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Plant Growth, Yield, and Quality of Containerized Heirloom Chile Pepper Cultivars Affected by Three Types of Biostimulants

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Abstract: Peppers (*Capsicum annuum*) are one of the most widely cultivated vegetable crops, with per capita consumption of bell and chile peppers being 11.4 and 7.7 pounds in 2017. Biostimulants are an emerging sustainable alternative to enhance plant health by increasing photosynthetic activity, stress tolerance, and nutrient uptake through various modes of action. The effects of different biostimulant applications largely remain unknown in containerized heirloom pepper production. This study evaluated plant growth, yield, and fruit quality of nine heirloom chile pepper cultivars, including 'Anaheim Chili', 'Ancho or Poblano', 'Big Jim', 'Cayenne Purple', 'Chile de Arbol', 'Jamaica Hot Red', 'Mulato Isleno', 'Padron', and 'Pasilla Bajio' in a container production system throughout the years of 2020 and 2021. Each cultivar was treated with three types of biostimulants, including Tribus® Original (a mixture of *Bacillus* bacterias), Vitazyme (containing plant growth regulators and B vitamins), C-Bio CPS (seaweed extract of *Ascophyllum Nodosum*), and water as control. Pepper cultivars varied in yield and quality, including fruit length, diameter, single fruit weight, and fruit color in both years. 'Anaheim Chili', 'Big Jim', and 'Jamaica Hot Red' produced highest marketable yields similarly, with 'Chile de Arbol' and 'Pasilla de Bajio' producing the lowest marketable yields in both years. Biostimulant application did not affect marketable yield either in 2020 or 2021 but enhanced fruit quality, including fruit length, diameter, and green coloration.

Keywords: *Capsicum annuum*; chili; container production; yield; fruit quality

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1. Introduction

Peppers (*Capsicum* spp.) are one of the most widely cultivated vegetable crops in the world [1]. The total market value of peppers, including chile and bell peppers, in the United States, was approximately 785 million US dollars (USD) in 2017. In the same year, 4.7 million pounds of chile peppers were produced from 18,900 acres in the US, with a per capita consumption of 7.7 pounds [2]. Peppers were produced on 265 acres of land in Mississippi [3]. Nationwide, most chili peppers are grown to be processed, with a small portion harvested for fresh consumption [4]. In Mississippi, most chile peppers were harvested for fresh consumption and marketed locally through farmer's markets, on-farm stands, local restaurants, and community-supported agriculture (CSA) [5,6].

C. annuum has been bred to be desirable for high-input commercial production, resulting in a number of F1 hybrids with increased plant vigor, uniformity, disease resistance, yield, and fruit size but reduced genetic diversity [7,8]. There has been a renewed interest in heirloom peppers that have been used for 50 to 100 years, open-pollinated, and produced 'true-to-type' seeds [9,10]. Heirloom cultivars are valued for their superior flavor, eating quality, and higher nutritional values. There is often a market premium for high-quality heirloom produce through local market outlets or high-end grocery stores. Selection of suitable heirloom cultivars for the local climate in a certain production system requires research investigation.

Biostimulants are complex products of biological origin that affect plant productivity through various modes of action, including mitigating abiotic stress, modifying soil microbiome, activating metabolism, and/or increasing nutrient absorption [11,12]. They have been considered an innovative method to enhance the plant health and productivity of horticultural and agronomic crops, with a rapidly expanding global market size projected to reach 6.79 billion USD by 2030 [13]. The increasing demand for biostimulants has been driven by growers' demand for high-quality products with sustainable and eco-friendly alternatives.

General categories of biostimulants include seaweed extracts, complex organic materials, inorganic salt, beneficial microorganisms, etc. [11,14,15]. Seaweed extracts are the most commonly used biostimulants, comprised of macroscopic and multicellular marine algae of various colors, often applied through foliar sprays, shown to enhance plant growth, abiotic stress tolerance, photosynthetic activity, resistance to pathogens, and increase yield and quality of horticultural crops [12,16–21]. Biostimulants made from beneficial microorganisms, including bacteria, yeast, and fungi, were reported to modify the hormonal status, increase productivity, affect plant response to abiotic stresses, and increase plant nutrient uptake from the soil by nitrogen fixation or solubilizing nutrients [12,22–25].

Biostimulants are promising sustainable products that enhance plant performance and reduce inputs in pepper production. The effects of different types of biostimulants on plant growth, yield, and quality of heirloom chile pepper cultivars in a container production system remain unclear. The objectives of this study were to: (1) evaluate vegetative plant growth as well as fruit yield and quality of nine heirloom chile pepper cultivars grown in containers; (2) compare the effect of three biostimulant treatments on growth and fruit production of selected pepper cultivars.

2. Materials and Methods

2.1. Plant Cultivation and Experiment Setup

The experiment was conducted outdoors in full sun at the R.R. Foil Research Center of Mississippi State University (lat. 33.45° N, long. 88.79° W; USDA hardiness zone 8A) throughout two growing seasons in 2020 and 2021. Nine heirloom chile pepper cultivars, including 'Anaheim Chili', 'Ancho or Poblano', 'Big Jim', 'Cayenne Purple', 'Chile de Arbol', 'Jamaica Hot Red', 'Mulato Isleno', 'Padron', and 'Pasilla Bajio' were evaluated for plant growth, pepper yield, and quality in a container production system in response to three types of biostimulants. Each selected cultivar produces a unique pod type, as described by Boseland and Votava [1].

