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Phosphorus Efficiency of Some Pomegranate Cultivars Inoculated by Mycorrhizal Fungi and Phosphate Solubilizing Bacteria under Calcareous Soil Conditions

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ABSTRACT: The present study was conducted to evaluate the effect of A. mycorrhizal fungi (AMF) and phosphorous-dissolving bacteria (PDB) as a bio fertilization inoculation under three phosphate fertilizer rates was added before planting, on the yield and fruit quality of three pomegranate cultivars Wonderful, H116 and Manfaloy under calcareous soil conditions. For this purpose, a field experiment designed in split split plot design was conducted in a private pomegranate orchard in Abd El Baset village, Burg Al Arab, Alexandria, Egypt during the 2019 growing season, where the three pomegranate cultivars were placed in the main plots and phosphorus levels were allocated in sub-plots. While, AMF, PDB and AMF + PDB in addition to the control were randomly distributed in the sub sub-plots. The obtained results confirmed that all pomegranate fruit physical and chemical properties as well as fruit yield and yield components were significantly affected by cultivars, phosphorus levels and biofertilization. Wonderful cv. had the highest; fruit length, fruit diameter, fresh fruit weight, dry fruit weight, fruit number tree^{-1}, fruit yield tree^{-1}, fruit contents of acidity, total sugars, vitamin C and anthocyanin. In addition to the lowest fruit TSS and TSS/acidity ratio. pomegranate trees that were fertilized with the full dose of phosphorus expressed the highest plant growth fruit length, fruit diameter, fresh fruit weight, dry fruit weight, fruit number tree^{-1}, fruit yield tree^{-1}, fruit contents of acidity, total sugars, vitamin C and fruit content of anthocyanin. Applied AMF in combined with PDB showed the highest fruit length, fruit diameter, fresh fruit weight, dry fruit weight, fruit number tree^{-1}, fruit yield tree^{-1}, acidity, total sugars, vitamin C and anthocyanin In addition to the lowest fruit content of TSS as well as less rate of fruit ripening. Wonderful fertilized with the full dose of phosphorous in the presence of the combined biofertilization treatment AMF + PDB had the highest fruit contents of acidity, total sugar, ascorbic acid anthocyanin in addition to the lowest TSS and TSS/acidity ratio. The results confirmed that used of AMF + PDB resulted in a large increase in soil phosphorus availability under all phosphorus levels.

Keywords: calcareous soil- biofertilization-phosphorus levels- pomegranate yield

INTRODUCTION

Pomegranate (*Punica granatum* L.) is an important commercial fruit crop in tropical and subtropical regions of the world. Pomegranate cultivation is very widespread in the Mediterranean and the Middle East, so this region contains a large number of genotypes that show high genetic diversity **(Holland et al., 2009)**. Pomegranate fruits have high nutritional values as they contain 78% water, 19% carbohydrates, 2% protein, 1% fat and 20% dietary fiber **(USDA 2019)**. Each 100g of pomegranate fruit provides 12% of the daily value for vitamin C, 16% for vitamin K, and 10% for folate **(USDA 2019)**. Pomegranate seeds are a rich source of powerful antioxidants and vitamins **(Parvizi and Sepaskhah, 2015)**. The cultivated area of pomegranate in Egypt is about 79.4 thousand feddans **(FAO Stat 2019)**, most of this area is spread in poor or marginal lands.

Pomegranate can grow in calcareous soils with pH ranged from 7.5 to 8.5 with a high content of calcium carbonate **(Marschner, 1995)**. Despite the success of pomegranate cultivation in calcareous soil, the yield declines in a way that cannot be overlooked, as the soil pH affects the availability of soil nutrients **(Rashid and Ryan 2004)**. The increase in Ca also leads to a strong decrease in the absorption of Mg and K, due to antagonistic interactions with each other, which ultimately affects the production **(Gransee and Führs, 2013)**.

Phosphorus (P) is one of the most important macro-essential elements which necessary for plant growth and reproduction (**Tigre *et al.*, 2014**). Phosphorus plays important role in all life forms because where it had a genetic function in nucleic acids and a role in biological energy transfer via ATP and ADP **(Zhou et al., 2014)**. Calcareous soils, $CaCO_3$ has a dominating influence due to its properties of relatively moderate solubility, higher buffer capacity, and alkalinity. In calcareous soils, phosphorus may be immobilized by several mechanisms **(FAO, 1984)**, which led to reduce the P availability. The low phosphorus availability in calcareous soils considers one of the main yield limiting factors **(Ozanne 1980)**. To overcome the problem of low yields in calcareous soils, there are several procedures must be followed. These procedures start by choosing the suitable genotype or cultivar. Genotypes differ in their ability to absorb and transport nutrients. Some genotypes can develop morphological, physiological, and biochemical mechanisms that help them absorb, transport and assimilate nutrients **(Miljković and Vrsaljko, 2009)**. It was found that the fertilizers that needs to pomegranate differ from one genotype to another **(Chater and Garner, 2018)**. Plant species may differ in phosphorus efficiency. Phosphorus efficiency of plants may arise from enhanced ability of roots to acquire P from the soil or/and from high ability of shoots to produce yield per unit P acquired (**Abou El-Seoud 2005)**. Plant species differ in their ability to grow under low available phosphorus in soil, where these species differ in their P efficiency **(Bhadoria et al., 2002)**. Phosphorus efficiency can be generally defined as the ability of plant to produce high yield in limiting phosphorus in root growing media **(Gourley et al., 1994)**. Also, phosphorus efficiency may be defined as the ability of a plant to produce a certain percentage of its maximum yield (80% of maximum yield) at low level of soil P **(Föhse, et al., 1988)**. Phosphorus efficiency can arise in two ways: i) The efficiency with which P is utilized to produce yield, i.e. the amount of P needed in the plant to produce one unit of dry matter. This is often called P utilization efficiency or internal P requirement and it is the P concentration in the plant to produce a given percentage of its maximum yield (for example 80-90% of maximum yield) **(Bhadoria et al., 2002)**. ii) The uptake efficiency of the plant, which is the ability of the root system to acquire P from the soil and accumulate it in the shoots **(Akter, 2003)**. Despite the importance of choosing the suitable genotype for those lands, it must be accompanied by improving soil physical and chemical characteristics of those lands by adding organic, bio and chemical fertilizers to provide plant needs from nutrition elements. Mineral fertilizers are always added in calcareous soils accompanied by the addition of biofertilization such as A. mycorrhizal (AMF) and/or element-dissolving bacteria.

