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# Effect of Some indigenous Bacilli and Cyanobacteria Strains inoculants on Growth Characteristics and Productivity of Sweet Pepper (*Capsicum frutescens*)

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**ABSTRACT: *Bacillus amyloliquifaciens (BA)*, *Bacillus megaterium (BM)*, and Cyanobacteria have all been identified as effective biofertilizers that positively impact various crops. Two successful field experiments were conducted at Al-Azhar University's Experimental Farm during the summer season 2020 and 2021 growing seasons to assess the effect of Bacilli strains and Cyanobacteria inoculation on the vegetative growth and growth parameters and plant chemical content of sweet pepper (*Capsicum frutescens*). In addition, the study investigates the ability of isolated bacterial strains from soil samples to improve plant growth, which is indicated by analyzing phytohormone production and phosphate solubilization. The obtained results indicated that mixed inoculation with both Bacillus strains and Cyanobacteria improved vegetative growth, plant chemical contents, and positive microbial activity in the soil Rhizosphere compared to un-inoculated plants. In addition, soil available nutrients (N, and K) increased significantly when *BA* and *BM* were combined with Cyanobacteria, while available phosphorus gave the most increase with *BM*.**

**Keywords:** *Bacillus amyloliquifaciens; Bacillus megaterium;* cyanobacteria; biofertilizers; sweet pepper *(Capsicum frutescens).*

## INTRODUCTION

Sweet pepper is one of the most important vegetable crops widely grown in Egypt for local consumption and export. Due to its high profit and nutritional advantages for human health, high cash crops like sweet pepper have acquired a prominent position in Egyptian and global agriculture (El Arnaouty *et al.* 2020). Because of its economic importance and health benefits, bell pepper *(Capsicum annuum L.)* is an important vegetable crop grown on all seven continents. The bell pepper fruits *(Capsicum annuum L.)* contain ascorbic acid and lycopene, a valuable chemical with antioxidant and anti-cancer properties. As a result, its cultivation, utility, and consumption are increasing yearly (Almadhoun 2021). As a result, significant efforts have been made over the last decade to replace chemical fertilizers with environmentally friendly bio-fertilizers (Mikhak et al., 2017).

Biofertilizer is a natural fertilizer that contains a large population of beneficial microorganisms that improve soil productivity by fixing atmospheric nitrogen, solubilizing soil phosphorus, or stimulating plant growth through the synthesis of growth-promoting substances or latent cells that activate the biological process rendering nutrients available to plants (Simarmata *et al.* 2017; Pandey *et al.* 2016; Bellm *et al.* 2018; Chittora *et al.* 2020). A plant's rhizosphere is a highly competitive ecosystem in which microorganisms compete for nutrients from the plant's root. Because they live within or around plant roots and promote plant growth, some bacteria are known as plant growth-promoting Rhizobacteria (PGPRs). Members of this genus can also survive in unsuitable surroundings for extended periods (Vejan et al., 2016). These biofertilizers are created by selecting beneficial soil microorganisms that have the highest efficiency in enhancing plant growth by providing nutrients in a readily absorbable form. Applying inoculants derived from these microorganisms increases the abundance of active and effective microorganisms in the root activity zone, increasing the plant's ability to absorb more nutrients. For example, bacillus megaterium, a phosphate-dissolving bacterium, can affect the solubility of poorly soluble inorganic acids (Chen et al. 2006). Other bacteria in the same group can release phosphorus from organic phosphorus compounds by producing phosphatase enzymes. Microbial cultures, bio inoculants, bacterial inoculants, and bacterial fertilizers are used to describe biofertilizers. *BM* generates endospores and is the most widely used soil bacterial biofertilizer for plant growth (PGPR) (Miljaković et al., 2020). Cyanobacteria have a unique potential and a vital function in boosting soil fertility and plant productivity in ecological conditions due to their significant feature of nitrogen fixation. Certain *Bacillus spp*. strains are known as plant growth-promoting Rhizobacteria (PGPR) and can be utilized as bio-fertilizer components (Zhang *et al.* 2011). *Bacillus* is a well-studied PGPR genus because of its well-characterized root colonization and sporulation abilities. *Bacillus sp.* inoculation as PGPR in the soil can help solubilize fixed soil P and enhance inorganic P availability to plants (Kumar and Narula 1999).

Plant-associated *B. amyloliquefaciens* (*BA*) has produced several secondary metabolites and enzymes involved in microbial antagonisms, such as chitinase, so indirectly aiding plant defense and growth through disease suppression. Phosphorus is essential for plant growth as it promotes root development, tillering, early blooming, and other metabolic activities, such as protein synthesis. Only monobasic or dibasic soluble forms are taken up. Phosphate-solubilizing bacteria (PSB) are soil microorganisms that play a significant role in solubilizing P for plants and permitting more efficient utilization of P fertilizers. *Bacillus megaterium* was chosen for this experiment because of its role in phosphorus transformation, and it was found to be one of the most powerful phosphates solubilizes (Mohamed et al., 2018). Based on these published facts and conclusions, biofertilizers are projected to reduce the usage of chemical fertilizers and pesticides. They restore the soil's natural nutrient cycle and increase soil organic matter, ensuring that the host plants receive sufficient nutrients and that their growth and physiology are correctly regulated. As a result, we're interested in comparing and studying the effects of applying *BA*, *BM*, and cyanobacteria as bio fertilizers to sweet pepper (*Capsicum frutescens)* crops in individual addition or varied mixes to regular chemical fertilization (Behera *et al.* 2012). So the current study aimed to investigate the effect of different isolated bacterial strains from soil samples to improve plant growth by analyzing phytohormone production and phosphate solubilization.

## MATERIALS AND METHODS

### Experiment design:

This study was carried out at Al-Azhar University's Experimental Farm (Cairo, Egypt) over two summer seasons, 2020 and 2021, to investigate the effect of selected *Bacilli* strains (*Bacillus amyloliquifaciens; Bacillus megaterium*)and Cyanobacteria as *(Nostoc muscorum*, *Anabaena oryzae* and *Spriulina platensis*) inoculation on sweet pepper *(capsicum frutescens*) cv. California Wonder Crop growth. Agriculture Microbiology Department- Soils, Water & Environmental Research Institute, Agriculture Research Centre (ARC), Giza, Egypt, prepared both *Bacilli* strains and Cyanobacteria under investigation. Seeds were sown in seedbeds on January 16th and 20$^{th}$. Prior to seed sowing, microorganism strains were cultured on the slant, inoculated into a nutrient medium, and turned into liquid seed following shaking culture at 200 r/min for 24 hours on a constant temperature shaking at 32 °C. Seedlings were transplanted after 45 days in the open field. Plant spacing in the open field took place 80 cm between rows and 40 cm between plants in the row. Each replicate consisted of 5 rows where each row was 6 m long, establishing an area of 24 $m^2$ for each plot. Agricultural practices were done whenever it was necessary. The experiment included 3 replicates, arranged in a randomized complete block design. Each plot was fertilized by ammonium sulphate (400 kg/fed), super calcium phosphate (300 kg/fed) and potassium sulphate (200 kg/ fed). These quantities of fertilizers were added as follows, 50 kg of each fertilizer during the preparation of the soil for planting. The rest of the quantities were divided into 3 equal parts, the first of which was added three weeks after planting, the second at the beginning of flowering, and the third after the first harvest. Where the first section served as a control line and the second part was immersed in cyanobacteria powder. After the seeds had been sown, the *Bacilli* strains were added as a soil drench. The experiment's treatments were as follows: Control; sweet pepper (*Capsicum frutescens)* seeds dressed cyanobacteria powder; sweet pepper (*Capsicum frutescens*) seeds drenched with BM; sweet pepper seeds drenched with *BM* and *BA*; sweet pepper seeds dressed cyanobacteria powder and drenched with BA; sweet pepper seeds dressed cyanobacteria powder and drenched with *BM* and *BA.*

