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# Enhancing *Cucurbita pepo* Growth, Productivity, and Fruit Quality using Bacilli Strains and Cyanobacteria Treatments

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*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

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## ABSTRACT

Two successful field experiments were carried out during 2020and 2021 growing seasons to evaluate the effect of bio fertilizers; *Bacillus amyloliquifaciens* (BA), *Bacillus megaterium* (BM) and cyanobacteria inoculation on the vegetative growth, growth parameters and plant chemical content of Cucurbita pepo (Squash) crop. The study of mixed inoculation with *both Bacillus strains*, and *cyanobacteria* was found to improve vegetative growth, plant chemical contents and positive microbial activity in the soil Rhizosphere in comparison to un-inoculated plants. Soil available nutrients (N and K) increased significantly with *BA* and *BM* combined with cyanobacteria while available phosphorus gave most increase with *BM.*

*Keywords: Bacillus amyloliquifaciens; Bacillus megaterium; cyanobacteria; bio fertilizers; Squash plant.*

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## 1. INTRODUCTION

The importance of the use of bio fertilizers in improving plant growth and yield was reported [1]. Bio fertilizers are considered as environmentally friendly and cost-effective alternative to chemical fertilizers, enhancing crop productivity and soil health in a long-term manner [2]. The microorganism beneficial nutrients, plant growth-promoting Rhizobacteria, $N_2$-fixing, P-solubilizing, P-mobilizing bio fertilizers are also of high importance [3].Through an immobilization process on carrier material, these microbial processes may aid plants in increasing nutrient uptake efficiency and increasing the availability of surface area and cell count of such microorganisms [4].The Rhizosphere of a plant is a highly competitive ecosystem in which microorganisms compete for nutrients supplied by the plant root. Some of these bacteria are known as "PGPRs," or plant growth promoting Rhizobacteria, because they live within or around plant roots and encourages plant growth. Members of this genus can also persist for a long period in inappropriate environments [5]. Genetics and environmental factors have an impact on crop productivity. Plant-friendly microorganisms are employed in place of artificial fertilizers and pesticides to boost crop productivity. Rhizobacteria that promote plant development includes Bacillus species, where they create a number of compounds that help plants develop faster while also reducing pathogen infestation [6]. Bacillus modulates intracellular phytohormones metabolism and promotes plant stress tolerance by producing indole-3-acetic acid, gibberellic acid, and 1-aminocyclopropane-1-carboxylate (ACC). Furthermore, the manufacture of exopolysaccharides and siderophores, which prevent harmful ions from moving through plant tissues and regulate ionic balance and water transport while suppressing pathogenic microbial populations [7]. As a result, the usage of new biotechnological products that are both environmentally friendly and sustainable, such as microbial bio fertilizers PGPRs, is steadily expanding [8]. Plant metabolism was disrupted by unfavourable environmental circumstances, resulting in reduced crop growth and yield. Bacillus-induced physiological changes (i.e. regulation of water transport, nutrient uptake, activation of the antioxidant and defence systems) reduce biotic and abiotic stress factors that harm crops. By changing stress-responsive genes, proteins, phytohormones, and associated metabolites, the Bacillus association boosts plant immunity to stressors [9]. Bacillus species are gaining popularity as a bio fertilizer or bio-pesticide due to their persistence and spore-forming ability. *BA* is a form of PGPR with a high vitality and capacity to be planted in the field [2]. It serves as a biological insecticide as well as a biological fertilizer. It was discovered that *BA* could help plants develop faster and more evenly. The proposed action mechanisms are as follows: first, several amino acids and fatty compounds produced by *BA* may aid plant growth and development, as well as the balance of soil minerals, such as phytase produced during metabolism [10], which could aid in the conversion of un absorbable organic phosphorus in the soil to absorbable phosphorus, thereby improving plant phosphorus absorption efficiency [11]. Furthermore, numerous plant growth-promoting bacteria have been found to create plant growth regulators in the Rhizosphere, such as IAA, CTK, and other plant hormones, in order to boost plant growth and yield [12]. *BM* is a gram-positive, rod-shaped bacterium that produces endospores. It is regarded to be one of the most common soil bacterial bio fertilizers that promotes plant development (PGPR) [13] and a soil inoculant that has the potential to solubilize phosphorus, which is beneficial to plants. Cyanobacteria have been shown to create connections with both vascular and non-vascular plants and produce growth-promoting chemicals. The presence of cyanobacteria in the Rhizosphere can aid in the digestion of organic compounds, and their interaction with agricultural plants is beneficial to crop establishment, growth, and yield [14].The application of *BM* fermentation broth exhibited a considerable increase in the germination and growth of several crops and inhabitation of the growth of approximately 20 different plant diseases [15]. Cyanobacteria are Gram-negative photosynthetic prokaryotes that can live in a variety of aquatic and terrestrial habitats. They can be found on their own or in symbiotic relationships with a wide range of lower and higher plants, as well as in microbial mats. They are well-known for their ability to fix nitrogen [16]. These organisms have the ability to fix nitrogen, produce various plant growth regulators (auxins, gibberellins, cytokines, etc. ), 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 [17]. The decomposed cyanobacteria organic matter mixes with the soil and functions as a binding mucilaginous agent, increasing the humus level and making the soil more suitable for other plant growth [18]. The biological soil crusts are degraded by natural and anthropogenic disturbances, and full recovery under natural conditions could take decades. Inoculation with cyanobacteria, on the other hand, substantially accelerates the recovery process, and biological crusts like these found in semiarid and arid regions of the world serve a vital role in maintaining and rebuilding the ecosystem [19]. Regarding to these reported facts and findings, bio fertilizers can be expected to reduce the use of chemical fertilizers and pesticides. They simply restore the soil's natural nutrient cycle and build soil organic matter, ensure that the host plants receive an adequate supply of nutrients and that their growth and physiology are properly regulated. Therefore, it's of our interest to study the effect of applying *BA*, *BM* and cyanobacteria as bio fertilizers in individual addition or in different mixtures on Cucurbita pepo (CP) crop in comparison with regular chemical fertilization (control).

