

Mitigation of CaCO_3 influences on *Ipomoea batatas* plants using *Bacillus megaterium* BSM 2894 var.

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Abstract

Under Egyptian soil conditions, when phosphorus fertilizers were applied to the soil, it gets fixed and converts to unavailable form, leading to low solubility for the plant. This study were fulfilled on sweet potato (cv. Beauregard) under undesirable soil properties (CaCO_3 10.8 vs 11.3%) using *Bacillus megaterium* DSM 2894 strain under different five mono calcium phosphate (CSP) levels [(69(CSP₂₀); 138(CSP₄₀); 207 (CSP₆₀); 276 (CSP₈₀) and 345 ((CSP₁₀₀) kg ha⁻¹ of calcium superphosphate (CSP)] to arise the potential efficiency of some nutrients uptake and decrease the applied total amount of CSP in 2019 and 2020 seasons. The results mentioned that highest values were obtained by inoculated plants with DSM2894 strain under 20, 60 and 100% of CSP for all studied nutrients content in both seasons, except Mn content in the 2019. Also, inoculated plants with DSM2894 under previous treatments for all tuberous root nutrients content, except Fe and Zn contents in both seasons, in addition protein and anti-radical power and total yield. Statistically, leaf nutrients uptake and tuberous root content were highly significant affected by DSM and CSP combination. Applying of phosphorus fertilizer with DSM2894

mixture was gave the best values as compared with phosphorus fertilizer or DSM2894, individually.

Keywords: Sweet potato, Phosphorus fertilizer, *Bacillus megaterium* DSM2894, leaf and tuberous root nutrients contents, Tuberous root yield.

1 Introduction

The increasing demand for food production focuses new challenges in agriculture, Egypt is one of the developing countries that suffer from increasing population growth that hinder the sustainable agricultural developing and expanse the gap between population growth and agricultural products. Due to some unsuitable agricultural practices as well as intensive cropping, excessive water irrigation and extensive use of traditional or soluble chemical fertilizers (TCF vs SCF) and amongst others which lead to soil degradation and covert it to in-arable lands under salinity and calcity soils. Wherefore, it is necessary to recovery the degraded soils and conserving it's all chemical, physical and biological properties related to plant growth in order to get the maximizing yield. In addition, the growing prices of most of (CF) which are unsuitable to economic status of most of Egyptian farmers and reduce or prevent the use of these CF especially, mono calcium phosphate (MCP) that is prohibited in organic cropping as a result of is one of the most common polluting fertilizers to decrease the negative effects on humans, animals, soils, ground water and environment as well as, achieving the food security and producing healthy products are free of chemical pollutants.

According to [1], calcareous soils are generally defined as a soils have a high contents of calcium carbonate (CaCO_3), and characterized by the high soil acidity (pH), low cations exchange capacity (CEC), high salinity (ECe), and soil-water retentions due to presence of surface hard and impermeable

crusting layers of CaCO_3 that prevent the water percolation to the subsurface layers and increase the loss of nutrients [2], in addition slow nutrients release especially, phosphorus (P), calcium (Ca) and most of micronutrients and the out-of- proportion their content [3]. Also, calcareous soils suffer from strictly shortage of organic matter (OM) that affect growth of croplands, as a result of these, cultivation of calcareous soils focus a hardness challenges. Statistically, calcareous soils are common in the arid and semi-arid regions where precipitation is scarce, whereas, occupying > 30% of the earth surface [2] and CaCO_3 content is vary widely from a few percentage and 95% [4]. In Egypt, calcareous soils are occupying around 270.833 hectares are located in the northern region of the Western Desert and Eastern Desert [5]. Under calcareous soil conditions, P is the least mobile and available among all the nutrients for plants in soil due to P anions react with Ca and magnesium (Mg) whereas, high pH values to form insoluble phosphate compounds and it imbalance between all elements [6], due to P is greatly affected by all calcareous soils properties as above mentioned.

From the point of view of plant nutrition researchers, P is the essential nutrient, whereas, it is a main element for many organic constituents which are essential for many metabolic and physiological processes such as, root elongation and division, photosynthetic ratio, nitrogen fixation enhancement, flowering and fruit maturing [7]. In the same vein, it is fundamental element in energy-rich compounds of the living cells such as, adenosine tri-phosphate (ATP), adenosine di-phosphate (ADP) and adenosine mono-phosphate (AMP), in addition, the seats of genetic inheritance such as deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) that are responsible for protein synthesis in plant tissues, so, neither plant or animal can grow without it. Furthermore, its

role in phospholipids that play vital role in cellular membranes. P unavailability is one of the major growth-limiting factors affected plant growth and development because high soil pH, whereas, the phosphate ions convert directly to unavailable forms and become is not available for uptake by plants [8].

Bacillus megaterium is one of subset of plant growth-promoting rhizobacteria (PGPR) which is widespread in soils and is member of the micro biome of several plant hosts [9]. *B. megaterium* effectively colonize soils and live inside plant tissues to enhance plant growth regulation (PGR) and protection via many mechanisms directly or indirectly including increasing phosphorus solubility, nitrogen availability, modulation of plant hormones, production of some antimicrobial compounds and produce a wide range of bioactive compounds [10,11]. *B. megaterium* is also a source for a wide range of metabolites and enzymes that are involved in PGR [11]. One of the most important characteristics of *B. megaterium* is its ability to improve the roots development, thus enhance the micronutrients availability [12], increase the ability of plants to secrete a several array of organic compounds which attract some soil microbes [13], and to maintain mutualistic interactions with roots which increases the ability of crops to grow well and overcome the stressful conditions [14,15]. Furthermore, its ability to form spores, thereby, increasing their capability for resistance against biotic and abiotic stress conditions [16].

Sweet potato (*Ipomoea batatas* L. Lam.) was selected because is a staple food in most of the developing countries, short period growth and its high ability for adaption, obtaining a high yield under various climatic changes and low input requirements [17,18]. Recently, it is regarded a strategic crop due to its importance for exportation and it considered the food security crop as is

superb source for carbohydrates, vitamins and minerals, in addition, recovery diseases related to malnutrition in developing world such as Egypt [19], as well as a raw material for many industries such as flour and starch that easily converts to sugars as compared with another crops. Potato is subtropical plant which is vegetatively belong to convolvulaceae. According to international potato center, 2018, potato is regarded the 6th most important food crop in the world. Ultimately, in this context, the present study was fulfilled to arise the use phosphorus efficiency and reduce the applied phosphorus fertilizers amounts using *B. megaterium* DSM 2849 to enhance the availability of phosphorus and other micro nutrients, in addition to produce food free from chemical pollutants and increase the productivity of sweet potato grown under calcic conditions.

2 Materials and Methods

2. 1. Study location, weather conditions and soil sampling

Two field experiments were fulfilled on sandy loam and sandy clay loam in both seasons, respectively at the experimental farm in Fayoum district (29° 17'N; 30° 53'E), Egypt, in two consecutive summer seasons 2019 and 2020 on standard variety of sweet potato (*Ipomoea batatas* L. cv. Beauregard) plants. Transplanting was carried out on 10 April 2019 and 19 April 2020 by utilizing vine cutting. Vines cutting of cv. Beauregard were collected from AGROFOOD farm at Nubaria district, on the Alexandria-Cairo Desert road, Egypt. The weather data (April to August) of the study region are provided in Table 1 as an average of both growing seasons. The soil samples of 0-30 cm surface layer are taken at random from different locations of the study area before transplanting for determine some physical and chemical properties according [20]. Soil analyses are shown in Table 2.

Table 1. Average weather data for the study region, Egypt over the sweet potato growing seasons

Month	Average Day Temperature (° C)	Average Night Temperature (° C)	Average relative Humidity (%)	Average Speed of Wind (ms ⁻¹)	Average of Measured Pan Evaporation Class A (mm d ⁻¹)	Average Precipitation (mm d ⁻¹)
April	32.9	16.1	32.3	1.87	5.58	0.02
May	35.8	18.9	34.1	1.88	6.87	0.00
June	40.0	19.8	38.4	1.49	7.56	0.00
July	41.1	24.8	37.9	2.03	6.88	0.02
August	41.3	25.6	36.8	1.78	6.78	0.00

Table 2 Some physical and chemical soil characters of the experimental sites before transplanting in seasons 2019 and 2020

Soil property	2019 season	2020 season
Particles size distribution		
Sand %	63.6	66.8
Silt %	7.8	16.5
Clay %	28.6	16.7
Soil texture class	Sandy loam	Sandy Clay loam
pH in soil paste	7.19	7.77
ECe (dSm ⁻¹) in soil paste extracted	3.95	4.24
Soluble ions mmol L⁻¹		
CO ₃ ²⁻	---	---
HCO ₃ ⁻	2.03	2.70
Cl ⁻	21.1	25.6
SO ₄ ²⁻	20.3	18.3
Na ⁺	31.6	31.3
K ⁺	0.65	0.88
Ca ⁺⁺	7.11	7.47
Mg ⁺⁺	4.03	6.98
Organic matter (OM) %	0.90	1.03
CaCO ₃ (%)	10.8	11.3
Total N (mg kg ⁻¹)	450	515

Available-P, mg kg ⁻¹ (Extractable with NaHCO ₃ (pH=8.5)	3424	4013
Available K mg kg ⁻¹ (Extractable with NH ₄ AOC)	1816	1237
Fe, mg kg ⁻¹ (Extractable with DPTA)	6.03	4.15
Mn, mg kg ⁻¹ (Extractable with DPTA)	18.2	10.7
Zn, mg kg ⁻¹ (Extractable with DPTA)	0.07	0.04
Cu, mg kg ⁻¹ (Extractable with DPTA)	0.68	0.40

2. 2. Treatments and agricultural practices

Each of the two field experiments included 5 levels of single calcium superphosphate (15.5% P₂O₅) as a phosphorus fertilizer at 20, 40, 60 and 80% and 100% of a recommended calcium superphosphate fertilizer (RCSF; 345 kg ha⁻¹) without and with inoculation with *Bacillus megatherium* strain as shown in Table 3. Therefore, the total number of treatments in each experiment was 10 treatments in 3 replicates (30 plots). The agricultural practices for sweet potato cultivation was applied according to the bulletin of the Egyptian Ministry of Agriculture (No.1020/2006), however the ammonium sulfate (N%≈20.6) as a nitrogen source was added with 432 kg N ha⁻¹ at three equal portions, in second and seventh week after transplanting (WAT). While, the potassium fertilizer was added with rate 230.4 kg of K₂O ha⁻¹ in form potassium sulfate as potassium source was applied at two equal portions in 7th and 9th WAT.

