

Data showed that when compared to the control in table (6), the fruit weight was impacted by various treatments. The trees treated with 2 kg of rock phosphate plus bacteria at 2 times (T3) had the highest values (7.91 and 7.35 g) during both seasons. The same table's results demonstrated that, during the study seasons, various additions significantly increased the fruit volume. In the first season, soil application containing 2 kg of rock phosphate plus bacteria twice (T3) yielded the highest value (8.00 cm³). Furthermore, the highest moral values were demonstrated by T4 and T5, as they were 7.75 and 7.75 cm³, respectively, during the second season.

The majority of the additions in the table (7) had a significant impact on fruit length. The maximum length of fruit was cleared by trees treated with 3 kg of rock phosphate plus bacteria twice (T5), 2 kg of rock phosphate plus bacteria three times (T4), and 1 kg of rock phosphate plus bacteria three times (T2), as there were (2.65, 2.66, and 2.64 cm, respectively), in the first season. Furthermore, the highest values measured with T5 during the second season were 2.53 cm. Findings shown in the same table indicate that various soil treatments had an impact on fruit diameter. During the study seasons, trees treated with 2 kg of rock phosphate plus bacteria twice (T3) had the largest diameters (2.24 and 2.13 cm).

Table 7. Effect of rock phosphate soil application and nutrients solubilizing bacteria on fruit length, diameter and shape index of Picual olive trees in 2022 and 2023 seasons.

Treat.	Fruit length (L cm)		Fruit diameter (D cm)		Fruit shape index (L/D)	
	2022	2023	2022	2023	2022	2023
T0	2.57 b	2.45 d	2.20 ab	2.10 b	1.17 c	1.11 c
T1	2.55 b	2.43 e	2.17 bc	2.05 d	1.18 c	1.13 bc
T2	2.64 a	2.52 b	2.10 e	2.00 e	1.26 a	1.20 a
T3	2.60 ab	2.48 c	2.24 a	2.13 a	1.16 c	1.11 c
T4	2.66 a	2.51 b	2.19 bc	2.08 c	1.21 b	1.15 b
T5	2.65 a	2.53 a	2.11 de	2.01 e	1.26 a	1.20 a
T6	2.47 c	2.35 f	2.15 cd	2.04 d	1.15 c	1.10 c

Mean in each column with similar letter(s) are not significantly different at 5 % level.

The results showed that the treatments carried out had an impact on the fruit shape index in Table (7). The trees that received treatments of 1 kg rock phosphate + bacteria three times (T2) and 3 kg rock phosphate + bacteria twice (T5) were able to consistently clear the highest values (1.26, 1.26) and (1.20, 1.20) during the experimental seasons. The data in Table 8 indicates that the treatments that were applied had an impact on the weight of the pulp. The maximum values (6.92 and 6.90 g) in the first season were cleared by soil application using 2 kg rock phosphate + bacteria twice (T3) and three times (T4), respectively. Furthermore, in the second season, the trees planted by T4 had the highest pulp weight (6.58 g). Table (8) regarding seed weight indicated that various additions had an impact on seed weight. The trees with the lowest weight (weights of 0.77 and 0.74 g in the first and second seasons, respectively) were those treated with 2 kg of rock phosphate plus bacteria three times (T4). The pulp/seed ratio was found to be impacted by the treatments that were carried out, as shown in table (8). The trees that were treated with 2 kg of rock phosphate plus bacteria three times (T4) cleared the highest ratios, with values of 8.92 and 8.51 in the two seasons that followed, respectively.

Table 8. Effect of rock phosphate soil application and nutrients solubilizing bacteria on pulp weight, seed weight and pulp/seed ratio of Picual olive trees in 2022 and 2023 seasons.

Treat.	Pulp weight (g)		Seed weight (g)		Pulp/Seed ratio	
	2022	2023	2022	2023	2022	2023
T0	6.39 bc	6.09 d	0.95 b	0.91 c	6.71 d	6.39 e
T1	6.56 b	6.25 c	0.91 c	0.87 d	7.20 c	6.86 c
T2	6.17 cd	5.88 e	0.79 e	0.75 f	7.84 b	7.47 b
T3	6.92 a	6.41 b	1.00 a	0.94 a	6.94 cd	6.50 d
T4	6.90 a	6.58 a	0.77 e	0.74 g	8.92 a	8.51 a
T5	6.38 bc	6.09 d	0.97 ab	0.93 b	6.56 d	6.25 f
T6	5.40 d	5.66 f	0.82 d	0.79 e	7.21 c	6.87 c

Mean in each column with similar letter(s) are not significantly different at 5 % level.

[14] Discussed the advantages of using rock phosphate on crops in terms of growth and leaf mineral content. They concluded that *Penicillium oxalicum* combined with rock phosphate can replace chemical fertilizers in alkaline soil and increase crop yields. Moreover, the production of crops increases significantly with increase both phosphorus and sulfur levels as well as content of protein increase by increasing the level of sulfur fertilization [15]. Phosphorous is included in the composition of living cells, the synthesis of DNA and energy compounds (ATP) in the cells, as well as it has a role in the process of cell division in all parts of the plant, especially the meristematic tissues, thus leads to directly affects the development and growth of roots and leaves, the growth of fruits, early ripening of fruits and the growth of embryos in the seeds of most crops [16]. Furthermore, rock phosphate fertilizers increased dry matter and yield over those of the untreated that did not receive phosphorus fertilizer, as demonstrated by [17], who introduced rock phosphate fertilizer as a naturally occurring source of phosphorus (P) and its relatively low cost. On the other side, the addition of bio-fertilizers increased the rate of decomposition of rock phosphate and release phosphorous element from it, and thus enhanced fruit quality, yield, and vegetative growth [6]. The obtained results in yield and physical properties of fruits, are in agreement with [18] on olives, [19] on oil palm.

4 Conclusion

In general, it can be said that improving the vegetative growth, leaf mineral content, yield, and fruit quality of olive trees (cv. Picual) can be achieved by applying rock phosphate and

bacteria on plants that are thought to be environmentally friendly. Applications by 2 kg rock phosphate per tree + phosphorus and sulfur release bacteria for three times of are recommended due to its clear effect among all treatments on the abovementioned parameters. Therefore, Phosphate rock can be recommended as a natural substitute for phosphorous, as the study showed.

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