Seeds of the tested cultivars were purchased from Eden Brothers (Arden, NC, USA). Pepper transplants were prepared in a greenhouse at Mississippi State University during the first week of March 2020 and 2021 and transplanted into 3-gallon containers (O30, Nursery Supplies, Inc., Chambersburg, PA, USA) on 13 May 2020 and 17 May 2021, 40 to 45 days old, respectively. Pepper seedlings were hardened off one week prior to transplanting. A soilless substrate containing approximately 50–60% composted pine bark, 35-40% sphagnum peat moss, and 15–20% perlite (Metro-Mix 852, SunGro Horticulture, Agawam, MA, USA) was used in this study. Each plant was top-dressed with 65 g of slow-release fertilizer (Osmocote® plus 15N-3.9P-10K, 5–6 months; ICL Specialty Fertilizers, Summerville, SC, USA) at the time of transplanting. All plants were drip irrigated daily as needed. During establishment, pepper plants were fertigated with 100 ppm of 20N-8.7P-16.6K water-soluble fertilizer (Peters® Profession 20-20-20 General Purpose; ICL Specialty Fertilizers) through an injector (D14MZ2; Dosatron Intl. Inc., Clearwater, FL, USA). Plants were also fertigated with another 5K-5.2P-21.5K water-soluble fertilizer (5-12-26, JR Peters Inc., Allentown, PA, USA) at a rate of 50 ppm N from flower production to fruit harvest. Pesticide zeta-cypermethrin was applied once, according to the manufacturer's label, in late June in both years to control three-cornered alfalfa hopper (*Spissistilus festinus*). Outdoor air temperature within the experiment duration in both years was recorded at one-hour intervals using a temperature, and relative humidity sensor (HOBO S-THB-M002; Onset Computer Corp., Bourne, MA, USA) connected to a data logger (HOBO Micro Station H21-002; Onset Computer Corp.) and presented in Figure S1.

2.2. Plant Vegetative Growth

Each plant was measured for plant height, width, and leaf relative chlorophyll content measured as soil plant analysis development (SPAD) reading once at 36 days after transplanting (DAT) in 2020 and twice at 38 DAT and 56 DAT in 2021. Plant height was measured from the substrate surface to the growing tip of each plant. Plant widths were measured in two perpendicular directions. The plant growth index (PGI) was estimated as the average plant height and two widths. Relative leaf chlorophyll content SPAD was measured from three fully expanded leaves using a chlorophyll meter (SPAD 502 Plus; Konica Minolta, Inc., Osaka, Japan). The three readings from the three individual leaves were averaged to represent the leaf SPAD for each plant.

2.3. Biostimulant Treatments

Each pepper cultivar was treated with one of three biostimulants, including Tribus (Tribus® Original, Impello Biosciences, Fort Collins, CO, USA), Vitazyme (Vital Grow Distribution LLC, Waterville, WA, USA), and C-BIO CPS (C&B Agri Enterprises Ltd., Donegal, Ireland). Tribus is a microbial inoculant containing a mixture of *Bacillus subtilis* (4.0×10^9 $CFU{\cdot}ml^{-1}$), *Bacillus pumilus* (4.0×10^9 $CFU{\cdot}ml^{-1}$), and *Bacillus amyloliquefaciens* (2.0×10^9 $CFU{\cdot}ml^{-1}$). Vitazyme is derived from fermented plant materials with active ingredients, including 1-triacontanol, brassinosteroids, and B-vitamins. C-BIO CPS is a seaweed extract (*Ascophyllum Nodosum*). Each of the biostimulant treatments was either sprayed or manually fertigated in compliance with the manufacturer's instructions. At each application, Tribus and Vitazyme of 240 mL were manually applied to each plant at rates of 0.53 $mL{\cdot}L^{-1}$ and 4 $mL{\cdot}L^{-1}$, respectively. C-BIO CPS was applied as a foliar spray at the rate of 7.8 $mL{\cdot}L^{-1}$ to the point of runoff using a 2-gallon plastic handheld sprayer. The water of 240 mL was applied to plants as a control. The biostimulant treatments were applied three times at 22 DAT, 37 DAT, and 57 DAT in 2020 and at 18 DAT, 33 DAT, and 49 DAT in 2021.

2.4. Pepper Harvest

Peppers from both years were harvested three times during early, mid-, and late seasons in mid-July, August, and September, respectively. The three harvests were 63 DAT, 84 DAT, and 119 DAT in 2020 and 64 DAT, 86 DAT, and 113 DAT in 2021. At each harvest, all pepper fruits reaching the mature green stage or above and deemed as marketable size were harvested as described by Boyhan et al. [26]. Unmarketable fruits with any disease, insect damage, blossom end rot, or sunscald were removed. Marketable fruit yield was measured for each plant at each harvest. Single fruit weight was estimated by dividing marketable fruit yield by the number of fruits.