## 2. MATERIALS AND METHODS

### 2.1 Experiment Design

Bacilli strains (*BA* and *BM*) and Cyanobacteria were provided by Agriculture Microbiology Department- Soils, Water & Environmental Research Institute, Agriculture Research Centre (ARC), Giza, Egypt. The work was conducted during two seasons 2020 and 2021 at the Experimental Farm of Al-Azhr University (Cairo, Egypt) to study the effect of selected Bacilli strains and *cyanobacteria* inoculation on *CP* crop. Culture of microorganism strains saved on the slant were inoculated into nutrient medium, and were made into liquid seed after shaking culture at 200 r/min for 24 h in the constant temperature shaking table of 32 °C, prior to seed sowing. The farm was fertilized with superphosphate (15% $P_2O_5$), 1.25 g ammonium sulphate (20.5% N) and 0.16 g potassium sulphate (48% $K_2O$). Seeds of *CP* were divided into two parts, where the first part was planted directly as control line and the second part was immersed into powder of cyanobacteria. The Bacilli strains were added as soil drench after seeds had been planted. Treatments of the experiment were as follow: Control; *CP* seeds dressed *cyanobacteria* powder; *CP* seeds drenched with *BA*; *CP* seeds drenched with *BM*; *CP* seeds drenched with *BM* and *BA*; *CP* seeds dressed *cyanobacteria* powder and drenched with *BA*; *CP* seeds dressed *cyanobacteria* powder and drenched with *BM*; *CP* seeds dressed *cyanobacteria* powder and drenched with *BM* and *BA*.

