Original Research Article

Enhancing *Cucurbita pepo* Growth, Productivity, and fruit quality using *Bacilli* strains and *Cyanobacteria* treatments.

Abstract

Bacillus amyloliquifaciens, *Bacillus megaterium* ,and cyanobacteria were reported as an efficient bio fertilizers that had a significant effect on different crops .Two successful field experiments were carried out during 2020and 2021 growing season at the Experimental Farm of Al-Azhr University to evaluate the effect of both Bacilli strains (*Bacillus megaterium* and *Bacillus amyloliquefaciens*), and cyanobacteria inoculation on the vegetative growth, growth parameters and plant chemical content of Cucurbita pepo. This study was aimed to identify plant growth promoting bacterial isolates from soil samples and to investigate their ability to improve plant growth by analysing phytohormones production and phosphate solubilisation. The following are some of the most important results: The results showed that mixed inoculation with *Bacillus amyloliquefaciens*, *Bacillus megaterium*, and *cyanobacteria* improved vegetative growth as plant height, number of leaves, number of fruits, fruit diameter, and fruit firmness in comparison to un inoculated plants. Fruit length, fruit fresh weight, fruit dry weight, and fruit size gave the highest increase with *Bacillus megaterium.* Double inoculation with *Bacillus megaterium and cyanobacteria* gave the highest value of total soluble solids (T.S.S), and the same increase recorded with *Bacillus megaterium* individually. Mixture of *Bacillus amyloliquefaciens, Bacillus megaterium, and cyanobacteria* provided the greatest increase in Ascorbic acid, total sugar, total chlorophyll, and total N, P and K in plant. The results revealed clearly positive and significant microbial activity with *Bacillus amyloliquefaciens*, *Bacillus megaterium,* and *cyanobacteria* in the soil Rhizosphere, as expressed by the activity of dehydrogenase and phosphatase enzymes. Soil available nutrients (N and K)increased significantly with *Bacillus amyloliquefaciens*, *Bacillus megaterium*, as combined with cyanobacteria while available phosphorus gave most increase with *Bacillus megaterium* .combined inoculation with *Bacillus amyloliquefaciens*, *Bacillus megaterium* mixed by cyanobacteria is the best addition followed by *Bacillus amyloliquefaciens, Bacillus megaterium* separately or in mixture and also mixed each of them with cyanobacteria.

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

1-**Introduction**

A bio fertilizer is a prepared product containing one or more microorganisms that improves nutrient status by improving plant availability of nutrients or increasing plant access to nutrients, resulting in increased plant growth and yield. (Malusa & Vassilev 2014). Bio fertilizer is also a one-of-a-kind, environmentally friendly and cost-effective alternative to chemical fertilizers, enhancing crop productivity and soil health in a long-term manner. (Bisen *et al*. 2015). These bio fertilizers may also be used. Bio fertilizers for microorganism beneficial nutrients and plant growth-promoting rhizobacteria, N2-fxing bio fertilizers, P-solubilizing bio fertilizers, P-mobilizing bio fertilizers, bio fertilizers for microorganism beneficial nutrients, and bio fertilizers for plant growth-promoting Rhizobacteria (Singh et al. 2014).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. (Kulkarni et al. 2018).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.(Vejan *et al.* 2016). 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 include Bacillus species. (Kilian *et al.* 2000). *Bacillus spp.* creates a number of compounds that help plants develop faster while also reducing pathogen infestation. Bacillus modulates intracellular phytohormone 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. (Saghafi *et al.* 2019). 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.(Kumari & Singh 2020). Plant metabolism was disrupted by unfavorable environmental circumstances, resulting in reduced crop growth and yield. Bacillus-induced physiological changes, such as the regulation of water transport, nutrient uptake, and the activation of the antioxidant and defense 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.(Etesami 2020). Bacillus species are gaining popularity as a bio fertilizer or bio-pesticide due to their persistence and spore-forming ability. *Bacillus amyloliquefaciens* is a form of PGPR with a high vitality and capacity to be planted in the field. (Bisen *et al.* 2015). *B. amyloliquefaciens* serves as a biological insecticide as well as a biological fertilizer. *Bacillus megaterium* is a gram-positive, rod-shaped bacterium that produces endospores. *Bacillus megaterium* is a soil inoculant that has the potential to solubilize phosphorus, which is beneficial to plants. *Bacillus megaterium* is regarded to be one of the most common soil bacterial bio fertilizers that promotes plant development (PGPR).(Miljaković *et al*. 2020). The application of *B. amyloliquefaciens* fermentation broth exhibited a considerable increase in the germination and growth of several crops, according to the study. *B. amyloliquefaciens* also inhibits the growth of approximately 20 different plant diseases. (Jiao *et al*. 2020). 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. Cyanobacteria are well-known for their ability to fix nitrogen. (Prasanna *et al.* 2013b). These organisms can fix nitrogen, produce various plant growth regulators (auxins, gibberellins, cytokinins, 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.(Meena *et al.* 2020).*Bacillus amyloliquifaciens, Bacillus megaterim* and cyanobacteria were reported as efficient bio fertilizers that had a significant effect on different crops. Therefore, the objectives of this study that comparison between regular chemical fertilization (control), and the effect of using *Bacilli* bacterial strains and cyanobacteria as bio fertilizers in individual addition or in different mixture of them.