2.5. Pepper Quality Evaluations

Three marketable peppers from each plant were measured for fruit length, diameter, and color at each harvest. Fruit length was measured from the tip to the calyx. Fruit diameter was measured at the widest point from two perpendicular directions. The two readings were then averaged to represent the diameter of a given fruit. Fruit color was measured using a chroma meter (CR-400, Konica Minolta Sensing Americas Inc., Ramsey, NJ, USA) with one reading per fruit. Each fruit was measured for absolute colors using the L*, a*, b* coordinates, where L* indicates lightness, a* is the red/green coordinate, and b* is the yellow/blue coordinate [27].

2.6. Experimental Design and Data Analyses

This experiment was conducted in a randomized complete block design with a factorial arrangement of treatments and five replications. This experiment had two experimental factors: pepper cultivar (9) and biostimulant treatment (4), resulting in 36 treatment combinations. Within each replication, each treatment combination had two single plant subsamples. Data from this study were analyzed by the two-way analysis of variance (ANOVA) using the PROC GLIMMIX procedure in SAS (version 9.4; SAS Institute, Cary, NC, USA). Means were compared by Tukey's Honest Significance Test (HSD) at $\alpha < 0.05$ to detect the effect of the treatments on the response variables under scrutiny.

3. Results

3.1. Plant Growth Index and Leaf SPAD

Plant growth index (PGI) varied among pepper cultivars for the three measurements taken throughout two years in this study (Table 1). Biostimulants did not affect PGI in any measurement. In 2020, the cultivar 'Jamaica Hot Red' had the greatest PGI of 57.9 among cultivars. Plant growth index among cultivars ranged from 45.5 for 'Pasilla Bajio' to 50.1 for 'Anaheim Chili', with 'Ancho or Poblano', 'Big Jim', 'Chile de Arbol', 'Mulato Isleno', and 'Padron' having similar PGIs.

Table 1. Plant size and leaf SPAD of nine heirloom chile pepper cultivars were measured once in 2020 and twice in 2021.

	2020		2021			
	36 DAT [1]		38 DAT		56 DAT	
Cultivar	**PGI**	**SPAD**	**PGI**	**SPAD**	**PGI**	**SPAD**
Anaheim Chili	50.1 b	61.7 b	49.4 bc	56.9 c	62.7 bc	58.6 b
Ancho or Poblano	49.3 b–d	62.0 b	49.9 b	60.6 b	65.9 b	58.2 b
Big Jim	48.0 b–e	57.8 c	46.4 c	55.1 cd	56.7 de	58.2 b
Cayenne Purple	46.4 de	54.2 de	41.8 d	51.9 de	60.5 cd	56.0 bc
Chile de Arbol	47.0 c–e	56.1 cd	46.8 bc	52.1 de	54.6 e	53.3 cd
Jamaica Hot Red	57.9 a	51.5 e	59.6 a	48.7 e	77.4 a	46.8 e
Mulato Isleno	49.6 bc	56.6 cd	49.7 b	52.3 d	62.6 bc	53.5 cd
Padron	47.2 b–e	59.4 bc	47.9 bc	51.8 de	64.7 b	52.9 d
Pasilla Bajio	45.5 e	73.9 a	41.6 d	65.3 a	64.0 bc	67.8 a
p-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

[1] Different lower-case letters suggest significant differences among means within a column indicated by Tukey's HSD test at $p \leq 0.05$.

In 2021, 'Jamaica Hot Red' also produced the highest PGI of 59.6 at 38 DAT and 77.4 at 56 DAT among all cultivars. The cultivars 'Cayenne Purple' and 'Pasilla Bajio' produced the lowest PGI of 41.8 and 41.6 at 38 DAT, respectively. The other six cultivars generally produced similar PGIs at 38 DAT. At 56 DAT, 'Big Jim' and 'Chile de Arbol' produced the lowest PGI of 56.7 and 54.6, respectively. The other six cultivars generally produced a similar PGI of 60.5 to 65.9.

Leaf SPAD readings varied among pepper cultivars but were not affected by biostimulant treatment at any measurement in 2020 or 2021 (Table 1). 'Pasilla Bajio' produced the highest leaf SPAD of 73.9, 65.3, and 67.8, and 'Jamaica Hot Red' produced the lowest leaf SPAD of 51.5, 48.7, and 46.8 among cultivars at 36 DAT in 2020, 38 DAT, and 56 DAT in 2021, respectively. The cultivars 'Cayenne Purple', 'Chile de Arbol', 'Mulato Isleno', and 'Padron' generally produced similar leaf SPAD in both growing seasons.

3.2. Marketable Pepper Yield

Marketable pepper yield in July, August, and September, and total marketable yield in 2020 and 2021 varied among cultivars and were not affected by biostimulants (Tables 2 and 3).

Table 2. Marketable yield of nine heirloom chile pepper cultivars grown in a container production system in Starkville, Mississippi, during the 2020 growing season.

