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Influence of Commercial Seaweed Extract and Microbial Biostimulant on Growth, Yield, Phytochemical Content, and Nutritional Quality of Five *Abelmoschus esculentus* Genotypes

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Abstract: Biostimulant application during the cultivation of underutilized crops is an environmental-friendly approach for their production and utilization to promote food security and human health. This study investigated the effect of two commercial biostimulants (a seaweed-based extract, Kelpak® (1:100, 1:40, and 1:20, dilutions), and plant growth promoting rhizobacteria, PGPR (1:5, 1:10, and 1:15, dilutions)) on the growth, yield, phytochemical content, and nutritional quality of five selected *Abelmoschus esculentus* genotypes. Biostimulant application significantly influenced vegetative growth and yield in a dose-dependent manner. Plant height, chlorophyll content, stem diameter, number of pods, and total pod fresh and dry weights increased with a decrease in dilution of the biostimulants. The application of PGPR (1:5) significantly promoted both the vegetative growth (plant height, chlorophyll content, and stem diameter) and yield (number of pods, total fresh weight, and total dry weight) when compared to the control (untreated plants) and other biostimulant dilutions. Genotype and biostimulant application had an interactive effect on all the phytochemical (total phenolics, flavonoids, and condensed tannins) and nutritional (β-carotene, vitamin C, calcium, iron, potassium, magnesium, sodium, and zinc) qualities evaluated. This study demonstrated the differential effect of biostimulant application on *A. esculentus* genotypes. These biostimulants can be used to enhance growth, yield, biochemical, and nutritional contents of underutilised crops such as *A. esculentus*, depending on the crop genotype, in order to improve crop productivity and combat food insecurity especially in food insecure communities.

Keywords: algal extract; biochemicals; crop production; Kelpak®; okra; okro; plant growth; rhizobacteria; seaweed biostimulants

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1. Introduction

The increasing world population amplifies the urgent need for enhanced food production to ensure food security. In addition, alternative nutritious diet supplements to be implemented in a regular diet have become a priority. For a well-balanced diet, a minimum of 400 g of fruits and vegetables is recommended daily, which is currently not attained

in most rural communities [1]. As a result, researchers have studied several African indigenous plants for their nutritional content to mitigate food insecurity, especially at the household level [2,3].

Abelmoschus esculentus (L.) Moench (family: Malvaceae) is an annual herb that is native to Africa, mostly cultivated worldwide in tropical to subtropical regions for its delicious tender fruits [4,5]. This species is commonly known as lady's finger, okra, okro, or gumbo [6]. *Abelmoschus esculentus* is considered a summer crop, however in India, it has been reported to flower throughout the year [7,8]. *Abelmoschus esculentus* is a well-known nutraceutical that is rich in proteins and tryptophan amino acids, vitamins (A, C, E, and K), thiamine (B_1), riboflavin (B_2), calcium, iron, magnesium, potassium, and zinc [9]. Furthermore, *A. esculentus* is also a rich source of oil which consists of up to 47.2% linoleic acid and possess components that are valued in the treatment of various diseases [10,11]. For instance, *A. esculentus* is used in Ayurveda's traditional system and is prepared as an edible infusion for its diuretic effect [12]. It has been widely used as an antidiabetic, anticancer and antimicrobial agents [13]. In Indian ethnomedicine, *A. esculentus* is used as antipyretic and for plasma replacement [12]. *Abelmoschus esculentus* is rich in flavonoids, pectin, oxalic acid, tannins, phenolic compounds, and carotenoids [12,14].

After seed germination, the next development step is seedling emergence and growth. Seedling emergence and stand establishment are dependent on environmental conditions as well as soil physical properties. However, soil infertility leads to poor soil physical properties and poor soil functions and characteristics including nutrient holding capacity, available plant nutrients, water filtration, water holding capacity, and aggregation (porosity). Biostimulants can modify root morphology directly, ameliorate nutrient transport in plants, or change soil structure and nutrient solubility to facilitate increased nutrient uptake and thus lead to enhanced plant growth and yield [15]. For instance, the application of Kelpak® using the soil drenching method significantly increased the shoot fresh weight of *Amaranthus hybridus* L. while the foliar application had no observable effects [16]. Based on the study by Román-Ponce et al. [17], an increase in root architecture (secondary roots and hair generation) of *Brassica nigra* under unfavourable conditions (heavy metals, As, Cu, Pb, and Zn) was evident following the application of plant growth-promoting rhizobacteria (PGPR). Few biostimulants have been reported to influence the biochemical (including nutritional and phytochemical) contents of plants. Seaweed extract application enhanced nutritional quality through direct plant provision of both macro- and micronutrients [18]. The mineral element concentration (nitrogen and potassium) of *Glycine max* straw remained unaffected by seaweed extract application while phosphorus content was significantly enhanced at 5, 7.5, and 10% (*v*/*v*) when measured against the control [19]. The application of TAM®—a commercial seaweed extract—improved growth, yield, and bioactive chemical contents of hot pepper, cucumber, and rocket [20–22]. The application of a plant biostimulant based on seaweed and yeast extract increased tomato fruit nutritional quality [23]. Plant growth-promoting rhizobacteria treatments significantly reduced the proline content of *Mentha piperita* while increasing total phenolic content when compared to the control [24]. Research on the effect of biostimulants on the phytochemistry of plants has been conducted; however, limited research has been conducted on *Abelmoschus esculentus*, an underutilised multipurpose crop. Recently, we established the potential stimulatory effect of biostimulants (especially Kelpak®) on the germination of *A. esculentus* seeds [25]. The current study was aimed at evaluating the effect of Kelpak® (seaweed-based biostimulants) and plant growth promoting rhizobacteria (PGPR) on the growth, yield, phytochemical content, and nutritional quality of five *A. esculentus* genotypes.