### Soil analysis

Using a Gallenkamp pH meter (A. Gallenkamp Co.& Ltd., UK), the soil acidity (pH) in the soil paste was determined, and electric conductivity (EC) in a 1: 2.5 soil: water extract was determined according to the described procedures (Sahlemedhin and Taye 2000). The international pipette method was used to determine the mechanical analysis of soil (Table 1), with NaOH as a depressing agent (Wirth 1946). The Devarda alloy method of steam distillation was used to extract available nitrogen (extracted using a 1% $K_2SO_4$ soln.) (Pramer and Schmidt 1964; Black *et al.* 1965). At a wavelength of 650 nm, spectrophotometry of accessible phosphorus (extracted using a $NaHCO_3$ 500 mM solution with a pH of 8.5) was determined (Olsen 1954). Using Beckman Du 7400 spectrophotometer (GMI Co., MN, and USA). A Corning flame photometer was used to measure available potassium (Dewis and Freitas 1970). Using a 1 N ammonium acetate soln. (pH = 7.0). The organic matter was determined using the Walkley and Black chromic acid wet oxidation method (Hesse 1971). Diethylene thiamine penta acetic acid (DTPA) soln. was used to extract available micronutrients from soil samples (Lindsay and Norvell 1978). The atomic absorption spectrophotometer was used to determine the results. According to the described procedure, the saturation percentage (SP%) was calculated (Aali *et al.* 2009), and Smith's standard method was used to determine the hydraulic conductivity (K) values of the soil sample columns (Smith 2000).

### Phosphatase enzyme and dehydrogenase activity analysis

Tabatabai and Bremner's approach was used to assess phosphatase activity (Tabatabai and Bremner 1969). Casida's method for determining dehydrogenase activity examined the samples (Casida Jr *et al.* 1964).

### Plant analysis

### Total chlorophyll and Carotenoids

Pigments were extracted from 0.5 g fresh young leaves in a dimethylformamide (DMF) soln. Overnight at 4 C° to estimate the mass of chlorophyll a, chlorophyll b, total chlorophyll and Carotenoids per leaf. At wavelengths of 663, 470, and 647 nm, the pigments were calculated using Moran's equation and a spectrophotometer Beckman Du 7400 (Lichtenther 1987).

### Plant growth parameters and chemical Contents

A kilogram balance was used to determine the weight of fresh fruit per patch. Magness and Ballauf pressure testers were used to determine fruit hardness in kg/cm$^2$ (D. Ballauf Manufacturing Co., MD, and USA) and Ballauf pressure testers were used to determine fruit hardness in kg/cm$^2$ By immersing the fruit in a container filled with water and measuring the displaced water with a graduated jar, the fruit's size was measured in cm$^3$. Drying 100g of fresh weight in a 70 °C oven until it reached a consistent weight was used to compute fruit dry weight as g/100g fresh weight. Plant height (cm) was measured from the first node to the plant top, and the number of leaves per plant and the total number of fruits per plot were recorded. The length and diameter of the fruit were measured in cm (using a Vernier caliper). Sulphuric and perchloric acid mixtures were used to wet digest plant samples (Chapman and Pratt 1978). The Kjeldahl method was used to determine plant nutrients in the aliquot for nitrogen (Jackson 1973) stannous chloride reduced molybdo-phosphoric blue colour method for phosphorus (Jackson 1973) and potassium using flame photometer (Jackson 1973). An Abbe refractometer was used to calculate percentages of total soluble solids (T.S.S %.) (Sparks *et al.* 2020). Ascorbic acid was measured as mg/100 g fresh weight using the dye 2, 6- dichlorophenol indophenol technique (Hernández *et al.* 2006). Dubois calculated total sugars as g/100g dry weight (Dubois *et al.* 1956). Titratable acidity content was determined analytically in mg/100g plant fresh weight by using a 0.1N standard NaOH solution (AOAC International Official methods of analysis 2005).

### Statistical Analysis

An appropriate analysis of variances was performed using COSTATE V 6.4 (2005) for Windows (CoStat 2005). The Least Significant Differences test at the 0.05 level of probability was used to compare the differences among the means of the various treatment combinations as illustrated by a computer software program based on significant differences among the mean of various treatments as determined by the Least Significant Differences test (Duncan 1955; Gomez and Gomez 1984). The soil's physical and chemical properties under experiment were measured according to the described methods (c.f. experimental section) and presented in Table 1.

**Table 1.** Physical and chemical properties of experimental soil**.**

| *Physical properties* | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Soil Type | Fine sand % | Coarse sand % | Silt % | Clay % | Wilting point (% v/v) | SP % | Field capacity (% v/v) | Hydraulic conductivity (cmhr$^{-1}$) |
| Sandy clay loam | 35.04 | 19.44 | 10.0 | 28.31 | 34.50 | 23.23 | 44.33 | 2.33 |
| Available water (% v/v) | | H.W% | | Bulk density ( Mg m$^{-3}$) | | | Total porosity % | |
| 8.40 | | 7.2 | | 1.66 | | | 48.4 | |
| *Chemical properties* | | | | | | | | |
| pH in suspension 1:2.5 | Organic matter (O.M %) | Available nutrients (ppm) | | | | | | |
| 7.76 | 0.59 | N | P | K | Fe | Mn | Zn | Cu |
| | | 28 | 12 | 98 | 14.00 | 8.9 | 1.7 | 0.89 |
| Soluble cation**(meq/ L) | | | | | Soluble anions**(meq/ L) | | | |
| $Ca^{++}$ | $Mg^{++}$ | $Na^{+}$ | $K^{+}$ | $CO_3^{--}$ | $HCO_3^{-}$ | $Cl^{-}$ | $SO_4^{--}$ | |
| 5.51 | 2.75 | 10.69 | 1.03 | 7.70 | 3.20 | 14.80 | 2.91 | |
| SAR | | ESP | | $CaCO_3$ -% | | | EC (ds/m) | |
| 1.87 | | 3.44 | | 5.88 | | | 2.33 | |

pH$^*$ in suspension 1:2.5,EC** (ds/m), soluble cation ** and anions (meq/L): in saturated past extract,EC: Electric conductivity; HW: Hygroscopic water; HC: hydraulic conductivity.

## RESULTS

### Plant and soil analysis:

**Growth parameters:**

Tables 2 and 3 demonstrated the effect of *BM*, *BA*, and Cyanobacteria on some sweet pepper growth parameters. Selected bacteria and cyanobacteria significantly improved plant growth characteristics, such as plant height (cm), number of leaves/plant, and number of fruits/plots (Table 2).