	Marketable Yield in 2020 (g per Plant)			
Cultivar	**July** [1]	**August**	**September**	**Total Yield**
Anaheim Chili	600.2 a	518.8 bc	138.1 d	1218 a
Ancho or Poblano	238.0 cd	409.1 c–e	193.9 cd	797.3 bc
Big Jim	452.2 b	702.5 a	317.5 b	1437 a
Cayenne Purple	130.9 d–f	374.0 c–e	265.5 bc	753.4 bc
Chile de Arbol	78.8 f	267.7 e	260.6 bc	593.8 c
Jamaica Hot Red	106.9 ef	637.8 ab	453.5 a	1181 a
Mulato Isleno	393.3 b	335.1 de	154.0 d	878.5 b
Padron	270.8 c	443.5 cd	225.9 b–d	909.1 b
Pasilla Bajio	201.0 c–e	386.1 c–e	151.0 d	711.6 bc
p-value	<0.0001	<0.0001	<0.0001	<0.0001

[1] Different lower-case letters suggest significant differences among means within a column indicated by Tukey's HSD test at $p \leq 0.05$.

Table 3. Marketable yield of nine heirloom chile pepper cultivars grown in a container production system in Starkville, Mississippi, during the 2021 growing season.

	Marketable Yield in 2021 (g per Plant)			
Cultivar	**July** [1]	**August**	**September**	**Total Yield**
Anaheim Chili	615.8 a	762.7 a	494.5 c	1840 a
Ancho or Poblano	381.9 cd	567.6 cd	482.2 c	1379 cd
Big Jim	524.8 ab	812.6 a	630.3 b	1878 a
Cayenne Purple	308.5 de	469.9 d	420.3 c	1165 de
Chile de Arbol	385.0 cd	323.8 e	246.0 d	929.4 ef
Jamaica Hot Red	231.8 ef	733.3 ab	867.3 a	1765 a
Mulato Isleno	615.6 a	613.0 bc	481.5 c	1667 ab
Padron	457.5 bc	573.9 cd	486.1 c	1491 bc
Pasilla Bajio	132.1 f	476.5 cd	285.0 d	890.2 f
p-value	<0.0001	<0.0001	<0.0001	<0.0001

[1] Different lower-case letters suggest significant differences among means within a column indicated by Tukey's HSD test at $p \leq 0.05$.

In 2020, 'Anaheim Chili' produced the greatest marketable yield of 600.2 g per plant among cultivars, with 'Big Jim' and 'Mulato Isleno' producing the next highest early season yields of 452.2 g and 393.3 g per plant in July. 'Cayenne Purple', 'Chile de Arbol', and 'Jamaica Hot Red' produced the lowest early season yield of 78.8 g to 130.9 g per plant. The cultivars 'Big Jim' and 'Jamaica Red Hot' had the highest marketable yields of 702.5 g and 637.8 g per plant during mid-season in August. Cultivars including 'Ancho or Poblano', 'Cayenne Purple', 'Chile de Arbol', 'Mulato Isleno', 'and 'Pasilla Bajio' had similarly low mid-season yields of 267.7 g to 409.1 g per plant. During late season in September, 'Jamaica Red Hot' produced the highest marketable yield of 453.5 g among cultivars, with 'Anaheim Chili', 'Mulato Isleno', and 'Pasilla Bajio' producing the lowest marketable yields of 138.1 g to 154.0 g per plant. For total yield in 2020, 'Anaheim Chili', 'Big Jim', and 'Jamaica Hot Red' produced similar highest total marketable yields of 1181 g to 1437 g per plant, with the other six cultivars producing generally similar total marketable yields of 593.8 g to 909.1 g per plant.

In 2021, early season yield in July ranged from 132.1 g to 615.8 g per plant, with 'Anaheim Chili', 'Big Jim', and 'Mulato Isleno' producing the greatest marketable yields, and 'Jamaica Hot Red' and 'Pasilla Bajio' producing the lowest yields among cultivars (Table 3). The cultivars 'Anaheim Chili', 'Big Jim', and 'Jamaica Hot Red' produced highest marketable yields of 762.7 g, 812.6 g, and 733.3 g per plant similarly during the middle season in August 2021, respectively. 'Chile de Arbol' had the lowest marketable yield of

323.8 g per plant among cultivars during this time. During the late season in September, 'Jamaica Red Hot' and 'Big Jim' produced the highest and second highest marketable yields of 867.3 g and 630.3 g per plant, higher than any other cultivar. The cultivars 'Chile de Arbol' and 'Pasilla Bajio' produced the lowest marketable yields of 246 g and 285 g per plant during the late season, respectively. The cultivars 'Anaheim Chili', 'Ancho or Poblano', 'Cayenne Purple', 'Mulato Isleno', and 'Padron' produced similar intermediate marketable yields ranging from 420.3 g to 494.4 g per plant. Total marketable yield in 2021 ranged from 890.2 g to 1878 g per plant, with 'Anaheim Chili', 'Big Jim', 'Jamaica Hot Red', and 'Mulato Isleno' producing the highest and 'Chile de Arbol' and 'Pasilla Bajio' producing the lowest total marketable yields.

3.3. Single Fruit Weight

Single fruit weight measured in July, August, and September 2020 and 2021 varied among cultivars and was not affected by biostimulant treatments (Table 4).

Table 4. Single fruit weight of nine heirloom chile pepper cultivars grown in a container production system in Starkville, Mississippi, during two growing seasons.