2-Materials and Methods:

2-1-Experiment design:

Bacilli strains and Cyanobacteria were provided by Agric. Microbiology. Dept., Soils, Water & Environ. Res. Inst., ARC, Giza, Egypt. The present work was conducted at the Experimental Farm of Al-Azhr University during two seasons 2020 and 2021 to study the effect of both Bacilli strains (*Bacillus amyloliquifaciens, Bacillus megaterium*),and *cyanobacteria* inoculation on squash (*Cucurbita pepo).*Culture of *Bacillus amyloliquefaciens* ,and *Bacillus megaterium* 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 ℃, prior to seed sowing, the farm was fertilized with superphosphate (15 % P2O5), 1.25 g ammonium sulphate (20.5% N) and 0.16g potassium sulphate (48% K2O), seeds of *Cucurbita pepo* were divided into two parts, first part was planted directly as control line, second part was immersed into powder of cyanobacteria, but Bacilli strains were added as soil drench after seeds planted. Thus the experiment included the following treatments: Control (with recommended, *Cucurbita pepo* seeds dressed *cyanobacteria* powder, *Cucurbita pepo* seeds drenched with *Bacillus amyloliquifaciens*. *Cucurbita pepo* seeds drenched with *Bacillus megaterium*, *Cucurbita pepo* seeds drenched with *Bacillus megaterium* and *Bacillus amyloliquifaciens*, *Cucurbita pepo* seeds dressed *cyanobacteria* powder and drenched with *Bacillus amyloliquifaciens*, *Cucurbita pepo* seeds dressed *cyanobacteria* powder and drenched with *Bacillus megaterium*, *Cucurbita pepo* seeds dressed *cyanobacteria* powder and drenched with *Bacillus megaterium* and *Bacillus amyloliquifaciens*.

2-2-Soil analysis:

Soil analysis was conducted as follows:

Data in table (1) showed the analysis of studied soil as follow**.** Mechanical analysis was determined following the international pipette method using NaOH as a depressing agent (Wirth 1946).PH value 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 (Sahlemedhin & Taye 2000). Available nitrogen (extracted using a 1 per cent potassium sulphate solution using the Devarda alloy method by steam distillation) (Pramer & Schmidt 1964)as described by (Black et al. 1965). Spectrophotometry of available phosphorus (extracted using a $NaHCO_3$ 0.5 M solution with a pH of 8.5) at wavelength 650 nm. (Olsen 1954). Available potassium was flame photo metrically measured using a Corning flame photometer, as described by (Dewis & Freitas 1970) using a 1N ammonium acetate solution with a pH of 7.0.Organic matter was determined according to Walkley & Black chromic acid wet oxidation method (Hesse 1971). Available micronutrients in soil samples were extracted by diethylene triamine pentaacetic acid (DTPA) solution (Lindsay & Norvell 1978) and determined using the atomic absorption spectrophotometer. Saturation percentage (SP%) was determined according to reported procedure (Aali et al. 2009). Hydraulic conductivity (K) values of the soil samples columns were determined according to (Smith 2000).

2-3-Plant analysis:

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 form amide (DMF) solution 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 (Moran 1982).

Phosphatase enzyme analysis

The activity of phosphatase was measured using technique of (Tabatabai & Bremner 1969).

