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Chemical, Anatomical, and Productivity Responses of Cowpea (*Vigna unguiculata* L.) to Integrated Biofertilizer Applications with PGPR, Cyanobacteria, and Yeast

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Abstract: Integrated biofertilizers such as Plant Growth-Promoting Rhizobacteria (PGPRs), cyanobacteria, and yeast can considerably improve the growth, integrity, and overall health of crops, including cowpea. In this study, we assess the benefits of applying microbial fertilizers as an eco-friendly approach to partially substitute chemical fertilizers while maintaining growth and yield characteristics in cowpea plants. We investigated the role of the three microorganisms, *Bacillus amyloliquefacien* (B), *Nostoc mucorum* (C), and *Saccharomyces cerevisiae* (Y), individually and in four possible combinations (B + C, B + Y, C + Y, and B + C + Y) as integrated bio-fertilizers on the microbial enzyme activities, plant growth parameters, and yield characteristics of cowpea. Plants inoculated with B + C + Y mixture resulted in significant improvement in dehydrogenase enzyme activity by 390%, chlorophyll by 180%, plant dry weight by 130%, and in the pod length and dry weight by 68% and 190%, respectively, compared to non-inoculated plants. The grain total carbohydrates increased by 170% over the control due to treatment with B + C + Y. The B + C + Y treatment also positively influenced the anatomy of the terminal leaflet with a 16.6% higher thickness of the midrib zone, 22.6% increase in vascular bundle length, and 42.4% and 33.5% increases in upper and lower epidermal leaf layers, respectively. Additionally, palisade and spongy tissues increased by 36.9% and 26.5%, respectively, compared to the control. An integrated nutrient management program using biofertilizers is recommended for achieving higher yields and environmentally safe cowpea production.

Keywords: cowpea; bio-fertilizers; anatomy; *Bacillus amyloliquefaciens*; *Nostoc mucorum*; *Saccharomyces cerevisiae*

1. Introduction

The cowpea, or *Vigna unguiculata*, in the *Phaseoleae* tribe of the *Leguminosae* family, is one of the oldest sources of sustenance for people. Cowpea cultivation is of growing

interest as an orphan crop because of its nutritive value and capacity to survive in arid soils, even though this product still has a restricted value chain [1,2]. Cowpea is a versatile, underutilized legume commonly grown in arid tropical regions [3,4]. Additionally, it is a reliable and important commodity that brings in money for sellers and farmers [5,6]. Because of its capacity to restore soil fertility for future cereal crops planted in rotation with it, cowpea is an essential part of the agricultural system in many places [7–9]. Dry grain for human food is the main product of the cowpea crop, while in some local contexts, fresh or dried leaves, fresh peas, and even fresh green pods are more crucial. In many areas of West Africa, cowpea hay is essential for livestock feed during the dry period [10].

Additionally, leaves are abundant in fiber, phytonutrients, calcium, zinc [11], beta-carotene, iron, and protein. These are highly deficient among the vulnerable populations in arid regions [12,13]. Therefore, cowpeas are an essential source of dietary protein that millions of people in Africa and other developing nations rely on as a nutritious supplement to traditional low-protein crops and tubers. Cowpeas are very nutritious since they include a high content of protein as well as carbohydrates [14].

Growth-promoting microorganisms, including PGPRs (Plant Growth-Promoting Rhizobacteria), may be used for sustainable agriculture due to their ability to fix nitrogen and produce growth-regulating plant hormones, such as auxins, gibberellins, and cytokinins. They also add organic compounds, improve soil fertility, degrade various agrochemicals (pesticides or herbicides), and regulate the pathogenic effects of many other microorganisms related to soil and plants [15,16]. It is suggested that using multiple beneficial bacteria in the biocontrol of pathogenic bacteria is superior to using individual agents of bacterial species [17]. According to reports from multiple researchers, soil microorganism inoculations generally promote plant growth more effectively than individual inoculations because each strain complements the others' positive characteristics.

Chemical fertilizers have been used more frequently because of the rising global food demand to obtain a high and considerable increase in crop output. However, negligent and excessive use of these fertilizers is causing soil and water pollution [18]. A bio-fertilizer is a type of organic fertilizer that contains a lot of beneficial microorganisms [19]. Plants continue to interact closely with the soil's microorganisms. These microorganisms are often helpful to plants and mutualistic. Exudates from the root system have an impact on the development of soil microorganisms [20]. Several soil microorganisms influence plant growth, including bacteria, actinomycetes, fungi, algae, and protozoa [21]. These rhizospheric capable and advantageous bacterial species primarily consist of rhizobacteria that promote plant growth [22].

Bacteria directly assist plants by facilitating development through nutrient solubilization [23], nitrogen fixation, and the production of growth hormones such as indoles, i.e., Indole Acetic Acid (IAA), Gibberellic (GA_3), and cytokinins, along with 1-aminocyclopropane, 1-carboxylate (ACC) deaminase production. Moreover, PGPRs indirectly affect plant growth by forming siderophores, chitinases, cyanide poisoning occasionally, antibiotics, b-1-3glucanase, and induce systemic resistance. *Bacillus* species are rhizobacteria that assist plant growth by producing a variety of chemicals that hasten plant growth and lower pathogen infection. By generating IAA, GA_3, and ACC, *Bacillus* modifies intracellular phytohormone metabolism and encourages plant stress tolerance. Additionally, the production of siderophores and exopolysaccharides inhibits harmful ions from passing through plant tissues and controls water transport and ionic equilibrium while reducing pathogenic microbial populations. By regulating water transport and nutritional intake and activating the antioxidant and defense systems, *Bacillus*-induced physiological changes reduce biotic and abiotic stress factors [24,25].

Furthermore, *S. cerevisiae* has emerged as a potential replacement for chemical fertilizers for the safety of people, animals, and the environment [26,27]. Due to its high concentration of proteins, carbohydrates, nucleic acids, lipids, vitamins, and other minerals, yeast is a natural growth enhancer [28], and it improves the plant roots' ability to absorb phosphorus and manganese [29]. Recently, researchers have looked into the possibility of

using yeast as plant growth stimulants and bio-control agents for soil-borne diseases of plants [30,31]. Chemical fertilizers can be replaced with cyanobacteria as an eco-friendly, cost-effective solution that both directly and indirectly improves soil productivity [32,33]. Cyanobacteria are Gram-negative, photosynthetic prokaryotes that can develop in terrestrial and aquatic habitats. They can be found naturally, in microflora, or in symbiotic relationships with a variety of lower and higher plants. As bio-fertilizers, cyanobacteria are valuable to agriculture because of their ability to fix atmospheric nitrogen dioxide (N_2), which enables them to thrive in environments with low or no accessible combined nitrogen [32]. The ability of cyanobacteria to fix nitrogen, as well as their other potential agricultural benefits, are widely known.

The available literature shows that the role of integrated biofertilizers is less studied in cowpea plants. Hence, there is a need to assess the integrated effect of biofertilizers in the sustainable production of cowpea crops. This study contributes to this need by assessing the response of cowpea plants to inoculation with growth-promoting microorganisms, *B. amyloliquefaciens* (B), yeast (*S. cerevisiae*) (Y), and cyanobacteria (*N. mucorum*) (C), as biofertilizers, including their combinations: B + C, B + Y, C + Y, and B + C + Y. Additionally, the goal of this experimental research is to highlight the synergistic effects of integrated treatments using a mixture of microorganisms, as well as singular treatments and control. In addition, the experiments aim at evaluating the potential for partial substitution of NPK mineral fertilizers by bio-fertilizers without affecting cowpea growth and production. We hypothesize that combined biofertilizers promote growth and improve yield characteristics in cowpea compared to adding individual microbes and untreated ones.

2. Materials and Methods

2.1. Materials

2.1.1. Plant Materials

Cowpea (*V. unguiculata*) cultivar Dokki 126, used in this study, was provided by the Legume Research Department, Field Crops Research Institute, ARC, Giza, Egypt.

2.1.2. Plant Growth-Promoting Rhizobacterial, Yeast, and Cyanobacteria Strains

Rhizobacteria (*Bacillus amyloliquefaciens*), cyanobacteria (*Nostoc mucorum*, EMCCN 3075), and yeast (*Saccharomyces cerevisiae*), strains from the Soil, Water, and Environment Research Institute's Microbiology Department, ARC, Giza, Egypt, were used in this investigation.

2.1.3. Soil

Sand–clay loam soil was used in the present research. Table 1 illustrates the physical properties and chemical characteristics of the soil.

Table 1. The soil's physical and chemical characteristics.