A. mycorrhizal fungi (AMF) is a symbiotic fungi that associated with a green plant where the plant makes organic molecules such as sugars by photosynthesis and supplies them to the fungus, and the fungus supplies to the plant water and mineral nutrients, such as phosphorus that taken from the soil **(Kottke and Nebel 2005)**. Mycorrhizal can enhance growth through nutrients uptake by external hyphae that can reach more than 10 cm from the root **(Marschner and Dell 1994)**. Plants depend heavily on mycorrhizal fungi in low P soil for improving P and N uptake. AMF supply host plants with about 25% of the total plant N_2 compared with 3.5% of N_2 supplied via non-inoculated plants **(Fulton 2011)**. In addition to the role of the A. mycorrhizal fungus in supplying plants with nutrients, many bacterial genera can dissolve nutrients, allowing them to be easily available for absorption by the plant. One of these types is phosphate-dissolving bacteria (PDB). **Abou El Seoud et al. (2017) and Abou El Seoud et al. (2020)** found that all mycorrhizal species were effective on plant growth and P content more than untreated plants.

Phosphate dissolving bacteria (PDB) are capable of hydrolyzing organic and inorganic phosphorus from insoluble compounds. They are commonly found in rhizoplane and rhizosphere **(Estrada et al. 2013)**. The inoculation of PDB were found to increase plant growth parameters (plant height, leaf area, dry matter, content and chlorophyll content), microbial population, enzymatic activity (Dehydrogenase, Phosphatase, and Urease), nutrient uptake (Nitrogen, Phosphorus and Potassium) and yield **(Yallappa 2012)**. These increased in plant growth and yield may be due to that bacteria could improve phosphorus and potassium availability in the soil by producing organic acid and other chemicals by stimulating growth and mineral uptake of plants **(Park et al. 2003)**.

Therefore, the current study aims to evaluate the complementary role between mycorrhizal fungi and phosphate-dissolving bacteria in improving the properties of calcareous soil and their impact on the growth and yield of pomegranate cultivars.

MATERIALS AND METHODS

To evaluate the effect of mycorrhizal fungi (AMF) and phosphorous-dissolving bacteria inoculations under three phosphate fertilizer rates P0 (without P fertilizer), P1 (50% of recommended P fertilizer 75 Kg calcium superphosphate (15.5% P_2 O_5)/fed.)), and P2 (100% of recommended P fertilizer (150 Kg calcium superphosphate/fed.)) was added before planting, on the yield and fruit quality of three pomegranate cultivars Wonderful, H116 and Manfaloy under calcareous soil conditions. For this purpose, a field experiment designed in split split plot design was conducted a private pomegranate orchard in Abd El Baset village, Burg Al Arab, Alexandria, Egypt during the 2019 growing season, where the three pomegranate cultivars were placed in the main plots and phosphorus levels were allocated in sub-plots. While, AMF, PDB and AMF + PDB in addition to the control were randomly distributed in the sub sub-plots. The experimental soil physical and chemical properties are presented in Table 1. The soil properties were determined according to the methods describing by **(Black, 1965)**

Table 1: The experimental soil physical and chemical properties

Parameters	Amount
Mechanical analysis	
Clay %	23.80
Silt %	8.48
Sand %	67.72
Soil texture	Sand clay Loam
pH	8.05
EC dSm^{-1}	1.43
Total $CaCO_3$%	20.00
Organic matter (%)	0.65
Soluble Cations and Anions (meq/ L)	
Calcium	2.21
Magnesium	2.39
Sodium	6.63
Potassium	0.99
Bicarbonate	1.97
Chloride	4.85
Sulphate	4.44
Available macronutrients, (mg/ kg soil)	
Nitrogen	117.83
Phosphorus	8.27
Potassium	225.40

The experimental procedure:

Experimental parameters were applied to 108 trees of the three pomegranate cultivars, where 36 trees of each cultivar were selected, uniform in shape and size, free from fungal and insect diseases at the age of two years. Those trees were divided into three replicates, where the experimental plot included one tree. All trees received all recommended practices from irrigation, nitrogen and potassium fertilization, according to the recommendations of the Egyptian Ministry of Agriculture regarding the pomegranate crop. Where, N and k fertilizers Where nitrogen and potassium were added as soil doses in January at a rate of 150 N kg/fed as NH_4NO_3 and 150 kg K as K_2SO_4 /fed. Three levels of phosphorus fertilizer were added to obtain P0 (without P fertilizer), P1 (50% of recommended P fertilizer (75 Kg calcium superphosphate (15.5% P_2O_5)/fed.)), and P2 (100% of recommended P fertilizer (150 Kg calcium superphosphate/fed.)) were added with the beginning of buds emergency in January.

Arbuscular Mycorrhiza fungi (AMF): mycorrhizal specie (***Arbuscular mycorrhiza***) which is used in this experiment, was obtained from Germany and activated in the soil microbiology lab, Soil and Agriculture Chemistry Department, Faculty of Agriculture, Saba Basha, Alexandria University, Alexandria, Egypt. Pomegranate trees were treated with mycorrhizal by inoculating the soil around the root in January, just before irrigation. The soil around the tree at 1m diameter was mixed with 100 ml Mycorrhizal inoculum one day before irrigation.