Table 3 The tested treatment in the study

Treatment	Description
CSP ₂₀ xDSM ₀	69 kg ha ⁻¹ of calcium superphosphate (20% of RPF) with non-inoculated plants by <i>Bacillus megatherium</i> DSM 2894 var.
CSP ₂₀ xDSM ₁	69 kg ha ⁻¹ of calcium super phosphate (20% of RPF) with inoculated plants by <i>Bacillus megatherium</i> DSM 2894 var.
CSP ₄₀ xDSM ₀	138 kg ha ⁻¹ of calcium super phosphate (40% of RPF) with non-inoculated plants by <i>Bacillus megatherium</i> DSM 2894 var.
CSP ₄₀ xDSM ₁	138 kg ha ⁻¹ of calcium super phosphate (40% of RPF) with inoculated

	plants by <i>Bacillus megaterium</i> DSM 2894 var.
CSP ₆₀ xDSM ₀	207 kg ha ⁻¹ of calcium super phosphate (60% of RPF) with non-inoculated plants by <i>Bacillus megaterium</i> DSM 2894 var.
CSP ₆₀ xDSM ₁	207 kg ha ⁻¹ of calcium super phosphate (60% of RPF) with inoculated plants by <i>Bacillus megaterium</i> DSM 2894 var.
CSP ₈₀ xDSM ₀	276 kg ha ⁻¹ of calcium super phosphate (80% of RPF) with non-inoculated plants by <i>Bacillus megaterium</i> DSM 2894 var.
CSP ₈₀ xDSM ₁	276 kg ha ⁻¹ of calcium super phosphate (80% of RPF) with incubated plants by <i>Bacillus megaterium</i> DSM 2894 var.
CSP ₁₀₀ xDSM ₀	345 kg ha ⁻¹ of calcium super phosphate (100% of RPF) with incubated plants by <i>Bacillus megaterium</i> DSM 2894 var.
PF ₁₀₀ *BM ₁	345 kg ha ⁻¹ of calcium super phosphate (100% of RPF) with incubated plants by <i>Bacillus megaterium</i> DSM 2894 var.

*PF = Phosphorus fertilizer

** BM = *Bacillus megaterium*

***RPF = Recommended phosphorus fertilizer

2. 3. Experimental design

Studied treatments of each experiment were arranged in the experimental units in a split-plot structure in a randomized complete block design (RCBD) arrangement with three replicates. Phosphorus fertilizer levels were in arranged the main plots (A), while the inculcation treatments with *Bacillus megaterium* DSM 2894 were arranged in sub plots (B). The experimental plot area was 10.5 m² (3.5m x 3.0m) and consisted of 4 rows. Nearly similar top slips (cuttings), 20 cm length were manually planted on the third top of slope ridge at 25 cm apart. *B. megaterium* solution with rate 20 ml was added to cuttings during transplanting process.

2. 4. Bacterial strain and medium used

Bacillus megaterium DSM 2894 was obtained from Cairo Microbiological Resources Centre (Cairo MIRCEN), Faculty of Agriculture, Ain Shams University, Cairo, Egypt.

Nutrient agar [21] was used for the maintenance of *B. megaterium* DSM 2894 strain. It has the following composition (g L⁻¹): 5, peptone; 20, agar and pH adjusted to 7.0 Nutrient broths medium was the same nutrient agar without adding agar. Glucose broth medium [22] was used for fermentation process. It has the following composition (g L⁻¹): 10, glucose, peptone; 3, beef extract and pH adjusted to 7.0

2. 5. Inoculum preparation and fermentation process

For preparation of standard inoculum, a loop of a single colony of the cultural strain was cultured in 500 ml plugged Erlenmeyer flask containing 250 ml of nutrient broth and incubated at 30 °C for 24 h on rotary shaker at 120 rpm. One milliliter of this culture contained 4.8×10⁶ colony forming unit (CFU) and was used as standard inoculum for fermentation process. Fermentation process was carried out in plugged Erlenmeyer flasks (10 L in volume) containing 8.5 L of glucose broth medium and inoculated with 2% of *B. megaterium* DSM 2894 standard inoculum. The inoculated flasks were incubated at 30 °C for 48 h on a rotary shaker at 120 rpm.

2. 6. Data of recorded

2. 6. 1. Leaves content of nutrients

At 90 days of transplanting, a random leaves sample (leaves of 5 plants) of each experimental unit was collected, washed by distilled water, weighted, oven dried at 70° C and weighted and used to determine the contents of nitrogen, phosphorus and potassium according to the methods described in [23]. The total content of Fe, Mn, Zn and Cu were determined using the inductively coupled plasma–optical emission spectrometry (ICP-OES, Perkin-Elmer OPTIMA-2100 DV, Norwalk, CT, USA) according to the methods described by [24]. The calculation $N \times 5.79$ ($1/0.172 = 6.25$) was used to convert

nitrogen content to protein content. The determined macro and micro nutrients were carried out in the leaves digest which prepared as pointed out by [25] as well as all determinations were carried according to [26].

2. 6. 2. Yield and its components

At harvest time (120 days from transplanting), all tuberous roots of plants grown in the rows of each sub-plot were collected, weighted (kg) and data were calculated as total yield ton ha⁻¹. Tuberous root sample (10 roots) was randomly chosen from each experimental unit to chemically analyze of tuberous roots.

2. 6. 3. Nutrients content of tuberous roots

At the harvest (120 days from transplanting), ten uniforms sized of tuberous roots from each replicate were collected, cleaned, cutted, dried, ground and analyzed to its content of N, P, and K according to the methods described by [23]. The total content of Fe, Mn, Zn and Cu were determined as mentioned above. Antioxidant contents were determined according to [27]. As mentioned with leaves before that all determinations were carried out in tuber digests prepared as described by [25].

2. 7. The statistical analysis

The analysis of variance (ANOVA) and LSD ($P \leq 0.05$ and 0.01) were calculated by using GENSTAT statistical package, version12.1 [28].

3 Results

3. 1. Leaves content of some macro and micronutrients

3. 1. 1 Effect of phosphorus fertilizer on some macro and micronutrient contents of sweet potato leaves

The influence of different five levels of mono and di calcium phosphorus fertilizer (MDCPF) is known as calcium superphosphate (CSP)

and inoculation with *Bacillus megaterium* DSM 2894 strain (DSM 2894) and their interactions on leaf nitrogen (N), phosphorus (P) and calcium (Ca) contents of sweet potato plant grown under calcic conditions (CaCO_3 10.8 vs 11.3% as shown in Table 2) during 2019 and 2020 seasons, respectively is presented in Table, 4. The CSP individually had a high significantly increase on sweet potato leaf P and Ca contents in both seasons and N content in the second season, only. The results indicate that applying of 207 and 345 kg ha⁻¹ of CSP [(CSP₆₀) vs (CSP₁₀₀)] have a superior effect on the leaf N ($3.97\text{a}\pm0.13$ vs $3.98\text{a}\pm0.09\%$) and P ($2.96\text{a}\pm0.04$ vs $2.94\text{a}\pm0.01\%$) contents than the other applied levels in both seasons, respectively. On the other hand, fertilized plants by 69 kg ha⁻¹ of CSP (CSP₂₀) gave the highest values for leaf Ca ($1.008\text{a}\pm0.02$ vs $1.006\text{a}\pm0.02\%$) contents in both seasons, respectively. It was mentioned that the incremental percentages were [(8.47 vs 8.45%); (29.26 vs 27.27) and (34.67 vs 26.25%) for N; P and Ca, respectively] as compared with the lowest values ($3.66\text{b}\pm0.09$ vs $3.67\text{d}\pm0.09$ and $2.29\text{d}\pm0.04$ vs $2.31\text{e}\pm0.01$) which recorded with CSP₂₀ treatment for leaf N and P contents in both seasons and ($0.75\text{d}\pm0.04$ vs $0.80\text{e}\pm0.03$) CSP₁₀₀ and CSP₆₀ treatments for leaf Ca contents in 2019 and 2020 growth seasons, respectively.

The results depicted in Table, 5 clearly indicate that enhancements of sweet potato leaf contents of some micronutrients such as iron (Fe), manganese (Mn), zinc (Zn), and copper (Cu) contents were achieved with individual applications of phosphorus fertilizer. The greatest content values of Fe were ($480.92\text{a}\pm5.81$ vs $479.30\text{a}\pm3.06$ mg kg⁻¹) and Cu ($23.10\text{a}\pm1.49$ vs $24.37\text{a}\pm0.64$ mg kg⁻¹) were recorded with applying the minimum rate of CSP (MCP₂₀) in both seasons, respectively. The same treatment (CSP₂₀) gave inconsistent results for both Mn and Zn contents, whereas the superiority

values (91.95 ± 0.42 vs 100.29 ± 1.27 mg kg⁻¹) of leaf Mn contents were recorded with applying 138 kg ha⁻¹ of CSP (PF₄₀) and PF₁₀₀ treatments as well as leaf Zn (26.07 ± 0.91 vs 29.02 ± 0.07 mg kg⁻¹) contents that found in the plants treated with CSP₆₀ and CSP₂₀ in 2019 and 2020 seasons, respectively. The percentages of increasing were [(21.71 vs 18.45) for Fe; (11.24 vs 15.96) for Mn; (17.80 vs 34.85) for Zn and (31.40 vs 22.03%) for Cu], respectively as compared with the lowest values in both seasons.

3. 1. 2 Effect of *Bacillus megaterium* BSM 2894 strain on some nutrient contents of sweet potato leaves

The influences of *Bacillus megaterium* BSM 2894 strain (DSM 2894) inoculation individually demonstrated a significant increases for the sweet potato leaf N, P and Ca contents in 2019 and 2020 seasons as shown in Table 4. The inoculated plants caused in significant increases for all studied macronutrient contents, except, Ca values in the 2019 season, however, the maximum values [(4.04 ± 0.08 vs $4.11 \pm 0.08\%$) and (2.93 ± 0.02 vs $2.99 \pm 0.02\%$)] of leaf N and P content were recorded in inoculated plants with DSM 2894 strain (DSM₁). On the other hand, the highest Ca contents (0.85 ± 0.06 and $0.88 \pm 0.02\%$) was recorded in non-inoculated sweet potato leaf (DSM₀) in both growth seasons, respectively. The percentages of increase were [(16.09 vs 16.10) for N and (33.18 vs 35.91% for P)]. on the other wise, the decrease percentages were (1.17 vs 8.59%) for leaf Ca contents as a compared with the lowest values for studied three nutrients in two seasons, respectively. There was high significantly differences for N, P and Ca values in both seasons between inoculated (DSM₁) and non-inoculated plants (DSM₀) in both seasons.