Property	Value	Property	Value
pH (in suspension 1:2.5)	7.81	N (ppm)	25
		P (ppm)	9
Saturation Percentage (SP%)	21.12	K (ppm)	87
		Fe (ppm)	12.4
EC (ds/m)	0.59	Mn (ppm)	9.8
Organic Carbon (O. C. %)	0.314	Zn (ppm)	1.6
		Cu (ppm)	0.87
Soluble cations (mmole kg^{-1})		Soil Type	Sandy clay loam
Ca^{++}	5.51	Particle size distribution (%)	

Table 1. *Cont.*

Property	Value	Property	Value
Mg^{++}	2.75	Fine sand%	34.07
Na^{+}	10.69	Coarse sand%	18.68
K^{+}	1.03	Silt%	9.0
Soluble anions (mmole kg^{-1})		Clay%	27.3
CO_3^{-}	7.7	Physical properties	
HCO_3^{-}	3.2	% Field Capacity	40
CI^{-}	14.9	(v/v) % Wilting point (v/v)	32
SO_4^{-}	2.9	Hydraulic conductivity (cmh^{-1})	1.35
		Available water (% v/v)	8.4

2.2. Methods

2.2.1. Soil Analysis

The soil samples were taken between 0 and 30 cm depths before cultivation to determine the physical and chemical characteristics of the investigated soil, according to Page et al. [34]. In the soil analysis, soil pH was determined in a 1:2.5 soil:water suspension using a Beckman glass electrode [35]. The soil moisture contents at the water holding capacity and wilting point were determined according to the methods of Veihmeyer and Hendrickson [36]. Electrical Conductivity (EC) in saturated soil paste extract was determined following Jackson [37], and organic carbon % (m mole. of $K_2Cr_2O_7$ added − m mole of Fe_2SO_4 used) × 0.336/(wt of sample in g) as described by Black [38].

Ca^{2+} and Mg^{2+} were assessed using the versenate method [35], while Na^{+} and K^{+} were measured using a flame photometer [37]. While CO_3^{2-}, HCO_3^{-}, and Cl^{-} in soil-saturated paste extract were determined through the use of titration with HCl [35]. The SO_4^{-} levels were calculated by subtracting the total determined soluble anions from the total determined soluble cations.

The available soil nitrogen (N) was determined using 2N KCl in a soil sample of 5 g, which was mixed with 50 mL of KCl solution and shaken for 30 min. This was then filtered, and the available $N\text{-}NH_4^{+}$ and $N\text{-}NO_3^{-}$ were determined using the Technicion Auto Analyzer [39]. Available phosphorus (P), potassium (K), iron (Fe), manganese (Mn), zinc (Zn), and copper (Cu) were extracted via the method of Soltanpour [40], using Inductively Coupled Plasma (ICP) Spectrometry (Ultima 2 JY Plasma), while (K) was determined using a flame photometer.

The soil particle size distribution was estimated using the dry sieving method, while calcium carbonate was determined using the calcimeter, following the procedures of Piper [41]. The cation exchange capacity was determined using 1 M ammonium acetate pH 7.0 [35].

2.2.2. Field Experiment

During the two growing seasons of 2021 and 2022, experiments were conducted at Al-Azhar University Agricultural Farm in Cairo, Egypt, in the open field to assess the impact of the tested yeast, cyanobacteria, and *B. amyloliquefaciens* inoculation on cowpea growth, anatomy, and yield under field conditions. Healthy seeds of cowpea cultivar Dokki 126 were sown on 2 and 4 March in the first and second seasons, respectively. Only seeds of the same size and color were chosen to ensure uniformity.

Average monthly temperatures during the growing season were 18.23, 21.86, 27.97, 27.68, and 30.74 °C for March to July, respectively, in the 2021 season. For these months (March–July) in 2022, temperatures recorded were 15.67, 23.22, 25.39, 28.78, and 29.21 °C, respectively.

In accordance with ARC, Giza, Egypt guidelines, a full dose of NPK fertilizer was applied during the tillage and growing stages of plants. Ammonium sulphate (200 kg/feddan),

the maximal recommended dose of mineral fertilizers (100% NPK), is employed as a control, while the biological amendments, *B. amyloliquefaciens*, yeast, cyanobacteria and their combination were applied with mineral nitrogen fertilization (50% N), calcium superphosphate (150 kg/feddan) and potassium sulfate (75 kg/feddan) (feddan = 0.42 Hectare). These quantities were added in two batches, the first at sowing and the second at the beginning of flowering. The Agriculture Research Center's recommendations for all other agricultural techniques were followed for cultivating legumes.

Three replicates were used in the experiment's completely randomized block design. There was a 40 cm distance between plants in each row and a distance of 100 cm between rows. The 40 m^2 area for each plot was created by dividing each of the tri-replicates into 8 rows, each of which was 5 m long. Seeds for all treatments and control were coated with rhizobial inoculation (*Bradyrhizobium* sp.) in sterilized vermiculite carrier bags (150 g/bag). A solution of Arabian gum was used to increase the carrier's adherence, and treated seeds were spread out on clean plastic sheets to air dry for one hour under shade before planting. The control plots with rhizobia (without inoculum) served as control. Three cowpea seeds were planted per planting hole.

B. amyloliquefaciens and yeast strains were streaked on nutrient agar plates (3.0 g/L beef extract, 5.0 g/L peptone, 20.0 g/L agar, 1.0 g/L glucose, 0.5 g/L yeast extract). Just one colony was chosen for inoculation in 250 mL of Luria Bertani broth (10.0 g/L NaCl, 10.0 g/L tryptone, 5.0 g/L yeast extract) and standard yeast medium (10 g/L yeast extract, 20 g/L peptone, 200 g/L D-glucose) [42], incubated overnight for about 12 h at 30 °C while being shaken. The mother cultures' turbidity was measured using the spectrophotometer at 600 nm and adjusted to ~0.3 × 10^9 CFU/mL to be used for liquid inoculation. Liquid BG110 media was used to sustain and grow the cyanobacteria strain until it reached the stationary phase [43].

These mother cultures were used twice as a soil drench application at a rate of 10 L/feddan of *B. amyloliquefaciens* suspension. The treatment was added to the soil immediately after sowing and after 30 days from sowing. For yeast and cyanobacteria, 50 L/feddan of cultural suspension was applied immediately following sowing and 30 days after sowing. The experimental trial included eight treatments as follows: control, B, Y, C, and a mixture of microorganisms as B + C, B + Y, C + Y, and B + C + Y.

At the flowering stage, samples of soil and leaves were collected. The soil sample was used to determine dehydrogenase activity, while leaves were immediately rinsed and dried between two filter papers to measure photosynthetic pigments. At harvest, some plant growth productivity parameters were measured, such as plant height, number of leaves, number of branches/plant, plant fresh weight, and plant dry weight. In addition, some pod quality parameters, such as length, diameter, fresh and dry weights, chemical content, and carbohydrate, were determined.

2.2.3. Analysis of Soil: Dehydrogenase Enzyme Activity (DHA)

Dehydrogenase activity (EC1.1) in the rhizospheric soil was assayed following the procedure of Glathe and Thalmann [44], in which samples of rhizospheric soil (2 g) were taken into test tubes. Then, 2 mL aliquots of T.T.C. (Triphenyl Tetrazolium Chloride) solutions with increasing concentrations (0.5%, 2%, 3%, and 5%) with Tris buffer (pH 7.8) as a solvent were thoroughly mixed. The T.T.C solution slightly submerged the saturated soil samples. Each tube was sealed with a silicon stopper, and then the tubes were incubated in the dark at 30 °C for 24 h. After that, absolute acetone (10 mL) was added to the tubes individually. With continuous shaking for two hours, all tubes were kept in the dark to extract the triphenyl formazan (T.P.F.) that had a pink-color formation. Then, the suspension was filtered using filter papers. The strength of the formed pink color in the filtrates was estimated spectrophotometrically at 485 nm using Spekol UV-vis Light (Westburg, The Netherlands). A standard curve was used to calculate formazan concentration and expressed in µg TPF/g dry soil/day. A blank sample included all additives with no soil.