Phosphate dissolving bacteria (PDB): Phosphate dissolving bacteria (PDB) which used in the experiment included by bacterial isolates (*Pseudomonas fluorescens* and *Bacillus megaterium*) were obtained from Laboratory of Phytopathology, Agricultural Botany, Faculty of Agriculture ,Saba Basha, University of Alexandria, Egypt. The PDB was added at the rate of 50 ml/tree (200g powder/100 L water) during the first irrigation in January.

Data recorded:

Fruit yield and some of its physical traits:

At harvest ten random fruits from each treatment were used to determine the average of fruit length (cm), fruit diameter (cm), fresh and dry fruit weight (g) while, fruits number and yield per tree were estimated as the total number and weight (kg) of fruits per tree. Also, Artificial dried in an oven at 70ºC for 48hr was used to determined fruit dry weight (g)

Fruit chemical properties:

Total Soluble solids (TSS): Fruit juice total soluble solids content (TSS) was measured using a hand refractometer, 0-32 scale (ATAGO N-1_E, Japan) and expressed in °Brix after making the temperature correction at 20°C.

Titratable acidity (TA): For juice titratable acidity (TA), an aliquot of fruit juice was taken and titrated against 0.1 N NaOH in the presence of phenolphthalein as an indicator to the end point and expressed as a percentage of citric acid **(Garner *et al.*, 2003).**

Fruit TSS/TA ratio: Fruit TSS/TA ratio was calculated from the values recorded for fruit juice TSS and TA percentages determined.

Total soluble sugar: total sugars percentage was determined according to the method described by **A.O.A.C (1990)**.

Ascorbic acid: samples of fruit juice were used, oxalic acid solution was added to each sample and titrated with 2,6-dichlorophenol-indophenol dye solution and expressed as a milligram of ascorbic acid and was calculated as mg/100 ml of the juice..

Anthocyanins concentration: was determined by using HCl (1.5N) by a spectrophotometer as described in **A.O.A.C (1990).**

Pomegranate leaves chemical constituents:

Leaf and fruit minerals contents were determined macro nutrients. The leaves were dried at 70°C for 48 hours **(Steyn, 1959)**, then grounded in a mill. Powder of plant material was wet -digested with H_2SO_4-H_2O_2 **(Lowther, 1980)** and the following determinations were carried out in the digested solutions: -

- Total N was determined calorimetrically by Nessler method **(Chapman and Pratt, 1978)**.

-Total P was determined using vanadomoly bdophosphoric method **(Jackson, 1967)**.

-Total K was determined using Flame Spectrophotometer **(Jackson, 1967)**.

- Available phosphorus was extracted from the soil samples at harvest time by the sodium bicarbonate (0.5 N) method according to **Olsen et al, (1954)** and determined by the ascorbic acid – molybdenum blue method at wavelength of 406 nm as described by **Murphy and Riley (1962)**.

Statistical Analysis: All collected data were subjected to three ways ANOVA according to **Gomez and Gomez (1984)** then means were compared using lest significant difference (LSD) at 5% level of probability.

RESULTS AND DISCUSSION

-Effect of pomegranate cultivars, phosphorus levels and bio-fertilizers on pomegranate fruit yield and some fruit physical properties.

The presented data in Table 2 confirmed that all pomegranate fruit physical properties as well as fruit yield and yield components significantly affected by cultivars, phosphorus levels and bio-fertilizers.

1. **Cultivars effect:**

Results in table 2 illustrated the significantly differ among the three pomegranate cultivars in fruit physical properties and fruit yield tree^{-1}. These differ cleared the wide genetic diversity and country of origin of the three tested cultivars. Also, the data showed that Wonderful cv. had the highest fruit length (9.79cm), fruit diameter (12.16 cm), fresh fruit weight (435.68 g), dry fruit weight (117.63 g), fruit number tree^{-1} (24.21) and fruit yield tree^{-1} (10.95 kg). On the other side Manfaloty cv showed the lowest fruit length (7.42 cm) and fruit diameter (10.74 cm) while, H116 cv expressed the lowest values for fresh fruit weight (268.92 g), dry fruit weight (72.61 g), fruit number tree^{-1} (21.29) and fruit yield tree^{-1} (5.93 kg). The results of this study showed a significant difference in the fruit yield as well as fruit physical and chemical characteristics of the three pomegranate cultivars, which was clearly demonstrated by the superiority of Wonderful cultivar over H116 and Manfalouty in all traits. The variation in traits between pomegranate cultivars is mainly due to genetic variation in terms of the pedigree and country of origin. These differences were the subject of great research by many researchers such as **Haleem et al. (2020)** who found a significant difference in fruit yield of the three pomegranate cultivars: Manfalouty, Nab - Elgamal, Wonderful and Wardi. They also confirmed the superiority of Wonderful and Nab-Elgamal in all characteristics of yield and quality of fruits.

2. **Phosphorus levels effect:**

Data in table 2 confirmed that pomegranate fruit physical properties and fruit yield gradually increased with the increase of phosphorus levels. Pomegranate tree the fertilized with the recommended dose of phosphorus (P2) gave the highest fruit length (10.64 cm), fruit diameter (13.60 cm), fresh fruit weight (415.40 g), dry fruit weight (112.16 g), fruit number tree^{-1} (27.44) and fruit yield tree^{-1} (11.78 kg). in the contrast of this, pomegranate tree under the control treatment (P0) had the lowest fruit length (7.11 cm), fruit diameter (9.62 cm), fresh fruit weight (292.77 g), dry fruit weight (75.67 g), fruit number tree^{-1} (19.12) and fruit yield tree^{-1} (5.82 kg).