Determination of N, K and P Contents of plants:

Plant samples were wet digested using sulphuric acid and per chloric acids mixture (Chapman & Pratt 1978) and plant nutrients were determined in the aliquot as follows:

- Nitrogen using kjeldahl method (Jackson 1973).
- Phosphorus was determined using stannous chloride reduced molybdo-phosphoric blue colour method, (Jackson 1973).
- Potassium by flame photometer (Jackson 1973).

1- Plant height was estimated in cm. from the first node to the plant top.

2- Leaf number was counted per plant.

3- Total number of fruits / plot was counted.

4- Fruit length was estimated in cm.

5- Fruit diameter in cm was estimated using a vernier caliper.

6-Fresh Fruit weight / plot were determined by a balance in kg.

7- Fruit firmness was measured in kg/cm² by Magness and Ballauf pressure tester.

8-- Fruit size was measured in centimetre by immersing the fruit in a container filled with water and measuring the displaced water with a graduated jar.

9- T.S.S %: Total soluble solids (T.S.S.) were determined as percentage by Abbe refract meter (Sparks et al. 2020).

10- The dye 2.6 dichlorophenol indophenol method was used to determine ascorbic acid as mg/100 g fresh weight. (Hernández *et al*. 2006).

11- Total sugars were determined as g/100g dry weight according to (Dubois et al. 1956).

12- 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.

13- The samples were analyzed for dehydrogenase activity according to the method described by (Casida Jr et al. 1964).

Statistical Analysis:

Appropriate analysis of variance 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)and **(**Gomez & Gomez 1984).

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)						
		N	P	K	Fe	Mn	Zn	Cu
7.84	0.541	25	9	88	12.4	9.9	1.5	0.88
Soluble cations**(meq/L)					Soluble anions**(meq/L)			
Ca^{++}	Mg^{++}	Na^{+}	K^{+}	Co_3^{--}	Hco_3^{-}	CI^{-}	$SO_4^{=}$	
5.51	2.75	10.69	1.03	7.70	3.20	14.80	2.91	
SAR		ESP		$CaCO_3$ ⁻%			EC (ds/m)	
1.64		3.34		5.95			2.05	

pH *: in suspension 1:2.5

EC** (ds/m), soluble cations ** 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:

3-1-1-plant height (cm), number of leaves/plant, and number of fruits/ plot:

The collected data in Table (2) illustrated the influence of *B. megaterium*, *B. amyloliquifaciens* and cyanobacteria on some growth parameters of squash plants.

Table (2): Effect of inoculation with *B. megaterium*, *B. amyloliquifaciens* and cyanobacteria on growth of squash plants at harvest during the seasons of 2020 and 2021.

Treatments	Plant height (cm)		Number of leaves/plant		Number of fruits / plot	
	2020 Season	2021 Season	2020 Season	2021 Season	2020 Season	2021 Season
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	73.03b	78.33b	22.00a	23.33a	616.67a	645.00b
Ba+Cyano	72.63bc	77.03c	19.33b	21.67b	605.67b	633.00c
Bm+Cyano	71.77c	75.50d	18.33bc	19.67c	589.33c	593.33d
Ba+Bm+Cyano	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**

The growth parameters of *Cucurbita pepo* plants as Plant height (cm), Number of leaves/plant, and Number of fruits / Plot were significantly increased by different type of bacteria and fungus (*bacillus megaterium*, *bacillus* amyloliquifaciens, and *cyanobacteria* under consecration (table2).The highest stimulatory effect and the maximum enhancement were exerted in plant parameters with *B.megaterium* mixed with each of *B. amyloliquifaciens*and and cyanobacteria followed by *B.amyloliquifaciens* mixed with B.megaterium compared to control in both seasons. There is highest significant increase in Plant height with *B.megaterium* (75.17, and79.90cm) respectively in the first and second season. *B.megaterium + B. amyloliquifaciens gave* the same highest values with number of leaves/plant in 1st and2nd season (22.0 amd23.67),and also the addition of *B. megaterium, B. amyloliquifaciens* and cyanobacteria gave the same values (22.0 and 23.33).Number of fruits /plot in *Cucurbita pepo* as affected by *B.megaterium and B. amyloliquifaciens* and *cyanobacteria* showed highest value with the mixture of *B.megaterium, B. amyloliquifaciens and cyanobacteria* in two seasons (620.67an653.00*)* these followed by addition of *B.megaterium, B. amyloliquifaciens,* while the lowest data recorded with cyanobacteria(466.67and459.33) respectively in both two seasons as compared to control.