### 2.2 Soil Analysis

Mechanical analysis of soil (Table 1) was determined by the international pipette method using NaOH as a depressing agent [20]. The soil acidity (pH) was determined in the soil paste using a Gallenkamp pH meter (A. Gallenkamp Co.& Ltd., UK), and electric conductivity (EC) in 1: 2.5 soil: water extract was determined according to the reported procedures [21]. Available nitrogen (extracted using a 1% potassium sulphate solution) using the Devarda alloy method by steam distillation [22], [23]. Spectrophotometry of available phosphorus (extracted using a $NaHCO_3$ 500 mM solution with pH of 8.5) was determined at wavelength 650 nm [24] using spectrophotometer Beckman Du 7400 (GMI Co., MN, USA). Available potassium was flame photo metrically measured using a Corning flame photometer [25] using a 1 N ammonium acetate soln. (pH= 7.0). Organic matter was determined according to Walkley & Black chromicacid wet oxidation method [26]. Available micronutrients in soil samples were extracted by diethylene triamine pentaacetic acid (DTPA) soln. [27] and determined using the atomic absorption spectrophotometer. Saturation percentage (SP%) was determined according to reported procedure [28] and Hydraulic conductivity (K) values of the soil samples columns were determined according to standard method of Smith [29].

### 2.3 Plant Analysis

#### 2.3.1 Determination of total chlorophyll

To estimate the mass of chlorophyll a, chlorophyll b, and total chlorophyll per leaf, pigments were extracted by soaking 0.5 g fresh and young leaves in a dimethyl formamide (DMF) soln. overnight at 4 °C. The pigments were computed using equation of Moran and a spectrophotometer Beckman Du 7400 at wavelengths of 663, 470, and 647 nm [30].

### 2.3.2 Phosphatase enzyme analysis

The activity of phosphatase was measured using the technique of Tabatabai & Bremner [31].

### 2.3.3 Determination of plant growth parameters and chemical Contents

Plant height (cm) was measured from the first node to the plant top, Leaf number per plant and total number of fruits/ plot was counted. Fruit length and diameter (using a vernier caliper) was measured in cm. Fresh Fruit weight/ plot was determined by a balance in kg. Fruit firmness was measured in kg/cm² by Magness and Ballauf pressure tester (D. Ballauf Manufacturing Co., MD, USA). Fruit size was measured in $cm^3$ by immersing the fruit in a container filled with water and measuring the displaced water with a graduated jar. Fruit dry weight was calculated as g/100g fresh weight by drying 100g of fresh weight in a 70 °C oven until it achieved a consistent weight. The samples were analyzed for dehydrogenase activity according to the method described by Casida [32]. Plant samples were wet digested using sulphuric acid and per chloric acid mixture [33] and plant nutrients were determined in the aliquot using kjeldahl method [34] for nitrogen, stannous chloride reduced molybdo-phosphoric blue colour method [34] for phosphorus and flame photometer [34] for potassium. Percentages of total soluble solids (%T.S.S.) were determined by Abbe refract meter [35]. The dye 2,6- dichlorophenol indophenol method was used to determine ascorbic acid as mg/100 g fresh weight [36]. Total sugars were determined as g/100g dry weight according to Dubois [37].

### 2.3.4 Statistical analysis

Appropriate analysis of variance was performed using COSTATE V 6.4 (2005) for Windows [38]. 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 [39], [40].

**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** | 34.06 | 18.69 | 9.00 | 27.31 | 31.90 | 21.23 | 40.29 | 1.36 |
| **Available water (% v/v)** | | **H.W%** | | **Bulk density ( Mg m-3)** | | | **Total porosity%** | |
| 8.39 | | 6.3 | | 1.52 | | | 47.5 | |
| **Chemical properties** | | | | | | | | |
| **pH in suspension 1:2.5** | **Organic matter (O.M%)** | **Available nutrients (ppm)** | | | | | | |
| 7.84 | 0.541 | N<br>25 | P<br>9 | K<br>88 | Fe<br>12.4 | Mn<br>9.9 | Zn<br>1.5 | Cu<br>0.88 |
| **Soluble cation**(meq/ L)** | | | | **Soluble anions**(meq/ L)** | | | | |
| **Ca++** | **Mg++** | **Na+** | **K+** | **Co3--** | **Hco3-** | **Cl-** | **SO4=** | |
| 5.51 | 2.75 | 10.69 | 1.03 | 7.70 | 3.20 | 14.80 | 2.91 | |
| **SAR** | | **ESP** | | **CaCO3%** | | | **EC (ds/m)** | |
| 1.64 | | 3.34 | | 5.95 | | | 2.05 | |