	Single Fruit Weight (g)					
	2020 [1]			2021		
Cultivar	**July**	**August**	**September**	**July**	**August**	**September**
Anaheim Chili	44.5 a	27.7 b	18.2 b	26.7 b	25.3 c	22.0 b
Ancho or Poblano	31.2 ab	27.6 b	21.7 a	26.5 b	29.9 b	20.9 b
Big Jim	41.7 a	35.8 a	24.5 a	34.1 a	34.7 a	27.9 a
Cayenne Purple	4.0 d	3.4 e	2.4 e	4.3 f	4.0 f	3.6 e
Chile de Arbol	2.5 d	2.0 e	1.3 e	2.4 f	2.5 f	1.9 e
Jamaica Hot Red	15.8 cd	13.1 c	12.5 c	14.8 d	15.4 d	13.0 c
Mulato Isleno	10.0 cd	8.2 d	5.9 d	8.6 e	8.3 e	7.0 d
Padron	15.9 cd	14.7 c	10.2 c	18.0 c	15.5 d	12.6 c
Pasilla Bajio	18.2 bc	13.7 c	10.2 c	13.4 d	15.8 d	11.1 c
p-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

[1] Different lower-case letters suggest significant differences among means within a column indicated by Tukey's HSD test at $p \leq 0.05$.

In July 2020, 'Anaheim Chili', 'Ancho or Poblano', and 'Big Jim' produced fruits of similar largest sizes from 31.2 g to 44.5 g higher than any other cultivar except for 'Pasilla Bajio.' 'Cayenne Purple', 'Chile de Arbol', 'Jamaica Hot Red', 'Mulato Isleno', and 'Padron' produced fruits of similar sizes, from 2.5 g to 15.9 g per fruit. The trend of single fruit weight among cultivars was similar in August, and September 2020 and in July, August, and September 2021, where 'Big Jim' produced the greatest single fruit weight among the nine tested cultivars except for being similar to 'Ancho or Poblano' in September 2020. Ranking of single fruit weight among cultivars in August 2020, and all three harvests in 2021 followed: 'Big Jim' > 'Anaheim Chili' or 'Ancho or Poblano' > 'Jamaica Hot Red', 'Padron', or 'Pasilla Bajio' > 'Mulato Isleno' > 'Cayenne Purple' or 'Chile de Arbol' (Table 4).

3.4. Fruit Length

Fruit length generally varied among cultivars in both years (Table 5). The ranking of fruit length among cultivars was generally similar at all six harvests in two growing seasons. The cultivars 'Big Jim', 'Anaheim Chili', and 'Pasilla Bajio' produced fruits with the greatest lengths among cultivars. 'Big Jim' produced longer fruits of 138.0 mm, 138.6 mm, and 117.6 mm in August 2020, August, and September 2021 than any other cultivar. 'Big Jim' produced longest fruit similarly to 'Anaheim Chili' in July 2020 and 2021, and similarly longest fruit to 'Pasilla Bajio' in September 2021. 'Jamaica Hot Red' produced fruits with the lowest length due to the squash resembling the shape of the fruit in all six harvests ranging from 37.3 mm to 45.3 mm. The five cultivars 'Ancho or Poblano', 'Cayenne Purple', 'Chile de Arbol', 'Mulato Isleno', and 'Padron' produced intermediate fruit lengths among cultivars.

Table 5. Fruit length of nine heirloom chile pepper cultivars grown in a container production system in Starkville, Mississippi, during two growing seasons.

	Fruit Length (mm)					
		2020 [2]			2021	
Cultivar	**July**	**August**	**September**	**July**	**August**	**September**
Anaheim Chili	147.4 a	129.5 b	89.6 b	127.9 a	119.2 b	105.9 c
Ancho or Poblano	73.1 c	68.8 c	58.7 c	65.1 c	72.6 c	60.2 ef
Big Jim	144.0 ab	138.0 a	107.1 a	133.0 a	138.6 a	117.6 a
Cayenne Purple	60.6 e	55.4 e	49.1 ef	67.2 c	63.9 cd	56.4 fg
Chile de Arbol	65.2 de	63.7 d	53.5 de	63.4 c	60.7 d	54.2 g
Jamaica Hot Red	37.3 f	42.7 f	44.1 f	37.5 d	45.3 e	44.1 h
Mulato Isleno	76.9 c	66.9 cd	61.6 c	67.9 c	63.1 cd	61.4 e
Padron	66.9 d	69.2 c	57.8 cd	67.0 c	72.7 c	66.1 d
Pasilla Bajio	142.4 b	129.6 b	102.4 a	114.5 b	125.1 b	111.9 b
			p-value			
Cultivar	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Biostimulant [1]	0.62	0.037	0.39	0.48	0.96	0.11
Cultivar × Biostimulant	<0.0001	0.078	0.0062	0.10	0.15	<0.0001

[1] Each cultivar was treated with three types of biostimulants: Tribus® Original, Vitazyme, C-Bio CPS, and water as the control. [2] Different lower-case letters suggest significant difference among means within a column indicated by Tukey's HSD test at $p \leq 0.05$.

Fruit length was also affected by the biostimulant treatment in August 2020 without interaction with the cultivar (Table 6), when the seaweed extract C-Bio CPS resulted in a higher fruit length compared with Tribus but similar to Vitazyme or control.