2. Materials and Methods

2.1. Source of Biostimulants, Seeds and Chemicals

Kelpak® was obtained from Kelp Products (Pty) Ltd., Simon's Town, South Africa. Plant growth promoting rhizobacteria (commercial solution) (a mixture of organic acids, *Bacillus* sp., amino/fulvic acid, and soil bacteria) was purchased from Agriman (Pty) Ltd.,

South Africa. Five genotypes (VI037996, VI046567, VI055421, VI050956, and VI033796) of *A. esculentus* seeds were obtained from the Agricultural Research Council, Vegetables, Industrial and Medicinal Plants (ARC-VIMP), Pretoria, South Africa. These genotypes were originally imported from the World Vegetables Center, Taiwan, and are maintained in the ARC-VIMP Genebank. The experiments were conducted in the glasshouse at the Agricultural Research Council, Vegetables, Industrial and Medicinal Plants, Pretoria, South Africa. All the chemicals and reagents used in the current study were of analytical grade and purchased from companies such as Sigma-Aldrich (St. Louis, MO, USA) and Merck (Darmstadt, Germany).

2.2. Seed Soaking Using Biostimulants Prior to Planting

Abelmoschus esculentus seeds were surface sterilised with a 1% sodium hypochlorite for 5 min and rinsed thoroughly with distilled water. The seeds of the five genotypes (VI037996, VI046567, VI055421, VI050956, and VI033796) were soaked in biostimulants for 24 h (room temperature) at varying dilutions (Kelpak® solution (1:100, 1:40, and 1:20 v/v) and PGPR (1:5, 1:10, and 1:15 v/v)) while distilled water was used as the control.

2.3. Planting, Seedling Growth and Yield

The experiment was established in potting soil, which consisted of 12% clay and both macro- and micronutrients with well-defined chemical and physical properties (Table S1). Two factors—effect of biostimulant application and genotypes—were considered. *Abelmoschus esculentus* seeds were sown directly into 25 cm diameter pots in a glasshouse, with a temperature of 25 °C. Treatments were arranged in a completely randomised block design, replicated five times. Pots were monitored daily and irrigated at regular intervals. Consequently, 100 mL of each biostimulant treatment per plant ((Kelpak® solution (1:100, 1:40 and 1:20) and plant growth-promoting rhizobacteria (PGPR) (1:5, 1:10 and 1:15 v/v)) or distilled water (used as a control) was applied through soil drenching after every two weeks until termination. After successful establishment (two months after planting), growth parameters (plant height, number of leaves, stem diameter, and chlorophyll content using SPAD) were measured weekly. The experiment commenced on 21 September 2019. Harvesting was done after five months of planting. Upon harvesting, fresh and dry weights of the pods were recorded. After harvesting, plant samples were weighed, frozen in a −80 °C freezer and lyophilised. Subsequently, the freeze-dried plant materials were ground into fine powders and used for further analysis.

2.4. Phytochemical Analysis

2.4.1. Determination of Total Phenolic (TP) Content

Total phenolic (TP) content was determined using the Folin–Ciocalteu method as described by Makkar [26]. Ground plant sample (0.2 g) was extracted using 10 mL of 50% methanol and sonicated for 20 min. In triplicates, 50 µL of plant extract was transferred into reaction tubes followed by the addition of 450 µL of distilled water, 250 µL of 1 N Folin-Ciocalteu reagent, and 1250 µL of Na_2CO_3 (2%) solution. The reaction mixture was sonicated and incubated under dark condition for 40 min at room temperature. Absorbance was measured at 725 nm and a blank was prepared in a similar manner, except that the plant extract was replaced with a solvent (50% methanol). We calculated the TP content based on calibration curve using gallic acid as a standard and the result was expressed in mg gallic acid equivalents per gram dry weight (DW).

2.4.2. Determination of Flavonoid Content

Flavonoid content was determined according to the method described by Marinova et al. [27]. A ground sample of 0.2 g was extracted using 10 mL of 50% methanol and sonicated for 20 min. In triplicates, an aliquot of 250 µL plant extract was added into a reaction tube. Thereafter, 1 mL of distilled water and 75 µL of 5% $NaNO_2$ were added. After 5 min, 75 µL of 10% $AlCl_3$, 0.5 mL of 1 M NaOH, and 0.6 mL of distilled water were added.

The reaction mixture was vortexed and measured for absorbance at 520 nm. A blank was prepared in a similar manner except that plant extract was replaced with a solvent, 50% methanol. Flavonoid content was calculated based on a calibration curve using catechin as a standard and the result was expressed as mg catechin equivalents per gram DW.

2.4.3. Determination of Condensed Tannins (CT)

Condensed tannins (CT) were determined using the HCl–butanol method as described by Makkar [26] with slight modifications. A ground sample of 0.2 g was extracted using 10 mL of 50% methanol and sonicated for 20 min. In triplicates, plant extract (500 µL) was added into reaction tubes, followed by 3000 µL of butanol-HCl added into the tube and 100 µL of ferric reagent. The mixture was vortexed to mix thoroughly. The heated and unheated blanks were then prepared. The unheated blank was prepared by adding 0.5 mL of the extracted sample with 3 mL of butanol–HCl reagent and 0.1 mL of ferric reagent, while the heated blank was prepared by adding 0.5 mL of the extracted sample with 3 mL of butanol and 0.1 mL of ferric reagent. The heated blanks and samples were incubated at 100 °C for 60 min and were then cooled at room temperature. The absorbance readings of both blanks were recorded at 550 nm. Condensed tannins were calculated based on a calibration curve using cyanidin chloride as a standard and the result was expressed as mg cyanidin chloride equivalents per gram DW.