*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.*

## 3. RESULTS AND DISCUSSION

### 3.1 Plant Growth Parameters

The collected data in Tables (2 and 3) illustrated the influence of *BM*, *BA* and cyanobacteria on some growth parameters of squash plants.

Selected bacteria and fungi (*BM, BA*, and *cyanobacteria*) are greatly boosted the growth characteristics of CP plants, such as plant height (cm), number of leaves/plant, and number of fruits/ plot (Table2).

In both seasons, treatment with *BM* combined with each of *BA* and/ or cyanobacteria had the best stimulatory impact and maximal augmentation in plant parameters compared to control, followed by treatment with *BA* mixed with *BM*. As indicated by revealed data (Table 2), it' obviously clear that *BM* had the greatest significant growth in plant height (75.17 and 79.90 cm, respectively) in both seasons. Furthermore, *BM/ BA* mixture produced the same highest values with number of leaves/ plant (22.0 and 23.67), and the addition of *BM, Ba* and cyanobacteria produced the same results (22.0 and 23.33). The blend of *BM, BA,* and *cyanobacteria* produced the largest number of fruits per plot in CP crop (620.67and 653.00), followed by *BM and BA.* On the other hand, Table 3 shows the growth and some yield parameters as affected by the individual addition or combined effect of *BM, BA,* and *cyanobacteria*.

The highest values of fruit length in both seasons are recorded with the mixture of *BA/BM/Cyanobacteria* (12.33 and11.17cm), followed by *BA/BM* mixture (11.90 and11.0cm) and *BA/ Cyano* mixture (11.03and10.43cm) comparing to the control. The effect of two types of bacteria and cyanobacteria on fruit diameter is recorded in the order *BA/BM/Cyano* mixture (2.78cm) in the first season and nearly the same with *BA/BM* mixture (2.77cm) in the second season, while the highest value is recorded on treatment with *BA/BM/Cyano* mixture (3.53cm) in the second season.

Fruit fresh weight yielded the highest values in both seasons with the addition of *BM* (38.62 and 37.11 g) followed by *BM/Cyano* (37.52 and 34.70 g) as compared with control. The same pattern is observed in fruit dry weight, where treatment with *BM* (3.411 and 3.25 g) recorded the highest values in both seasons, followed by BM/Cyano mixture (2.84and 2.89g) in both seasons.

Fruit firmness and fruit size are among the yield metrics included in the same table. Fruit firmness data shows that the highest values are obtained with the combined application of *BA/ BM/Cyano* (7.67 and 7.80 kg/cm$^2$) and the lowest values were obtained with *Cyanobacteria* (6.21 and 6.34 kg/cm$^2$) in the two seasons, respectively.

**Table 2. Effect of inoculation with *BM, BA* and *cyanobacteria* on growth parameters of squash plants at harvest time during the seasons of 2020 and 2021**

| Treatments | Plant height (cm) | | Number of leaves/plant | | Number of fruits/ plot | |
|---|---|---|---|---|---|---|
| | Season | | | | | |
| | 2020 | 2021 | 2020 | 2021 | 2020 | 2021 |
| **Control** | 58.57g | 60.23g | 13.00e | 14.33e | 337.33f | 442.67h |
| **BA** | 70.60d | 73.70e | 17.67cd | 18.67c | 579.00d | 583.33e |
| **BM** | 69.00e | 71.30f | 17.67cd | 17.33d | 469.00e | 567.33f |
| **Cyanobacteria** | 65.90f | 75.97d | 16.67d | 16.33d | 466.67e | 459.33g |
| **BA/BM mixture** | 73.03b | 78.33b | 22.00a | 23.33a | 616.67a | 645.00b |
| **BA/Cyano mixture** | 72.63bc | 77.03c | 19.33b | 21.67b | 605.67b | 633.00c |
| **BM/Cyano mixture** | 71.77c | 75.50d | 18.33bc | 19.67c | 589.33c | 593.33d |
| **BA/BM/ Cyano mixture** | 75.17a | 79.80a | 22.00a | 23.67a | 620.67a | 653.00a |
| **L.S.D. (0.05)** | **0.93** | **0.59** | **1.38** | **1.01** | **7.80** | **4.73** |

