American-Eurasian J. Agric. & Environ. Sci., 21 (2): 126-139, 2021
ISSN 1818-6769

DOI: 10.5829/idosi.aejaes.2021.126.139

Role of Arbuscular Mycorrhizal Fungi and Phosphate-Solubilizing Bacteria in Improving Rock-Phosphate Availability for Superior Grapevines

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Abstract: This trial was performed in a private vineyard located at 58 km on the Cairo-Alexandria desert road for three consecutive seasons (2017, 2018 & 2019), to study the possibility of improving the availability of rock-phosphate by using the inoculation with arbuscular mycorrhizal fungi and phosphate-solubilizing bacteria of Superior grapevines. The chosen vines were twelve years old, spaced 2 X 3 meters apart, grown in sandy loam soil and irrigated by the drip irrigation system. The vines were trellised using the Spanish Parron pattern and cane-pruned during the first week of January to maintain a load of 120 buds/vine. Calcium super phosphate (15.5% P_2O_5) was added as a source of mineral phosphate fertilizer (MP) (control) at the rate of 30 units phosphorus/Feddan during the second week of January. Rock-phosphate (RP) (20.4% P_2O_5) was applied to the equivalent of the mineral phosphate fertilizer based on its phosphorous content, once during winter service at the second week of January. Arbuscular mycorrhizal fungi (AMF) as well as a strain of phosphate solubilizing bacteria (*Bacillus megaterium*) (PSB) were used as bio-inoculants. AMF was applied at the rate of 250g inoculum /vine once during winter service at the second week of January. On the other hand, PSB was applied at the rate of 20cm or 40cm/vine twice to the soil amended with rock-phosphate: the first addition was added during winter service and the other 35 days after the first addition. Six treatments were conducted as follows: MP (control), RP + AMF, RP + PSB at 20cm/vine, RP + PSB at 40cm/vine, RP + AMF + PSB at 20cm/vine and RP + AMF + PSB at 40cm/vine. The results showed that dual inoculation arbuscular mycorrhizal fungi plus phosphate solubilizing bacteria at 40cm/vine in soil amended with rock-phosphate resulted in the best vegetative growth aspects, increased leaf content of total chlorophyll and percentages of total nitrogen, phosphorus and potassium and cane content of total carbohydrates, ensured the highest yield and good components and improved bunch quality attributes namely the physical and chemical characteristics of berries as well as increased the microbiological and enzyme activity in the rhizosphere including populations of total microorganism, total count of phosphate solubilizing bacteria, the percentage of AMF infection, dehydrogenase and alkaline phosphatase enzyme activity in the rhizosphere of Superior grapevines.

Key words: Rock-phosphate · Arbuscular mycorrhizal fungi · *Phosphate solubilizing bacteria* · Yield · Grapevines

INTRODUCTION

Phosphorous (P) is one of the most important macronutrients for plant growth and development. It plays a role in critical plant metabolic activities as cellular energy storage, photosynthesis and respiration [1]. However, fruit tree output is mostly reliant on synthetic fertilizers as a supply of plant nutrients. This approach not only increases production costs, but it also has the potential to pollute the environment. Because of the

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significant absorption to and fixation by metal oxides in the soil matrix, the application of phosphorus (P) fertilizer is similarly inefficient. As a result, soil P exists in insoluble, immobilized and/or precipitated forms that plants cannot use right away; this requires the need to find more efficient alternative sources of P, safer and more environmentally friendly [2]. As a consequence, the use of rock-phosphate as a fertilizer for phosphorus-deficient soils has piqued attention in recent years, owing to the fact that it is a natural, affordable and readily available fertilizer [3].

The fundamental goal of soil phosphorus management right now is to maximize agricultural productivity while minimizing phosphorus losses in soils through huge microbial populations that facilitate weathering of insoluble P compounds that can be solubilized by several mechanisms i.e. lowering pH by producing organic acids, phenolic compounds, phosphatase enzymes and complicating factors produced by soil microorganisms [4]. Among microbial groups that can solubilize mineral phosphates, allowing the sustainable use of phosphate fertilizers and improving plant phosphorus nutrition are arbuscular mycorrhizal fungi (AMF) and phosphate solubilizing bacteria (PSB) [5].

Arbuscular mycorrhizal fungi (AMF) are a type of soil microbe that forms symbiotic relationships with a wide range of plant species. AMF can help improve plant P nutrition and the nutritional status of other poorly mobile nutrients by releasing organic acid to solubilize less soluble phosphate [6].

Phosphate-dissolving bacteria (PSB) are a group of soil organisms common to the root zone of many plants. This group is known for secreting organic acids and phosphatases to convert insoluble phosphate to soluble forms [7].

Interactions between AMF and PSB have often been described as synergistic, dual inoculation of AMF and PSB could improve plant P acquisition, stimulated plant growth and increased yield to a greater extent than inoculation with any of the microorganisms alone. PSB solubilize P and enhance P availability, which is then taken up by the AMF and supplied to the plant, according to one possible mechanism for interacting (synergistic) effects [8].

The principal goal of this investigation is to estimate the validity and efficiency of arbuscular mycorrhizal fungi (AMF) and phosphate solubilizing bacteria (PSB) inoculations in soil amended with rock-phosphate on phosphorus-releasing capacity in improving soil properties and increasing yield and fruit quality attributes of Superior grapevines.

MATERIALS AND METHODS

This trial was performed in a private vineyard located at 58 km on the Cairo-Alexandria desert road for three consecutive seasons (2017, 2018 & 2019), to study the possibility of improving the availability of rock-phosphate by using the inoculation with arbuscular mycorrhizal fungi and phosphate-solubilizing bacteria of Superior grapevines.

The chosen vines were twelve years old, spaced 2 X 3 meters apart, grown in sandy loam soil and irrigated by the drip irrigation system. The vines were trellised using the Spanish Parron pattern and cane-pruned during the first week of January to maintain a load of 120 buds/vine.

Soil samples under study were taken from the experimental area before the start of the experiment and in which the physicochemical and microbiological analysis of soil was done as presented in Table (1) according to analysis carried out by soils, water and environment research institute [9].