2.2.4. Analysis of Plant

a. Pigments of Photosynthesis:

Chlorophyll a and chlorophyll b, as well as carotenoids, were estimated in small leaf disks (2.5 cm^2/area). The disks were taken from the third leaf from the top of the branch. Dimethyl Formamide (D.M.F.) was used as a solvent to extract the photosynthetic pigments from the samples. The samples were kept at 5 °C overnight. Chlorophyll a and b, along with carotenoid contents, were measured at wavelengths 663, 647, and 470 nm, respectively, using a spectrophotometer. Calculations were carried out using the following equations and expressed as μg/g F.W. [45], as in the equations below:

$$\text{Chlorophyll a} = 12.70\ A663 - 2.79\ A647. \quad (1)$$

$$\text{Chlorophyll b} = 20.76\ A647 - 4.62\ A663. \quad (2)$$

$$\text{Total chlorophylls} = 17.90\ A647 + 8.08\ A663. \quad (3)$$

$$\text{Total carotenoids} = 1000 \times A470 - 3.72chl.a - 104chl.b/229. \quad (4)$$

b. Anatomical Studies:

A piece from the middle part of the terminal leaflet (1 cm^2) was taken throughout the second growing season of 2022. The samples were dehydrated in an increasing series of ethyl alcohol concentrations that ranged from 50% to 100%. The samples were then embedded in paraffin wax (m.p. 58–61 °C) using xylol as a solvent. Then, the specimens were sectioned using rotary Jung PM 2045 microtome at 15 μm, then mounted using egg albumen on glass slides. Next, xylol was used to dissolve the wax, and safranin and light green were used to stain the slides. After that, Canada balsam was used as a mounting agent to prepare permanent slides. The execution of the microtechnique was conducted following the method detailed by Nassar and El-Sahhar [46]. All measurements (μ), counts, and pictures were taken using a Nikon camera on a Carl Zeiss Jena microscope. Each value refers to 5 replicates (sections).

2.2.5. Yield Parameters

a. Productivity Criteria

At harvest, some plant growth productivity parameters were measured, such as the height of the plant (cm), number of leaves, branches number/plant, fresh weight of plants (g), and dry weight plant (g). In addition, some pod quality traits such as length (cm), diameter (cm), fresh weight (g), and the dry weight (g) of the pods.

b. Chemical Contents of Seed

The cowpea seeds were digested using a mixture of perchloric and sulphuric acids [47]. NPK contents were determined in the aliquot using the Kjeldahl method [48] for N content; stannous chloride reduced $H_3[PMo_{12}O_{40}]$ procedure [48] for P assay, and flame photometer [48] for K estimation.

c. Total Carbohydrates Contents

Total Carbohydrates contents were determined in dry seeds according to Herbert et al. [49].

2.3. Statistical Analysis

The response variables were plant height, number of leaves, no. of branches/plant, pod length, pod diameter, pod fresh weight, pod dry weight, plant fresh weight, plant dry weight, carbohydrates, DHA, nitrogen, phosphorus, potassium, chlorophyl A and B, and carotenoid levels. The explanatory variables were the PGPR treatments and control. The presented data are the mean values of the tri-replicate experiments to represent each

treatment. The results were analyzed using the one-way Analysis of Variance (ANOVA) following Snedecor and Cochran [50] using the CoStat software to measure the significance among means (CoHort software, Berkley, CA, USA). In addition, a post hoc test (LSD at 0.05 level) was performed and presented in the results.

3. Results

3.1. Analysis of Soil (Dehydrogenase Enzyme Activity (DHA))

The effect of inoculation on dehydrogenase activity (DHA) in the rhizosphere of cowpea in two growing seasons is illustrated in Figure 1. The inoculation improved enzymatic activity in the rhizospheric soil of treated cowpea, as evidenced by the results. DHA activity in the 2021 season was more responsive than in the 2022 season. In two seasons, inoculation with *B. amyloliquefaciens* + yeast + cyanobacteria mixture recorded the highest level of activity compared to the other treatments with 435 µg TPF/g soil/day and 430 µg TPF/g soil/day in the first and second seasons, respectively. While inoculation with yeast strongly increased the activity of the same enzyme in the first and second seasons by 240% and 219%, respectively, compared with un-inoculated plants. In addition, in the two seasons, inoculation with *B. amyloliquefaciens* + yeast mixture promoted the DHA activity by 160% and 150% in contrast to the control treatment.

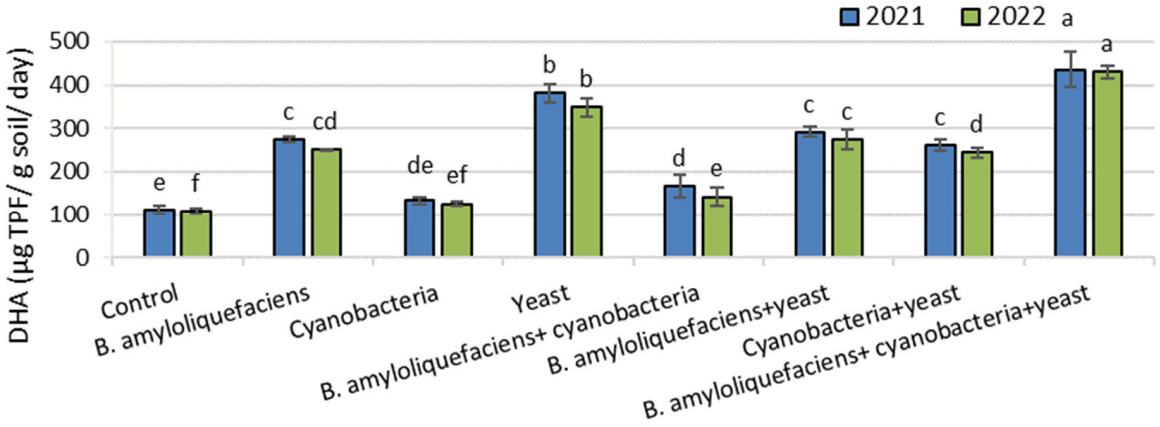

Figure 1. Effect of *B. amyloliquefaciens*, cyanobacteria, and yeast inoculation on the activity of DHA in soil rhizosphere of cowpea in 2021 and 2022 seasons. Significant differences among treatments are indicated by different alphabetic letters from a to f that represents significant levels ($p < 0.05$), i.e., a treatment with the letter "a" is significantly different from "b", and "b" is significantly different from "c", and so forth. However, if two treatments have the same letter, they are not significantly different. The LSD ($p < 0.05$) values for DHA are 34.569 and 25.933 for 2021 and 2022, respectively. The standard deviation (SD) values are presented as error bars.

3.2. Plant Analysis

3.2.1. Photosynthetic Pigments

Figure 2 illustrates how bio-fertilizers affect the photosynthetic pigments of cowpea plants. The data demonstrate that during the two seasons, the un-inoculated plants' leaves had the lowest levels of carotenoids, chlorophyll a, and b relative to those found in the inoculated plants. However, plants treated with *B. amyloliquefaciens* + yeast + cyanobacteria mixture and cyanobacteria individually in the first season demonstrated the greatest significant effects. Relative to the un-inoculated plants, there was a documented rise in the concentration of chlorophyll a by 81% and 66%, chlorophyll b by 64% and 47%, and carotenoids by 138% and 118%, respectively. Plants that had been inoculated with the *B. amyloliquefaciens* + yeast + cyanobacteria mixture contained higher levels of chlorophyll a, chlorophyll b, and carotenoids by 52%, 57%, and 188%, respectively, compared to the control. In the second season, photosynthetic pigments were less responsive than in the first season.