**Table 2:** The effect of inoculating sweet pepper plant with *BM*, *BA*, and Cyanobacteria on growth parameters at harvest time.

| Treatments | Plant height (cm) | | Number of leaves/plant | | Number of fruits/ plot | |
|---|---|---|---|---|---|---|
| | Seasons | | | | | |
| | 1st | 2nd | 1st | 2nd | 1st | 2nd |
| Control | 54.54h | 59.33g | 4.67f | 5.50f | 23.33g | 25.67g |
| *BA* | 67.75e | 72.02e | 7.37d | 7.17e | 31.33d | 29.67e |
| *BM* | 61.44g | 65.23f | 5.67e | 5.33f | 26.33f | 27.67f |
| Cyanobacteria | 65.40f | 70.34e | 6.33e | 6.67e | 28.33e | 31.00d |
| *BA/BM* mixture | 70.57d | 74.75d | 7.83d | 8.33d | 34.00c | 31.67d |
| *BA*/Cyano mixture | 81.50b | 84.18b | 10.67b | 11.33b | 37.00b | 40.00b |
| *BM*/Cyano mixture | 75.27c | 79.37c | 9.17c | 10.00c | 35.00c | 35.33c |
| *BA/BM/* Cyano mixture | 86.37a | 91.18a | 12.50a | 13.33a | 40.00a | 43.00a |
| L.S.D. (0.05) | 2.24 | 1.98 | 0.83 | 1.05 | 1.54 | 1.32 |

Treatments with *BM* combined with each of *BA* and/or Cyanobacteria exhibited the strongest stimulatory influence and maximum augmentation in plant parameters compared to control in both seasons, followed by BA mixed with cyanobacteria. As evidenced by the data (Table 2), *BM* had the most substantial increase in plant height (86.37 and 91.18cm, respectively) in both seasons. Furthermore, the *BM*/ *BA* and *Cyanobacteria* mixture produced the same highest values regarding to the number of leaves per plant. At the same time, the lowest values were recorded in the case of treatment with *BM*. In the Sweet pepper crop. This could be due to the experimental organisms secreting plant growth regulators. Table 3 demonstrates how adding *BM*, *BA*, and Cyanobacteria could affect the growth and several yield characteristics alone and in combination.

**Table 3:** Influence of inoculation with *BM*, *BA* and Cyanobacteria on sweet pepper fruit features during the seasons**.**

| Treatments | Fruit fresh weight (g) | | Fruit dry weight (g) | | Fruit length (cm) | | Fruit diameter (cm) | | Fruit size (cm) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Seasons | | | | | | | | | |
| | 1st | 2nd | 1st | 2nd | 1st | 2nd | 1st | 2nd | 1st | 2nd |
| Control | 9.22g | 8.42g | 5,83g | 5.69g | 4.27d | 3.60h | 3.56g | 3.11f | 39.26h | 44.75h |
| *BA* | 11.60de | 10.94de | 7.19d | 6.89d | 5.84b | 4.51e | 4.35d | 4.18d | 61.96e | 65.64e |
| *BM* | 10.09f | 9.54f | 6.37f | 6.17f | 4.68c | 3.87g | 3.79f | 3.63e | 48.99g | 55.88g |
| Cyanobacteria | 10.89e | 10.10e | 6.88e | 6.70e | 4.95c | 4.41f | 4.23e | 4.07d | 53.52f | 61.48f |
| *BA/BM* mixture | 12.03d | 11.71d | 7.58c | 7.20c | 5.65b | 4.97d | 4.45d | 4.20d | 67.86d | 71.05d |
| *BA*/Cyano mixture | 14.04b | 15.16b | 8.16b | 7.73a | 6.01b | 5.73b | 4.83b | 4.73b | 77.07b | 81.05b |
| *BM*/Cyano mixture | 13.40c | 13.39c | 7.98b | 7.46b | 5.81b | 5.36c | 4.64c | 4.43c | 73.80c | 77.70c |
| *BA/BM*/Cyano *mixture* | 15.83a | 16.50a | 9.08a | 7.88a | 6.43a | 6.13a | 5.30a | 5.49a | 79.78a | 84.74a |
| L.S.D.(0.05) | 0.75 | 0.91 | 0.23 | 0.16 | 0.39 | 0.23 | 0.12 | 0.21 | 2.31 | 2.06 |

In treatment with *BM*, the fruit fresh weight yielded the greatest results in both seasons (15.83 and 16.50 g), followed by *BA/*Cyano (14.04 and 15.16 g) compared with control. Fruit dry weight exhibits a similar pattern with *BA/BM/*Cyanobacteria mixture (9.08 and 7.88 g), recording the highest values in both seasons, followed by *BA/*Cyano mixture (8.16 and 7.73g). A mixture of *BA/BM/*Cyanobacteria has the highest fruit length values in both seasons (6.43 and 6.13cm), followed by *BA/*Cyanobacteria mixture (7.73 and 6.01cm) and *BM/*Cyano mixture (5.81 and 5.36cm). Fruit diameter was measured in the order *BA/BM/*Cyano mixture (5.30cm) in the first season and nearly the same with *BA/BM* mixture (5.49cm) in the second season. Fruit size is the penultimate characteristic in the same Table, and it increased the most with the addition of the mixture of *BA/BM* mixed with Cyanobacteria *(*79.78 and 84.74cm, respectively) in both seasons.

**Chemical constituents and some fruit quality parameters:**

Figures 1-3 showed the effects of two types of Bacillus strains, either alone or in combination with Cyanobacteria, on some chemical fruit quality parameters of sweet pepper crop, including total soluble solids (TSS) endogenous phytohormones content (i.e. ascorbic acid) chemical content (i.e. total sugar)**.** For instance, inoculation with *BA/BM* (5.38 and 5.75 %) or in conjunction with Cyanobacteria resulted in a significant increase of total soluble solids (T.S.S), followed by mixed inoculation with *BA/Cyano* (4.73 and 5.04 %) in both seasons as compared to control (Figure 1).

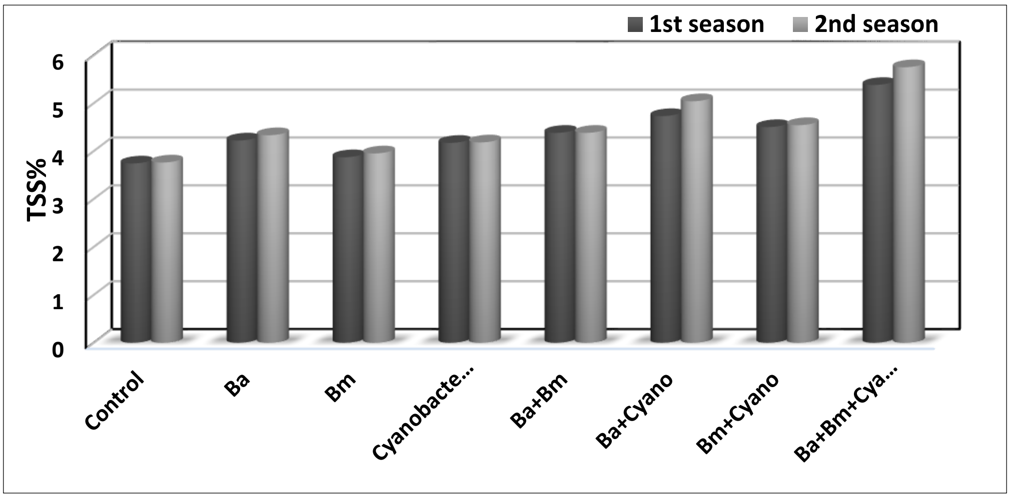

Fig.1. Inoculation effect with *BM*, *BA* and Cyanobacteria affected TSS% of sweet pepper plant during the seasons.