One hundred and forty-four uniform vines were selected as indirect estimates of vine vigour based on the prunings weight. Each six vines acted as a replicate, with each of the four replicates were treated by one of the following treatments:

- Mineral phosphate (control)
- Rock-phosphate (RP) + arbuscular mycorrhizal fungi (AMF)
- RP + phosphate solubilizing bacteria (PSB) at 20cm/vine
- RP + phosphate solubilizing bacteria (PSB) at 40cm/vine
- RP + AMF + PSB at 20cm/vine
- RP + AMF + PSB at 40cm/vine

Calcium superphosphate (15.5% P_2O_5) as a source of mineral phosphate fertilization (control) was added at the rate of 30 units phosphorus/Feddan once during winter service at the second week of January of each season.

Rock-phosphate (20.4% P_2O_5) was purchased from Al-Ahram Company for Natural fertilizers, Giza, Egypt. Rock-phosphate was applied to the equivalent of the mineral phosphate fertilizer based on its phosphorous content, once during winter service at the second week of January.

Table 1: Physical, chemical and microbiological analysis of the soil

Sand (%)	72.9
Silt (%)	2.4
Clay (%)	24.7
Texture	Sandy loam
PH	7.43
EC (ds/m)	1.51
Organic matter (%)	0.62
Available N (mg/kg soil)	113
Available P (mg/kg soil)	6
Available K (mg/kg soil)	65
Total microbial count (cfu/g soil)	22 x 10^5
AMF infection (%)	6.9

Spores of arbuscular mycorrhizal fungi (AMF) were obtained from Egyptian soils. The confused seeds of AMF genera were got ready and used with sand as a transporter (50 spore/g inoculum) and then merged with the soil at a dose (100g inoculum/ m long) so that each 2.5 m vine needs 250 g inoculum/vine once during winter service at the second week of January.

Phosphate solubilizing bacteria (*Bacillus megaterium*) were grown on a medium [10] and enriched on a nutrient growth medium [11]. Phosphate solubilizing bacteria was applied at the rate of 20cm or 40cm/vine twice to the soil amended with rock-phosphate: the first addition was added during winter service and the other 35 days after the first addition.

The considered treatments were evaluated through the following estimations:

Morphological Characteristics of Vegetative Growth: In the first week of June, the following morphological studies were carried out on four fruitful shoots/the conducted vines: the average of shoot length (cm) and diameter, number of leaves/shoot and leaf area (cm^2).

Chemical Characteristics of Vegetative Growth: During the second week of June, leaf samples were obtained from the 5^{th} and 6^{th} apical leaves on the main shoot/vine and the following estimates were determined:

Total chlorophyll: it was determined using the Minolta non-destructive chlorophyll meter SPAD 502 according to Wood *et al.* [12].

Macro-Elements: Nitrogen was estimated using the modified micro-Kjeldahl method according to Pregl [13], phosphorus was calorimetrically measured according to Snell and Snell [14] and potassium was determined according to Jackson [15] using a Flame photometry instrument.

Cane Total Carbohydrates Content: During winter pruning (4^{th} week of January), cane samples are collected in and analyzed according to Smith *et al.* [16].

Yield and Physical Characteristics of Bunch: According to Tourky *et al*. [17], representative random samples of 6 bunches/vine were harvested at maturity when TSS reached about 16-17%.

Yield/vine (kg) was determined by multiplying the number of bunches/vine by the average bunch weight. Average bunch dimensions (cm) were also measured.

Berry Physical Properties: Average berry weight (g), berry size (cm^3) and berry dimensions (length and diameter) (cm) were estimated.

Berry Chemical Properties: Total soluble solids (TSS) using hand refractometer and total titratable acidity as tartaric acid in berry juice were determined [18]. Then the TSS /acid ratio was calculated.

Microbiological Studies

Samples Were Brought for Conducting the Following Estimations: Total microbial count (-x10^5 colony forming unit (cfu)/g soil) was determined as outlined by Esher and Jensen [19].

Total count of phosphate solubilizing bacteria (PSB) (-x10^5 colony forming unit (cfu)/g soil) was determined outlined by Pikovskayas Agar was modified by Sundara Rao and Sinha [20]. PSB will grow on this medium and form a clear zone around the colony, formed due to phosphate solubilization in the vicinity of the colony.

AMF root colonization (%) was determined outlined by Phillips and Hyman [21] colored segments were randomly chosen, microscopically inspected and AMF root colonization rate was estimated according to Trouvelot *et al.* [22].

Enzyme Activities: Dehydrogenase activity (igTPF.g^{-1} dry soil day^{-1}) was determined according to Salman [23].

Phosphatases were measured by using method of Senwo *et al.* [24] where alkaline phosphatase (alPase) activity at pH 11.0.

Experimental Design and Statistical Analysis: For the experiment, the complete randomized block design was performed. According to Snedecor and Cochran [25], the statistical analysis of the present data was performed. Averages were compared using the new LSD values at a 5% level [26].

RESULTS AND DISCUSSION

Morphological Characteristics of Vegetative Growth: As shown in Table (2), all vegetative growth traits expressed as shoot length, shoot diameter, number of leaves/shoot and leaf area were significantly improved by inoculation with arbuscular mycorrhizal fungi (AMF) and/or two different doses of the phosphate-solubilizing bacteria (PSB) in soil amended with rock-phosphate (RP) compared to the control during the three seasons.

Dual inoculation of AMF plus PSB at 40cm/vine attained significantly the highest values of these parameters followed by dual inoculation of AMF plus PSB at 20cm/vine, while control had the lowest values of these ones during the three seasons.

The positive influence of AMF and PSB inoculations in soil amendment with rock-phosphate on vegetative growth parameters could be explained by enzymes produced from AMF that enhance root respiration, improve elements uptake and production of growth-promoting substances and stimulate microbial activity in the root zone [27, 28]. Moreover, growth promotion by PSB may also be related to its ability to produce the hormone, especially IAA [29].