With respect to influence of inoculated plants with DMS 2894 strain on all abovementioned micronutrients were shown in Table, 5. The results

indicate that micronutrients values in non-inoculated plants were higher than their contents in inoculated plants. The maximum values [(491.40a±19.09 vs 497.44a±1.96 for Fe and (24.67a±2.03 vs 24.03a±0.41 mg kg⁻¹ for Zn)] were produced by non-inoculated plants with DSM 2894 strain (DSM₀) in both seasons, respectively. On the contrast, DSM₁ treatment gave the maximum values (90.26a±0.70 vs 96.88a±0.95 mg kg⁻¹) of sweet potato leaf Mn contents in 2019 and 2020 season, respectively. For leaves Cu values, the results were differed from season to another, where the greatest values of its content were 20.00a±1.28 vs 23.69a±0.63 mg kg⁻¹ which obtained in the inoculated and non-inoculated plants in both seasons, respectively. The main effect of *B. megaterium* inoculation was statistically significant ($P \leq 0.01$) on leaf contents of Fe in both seasons, furthermore, Zn, Mn and Cu contents in the second season, only.

3. 1. 3 Effect of phosphorus fertilizers and *Bacillus megaterium* BSM 2894 strain Interaction on some nutrient contents of sweet potato leaves

It is clear from the data given in Table 4 that some studied macronutrient contents were high significantly affects by the CSP and DSM interaction in two growth seasons. The obtained results indicate that application of 207 kg ha⁻¹ of CSP with *B. megaterium* DSM 2894 strain inoculation (CSP₆₀×DSM₁) treatment gave the highest values (4.60a±0.22 vs 4.46a±0.08%) of leaf N contents, while fertilized plants with 345 kg ha⁻¹ of CSP and inoculated with *B. megaterium* (CSP₁₀₀×DSM₁) treatment produced the maximum values (3.58a±0.07 vs 3.57a±0.01%) for leaf P values. While fertilized plants with minimum level of CSP with non-inoculated plants (CSP₂₀×DSM₀) treatment gave the greatest values (1.03a±0.02 vs 1.02a±0.01%) for leaf Ca content.

In this context, above mentioned results were corroborated between CSP and DSM integration on previous micronutrients contents as depicted in Table 5. The highest values of sweet potato leaf micronutrients values reached to [(531.80a±9.12 vs 532.00a±1.62 for Fe; 103.58a±11.16 vs 110.13a±6.11 for Mn and 30.22a±0.27 vs 30.30a±0.31 mg kg⁻¹ for Cu)] which recorded with using (CSP₂₀×DSM₀); (CSP₆₀×DSM₀) and (CSP₂₀×DSM₁) treatments in 2019 and 2020 seasons, respectively. For Zn values, there were clear differences in the results of two seasons. The greatest value (28.70a±1.79 vs 31.48a±0.07 mg kg⁻¹) of leaf Zn contents were achieving with applying 60% and 20% of CSP in non-inoculated plants [(CSP₆₀×DSM₀) and (CSP₂₀×DSM₀)] treatments in the first and second seasons, respectively. The analysis of variance indicates that there were high significantly variations for the mentioned micronutrients content in both seasons.

Table 4 Influence of phosphorus fertilizer, *Bacillus megaterium* DSM 2894 strain and their interactions on some leaf nutrients content of CaCO₃-stressed sweet potato plants in 2019 and 2020

Treatment	N			P			Ca		
Ca(H ₂ PO ₄) ₂	Leaves (%)								
	DSM ₀	DSM ₁	Mean	DSM ₀	DSM ₁	Mean	DSM ₀	DSM ₁	Mean
2019 season									
CSP ₂₀	3.48de±0.15	3.85bc±0.03	3.66b±0.09	1.83e±0.06	2.74b±0.02	2.29d±0.04	0.99b±0.01	1.03a±0.02	1.01a±0.02
CSP ₄₀	3.40de±0.06	4.09b±0.05	3.75ab±0.06	2.10d±0.04	2.75b±0.03	2.42cd±0.04	0.89c±0.02	0.91c±0.02	0.90b±0.02
CSP ₆₀	3.34e±0.04	4.60a±0.22	3.97a±0.13	2.37c±0.01	2.75b±0.06	2.56bc±0.04	0.80d±0.02	0.79d±0.02	0.79c±0.02
CSP ₈₀	3.50de±0.03	3.95bc±0.03	3.73b±0.03	2.36c±0.01	2.81b±0.20	2.58b±0.02	0.79d±0.03	0.75e±0.01	0.77cd±0.02
CSP ₁₀₀	3.67cd±0.04	3.68cd±0.06	3.68b±0.05	2.35c±0.01	3.58a±0.07	2.96a±0.04	0.79d±0.04	0.71f±0.03	0.75d±0.04
Mean	3.48b±0.04	4.04a±0.08	3.76±0.71	2.20b±0.02	2.93a±0.02	2.56±0.05	0.85a±0.06	0.84a±0.02	0.85±0.04
LSD _{0.05}	CSP	DSM	CSPxDSM	CSP	DSM	CSPxDSM	CSP	DSM	CSPxDSM
	0.23**	ns	0.28**	0.10**	0.15**	0.22**	0.01*	0.02**	0.03**
2020 season									
CSP ₂₀	3.37g±0.11	3.97c±0.06	3.67d±0.09	1.91i±0.01	2.72e±0.01	2.31e±0.01	0.99b±0.03	1.02a±0.01	1.01a±0.02
CSP ₄₀	3.38fg±0.07	4.23b±0.09	3.81c±0.08	2.12h±0.01	2.73d±0.02	2.43d±0.02	0.89d±0.02	0.91c±0.01	0.90b±0.02
CSP ₆₀	3.51f±0.09	4.46a±0.08	3.98a±0.09	2.33f±0.01	2.75c±0.03	2.54c±0.02	0.79g±0.02	0.80g±0.03	0.80e±0.03
CSP ₈₀	3.65e±0.08	4.11b±0.08	3.88b±0.08	2.32f±0.01	3.16b±0.01	2.74b±0.01	0.79h±0.02	0.83f±0.02	0.81d±0.02
CSP ₁₀₀	3.80d±0.06	3.79d±0.07	3.80c±0.07	2.31g±0.01	3.57a±0.01	2.94a±0.01	0.78i±0.01	0.86e±0.02	0.82c±0.02
Mean	3.54b±0.08	4.11a±0.08	3.83±0.08	2.20b±0.01	2.99a±0.02	2.59±0.02	0.85b±0.02	0.88a±0.02	0.87±0.02
LSD _{0.05}	CSP	DSM	CSPxDSM	CSP	DSM	CSPxDSM	CSP	DSM	CSPxDSM
	0.06**	0.05**	0.13**	0.01**	0.01**	0.01**	0.00	0.00	0.01

Mean values (\pm SE) with different letters in each columns are significant ($p \leq 0.05$). CSP₂₀ = 69 kg of Ca(H₂PO₄)₂, CSP₄₀ = 138 kg of Ca(H₂PO₄)₂, CSP₆₀ = 207 kg of Ca(H₂PO₄)₂, CSP₈₀ = 276 kg of Ca(H₂PO₄)₂, CSP₁₀₀ = 345 kg of Ca(H₂PO₄)₂, DSM₀ = non-inoculated with *Bacillus megaterium* DSM 2894 strain, DSM₁ = inoculated with *Bacillus megaterium* DSM 2894 strain, N = nitrogen content, P = phosphorus content and Ca = calcium content

Table 5 Influence of phosphorus fertilizer, *Bacillus megaterium* DSM 2894 strain and their interaction on some leaf micronutrients content of sweet potato plants grown on calcareous saline soil in 2019 and 2020 seasons

2019 season												
Treatment		Leaves (mg kg ⁻¹)										
Ca(H ₂ PO ₄) ₂	Fe			Mn			Zn			Cu		
	BSM ₀	BSM ₁	Mean	BSM ₀	BSM ₁	Mean	BSM ₀	BSM ₁	Mean	BSM ₀	BSM ₁	Mean
CSP ₂₀	531.80a±9.12	430.05b±2.51	480.92a±5.82	74.40bc±0.22	100.02a±0.22	87.21a±0.22	20.77e±1.24	26.46ab±0.01	23.61bc±0.63	15.99cd±2.71	30.22a±0.27	23.10a±1.49
CSP ₄₀	406.90b±47.40	383.39b-d±1.99	395.14ab±24.70	95.40a±0.19	88.49ab±0.65	91.95a±0.42	24.95bc±0.21	24.95bc±0.02	24.95ab±0.12	21.68bc±0.45	21.82bc±1.42	21.75ab±0.94
CSP ₆₀	506.80a±24.19	336.74d±1.47	421.77a-c±12.83	103.58a±11.16	76.96bc±1.09	90.27a±6.13	28.70a±1.79	23.44cd±0.02	26.07a±0.91	24.35ab±1.97	13.42d±2.57	18.89ab±2.27
CSP ₈₀	507.20a±13.18	362.03cd±7.60	434.62bc±10.39	87.89ab±4.84	87.23ab±0.37	87.56a±2.61	25.95b±0.80	22.35de±0.04	24.15b±0.42	20.22b-d±2.57	15.99cd±0.44	18.11b±1.51
CSP ₁₀₀	504.30a±1.56	405.40bc±0.58	454.85c±1.07	66.70c±7.98	98.61a±1.17	82.66a±4.58	23.00c-e±0.02	21.26de±0.06	22.13c±0.04	16.90cd±3.14	18.56b-d±1.69	17.58b±2.42
Mean	491.40a±19.09	383.52b±2.83	437.46±10.96	85.59a±4.88	90.26a±0.70	87.93±2.79	24.67a±2.03	23.69a±0.08	24.18±1.06	19.77a±2.17	20.00a±1.28	19.88±
LSD _{0.05}	CSP	DSM	CSPxDSM	CSP	DSM	CSPxDSM	CSP	DSM	CSPxDSM	CSP	DSM	CSPxDSM
	23.97**	47.62*	53.59**	ns	ns	14.84**	ns	1.82	2.34	ns	ns	6.45**
2020 season												
CSP ₂₀	532.00a±1.62	426.60e±4.50	479.30a±3.06	73.80f±1.06	99.18c±0.70	86.49d±0.88	31.48a±0.07	26.56b±0.07	29.02a±0.07	18.44e±0.97	30.30a±0.31	24.37a±0.64
CSP ₄₀	498.20b±0.12	382.63g±2.43	440.41c±1.28	94.98cd±0.06	88.68d±0.54	91.83c±0.30	25.27c±0.03	24.97c±0.03	25.12b±0.03	23.30cd±0.48	21.33d±1.14	22.32c±0.81
CSP ₆₀	470.60d±6.35	338.66i±0.36	404.63e±3.36	110.13a±6.11	78.18ef±0.38	94.15bc±3.25	19.65g±0.59	23.38d±0.01	21.52c±0.31	27.58b±0.58	12.36f±1.96	19.97e±1.27
CSP ₈₀	483.60c±1.04	369.95h±1.09	426.77d±1.07	98.61c±0.63	98.85c±0.92	98.73ab±0.78	20.93f±0.04	22.24e±0.02	21.58c±0.03	25.76bc±0.38	17.54e±1.34	21.65d±0.86
CSP ₁₀₀	502.80b±0.69	401.24f±1.82	452.02b±1.26	81.06d±0.31	119.51a±2.22	100.29a±1.27	22.80de±0.09	21.10f±0.03	21.95c±0.06	23.36cd±0.76	22.72d±0.72	23.04b±0.74
Mean	497.44a±1.96	440.41b±2.04	440.63±2.00	91.72b±4.09	96.88a±0.95	94.30±2.52	24.03a±0.41	23.65b±0.08	23.84±0.25	23.69a±0.63	20.85b±1.09	22.27±0.86