2.5. Nutritional Analysis

2.5.1. Determination of β-Carotene Content

Beta-carotene content was determined using a method described by Biehler et al. [28] with the modifications detailed by Moyo et al. [29]. Ground samples (0.2 g) was extracted using 10 mL of ice-cold hexane: acetone (1:1). A total of 15 mL of saturated NaCl was added to the reaction mixture. The mixture was vortexed and centrifuged (HERMLE Z513, Wehingen, Germany) (at 2000 rpm) for about 2 min each, to achieve phase separation to form a distinct aqueous polar layer and a non-polar layer. Aliquots of 20 µL extracts from the top layer of the nonpolar phase were withdrawn and filtered through a syringe filter (0.45 µm) and were injected into a High-Performance Liquid Chromatography (HPLC) system (LC-2030C 3D, Shimadzu Corporation, Kyoto, Japan) with a photodiode array detector. *Beta*-carotene content of samples was calculated from peak area generated from β-carotene standard calibration curve.

2.5.2. Determination of Vitamin C Content

Vitamin C content was determined using a method described by Odriozola-Serrano et al. [30] with the modifications detailed by Moyo et al. [29]. Extraction was done by adding 10 mL of 4.5% metaphosphoric acid into 0.2 g of sample in reaction tubes. The tubes were vortexed, ice-cold sonicated, and centrifuged. The mixture was then filtered through a syringe filter (0.45 µm) and 20 µL of each sample injected into a HPLC. The standard curve calibrated using ascorbic acid was used to quantify vitamin C content in the samples.

2.5.3. Determination of Mineral Element Content

The mineral elements were quantified using an inductively coupled plasma, optical emission spectrometry (ICP-OES) (ICPE-9820, Shimadzu Corporation, Kyoto, Japan) as described by Ang and Lee [31]. Approximately 0.5 g of finely ground dried samples was wet digested using a mixture of nitric acid (65%) and hydrochloric acid (37%) (1:3 v/v). Digestion was conducted on a 95 °C hot plate. Each sample was digested in triplicates. Mineral elements in the digested plant materials were determined using the ICP-OES.

2.6. Data Analysis

Data were subjected to a two-way analysis of variance (ANOVA) using Genstat 64-bit Release 18.2 (PC/Windows 8, Hertfordshire, UK). For statistical significance ($p \leq 0.05$), mean values were separated using Fischer's least significant difference (LSD) test.

3. Results

3.1. Growth and Yield of *Abelmoschus esculentus*

Application of biostimulants had a significant effect on the growth (plant height, chlorophyll content and stem diameter) and yield parameters (number of pods, total pod fresh weight, and total pod dry weight) (Table 1). In general, biostimulant effect was dose-dependent (Table 2). Plant height, chlorophyll content, stem diameter, number of pods, as well as total pod fresh and dry weights increased with a decrease in dilution (or an increase in concentration) of the biostimulants (Table 2). Plants treated with PGPR (1:5) generally had the highest plant height, chlorophyll content, and stem diameter (Table 2). This same treatment gave significantly high yield parameters (number of pods, and total pod fresh and dry weights) when compared to the Kelpak® treatments and the control (without any biostimulant application).

Table 1. Analysis of variance for the effect of biostimulant and genotype on *Abelmoschus esculentus* growth and yield. df = Degree of freedom.

Source of Variation	df	Mean Square						
		Plant Height	Number of Leaves	Chlorophyll Content	Stem Diameter	No. of Pods	Total Pod Fresh Weight	Total Pod Dry Weight
Genotype (G)	4	102,823 *	39 *	6 n.s	7 n.s	33 n.s	930 n.s	18 n.s
Biostimulant (B)	6	225,161 ***	19 n.s	143 ***	35 ***	94 ***	8696 ***	256 ***
G × B	24	28,253 n.s	13 n.s	21 n.s	2 n.s	24 n.s	711 n.s	16 n.s
Residual	140	34,271	15	18	3	16	740	13
Total	174							

* = $p \leq 0.05$, *** = $p \leq 0.001$, n.s = not significant.

Table 2. Effect of biostimulant treatment on *Abelmoschus esculentus* growth and yield. In each column, values followed by different letters indicate statistically significant ($p \leq 0.05$) differences. KLP = Kelpak® dilutions, PGPR = plant growth-promoting rhizobacteria.

Treatment	Plant Height (mm)	Chlorophyll Content (SPAD)	Stem Diameter (mm)	Number of Pods	Total Pod Fresh Weight (g)	Total Pod Dry Weight (g)
Control	460.0 ^{d}	35.4 ^{cd}	7.5 ^{b}	5.8 ^{c}	32.4 ^{e}	3.9 ^{e}
KLP 1:20	594.4 ^{bc}	35.3 ^{cd}	8.4 ^{b}	8.3 ^{b}	55.7 ^{cd}	6.9 ^{cd}
KLP 1:40	632.8 ^{abc}	34.4 ^{cd}	8.3 ^{b}	7.9 ^{bc}	47.6 ^{d}	5.5 ^{de}
KLP 1:100	530.8 ^{cd}	33.4 ^{d}	7.8 ^{b}	5.7 ^{c}	46.7 ^{de}	5.1 ^{de}
PGPR 1:5	727.2 ^{a}	40.5 ^{a}	10.5 ^{a}	9.7 ^{ab}	88.5 ^{a}	13.1 ^{a}
PGPR 1:10	698.4 ^{a}	38.2 ^{ab}	10.1 ^{a}	10.7 ^{a}	72.9 ^{b}	9.8 ^{b}
PGPR 1:15	666.4 ^{ab}	35.8 ^{bc}	9.5 ^{a}	9.7 ^{ab}	64.2 ^{bc}	8.8 ^{bc}
LSD ($p \leq 0.05$)	103.5	2.4	1.0	2.3	15.2	2.1