Vitamin C, also known as ascorbic acid, is essential for many physiological processes in plants; highest increase is found in the first season with the *BA/BM/*Cyano mixture (127.19 mg/100 g F.W), followed by the *BM/*Cyano mixture (121.29mg/100 g F.W). On the other hand, the highest value of ascorbic acid was recorded by adding BA/Cyano (126.37 mg/100g fresh weight) followed by *BA/BM/*Cyano in the same Table, and we studied the effect of Bacillus strains and Cyanobacteria (Figure 2).

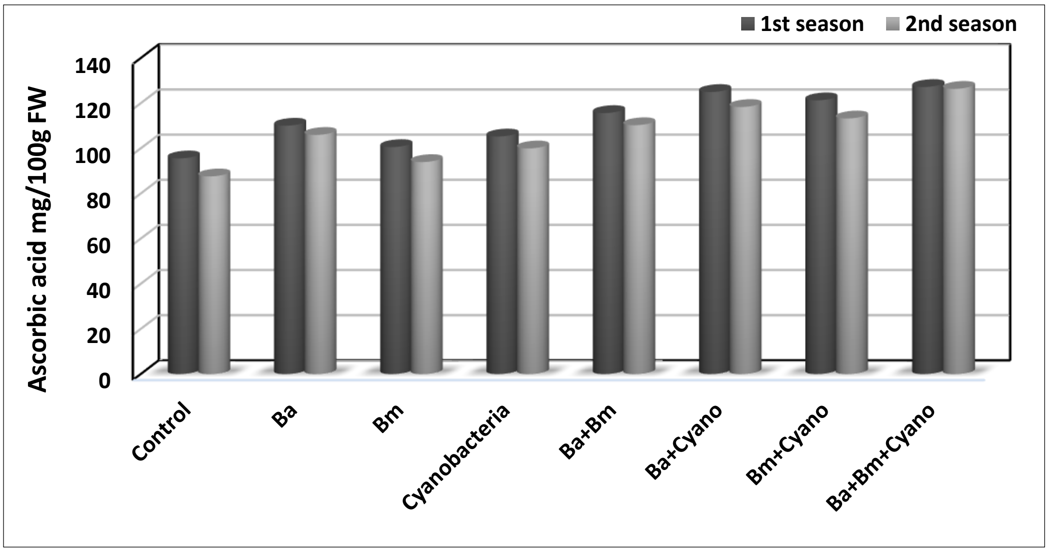

Fig 2. Inoculation with *BM*, *BA* and Cyanobacteria and their effect on ascorbic acid mg/100g of sweet pepper plants during two seasons.

Data revealed a highly significant increase in total sugar with *BA/*Cyano *(*9.04 g/100 GFW*)* in the first season, but in the second season, the highest increase was recorded with *BA/BM/*Cyano. *BM* has the lowest value in both seasons (Figure 3).

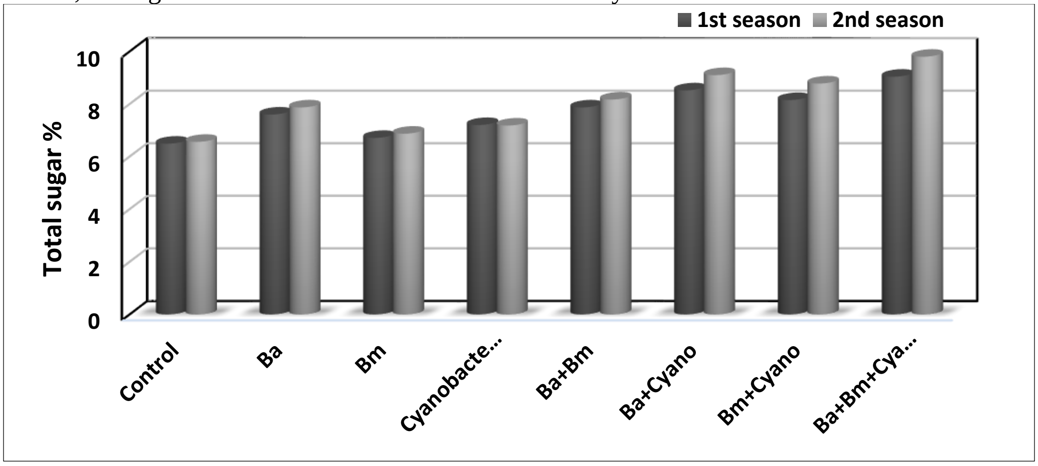

Fig.3. Effect of inoculation with *BM*, *BA* and Cyanobacteria on total sugar % of sweet pepper plants during two seasons.

**Total chlorophyll, Carotenoids and Total acidity:**

In terms of fruit quality, presoaking sweet pepper seeds in *BA/BM/*Cyano mixture resulted in significant increases in photosynthetic pigments (Total chlorophyll mg100g FW), Carotenoids (mg/100g FW) and acidity mg100g FW) in the leaves ($P < 0.05$) as shown in Figure 4.

**Table 4.** Influence of inoculation with *BM*, *BA* and Cyanobacteria on sweet pepper fruits features during two seasons

| Treatments | Acidity mg100g/FW | | Total chlorophyll mg/100gFW | | Carotenoids mg/100gFW | |
|---|---|---|---|---|---|---|
| | Seasons | | | | | |
| | 1st | 2nd | 1st | 2nd | 1st | 2nd |
| Control | 3.47h | 3.76h | 6.49e | 6.56h | 96.56e | 87.58h |
| *BA* | 4.22e | 4.33e | 7.60c | 7.87e | 110.04d | 105.95e |
| *BM* | 3.87g | 3.95g | 6.71d | 6.87g | 100.57d | 93.92g |
| Cyanobacteria | 4.17f | 4.18f | 7.20d | 7.18f | 105.26d | 99.98g |
| *BA/BM* mixture | 4.38d | 4.40d | 7.87c | 8.17d | 115.65cd | 110.26d |
| *BA/*Cyano mixture | 4.73b | 5.04b | 8.52b | 9.80a | 127.19a | 118.32b |
| *BM/*Cyano mixture | 4.50c | 4.54c | 8.15b | 8.78c | 121.29bc | 113.25c |
| *BA/BM/*Cyano mixture | 5.38a | 5.75a | 9.04a | 9.10b | 124.98 | 126.37a |
| L.S.D. (0.05) | 1.61 | 2.32 | 3.05 | 2.28 | 3.49 | 1.11 |

Data in Table(4)and fig4 showed a positive increase in acidity (mg100gFW) with inoculation by *BA /BM/*Cyano (5.38 and 5.75 mg100g FW) in both seasons, followed by mixed inoculation by *BA*+ Cyano (4.73 and 5.04 mg100g FW) in both seasons. Plants inoculated with *BA/BM* mixed with Cyanobacteria produced the maximum photosynthetic pigments and nutritional content in the first season (9.04 mg/100 g FW). In contrast, in the second season, the highest increase was recorded with *BA/Cyanobacteria* (9.80 mg/g100g FW) as evidenced by the acquired results. Also, the highest increase was recorded in carotenoids in the first season with *BA/*Cyano, but in the second season, the highest was recorded by adding *BA/BM/*Cyano.