The obtained results are in harmony with those achieved by Abd El-Wahab *et al.* [30]; Rizk-Alla and Tolba [31] and Abd El-Wadoud [32] on some table grape cultivars they showed that AMF enhance vegetative growth.

As for the effect of PSB, Abd El-Wahab *et al.* [33] on Thompson Seedless grapevines, Dakhly and Abada [34] on Superior grapevines and Mekawy and Abd El-Hafeez [35] on Red Globe grapevines pointed out that PSB inoculation increased significantly main shoot length and improved vine vigour in comparison with the non-inoculated vines (control).

Chemical Characteristics of Vegetative Growth: Data presented in Table (3) indicated that inoculation with arbuscular mycorrhizal fungi (AMF) and/or two different doses of the phosphate-solubilizing bacteria (PSB) in soil amended with rock-phosphate (RP) greatly affected leaf content of total chlorophyll and macro-elements expressed total nitrogen, phosphorus and potassium as well as cane content of total carbohydrates during the three seasons.

Concerning leaf content of total chlorophyll, dual inoculation of AMF plus PSB at 40cm/vine achieved significantly the highest values of this estimation followed by dual inoculation of AMF plus PSB at 20cm/vine. On the other hand, the lowest values were obtained from control during the three seasons.

The positive influence of AMF and PSB inoculations in soil amendment with rock-phosphate on chlorophyll index could be explained by increasing photosynthesis in inoculated plants and this could also be attributed to the increased uptake of magnesium and iron, which are essential for chlorophyll biosynthesis rate [36].

The obtained results are in agreement with those achieved by Abd El-Wahab *et al.* [30]; Rizk-Alla and Tolba [31] and Abd El-Wadoud [32] on some table grape cultivars they found that AMF improve leaf chlorophyll content.

As for the effect of PSB, Abd El-Wahab *et al.* [33] on Thompson Seedless grapevines, Dakhly and Abada [34] on Superior grapevines and Mekawy and Abd El-Hafeez [35] on Red Globe grapevines pointed out that PSB inoculation increased significantly the leaf content of total chlorophyll in comparison with the non-inoculated vines (control).

Regarding leaf content of and macro-elements *i.e.* total nitrogen, phosphorus and potassium, increasing N, P and K uptake was obtained from dual inoculation of AMF plus PSB at 40cm/vine followed by dual inoculation of AMF plus PSB at 20cm/vine, whereas control had the lowest values of these ones during the three seasons.

The influence of AMF and PSB on improving nutrient uptake may be due to its solubilizing phosphorus from its rock-phosphate by production of growth promoting materials or organic acids [37].

These findings are supported by other reports showing that acquisition of mineral nutrients particularly P, N and K, was enhanced in grapevines inoculated with AMF [30-32].

As for the effect of PSB, Abd El-Wahab *et al.* [33] on Thompson Seedless grapevines, Dakhly and Abada [34] on Superior grapevines and Mekawy and Abd El-Hafeez [35] on Red Globe grapevines pointed out that PSB inoculation increased significantly the leaf content of nutrients in comparison with the non-inoculated vines (control).

With respect to cane content of total carbohydrates, compared to control, dual inoculation of AMF plus PSB at 40cm/vine had significantly the highest values of this estimation followed by dual inoculation of AMF plus PSB at 20cm/vine during the three seasons.

Table 2: Effect of inoculation with arbuscular mycorrhiza fungi and phosphate solubilizing bacteria in rock phosphate amended soil on vegetative growth characteristics of Superior grapevines during 2017, 2018 and 2019 seasons

	Characteristics											
	Average shoot length (cm)			Average shoot diameter (cm)			Average number of leaves/shoot			Average leaf area (cm^2)		
Treatments	2017	2018	2019	2017	2018	2019	2017	2018	2019	2017	2018	2019
MP (control)	147.2	153.6	158.3	1.39	1.43	1.47	23.7	24.5	25.3	160.4	168.3	172.6
RP + AMF	168.3	176.1	180.4	1.51	1.52	1.58	26.2	27.6	28.3	171.6	180.5	185.1
RP + 20cmPSB	149.6	157.7	161.7	1.43	1.47	1.49	24.2	25.3	25.9	164.9	170.7	177.4
RP + 40cm PSB	162.1	169.9	174.3	1.48	1.49	1.54	25.6	26.9	27.6	167.8	175.1	182.7
RP+AMF+20cmPSB	181.6	189.3	194.3	1.53	1.56	1.60	27.7	29.1	29.9	177.7	184.3	190.4
RP+AMF +40cmPSB	185.9	193.7	199.1	1.57	1.59	1.64	28.3	29.8	30.4	181.3	188.2	193.8
New LSD at 0.05 =	3.7	4.1	4.4	0.03	0.01	0.02	0.5	0.4	0.3	3.4	3.1	2.7

Mineral phosphate (MP) Rock phosphate (RP) Arbuscular mycorrhizal fungi (AMF) Phosphate solubilizing bacteria (PSB)

Table 3: Effect of inoculation with arbuscular mycorrhiza fungi and phosphate solubilizing bacteria in rock phosphate amended soil on leaf content of total chlorophyll and mineral elements and cane content of total carbohydrates of Superior grapevines during 2017, 2018 and 2019 seasons

	Characteristics														
	Total chlorophyll (SPAD)			Nitrogen (%)			Phosphorus (%)			Potassium (%)			Total carbohydrates (%)		
Treatments	2017	2018	2019	2017	2018	2019	2017	2018	2019	2017	2018	2019	2017	2018	2019
MP (control)	30.33	33.26	33.26	1.39	1.47	1.50	0.18	0.23	0.26	0.30	0.34	0.34	24.97	27.87	26.23
RP + AMF	37.76	37.75	38.89	1.57	1.65	1.68	0.25	0.31	0.35	0.36	0.38	0.39	30.73	32.31	33.28
RP + 20cmPSB	33.82	34.94	35.99	1.42	1.47	1.52	0.21	0.24	0.28	0.33	0.35	0.36	27.76	28.47	28.81
RP + 40cm PSB	35.49	36.58	37.67	1.45	1.50	1.55	0.24	0.28	0.32	0.35	0.36	0.38	28.40	29.79	30.76
RP+AMF+20cmPSB	37.91	38.21	39.36	1.60	1.67	1.72	0.28	0.35	0.38	0.37	0.39	0.41	33.24	34.57	35.61
RP+AMF +40cmPSB	38.43	38.78	39.94	1.62	1.70	1.75	0.32	0.38	0.43	0.40	0.41	0.44	36.28	37.92	38.57
New LSD at 0.05 =	0.51	0.53	0.57	0.01	0.02	0.01	0.03	0.01	0.02	0.02	0.01	0.02	2.67	2.78	2.83