Relative to the control, Kelpak® (1:20) treatment significantly improved plant height, number of pods, as well as the fresh and dry weights of the pods (Table 2). However, no significant effect was recorded in terms of the chlorophyll content and stem diameter between the control and Kelpak® treatments.

Genotype had significant effect on the plant height and number of leaves (Table 1). The highest height and number of leaves were recorded in genotypes VI033796 and VI046567, respectively (Table 3). Genotype did not significantly affect the chlorophyll content, stem diameter, number of pods, as well as total pod fresh and dry weights among the five genotypes (Table 1). There was also no significant interaction effect between the biostimulants and genotypes on all the growth and yield parameters (Table 1).

Table 3. Effect of genotype on *Abelmoschus esculentus* plant height and number of leaves. In each column, values followed by different letters indicate statistically significant ($p \leq 0.05$) differences.

Genotype	Plant Height (mm)	Number of Leaves
VI037996	599.4 ^{abc}	9.1 ^{a}
VI046567	657.7 ^{ab}	9.2 ^{a}
VI055421	592.3 ^{bc}	7.1 ^{b}
VI050956	546.9 ^{c}	8.6 ^{ab}
VI033796	682.3 ^{a}	7.1 ^{b}
LSD ($p \leq 0.05$)	87.5	1.8

3.2. Nutritional and Phytochemical Contents of Abelmoschus Esculentus

Application of biostimulants and genotypes significantly influenced the nutritional profiles and phytochemical content individually and interactively (Table 4). The effect of biostimulant application on β-carotene content was genotype-dependent (Table 5). Biostimulant application resulted in an increased β-carotene content in genotypes VI050956 and VI033796, whereas the opposite is generally the case with genotypes VI037996, VI046567, and VI055421 (Table 5). Similarly, biostimulant application increased the vitamin C content in genotype VI046567 but reduced vitamin C content in genotypes VI050956 and VI033796 (Table 5). Application of biostimulant low dilutions increased vitamin C content in genotype VI037996 whereas the highest biostimulant dilutions increased vitamin C content in genotype VI055421 (Table 5). The effect of biostimulant application on mineral element content was dependent on the biostimulant type and dilution as well as genotype. In genotype VI050956, application of Kelpak® 1:100 significantly improved all the mineral element content, when compared to the control. Similarly, the application of Kelpak® (1:20 and 1:40 dilutions) and PGPR (1:5) significantly increased all the mineral element content in comparison to the control in genotype VI033796. Biostimulant application did not significantly improve mineral element content in genotypes VI055421 and VI037996 (with the exception of PGPR 1:5 that increased zinc content). Similarly in genotype VI046567, biostimulant application did not significantly improve mineral element content except with PGPR 1:10 treatment, which significantly increased iron, potassium, magnesium, sodium, and zinc contents, in comparison to the control (Table 5).

Genotypes and biostimulants had a significant effect on the TP, flavonoids, and CT contents (Table 4). The highest concentrations of these aforementioned phytochemicals were induced by interactive effect of genotype and biostimulant (Table 5). Genotype VI050956 treated with PGPR (1:5) had the highest concentration of TP, while Kelpak® at 1:40 and 1:20 dilutions corresponded to the highest concentration of flavonoids (VI046567) and CT (VI037996), respectively. Stimulatory effects on the TP were evident in genotype VI055421 for the majority of biostimulant (PGPR and Kelpak®) treatments. On the other hand, significant inhibitory effect was observed in a few biostimulant treatments. Relative to the control, this was evident in lower concentrations of TP quantified in PGPR (1:5; 1:10; 1:15) and Kelpak® at 1:20 for genotype VI046567. Likewise, reduced flavonoids in genotype VI055421 and CT in genotype VI045667 treated with PGPR (1:5 and 1:15) was recorded when compared to the control.

Table 4. Analysis of variance for the effect of biostimulant and genotype on *Abelmoschus esculentus* nutritional and phytochemical contents. df = Degree of freedom, BCB = β-carotene, Vit C = Vitamin C, TP = Total phenolics, Flav = Flavonoids, CT = Condensed tannins, Ca = Calcium, Fe = Iron, K = Potassium, Mg = Magnesium, Na = Sodium, Zn = Zinc.