**Table 3. Effect of inoculation with BM, BA and cyanobacteria on CP fruit features during the seasons of 2020 and 2021**

| Treatments | Fruit length (cm) | | Fruit diameter (cm) | | Fruit fresh weight (g) | | Fruit dry weight (g) | | Fruit firmness (kg/ cm2 ) | | Fruit size (cm) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Season | | | | | | | | | | | |
| | **2020** | **2021** | **2020** | **2021** | **2020** | **2021** | **2020** | **2021** | **2020** | **2021** | **2020** | **2021** |
| ***Control*** | 8.67g | 7.73d | 1.60d | 2.10f | 21.86h | 20.91g | 2.00f | 2.13f | 5.26h | 5.37h | 21.97f | 21.23h |
| ***BA*** | 9.93e | 10.03b | 2.40b | 2.97d | 30.16e | 34.29b | 2.14f | 2.25e | 6.40e | 6.62e | 34.40b | 42.93b |
| ***BM*** | 9.80e | 8.83c | 2.13c | 2.90de | 38.62a | 37.11a | 3.41a | 3.25a | 6.31f | 6.53f | 43.30a | 45.87a |
| ***Cyanobacteria*** | 9.20f | 8.50c | 2.10c | 2.77e | 33.19d | 29.81d | 2.63c | 2.43d | 6.21g | 6.34g | 31.17c | 29.73e |
| ***BA/BM mixture*** | 11.90b | 11.00a | 2.77a | 3.37b | 26.58f | 26.46e | 2.45d | 2.33e | 7.25b | 7.61b | 42.07a | 27.03f |
| ***BA/Cyano mixture*** | 11.03c | 10.43b | 2.70a | 3.17c | 24.50g | 24.95f | 2.29e | 2.28e | 6.94c | 7.22c | 25.17e | 22.83g |
| ***BM/Cyano mixture*** | 10.40d | 10.10b | 2.40b | 3.03cd | 37.52b | 34.70b | 2.73bc | 2.60c | 6.51d | 6.76d | 28.07d | 38.23c |
| ***BA/BM/Cyano mixture*** | 12.33a | 11.17a | 2.87a | 3.53a | 35.87c | 31.68c | 2.84b | 2.89b | 7.67a | 7.80a | 34.40b | 34.13d |
| ***L.S.D.(0.05)*** | **0.31** | **0.44** | **0.19** | **0.15** | **0.95** | **1.25** | **0.15** | **0.09** | **0.07** | **0.04** | **1.33** | **1.04** |

**Table 4. Effect of inoculation with *B. megaterium*, *B. amyloliquifaciens* and cyanobacteria on squash fruits features during the seasons of 2020 and 2021**