Mineral phosphate (MP) Rock phosphate (RP) Arbuscular mycorrhizal fungi (AMF) Phosphate solubilizing bacteria (PSB)

The obtained results are in agreement with those achieved by Abd El-Wahab *et al.* [30], Rizk-Alla and Tolba [31] and Abd El-Wadoud [32] on some table grape cultivars they found that AMF increase cane total carbohydrates content.

As for the effect of PSB, Abd El-Wahab *et al.* [33] on Thompson Seedless grapevines, Dakhly and Abada [34] on Superior grapevines and Mekawy and Abd El-Hafeez [35] on Red Globe grapevines pointed out that PSB inoculation increased significantly the cane content of total carbohydrates in comparison with the non-inoculated vines (control).

In general, the positive effect of AMF and PSB inoculations and rock-phosphate amendment on vine nutritional status could be explained by that the interaction of phosphate-solubilizing bacteria and arbuscular mycorrhizal fungi can also promote better establishment of arbuscular mycorrhizal fungi by phosphorus ions released from rock-phosphate which are taken up by the AMF mycelium, thereby maintaining a low soluble phosphorus concentration in the discrete soil microhabitats where the rock-phosphate particles are attacked by the phosphate-solubilizing bacteria and thus favoring a continuous phosphorus release [33].

Yield and Physical Characteristics of Bunch: As referenced in Table (4), yield and physical characteristics of bunch expressed average of bunch weight, length and width were significantly improved by inoculation with arbuscular mycorrhizal fungi (AMF) and/or two different doses of the phosphate-solubilizing bacteria (PSB) in soil amended with rock-phosphate (RP) compared to the control during the three seasons.

The highest values of these parameters were obtained from dual inoculation of AMF plus PSB at 40cm/vine followed dual inoculation of AMF plus PSB at 20cm/vine, while control had the lowest values of these ones during the three seasons.

The positive influence of AMF and PSB inoculations in soil amendment with rock-phosphate on yield and its attributes could be explained that AMF are able to absorb and translocate elements to host root tissues, besides,

Table 4: Effect of inoculation with arbuscular mycorrhiza fungi and phosphate solubilizing bacteria in rock phosphate amended soil on yield and bunch physical characteristics of Superior grapevines during 2017, 2018 and 2019 seasons

	Characteristics											
	Yield/vine (kg)			Average bunch weight (g)			Average bunch length (cm)			Average bunch width (cm)		
Treatments	2017	2018	2019	2017	2018	2019	2017	2018	2019	2017	2018	2019
MP (control)	14.01	15.19	16.04	526.7	542.6	560.8	24.5	25.3	25.4	13.1	13.8	13.7
RP + AMF	16.04	17.52	18.43	594.2	612.7	633.4	25.4	26.0	25.9	13.7	14.1	14.3
RP + 20cmPSB	14.82	16.13	16.96	552.9	569.8	588.9	24.8	25.4	25.6	13.3	13.9	13.9
RP + 40cm PSB	15.48	16.85	17.76	575.6	593.4	612.3	24.9	25.6	25.7	13.4	13.9	14.0
RP+AMF+20cmPSB	16.52	18.03	19.02	609.5	628.4	649.2	25.7	26.1	26.2	13.9	14.2	14.4
RP+AMF +40cmPSB	16.97	18.59	19.67	621.6	643.1	664.6	25.9	26.4	26.4	14.2	14.4	14.7
New LSD at 0.05 =	0.41	0.48	0.53	9.4	13.1	14.7	0.1	0.2	0.1	0.2	0.1	0.2

Mineral phosphate (MP) Rock phosphate (RP) Arbuscular mycorrhizal fungi (AMF) Phosphate solubilizing bacteria (PSB)

Table 5: Effect of inoculation with arbuscular mycorrhiza fungi and phosphate solubilizing bacteria in rock phosphate amended soil on berry physical properties of Superior grapevines during 2017, 2018 and 2019 seasons

	Characteristics											
	Average berry weight (g)			Average berry size (cm^3)			Average berry length (cm)			Average berry diameter (cm)		
Treatments	2017	2018	2019	2017	2018	2019	2017	2018	2019	2017	2018	2019
MP (control)	3.68	3.81	3.93	3.59	3.74	3.86	2.14	2.16	2.19	1.70	1.72	1.73
RP + AMF	4.07	4.21	4.34	3.99	4.11	4.26	2.19	2.22	2.23	1.73	1.76	1.77
RP + 20cmPSB	3.84	3.96	4.08	3.75	3.91	4.01	2.16	2.17	2.21	1.72	1.73	1.74
RP + 40cm PSB	3.98	4.09	4.23	3.87	4.02	4.17	2.17	2.19	2.22	1.72	1.74	1.75
RP+AMF+20cmPSB	4.19	4.31	4.46	4.10	4.24	4.37	2.20	2.23	2.25	1.74	1.77	1.77
RP+AMF +40cmPSB	4.24	4.39	4.53	4.17	4.33	4.46	2.23	2.25	2.28	1.76	1.78	1.79
New LSD at 0.05 =	0.05	0.07	0.06	0.04	0.05	0.07	0.02	0.01	0.02	0.02	0.01	0.02

Mineral phosphate (MP) Rock phosphate (RP) Arbuscular mycorrhizal fungi (AMF) Phosphate solubilizing bacteria (PSB)

they can also break down and make available to their hosts certain complex minerals and organic matter in the soil [38].

The obtained results are in harmony with those achieved by Abd El-Wahab *et al.* [30]; Rizk-Alla and Tolba [31] and Abd El-Wadoud [32] on some table grape cultivars they found that AMF improve yield and its attributes.