Source of Variation	df	Mean Square										
		BCB	Vit C	TP	Flav	CT	Ca	Fe	K	Mg	Na	Zn
Genotype (G)	4	3.07 ***	17.79 ***	17.84 ***	9.18 ***	3.42 ***	57,997,755 ***	27,175 ***	7.8×10^9 ***	79,595,816 ***	15,911,508 ***	9086 ***
Treatment (T)	6	1.31 ***	37.38 ***	7.34 ***	5.49 ***	0.43 ***	57,00,772 ***	3781 ***	4.9×10^8 ***	2,373,265 ***	401,018 ***	372 ***
G × T	24	1.27 ***	34.95 ***	12.71 ***	7.96 ***	0.31 ***	6,410,035 ***	7071 ***	1.15×10^9 ***	10,056,126 ***	1,522,153 ***	1388 ***
Residual	70	0.00	0.17	0.47	0.12	0.01	340,822	113	6.22×10^7	375,455	50,733	36
Total	104											

*** = $p \leq 0.001$.

Table 5. Interaction effect of genotypes and biostimulants on *Abelmoschus esculentus* nutritional and phytochemical contents. In each column, values followed by different letters indicate statistically significant ($p \leq 0.05$) differences. KLP = Kelpak® dilutions, PGPR = plant growth promoting rhizobacteria, BCB = β-carotene (mg/100 g sample), Vit C = Vitamin C (mg/100 g sample), TP = Total phenolics (mg GAE/g), Flav = Flavonoids (mg CE/g), CT = Condensed tannins (mg CCE/g), Ca = Calcium (mg/100 g), Fe = Iron (mg/100 g), K = Potassium (mg/100 g), Mg = Magnesium (mg/100 g), Na = Sodium (mg/100 g), Zn = Zinc (mg/100 g), GAE = gallic acid equivalents, CE = catechin equivalents, CCE = cyanidin chloride equivalents.

Genotype	Treatment	BCB	Vit C	TP	Flav	CT	Ca	Fe	K	Mg	Na	Zn
VI037996	Control	4.57 ^{d}	13.80 ^{j}	10.13 $^{d–k}$	4.83 $^{h–l}$	1.74 ^{d}	6287 ^{def}	88.40 $^{d–h}$	51,933 ^{e}	6020 $^{i–l}$	2327 ^{h}	60.53 ^{jk}
	KLP 1:100	3.15 ^{t}	11.98 $^{m–p}$	9.41 $^{i–l}$	4.30 $^{l–o}$	1.41 ^{fg}	3240 ^{nop}	45.13 $^{m–p}$	27,667 ^{gh}	3144 ^{opq}	1336 ^{lm}	35.45 $^{n–q}$
	KLP 1:40	3.56 ^{lm}	8.91 ^{x}	11.97 ^{b}	5.87 ^{def}	1.84 ^{cd}	5493 ^{fgh}	68.60 ^{ijk}	54,733 ^{e}	5513 ^{klm}	2567 ^{gh}	57.20 ^{kl}
	KLP 1:20	3.01 ^{u}	17.85 ^{de}	9.48 $^{i–l}$	5.19 $^{g–h}$	2.52 ^{a}	5393 ^{fgh}	75.47 ^{hij}	51,600 ^{e}	4987 ^{m}	2443 ^{h}	51.60 ^{kl}
	PGPR 1:5	3.16 st	14.02 ^{ij}	10.70 $^{c–h}$	5.88 ^{de}	1.61 ^{e}	3933 $^{k–n}$	81.62 $^{f–i}$	42,860 ^{ef}	4917 ^{mn}	2514 ^{h}	73.93 ^{ghi}
	PGPR 1:10	3.45 ^{o}	14.15 ^{hij}	11.23 ^{bcd}	6.39 $^{c–d}$	1.89 ^{c}	3080 ^{nop}	48.67 ^{mn}	32,540 ^{fg}	3668 ^{op}	1498 $^{j–m}$	39.13 ^{mno}
	PGPR 1:15	4.07 ^{gh}	10.60 $^{s–v}$	10.01 $^{e–k}$	4.66 $^{j–n}$	2.34 ^{b}	2517 ^{opq}	40.93 ^{nop}	26,380 ^{gh}	2904 ^{pq}	1363 ^{lm}	35.40 $^{n–q}$
VI046567	Control	3.69 ^{k}	9.83 ^{w}	11.10 $^{b–e}$	4.18 ^{no}	1.23 ^{i}	7433 ^{bc}	105.20 ^{cd}	68,467 ^{d}	7147 ^{fgh}	3240 ^{def}	90.27 ^{cde}
	KLP 1:100	3.53 ^{mn}	12.25 $^{l–o}$	10.36 $^{c–j}$	4.44 $^{k–o}$	0.48 ^{r}	6107 ^{efg}	74.53 ^{hij}	72,000 ^{cd}	5767 $^{j–m}$	3350 ^{de}	67.53 ^{ij}
	KLP 1:40	3.48 ^{no}	10.38 ^{uvw}	10.20 $^{c–k}$	11.95 ^{a}	1.09 $^{j–n}$	5267 ^{ghi}	78.93 $^{f–j}$	55,133 ^{e}	6707 $^{g–j}$	2540 ^{gh}	80.67 $^{e–h}$
	KLP 1:20	4.09 ^{g}	14.50 ^{hi}	7.79 ^{n}	4.69 $^{j–n}$	0.80 ^{q}	7673 ^{bc}	86.87 $^{e–h}$	79,267 ^{bcd}	8040 ^{def}	3020 ^{ef}	89.20 ^{cde}
	PGPR 1:5	2.96 ^{uv}	10.90 $^{r–u}$	8.10 ^{mn}	4.71 $^{j–n}$	0.95 ^{op}	3720 $^{k–n}$	62.13 $^{j–m}$	34,400 ^{fg}	3933 ^{no}	1959 ^{i}	40.93 ^{mn}
	PGPR 1:10	3.53 ^{mn}	11.41 ^{pqr}	5.34 ^{o}	2.63 ^{r}	1.38 ^{fgh}	7953 ^{b}	262.67 ^{a}	100,667 ^{a}	9207 ^{abc}	3973 ^{b}	100.40 ^{ab}
	PGPR 1:15	3.23 ^{r}	20.03 ^{b}	9.28 ^{jkl}	4.02 ^{op}	1.07 $^{k–o}$	7127 ^{bcd}	276.87 ^{a}	86,400 ^{b}	8233 ^{cde}	3567 ^{cd}	91.67 ^{bcd}