Table 6. Fruit length, diameter, and color of chile pepper cultivars are affected by three types of biostimulants.

Biostimulant [1]	Fruit Length (mm) [2] Aug. 2020	Fruit Diameter (mm) July 2021	Fruit Diameter (mm) Sep. 2021	Color a* Aug. 2020	Color a* Sep. 2021
C-Bio CPS	86.5 a	29.1 a	26.82 a	−6.46 ab	−9.83 b
Vitazyme	84.8 ab	29.0 a	26.40 b	−6.10 ab	−7.83 ab
Tribus	81.8 b	27.9 b	26.43 ab	−7.21 b	−7.53 ab
Control	85.6 ab	28.2 ab	26.67 ab	−4.30 a	−6.31 a
p-value	0.037	0.0006	0.041	0.038	0.015

[1] Each cultivar was treated with three types of biostimulants: Tribus® Original, Vitazyme, C-Bio CPS, and water as the control. [2] Different lower-case letters suggest significant difference among means within a column indicated by Tukey's HSD test at $p \leq 0.05$.

Fruit length was affected by the interaction between cultivar and biostimulant treatments in July and September 2020 and in September 2021. The seaweed extract C-Bio CPS increased the fruit length of 'Big Jim' compared with Vitazyme or Tribus. With the interaction in September 2021, C-Bio CPS increased the fruit length of 'Anaheim Chili' compared with Vitazyme, Tribus, or control. The three biostimulants resulted in similar fruit lengths to control in other cultivars.

3.5. Fruit Diameter

Fruit diameter in every harvest in both years varied among cultivars (Table 7). In 2020, fruit diameter ranged from 9.0 mm to 46.3 mm, 9.4 mm to 43.8 mm, and 8.5 mm to 44.2 mm in July, August, and September, respectively, with 'Jamaica Hot Red' producing the largest fruit diameter and 'Chile de Arbol' producing the smallest diameter, except that 'Ancho or

Poblano' produced similar fruit diameter to that of 'Jamaica Hot Red' in July. Ranking of fruit diameter generally followed: 'Jamaica Hot Red' > 'Ancho or Poblano' > 'Big Jim', or 'Padron' > 'Anaheim Chili' > 'Mulato Isleno' or 'Pasilla Bajio' > 'Cayenne Purple' > 'Chile de Arbol' with some variations in the July harvest.

Table 7. Fruit diameter of nine heirloom chili pepper cultivars grown in a container production system in Starkville, Mississippi, during two growing seasons.

	Fruit Diameter (mm)					
	2020 [2]			2021		
Cultivar	July	August	September	July	August	September
Anaheim Chili	33.5 c	29.8 d	25.9 d	28.6 e	29.4 e	27.8 e
Ancho or Poblano	46.1 a	42.6 b	38.4 b	45.4 b	46.6 b	41.2 b
Big Jim	35.4 b	32.1 c	29.8 c	31.6 d	32.1 d	30.2 d
Cayenne Purple	14.0 f	13.1 f	13.5 g	15.8 h	14.3 h	14.0 h
Chile de Arbol	9.0 g	9.4 g	8.5 h	9.0 i	9.2 i	9.1 i
Jamaica Hot Red	46.2 a	43.8 a	44.2 a	48.8 a	48.9 a	44.5 a
Mulato Isleno	22.7 d	20.2 e	21.5 e	21.7 f	22.4 f	22.2 f
Padron	34.8 bc	32.4 c	29.8 c	35.7 c	33.3 c	32.7 c
Pasilla Bajio	20.8 e	19.7 e	18.7 f	19.0 g	19.0 g	17.5 g
	p-value					
Cultivar	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Biostimulant [1]	0.49	0.40	0.053	0.0006	0.66	0.041
Cultivar × Treatment	0.0316	0.0008	<0.0001	0.0001	<0.0001	<0.0001

[1] Each cultivar was treated with three types of biostimulants: Tribus® Original, Vitazyme, C-Bio CPS, and water as the control. [2] Different lower-case letters suggest significant difference among means within a column indicated by Tukey's HSD test at $p \leq 0.05$.

In 2021, fruit diameter ranged from 9.0 mm to 48.8 mm, from 9.2 mm to 48.9 mm, and from 9.0 mm to 44.5 mm in July, August, and September, respectively (Table 7). The trend of fruit diameter among cultivars was similar, with 'Jamaica Hot Red' producing the largest fruit diameter and 'Chile de Arbol' producing the smallest diameter in all three harvests. The ranking in each harvest of 2021 followed: 'Jamaica Hot Red' > 'Ancho or Poblano' > 'Padron' > 'Big Jim' > 'Anaheim Chile' > 'Mulato Isleno' > 'Pasilla Bajio' > 'Cayenne Purple' > 'Chile de Arbol.'

The C-Bio CPS and Vitazyme biostimulants resulted in higher fruit diameter than Tribus but similar to the control in July 2021 (Table 6). C-Bio CPS extract also increased fruit diameter compared with Vitazyme in September 2021.