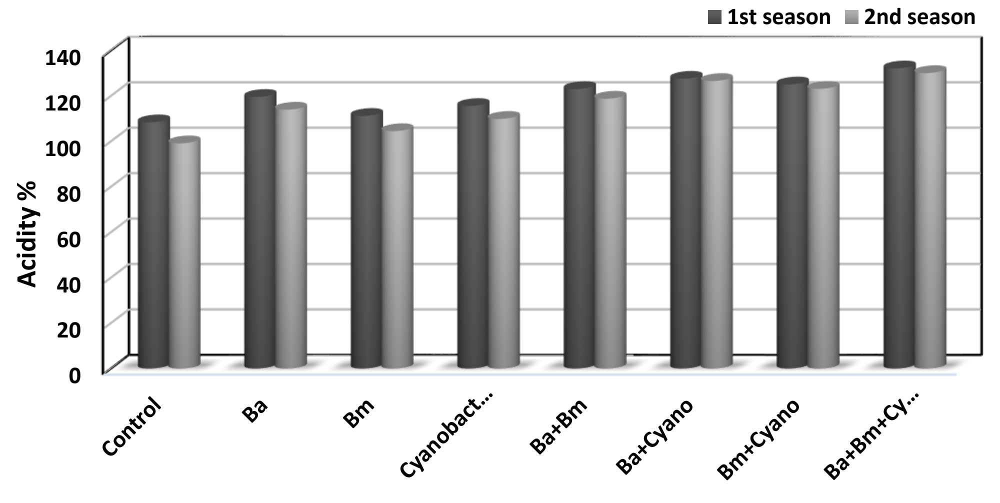

Fig.4. Effect of inoculation with *BM, BA* and Cyanobacteria on acidity % of sweet pepper plants during the seasons

**Plant chemical contents:**

**Effect of inoculation with BM, BA and cyanobacteria on sweet pepper (*capsicum frutescens*) chemical content**

Table 5 summarizes the effects of *BM*, *BA*, and Cyanobacteria on chemical contents of sweet pepper.

**Table 5.** Effect of inoculation with *BM, BA* and Cyanobacteria on chemical contents of sweet pepper plant at harvest during two seasons

| Treatments | N% | | P% | | K% | |
|---|---|---|---|---|---|---|
| | Season | | | | | |
| | 1st | 2nd | 1st | 2nd | 1st | 2nd |
| Control | 1.70g | 1.51h | 0.147h | 0.183h | 1.59g | 1.73h |
| *BA* | 2.15d | 1.94e | 0.217e | 0.257e | 1.82d | 1.87e |
| BM | 1.88f | 1.63g | 0.173g | 0.203g | 1.68f | 1.82f |
| Cyanobacteria | 2.00e | 1.77f | 0.193f | 0.227f | 1.74e | 1.78g |
| *BA/BM* mixture | 2.25c | 2.13d | 0.247d | 0.290d | 1.87cd | 1.90d |
| *BA/*Cyano mixture | 2.61b | 2.36b | 0.300b | 0.347b | 1.96b | 1.97b |
| *BM/Cyano mixture* | 2.53b | 2.23c | 0.267c | 0.323c | 1.91c | 1.93c |
| *BA/BM/*Cyano mixture | 2.71a | 2.54a | 0.340a | 0.380a | 2.12a | 2.11a |
| L.S.D.(0.05) | 0.08 | 0.06 | 0.012 | 0.012 | 0.05 | 0.02 |

According to Table 5 nitrogen content of sweet pepper plant by adding *BA/BM* mixed with Cyanobacteria increased in both seasons (2.71 and 2.54%), followed by *BM/*Cyanobacteria and *BA/Cyanobacteria* gave the same value in the first season, and the highest value in the second season was recorded with *BA/*Cyanobacteria while using *BM* separately resulted in the smallest increase in nitrogen content. Plant phosphorus content increased the most in the first and second seasons with a *BA/BM/*Cyano mixture (0.340 and 0.380%, respectively), followed by a *BA/Cyano mixture* (0.300 and 0.347%). The addition of *BA/BM/*Cyano has resulted in the highest potassium contents of the sweet pepper plant in both seasons (2.12 and 2.11%, respectively). These are followed by the treatment blend *BA/*Cyanobacteria (1.96 and 1.97%) and hence with *BA* (1.68and 1.82%), which shows the most minor decrease in both seasons.

### Soil analysis:

### Available N, P and K in soil

Applying bio-fertilizer formulations increased the nitrogen, potassium, and phosphorus contents. Individually inoculated sweet pepper plants with *BA or BM* or *Cyanobacteria* showed a remarkable increase in available N, P, and K compared to un-inoculated plants, as shown in Table 6.

**Table 6.** Effect of inoculation with *BM, BA* and Cyanobacteria on sweet pepper fruits features during two seasons

| Treatments | Available N(ppm) | | Available P(ppm) | | Available K(ppm) | |
|---|---|---|---|---|---|---|
| | Season | | | | | |
| | 1st | 2nd | 1st | 2nd | 1st | 2nd |
| Control | 26.67g | 39.33h | 12.43d | 15.34f | 181.33f | 359.67b |
| *BA* | 81.00d | 91.67e | 16.30b | 18.18c | 250.00e | 295.33cd |
| *BM* | 66.00e | 82.67f | 16.94ab | 19.55a | 278.00d | 305.33c |
| Cyanobacteria | 56.33f | 71.00g | 14.33c | 16.43e | 291.67d | 270.67cde |
| *BA/BM* mixture | 140.00a | 142.33a | 17.00ab | 18.40b | 355.00b | 352.00b |
| *BA/*Cyano mixture | 101.00c | 116.00d | 16.84ab | 17.67d | 288.00d | 258.33de |
| *BM/*Cyano mixture | 107.00b | 120.00c | 16.97ab | 17.99cd | 325.00c | 247.00e |
| *BA/BM/*Cyano mixture | 100.33c | 134.00b | 17.27a | 17.76a | 459.67a | 469.33a |
| L.S.D.(0.05) | 3.46 | 2.82 | 0.75 | 0.365 | 26.64 | 46.66 |

The mixture of *BA*, and *BM* produced the greatest increases in ammoniacal and nitrate nitrogen contents, while the combination of *BM* and *BA* mixed with *Cyanobacteria* produced the highest values of available-P and K in both seasons, either without or with boron application. The combination of *BA* and *BM* resulted in the highest increase in nitrogen availability (140.00, and 142.00 ppm) in both seasons, followed by *BM* mixed with *Cyanobacteria* (107.00, and 120.00 ppm). When sweet pepper plants were inoculated with *BA/BM/Cyanobacteria*, the highest increase in available K was observed (459.67, and 469.33 ppm), followed by a mixture of *BA/ BM* (355.00, and 352 ppm).