The findings showed that *B.amyloliquefaciens* as a bio fertilizer may not only increase plant development but also limit pathogen infection. The application of *B.amyloliquefaciens* as bio fertilizer could greatly improve tea yield and quality by increasing 100-bud weight when compared to treatments with no fertilizer application. It was discovered that *B.amyloliquefaciens* could help plants develop faster and more evenly. The mechanisms are as follows: first, several amino acids and fatty compounds produced by *B.amyloliquefaciens* may aid plant growth and development, as well as the balance of soil minerals, such as phytase produced during metabolism. (Chen et al. 2007). which could aid in the conversion of un absorbable organic phosphorus in the soil to absorbable phosphorus, thereby improving plant phosphorus absorption efficiency (Fan & He 2006) Second, *B.amyloliquefaciens* has an inhibitory effect on various plant diseases and insect pests, as well as tea fungus, which could reduce pests and diseases while also adjusting the micro flora environment around the tea root system and balancing soil nutrient elements, providing a suitable growth environment for tea plants that is conducive to root development and nutrient absorption of tea plants. 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. (Bent et al. 2001). When the function range of *B.amyloliquefaciens* was determined in this study, the growth-promoting effect improved with increasing concentration, and tea yield and quality rose as well. The benefits were diminished if the concentration was too high. 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. (Prasanna *et al.* 2013a).

3-1-2 Fruit length(cm), Fruit diameter(cm), Fruit fresh weight(gm.), Fruit dry weight(gm.), Fruit firmness(kg / cm2),and Fruit size(cm):

Table (3) Effect of inoculation with B. megaterium, B. amyloliquifaciens and cyanobacteria on Cucurbita pepo fruit features during the seasons of 2020and2021.

Treatments	Fruit length (cm)		Fruit diameter (cm)		Fruit fresh weight (gm.)		Fruit dry weight (gm.)		Fruit firmness (kg / cm^2)		Fruit size (cm)	
	2020 Season	2021 Season	2020 Season	2021 Season	2020 Season	2021 Season	2020 Season	2021 Season	2020 Season	2021 Season	2020 Season	2021 Season
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	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	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	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	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**

Growth parameters and some yield parameters as affected by the individual addition or combined effect of *B. megaterium, B. amyloliquifaciens* and *cyanobacteria* was conducted in table (3).data showed that the highest values of fruit length (cm) in both seasons recorded with the mixed of *Ba+Bm+Cyano (*12.33 and11.17cm) followed by *Ba+Bm (*11.90 and11.0cm) and *Ba+Cyano (*11.03and10.43cm) in both two seasons as compared to control. Data in the same table showed the effect of two kinds of bacteria and *cyanobacteria* on fruit diameter the highest values in two seasons were recorded with *Ba+Bm+Cyano* (2.78cm) in the first season and the same recorded with *Ba+Bm* (2.77cm) while in the second season the highest value recorded with *Ba+Bm+Cyano (3.53cm)* followed by *Ba+Bm (3.37cm).*

While fruit fresh weight gave the highest values in both seasons with the addition of *Bm (38.62and37.11 gm.)followed by Bm+Cyano(*37.52and34.70gm.) as compared to control .the same trend was found in fruit dry weight the highest values were recorded with *Bm*(3.41and 3.25gm)in both seasons followed by *Bm+Cyano (*2.84and2.89gm)in two seasons.

Some of yield parameters like Fruit firmness and fruit size were recorded in the same table. The data of fruit firmness appears the highest values as combined application of *Ba+Bm + Cyano (*7.67 and 7.80kg/cm^2) respectively in two seasons while the lowest values recorded with Cyanobacteria(6.21 and 6.34kg/cm^2).in both seasons. The last parameter in this table was fruit size and it gave the highest increase with the addition of *Bm and also with Ba+Bm(*43..30and42.07cm) respectively in the first season while in the second season the highest value was found with *Bm* (45.87cm) followed by *Ba(42.93cm).*