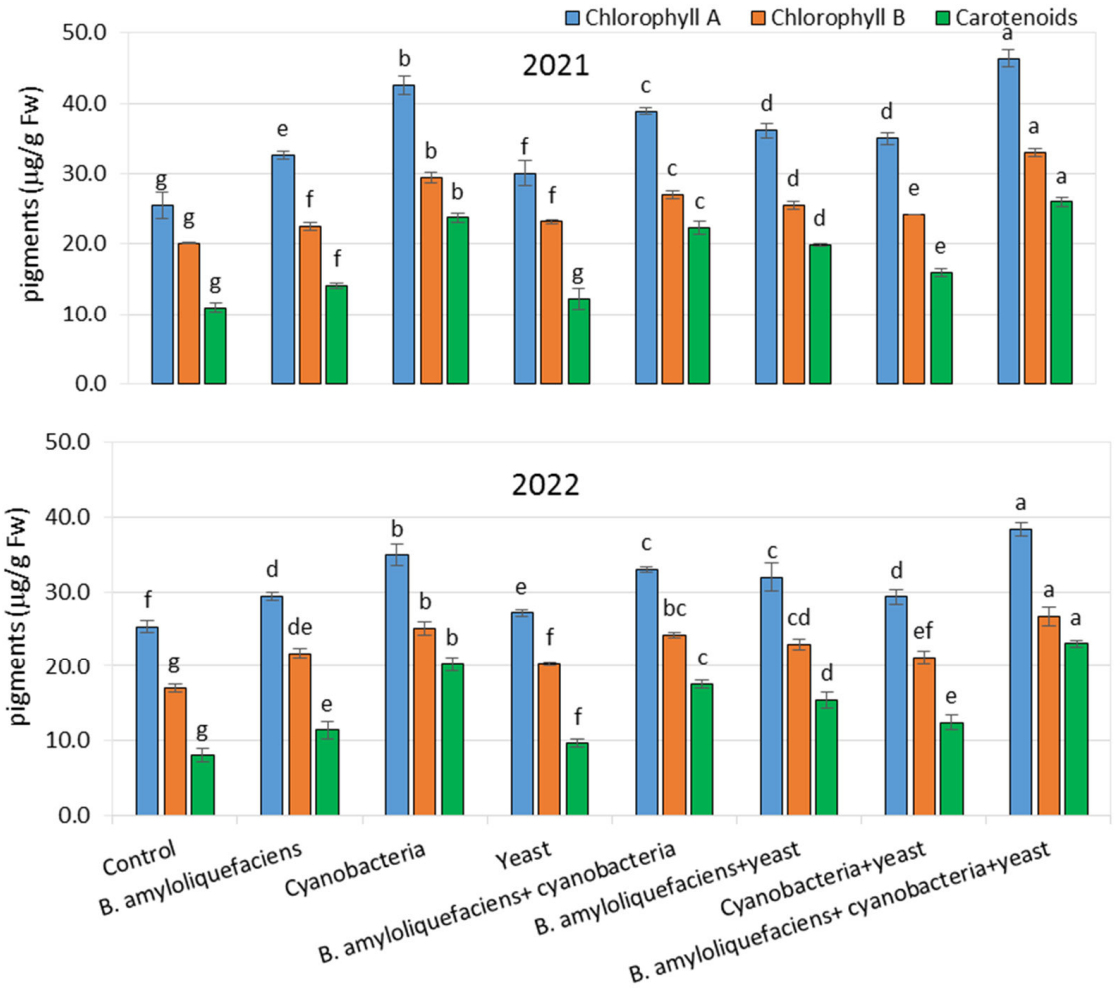

Figure 2. Effect of *B. amyloliquefaciens*, cyanobacteria, and yeast inoculation on pigment contents (µg/g FW) of cowpea in 2021 and 2022 seasons. Significant differences among treatments are indicated by different alphabetic letters from a to g that represents significant levels ($p < 0.05$), i.e., a treatment with the letter "a" is significantly different from "b", and "b" is significantly different from "c", and so forth. However, if two treatments have the same letter, they are not significantly different. The LSD ($p < 0.05$) values are 2.136, 0.832, and 1.381 for chlorophyll a, chlorophyl b, and carotenoids, respectively, in the first season. These values are 1.813, 1.294, and 1.495 for chlorophyll a, chlorophyl b, and carotenoids, respectively, in the second season. The standard deviation (SD) values are presented as error bars.

3.2.2. Anatomical Studies

Certain anatomical characteristics were measured at the microscopic level in transverse sections in cowpea terminal leaflets as compared to the control. Microphotographs in Figures 3 and 4 show that the application of *B. amyloliquefaciens* + yeast + cyanobacteria recorded the greatest increase in the midrib zone's thickness and vascular bundle length by 16.6% and 22.6%, respectively, compared to untreated plants. In comparison, the application of cyanobacteria resulted in a slight increase in the midrib zone by 3.3% and the length of the vascular bundle by 7.1% (Figure 3) in comparison with the control. Such an effect relates to the clearly developed and differentiated elements of the main vascular, including xylem vessels. Furthermore, the area that is occupied by collenchymatous cells that are located behind the main vascular bundle has a larger size and multiple layers of collenchymatic cells (Figure 4B,C), respectively, in contrast to untreated plants (Figure 4A).

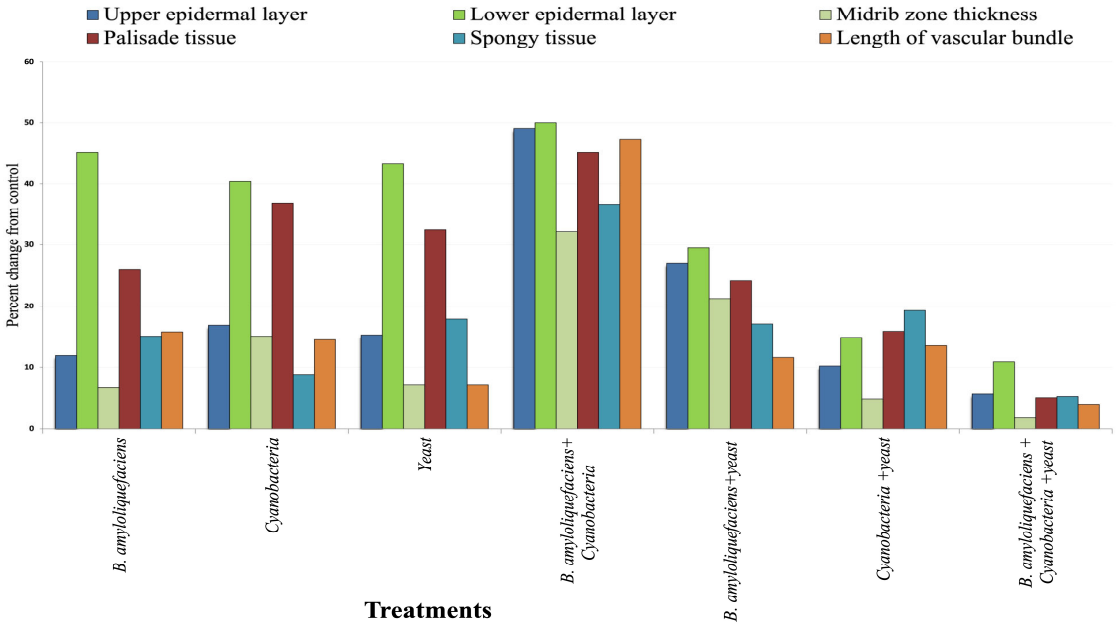

Figure 3. Effect of application of *B. amyloliquefaciens*, cyanobacteria, and yeast on anatomical structure of cowpea (*V. unguiculata* cv. Dokki 126) the terminal leaflet.

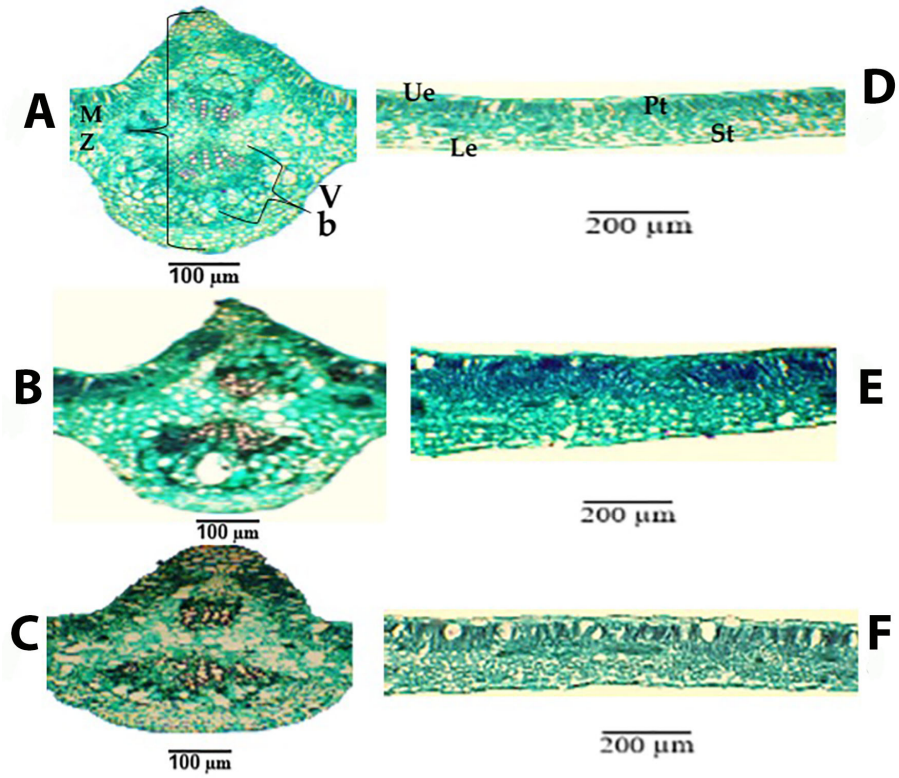

Figure 4. (**A**–**F**) Cross-sections microphotographs of the terminal leaflet blade in the compound leaf at the median portion of cowpea main stem (*V. unguiculata* cv. Dokki 126) terminal leaflet. (**A**,**D**) control, (**B**,**E**) inoculated with *B. amyloliquefaciens* + yeast + *cyanobacteria*, and (**C**,**F**) inoculated with *cyanobacteria*. Vb = vascular bundle; Mz = midrib zone; Ue = upper epidermis; Le = lower epidermis; Pt = palisade tissue; and St = spongy tissue.