### Phosphatase and Dehydrogenase activity:

Multiple inoculations, particularly with *BM/BA* and Cyanobacteria, resulted in the highest phosphatase activity. *Bacillus* and Cyanobacteria work well together as a co-inoculation system. The highest increase is seen in the *BA/BM/*Cyano mixture (37.54, and 50.08 µ inorganic phosphorus/g dry soil/day) followed by *BM/*Cyano (36.06 and 47.29 µ inorganic phosphorus/g dry soil/day), respectively in both seasons as indicated in Figure 5. In terms of phosphatase activity, using soluble P fertilizer in un-inoculated treatments reduced phosphatase activity compared to nitrogen-fixing bacteria.

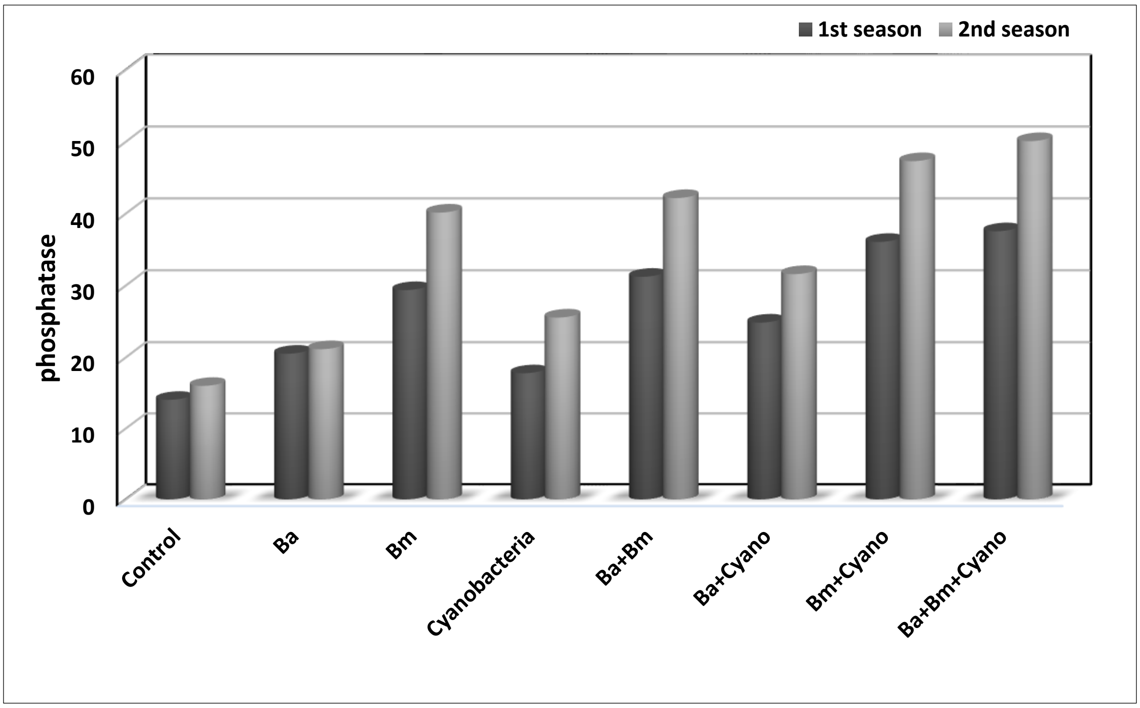

Fig.5. Effect of inoculation with *BM, BA* and Cyanobacteria on phosphatase of sweet pepper plants during the two seasons

Dehydrogenase enzyme activity (DHA) as an energy transfer function is considered an indicator of overall microbial activity in the soil. Figure 6 shows the determination of enzyme activity in the Rhizosphere of sweet pepper plants. *BM* inoculation resulted in higher DHA levels than un-inoculated controls. This could be because *BM* acts as a plant growth promoting Rhizobacteria via P-solubilization.

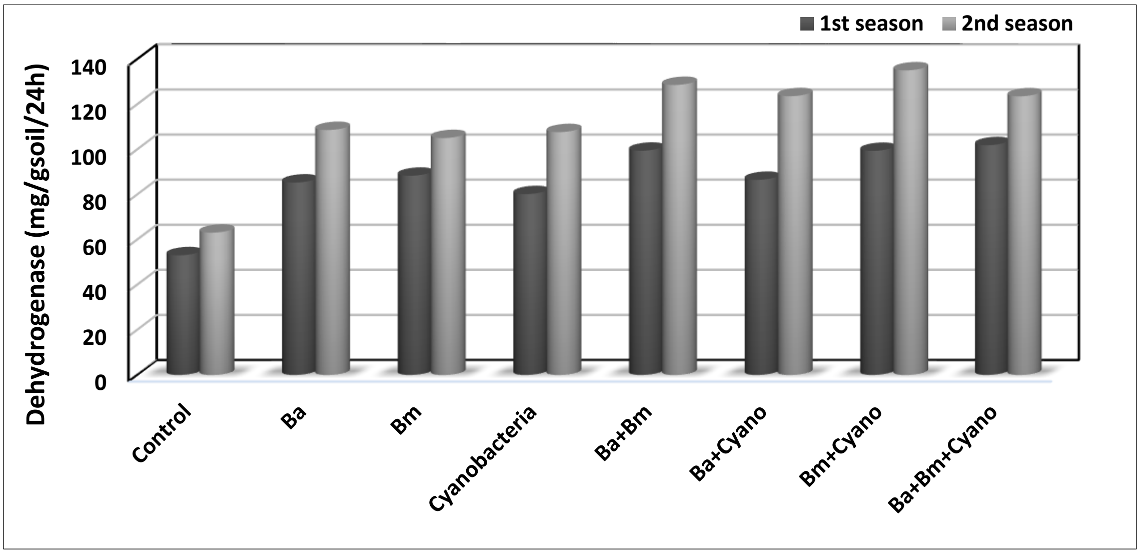

Fig.6. inoculation effect with *BM, BA* and Cyanobacteria and their influence on dehydrogenase (mg/g soil/24h) of sweet pepper plants during two seasons

Alternatively, the data showed that inoculation with *BM* increased DHA levels separately or in combination with *BA* and Cyanobacteria when compared to control samples. The *BA/BM/Cyano* mixture exhibited the greatest positive increase (101.88mg/g soil/24h) in the first season, while in the second season the highest increase was recorded by the addition of *BM* mixed with *Cyanobacteria*. *BM/ Cyano* (135.06 mg/g soil/24h) and *BA/BM/*cyanobacteria comes next.