In many fields, nitrogen is the second limiting factor for plant growth, and fertilizers are used to compensate for a lack of this element(Rashid et al. 2017)As a natural bio fertilizer, cyanobacteria play a significant role in soil fertility maintenance and build-up, resulting in increased rice growth and production. (Song et al. 2005). Blue green algae (BGA) are free-living photosynthetic nitrogen fixers they can be found in large quantities. They, too, offer growth-promoting chemicals like vitamin B12, which boost soil aeration and water holding capacity while also adding biomass when degraded at the end of the life cycle. Azolla is an aquatic fern that can be found in rice fields and tiny, shallow water bodies. They discovered that applying Nostoc to the surface soil boosted the organic C and N content, as well as plant growth and ion uptake. They found that these microbes could provide the microelements required for plant growth. Furthermore, polysaccharides released by cyanobacteria contribute to soil structural stability, increased soil C and N levels, and plant growth stimulation.(Rossi & De Philippis 2015).Previously; (Nanda et al. 1991) revealed that spraying westiellopsis prolific Janet extracts on pumpkin (*Cucurbita pepo L.*) and cucumber (*Cucumis sativus L.*) seedlings during cultivation resulted in considerable increases in growth and development of both crops. They claimed that the seed's supply of nitrogenous nutrients is critical. The effect of cyanobacteria extract on potato (*Solanum tuberosum L.*) tissue culture was investigated by (Shanab et al. 2003). Demonstrates that the rise in agricultural yields is not solely attributed to cyanobacteria's nitrogen-fixing ability, but also to the growth-regulating chemicals produced endogenously by these algae. The fact that non-nitrogen-fixing species like *Oscillatoria sp.* and *Phormedium sp*. encouraged the growth of plants like rice backs up this theory.(Mona et al. 2020).Another cause for the increased plant growth and output in treated plants could be the algae's synthesis of growth-promoting chemicals and vitamins (Vitamin B12, folic acid, nicotinic acid, and pantothenic acid).(Sharma & Sharma 2017) Furthermore, cyanobacteria can boost secondary metabolite production, and these mechanisms may be influenced or regulated by hormones. (Saker et al. 2000); (Shanab 2001).

3-2-Photosynthetic pigments, chemical constituents, some chemical fruit quality parameters and some fruit quality parameters:

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/ gm.)	
	2020 Season	2021 Season	2020 Season	2021 Season	2020 Season	2021 Season	2020 Season	2021 Season
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	5.57b	4.64d	29.04b	30.59a	29.17b	33.50a	1.356b	1.466b
Ba+Cyano	5.67a	5.74a	28.14c	25.92b	28.00bc	30.63b	1.243c	1.387c
Bm+Cyano	5.63ab	5.71bc	26.61d	23.57c	28.87c	28.10c	1.233d	1.249d
Ba+Bm+Cyano	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**

Regarding to the effect of two kinds of bacillus singly or in combination with Cyanobacteria on Some chemical fruit quality parameters of squash (Total soluble solids), Endogenous phytohormones content of squash (Ascorbic acid), chemical content(total sugar),and photosynthetic pigments(Total chlorophyll) were showed in table(4). Concerning the fruit quality the data showed the positive increase in total soluble solids (T.S.S) with inoculation by *Ba* individually(5.67%) or in combined with Cyanobacteria (5.67%),this followed by mixed inoculation by *Ba+Bm(5.57%)* in the first season. While in the second season the highest increase recorded with *Ba+Cyano* (5.74%) followed by adding *Bm* individually (5.72%).Data in the same table of Ascorbic (acid Vitamin C) is very popular for its antioxidant properties ,and it also considered as one of chemical fruit quality parameters .The highest increase was recorded with *Ba+Bm +Cyano (*29.88and31.16 mg/ 100 g F.W*)* in both seasons this followed by *Ba+Bm* (29.04and30.59 mg/ 100 g F.W).While Cyanobacteria gave the lowest increase in both seasons .Maximum increase of total sugar with *Ba+Bm +Cyano* (32.10and34.93 mg/g FW),respectively in the first and second season .These followed by *Ba+Bm* (29.17and33.50 mg/g FW). Plants inoculated with *Ba* and *Bm* mixed with cyanobacteria produced the highest increase of photosynthetic pigments, as evidenced by the acquired data, while the lowest increase recorded with cyanobacteria. 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.(El-Yazeid et al. 2007)

By conserving organic matter, nitrogen, phosphate, and moisture in soil, cyanobacteria supplementation improved soil quality. 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. (Maqubela et al. 2009). 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.(Wang et al. 2009).