Our findings revealed that the recommended phosphorus level resulted in significant increase in pomegranate tree yield compared with the low phosphorus level. Plants growth and yield is highly influenced by presences and absences of P **(Hussain et al .**

2006) where Phosphorus plays vital role in major process of plant like photosynthesis, cell division and nucleus formation **(Ayub et al ., 2002)**. P is needed for utilization of starch and sugar where reproduction and growth energy is stored in phosphate compounds after photosynthesis. **Ali et al . (2002)** revealed that P can easily transport within plant form tissues of older age to younger through cell of root, stem or leaves.

3. **Bio-fertilizers effect:**

The obtained results in Table 2 revealed that all used bio-fertilizers significantly exceeded the control treatment in increase pomegranate fruit physical properties and fruit yield. Applied Mycorrhizal in combined phosphorus dissolved bacteria showed the highest fruit length (9.82 cm), fruit diameter (12.66 cm), fresh fruit weight (392.97 g), dry fruit weight (106.10 g), fruit number tree^{-1} (26.18) and fruit yield tree^{-1} (10.83 kg) followed by the individual treatments of PDB then Mycorrhizal in all previous traits. On the other hand pomegranate trees under the control treatment had the lowest fruit length (67.95 cm), fruit diameter (9.77 cm), fresh fruit weight (304.73 g), dry fruit weight (82.28 g), fruit number tree^{-1} (19.84) and fruit yield tree^{-1} (6.47 kg). **Abou El Seoud (2008)** found that tagetes plants which treated with AM fungi strains achieved more than 80% of its maximum yield at the lower level of P in soil.

-Interactions effects:

The results presented in Table 2 showed that there was no significant effect for the interactions of cultivar x phosphorous levels, cultivars x bio fertilizers, phosphorous levels x bio fertilizers and cultivars x phosphorous levels x bio fertilizers on all yield and components traits in addition to the physical properties of pomegranate fruits except cultivar x phosphorous levels for the fruit yield Tree^{-1}. The insignificant effects of the interaction between the three factors in the study is a clear evidence of the independent effect of these factors, which means a positive effect of adding each factor individually on these traits. However, the response of pomegranate cultivar Wonderful to all levels of phosphorus in the presence of mycorrhizal and phosphorous-dissolving bacteria was higher than the other two cultivars. Wonderful fertilized with the full dose of phosphorous in the presence of the combined bio-fertilizer treatment mycorrhizal + PDB gave the highest values for fruit length (13.94 cm), fruit diameter (16.50 cm), fresh fruit weight (550.00 g), dry fruit weight (148.50 gm), number of fruits/tree (30.56) and fruits yield tree^{-1} (17.30 kg).

Our results confirmed that inoculating pomegranate tree with A. Mycorrhizal in combined with PDB resulted in significant increase in fruit yield. PDB works to dissolve phosphorous and make it suitable and easy for plants to absorb. However, plants growing in calcareous soils face a problem in accessing to the available phosphorous in the soil due to the weak growth of roots and the lack of their spread in the soil due to the presence of hard scales of calcium carbonate in the surface layer, but in the presence of Mycorrhizal The problem of poor root growth can be overcome, as the fungal growths on the roots of the plant play a mediating role in transferring the phosphorous available in the soil area surrounding the root to the inside of the plant. the effect bio-fertilizers on plant growth and yield were described by many authors before such as; **Abd Ella (2006)** who found that microbial bio-fertilizers significantly increased pomegranate yield parameters (number and weight of fruit). 100% NPK + arbuscular mycorrhizal and 75% NPK + arbuscular mycorrhizal treatments recorded the highest fruit weight and number per tree. At the same time, 75% NPK + arbuscular mycorrhizal recorded the highest average fruit weight, length and width. Similar results were obtained by **Suri and Choudhary, (2010)** who indicated that seed inoculation with mycorrhizal fungi combined with PSB in integration with 50 or 75% from recommended rate in alkaline soils increased yield and yield components of wheat by 15.7% compared with control. **Lone et al. (2011)** found that PSB (*Bacillus spp*) with 60 kg/ha phosphate significantly improve yield and yield components of rice compared with the control. **Panhwar et al. (2011)** reported that application PDB and mycorrhizal fungi with 45 P 2 O5/ha. increased yield and yield component s of wheat in semi and tropical soils. While, **Zaefarian et al. (2011)** found that application of four mycorrhizal strains included of (*Glomus mosseae*, *G. etanicatum*, *G. intraradices* and mixed strains (combination of *Glomus mosseae*, *G. igaspora hartiga* and G. *fasciculatum*) improved grain yield of barley. Also,

Table 2: Effect of pomegranate cultivars, phosphorus levels and bio-fertilizers types on pomegranate fruit yield and some fruit physical properties.

Factors	Fruit	fruit	Fresh	Dry fruit	Number	fruit
Cultivars						
Wonderful	9.79 a	12.16 a	435.68 a	117.63 a	24.21 a	10.95 a
H116	8.18 b	10.96 b	268.92 c	72.61 c	21.29 b	5.93 b
Manfaloty	7.42 c	10.74 b	329.99 b	89.10 b	22.37 ab	7.92 b
LSD 5%	**0.21**	**0.82**	**55.67**	**15.03**	**2.72**	**2.66**
P levels						
P0	7.11 c	9.62 c	292.77 c	75.67 c	19.12 c	5.82 c
P1	7.64 b	10.64 b	326.42 b	88.13 b	21.31 b	7.20 b
P2	10.64 a	13.60 a	415.40 a	112.16 a	27.44 a	11.78 a
LSD 5%	***0.41***	***0.79***	***25.14***	**6.79**	***1.33***	***1.06***
Bio- fertilizers						
Control	6.95 c	9.77 c	304.73 c	82.28 c	19.84 c	6.46 c
M	8.47 b	11.07 b	339.83 b	91.35 b	22.18 b	7.82 b
PDB	8.61 b	11.64 b	341.91 b	92.72 b	22.28 b	7.95 b
M +PDB	9.82 a	12.66 a	392.97 a	106.10 a	26.18 a	10.83 a
LSD 5%	***0.43***	***0.70***	***25.24***	**6.83**	***1.53***	***1.22***
LSD 5% Cult x P levels	**0.58**	**1.12**	**35.61**	**10.42**	**1.88**	**1.50 ***
LSD 5% Cults x Bio-ferti.	**0.62**	**1.02**	**36.44**	**12.85**	**2.21**	**1.76**
LSD 5% P levels x Bio-ferti.	**0.62**	**1.02**	**36.44**	**12.85**	**2.21**	**1.76**
LSD 5% Cult. x P. levels x Bio-	**1.07**	**1.76**	**63.12**	**17.09 (ns**	**3.83**	**3.05**

[M= Mycorrhizal, B= Phosphate dissolved Bactria]

Effect of pomegranate cultivars, phosphorous levels and bio-fertilizers on some chemical properties of pomegranate fruits.