## DISCUSSION

A sustainable agriculture system requires renewable inputs that maximize ecological advantages while minimizing environmental hazards. This study assesses the impact of adding microbial fertilizer to sweet pepper growth characteristics and productivity by soil inoculation with different rates of *BM* and *BA* alone or combined with Cyanobacteria extracts on sweet pepper growth parameters and productivity. *Bacillus megaterium* was tested as solubilize insoluble phosphate while *Bacillus amyloliquifaciens* and cyanobacteria can fix nitrogen, produce plant growth regulators (auxins, gibberellins, cytokines, and so on), improve soil fertility by adding organic matter, nitrogen, and phosphorus to soil, degrade various agrochemicals (pesticides and herbicides), and control pathogenic effects of other microorganisms and plants, all of which can be used to boost agriculture this finding in harmony of (Meena *et al.* 2020). Many *Bacillus amyloliquefaciens*, *Bacillus megaterium*, and *Bacillus subtilis* strains have been identified to interact with plants and provide favorable effects such as plant resistance, protection against plant diseases, and plant growth promotion features such as siderophore synthesis (Choudhary and Johri 2009). The study suggested that the mixture of *Bacillus amyloliquifaciens*, *Bacillus megaterium* and cyanobacteria increased the sweet pepper plant growth parameters (plant height cm, number of leaves/plant) and number of fruits/plot as compared to the control (Choudhary and Johri 2009). The long-term survival of *B. amyloliquefaciens* in the soil makes inocula highly necessary to ensure a persistent synergistic connection. Our findings reveal that mineral fertilization and bacterial inoculation increased microbial biomass (total cultural bacteria, PSB, and fungus) and modified the community structure consistently depending on the treatments used. This is comparable to the findings of (Jamal *et al.* 2018).

In this study, the *BA/BM/*Cyano mixture has the most significant positive increase in DHA in the first season, while in the second season the highest value recorded with *BM/*Cyano. This could be because BM promotes plant development by P-solubilizing Rhizobacteria. Inoculation with *BM*, alone or in combination with *BA* and *cyanobacteria* raised (DHA) levels compared to those not infected. Concerning the activity of dehydrogenase activity, data revealed a close relationship between dehydrogenase activity and microbial populations (Luo et al., 2015). Data in our study showed that The *BA/BM/*Cyano mixture produces the greatest increase in the first season. Then comes the *BM/*Cyano combination. Vitamin C, also known as ascorbic acid, is essential for many physiological processes in plants, including enzymatic activity, gene expression, environmental signalling, and transport. Furthermore, it is a compound with proven antioxidant activity that is commonly found in bell peppers. Another critical factor to consider is that vitamin C cannot be synthesized by the human body and must be obtained through diet, so any improvement in agricultural practices is beneficial (Flores-Félix *et al.* 2015; Kim et al., 1997). In their studies (Zhou *et al.* 2012) found that PGPR promotes plant growth indirectly by generating a fungal cell wall degrading enzyme that protects plants from infections. Microorganisms can have a significant impact on the availability of phosphorus in the soil. The plant takes more phosphorus when phosphate dissolving microorganisms are present (Kumar *et al.* 2007). Our research found that inoculating sweet pepper with Bacillus sp. and cyanobacteria increased plant growth because they are capable of directly enhancing plant growth through the production of plant growth regulators (Ahemad and Kibret 2014). Soil microorganisms became major determinants of nutrient cycling and plant growth in organic management systems that did not use synthetic chemicals, and interactions between biological and chemical fertilizers caused significant differences in yield (van der Putten et al., 2016). Ascorbic acid (AA) has long been thought to be a vital nutritional component of peppers. Vitamin C in pepper flesh varies depending on cultivar, environmental conditions, and cultural practices. This could be because P cycle enzyme activities are inversely related to P availability, and when P is a limiting nutrient, demand increases, resulting in higher phosphatase activity in the presence of P-solubilizes. Phosphatase activity is also increased following dual inoculation, particularly with *Paenibacillus Polymyxa (PP)* and *BM* and *Mycorrhizal* bacteria, which work well together as a co-inoculation system (Ju *et al.* 2020).

## CONCLUSION

The effects of some biofertilizers (*BM*, *BA*, and Cyanobacteria) on the growth parameters and chemical contents of the sweet pepper (*Capsicum frutescens*) plant were investigated in two successive seasons. Plants treated with the combination *BM*/*BA*/Cyanobacteria have shown the highest increase in photosynthetic pigments, increasing the total carbohydrates and sugar levels in the leaves. That resulted in vigorous growth as measured by plant stem length, stem diameter, number of leaves per plant, leaf area/plant, and leaf dry weight/plant. In addition, *bacillus* species were found to promote biological nitrogen fixation and the breakdown of insoluble complex organic matter into simpler forms, making them biologically available to plants. It also enhances soil moisture retention, plant nutrient availability (nitrogen and phosphorus), soil microbial health, soil aeration, and natural fertilization.

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## AUTHOR CONTRIBUTION

All the authors contributed equally to this work.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## REFERENCES

Aali K.A., Parsinejad M. and Rahmani B., 2009. Estimation of Saturation Percentage of Soil Using Multiple Regression, ANN, and ANFIS Techniques. Computer and Information Science 2 (3):127-136. DOI:https://doi.org/10.5539/cis.v2n3p127

Ahemad M. and Kibret M., 2014. Mechanisms and applications of plant growth promoting rhizobacteria: current perspective. Journal of King saud University-science 26 (1):1-20.

Almadhoun H.R., 2021. Bell pepper classification using deep learning.

AOAC International Official methods of analysis t.e. (2005) Current through revision 2, 2007 (On-line). AOAC International Gaithersburg, MD,

Behera K.K., Alam A., Vats S., Sharma H.P. and Sharma V. (2012) Organic farming history and techniques. In: Agroecology and strategies for climate change. Springer. pp 287-328,

Bellm E.C., Kulkarni S.R., Graham M.J., Dekany R., Smith R.M., Riddle R., Masci F.J., Helou G., Prince T.A. and Adams S.M., 2018. The zwicky transient facility: System overview, performance, and first results. Publications of the Astronomical Society of the Pacific 131 (995):018002.

Black C.A., Evans D. and White J. (1965) Methods of soil analysis: chemical and microbiological properties. ASA,

Casida Jr L., Klein D.A. and Santoro T., 1964. Soil dehydrogenase activity. Soil science 98 (6):371-376.

Chapman H. and Pratt P. (1978) Methods of analysis for soils, plant and water. . In, vol 50. "University of California Division of Agricultural Sciences" Sciences. Priced Publication. doi:https://doi.org/10.2136/sssaj1963.03615995002700010004x

Chen Y., Rekha P., Arun A., Shen F., Lai W.-A. and Young C.C., 2006. Phosphate solubilizing bacteria from subtropical soil and their tricalcium phosphate solubilizing abilities. Applied Soil Ecology 34 (1):33-41.

Chittora D., Meena M., Barupal T., Swapnil P. and Sharma K., 2020. Cyanobacteria as a source of biofertilizers for sustainable agriculture. Biochemistry and biophysics reports 22:100737.

Choudhary D.K. and Johri B.N., 2009. Interactions of Bacillus spp. and plants–with special reference to induced systemic resistance (ISR). Microbiological research 164 (5):493-513.

CoStat V. (2005) Cohort software798 light house Ave. PMB320. USA, DownloadCoStatPart2. html

Dewis J. and Freitas F., 1970. Physical and chemical methods of soil and water analysis. FAO Soils Bulletin (10):275.

Dubois M., Gilles K.A., Hamilton J.K., Rebers P.t. and Smith F., 1956. Colorimetric method for determination of sugars and related substances. Analytical Chemistry 28 (3):350-356.

Duncan D.B., 1955. Multiple range and multiple F tests. Biometrics 11 (1):1-42.

El Arnaouty S., El-Heneidy A., Afifi A.I., Heikal I. and Kortam M.N., 2020. Comparative study between biological and chemical control programs of certain sweet pepper pests in greenhouses. Egyptian Journal of Biological Pest Control 30 (1):1-7.