LSD _{0.05}	CSP	DSM	CSPxDSM	CSP	DSM	CSPxDSM	CSP	DSM	CSPxDSM	CSP	DSM	CSPxDSM
	3.62**	6.39**	8.10**	2.90**	5.20**	6.48**	0.27**	0.44**	0.61**	1.09**	0.59**	2.44**

Mean values (\pm SE) with different letters in each columns are significant ($p \leq 0.05$). CSP₂₀ = 69 kg of Ca(H₂PO₄)₂, CSP₄₀ = 138 kg of Ca(H₂PO₄)₂, CSP₆₀ = 207 kg of Ca(H₂PO₄)₂, CSP₈₀ = 276 kg of Ca(H₂PO₄)₂, CSP₁₀₀ = 345 kg of Ca(H₂PO₄)₂, DSM₀ = non-inoculated with *Bacillus megaterium* DSM 2894 strain, DSM₁ = inoculated with *Bacillus megaterium* DSM 2894 strain, N = nitrogen content, P = phosphorus content and Ca = calcium content

3. 2 Tuberous root nutrient contents

3. 2. 1 Effects of phosphorus fertilizer on some nutrient contents of sweet potato tuberous roots

Results pertaining to effect of phosphorus fertilizer individually, or with inoculated plants with *B. megaterium* presented in Table, 6. In this study, the best effect for CSP fertilizer, alone indicate that the maximum improvement ($2.19a \pm 0.06$ vs 2.44 ± 0.08) for tuberous root N contents in the plants treated with MCP₁₀₀ and 276 kg ha⁻¹ of CSP (CSP₈₀) treatments in both seasons, respectively. Regarding the uptake of P and Ca, the results revealed those highest values ($4.43a \pm 0.01$ vs $2.94a \pm 0.01\%$) for P contents was obtained as a result of CSP₁₀₀ treatment, on the other side, for sweet potato tuberous root Ca contents were recorded ($0.35a \pm 0.04$ vs $1.01a \pm 0.02\%$) with three levels of CSP *i.e.* CSP₂₀; CSP₄₀ and CSP₆₀ treatments in 2019 season and CSP₂₀ treatment in 2020 only. The incremental percentages reached to [(12.88 vs 7.96; 40.75 for N and 40.64 and 27.27 for P and 12.90 vs 26.25% for Ca)], respectively in 2019 and 2020 seasons. The phosphorus fertilizer had high significantly increases on all determined nutrients, except sweet potato tuberous root N contents in the first season, only.

The main effect of phosphorus fertilizer on tuberous root accumulations of Fe, Mn, Zn and Cu was significantly ($P \leq 0.05$) which was shown in Table 7. The greatest accumulation values were [(836.50a \pm 1.56 vs 853.30a \pm 5.25 for Fe; (14.60a \pm 0.35 vs 14.41a \pm 0.38 for Mn ; 28.37a \pm 1.01 vs 28.44a \pm 0.05 for Zn and 27.91a \pm 1.54 vs 24.67a \pm 0.80 mg kg⁻¹ for Cu)] which produced with CSP₂₀; CSP₆₀; CSP₂₀ and CSP₁₀₀ treatments in 2019 and 2020 seasons, respectively. The lowest accumulation values [(566.40d \pm 0.79 vs 565.20d \pm 1.39 for Fe and 8.83d \pm 0.43 vs 9.06d \pm 0.21 for Mn; 17.29e \pm 0.37 vs 17.08e \pm 0.08 for Zn and 16.39d \pm 1.31 vs

14.67 \pm 1.96 for Cu)] which were produced with CSP₁₀₀ treatment for both Fe and Mn; CSP₆₀ for Zn in two seasons and CSP₂₀ and CSP₆₀ treatments for Cu in both seasons, respectively. All previous treatments gave incremental percentages [(47.69 vs 50.97 for Fe; 65.35 vs 59.05 for Mn; 64.08 vs 66.51 for Zn and 70.29 vs 68.17% for Cu)] as compared with the lowest values in both seasons, respectively.

Table 6 Influence of phosphorus fertilizer, *Bacillus megaterium* DSM 2894 strain and their interactions on some tuber nutrients accumulation of CaCO₃-stressed sweet potato plants in 2019 and 2020

Treatment	N			P			Ca		
Ca(H ₂ PO ₄) ₂	Tubers (%)								
	DSM ₀	DSM ₁	Mean	DSM ₀	DSM ₁	Mean	DSM ₀	DSM ₁	Mean
2019 season									
CSP ₂₀	2.16b±0.01	1.73d±0.12	1.94d±0.07	2.59de±0.00	3.70d±0.01	3.15d±0.01	0.27j±0.03	0.43a±0.02	0.35a±0.03
CSP ₄₀	2.19b±0.04	1.82d±0.06	2.01c±0.05	2.72g±0.02	3.64de±0.02	3.18d±0.02	0.27ic±0.04	0.42b±0.03	0.35a±0.04
CSP ₆₀	2.15b±0.01	1.99c±0.04	2.07b±0.03	2.94f±0.10	3.59g±0.03	3.27c±0.07	0.28h±0.02	0.41c±0.06	0.35a±0.04
CSP ₈₀	2.15b±0.06	2.15b±0.04	2.15a±0.05	3.53e±0.10	4.21b±0.02	3.87b±0.06	0.30g±0.03	0.36d±0.03	0.33b±0.03
CSP ₁₀₀	2.07bc±0.07	2.32a±0.04	2.19a±0.06	4.03c±0.01	4.83a±0.01	4.43a±0.01	0.31e±0.03	0.30f±0.03	0.31c±0.03
Mean	2.14a±0.04	2.00b±0.06	2.07±0.05	3.16b±0.02	3.99a±0.02	3.58±0.04	0.29b±0.06	0.39a±0.02	0.85±0.04
LSD _{0.05}	CSP	DSM	CSPxDSM	CSP	DSM	CSPxDSM	CSP	DSM	CSPxDSM
	0.23**	ns	0.28**	0.10**	0.15**	0.22**	0.01*	0.02**	0.03**
2020 season									
CSP ₂₀	2.39a±0.11	2.13bc±0.06	2.26b±0.09	2.57j±0.01	3.77d±0.01	3.17c±0.01	0.99b±0.03	1.02a±0.01	1.01a±0.02
CSP ₄₀	2.31abc±0.07	2.15bc±0.09	2.23b±0.08	2.69i±0.01	3.68e ±0.02	3.18c±0.02	0.89d±0.02	0.91c±0.01	0.90b±0.02
CSP ₆₀	2.38ab±0.09	2.31abc±0.08	2.34ab±0.09	2.80h±0.01	3.58f±0.03	3.19c±0.02	0.79g±0.02	0.80g±0.03	0.80e±0.03
CSP ₈₀	2.44a±0.08	2.43a±0.08	2.44a±0.08	3.41g±0.01	4.21b±0.01	3.81b±0.01	0.79h±0.02	0.83f±0.02	0.81d±0.02
CSP ₁₀₀	2.28abc±0.06	2.48a±0.07	2.38ab±0.07	4.01c±0.01	4.84a±0.01	4.42a±0.01	0.78i±0.01	0.86e±0.02	0.82c±0.02
Mean	3.54b±0.08	4.11a±0.08	3.83±0.08	3.10b±0.01	4.01a±0.02	3.56±0.02	0.85b±0.02	0.88a±0.02	0.87±0.02
LSD _{0.05}	CSP	DSM	CSPxDSM	CSP	DSM	CSPxDSM	CSP	DSM	CSPxDSM
	0.06**	0.05**	0.13**	0.01**	0.01**	0.01**	0.00	0.00	0.01

Mean values (\pm SE) with different letters in each columns are significant ($p \leq 0.05$). CSP₂₀ = 69 kg of Ca(H₂PO₄)₂, CSP₄₀ = 138 kg of Ca(H₂PO₄)₂, CSP₆₀ = 207 kg of Ca(H₂PO₄)₂, CSP₈₀ = 276 kg of Ca(H₂PO₄)₂, CSP₁₀₀ = 345 kg of Ca(H₂PO₄)₂, DSM₀ = non-inoculated with *Bacillus megaterium* DSM 2894 strain, DSM₁ = inoculated with *Bacillus megaterium* DSM 2894 strain, N = nitrogen content, P = phosphorus content and Ca = calcium content

Table 7 Influence of phosphorus fertilizer, *Bacillus megaterium* DSM 2894 strain and their interaction on some tuber micronutrients accumulation of sweet potato plants grown on calcareous saline soil in 2019 and 2020 seasons