As for the effect of PSB, Dakhly and Abada [34] on Superior grapevines and Mekawy and Abd El-Hafeez [35] on Red Globe grapevines pointed out that PSB inoculation increased significantly bunch weight and yield/vine in comparison with the non-inoculated vines (control).

Physical Properties of Berries: Data presented in Table (5) indicated that inoculation with arbuscular mycorrhizal fungi (AMF) and/or two different doses of the phosphate-solubilizing bacteria (PSB) in soil amended with rock- phosphate (RP) greatly affected all physical properties of berries expressed average of berry weight, size and dimensions compared to the control during the three seasons.

Dual inoculation of AMF plus PSB at 40cm/vine attained significantly the highest values of these parameters followed by dual inoculation of AMF plus PSB at 20cm/vine, whereas control had the lowest values of these ones during the three seasons.

The positive effect of AMF inoculation on improving berry physical properties can be explained that AMF are able to absorb and translocate elements to host root tissues, in addition, they can also break down certain complex minerals and organic substances in the soil and make them available to their hosts [38].

The obtained results are in agreement with those achieved by Abd El-Wahab *et al.* [30]; Rizk-Alla and Tolba [31] and Abd El-Wadoud [32] on some table grape cultivars they found that AMF improve berry physical properties.

As for the effect of PSB, Dakhly and Abada [34] on Superior grapevines and Mekawy and Abd El-Hafeez [35] on Red Globe grapevines pointed out that PSB inoculation increased significantly the physical properties of berries i.e. weight, size, length and diameter in comparison with control.

Table 6: Effect of inoculation with arbuscular mycorrhiza fungi and phosphate solubilizing bacteria in rock phosphate amended soil on berry chemical properties of Superior grapevines during 2017, 2018 and 2019 seasons

	Characteristics								
	TSS (%)			Acidity (%)			TSS/acid ratio		
Treatments	2017	2018	2019	2017	2018	2019	2017	2018	2019
MP (control)	15.88	15.81	15.88	0.68	0.64	0.62	23.35	24.70	25.61
RP + AMF	16.24	16.22	16.27	0.62	0.58	0.55	26.19	27.97	29.58
RP + 20cmPSB	15.93	15.89	15.93	0.66	0.61	0.60	24.14	26.05	26.55
RP + 40cm PSB	16.05	16.01	16.15	0.63	0.59	0.57	25.48	27.14	28.33
RP+AMF+20cmPSB	16.48	16.51	16.59	0.60	0.56	0.54	27.47	29.48	30.72
RP+AMF +40cmPSB	16.74	16.82	16.88	0.54	0.51	0.48	31.00	32.98	35.17
New LSD at 0.05 =	0.23	0.29	0.31	0.05	0.03	0.02	2.81	2.89	2.94

Mineral phosphate (MP) Rock phosphate (RP) Arbuscular mycorrhizal fungi (AMF) Phosphate solubilizing bacteria (PSB)

Chemical Properties of Berries: As shown in Table (6), all chemical properties of berries i.e. TSS, acidity and TSS/acid ratio were significantly improved by inoculation with arbuscular mycorrhizal fungi (AMF) and/or two different doses of the phosphate-solubilizing bacteria (PSB) in soil amended with rock-phosphate (RP) compared to the control during the three seasons.

The highest values of TSS and TSS/acid ratio and the lowest values of acidity were obtained from dual inoculation of AMF plus PSB at 40cm/vine followed dual inoculation of AMF plus PSB at 20cm/vine, while control resulted in the lowest values TSS and TSS/acid ratio and the highest values of acidity during the three seasons.

The positive effect of AMF inoculation on improving berry chemical properties can be explained that AMF are able to absorb and translocate elements to host root tissues, besides, they can also break down and make available to their hosts certain complex minerals and organic matter in the soil [38].

The obtained results are in agreement with those achieved by Abd El-Wahab *et al.* [30]; Rizk-Alla and Tolba [31] and Abd El-Wadoud [32] on some table grape cultivars they found that AMF improve berry chemical properties.

As for the effect of PSB, Dakhly and Abada [34] on Superior grapevines and Mekawy and Abd El-Hafeez [35] on Red Globe grapevines they pointed out that PSB inoculation increased significantly TSS% and TSS/acid ratio and decreased total acidity% of the berry juice in comparison with control.

Microbiological Studies: The Effect of fertilization treatments on microbial and enzyme activity in the rhizosphere of Superior grapevines were evaluated during the 2017, 2018 and 2019 seasons are shown in Tables (7 & 8) and Figure (1 & 2).

Soil Microbial Properties: The amount and kind of different substances entering soil through plant litter, root exudates and management factors such as mineral and organic or inorganic fertilizers influence microbial population diversity and numbers in the soil. This in turn affects crop production and sustainability of soil health. Therefore, we tested population density of microorganisms, especially phosphate solubilizing bacteria (*B. megaterium*) *and arbuscular mycorhyzea fungi* enrichment in soil.

Total Microbial Count: It is obviously from data in Table (7) that the total microbial count of inoculant plants significantly increase during three seasons (2017, 2018 & 2019). The most observed data at RP + AMF + PSB at 20cm/vine & RP + AMF + PSB at 40cm/vine, which co-inculcated with AMF and phosphate solubilizing bacteria, the highest value of total microbial count has achieved the expected results. Essentially, the result of the second season (12.3&12.6 × 10^5 cfu/ g soil). However, in the third season of the experiment 2019, there is a slight increase in total microbial count compared was observed (12.6 & 13.2 × 10^5 cfu/g soil), This result explained as pH tends to slightly decrease in the soil treated with different rates of inorganic phosphours source (rock-phosphate) combined with biofertilizer than other treatments. The obtained results are identical with results that achieved by Elfstrand *et al.* [39] who explained that the increase in population of rhizospheric microorganism in the roots of most plants are influenced by a combined inoculation of microorganism and AMF fungi.