It was clear from the results presented in Table 3 that all chemical properties of pomegranate fruits significant affected by cultivars, phosphorous levels and bio-fertilization.

1. The effect of cultivars:

The results in Table 3 confirmed the presence of significant difference in pomegranate fruits chemical properties in the three pomegranate cultivars. Wonderful cv. had the highest fruit contents of acidity (1.38%), total sugars (127.96 mg/g), vitamin C (0.12 mg/g) and anthocyanin (0.46 mg/g). Whereas, cultivar H116 fruits had the highest total soluble solids content (11.41%) and fruit ripening (TSS/Acidity) (12.08). The results also showed that fruit ripening of H116 cv. did not differ significantly with that of Manfaloty cv. The results of this study showed a significant difference in fruit physical and chemical characteristics of the three pomegranate cultivars, which was clearly demonstrated by the superiority of Wonderful cultivar over H116 and Manfalouty in all traits. The variation in traits between pomegranate cultivars is mainly due to genetic variation in terms of the pedigree and country of origin. These differences were the subject of great research by many researchers such as **Haleem et al. (2020)** who found a significant difference in fruit physical and chemical properties of the three pomegranate cultivars: Manfalouty, Nab - Elgamal, Wonderful and Wardi. They also confirmed the superiority of Wonderful and Nab-Elgamal in all characteristics of quality of fruits. Also, **Hegazi et al., (2014)** recorded the highest values of SSC, total acidity and SSC/acid ratio Wonderful. While, the highest anthocyanin content in juice and peel were recorded in Manfalouty.

2. Effect of phosphorus levels:

Results in Table 3 showed a significant increase in all fruit chemical properties with the increase of phosphorus level except TSS and fruits ripening (TSS/TA). In general, pomegranate trees that fertilized with the full dose of phosphorus expressed the highest fruit contents of acidity (1.46%), total sugars (155.03 mg), vitamin C (0.15 mg / g) and fruit content of anthocyanin (0. 55 mg / g). On the other side, pomegranate trees under the control treatment gave of the lowest fruit content of TSS (12.75%) as well as the highest TSS/acidity ratio (14.50).

Our findings revealed that the recommended phosphorus level resulted in significant increase in pomegranate improve all fruit physical properties (fruit length, diameter and weight) as well as fruit chemical properties (Acidity, total sugar, ascorbic acid and anthocyanin) compared with the low phosphorus level. Plants growth and yield is highly influenced by presences and absences of P **(Hussain et al . 2006)** where Phosphorus plays vital role in major process of plant like photosynthesis, cell division and nucleus formation **(Ayub et al ., 2002)**. P is needed for utilization of starch and sugar where reproduction and growth energy is stored in phosphate compounds after photosynthesis. **Ali et al . (2002)** revealed that P can easily transport within plant form tissues of older age to younger through cell of root, stem or leaves. The affected representation and construction process in plant on food representation affects the quality of fruits, physical and chemical properties as well as their ability to storage.

3. Effect of bio-fertilizers:

The bio-fertilization of Pomegranate trees with mycorrhizal, PDB or both led significantly improve all fruits chemical properties, where these treatments led to an increase in acidity, total sugars, vitamin C and fruits content of Anthocyanin (Table 3). These treatments also resulted in desirable decrease in fruit content of TSS as well as fruit repining compared to control. Pomegranate trees that treated with mycorrhizal + PDB had the highest fruit contents of acidity (1.32%), total sugars (143.06 mg / g), vitamin C (0.14 mg / g) and anthocyanin (0. 50 mg / g) In addition to the lowest fruit content of TSS (9.40%) as well as less rate for fruit ripening (8.13%).

-Interactions effects:

Data in Table 3 confirmed the presence highly significant effect for the interactions of cultivar x phosphorous levels, cultivars x bio fertilizers, phosphorous levels x bio fertilizers and cultivars x phosphorous levels x bio fertilizers on all pomegranate fruit chemical properties except cultivar x bio-fertilizers for fruit contents of TSS and ascorbic acid. The significant effects of the interaction between the three factors in the study consider clear evidence about the wide diversity among the three pomegranate cultivars and these cultivars will differ in their response to both phosphorus levels and bio-fertilizers. Also, these significant refer to the effect three bio-fertilizers on pomegranate fruit chemical properties will differ according to phosphorus levels. The response of pomegranate cultivar Wonderful to all levels of phosphorus in the presence of mycorrhizal and phosphorous-dissolving bacteria was higher than the other two cultivars. Wonderful fertilized with the full dose of phosphorous in the presence of the combined bio-fertilizer treatment mycorrhizal + PDB had the highest fruit contents of acidity (1.98%), total sugar (215.93 mg/g), ascorbic acid (0.19 mg/g) anthocyanin (0.82 mg/g) in addition to the lowest TSS (7.27%) and TSS/acidity ratio (3.67).