Flores-Félix J.D., Silva L.R., Rivera L.P., Marcos-García M., García-Fraile P., Martínez-Molina E., Mateos P.F., Velázquez E., Andrade P. and Rivas R., 2015. Plants probiotics as a tool to produce highly functional fruits: the case of Phyllobacterium and vitamin C in strawberries. PLoS ONE 10 (4):e0122281.

Gomez K.A. and Gomez A.A. (1984) Statistical procedures for agricultural research. John Wiley & Sons, https://books.google.com.sa/books?hl=en&lr=&id=PVN7_XRhpdUC&oi=fnd&pg=PA1&dq=Statistical+procedures+for+agricultural+research&ots=Hs698nsmk5&sig=Fy9BWEHuRnbatuPahoGeswLCFxw&redir_esc=y#v=onepage&q=Statistical%20procedures%20for%20agricultural%20research&f=false

Hernández Y., Lobo M.G. and González M., 2006. Determination of vitamin C in tropical fruits: A comparative evaluation of methods. Food Chemistry 96 (4):654-664.

Hesse P.R. (1971) A Text Book of Soil Chemical Analysis vol 528. Soil Chemical Analysis. John murry, London, UK,

Jackson M. (1973) Soil chemical analysis: Advanced Course vol 498. Soil Chemistry. UW-Madison Libraries Parallel Press, USA,

Jamal Q., Lee Y.S., Jeon H.D. and Kim K.Y., 2018. Effect of plant growth-promoting bacteria Bacillus amylliquefaciens Y1 on soil properties, pepper seedling growth, rhizosphere bacterial flora and soil enzymes. Plant Protection Science 54 (3):129.

Ju W., Jin X., Liu L., Shen G., Zhao W., Duan C. and Fang L., 2020. Rhizobacteria inoculation benefits nutrient availability for phytostabilization in copper contaminated soil: drivers from bacterial community structures in rhizosphere. Applied Soil Ecology 150:103-150.

Kim K., Jordan D. and McDonald G., 1997. Effect of phosphate-solubilizing bacteria and vesicular-arbuscular mycorrhizae on tomato growth and soil microbial activity. Biology and Fertility of Soils 26 (2):79-87.

Kumar A., Kumar J. and Ram B., 2007. Effect of inorganic and bio-fertilizers on growth, yield and quality of tomato (Lycopersicon esculentum Mill.). Progressive Agriculture 7 (1and2):151-152.

Kumar V. and Narula N., 1999. Solubilization of inorganic phosphates and growth emergence of wheat as affected by Azotobacter chroococcum mutants. Biology and Fertility of Soils 28 (3):301-305.

Lichtenther H., 1987. Chlorophylls and carotenoides: Pigments of photosynthesis. Methods in Enzymology INRA, EDP Sci 57:245-250.

Lindsay W.L. and Norvell W.A., 1978. Development of a DTPA soil test for zinc, iron, manganese, and copper. Soil science society of America journal 42 (3):421-428. DOI: https://doi.org/10.2136/sssaj1978.03615995004200030009x

Luo P., Han X., Wang Y., Han M., Shi H., Liu N. and Bai H., 2015. Influence of long-term fertilization on soil microbial biomass, dehydrogenase activity, and bacterial and fungal community structure in a brown soil of northeast China. Annals of microbiology 65 (1):533-542.

Meena R.S., Kumar S., Datta R., Lal R., Vijayakumar V., Brtnicky M., Sharma M.P., Yadav G.S., Jhariya M.K. and Jangir C.K., 2020. Impact of agrochemicals on soil microbiota and management: A review. Land 9 (2):34.

Mikhak A., Sohrabi A., Kassaee M.Z. and Feizian M., 2017. Synthetic nanozeolite/nanohydroxyapatite as a phosphorus fertilizer for German chamomile (Matricariachamomilla L.). Industrial crops and products 95:444-452.

Miljaković D., Marinković J. and Balešević-Tubić S., 2020. The significance of Bacillus spp. in disease suppression and growth promotion of field and vegetable crops. Microorganisms 8 (7):1037.

Mohamed E.A., Farag A.G. and Youssef S.A., 2018. Phosphate solubilization by Bacillus subtilis and Serratia marcescens isolated from tomato plant rhizosphere. Journal of Environmental Protection 9 (03):266.

Olsen S.R. (1954) Estimation of available phosphorus in soils by extraction with sodium bicarbonate. vol 939. US Department of Agriculture,

Pandey V.C., Bajpai O. and Singh N., 2016. Energy crops in sustainable phytoremediation. Renewable and Sustainable Energy Reviews 54:58-73.

Pramer D. and Schmidt E., 1964. Experimental soil microbiology burges publishing company Minneapolis, 15. Minnesota, USA Sons Ltd Beecles and London:31-32.

Sahlemedhin S. and Taye B., 2000. Procedures for soil and plant analysis. Technical paper 74:110.

Simarmata T., Turmuktini T., Fitriatin B.N. and Setiawati M.R., 2017. Bioameliorant and Biofertilizers Application to Increase the Soil Health and Rice Productivity. HAYATI Journal of Biosciences.

Smith K.A. (2000) Soil and environmental analysis Physical methods, revised and expanded, 2nd edn. CRC Press, https://books.google.com.sa/books?hl=en&lr=&id=lGMFYp2CA2EC&oi=fnd&pg=PP1&dq=Soil+and+environmental+analysis&ots=DfxLo81FJH&sig=lUqtyxXp-D5Hx6hd-80K14Xpklw&redir_esc=y#v=onepage&q=Soil%20and%20environmental%20analysis&f=false

Sparks D.L., Page A.L., Helmke P.A. and Loeppert R.H. (2020) Methods of soil analysis, part 3: Chemical methods, vol 14. John Wiley & Sons,

Tabatabai M.A. and Bremner J.M., 1969. Use of p-nitrophenyl phosphate for assay of soil phosphatase activity. Soil Biology and Biochemistry 1 (4):301-307.

van der Putten W.H., Bradford M.A., Pernilla Brinkman E., van de Voorde T.F. and Veen G., 2016. Where, when and how plant–soil feedback matters in a changing world. Functional Ecology 30 (7):1109-1121.

Vejan P., Abdullah R., Khadiran T., Ismail S. and Nasrulhaq Boyce A., 2016. Role of plant growth promoting rhizobacteria in agricultural sustainability—a review. Molecules 21 (5):573.

Wirth E.H., 1946. Soil and Plant Analysis, by C. S. PIPER. Interscience Publishers, Inc., New York. Journal of the American Pharmaceutical Association (Scientific ed) 35 (6):192. DOI:https://doi.org/10.1002/jps.3030350611

Zhang F., Pant D. and Logan B.E., 2011. Long-term performance of activated carbon air cathodes with different diffusion layer porosities in microbial fuel cells. Biosensors and Bioelectronics 30 (1):49-55.

Zhou T., Chen D., Li C., Sun Q., Li L., Liu F., Shen Q. and Shen B., 2012. Isolation and characterization of *Pseudomonas brassicacearu*m J12 as an antagonist against Ralstonia solanacearum and identification of its antimicrobial components. Microbiological research 167 (7):388-394.