On the other hand, it was clear that a cowpea leaflet's upper and lower epidermal layer thickness increased by 42.4% and 33.5%, respectively, under treatment with application *B. amyloliquefaciens* + yeast + cyanobacteria compared to the control (Figure 3). In

comparison, the application of cyanobacteria led to a slight increment in both epidermal layers' (upper and lower) thickness by 4.5% and 4.1%, respectively (Figure 3), in comparison with the control. Additionally, the palisade and spongy tissues showed the largest increase, by 36.9% and 26.5%, respectively, under treatment with *B. amyloliquefaciens* + yeast + cyanobacteria, while application with cyanobacteria only led to a slight increase in palisade as well as spongy layer by 5.1% and 5.3%, respectively, more than the control (Figure 3). Furthermore, the upper and lower epidermal layers' parenchymatous cells, as well as the palisade and spongy layer, are larger and more rounded than normal. Leaflet blade thickness increased mainly due to a slight increase in the palisade, spongy tissues, and both epidermal layers. An increase in leaflet blade thickness was observed, resulting from increases in the thickness of some leaflet structures such as the palisade, spongy tissues, both epidermal layers of treated (Figure 4E,F) and untreated plants (Figure 4D).

3.3. Yield Parameters

a. Productivity Criteria

Table 2 shows the effects of bio-fertilizer inoculation with yeast, cyanobacteria, and *B. amyloliquefaciens* on the yield criteria of cowpea plants. The results clearly demonstrated that inoculating plants with a mixture of *B. amyloliquefaciens*, yeast, and cyanobacteria considerably increased plant biomass compared to the un-inoculated control or inoculation with individual strains, as indicated in the table. Additionally, individual inoculation increased plant biomass in comparison to the un-inoculated control. The impact of inoculation on the observed parameters varied during the first season. According to the findings, the un-inoculated treatment produced plants with the least height of 169 cm. This parameter was considerably enhanced with the combined treatment of *B. amyloliquefaciens* + yeast + cyanobacteria mixture and yeast + cyanobacteria mixture to average plant heights of 231 cm and 221 cm, respectively. Similarly, the number of leaves was raised by inoculation with both treatments by 90% and 79%, respectively, compared with the un-inoculated counterparts. Additionally, the greatest value of branch number per plant was obtained in the mixture treatment, which was increased two-fold relative to the un-inoculated ones. Likewise, during the second season, inoculated treatments with *B. amyloliquefaciens* + yeast + cyanobacteria mixture enhanced all productivity parameters compared to their untreated counterparts. Inoculations in the first season showed comparable improvements, but inoculation in the second season had a better response than the un-inoculated control.

b. Pod Features Parameters

The effects of inoculation with *B. amyloliquefaciens*, yeast, and cyanobacteria on pod characteristics of cowpea plants during growing seasons (2021 and 2022) are demonstrated in Table 3. All treatments showed superiority over the control in both growing seasons, and the most significant increase in pod length was recorded in the case of *B. amyloliquefaciens* + yeast + cyanobacteria mixture treatment by 35% and 46%, respectively. Similar significant results were obtained for the pod diameter, which was also increased in plants inoculated with yeast + cyanobacteria mixture by 39% in the 2021 season and 41% in the 2022 season, compared to the control. In addition, plants treated with a mixture of *Bacillus amyloliquefaciens* + yeast + cyanobacteria in both seasons had significantly higher values of pod fresh and dry weight over the control, by 44% and 102%, respectively. All other treatments had similar plant responses in both seasons.

c. Seed Chemical Content

One of the first observable results of the chemical content (nitrogen, phosphorus, and potassium) of the yielded seed of cowpea is the rise in the inoculated treatments, as shown in Figure 5. Inoculation with *B. amyloliquefaciens* + yeast + cyanobacteria mixture in both seasons showed a significant increase in the NPK contents compared to the control. There was also a significant increase in plants treated with cyanobacteria individually. Cyanobacterial inoculation resulted in an increase in nitrogen contents. The inoculation

with *B. amyloliquefaciens* + yeast + cyanobacteria mixture had the highest increase by 1.45- and 1.6-fold with respect to un-inoculated counterparts in the first season.

Table 2. Effect of *B. amyloliquefaciens*, cyanobacteria, and yeast inoculation on cowpea yield characteristics in 2021 and 2022 seasons.

	Height of Plant (cm)		**Leaf Number**		**Branches Number/Plant**		**Fresh Weight of Plant (g)**		**Dry Weight of Plant (g)**	
					Seasons					
Treatment	2021 *	2022 *	2021 *	2022 *	2021 *	2022 *	2021 *	2022 *	2021 *	2022 *
Control	169 ± 6^{h}	162 ± 2.5^{h}	42 ± 1.5^{h}	38 ± 1.0^{h}	3 ± 0.58^{f}	4 ± 0.10^{h}	327 ± 5.7^{h}	319 ± 2.1^{h}	82.5 ± 1.4^{h}	78.2 ± 1.2^{h}
B. amyloliquefaciens	181 ± 1.7^{g}	176 ± 2.5^{g}	50 ± 1.0^{g}	44 ± 0.6^{g}	4 ± 0.29^{f}	4 ± 0.06^{g}	340 ± 1.5^{g}	327 ± 0.7^{g}	85.9 ± 1.5^{g}	80.8 ± 1.2^{g}
Cyanobacteria	186 ± 0.9^{f}	180 ± 0.9^{f}	56 ± 1.0^{f}	49 ± 1.5^{f}	4 ± 0.06^{e}	4 ± 0.06^{f}	351 ± 1.5^{f}	333 ± 1.6^{f}	89.8 ± 1.1^{f}	83.1 ± 1^{f}
Yeast	195 ± 2.7^{e}	185 ± 2.5^{e}	61 ± 1.0^{e}	55 ± 0.6^{e}	5 ± 0.10^{d}	5 ± 0.06^{e}	359 ± 0.7^{e}	343 ± 1.5^{e}	95.4 ± 1.1^{e}	87.5 ± 0.6^{e}
B. amyloliquefaciens + cyanobacteria	204 ± 2.5^{d}	194 ± 1.8^{d}	64 ± 0.6^{d}	61 ± 1.0^{d}	5 ± 0.12^{c}	5 ± 0.06^{d}	367 ± 3.7^{d}	350 ± 1.6^{d}	98.5 ± 0.4^{d}	92.2 ± 1^{d}
B. amyloliquefaciens + yeast	213 ± 2.9^{c}	199 ± 1.7^{c}	68 ± 1.0^{c}	64 ± 0.6^{c}	$6 \pm 0.12^{b\,c}$	5 ± 0.06^{c}	375 ± 0.8^{c}	355 ± 0.7^{c}	101.5 ± 0.5^{c}	95.8 ± 0.3^{c}
Cyanobacteria + yeast	221 ± 0.8^{b}	209 ± 1.9^{b}	75 ± 1.0^{b}	72 ± 0.6^{b}	6 ± 0.17^{b}	6 ± 0.17^{b}	383 ± 2.5^{b}	361 ± 2.0^{b}	103.9 ± 0.8^{b}	99.7 ± 0.5^{b}
B. amyloliquefaciens + cyanobacteria + yeast	231 ± 1.7^{a}	220 ± 1.7^{a}	80 ± 1.0^{a}	78 ± 1.5^{a}	6 ± 0.06^{a}	6 ± 0.10^{a}	390 ± 1.4^{a}	369 ± 1.0^{a}	105.7 ± 0.8^{a}	103.1 ± 0.8^{a}
LSD at 0.05	3.506	3.507	1.802	1.731	0.428	0.158	4.735	2.592	1.758	1.522

* Significant differences among treatments are indicated by different alphabetic letters from ^{a} to ^{h} that represents significant levels ($p < 0.05$), i.e., a treatment with the letter "^{a}" is significantly different from "^{b}", and "^{b}" is significantly different from "^{c}", and so forth. However, if two treatments have the same letter, they are not significantly different. The standard deviation (SD) values are presented after the means (+/−).