3-3-Plant chemical contents:

3-3-1- Effect of inoculation with *B. megaterium*, *B. amyloliquifaciens* and cyanobacteria on squash chemical content plants.

The effect of inoculation *with B. megaterium, B. amyloliquifaciens* and *cyanobacteria* on squash plants was mentioned in table (5).

Table (5): Effect of inoculation with *B. megaterium*, *B. amyloliquifaciens* and cyanobacteria on chemical content of squash plants at harvest during the seasons of 2020 and 2021.

Treatments	N% in Plants		P% in Plants		K% in Plants	
	2020 Season	2021 Season	2020 Season	2021 Season	2020 Season	2021 Season
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	2.13c	2.88b	0.715a	0.745b	4.20b	4.32a
Ba+Cyano	2.19c	2.84b	0.653b	0.742b	3.73c	3.16bc
Bm+Cyano	2.57b	2.88b	0.454c	0.651c	3.36d	3.20b
Ba+Bm+Cyano	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**

Results in table (5) showed that the highest significant values of plant nitrogen %were clearly indicated with the application of *Ba+Bm+Cyano(*2.75,and3.43%),these followed by *Bm+Cyano(*2.57and2.88%). While *Bm* gave the lowest increase in nitrogen content .also plant phosphorus content take the same highest increase with mixture of *Ba+Bm+Cyano* in the first and second seasons (0.726and0.813%) respectively, these followed *Ba+Cyano (0.653and0.742%).*the potassium content in squash plant was presented in the same table. the highest values in both two seasons as the result of adding *Ba+Bm+Cyano*

(4.44and4.26%).these followed by *Ba+Bm (4.20and4.32%)* while the lowest decrease was recorded with cyanobacteria (2.42and2.25%) in both seasons. In a recent study, (Prasanna et al. 2013a). It was discovered that treatment with cyanobacteria formulations increased the content of nitrogen, potassium, phosphorus. 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.(Wang et al. 2009). So the combinations of cyanobacteria strains (*Anabaena doliolum*, *A. torulosa*, *Nostoc carneum*, *N. piscinale*, *Oscillatoria*, *Plectonema*, *Schizothrix*, etc.) enhanced soil microbial biomass, nitrogen, carbon and humus content, thus retaining moisture and aiding in soil formation. (Prasanna et al. 2013a). Cyanobacteria improved plant development parameters such as plant height, dry weight, and grain yields in addition to soil fertility. (Joshi et al. 2012). Cyanobacteria have been shown in several studies to increase plant growth through enhancing soil structure by secreting extracellular polysaccharides (EPS), which aid in soil aggregation and water retention. (Maqubela et al. 2009).

3-3-2-Soil analysis:

Available N, P and K in Squash Rhizosphere:

When squash plants were inoculated with *Ba* individually or with either *Bm* or cyanobacteria, significant increases in available N, P, and K were observed when compared to un inoculated plants, as shown in Table (6). The combination of *Ba, Bm*, and cyanobacteria produced the greatest increase in available nitrogen content (137.67and142.00%), followed by the combination of *Ba* and *Bm* (128.67and136.67%). The greatest available-P value was obtained in the two seasons when *Bm* was used alone or in combination with *Ba* and cyanobacteria. When squash plants were inoculated with Ba+ Bm+ Cyanobacteria, the most significant increase in available K was observed (517.33and528.67%), followed by a mixture of *Ba+ Bm* (367.33and378.33%). Bio fertilizers can be expected to reduce the use of chemical fertilizers and pesticides. The microorganisms (*Azotobacter, Blue green algae, Rhizobium Azospirillum*) in bio fertilizer restore the soil's natural nutrient cycle and build soil organic matter. The microorganisms in bio fertilizer ensure that the host plants receive an adequate supply of nutrients and that their growth and physiology are properly regulated. Only those living microorganisms that have specialized roles to improve plant growth and reproduction are employed in the manufacturing of bio fertilizer. For the plants' availability, microorganisms convert complicated nutrients to simple nutrients. If bio fertilizer is used correctly, crop yield can be boosted by 20% to 30%. Plants can be protected from soil-borne diseases by using bio fertilizer to some extent. For two reasons, the usage of bio fertilizer has become necessary. First, increasing fertilizer use leads to higher crop yield; second, increased chemical fertilizer use damages soil texture and causes other environmental issues.(Sahu *et al.* 2012). As a natural bio fertilizer, cyanobacteria play a significant role in maintaining and building soil fertility, resulting in increased rice growth and output. (Song *et al*. 2005). The following are some of the actions of these algae: (1) Increase in soil pores due to filamentous structure and synthesis of sticky compounds. (2) Hormones (auxin, gibberellin), vitamins, and amino acids are excreted to promote growth. (Rodríguez et al. 2006) (3) Due to their jelly structure, they have a greater capacity to hold water. (Saadatnia & Riahi 2009)(4) Salinity of the soil is decreasing. (Saadatnia & Riahi 2009)(5) Stopping the spread of weeds (Saadatnia & Riahi 2009)(6) Organic acid excretion causes an increase in soil phosphate. (Wilson 2006). Plant growth-promoting Rhizobacteria, i.e., Rhizobium, blue-green algae (BGA), the fungal mycorrhizae, and bacterial Azotobacter, Azospirillum, phosphate-solubilizing bacteria such Pseudomonas sp. made up the majority of the bio fertilizer. *Bacillus sp.,* which increase biological nitrogen fixation and solubilisation of insoluble complex organic matter to a simpler form, making them biologically available to plants, and *Bacillus sp*, which increase nutrient supply to crops by increasing biological nitrogen fixation and solubilisation of insoluble complex organic matter to a simpler form, making them biologically available to plants. It improves soil moisture retention, soil nutrient (nitrogen and phosphorus) availability to plants, and soil microbial status, as well as soil aeration and natural fertilization. (Itelima et al. 2018).

Table (6): Effect of inoculation with *B. megaterium, B. amyloliquifaciens* and cyanobacteria on squash fruits features during the seasons of 2020 and 2021.

Treatments	**N (in soil)**		**P (in soil)**		**K (in soil)**	
	2020 Season	**2021 Season**	**2020 Season**	**2021 Season**	**2020 Season**	**2021 Season**
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	128.67b	136.67a	16.17e	17.31c	367.33b	378.33b
Ba+Cyano	101.00d	116.00b	16.63cd	17.54c	345.33c	356.00c
Bm+Cyano	108.00c	116.33b	16.70c	17.60bc	325.00d	352.00c
Ba+Bm+Cyano	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**

Some microbial activity:

Phosphatase and Dehydrogenase activity:

The enzyme activity in the Rhizosphere of squash plants was determined, as shown in Table (7). Dehydrogenase activity (DHA) is an indicator of overall microbial activity in the soil since it indicates the energy transfer. Inoculation with *Bm* raised (DHA) levels separately or in combination with Ba and cyanobacteria compared to those who were not infected. The most positive increase was recorded with *Ba+ Bm +Cyano* (126.23and143.83 mg/g soil/24h).followed by *Ba+ Cyano (*112.27and135.06 mg/g soil/24h). In addition, multiple inoculations, particularly with *Bm, Ba*, and cyanobacteria, resulted in the highest phosphatase activity. As a good co-inoculation system, bacteria like Bacillus and cyanobacteria have favorable interactions. The highest increase was recorded with *Ba+ Bm +Cyano (*34.35and46.67 µ inorganic phosphorus/g dry soil/day) followed by *Bm+Cyano* (33.10and42.40 µ inorganic phosphorus/g dry soil/day).

Table (7) some microbial activities in Rhizosphere of squash plants as affected by interactions between *B. megaterium, B. amyloliquifaciens* and *cyanobacteria* during the seasons of 2020 and 2021.

Treatments	**Phosphatase(µ inorganic phosphorus/g dry soil/day**		**Dehydrogenase (mg/g soil/24h)**	
	2020 Season	**2021 Season**	**2020 Season**	**2021 Season**
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	29.20b	39.45c	103.99c	127.38c
Ba+Cyano	23.07d	29.32e	95.44d	123.61c
Bm+Cyano	33.10a	42.40b	112.27b	135.06b
Ba+Bm+Cyano	34.35a	46.67a	126.23a	143.83a
L.S.D.(0.05)	**1.34**	**1.97**	**5.30**	**5.12**

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 because 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 was also highest after dual inoculation, notably with P. polymyxa and *bacillus megaterium. Bacillus* and mycorrhizae work well together as a co-inoculation system.(Ju *et al*. 2020).