Per contra the results announced that total microbial count of non- AM plants grown with rock-phphosphate gave the lowest value of the study (4.5, 4.2× 10^5cfu/ g soil) and (4.8 × 10^5 CFU/ g soil) through three seasons.

Table 7: Effect of inoculation with arbuscular mycorrhiza fungi and phosphate dissolving bacteria in rock-phosphate amended soil on total microbial, phosphate dissolvers bacteria and colonization percentage of AMF in the rhizosphere of Superior grapevines during 2017, 2018 and 2019 seasons

	Characteristics								
	Total microbial count ($\times 10^6$CFU/g rhizosphere)			Phosphate dissolvers ($\times 10^6$CFU/g rhizospher)			AMF root colonization (%)		
Treatments	2017	2018	2019	2017	2018	2019	2017	2018	2019
MP (control)	4.5	4.2	4.8	2.31	4.12	6.21	13.84	16.00	14.79
RP + AMF	7.6	8.2	7.5	4.53	5.18	9.13	41.00	56.66	58.98
RP + 20cmPSB	6.1	6.5	6.9	11.10	11.50	12.80	15.76	25.00	23.00
RP + 40cm PSB	6.4	5.8	7.1	14.50	13.60	16.70	13.85	21.86	25.7
RP+AMF+20cmPSB	11.6	12.3	12.6	17.30	18.90	18.90	60.30	74.80	76.00
RP+AMF +40cmPSB	12.1	12.6	13.2	18.60	19.10	22.10	78.40	85.00	83.00
New LSD at 0.05 =	---	---	---	---	---	---	11.23	7.79	5.37

Mineral phosphate (MP) Rock phosphate (RP) Arbuscular mycorrhizal fungi (AMF) Phosphate solubilizing bacteria (PSB)

Table 8: Effect of inoculation with arbuscular mycorrhiza fungi and phosphate dissolving bacteria in rock-phosphate amended soil on enzymes activity in the rhizosphere of Superior grapevines during 2017, 2018 and 2019 seasons

	Characteristics					
	Dehydrogenase activity (µ g TPF g^{-1} dry soil day^{-1})			Alkaline phosphatase activity (mg PNP/g dry soil/hr)		
Treatments	2017	2018	2019	2017	2018	2019
MP (control)	18.12	23.00	29.64	0.35	0.41	0.40
RP + AMF	39.19	42.23	40.32	0.39	0.42	0.41
RP + 20cmPSB	32.55	28.00	38.87	0.45	0.48	0.46
RP + 40cm PSB	41.93	37.54	40.00	0.46	0.49	0.51
RP+AMF+20cmPSB	65.62	71.00	80.00	0.53	0.56	0.58
RP+AMF +40cmPSB	72.36	80.00	88.00	0.58	0.59	0.61
New LSD at 0.05 =	6.39	5.74	3.98	0.04	0.01	0.02

Mineral phosphate (MP) Rock -phosphate (RP) Arbuscular mycorrhizal fungi (AMF) Phosphate solubilizing bacteria (PSB)

The results in symmetry with these achieved by Hellal *et al.* [40] and Fetyan *et al*. [41] they demonstrated that the increment in populations of rhizospheric microorganisms in the roots of most plants was affected by a combined inoculation of microorganism with inorganic phosphorus source. In addition, Shams El-Deen *et al*. [42] provide a strong essential for *B. megaterium* strain evolution as bio inoculants to be accessed plant growth-promoting activity in horticulture.

Total Phosphate Solubilizing Bacteria Count: The phosphate solubilizing activity is significantly affected by mineral input. According to fermented results in Table (7) significantly high populations of phosphate dissolving bacteria were found in RP + AMF + PSB at 40cm/vine followed by RP + AMF + PSB at 20cm/vine and the lowest population was recorded in the control treatment.

Results in Table (7) and Figure (1) indicate that during consecutive seasons (2017, 2018 & 2019), the effect of rock-phosphate inoculated with AM fungi and 40cm/vine *B. megaterium* gave a marked increase in values of p-solubilizing populations where the count of PSB recorded (18.6, 19.1 and 22.1 $\times 10^6$ CFU /g soil) during three seasons, whereas treatments with single inoculation exhibited fewer counts both for *B. megaterium* and AM fungi recorded (11.1, 11.5 &12.8 $\times 10^6$ CFU /g soil for 20 cm/vine), (14.5, 13.6 &16.7 $\times 10^6$ CFU /g soil for 40 cm/vine) and (4.53, 5.18 &9.13 $\times 10^6$ CFU /g soil for AMF) in three seasons respectively.

The increase of phosphate dissolving population depended upon the capability of these bacteria to secrete some organic acids that helped in the release of phosphorus to be used for its growth and plant uptake [43].

As mentioned above, co-inoculation of phosphate soloublizing bacteria with AM fungi directly affected the microbial community in soils. Hellal *et al.* [40] also found that incorporation of inorganic fertilizer increased the available phosphorus (P) status at a maximum level. Therefore, there is a direct relationship between the effect of rock- phosphate as an inorganic phosphorus source inoculated with AM fungi and *B. megaterium* application on phosphorus uptake by grapevines is described in Table (3). This result concluded that rock-phosphate