2019 season												
Treatment		Leaves (mg kg ⁻¹)										
Ca(H ₂ PO ₄) ₂	Fe			Mn			Zn			Cu		
	BSM ₀	BSM ₁	Mean	BSM ₀	BSM ₁	Mean	BSM ₀	BSM ₁	Mean	BSM ₀	BSM ₁	Mean
CSP ₂₀	907.80a \pm 2.66	765.20d \pm 0.46	836.50a \pm 1.56	10.98d \pm 0.96	11.72cd \pm 0.62	11.35c \pm 0.79	28.94a \pm 0.01	27.80b \pm 2.00	28.37a \pm 1.01	18.08de \pm 0.05	14.70ef \pm 2.57	16.39d \pm 1.31
CSP ₄₀	591.03f \pm 1.47	827.40b \pm 2.08	709.50b \pm 1.78	10.69d \pm 0.43	15.33b \pm 0.04	13.01b \pm 0.24	21.86d \pm 0.02	23.58c \pm 1.02	22.72b \pm 0.52	20.85cd \pm 0.51	13.64ef \pm 1.76	17.25cd \pm 1.14
CSP ₆₀	286.70i \pm 2.02	889.60a \pm 4.62	588.15c \pm 3.32	10.26de \pm 0.16	18.94a \pm 0.54	14.60a \pm 0.35	15.22h \pm 0.69	19.36f \pm 0.05	17.29e \pm 0.37	23.46bc \pm 0.99	12.58f \pm 0.94	18.02c \pm 0.97
CSP ₈₀	362.87h \pm 2.51	797.90c \pm 2.60	580.38c \pm 2.56	9.79de \pm 0.32	13.57bc \pm 0.44	11.68c \pm 0.38	17.89g \pm 1.12	19.20f \pm 0.02	18.58d \pm 0.57	20.46cd \pm 0.25	25.39b \pm 0.47	22.92b \pm 0.36
CSP ₁₀₀	426.60g \pm 1.50	706.20e \pm 0.58	566.40d \pm 0.79	9.46de \pm 0.50	8.20e \pm 0.35	8.83d \pm 0.43	20.12e \pm 1.73	19.16f \pm 1.15	19.64c \pm 1.44	17.62de \pm 1.35	38.20a \pm 1.73	27.91a \pm 1.54
Mean	515.00b \pm 2.03	797.26a \pm 2.07	656.13 \pm 2.05	10.24b \pm 0.47	13.55a \pm 0.40	11.89 \pm 0.44	20.81b \pm 0.70	21.83a \pm 0.85	21.32 \pm 0.64	20.09a \pm 0.63	20.90a \pm 1.50	20.50 \pm 1.07
LSD _{0.05}	CSP	DSM	CSP \times DSM	CSP	DSM	CSP \times DSM	CSP	DSM	CSP \times DSM	CSP	DSM	CSP \times DSM
	8.37**	5.06**	8.40**	0.94**	0.42**	2.10**	0.28**	0.40**	0.62**	ns	1.56**	1.88**
2020 season												
CSP ₂₀	946.40a \pm 8.08	760.20e \pm 2.42	853.30a \pm 5.25	11.76c \pm 0.51	11.54c \pm 0.52	11.65c \pm 0.38	29.08a \pm 0.07	27.80b \pm 0.02	28.44a \pm 0.05	16.22c \pm 1.03	26.80ab \pm 4.41	21.51ab \pm 2.72
CSP ₄₀	607.77g \pm 2.42	828.20c \pm 1.62	717.99b \pm 2.02	10.96cd \pm 0.28	15.10b \pm 0.09	13.03b \pm 0.19	21.91d \pm 0.01	23.61c \pm 0.01	22.76b \pm 0.01	18.52c \pm 0.83	17.66c \pm 0.57	18.09bc \pm 0.70
CSP ₆₀	269.14j \pm 3.24	896.20b \pm 0.81	582.67c \pm 2.03	10.16de \pm 0.05	18.66a \pm 0.70	14.41a \pm 0.38	14.74j \pm 0.06	19.42f \pm 0.01	17.08e \pm 0.04	20.82bc \pm 0.64	8.52d \pm 3.28	14.67c \pm 1.96
CSP ₈₀	347.57i \pm 1.05	800.30d \pm 1.21	573.94cd \pm 1.13	9.61de \pm 0.11	13.86b \pm 0.28	11.74c \pm 0.20	17.42i \pm 0.03	19.32g \pm 0.01	18.37d \pm 0.02	19.46c \pm 0.85	19.88c \pm 1.37	19.67b \pm 1.11
CSP ₁₀₀	426.00h \pm 1.15	704.40f \pm 1.62	565.20d \pm 1.39	9.06e \pm 0.27	9.06e \pm 0.15	9.06d \pm 0.21	20.10e \pm 0.01	19.22h \pm 0.01	19.66c \pm 0.01	18.10c \pm 1.07	31.24a \pm 0.53	24.67a \pm 0.80
Mean	519.38b \pm 3.19	797.86a \pm 1.54	658.62 \pm 2.36	10.31b \pm 0.24	13.64a \pm 0.29	11.98 \pm 0.27	20.65b \pm 0.04	21.87a \pm 0.01	21.26 \pm 0.03	18.62a \pm 0.88	20.82a \pm 2.03	19.72 \pm
LSD _{0.05}	CSP	DSM	CSP \times DSM	CSP	DSM	CSP \times DSM	CSP	DSM	CSP \times DSM	CSP	DSM	CSP \times DSM

10.88**	14.20**	24.33**	0.58**	0.71**	1.29**	0.031**	0.103**	0.07**	ns	4.43**	6.41**
Mean values (± SE) with different letters in each columns are significant ($p \leq 0.05$). MCP ₂₀ = 69 kg of Ca(H ₂ PO ₄) ₂ , MCP ₄₀ = 138 kg of Ca(H ₂ PO ₄) ₂ , MCP ₆₀ = 207 kg of Ca(H ₂ PO ₄) ₂ , MCP ₈₀ = 276 kg of Ca(H ₂ PO ₄) ₂ , MCP ₁₀₀ = 345 kg of Ca(H ₂ PO ₄) ₂ , DSM ₀ = non-inoculated with <i>Bacillus megaterium</i> DSM 2894 strain, DSM ₁ = inoculated with <i>Bacillus megaterium</i> DSM 2894 strain, N = nitrogen content, P = phosphorus content and Ca = calcium content											

3. 2. 2 Effect of *Bacillus megaterium* DSM 2894 strain on some nutrient contents of sweet potato tuberous roots

The results indicate that DSM 2894 treatment was the best ever for sweet potato tuberous root P and Ca accumulation as mentioned in Table 6. The highest tuberous root ($3.99a \pm 0.02$ vs $4.01a \pm 0.02$ for P and $0.39a \pm 0.02$ vs $0.88a \pm 0.02$) for P and Ca accumulations which recorded in inoculated plants with DSM 2894 strain in two seasons. On the other side, non-inoculated plants gave the superior values ($2.14a \pm 0.04$ vs $2.36a \pm 0.08$) for N accumulations. The maximum increasing percentages reached to [(5.00 vs 2.61 for N; 10.83 vs 29.36 for P) and (34.48 vs 3.53% for Ca)] in both seasons, respectively. The ANOVA results showed that tuberous root P and Ca accumulations in both seasons and N values in the first seasons were significantly affected with inoculation with DSM 2894 bacteria.

The presented results in **Table 7** display that the influences of BSM 2894 strain were very excellent in both growth seasons. Inoculated plants with BSM 2894 strain have the superiority micronutrients accumulations as a compared with non-inoculated plants. Whereas, the maximum tuberous root [($797.26a \pm 2.07$ vs $797.86a \pm 1.54$; $13.55a \pm 0.44$ vs $13.64a \pm 0.29$; $21.83a \pm 0.85$ vs $21.87a \pm 0.01$ and $20.90a \pm 1.50$ vs $20.82a \pm 2.03$ mg kg⁻¹ for Fe; Mn; Zn and Cu, respectively)] which found in the inoculated plants during 2019 and 2020 seasons, respectively. Based on these results, the increasing percentages were [(54.81 vs 53.62; 32.32 vs 32.30; 4.90 vs 5.91 and 4.03 vs 11.86%)] for the determined abovementioned nutrients in both season, respectively. There was significant effect ($p \leq 0.01$) of the inoculation on tuber uptake of all abovementioned micronutrients.

3. 2. 3 Effect of phosphorus fertilizer and *Bacillus megaterium* DSM 2894 strain interaction on some nutrient accumulations of sweet potato tuberous roots

It is clear from statistical analysis results that total tuberous root N, P and Ca contents were high significantly differences ($P \leq 0.01$) due to the combined different levels of CSPF and DSM 2894 strain inoculation interactions on sweet potato tuberous root N, P and Ca accumulations presented in Table 6. The $CSP_{100} \times DSM_1$ treatment enhanced the accumulation of all studied nutrients as shown in the following values [(2.32a \pm 0.04 vs 2.48a \pm 0.07 for N and 4.83a \pm 0.01 vs 4.84a \pm 0.01% for P)], which produced with $CSP_{100} \times DSM_1$ treatment for both tuberous root N and P accumulations, together. On the contrast, maximum tuberous root Ca accumulations (0.43a \pm 0.02 vs 1.02 \pm 0.01%) were obtained with minimum fertilized plants with BSM 2894 inoculation ($CSP_{20} \times DSM_1$). In this connection, increasing percentages were 34.10 vs 16.43%; 86.95 vs 88.33% and 59.26 vs 30.77% for N; P and Ca, respectively when a comparison between highest and lowest accumulation values.

With regard to the interaction between different levels of CSPF and DSM 2894 strain, statistical analysis had high significantly increase effects on tuberous root Fe, Mn, Zn and Cu contents as presented in Table 7. It is evident that highest value accumulations were [(907.80a \pm 2.66 vs 946.40a \pm 8.08 for Fe, 18.94a \pm 0.54 vs 18.66a \pm 0.70 for Mn; 28.94a \pm 0.01 vs 29.08a \pm 0.07 for Zn and 38.20a \pm 1.73 vs 31.24a \pm 0.53 mg kg⁻¹ for Cu)] which were obtained with treated plants [($CSP_{20} \times DSM_0$; $CSP_{60} \times DSM_1$; $CSP_{20} \times DSM_0$ and $CSP_{100} \times DSM_1$)] treatment in 2019 and 2020 seasons, respectively. The results in this investigation indicate that increasing rates of tuberous roots nutrient accumulations for both Mn and Cu were clearly appeared resulting in mixture phosphorus fertilizer and DSM 2894 strain ($CSP_{60} \times DSM_1$) and ($CSP_{100} \times DSM_1$) treatments. On the other hand, the inoculation results with DSM 2894 did not appear effects on Fe and Zn accumulations, furthermore the highest values were recorded with ($CSP_{20} \times DSM_1$) treatment for both micronutrients.