Our results cleared that inoculating pomegranate tree with A. Mycorrhizal in combined with PDB resulted in significant increase. The effect bio-fertilizers on plant growth and yield were described by many authors before such as; **Abd Ella (2006)** who found that microbial biofertilizers significantly increased pomegranate yield parameters (number and weight) and fruit quality. 75% NPK + arbuscular mycorrhizal recorded the highest average fruit weight, length and width; moreover, it significantly improved TSS %, acidity % and anthocyanin content of fruit juice at harvest time in both seasons. Also, 75% NPK + arbuscular mycorrhizal improved V.C content and decreased tannins % of fruit juice without significant difference in comparison with control. Similar results were obtained by **Suri and Choudhary, (2010)** who indicated that seed inoculation with mycorrhizal fungi combined with PSB in integration with 50 or 75% from recommended rate in alkaline soils increased yield and yield quality of wheat compared with control. **Panhwar et al. (2011)** reported that application PDB and mycorrhizal fungi with 45 P 2 O5/ha. increased yield and yield quality of wheat in semi and tropical soils.

Table 3: Effect of pomegranate cultivars, phosphorous levels and bio-fertilizers on some chemical properties of pomegranate fruits.

Factors	TSS%	Acidit	TSS/Acidi	Total	VC	Anthocyan
Cultivars						
Wonderful	10.94 b	1.38 a	8.71 b	127.9	0.12 a	0.46 a
H116	11.41 a	1.03 b	12.08 a	113.1	0.11 b	0.43 b
Manfaloty	10.49 c	0.96 c	12.08 a	113.1	0.11 b	0.32 c
LSD 5%	**0.23**	**0.02**	**0.25**	**0.61**	**0.01**	**0.02**
P levels						
P0	12.75 a	0.91 c	14.50 a	95.07	0.10 b	0.31 c
P1	11.98 b	1.00 b	12.44 b	104.1	0.10 b	0.35 b
P2	8.11 c	1.46 a	5.93 c	155.0	0.15 a	0.55 a
LSD 5%	***0.06***	***0.02***	***0.19***	***0.34***	***0.01***	***0.01***
Bio-fertilizers						
Control	12.61 a	0.96 d	14.19 a	95.44	0.09 d	0.31 d
M	10.93 b	1.07 c	10.99 b	113.2	0.11 c	0.39 c
PDB	10.85 b	1.14 b	10.51 c	120.6	0.12 b	0.41 b
M +PDB	9.40 c	1.32 a	8.13 d	143.0	0.14 a	0.50 a
LSD 5%	***0.12***	***0.02***	***0.26***	***0.32***	***0.01***	***0.01***
LSD 5% Cult x P levels	**0.09****	**0.03****	**0.27****	**0.49***	**0.002***	**0.01****
LSD 5% Cults x Bio-ferti.	**0.17(n**	**0.03****	**0.38****	**0.46***	**0.003(n**	**0.01****
LSD 5% P levels x Bio-ferti.	**0.17 ****	**0.03****	**0.38****	**0.46***	**0.003****	**0.01****
LSD 5% Cult. x P. levels x Bio-	**0.29****	**0.04****	**0.65***	**0.79***	**0.01(ns)**	**0.01****

[M= Mycorrhizal, B= Phosphate dissolved Bactria]

-Effect of phosphorous levels and bio-fertilizers on soil of available phosphorus.

Data in table 3 and fig. 1 confirmed that phosphorus availability in soil gradually increased with the increase of phosphorus application rate under both presence and absence bio-fertilizers. Compared the low P fertilizer P0 and P1, low significant increase in available P was observed while, the high P fertilizer level (P2) resulted in highly significant increase in available P in soil. Similar results were obtained before by Similarly, **Abou- El -Seoud et al., (2017) and (2020)** who found that the available P in soil gradually increased with the increase of soil application with phosphorus.

The presented results in Table 3 and Fig. 1 cleared that inoculation pomegranate mycorrhizal or treated the root zone soil with phosphate dissolved bacteria led to large increase in available P in the root zone in the presence or absence of phosphorus application. In all cases the combined treatment mycorrhizal + phosphate dissolved bacteria exceeded the individual treatments of mycorrhizal and PDB as well as the control treatments in improve available phosphorus in the soil. At the low phosphorus application levels all used treatments showed low significant effect on available phosphorus while, highly significant differences were showed among all used treatments under high P level. In the previous study of **Abou-El - Seoud (1998)**, **Abou- El -Seoud et al., (2017)** and **Abou- El -Seoud et al., (2020)** it was found that available P significantly increased as a result of inoculation cotton, wheat, squash and tomato plants with AMF, that could be due to the hyphae of the A- mycorrhizal fungi produce organic acids and phosphates which catalyze the release of P from organic complexes which tend to improve the available P in soil **(Aono et al.,2004)**. In addition, in alkaline soils, mycorrhizal hyphae lead to decrease in alkalinity of the rhizosphere soil from 8.5 to 7.4 by organic acids exudation, which due to solubilizing immobile elements such as P **(Giri et al.,2005)**.

Table 3: Effect of phosphorous levels and biofertilization on soil content of available phosphorus.

P levels	P0	P1	P2
Control	8.31 d	10.44 d	15.72 d
M	11.19 b	13.24 b	17.76 c
B	12.24 b	14.54 b	18.09 b
M +B	15.27 a	18.51 a	27.58 a