Table 3. Effect of *B. amyloliquefaciens*, cyanobacteria, and yeast inoculation on pod characteristics of cowpea plants in 2021 and 2022 seasons. Significant differences among treatments are indicated by different alphabetic letters ($p < 0.05$).

	Length of Pods (cm)		**Diameter of Pods (cm)**		**Fresh Weight of Pod (g)**		**Dry Weight of Pod (g)**	
				Seasons				
Treatment	2021	2022	2021	2022	2021	2022	2021	2022
Control *	17.1 ± 0.69^{h}	14.7 ± 0.48^{g}	0.62 ± 0.01^{h}	0.59 ± 0.01^{h}	8.3 ± 0.2^{f}	7.7 ± 0.06^{h}	2.2 ± 0.05^{h}	2.4 ± 0.02^{h}
B. amyloliquefaciens	18.3 ± 0.46^{e}	16.6 ± 0.52^{f}	0.66 ± 0.02^{g}	0.63 ± 0.01^{g}	8.9 ± 0.03^{e}	8.5 ± 0.12^{g}	2.8 ± 0.16^{g}	2.9 ± 0.02^{g}
Cyanobacteria	19.4 ± 0.32^{d}	18.2 ± 0.5^{e}	0.71 ± 0.01^{f}	0.69 ± 0.01^{f}	$9.1 \pm 0.07^{d\,e}$	9 ± 0.11^{f}	3.1 ± 0.08^{f}	3.1 ± 0.04^{f}
Yeast	20.8 ± 0.06^{c}	19.5 ± 0.59^{d}	0.74 ± 0.01^{e}	0.72 ± 0.02^{e}	9.4 ± 0.19^{d}	9.3 ± 0.08^{e}	3.5 ± 0.18^{e}	3.3 ± 0.05^{e}
B. amyloliquefaciens + cyanobacteria	21.2 ± 0.06^{c}	20.5 ± 0.39^{c}	0.8 ± 0.01^{d}	0.77 ± 0.01^{d}	10.1 ± 0.04^{c}	9.8 ± 0.07^{d}	4 ± 0.08^{d}	3.7 ± 0.06^{d}
B. amyloliquefaciens + yeast	21.8 ± 0.15^{b}	$21.1 \pm 0.09^{b\,c}$	0.82 ± 0.01^{c}	0.8 ± 0.01^{c}	10.9 ± 0.08^{b}	10.2 ± 0.1^{c}	4.3 ± 0.02^{c}	3.9 ± 0.03^{c}
Cyanobacteria + yeast	22.3 ± 0.15^{b}	$21.8 \pm 0.15^{a\,b}$	0.86 ± 0.01^{b}	0.83 ± 0.01^{b}	11.2 ± 0.17^{b}	10.6 ± 0.06^{b}	4.6 ± 0.1^{b}	4.2 ± 0.06^{b}
B. amyloliquefaciens + cyanobacteria + yeast	23.0 ± 0.17^{a}	22.4 ± 0.25^{a}	0.9 ± 0.01^{a}	0.88 ± 0.02^{a}	12.1 ± 0.36^{a}	11.1 ± 0.12^{a}	4.8 ± 0.03^{a}	4.5 ± 0.04^{a}
LSD at 0.05	0.573	0.707	0.014	0.019	0.308	0.162	0.178	0.072

* Significant differences among treatments are indicated by different alphabetic letters from ^{a} to ^{h} that represents significant levels ($p < 0.05$), i.e., a treatment with the letter "^{a}" is significantly different from "^{b}", and "^{b}" is significantly different from "^{c}", and so forth. However, if two treatments have the same letter, they are not significantly different. The standard deviation (SD) values are presented after the means (+/−).

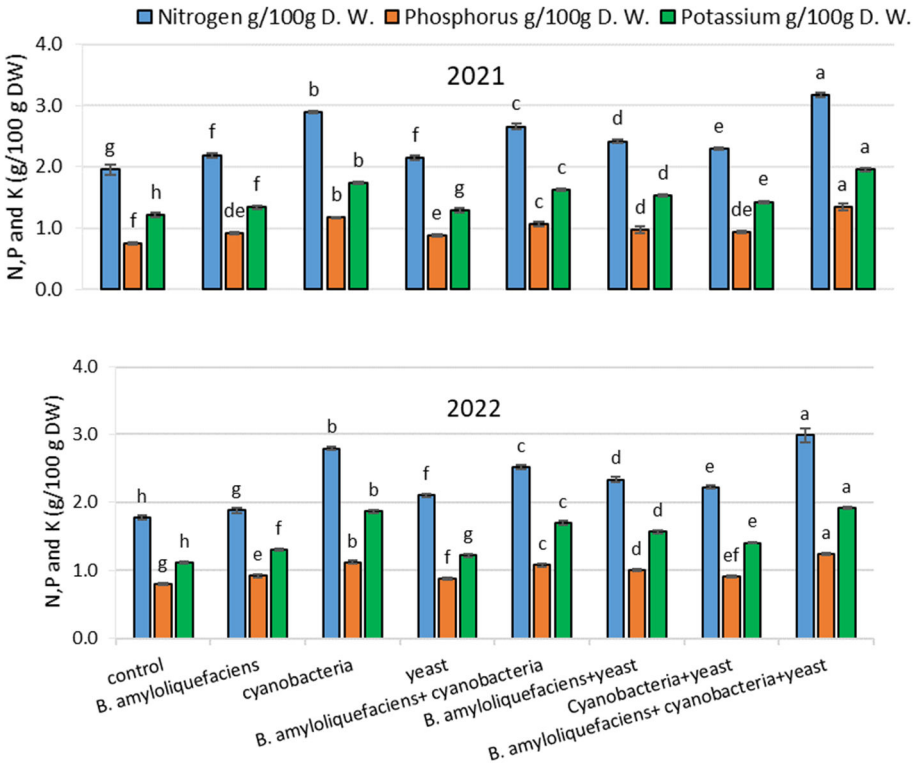

Figure 5. Effect of *B. amyloliquefaciens*, cyanobacteria, and yeast inoculation on the chemical contents N, P, and K (g/100 g DW) of the crop seeds of cowpea plant in 2021 and 2022 seasons. Significant differences among treatments are indicated by different alphabetic letters from a to h that represents significant levels ($p < 0.05$), i.e., a treatment with the letter "a" is significantly different from "b", and "b" is significantly different from "c", and so forth. However, if two treatments have the same letter, they are not significantly different. The LSD ($p < 0.05$) values are 0.075, 0.061, and 0.047 for N, P, and K, respectively, in the first season. These values are 0.078, 0.033, and 0.038 for N, P, and K, respectively, in the second season. The standard deviation (SD) values are presented as error bars.

Relative to the un-inoculated samples, phosphorus contents in seeds of the inoculated plants with *B. amyloliquefaciens* + yeast + cyanobacteria mixture and individual inoculation with cyanobacteria noticeably increased in the first season. In the first season, the greatest potassium content was found in yielded seeds inoculated with *B. amyloliquefaciens* + yeast + cyanobacteria mixture, which was 1.96 g/100 g DW, followed by cyanobacteria which was 1.73 g/100 g DW. The findings indicated no discernible difference between the two growing seasons.

d. Total Carbohydrate Contents

As shown in Figure 6, the total carbohydrates of seeds inoculated with *B. amyloliquefaciens* + cyanobacteria + yeast mixture were significantly higher than in other treatments. For the two seasons, the utilization of *B. amyloliquefaciens*+ cyanobacteria + yeast mixture significantly increased the carbohydrates by 71% and 72%, respectively, compared to the control. Moreover, in the first season, yeast + cyanobacteria, *B. amyloliquefaciens* + cyanobacteria mixture, and *B. amyloliquefaciens* + yeast mixture improved carbohydrate accumulation compared to individual inoculation as well as the un-inoculated counterparts and a similar pattern was noticed in the second season.