3.3 Tuberous root yield and quality

3.3.1 Effect of phosphorus fertilizers and *Bacillus megaterium* DSM 2894 strain and their interactions on Anti-radical power and protein contents of sweet potato tuberous root

As shown in Figures 1-6 that applying either different levels of CSPF or inoculants with DSM 2894 strain as well as their interaction affect tuberous root Anti-radical power (ARP) and protein of sweet potato plants. The maximum values [(173.79a±10.01 vs 188.89a±7.06 and 13.70a±1.80 vs 15.23a±2.33)] were obtained with plants fertilized with CSP₆₀ of CSPF individually for tuberous root ARP content in two seasons. For protein contents, the CSP₁₀₀ and CSP₈₀ treatments were the best treatments in 2019 and 2020, respectively. The different levels of MCP have a significant effect ($P \leq 0.01$) on the content of ARP in both seasons and protein contents in the first season only, while had no significantly differences for protein content in the second season.

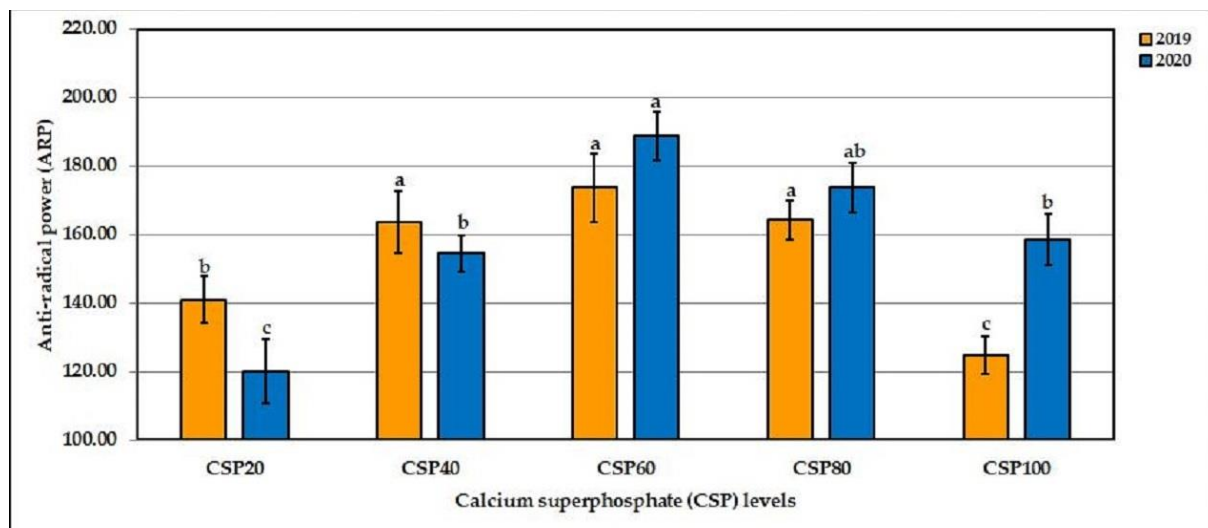


Figure 1. Influence of phosphorus fertilizer levels on anti-radical power (ARP) of tubers of CaCO₃-stressed sweet potato plants grown in 2019 and 2020. Bars with a different letter indicate significant different between treatments at $p \leq 0.05$

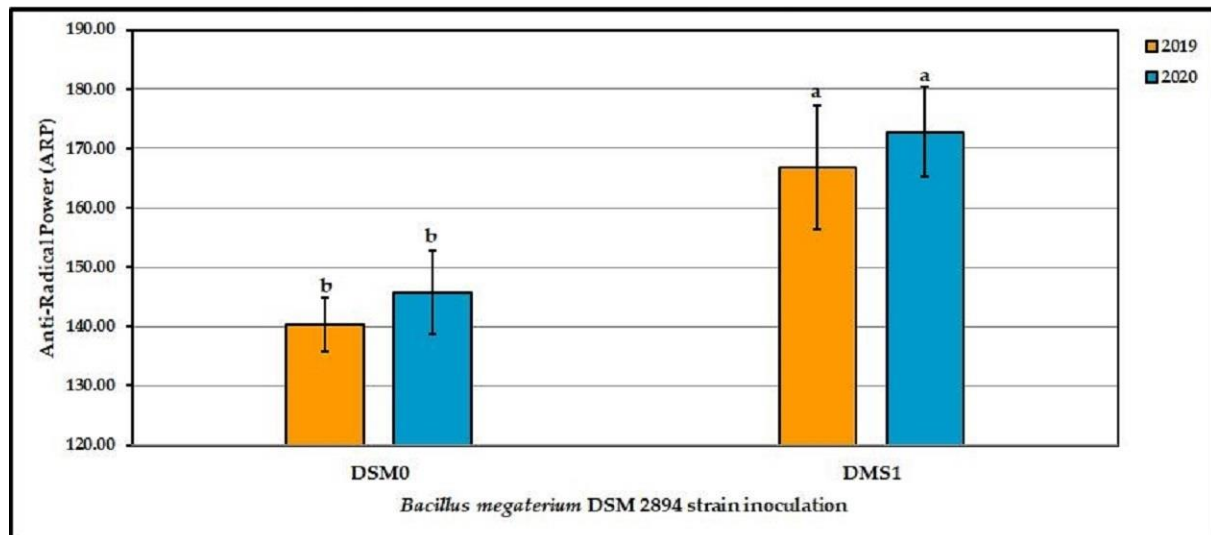


Figure 2. Influence of *Bacillus megaterium* DMS 2894 strain on anti-radical power (ARP) of tubers of *CaCO*₃-stressed sweet potato plants grown in 2019 and 2020. Bars with a different letter indicate significant different between treatments at $p \leq 0.05$

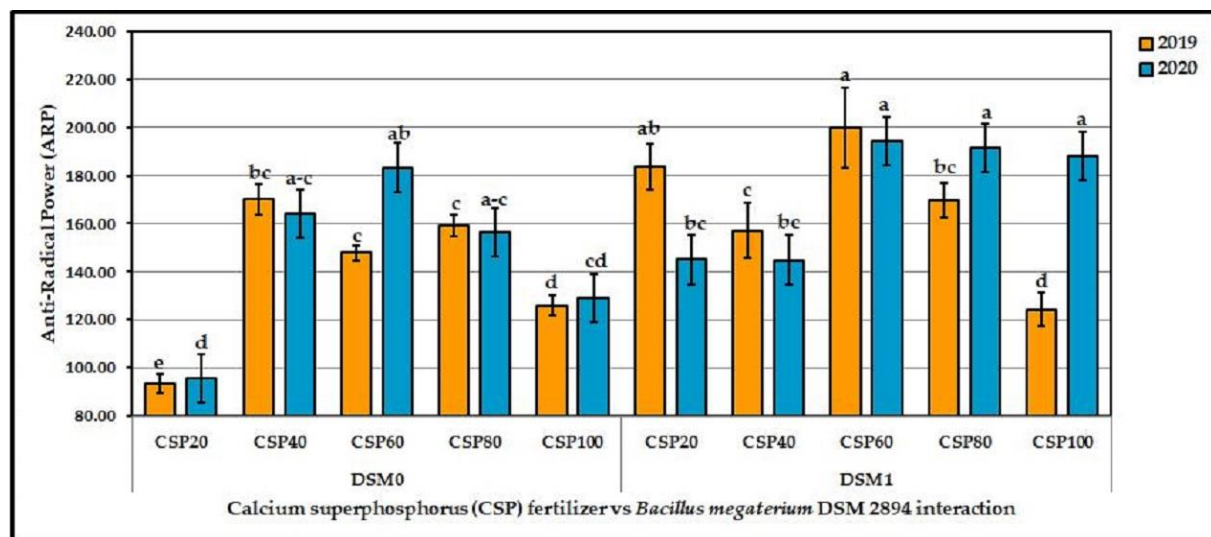


Figure 3. Influence of phosphorus fertilizer level, *Bacillus megaterium* DMS 2894 strain interactions on anti-radical power (ARP) of tubers of *CaCO*₃-stressed sweet potato plants grown in 2019 and 2020. Bars with a different letter indicate significant different between treatments at $p \leq 0.05$

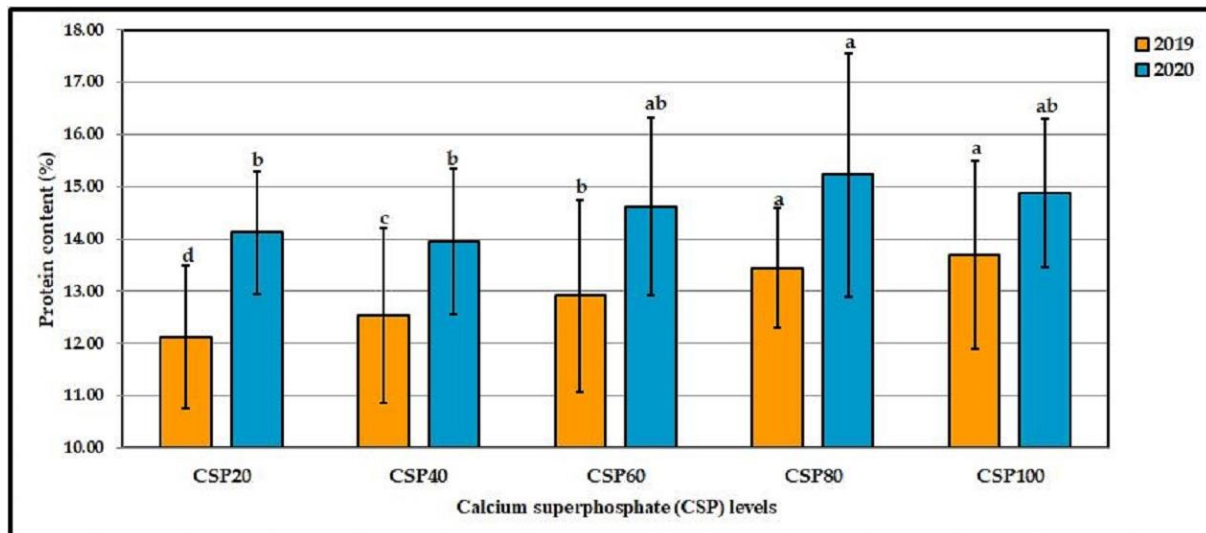


Figure 4. Influence of phosphorus fertilizer levels on protein content of tubers of CaCO_3 -stressed sweet potato plants grown in 2019 and 2020. Bars with a different letter indicate significant different between treatments at $p \leq 0.05$