The interaction between cultivar and biostimulant treatment was significant at each harvest in both growing seasons (Table S1). Vitazyme increased the fruit diameter of 'Jamaica Hot Red' compared with C-Bio CPS in July 2020. Tribus resulted in a higher fruit diameter of 'Ancho or Poblano' as opposed to control in August 2020. Vitazyme also resulted in a larger fruit diameter of 'Big Jim' than any other treatment in September 2020. In 2021, Vitazyme resulted in a higher fruit diameter of 'Jamaica Hot Red' than the control in July. Tribus resulted in a larger fruit diameter of 'Jamaica Hot Red' than C-Bio CPS or control. The three biostimulants resulted in similar fruit diameters to control other than those described above.

3.6. Fruit Color

Color coordinates L, a*, and b* varied among cultivars in July, August, and September in both years (Tables 8 and 9).

Table 8. Fruit color measurements of nine heirloom chile pepper cultivars treated with three types of biostimulants grown in a container production system in Starkville, Mississippi, in 2020.

Cultivar	2020 July L [2]	July a*	July b*	August L	August a*	August b*	September L	September a*	September b*
Anaheim Chili	39.1 e	−15.0 d	24.6 e	41.7 d	−14.2 de	28.0 e	40.7 d	−11.7 e	24.9 e
Ancho or Poblano	30.2 f	−9.08 c	11.5 f	33.0 e	−10.5 d	14.7 f	31.9 f	0.045 cd	13.9 g
Big Jim	47.1 c	−17.0 ef	34.7 c	49.5 b	−16.2 e	36.3 bc	46.1 c	−15.1 e	34.9 c
Cayenne Purple	31.4 f	2.99 a	7.27 g	29.6 f	7.97 b	6.57 g	26.5 g	8.52 b	4.00 i
Chile de Arbol	50.7 b	−19.2 g	38.5 ab	44.5 c	13.5 a	32.2 d	34.4 e	31.8 a	19.8 f
Jamaica Hot Red	53.7 a	−15.3 de	39.8 a	55.2 a	−15.2 de	45.1 a	53.0 a	3.45 bc	45.0 a
Mulato Isleno	49.9 b	−16.3 d–f	37.3 b	48.9 b	−4.48 c	38.1 b	50.0 b	−13.3 e	38.4 b
Padron	43.3 d	−17.9 fg	32.6 d	44.1 c	−12.9 de	33.9 cd	39.9 d	−2.54 cd	27.2 d
Pasilla Bajio	25.7 g	−3.21 b	4.41 h	27.1 g	−4.00 c	5.22 g	27.5 g	−3.17 d	6.41 h
					p-value				
Cultivar	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Biostimulant [1]	0.26	0.26	0.39	0.23	0.038	0.12	0.96	0.098	0.69
Cultivar*Biostimulant	0.0053	0.017	0.020	0.22	0.020	0.11	0.005	0.047	0.0024

[1] Each cultivar was treated with three types of biostimulants: Tribus® Original, Vitazyme, C-Bio CPS, and water as the control. [2] Different lower-case letters suggest significant difference among means within a column indicated by Tukey's HSD test at $p \leq 0.05$.

Table 9. Fruit color measurements of nine heirloom chile pepper cultivars treated with three types of biostimulants grown in a container production system in Starkville, Mississippi, in 2020.

Cultivar	2021 July L [2]	July a*	July b*	August L	August a*	August b*	September L	September a*	September b*
Anaheim Chili	39.8 e	−15.3 d	23.1 e	40.4 f	−15.6 d	25.8 f	38.7 e	−9.37 b-d	23.8 d
Ancho or Poblano	29.0 f	−7.29 c	8.60 f	31.8 g	−10.0 c	12.9 g	31.9 f	−5.76 bc	14.2 e
Big Jim	47.8 c	−17.7 f	34.7 c	47.4 d	−16.8 ef	34.8 d	46.7 c	−14.2 d	34.2 c
Cayenne Purple	29.1 f	1.81 a	5.03 g	29.5 h	1.69 a	6.61 h	29.9 g	4.50 a	9.61 f
Chile de Arbol	49.7 b	−19.4 g	36.9 b	49.9 c	−19.0 g	39.2 c	49.2 b	−13.5 d	38.5 b
Jamaica Hot Red	52.9 a	−16.6 e	39.8 a	56.4 a	−15.6 de	45.3 a	56.4 a	−5.11 b	47.8 a
Mulato Isleno	49.9 b	−19.6 g	38.0 b	51.7 b	−17.5 f	41.6 b	47.7 bc	−10.2 cd	36.6 b
Padron	42.2 d	−17.0 ef	27.7 d	44.2 e	−17.9 fg	32.0 e	43.2 d	−12.9 d	32.2 c
Pasilla Bajio	26.5 g	−2.53 b	3.28 h	26.8 i	−3.32 b	4.22 i	27.0 h	−4.42 b	6.03 g
					p-value				
Cultivar	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Biostimulant [1]	0.41	0.78	0.37	0.38	0.29	0.17	0.66	0.015	0.42
Cultivar × Biostimulant	<0.0001	<0.0001	<0.0001	0.023	0.04	0.0009	<0.0001	<0.0001	0.0003

[1] Each cultivar was treated with three types of biostimulants: Tribus® Original, Vitazyme, C-Bio CPS, and water as the control. [2] Different lower-case letters suggest significant difference among means within a column indicated by Tukey's HSD test at $p \leq 0.05$.