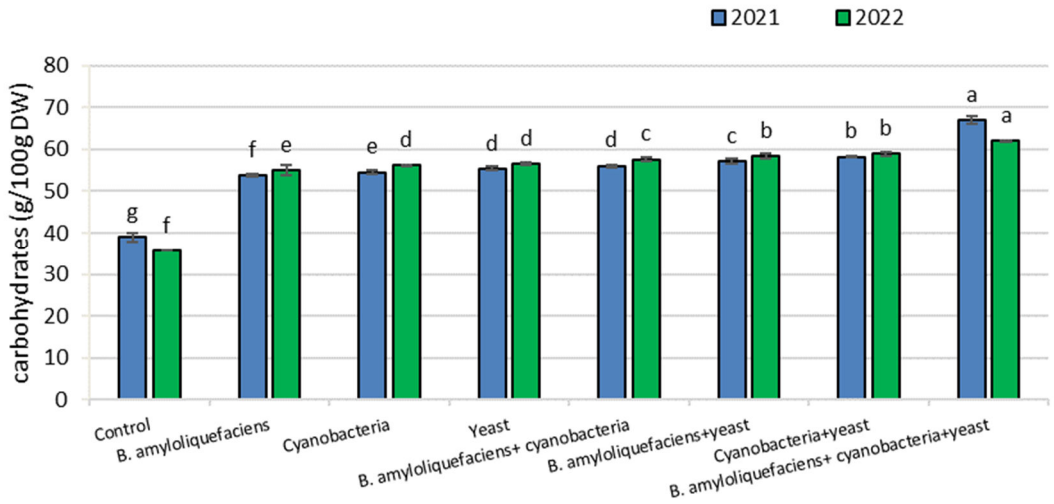

Figure 6. Effect of *B. amyloliquefaciens*, cyanobacteria, and yeast, inoculation on the total carbohydrates of the crop seeds of cowpea plant in 2021 and 2022 seasons. Significant differences among treatments are indicated by different alphabetic letters from a to g that represents significant levels ($p < 0.05$), i.e., a treatment with the letter "a" is significantly different from "b", and "b" is significantly different from "c", and so forth. However, if two treatments have the same letter, they are not significantly different. The LSD ($p < 0.05$) values for total carbohydrates are 0.704 and 0.718 for 2021 and 2022, respectively. The standard deviation (SD) values are presented as error bars.

3.4. Multivariate Correlation Analysis

Table 4 presents the multivariate correlation matrix of the studied morphological and yield characteristics of cowpea plants. It is observed that all characteristics were correlated at varying levels. A high level of positive correlation was observed between the number of leaves and pod characteristics, including the diameter and fresh and dry weights. In addition, plant dry weight was also positively correlated with leaf number. However, the DHA values showed lower levels of correlation to K and carotenoid contents.

Table 4. Multivariate correlation matrix among morphological and yield characteristics of cowpea plants.

	Plant Height	*No. of Leaves*	*No. of Branches/Plant*	*Pod Length*	*Pod Diameter*	*Pod Fresh Weight*	*Pod dry Weight*	*Plant Fresh Weight*	*Plant Dry Weight*	*Carbohydrates*	*DHA*	*Nitrogen*	*Phosphorus*	*Potassium*	*Chl A*	*Chl B*	*Carotenoids*
Plant height	1.00																
No. of leaves	0.92	1.00															
No of. branch/plant	0.87	0.94	1.00														
Pod length	0.84	0.96	0.86	1.00													
Pod diameter	0.91	0.99	0.94	0.96	1.00												
Pod fresh weight	0.92	0.97	0.92	0.93	0.97	1.00											
Pod dry weight	0.89	0.97	0.95	0.93	0.98	0.97	1.00										
Plant fresh weight	0.85	0.95	0.86	0.93	0.93	0.95	0.94	1.00									
Plant dry weight	0.86	0.98	0.90	0.96	0.97	0.96	0.95	0.97	1.00								
Carbohydrates	0.86	0.87	0.93	0.78	0.89	0.85	0.87	0.71	0.78	1.00							
DHA	0.63	0.64	0.62	0.60	0.60	0.63	0.60	0.59	0.60	0.60	1.00						
N	0.60	0.63	0.58	0.61	0.63	0.63	0.59	0.60	0.57	0.57	0.27	1.00					
P	0.62	0.59	0.59	0.55	0.60	0.62	0.58	0.55	0.52	0.60	0.34	0.95	1.00				
K	0.58	0.58	0.58	0.56	0.60	0.60	0.58	0.52	0.51	0.61	0.20	0.96	0.94	1.00			
Chl A	0.60	0.62	0.55	0.60	0.61	0.65	0.61	0.68	0.60	0.45	0.29	0.91	0.89	0.84	1.00		
Chl B	0.61	0.63	0.55	0.63	0.62	0.65	0.60	0.69	0.61	0.44	0.33	0.91	0.89	0.85	0.96	1.00	
Carotenoids	0.55	0.59	0.55	0.60	0.60	0.62	0.58	0.62	0.58	0.47	0.19	0.95	0.90	0.92	0.94	0.93	1.0

4. Discussion

In order to promote the growth of high-yield crops, fertilizers are necessary. Large quantities of nitrogen, phosphorus, calcium, sulphur, magnesium, potassium, iron, and zinc are needed by several plants in most soils as critical nutrients for good growth [51]. Large quantities of nutrients, including nitrogen, phosphorous, sulphur, and potassium, are mostly used as mineral fertilizers, either as processed naturally occurring minerals or synthetic chemicals [52]. Some advantages are that organic fertilizers have substantially maintained agriculture productivity and improved the crop quality of certain plants [53].

Regardless of the amount of nutrients in the crop, biostimulants are biological agents or microorganisms administered to plants to increase their health and nutrition efficiency, resistance to abiotic stress, and qualitative attributes. As a subclass of bio-stimulants, bio-fertilizers are microbial inoculants that can increase the nutritional effectiveness of plants by including active and/or inactive formulations of helpful microorganisms [54,55]. In the present study, the soil for growing cowpea plants was inoculated with the three biofertilizers: PGPR (*B. amyloliquefaciens*), yeast (*S. cerevisiae*), and cyanobacteria (*N. mucorum*) alone or in combination to evaluate growth and yield.

The dehydrogenase enzyme is an oxidoreductase that can only be found in living cells. It is thought to be a sensitive indicator of the soil's quality and has been suggested as a reliable biomarker to detect changes in the total bacterial count as a result of soil management modifications [56]. The findings of the study [56] also showed that, under salt stress, dehydrogenase activity is stimulated by inoculation with *B. amyloliquefaciens*, yeast, and cyanobacteria. Such an increase in dehydrogenase enzyme activity in the rhizosphere soils could be attributed to the availability of large amounts of biodegradable substances, which in turn improves their microbial activity [57]. The reason for the increase in dehydrogenase may be a result of the intra- and extracellular enzyme populations that the root exudates can promote [58]. The inoculation with PGPR has been associated with a similar rise in dehydrogenase activity, according to Rana et al. [59]. In addition, Sharma et al. [60] observed that *B. amyloliquefaciens* inoculated treatments increased the dehydrogenase activity of inoculated rice plants. Similarly, Tolba et al. [61] recorded an increase in microbial populations in the plant rhizosphere because of the effects of yeast and two cyanobacterial strains in the soil. In another study, it was observed that dehydrogenase activity increased in the soil [62]. The DHA activity in this study was more responsive in the 2021 season than in the 2022 season. This difference in effects might be attributed to environmental factors. For example, the first season temperature recorded in March was higher in 2021 by 2.56 °C compared to the corresponding month in 2022. In addition, the temperature was also higher in May and July months in 2021 compared to those months in 2022.

Photosynthesis is a crucial phenomenon that significantly contributes to the growth and development of plants, although a variety of physiological, biochemical, and molecular mechanisms regulate plant growth [63]. Our findings demonstrated that inoculation with a mixture of three biofertilizers caused an increase in the concentrations of chlorophyll a, chlorophyll b, and carotenoids. In this experiment, the first season had a slightly higher response in photosynthetic pigments than the second season. A relatively higher average monthly temperature was recorded in March, May, and July months in the first season compared to the same months in the second season, which might contribute to these effects. This might be because of environmental conditions on plant growth and biochemical processes. Nautiyal et al. [64] reported that salt stress caused a decrease in chlorophyll content, which demonstrated a relative recovery with the existence of *B. amyloliquifaciens* (SN13). Moreover, the results were also in accordance with the findings of Samaniego-Gámez et al. [65], who noted that inoculation of plants with *Bacillus* spp. (M9 strain, a combination of *B. subtilis* and *B. amyloliquefaciens*) can enhance photosynthesis rates and plant growth. Hence, the increased activity of the photosynthetic system was attributed to the enhancement in leaf chlorophyll content in plants treated with bacterial biofertilizers [66].

Furthermore, plants treated with biofertilizers had increased PSII operating efficiency. This variable measures the amount of light absorbed by PSII-associated chlorophyll, which is employed in photochemistry [67]. The increase in chlorophyll content was positively impacted by cyanobacterial inoculation [68] and provided evidence of the plant's total chlorophyll's favorable response to cyanobacterial inoculation. In response to various fertilization treatments, the cyanobacterial extract contains biologically active substances, including plant growth regulators that enhance leaf chlorophyll concentration, slowing the pace of senescence and transpiration [69]. The positive impact of yeasts on chlorophyll a and b is also consistent with the findings of Hayat [70] and Stino et al. [71], who found that an increase in chlorophyll a and b results in a corresponding rise in total carbohydrates. This is because yeast application might also enhance cell division and elongation function, generating further leaf area. *Saccharomyces* sp. has the ability to enhance photosynthesis, produce bioactive chemicals, including hormones and enzymes, and control soil diseases, and in turn, enhance crop growth and production [72].