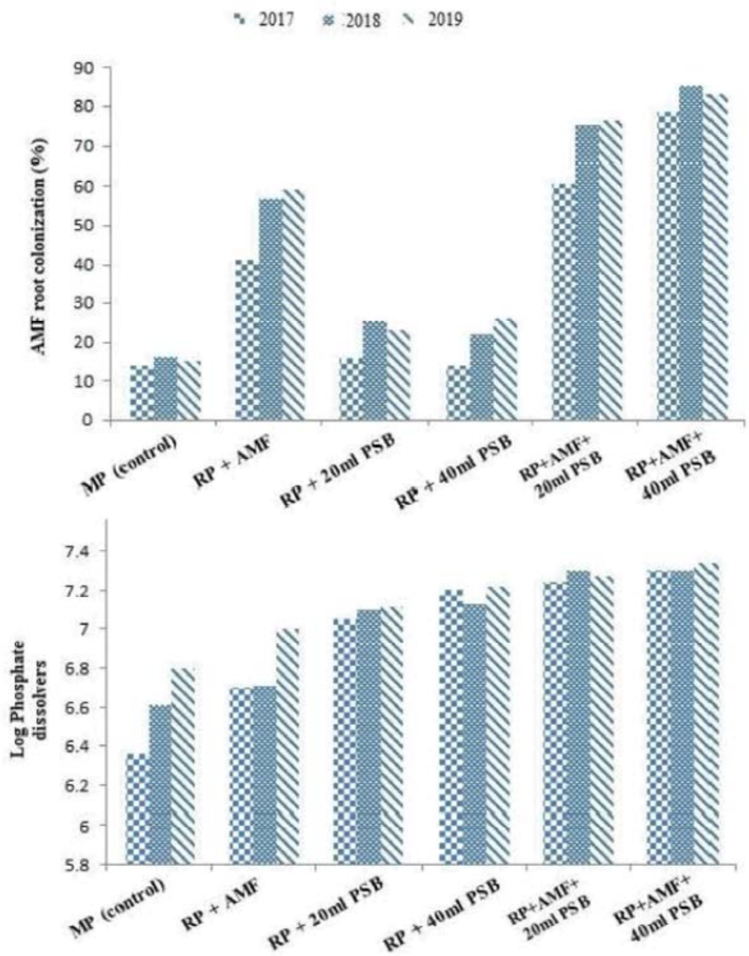

Fig. 1: Effect of inoculation with arbuscular mycorrhiza fungi and phosphate dissolving bacteria in rock-phosphate amended soil on colonization percentage of AMF and phosphate dissolving bacteria in the rhizosphere of Superior grapevines during 2017, 2018 and 2019 seasons

inoculated with *B. megaterium* and mycorrhizae proved to be the appropriate technology to use inorganic source of phosphorus for continuous crop production as well as supporting healthier environment these finding are in line with [44].

AMF Root Colonization (%): Data concerning with the effect of rock-phosphate (as inorganic p source) and soil co-inoculation with Arbuscular mycorrhizae fungi and *B. megaterium* on percentage of AM infection of superior grapevines through 2017, 2018 and 2019 seasons exists in Table (7).

Result presented in Table (7) and illustrated in Figure (1) clearly show that all treatments have a significant increase in AMF root colonization in comparison to control, while there is significant increase in dual inoculation AMF and *B. megaterium* exhibited the most performance in AM root colonization % at different concentration of *B. megaterium* 20 & 40 cm/ vine at the 1st, 2nd and 3rd growing seasons, it is obvious that single inoculation of AM fungi gradually decreased percentage of AM infection.

The best colonization percentage was recorded with RP + AMF + PSB at 40cm/vine (78.40, 85.00 and 83.00%) in three seasons, respectively. Inorganic phosphorus input led to the increase of native mycorrhizal colonization that enhanced the plants for phosphorus uptake. Synergistic effect of dual colonization of roots with AMF and *B. megaterium* on growth, nutrient uptake in many plants have been reported by Xavier and Germida [45] and Kowalski *et al.* [46]. Based on the results obtained in the second and third seasons, the increasing percentage of root colonization, the increase in grapevine yield and chemical characters of vegetative growth reinforce

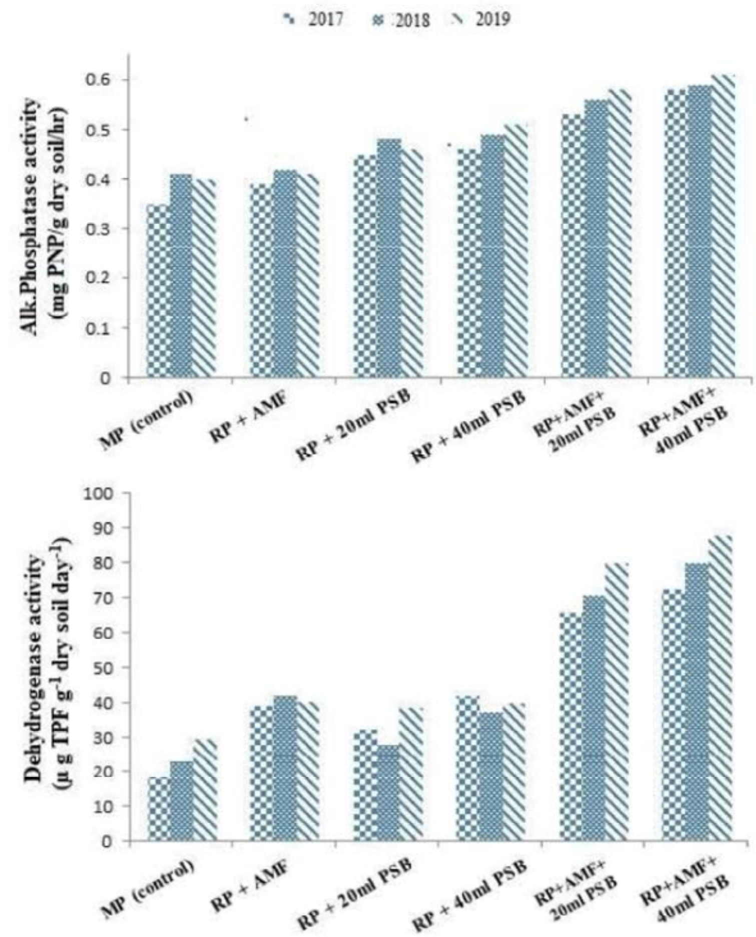

Fig. 2: Effect of inoculation with arbuscular mycorrhiza fungi and phosphate dissolving bacteria in rock-phosphate amended soil on enzymes activity in the rhizosphere of Superior grapevines during 2017, 2018 and 2019 seasons