| Treatments | Total soluble solids (%) | | Ascorbic acid (mg/ (100 g F.W.) | | Total sugar (mg/g FW) | | Total chlorophyll (Mg/ g) | |
|---|---|---|---|---|---|---|---|---|
| | Season | | | | | | | |
| | 2020 | 2021 | 2020 | 2021 | 2020 | 2021 | 2020 | 2021 |
| **Control** | 3.31d | 3.40g | 18.47h | 17.82e | 17.77f | 18.70f | 1.011h | 1.022g |
| **BA** | 5.61ab | 4.51e | 22.83e | 23.24c | 25.00d | 22.93d | 1.185e | 1.231e |
| **BM** | 5.67a | 5.72b | 21.90f | 22.84c | 23.57e | 20.83e | 1.168f | 1.123f |
| **Cyanobacteria** | 3.62c | 3.61f | 20.14g | 20.63d | 17.77f | 20.40ef | 1.155g | 1.122f |
| **BA/BM mixture** | 5.57b | 4.64d | 29.04b | 30.59a | 29.17b | 33.50a | 1.356b | 1.466b |
| **BA/Cyano mixture** | 5.67a | 5.74a | 28.14c | 25.92b | 28.00bc | 30.63b | 1.243c | 1.387c |
| **BM/Cyano mixture** | 5.63ab | 5.71bc | 26.61d | 23.57c | 28.87c | 28.10c | 1.233d | 1.249d |
| **BA/BM/Cyano mixture** | 5.63ab | 5.70c | 29.88a | 31.16a | 32.10a | 34.93a | 1.388a | 1.492a |
| **L.S.D.(0.05)** | **0.07** | **0.01** | **0.82** | **1.21** | **1.29** | **2.04** | **0.005** | **0.007** |

Fruit size is the penultimate characteristic in this table, and it increased the most with the addition of *BM* and also with the mixture of *BA/ BM* (43.30 and 42.07cm, respectively) in the first season, whereas in the second season, the greatest value was observed on treatment with the mixture of *BM* (45.87cm) followed by *BA* (42.93cm).

## 3.2 Photosynthetic Pigments, Chemical Constituents and Fruit Quality Parameters

Table 4 shows the effects of two types of bacillus, either alone or in combination with cyanobacteria on some chemical fruit quality parameters of squash crop such as total soluble solids(TSS), endogenous phytohormones content (i.e. Ascorbic acid), chemical content (i.e. total sugar), and photosynthetic pigments (i.e. Total chlorophyll).

In terms of fruit quality, data shows that inoculation with *BA* separately (5.67%) or in combination with *Cyanobacteria* (5.67%) resulting in a good rise in total soluble solids (T.S.S), followed by mixed inoculation with *BA/BM* (5.57%) in the first season. The biggest rise is obtained on treatment with mixture of *BA/Cyano* (5.74%) in the second season, followed by adding *BM* separately (5.72%). Ascorbic acid (Vitamin C) data is well-known for its antioxidant qualities, and it's also one of the chemical fruit quality factors. In both seasons, the largest increase is observed with *BA/BM/Cyano* mixture (29.88 and 31.16 mg/ 100 g F.W), followed by *BA/BM* mixture (29.04 and 30.59 mg/ 100 g F.W). *A cyanobacterium, on the other hand, has* the lowest value in both seasons. The maximum increase in total sugar is appeared on treatment with *BA/ BM/Cyano* blend (32.10 and 34.93 mg/g FW, respectively). Treatment with *BA/BM* blend (29.17 and 33.50 mg/g FW) comes in the second order.

## 3.3 Plant Chemical Contents

### 3.3.1 Effect of inoculation with *BM*, *BA* and *cyanobacteria* on squash chemical content

The effect of inoculation *with BM, BA* and *cyanobacteria* on squash plants is tabulated in Table 5.

The results in Table 5 show that the application of *BA/BM/Cyano* resulted in the highest significant values of plant nitrogen percent (2.75 and 3.43%), followed by *BM/Cyano* blend (2.57and 2.88%). While, applying of *BM* separately produced the smallest increase in nitrogen content. Plant phosphorus content increased the most with a mixture of *BA/BM/Cyano* in the first and second seasons (0.726 and 0.813%, respectively), followed by *BA/Cyano* mixture (0.653 and 0.742%). The potassium contents of the squash plant are displayed the highest values in both seasons as a result of the addition of *BA/BM/Cyano* (4.44 and 4.26%, repectively). These are followed by the treatment blend *BA/BM* (4.20 and 4.32%) and hence with *cyanobacteria* (2.42 and 2.25%) which showing the smallest decrease in both seasons.