Our extensive anatomical study showed variations in leaflet structure resulted from applying a *B. amyloliquefaciens*, yeast, and cyanobacteria mixture, which produced the highest increments in midrib zone thickness, vascular bundle length, upper and lower epidermis, palisade, and spongy tissues. The increase in leaflet blade thickness can be attributed to changes in the thickness of the palisade, spongy tissues, both epidermal layers and untreated plants (Figure 4A–C). However, the application of cyanobacteria only produced the smallest increases in the thickness of the aforementioned characters in treated cowpea plants compared to untreated plants. Inoculation with growth-promoting microorganisms resulted in a greater density of relatively small stomata, relatively thick palisade parenchyma, and a significant increase in mesophyll intercellular spaces in soybean leaves [73].

These changes could be attributed to alterations in leaf anatomical and functional characteristics encouraging leaf gas exchanges. In addition, the improved photosynthesis in treated plants had improved the thickness of the palisade parenchyma that stores most of the chloroplasts. Additionally, in soybean leaves, Pieruschka et al. [74] stated that characteristics, such as palisade thickness and the porosity of spongy parenchyma, influence the vertical and lateral gas transport inside the leaf lamina. Additionally, the anatomical characteristics of tomato leaflets were modified with the treatment with growth-promoting microbes, such as *B. amyloliquefaciens*, yeast, and cyanobacteria. The modifications showed increments in upper and lower epidermis thickness, the thickness of the palisade, and spongy parenchyma, along with changes in the thickness and dimension of the vascular bundles [75]. There are limited readily available studies on the impact of PGPR on micro and macro morphological components of cowpea leaves.

Nevertheless, several researchers supported the data presented here by utilizing certain growth regulators., e.g., salicylic acid on other field crops [74]; for instance, barley [76], *Phaseolus vulgaris* [77], and beans [78], as well as antioxidants on *Zea mays* L. [79] and *Lupinus termis* [80]. The improvement in the table beet's morphological traits correlated with the exogenous fertilizer application at various phosphorus and zinc levels [81]. The above-mentioned studies reported that applying salicylic acid increased the thickness of leaf midvein and lamina. A significant correlation was found between the tomato plant's vigorous growth and several anatomical responses of the leaves because tomato leaflet thicknesses of the midvein, lamina, upper epidermis, and lower epidermis, spongy tissue, and palisade tissue were increased.

Additionally, the length and width of vascular bundles, the thickness of the phloem and xylem tissues, and the number of vessels in the xylem all increase after treatment with 30 mL/L of yeast extract and 3 mL/L of amino acids [82]. In two seasons, inoculated cowpea plants with bio-fertilizer showed better growth than the un-inoculated plants. Plants inoculated with *B. amyloliquefaciens*, yeast, and cyanobacteria mixture exhibited maximum plant growth productivity such as the height of plants, leaves number, branches number/plant, and the fresh and dry weight of the plant. In addition, some pod qualities,

such as the length of the pod, the diameter of the pod, and the fresh and dry weight of the pod, have also increased. According to Malusà et al. [83], consortia of helpful microbes may be more effective than single-strain inoculants since they combine the various modes of action of the various microorganisms. Therefore, it is crucial to consider the plant's overall reaction in the field as multifactorial so that diverse communities of microorganisms with various traits might work in harmony and become more useful. Furthermore, the ability of helpful microbes to increase the production of crops can be significantly impacted by the host specificity of microbial strains and growth conditions (such as soil characteristics, native microbiota, and nutrition) [84].

According to research, phytohormones produced by cyanobacteria increase the availability of nutrients locally or make it easier for plants to absorb those nutrients. This increases plant height and productivity [85,86]. It has been demonstrated that cyanobacterial inoculation in tomato plants improved plant response against *Fusarium* wilt [87]. Researchers have compiled several reports on cyanobacteria that fix nitrogen, and there is unambiguous proof that the nitrogen they fix is available to plants or other small and large species in the environment [88].

PGPRs are known for increasing nutrient availability in the plant rhizosphere by producing siderophores and promoting the transfer of nutrients. This may be carried out by solubilizing inaccessible forms of nutrients [89]. Regarding its ability to promote plant growth and exercise biocontrol, the *B. amyloliquefaciens/subtilis* clade is the most extensively researched *Bacillus* species [90]. According to Salvatierra-Martinez et al. [91], when bacteria were administered to the roots, both strains dramatically enhanced the dry weight and plant height compared to control plants. Through direct and indirect methods, such as solubilizing vital soil components and synthesizing phytohormones, volatiles, and defense chemicals, economically beneficial bacteria increase plant vigor and growth [92,93]. As a result, the rise in the root-to-shoot ratio seen in plants treated with yeast may result from the plant utilizing organic forms of derived N and P and chemicals by yeast that promote plant growth.

In addition, Salim et al. [94] noted that bread yeast at concentrations of 8 and 12%, combined with some bio-fertilizers (*A. brasilense*, *P. fluorescens,* and *B. megaterium*), increased the growth traits of cucumber plants. This is in line with the findings of Sarhan [95], who attributed the improvement in potato growth to the yeast's capacity to stimulate plant growth hormones, such as auxins, cytokinins, and gibberellins, that enhance cell division and growth. Our results are consistent with those of Bevilacqua et al. [96], who noted that the table olives plant response to the yeast application could be a result of the contents of proteins, vitamins, free amino acids, and various elements such as P, K, Mn, Ca, Mg, B, Zn, and Fe in yeast. The yeast content may contribute to the growth promotion effects.

In our investigation, treatment with a *B. amyloliquefaciens*, yeast, and cyanobacteria mixture increased nitrogen, potassium, and phosphorus levels. This is corroborated by prior research that used cyanobacteria/bacteria and their mixtures and showed their important function in nitrogen cycling and biofortification of wheat crops [59,88,97]. N and P regulate the growth and development of plants, and productivity and yield are strongly correlated with their availability [98]. In the plant–soil relationship, beneficial soil microorganisms preserve soil organic N and other nutrients and facilitate their release [99]. The role of biostimulants was also observed in promoting the accumulation of NPK and other minerals [100,101]. Furthermore, Tolba et al. [61] recorded that plants and grains treated with yeast and humate had greater N, P, and K levels.

The higher NPK levels in plants could be explained by the occurrence of soil yeasts from various genera, such as *Candida*, *Saccharomyces*, *Geotrichum*, *Rhodotorula*, and *Williopsis* that contribute to the nitrogen and sulphur cycles in the soil [102]. Moreover, the yeast culture increases the nutrient components through the disintegration of *S. cerevisiae* cells that improve the formation of biologically produced CO_2 [103]. Additionally, these yeasts could solubilize insoluble phosphates, increasing the availability of these nutrients to

plants [104]. Finally, by producing plant growth regulators, yeast can directly increase plant growth and yield [105].

In this study, the number of leaves in cowpea was highly positively correlated with pod characteristics such as diameter and weight. In addition, plant dry weight was also highly correlated with the number of leaves. This positive correlation between leaf number and yield characteristics can be explained by a higher leaf area index [106], leading to improved photosynthesis and the resulting accumulation of assimilates, leading to improved nutrient storage that results in higher yield.

5. Conclusions

The mixture of *B. amyloliquefaciens*, yeast, and cyanobacteria was used as integrated treatments in the experiments and was compared to separate treatments of these microorganisms and the control. The results showed that the synergistic effects of microbial inoculation could be an alternative fertilization approach for the eco-friendly production of cowpea. The integrated treatment with the mixture of *B. amyloliquefaciens*, yeast, and cyanobacteria improved the photosynthetic pigments of plants grown in the treated soils. Additionally, soils treated with this mixture indicated a noticeable improvement in soil dehydrogenase activity and seed N, P, and K contents, including enhancements in all growth characteristics. The unique finding of this study is the superior effect of the combined application of biofertilizers that surpassed individual treatments and control in cowpea crops. This has relevance as an eco-friendly alternative method to ensure the sustainability of cowpea production. Future studies can focus on comparing the effects of application methods and timing on a large scale under field conditions. Additionally, soil microbial assessment of changes in the microbial community resulting from bio-fertilizer applications is suggested for a more comprehensive analysis in prospective studies.

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