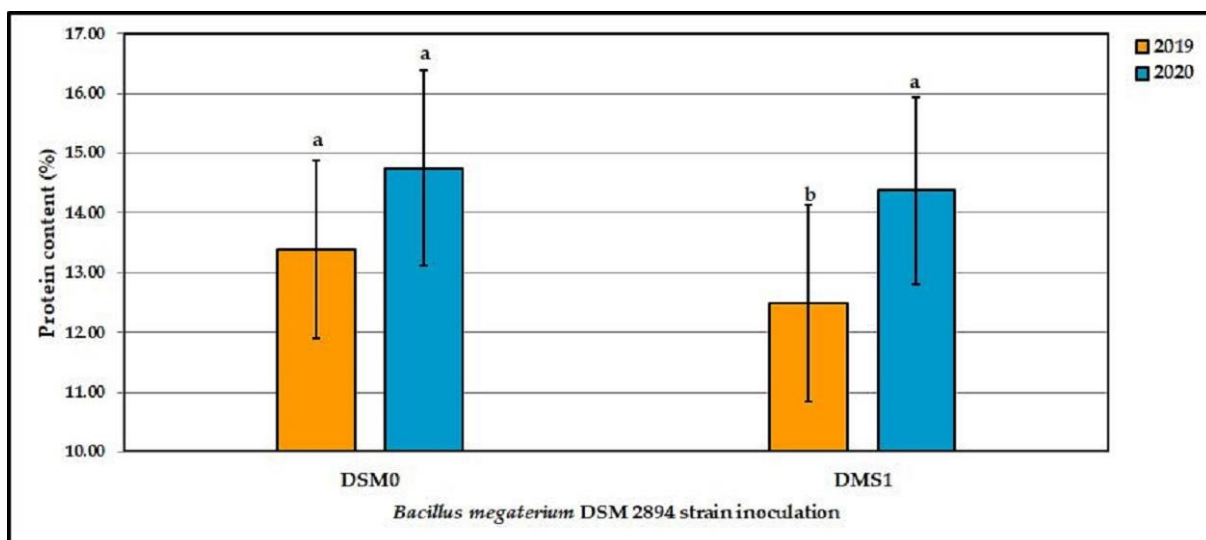


Figure 5. Influence of *Bacillus megaterium* DMS 2894 strain on protein content of tubers of CaCO_3 -stressed sweet potato plants grown in 2019 and 2020. Bars with a different letter indicate significant different between treatments at $p \leq 0.05$

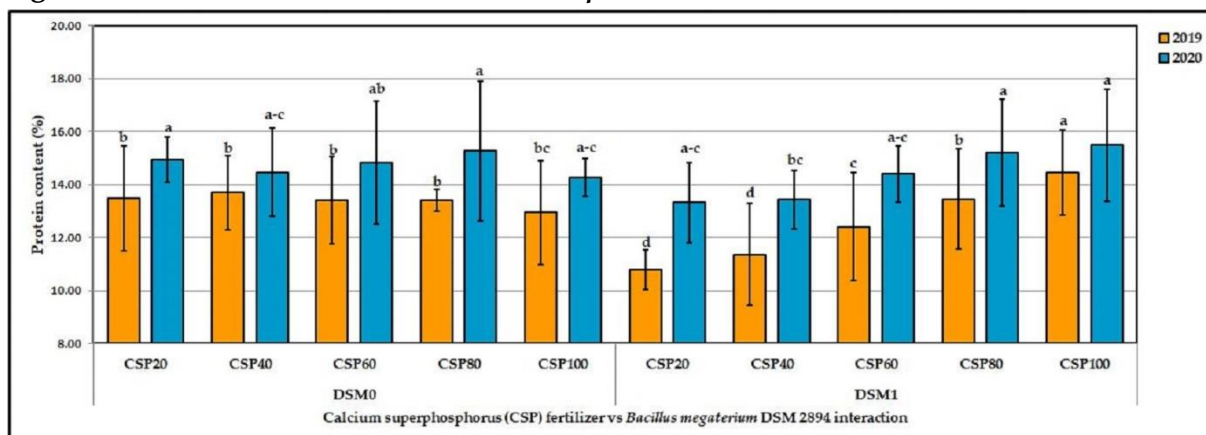


Figure 6. Influence of phosphorus fertilizer level, *Bacillus megaterium* DMS 2894 strain interactions on protein content of tubers of CaCO₃-stressed sweet potato plants grown in 2019 and 2020. Bars with a different letter indicate significant different between treatments at $p \leq 0.05$

With respect to the influence of DSM 2894 inoculation, it is clear from Figures 2 and 5 indicate that the superior values were [(166.89a±10.40 vs 172.80a±7.51 for ARP and 13.39a±1.48 vs 14.75a±1.64 for protein)] which were produced in the inoculated (DSM₁) for ARP and non-inoculated (DSM₀) plants for protein contents in 2019 and 2020, respectively. Inoculation treatment have a significant effects ($P \leq 0.01$) for ARP contents in both seasons and protein contents in the first season, while had no significantly differences for protein content in the second season.

The influence of mixture phosphorus fertilizer with *B. megaterium* in (Figs 4 and 7) mentioned that the superiority (MCP₆₀×BSM₁) treatment for ARP values in both seasons. But, the greatest values (14.47 vs 15.49%) were found with the treatment of (MCP₁₀₀×BSM₁) and (MCP₈₀×BSM₁) in both seasons, respectively. Statistical analysis of variance indicated that combined treatment have a high significant increase in antioxidant and protein contents in the first season, where it was non-significantly differences in the second season.

3. 5. 2 Effect of phosphorus fertilizers and *Bacillus megaterium* DSM 2894 strain and their interactions on tuberous root yield of sweet potato

All treatment had a significant effect on the tuberous root yield of sweet potato as a result different levels of CSPF. Results depicted in Figure 7 indicate that the maximum values [(14.95a±1.56 vs 15.78a±1.50 followed by 14.88a±1.75 vs 14.77ab±1.82 tons ha⁻¹)] of tuberous root yield of sweet potato which were obtained by using CSP₆₀ and CSP₁₀₀ treatments in 2019 and 2020 seasons, respectively. Furthermore, the incremental percentages were 40.11 and 47.06% as compared with the lowest values (10.67c±1.23 vs 10.73c±1.30) which were obtained with CSP₂₀

treatment in both seasons. Regarding influence of DSM 2894 on tuberous root yield as shown in Figure 8 pronounce that inoculated plants gave the highest values (16.07 ± 1.64 vs 16.37 ± 2.15 tons ha^{-1}) of tuberous roots in two seasons, respectively. when a comparison between inoculated and non-inoculated plants, the increasing percentages reached 47.16 vs 43.72% in both seasons, respectively.

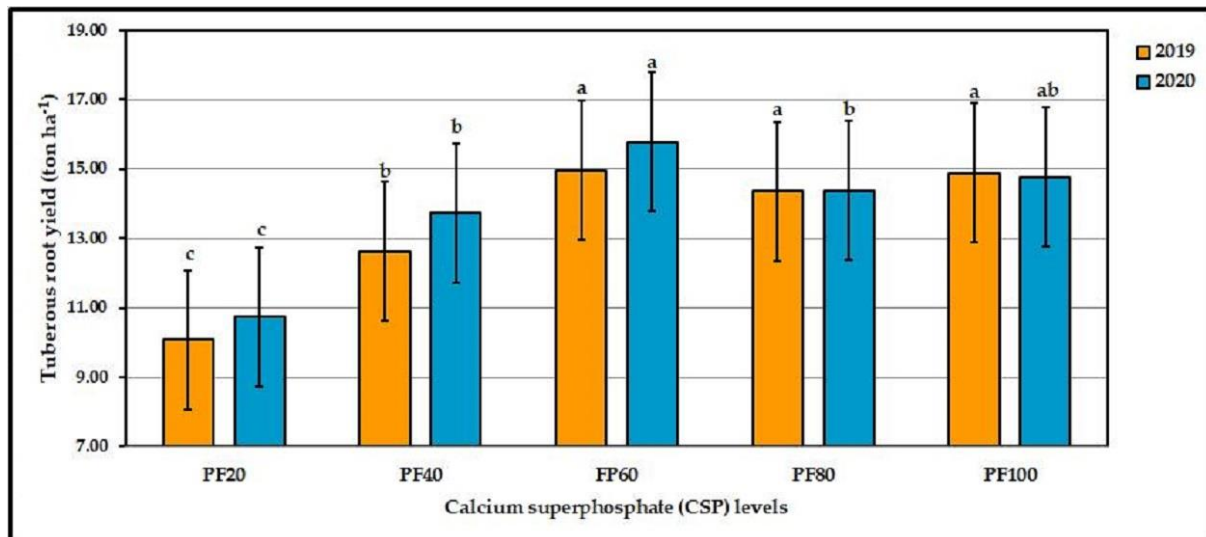


Figure 7. Influence of phosphorus fertilizer levels on total tubers yield of CaCO_3 -stressed sweet potato plants grown in 2019 and 2020. Bars with a different letter indicate significant different between treatments at $p \leq 0.05$