When put to the soil, living cells of microorganisms that are naturally present in the soil can assist agricultural plants in absorbing nutrients through their interactions in the Rhizosphere. Many biological mechanisms speed up specific microbial processes in the soil and mobilize nutrients from non-usable to useable form.(Alfa *et al.* 2014). Bacteria that produce acids or enzymes as metabolites can help to solubilize phosphorus. Acid production reduces the pH of the growth medium, affecting phosphorus solubilisation.(Behera *et al.* 2017). When phosphorus is released from the hydroxyapatite structure into the solution, it is available to plants in a form that they may use in their metabolism. *Bacillus megaterium*, a Gram-positive soil bacterium, has been employed in industrial biotechnology for decades. Because it lacks an exterior cell membrane, *B. megaterium* possesses a high secretion capacity directly into the surrounding medium.(Korneli *et al.* 2013). Due to the increased use of agrochemicals to maintain the same yield, environmentally friendly indigenous bacteria in the soil are becoming extinct. *Bacillus spp*., on the other hand, is a common source of various plant-friendly enzymes and growth factors. Phosphorus (P) is the second most important nutrient for crop growth, yet it is deficient in many fertile soils around the world (30–40%). (Ju et al. 2020).Despite the fact that most soils have significant total P stocks, only a small percentage (less than 1%) of the total inorganic and organic P is dissolved at any given moment. (Malik et al. 2017).

The limiting nutrient for biomass production in natural ecosystems is phosphorus, a necessary mineral for plant growth and development. It is commonly provided to the soil in the form of phosphate fertilizers, but plants only use a small portion of this nutrient, and the majority of phosphate is quickly transformed to insoluble complexes in the soil that plants cannot use. It is commonly applied to the soil in the form of phosphate fertilizers, but plants only use a small portion of this nutrient, and the majority of phosphate is quickly converted in the soil to insoluble complexes that plants cannot use. It is commonly applied to the soil in the form of phosphate fertilizers, but plants only use a small portion of this nutrient, and the majority of phosphate is quickly converted in the soil to insoluble complexes that plants cannot use. It is commonly applied to the soil in the form of phosphate fertilizers, but plants only use a small portion of this nutrient, and the majority of phosphate is quickly converted in the soil to insoluble complexes that plants cannot use. (Chen et al. 2006)). Cyanobacteria increased phosphate breakdown and mineralization, converting it to soluble organic phosphates/orthophosphates. Furthermore, cyanobacteria treatment in agricultural fields aids in the mobilization of inorganic phosphates via extracellular phosphatases and the excretion of organic acids. (Rana et al. 2012).

Plant growth hormone synthesis was stimulated by bio-fertilizers based on Bacillus .It released amyloclastic, celluloselytic, and proteoclastic enzymes from decaying soil organic matter, enriching plant tissue N and P concentrations and so enhancing biomass output .Bacillius-based microbial agents may play an essential role in the production of off-season potatoes in the field when air and soil temperatures are sub-optimal. (Itelima et al. 2018).

In order to study the possibilities of using environmentally acceptable alternative fertilizer for off-season potato production in coastal areas where considerably milder temperatures allow plant development during the winter, an equal blend of Bacillus strains was used. For off-season potato production, greater research with more cold-tolerant Bacillus bacterial strains, either alone or in combinations, is needed. The development of bacterial strains that sustain activity and survive in cold soils could open up new possibilities for using associative Rhizobacteria in off-season potato production. Microbial fertilizers alone achieved tuber yields similar to artificial fertilizers under favourable conditions, according to data from two seasons. Microbial fertilizers can also be utilized to grow organic potatoes. Even in the cooler spring months, SFA ensured increased tuber yields.(Ronald & Adamchak 2018).

Rhizobacteria that promote plant growth, such as *B.subtilis* and *B. amyloliquefaciens*, have bio protective, phytostimulant, and bio fertilization effects on potato plant growth and tuber yields. There was plenty of evidence that PGPR was beneficial to potato development. In our investigation, plots with B. subtilis and

B. amyloliquefaciens culture applied yielded the same amount as plots with chemical fertilizer applied in August. Bacillus amyloliquefaciens belongs to the gram-positive bacteria family and promotes plant growth. (Aloo *et al.* 2020).

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. 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.

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