the importance of co-inoculation arbuscular mycorrhizal fungi and phosphate-solubilizing bacteria in improving rock-phosphate availability for Superior grapevines. The increase of N, P and K uptake % in grapevines is considered as an indication of soil fertility and the plants systemically are able to uptake the essential macronutrients. The fertilization with rock-phosphate and biofertilizer led to an increase of macronutrients as available through 3 seasons (2017, 2018 and 2019) where RP + AMF + PSB at 20cm/vine and RP + AMF + PSB at 40cm/vine obtained the highest values of NPK content of plant compared to the control and single inoculation with AMF or *B. megaterium*. Kim *et al.* [47] showed that the production of organic acids in dissolving phosphorous by PSB is not the only reason for the increase in phosphorous concentration in the rhizospher. Furthermore, mycorrhizal fungi effectively extend plant roots, aiding crop phosphorus nutrition by increasing the volume of soil from which phosphate may be absorbed grapevines could uptake macronutrients and that reflected on the growth and yield of plants. This result confirmed that phosphate-solublizing bacteria was more potent P-solubilizer when co-inoculated with AMF. In contrary to organic fertilizers, which require microbial metabolism to make the majority of nutrients available to plants, inorganic fertilizers inoculated with PSB and AM fungi have a direct impact on crop output, which is the primary rationale for their use.

These facts its explanation goes back to Rigamonte *et al.* [48] who created the connotation of Mycorrhization Helper Bacteria (MHB). MHB is thought to have five main impacts on mycorrhizae: root mycobiont receptivity, root-fungus identification, fungal growth, rhizospheric soil change and germination of fungal propagate.

Soil Enzyme Activities: Soil enzyme activities are an indirect indication of microbial behavior that is linked to microbial dynamics in the soil. Enzyme activities in the soil ecosystem are considered to be a significant contributor to overall soil microbial activity [49, 50].

Dehydrogenase and Phosphatase Activity: Oxidoreductases, such like dehydrogenase, are involved in oxidative reactions in soils and their activity mainly depends on the metabolic state of the soil organisms; as a result, they are considered advantage indicators of soil microbial activity [51].

Results illustrated in Table (8) and Figure (2) show the effect of interaction between *B. megaterium*, AM fungi and rock-phosphate on Superior grapevines dehydrogenase and phosphatase activities under field condition. All treatments induced rhizosphere soil dehydrogenase and phosphatase activities with variable degrees compared to control. With respect to dual inoculation *B. megaterium* with AM fungi being the most effective recording (65.62, 71.00, 80.00 μ g TPF g^{-1} dry soil day^{-1} for20cm/vine PSB) & (72.36, 80.00, 88.00 μ g TPF g^{-1} dry soil day^{-1} for40 cm/vine PSB) for dehydrogenase activity, respectively followed by single AMF inoculation recording (39.19, 42.23, 40.32 μ g TPF g^{-1} dry soil day^{-1}) at three seasons, respectively. Our results are in agreement with Alguacil *et al.* [52] they stated that the increment in dehydrogenase activity in the rhizosphere soil of dual-inoculated plants reflects the rise in microbial activity. Hydrolases are associated to the minerlisation of essential nutrient components required for both plant and microbial growth, hence measuring them offers an early indication of changes in soil fertility. Moreover, the increment in dehydrogenase enzyme activity was due to the potent activity of microflora as a mixture of biomass than each singular one. The most distinguished increment in microbial respiration was recorded with the mixture of microorganisms in the soil [53].

Phosphatases are a group of enzymes that catalyze the hydrolysis of organic phosphorus compounds and convert them to an inorganic form that plants and microbes may utilize [54] in the harmony with the previous data alkaline phosphatase activity the same expected results was observed.

Data shown in Table (8) exhibited the presence of phosphatase enzyme activity between treatments showing microbial activity in the soil treated by AM fungi and *B. megaterium* in the presence of rock-phosphate as p source.

The alkaline phosphatase activity was higher in second and third season than it is the first one with all treatments. RP + AMF + PSB at 40cm/vine possessed the highest alkaline phosphatase being (0.58, 0.59 and 0.61 mg PNP/g dry soil/day) in three seasons, respectively. Single inoculation for both *B. megaterium* and AM fungi gave the lowest value in three seasons, respectively.

Increases in alkaline phosphatase could be linked to an increase in rhizospher microbial population as a result of inoculation treatments. Phosphatase secretion by phosphate-solubilizing bacteria and/or AM Fungi are a frequent technique of accelerating the conversion of insoluble forms of phosphorus to plant-available forms, hence increasing phosphorus intake of the plants [55].

Alorie *et al.* [56] stated that the activities of phosphatase and dehydrogenase in the rhizosphere increased with plant age. This could be due to the steady growth of a microbial population in the root zone. Moreover, due to the rise in total root surface area, the expansion of the root system with age may have resulted in an increased production of phosphatases of plant origin.

Our results are in agreement with Zaidi and Khan [57] and Frey *et al.* [58] they emphasize these interpretations with the increases available phosphorus utilization in soil and phosphorus uptake in grapevines may be partially related to the tested PGPR's generation of a range of organic acids, which decreased the soil pH, resulting in the conversion of non-available phosphorus into available form. Finally, AMF colonization of grape plants raised growth responses, alkaline phosphatases and dehydrogenase levels considerably. The fundamental contribution of these fungi to plant growth is the uptake of phosphorus and other elements by extra-radical mycorrhizal hyphae and the subsequent transfer of these elements to the root tissues [59].

In conclusion, it is easy to adopt practices that encourage AMF populations, because these beneficial fungi naturally colonize grape roots, but they will be most effective when inoculated with a plant growth promoting hormone such as phosphate-soluble bacteria (PSB). Application of AMF plus PSB by inoculating in soil amended with rock-phosphate (RP) was an effective technique to reduce mineral fertilizer in continuous cropped management on farms. Under Egyptian conditions, these together combined with rock-phosphate as the sole of phosphorus with being healthy, cheap and environmentally safe are of great importance in practicing integrated fertilizer management concept for sustainable and organic agriculture.

According to aforementioned results, it can be recommended the dual inoculation of AMF plus PSB (*Bacillus megaterium*) at 40cm/vine in soil amended with

RP to obtain it on the optimum results in terms of enhancement of microbial community and enzyme activity in the rhizosphere, which is indicated increasing mineral nutrients uptake, vegetative growth traits and yield besides improvement the fruit quality attributes of Superior grapevines.

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