**Table 5. Effect of inoculation with *BM, BA* and *cyanobacteria* on chemical content of squash plant at harvest during the seasons of 2020 and 2021**

| Treatments | N% | | P% | | K% | |
|---|---|---|---|---|---|---|
| | Season | | | | | |
| | 2020 | 2021 | 2020 | 2021 | 2020 | 2021 |
| **Control** | 1.44f | 1.83d | 0.252f | 0.336g | 1.47f | 1.67f |
| **BA** | 1.80d | 2.29c | 0.445c | 0.532d | 3.62c | 2.73cd |
| **BM** | 1.77d | 2.49c | 0.412d | 0.452e | 3.69c | 2.35de |
| **Cyanobacteria** | 1.61e | 2.43c | 0.359e | 0.352f | 2.42e | 2.25e |
| **BA/BM mixture** | 2.13c | 2.88b | 0.715a | 0.745b | 4.20b | 4.32a |
| **BA/Cyano mixture** | 2.19c | 2.84b | 0.653b | 0.742b | 3.73c | 3.16bc |
| **BM/Cyano mixture** | 2.57b | 2.88b | 0.454c | 0.651c | 3.36d | 3.20b |
| **BA/BM/Cyano mixture** | 2.75a | 3.43a | 0.726a | 0.813a | 4.44a | 4.26a |
| **L.S.D.(0.05)** | **0.14** | **0.24** | **0.011** | **0.008** | **0.20** | **0.39** |

**Table 6. Effect of inoculation with *BM*, *BA* and *cyanobacteria* on squash fruits features during the seasons of 2020 and 2021**

| Treatments | % N | | % P | | % K | |
|---|---|---|---|---|---|---|
| | Season | | | | | |
| | 2020 | 2021 | 2020 | 2021 | 2020 | 2021 |
| **Control** | 33.00g | 40.00e | 13.26g | 14.24e | 278.33e | 265.33d |
| ***BA*** | 79.00e | 93.33c | 16.32de | 17.55c | 325.00d | 345.33c |
| ***BM*** | 70.00f | 84.33d | 17.62a | 18.64a | 278.33e | 279.67d |
| ***Cyanobacteria*** | 66.67f | 81.67d | 14.36f | 15.52d | 318.67d | 341.33c |
| ***BA/BM mixture*** | 128.67b | 136.67a | 16.17e | 17.31c | 367.33b | 378.33b |
| ***BA/Cyano mixture*** | 101.00d | 116.00b | 16.63cd | 17.54c | 345.33c | 356.00c |
| ***BM/Cyano mixture*** | 108.00c | 116.33b | 16.70c | 17.60bc | 325.00d | 352.00c |
| ***BA/BM/Cyano mixture*** | 137.67a | 142.00a | 17.10b | 18.08b | 517.33a | 528.67a |
| **L.S.D.(0.05)** | **4.51** | **5.46** | **0.34** | **0.50** | **14.41** | **21.66** |

### 3.3.2 Soil analysis

*3.3.2.1 Available N, P and K percentages in Squash Rhizosphere*

Treatment with bio fertilizers' formulations increased the content of nitrogen, potassium, phosphorus [14]. Squash plants were individually inoculated with *BA* or with either *BM* or *cyanobacteria* showing significant increase in available N, P, and K comparing to un-inoculated plants, as detailed in Table 6.

The combination of *BA, BM,* and *cyanobacteria* resulted in the greatest increase in available nitrogen content (137.67 and 14.00%), followed by the combination of *BA* and *BM* (128.67and136.67%). In both seasons, the highest available phosphorus value (P%) was obtained when *BM* was used alone or in combination with *BA* and *cyanobacteria*. The most significant increase in available K (K%) was observed when squash plants were inoculated with *BA/ Bm/ Cyanobacteria* blend (517.33 and 528.67%), followed by a mixture of *BA/ Bm/ Cyanobacteria* (367.33and378.33%).

*3.3.2.2 Phosphatase and Dehydrogenase activity*

Dehydrogenase enzyme activity (DHA) is an indicator of overall microbial activity in the soil because it indicates energy transfer. The enzyme activity in the Rhizosphere of squash plants is determined and tabulated in Table 7.