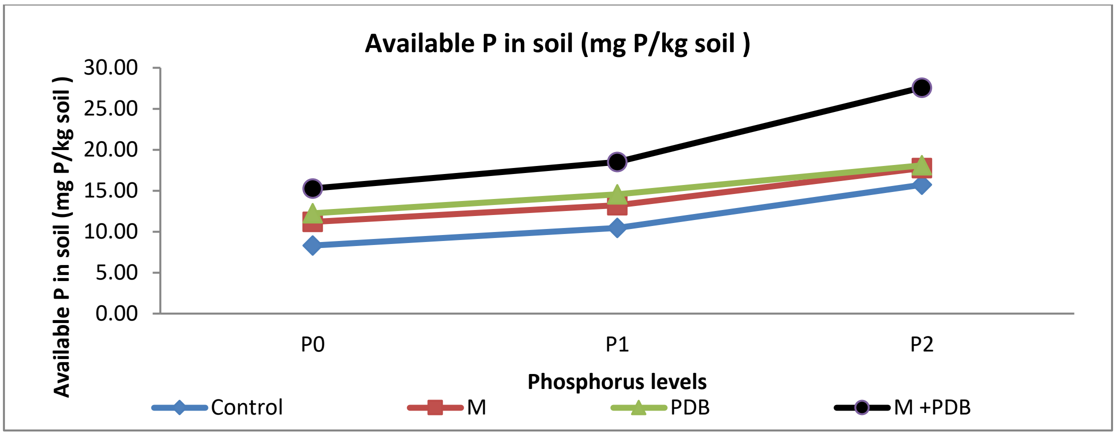

Fig. 1: Effect of phosphorous levels and bio-fertilizers on soil content of total and phosphorus. **[M= Mycorrhizal, B= Phosphate dissolved Bactria]**

-Effect of pomegranate cultivars, phosphorous levels and bio-fertilizers on pomegranate leaf, fruit and flesh contents of N, P and K.

It was clear from the obtained results in Table 4 that pomegranate leaves, fruits and flesh contents of N, P and K significant affected by cultivars, phosphorous levels and bio-fertilization.

1. The effect of cultivars:

The results in Table 3 confirmed that Wonderful cv. had the highest N (20.12, 15.93 and 11.25 mg g^{-1}), P (3.34, 2.64 and 1.85 mg g^{-1}) and K (15.67, 12.59 and 8.76 mg g^{-1}) contents of in leaf, fruit and flesh, respectively followed by H116 then Manfaloty. These differences were the subject of great research by many researchers such as **Haleem et al. (2020)** who found a significant difference in fruit physical and chemical properties of the three pomegranate cultivars: Manfalouty, Nab - Elgamal, Wonderful and Wardi. They also confirmed the superiority of Wonderful and Nab-Elgamal in all characteristics of quality of fruits.

2. Effect of phosphorus levels:

Results in Table 4 showed the increase of phosphorus fertilization rate resulted in gradually increase in nitrogen, phosphorus and potassium in pomegranate leaf, fruit and flesh. In general, pomegranate trees that fertilized with the full dose of phosphorus expressed the highest N (22.56, 18.00 and 12.30 mg g^{-1}), P (3.56, 2.88 and 1.99 mg g^{-1}) and P (17.84, 13.82 and 9.97 mg g^{-1}) contents in leaves, fruit and flesh. On the other side, pomegranate trees under the control treatment gave of the lowest fruit content of N, P and K in leaves, fruit and flesh.

Our findings revealed that the recommended phosphorus level resulted in significant increase in pomegranate improve all fruit physical properties (fruit length, diameter and weight) as well as fruit chemical properties (Acidity, total sugar, ascorbic acid and anthocyanin) compared with the low phosphorus level. Plants chemical statues highly influenced by presences and absences of P **(Hussain et al . 2006)** where Phosphorus plays vital role in major process of plant like photosynthesis and nucleus formation **(Ayub et al ., 2002)**. P is needed for utilization of starch and sugar where reproduction and growth energy is stored in phosphate compounds after photosynthesis.

3. Effect of bio-fertilizers:

The bio-fertilization of Pomegranate trees with AMF, PDB or both led significantly improve all fruits chemical properties, where these treatments led to an increase in leaf contents of N, P and K (Table 4). Pomegranate trees that treated with AMF + PDB had the highest N (20.56, 16.63 and 11.47 mg g^{-1}), P (3.28, 2.66 and 1.84 mg g^{-1}) and P (16.32, 13.37 and 9.19 mg g^{-1}).

-Interactions effects:

Data in Table 4 confirmed the presence highly significant effect for the interactions of cultivar x phosphorous levels, cultivars x bio fertilizers and phosphorous levels x bio fertilizers in in leaf, fruit and flesh contents of N, P and K except cultivar x P levels for leaf and fruit contents of K, cultivar x bio-fertilizers for fruit content of N and K as well as leaf content of K and P levels x bio-fertilizers for fruit content of N and K and flesh content of P. The significant effects of the interaction between the three factors in the study consider clear evidence about the wide diversity among the three pomegranate cultivars and these cultivars will differ in their response to both phosphorus levels and bio-fertilizers. Also, these significant refer to the effect three bio-fertilizers on pomegranate fruit chemical properties will differ according to phosphorus levels. The response of pomegranate cultivar Wonderful to all levels of phosphorus in the presence of AMF and PDB was higher than the other two cultivars. Wonderful fertilized with the full dose of phosphorous in the presence of the combined bio-fertilizer treatment AMF + PDB had the highest N(28.83, 17.23 and 16.13 mg g^{-1}), P(4.97, 2.77 and 2.80 mg g^{-1}) and K (22.23, 13.20

and 12.40 mg g^{-1}) contents in leaves, fruit and flesh. In the previous study of **Abou-El - Seoud (1998)**, **Abou- El -Seoud et al., (2017)** and **Abou- El -Seoud et al., (2020)** it was found that available P significantly increased as a result of inoculation cotton, wheat, squash and tomato plants with AMF, that could be due to the hyphae of the AMF fungi produce organic acids and phosphates which catalyze the release of P from organic complexes which tend to improve the available P in soil **(Aono et al.,2004)**. In addition, in alkaline soils, AMF hyphae lead to decrease in alkalinity of the rhizosphere soil from 8.5 to 7.4 by organic acids exudation, which due to solubilizing immobile elements such as P **(Giri et al.,2005)**.

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Table 4: Effect of phosphorous levels and biofertilization on pomegranate tree content of available phosphorus.