Table 5. *Cont.*

Genotype	Treatment	BCB	Vit C	TP	Flav	CT	Ca	Fe	K	Mg	Na	Zn
VI055421	Control	6.15 ^{a}	14.76 ^{h}	7.88 ^{n}	5.33 $^{e–h}$	0.50 ^{r}	9107 ^{a}	120.13 ^{bc}	100,333 ^{a}	10,200 ^{a}	4460 ^{a}	103.80 ^{a}
	KLP 1:100	3.21 ^{rs}	17.39 ^{ef}	8.81 ^{lmn}	4.52 $^{k–o}$	1.00 ^{nop}	6893 ^{cde}	83.20 $^{f–i}$	82,067 ^{bc}	7853 ^{ef}	3247 ^{def}	71.67 ^{hi}
	KLP 1:40	3.56 ^{lm}	11.23 ^{qrs}	10.93 $^{b–f}$	4.85 $^{h–l}$	1.241 i	5927 ^{fg}	83.60 $^{f–i}$	71,400 ^{cd}	7100 ^{fgh}	3000 ^{ef}	76.87 $^{f–i}$
	KLP 1:20	3.40 ^{p}	11.70 $^{n–q}$	9.47 $^{i–l}$	6.75 ^{c}	1.18 $^{i–m}$	7500 ^{bc}	92.87 $^{d–g}$	82,800 ^{bc}	8173 ^{de}	3727 ^{bc}	97.73 ^{abc}
	PGPR 1:5	5.04 ^{c}	12.36 ^{lmn}	9.09 ^{klm}	3.97 ^{op}	1.05 $^{l–p}$	4660 $^{h–k}$	74.60 ^{hij}	52,133 ^{e}	6353 $^{h–k}$	3019 ^{ef}	74.40 ^{ghi}
	PGPR 1:10	5.26 ^{b}	11.06 $^{q–t}$	9.59 $^{h–l}$	5.15 $^{g–j}$	0.96 ^{nop}	5467 ^{fgh}	95.40 ^{def}	70,867 ^{cd}	7353 ^{efg}	2953 ^{f}	90.20 ^{cde}
	PGPR 1:15	4.34 ^{e}	17.09 ^{f}	7.91 ^{n}	4.02 ^{op}	1.04 $^{m–p}$	7860 ^{b}	103.60 ^{cde}	90,267 ^{ab}	9527 ^{ab}	4433 ^{a}	97.60 ^{abc}
VI050956	Control	3.35 ^{pq}	23.00 ^{a}	10.42 $^{c–i}$	4.53 $^{k–o}$	1.21 ^{ij}	3015 ^{nop}	46.00 ^{mno}	33,867 ^{fg}	3060 ^{opq}	1161 ^{m}	30.47 $^{o–r}$
	KLP 1:100	4.13 ^{fg}	12.78 ^{kl}	11.13 ^{bcd}	5.21 $^{g–j}$	1.40 ^{fgh}	4373 $^{i–l}$	75.93 $^{g–j}$	70,467 ^{cd}	6820 ^{ghi}	2387 ^{h}	83.40 $^{d–g}$
	KLP 1:40	2.85 ^{w}	11.22 ^{qrs}	8.13 ^{mn}	3.53 ^{pq}	0.93 ^{pq}	1639 ^{qr}	30.47 ^{opq}	17,327 ^{hi}	1786 ^{rs}	765 ^{n}	29.03 ^{pqr}
	KLP 1:20	3.60 ^{l}	11.64 ^{opq}	9.78 $^{g–l}$	4.75 $^{i–m}$	1.29 ^{ghi}	3123 ^{nop}	50.21 ^{lmn}	32,360 ^{fg}	3032 ^{opq}	1445 ^{lm}	35.51 $^{n–q}$
	PGPR 1:5	4.15 ^{f}	15.72 ^{g}	18.80 ^{a}	5.31 $^{f–i}$	0.98 ^{nop}	1058 ^{r}	14.78 ^{q}	13,507 ^{i}	1453 ^{s}	639 ^{n}	16.09 ^{s}
	PGPR 1:10	3.83 ^{j}	12.64 ^{klm}	9.98 $^{f–k}$	3.18 ^{qr}	0.61 ^{r}	3407 ^{mno}	37.25 ^{nop}	35,800 ^{fg}	3780 ^{op}	1527 $^{j–m}$	40.93 ^{mn}
	PGPR 1:15	3.48 ^{no}	18.92 ^{c}	9.20 ^{klm}	4.26 ^{mno}	1.30 ^{ghi}	2367 ^{pq}	43.53 ^{nop}	27,200 ^{gh}	2500 ^{qr}	1677 $^{i–l}$	22.67 ^{rs}
VI033796	Control	2.94 ^{v}	18.28 ^{cd}	10.78 $^{c–g}$	6.55 ^{c}	1.85 ^{cd}	3513 ^{lmn}	37.60 ^{nop}	44,467 ^{ef}	3773 ^{op}	1479 ^{klm}	37.00 ^{nop}
	KLP 1:100	3.31 ^{q}	13.10 ^{k}	9.17 ^{klm}	6.49 ^{c}	1.18 ^{ijk}	3545 ^{lmn}	28.27 ^{pq}	28,933 ^{gh}	2573 ^{pr}	1340 ^{lm}	26.87 ^{qr}
	KLP 1:40	3.82 ^{j}	17.98 ^{de}	10.89 $^{b–g}$	5.52 ^{efg}	1.18 $^{i–l}$	7333 ^{bc}	73.67 ^{hij}	88,467 ^{ab}	6793 ^{ghi}	3113 ^{ef}	68.93 ^{ij}
	KLP 1:20	2.98 ^{uv}	12.58 ^{klm}	11.09 $^{b–f}$	6.87 ^{c}	2.37 ^{b}	5213 $^{g–j}$	67.53 $^{i–l}$	72,133 ^{cd}	6807 ^{ghi}	2887 ^{fg}	68.33 ^{ij}
	PGPR 1:5	4.16 ^{f}	11.56 ^{pqr}	9.29 ^{jkl}	4.88 $^{h–k}$	1.50 ^{ef}	7007 $^{b–e}$	129.93 ^{b}	82,533 ^{bc}	9033 ^{bcd}	3047 ^{ef}	101.67 ^{a}
	PGPR 1:10	4.02 ^{h}	10.18 ^{vw}	11.29 ^{bc}	7.83 ^{b}	1.48 ^{ef}	4613 $^{h–k}$	53.20 $^{k–n}$	49,800 ^{e}	5300 ^{lm}	1824 ^{ijk}	84.19 ^{def}
	PGPR 1:15	3.91 ^{i}	10.50 ^{tuv}	9.10 ^{klm}	5.36 $^{e–h}$	1.27 ^{hi}	4280 $^{j–m}$	51.07 ^{lmn}	44,800 ^{ef}	4907 ^{mn}	1862 ^{ij}	48.07 ^{lm}
LSD ($p \leq 0.05$)		0.06	0.66	1.11	0.56	0.13	950.7	17.32	12,845.1	997.8	366.8	9.72