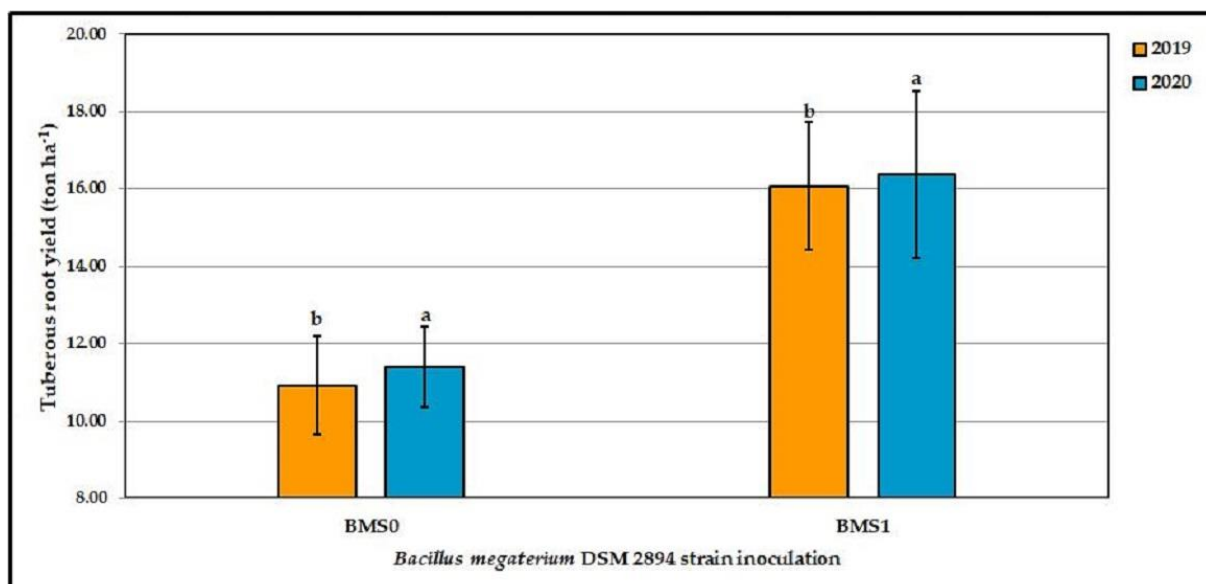


Figure 8. Influence of *Bacillus megaterium* DMS 2894 strain on total tubers yield of CaCO_3 -stressed sweet potato plants grown in 2019 and 2020. Bars with a different letter indicate significant different between treatments at $p \leq 0.05$

The results of different levels of CSPF with DSM 2894 strain interactions presented in Figure 9. The maximum tuberous root yield ($18.86a \pm 1.97$ vs $19.32a \pm 1.60$ ton ha⁻¹) which recorded via CSP₆₀ × DSM₁ treatment in both seasons, respectively. On the other hand, the lowest tuber roots yield ($9.03f \pm 1.29$ vs $8.90d \pm 0.53$ ton ha⁻¹) were recorded with MCP₂₀ × BSM₀ treatment in the first and second seasons. Results of statistical analysis showed that CSPF with DSM interactions had a significant ($P \leq 0.01$) effects on total tuberous root yield of sweet potato in both seasons.

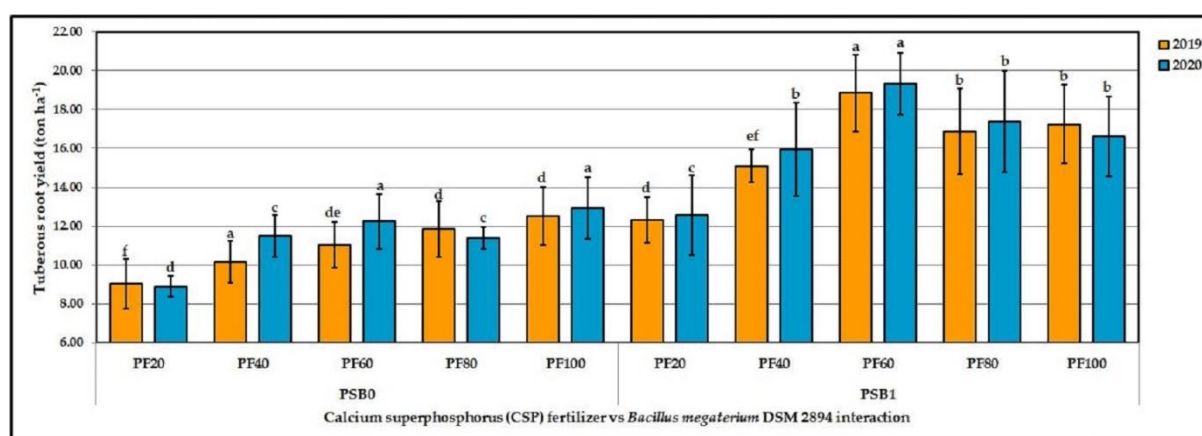


Figure 9. Influence of phosphorus fertilizer level, *Bacillus megaterium* DMS 2894 strain interactions on total tubers yield of CaCO₃-stressed sweet potato plants grown in 2019 and 2020. Bars with a different letter indicate significant different between treatments at $p \leq 0.05$

4 Discussions

The tested soil in this study has undesirable characters such as low content of soil organic matter [(SOM 0.90 vs 1.03%)], high pH (7.19 vs 7.77) and high calcium carbonate [(CaCO₃ 10.8 vs 11.3%)] and salinity [(ECe 3.95 vs 4.24 dSm⁻¹)] content as shown in (Table 2). Which result in low fertility with nutritional imbalance that affects nutrients uptake and performance of grown sweet potato plants. One of the most dominant rhizospheric bacteria, *Bacillus megaterium* BSM 2894 strain developed different mechanisms to enhance plant growth directly or indirectly via increasing

the solubility of phosphorus (P) and other micro nutrients [29]. Under Egyptian soils, tricalcium phosphate, dicalcium phosphate, hydroxy phosphate and rock phosphate are considered common insoluble inorganic phosphorus fertilizers [30,31], however the phosphorus is present in unavailable form (PO_4^{3-}). The strategy to solubilize the unavailable form to available form as monobasic (H_2PO_4^-) or dibasic (HPO_4^{2-}) ions [32]. BSM 2894 strain can improve plant growth via many mechanisms. In both mechanisms, *B. megaterium* or their metabolites alter the biotic and antibiotic components of the rhizosphere community to bring about plant growth promotion [33].

The most accepted mechanism of phosphate solubilizing depends on BSM 2894 strain is lowering of the pH in surrounding root due to production of H^+ ions excretion of some organic acids which are low molecular weights including, gluconic, formic; 2-ketogluconic; citric, oxalic; lactic isovaleric; succinic, glycolic and acetic acid which chelates cations with phosphate [34,35]. In addition to the direct or indirect effects for indole-3-acetic acid [36] or presence of extracellular oxidative of glucose to gluconic acid via pyrroloquinoline (PQQ) which result in the reducing of soil pH. To confirm these results, similar findings were observed by [37] due to produce some enzymes including phosphatase and phytases to soluble phosphorus from different sources that leading to decrease soil pH in surrounding roots.

As shown in Table 4, the enhanced sweet potato leaf nitrogen and phosphorus contents might be due to the fundamental role of BSM 2894 strain for the enhanced amino acids content which suggest the increasing of bioavailability of nutrients N and P in the soil and their accumulation in the plant [38]. These results were confirmed by [39] using PGPR bacterial consortia involved *B. megaterium* that enhanced protein contents in chilli and cauliflower. Also, the results pertained to N

and P uptakes might be explained that MSB 2894 strain in soil may be decreased soil pH in surrounding roots due to their production of some organic acid as a second metabolite which can overcome some of shown undesirable soil characters [40] as mentioned in Table 2. These results are in accordance with the findings of [41] on common bean plants. On the contrast, some leaf nutrient contents such as calcium (Ca) as presented in **Table 4**, depressed by higher levels of phosphorus fertilizer may be due to higher levels inhibited the production of gluconic acid by *B. megaterium* [36].

The direct effect of *B. megaterium* on some leaf micronutrients *i.e.* iron (Fe), manganese (Mn), zinc (Zn) and copper (Cu) contents may be involve biological processes as well as solubility of complex organic and inorganic nutrients, mobilization of Fe via production of siderophore and plant growth regulators production such as, indole acetic acid (IAA), gibberellin and cytokinins [33] which resulted in improving plant growth and nutrient uptakes either directly or indirectly mechanisms, in both mechanism and their metabolites alter the biotic and abiotic components of rhizosphere community to bring about plant growth promotion [33]. These results are consistent with those of other studies by [42] in terms of leaf Mn and Cu uptakes. These findings were explained by some researchers, many of microorganisms have abilities to produce some phytohormones including varieties of auxins *i.e.* indole-3-acetic acid (IAA), indole - 3-butyric acid (IBA), indole-3-pyruvic acid (IPA), indole lactic acid (ILA) and tryptophol (TOL); cytokinins and gibberellin acid which affect some physiological and morphological processes, regulating the plant growth via increasing the root volume with greater and modify root system resulting more nutrients uptake from the soil. These results are in line with [43] on kiwifruits; [44] on pea; [45] on strawberries. These results were confirmed by [46] whereas, the microorganisms

caused significant increases in roots (increase percentage exceeding 90%) while, shoots growth (increasing rate about 50%) as compared with non-inoculated plants which leading to increase of some nutrients uptakes. Antagonism between Fe x Mn and Fe x Zn were found in table 5 which also may be related to ferric-chelate reductase activity [47]. These results are agrees with other findings described by [48,14]. On the other hand, the synergistic interactions (Fe x P), (P x Zn), (Cu x Fe) and (Cu x P) were noticed may be due to the influence of some macronutrients on root reeducates activity [47].

5 Conclusion

Under these harsh soil conditions as presented in Table 2, including high CaCO_3 10.8 vs 11.3%, salinity 3.95 vs 4.24 dSm^{-1} and low SOM 0.90 vs 1.03%, it is difficult to obtain satisfied level of nutrient uptakes and yield of sweet potato plants (Beauguard cv.) without plant-soil biota which can covert nutrients from unavailable to available forms under these undesirable conditions. The role of soil microorganisms such as *Bacillus megaterium* BSM 2894 strain in the utilization of mono calcium phosphate (MCP) was described in this study. The obtained results can be concluded that, the best results of leaf nutrients uptake, their tuberous root content, protein and anti-radical power (ARP) contents in addition to total yield were obtained via applying of three levels of MCP (MCP₂₀; MCP₆₀ and MCP₁₀₀) of recommended phosphate fertilizer and *B. megaterium* mixture in 2019 and 2020 seasons, respectively. In this connection, improvement of nutritional status of sweet potato plants was resulted in tuberous root nutrients content as well as tuberous root yield, however results of integration was the better as compared with inoculation with *B. megaterium* or phosphorus fertilizer, individually.

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