Factors		N content (mg/g) in			P content (mg/g) in			K content (mg/g) in		
		Leaf	**Fruit**	**Flesh**	**Leaf**	**Fruit**	**Flesh**	**Leaf**	**Fruit**	**Flesh**
Cultivars										
	Wonderful	20.12 a	15.93 a	11.25 a	3.34 a	2.64 a	1.85 a	15.67 a	12.59 a	8.76 a
	H116	17.32 b	13.83 b	9.50 b	2.76 b	2.24 b	1.52 b	13.94 b	11.54 b	7.79 b
	Manfaloty	15.16 c	12.97 b	8.53 c	2.19 c	1.82 c	1.22 c	12.99 c	8.79 c	7.27 c
	LSD 5%	**0.36**	**1.08**	**0.47**	**0.29**	**0.21**	**0.17**	**0.71**	**0.93**	**0.24**
P levels										
	P0	14.44 c	11.72 c	8.05 c	2.26 c	1.83 c	1.25 c	11.81 c	9.08 c	6.58 c
	P1	16.09 b	13.01 b	8.93 b	2.46 b	1.98 b	1.36 b	12.94 b	10.02 b	7.27 b
	P2	22.07 a	18.00 a	12.30 a	3.56 a	2.88 a	1.99 a	17.84 a	13.82 a	9.97 a
	LSD 5%	***0.07***	***0.23***	***0.08***	***0.1***	***0.07***	***0.05***	***0.37***	***0.38***	***0.08***
Bio-fertilizers										
	Control	14.75 d	12.01 d	8.20 d	2.26 d	1.80 d	1.24 d	12.36 c	9.99 c	6.80 d
	M	17.28 c	14.00 c	9.61 c	2.69 c	2.18 c	1.49 c	14.21 b	11.56 b	7.93 b
	B	17.53 b	14.34 b	9.75 b	2.82 b	2.28 b	1.55 b	13.89 b	11.70 b	7.84 c
	M +B	20.56 a	16.63 a	11.47 a	3.28 a	2.66 a	1.84 a	16.32 a	13.37 a	9.19 a
	LSD 5%	***0.03***	***0.33***	***0.1***	***0.06***	***0.05***	***0.05***	***0.42***	***0.4***	***0.08***
Significant	***Cult x P levels***	**0.10 ***	**0.32 ****	**0.12 ****	**0.14 ***	**0.10 ****	**0.08 ***	**0.53 (ns)**	**0.54 (ns)**	**0.11 ****
	Cults x Bio-ferti.	**0.04 ****	**0.48 (ns)**	**0.15 ****	**0.09 ****	**0.07 ****	**0.07 ***	**0.61 (ns)**	**0.58 (ns)**	**0.11 ***
	P levels x Bio-ferti.	**0.04 ****	**0.48 (ns)**	**0.15****	**0.09 ****	**0.07 ****	**0.07 (ns)**	**0.61 ***	**0.58 (ns)**	**0.11 ****
	Cult. x P. levels x Bio-ferti.	**0.07 (ns)**	**0.83 (ns)**	**0.26 (ns)**	**0.15 (ns)**	**0.12 (ns)**	**0.13 (ns)**	**1.06 (ns)**	**1.01 (ns)**	**0.20 (ns)**

[M= Mycorrhizal, B= Phosphate dissolved Bactria]

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الملخص العربي

كفاءة الفسفور لبعض أصناف الرمان الملقحة بفطريات الميكوريزا والبكتيريا المذيبة للفوسفات تحت ظروف التربة الجيرية

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[2]مديرية الزراعة بدمنهور – وزارة الزراعة واستصلاح الأراضي مصر

أجريت هذه الدراسة لتقييم تأثير فطر الميكوريزا الداخلية AMF والبكتيريا المذيبة للفوسفور (PDB) حيث تمت إضافة التلقيح الحيوي تحت ثلاثة معدلات سماد فوسفاتي قبل الزراعة ، على محصول وجودة الثمار لثلاثة أصناف من الرمان. Wonderful، H116 and Manfaloy تحت ظروف الأرض الجيرية. أكدت النتائج المتحصل عليها أن جميع الخواص الفيزيائية والكيميائية لثمار الرمان وكذلك محصول الثمار ومكوناتها تأثرت معنويا بالصنف ومستويات الفسفور والتسميد الحيوي. بالإضافة إلى أقل نسبة من المواد الصلبة الذائبة والمواد الصلبة المذابة / الحموضة. أشجار الرمان التي تم معاملتها بالجرعة الكاملة من الفوسفور معبرة عن أعلى طول للفاكهة لنمو النبات ، قطر الثمار ، وزن الفاكهة الطازجة ، وزن الثمرة الجافة ، رقم شجرة الفاكهة $^{-1}$ ، شجرة محصول الفاكهة $^{-1}$ ، محتوى الفاكهة من الحموضة ، السكريات الكلية ، فيتامين ج ومحتوى الفاكهة من الأنثوسيانين. أظهر AMF المطبق مع PDB أعلى طول للفاكهة ، قطر الثمرة ، وزن الفاكهة الطازجة ، وزن الثمرة الجافة ، رقم شجرة الفاكهة $^{-1}$ ، شجرة محصول الفاكهة $^{-1}$ ، الحموضة ، السكريات الكلية ، فيتامين ج والأنثوسيانين بالإضافة إلى الفاكهة الأقل محتوى المواد الصلبة الذائبة وكذلك أقل معدل نضج الثمار. المعاملة بجرعة كاملة من الفوسفور في وجود معاملة التسميد الحيوي المركب AMF + PDB أعطى أعلى محتوى من الفاكهة من الحموضة والسكر الكلي وحمض الأسكوربيك الأنثوسيانين بالإضافة إلى أدنى نسبة TSS و TSS / الحموضة. أكدت النتائج أن استخدام AMF + PDB أدى إلى زيادة كبيرة في توافر الفسفور في التربة تحت جميع مستويات الفوسفور.