4. Discussion

4.1. Growth and Yield Response

Plant genotypes respond differently to varying environmental conditions thereby resulting in an array of metabolic functions, which ultimately affect plant growth [32]. Genotypes determine the expression of plant growth parameters in an environment which subsequently influence plant yield [33]. As indicated by Golorana et al. [34], genotypes had a significant effect on rice grain yield. Similarly, the storage organ and vine length of sweet potato cultivars were significantly influenced by genotypic differences [35]. In the current study, we established the importance of *A. esculentus* genotypes in terms of the phenotypic traits including plant height and number of leaves. In particular, genotype VI055421 and VI033796 had significantly lower number of leaves when compared to other *A. esculentus* genotype. Furthermore, genotype VI050956 had a relative shorter height. Despite these aforementioned variations, the yields were similar across the five genotypes.

Given that the application of biostimulants alters the nutritional, hormonal, and bioactive compound contents, the agronomical responses in plant genotypes often differ [33,36,37]. Biostimulants are well known for their stimulatory effect in many plants [38–41]. In the current study, the applied biostimulants, especially PGPR treatment, had remarkable stimulatory effects on the phenotypic traits and yield parameters evaluated. This is consistent with the response in *Capsicum annuum*, whereby the application of *B. amyloliquefaciens* and *B. cepacia* significantly enhanced plant height and number of leaves when compared to the control [42]. *Bacillus* spp. (strains M9 and K46) had no significant effect on plant height of *Capsicum annuum* while stem diameter and fresh weight were significantly enhanced by strain M9 relative to the control [43]. Strain M9 further significantly increased chlorophyll content when compared to strain K46 and control [43]. Root exudates further play a role in the efficacy of PGPR. Their interaction with PGPR can either impede or promote plant nutrient cycling and thus reduce the need for chemical fertilisers. Often PGPR is referred to as biofertilisers, rhizoremediators, and phytostimulators because of their role in plant growth [44]. Moreover, *Bacillus* spp. promote the production of lytic enzymes, secondary metabolites and phytohormones [45], which may facilitate the formation of lateral roots, root hairs, and primary root elongation. *Bacillus* spp. Further plays a role in enhanced nutrient absorption by plants [46,47]. Based on increasing evidence [44,46,48,49], PGPR promote plant growth through the synthesis of plant growth regulators, promoting symbiotic N_2 fixation, and solubilisation of mineral phosphate and other nutrients. The efficacy of PGPR is further dependent on environmental factors such as composition of microbial flora and soil characteristics [50].

Kelpak® treatment (1:20) significantly increased plant height and yield of *A. esculentus*. This is in agreement with the findings by Wang et al. [51] whereby the plant height of *Malus hupehensis* Rehd. seedlings was significantly enhanced by the application of brown seaweed extracts (*Lessonia nigrescens* and *Lessonia flavicans*) relative to the control. *Ecklonia maxima* extracts had no stimulatory effect on the leaf number of *Brassica rapa* L. subsp. *sylvestris* [52]. However, a significant increase in SPAD (Soil Plant Analysis Development) index and yield was observed when *Ecklonia maxima* extracts were applied to *Brassica rapa* L. subsp. *sylvestris* [52]. A brown seaweed *Sargassum vulgare*, significantly increased plant height, number of leaves, root diameter, yield, and the chlorophyll content of *Raphanus sativus*, relative to the control [53]. Kelpak® is a commercially available brown seaweed extract that is predominantly high in cytokinins, auxins, gibberellins, brassinosteroids, polyamines, phlorotannins, aliginates, amino acids, mannitol but low in abscisic acid, macro-, and microelements [54,55]. The presence of these compounds and their interaction with genotypes may contribute to the stimulatory effect of seaweed extracts on plant growth, development, and yield [19,54].