The data revealed that inoculation with *BM* increased (DHA) levels separately or in combination with *BA* and *cyanobacteria* compared to those who were not infected. The highest positive increase is observed with *BA/BM/Cyano* mixture (126.23 and 143.83 mg/g soil/24h). *BA/ Cyano* (112.27 and 135.06 mg/g soil/24h) comes next.

**Table 7. Some microbial activities in Rhizosphere of squash plants as affected by interactions between *BM, BA* and *cyanobacteria* during the seasons of 2020 and 2021**

| Treatments | Phosphatase (µ inorganic phosphorus/g dry soil/day) | | Dehydrogenase (mg/g soil/24h) | |
|---|---|---|---|---|
| | Season | | | |
| | **2020** | **2021** | **2020** | **2021** |
| ***Control*** | 11.73g | 14.57h | 63.54f | 72.65f |
| ***BA*** | 17.47e | 22.83f | 95.19d | 113.31d |
| ***BM*** | 27.15c | 37.42d | 99.00cd | 126.94c |
| ***Cyanobacteria*** | 15.77f | 19.13g | 89.78e | 107.68e |
| ***BA/BM mixture*** | 29.20b | 39.45c | 103.99c | 127.38c |
| ***BA/Cyano mixture*** | 23.07d | 29.32e | 95.44d | 123.61c |
| ***BM/Cyano mixture*** | 33.10a | 42.40b | 112.27b | 135.06b |
| ***BA/BM/Cyano mixture*** | 34.35a | 46.67a | 126.23a | 143.83a |
| ***L.S.D.(0.05)*** | **1.34** | **1.97** | **5.30** | **5.12** |

Multiple inoculations, especially with *BM/BA* and *cyanobacteria*, also resulted in the maximum phosphatase activity. As a co-inoculation system, Bacillus and cyanobacteria work well together. *BA/ BM/Cyano* mixture shows the highest increase (34.35 and 46.67 µ inorganic phosphorus/g dry soil/day), followed by *BM/Cyano* (33.10 and 42.40 µ inorganic phosphorus/g dry soil/day), respectively in both seasons. *BA/Cyano* (112.27 and 135.06 µ inorganic phosphorus/g dry soil/day) comes next. In terms of phosphatase activity, utilizing soluble P fertilizer in un- inoculated treatments reduced phosphatase activity as compared to nitrogen-fixing bacteria. This could be due to P cycle enzyme activities are inversely related to P availability, and when P is a limiting nutrient, demand rises, resulting in higher phosphatase activity in the presence of P-solubilizes. Phosphatase activity is also higher after dual inoculation, notably with *Paenibacillus Polymyxa (PP)* and *BM* and mycorrhizae bacteria which they work well together as a co-inoculation system [41].

## 4. CONCLUSION

It's of our interest to study the effect of some bio fertilizers (*BM, BA,* and *cyanobacteria*) on the growth parameters and chemical contents of squash plant for their great importance and validation for plant growth and production. According to the findings, plants inoculated with *BM and BA* combined with *cyanobacteria* produced the maximum increase in photosynthetic pigments, whereas cyanobacteria produced the lowest rise. These results could be attributed to the effect of such bio treatments on increasing photosynthetic pigments, which in turn helped to increase total carbohydrates and sugar levels in the leaves, resulting in vigorous growth as measured by plant stem length, stem diameter, number of leaves per plant, leaf area/ plant, and leaf dry weight/ plant. Combined inoculation with *Bacillus* species mixed by cyanobacteria is the best addition followed by *BA, BM* and *Cyanobacteria* individually and/ or in a mixture. *Bacillus* species increase biological nitrogen fixation and solubilisation of insoluble complex organic matter to a simpler form, making them biologically available to plants. It also improves soil moisture retention, soil nutrient (nitrogen and phosphorus) availability to plants, and soil microbial status, as well as soil aeration and natural fertilization.

## DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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