4.2. Biochemical and Mineral Element Content of Abelmoschus esculentus Genotypes

Biochemical characteristics of plants vary with genotypes and are considered to be amongst the important quality attributes in agricultural production and food security.

In the present study, genotypes of *A. esculentus* had varying biochemical composition (Table 5). Generally, this varying response has been reported for different plants [56,57]. The chemical composition (β-carotene and total phenolic content) in *Cannabis sativa* varied with genotypes [56]. For instance, *Cannabis sativa* genotype Futura had significantly higher β-carotene content while Tygra had the least quantity. In addition, Futura had the highest phenolic content followed by Finola and Felina [56]. Similar to the secondary metabolite content, mineral element concentration varied with genotypes used in the current study. Sokrab et al. [57] demonstrated that mineral element content is influenced by genotypes of *Zea mays*. Genotype Mugtama-45 had significantly high total Na content, while Hudiba-1 had the lowest quantity of Na [57]. Total K, Mg and Ca content was high in PAN-6480, TL-98 B-6225-9×TL617 and S-98 TLW-GHA, and least in S-98TLW-GHA, Banglore-9733 and Hudiba-1, respectively [57]. Furthermore, Fe and Zn was high in TL-98B-6225-9×TL617 and PAN-6480, respectively, while Mugtama-45 and S-98TLW-GHA had least Fe and Zn contents, respectively [57].

The effect of Kelpak® on the phytochemical and nutritional content varies across genotypes. In the current study, Kelpak® (1:40) significantly enhanced phenolic content of VI037996. Likewise, Kelpak® (at varying levels) affected the biochemical content of two common bean cultivars (var. Aura and Toska) [58]. In *Phaseleous vulgaris* (var. Toska), Kelpak® application (single spraying with 0.2 and 0.4% (*v*/*v*), double spraying with 0.2 and 0.4% (*v*/*v*)) had no significant effect on the total phenolic content [58]. However, single spraying of Kelpak® (0.2% *v*/*v*) significantly enhanced total phenolic content of the same cultivar. Kelpak treatments had no significant effect on both total phenolic and flavonoid content in *Phaseleous vulgaris* (var. Aura) [58]. In the current study, the mineral content of genotypes was affected by Kelpak® application at varying levels. Similar trends have been reported in other plant species [59,60]. Based on the findings by Ngoroyemoto et al. [59], the application of Kelpak® had diverse effects on the mineral composition of *Amaranthus hybridus*, while Kelpak® had no significant effect on Na, Zn, and Mg. However, it significantly reduced the accumulation of Ca, Mg, and Fe in *Amaranthus hybridus* [59]. Seaweed extracts are common in agriculture for their ability to influence absorption, translocation and retention of mineral nutrients [61]. Compounds (e.g., eckol and phloroglucinol) found in *Ecklonia maxima* enhanced the activity of enzymes (such as α-amylase and MDH) and increased secondary metabolite contents [62]. This enhanced metabolism improves the production and activity of enzymes involved in various biological processes including glycolysis and nitrogen assimilation, thereby increasing the production of secondary metabolites [53,63].

Despite the numerous studies on the effects of PGPR application on improving growth and yield in crop plants, there are only few reports on its effect on biochemical and nutritional parameters. In the current study, PGPR (1:5, 1:10, and 1:15) significantly enhanced the β-carotene and TPC of VI050956. Likewise, inoculation of *Cannabis sativa* 'Finola' with PGPR significantly enhanced the TPC when compared to the control [64]. Interestingly, the TPC of PGPR-inoculated plants was similar to that of nitrogen fertiliser-treated plants [64]. The application of PGPR to *A. esculentus* genotypes had varying effect on the mineral element composition. Orhan et al. [65] studied the effect of two *Bacillus* strains OSU-142 and M3 on *Rubus ideaeus* nutrient content and the findings indicated that Ca content in the plants inoculated with both strains (OSU-142 and M3) was significantly higher than other nutrients. Furthermore, *Bacillus* stain M3 significantly enhanced the Ca, Fe and Mg content relative to strain OSU-142 and control [65]. The interaction of plant and bacteria organic acids in the rhizosphere has the potential to maintain the soil pH and thus improve the availability of mineral elements [65]. PGPR have the ability to produce volatile organic compounds including antioxidants which can promote plant absorption and endophytic metabolic pathways leading to the production of volatile organic compounds [66]. Some plant genotypes support the stimulatory effect of PGPR through production of root exudates that act as substrates to the inoculants [38].

5. Conclusions

The current study demonstrated the remarkable effect of biostimulant types and concentration, as well as genotype on the plant growth, yield, biochemical, and mineral elements content of *A. esculentus*. Kelpak® and PGPR treatments had varying effects on the growth, yield, biochemical and mineral element content. In most cases, biostimulant treatments that enhanced the growth of *A. esculentus* genotypes also enhanced the yield of these genotypes. Compared to Kelpak® treatments, PGPR treatments enhanced one or more growth and/or yield parameters of almost all genotypes used in this study. Even though Kelpak® enhanced growth and yield at a lesser extent when compared to PGPR, it did not inhibit either the growth or yield parameters relative to control. Overall, PGPR application had positive impact on the growth and yield of *A. esculentus*. However, the efficacy of these biostimulants varied with genotype and concentration. This study further demonstrates that biostimulants may have neutral effect on growth and yield of plants, therefore, more studies need to be conducted that will focus on optimising the promontory effect of biostimulants on plant growth and yield. The varying effects observed in the current study suggest the need for further research to optimise the use of biostimulants for accumulation of important secondary metabolites in plants.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy12020428/s1, Table S1: Chemical and physical properties of potting soil used in the current study.

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