'Jamaica Hot Red' fruits had the greatest lightness in L values and yellow coloration in b* values in July, August, and September of 2020 and 2021, higher than any other cultivar except being similar to 'Chile de Arbol' in yellow coloration in July 2020 (Tables 8 and 9). 'Pasilla Bajio' produced dark green colored fruits with the lowest lightness in L values and lowest yellow coloration in b* values among cultivars at each harvest in both years except having similar yellow coloration to 'Cayenne Purple' in August 2020 and similar lightness to 'Cayenne Purple' in September 2020. 'Cayenne Purple' produced fruits with the highest red coloration in a* values in July 2020 and in all three harvests in 2021, whereas the other eight cultivars presented negative values and prevalent green coloration in 2021. In August and September 2020, 'Chile de Arbol' produced fruits with the highest red coloration in a* values, higher than any other cultivars, with 'Cayenne Purple' producing the second highest a* values.

The trend of yellow-blue coloration in b* values among cultivars was generally similar at each harvest in 2021, following 'Jamaica Hot Red' > 'Chile de Arbol' or 'Mulato Isleno' >

'Big Jim' or 'Padron' > 'Anaheim Chili' > 'Ancho or Poblano' or 'Cayenne Purple' > 'Pasilla Bajio' (Table 9).

The biostimulant treatment affected the red-green coloration a* in August 2020 and September 2021 (Table 6). Means in August 2020 and September 2021 were all negatives, suggesting the average coloration of pepper cultivars was green rather than red. In August 2020, Tribus resulted in the lowest a* value of −7.21 compared with control −4.30, suggesting increased green coloration from Tribus. In September 2021, C-Bio CPS resulted in the lowest a* value of −9.83 and enhanced green coloration compared with the control of −6.31 a* value.

The interaction between cultivar and biostimulant treatment was also significant in affecting color coordinates L, a*, b* at each harvest in both years except L or b* in August 2020 (Tables S2 and S3). Tribus increased lightness (L value) compared with Vitazyme and increased yellow coloration (b* value) compared with C-Bio CPS in 'Cayenne Purple' in July 2020. The seaweed extract C-Bio CPS decreased a* value compared with the control and decreased the red coloration of 'Chile de Arbol' in August 2020. In 2021, Tribus and Vitazyme increased the fruit lightness of 'Cayenne Purple' compared with C-Bio CPS in July. Vitazyme and C-Bio CPS also increased fruit lightness of 'Chile de Arbol' compared with control in September 2021.

4. Discussion

The trend for total marketable yield among cultivars for both years was, in general similar, with no effect from biostimulant application. The cultivars 'Anaheim Chili', 'Big Jim', and 'Jamaica Hot Red' produced highest marketable yields of 1181 g similarly to 1437 g per plant in 2020. In 2021, 'Anaheim Chili', 'Big Jim', 'Jamaica Hot Red', and 'Mulato Isleno' produced comparable highest marketable yields of 1667 g to 1878 g per plant. The repeated measure showed higher marketable pepper yield in 2021 than in 2020. For the timing of production, 'Anaheim Chili' and 'Mulato Isleno' were the early-fruiting cultivars, with the highest July yield in both years. 'Big Jim' and 'Jamaica Hot Red' produced the highest mid-season yields in August in both years. 'Jamaica Hot Red' was the most late-season cultivar producing the highest September yield in both years. The yield range of tested cultivars in our study was generally in agreement with reported hot pepper yields [28]. Hand harvest used in our study was also considered to improve marketable yield and quality with an appropriate maturity level compared to machine harvest [1].

The cultivar 'Big Jim' produced the largest fruit harvest during 2020 and 2021 in terms of single fruit weight and fruit length, with 'Anaheim Chili' producing similar or the second highest single fruit weight and fruit length. The large fruit sizes of 'Big Jim' and 'Anaheim Chili' have likely contributed to their high marketable yields. By comparison, the two small, fruited cultivars 'Chile de Arbol' and 'Cayenne Purple' are among the cultivars producing the lowest marketable yield. However, total marketable yield can be determined by both fruit size and number when cultivars with intermediate fruit sizes, including 'Jamaica Hot Red' and 'Mulato Isleno', were among the most productive cultivars due to high fruit number. When selecting pepper cultivars for container production, plant growth vigor and size also require consideration. In our study, 'Jamaica Hot Red' had the largest PGI for two years. The plants became top heavy during mid to late season, causing frequent falling over problems. In addition to productivity, a cultivar with dwarf and compact growth habits would be preferred in a container production system, as agreed by Butzler et al. [29].

Seaweed extracts have been applied to a number of vegetable and fruit crops to increase productivity and produce quality [16]. The chemical compositions of seaweed extract include complex polysaccharides, fatty acids, vitamins, phytohormones, and mineral nutrients [16]. Active ingredients in Vitazyme contain plant growth regulators of brassinosteroids and triacontanol, and B vitamins (including Thiamine, Riboflavin, and pyridoxine) that stimulate plant growth. Field trials of jalapeno peppers in Mexico showed a 16% increased yield with greater overall plant growth and enhanced root growth when Vitazyme was applied as root dip before transplanting and foliar spray twice at 35 and

57 DAT [30]. Tribus contains a mix of plant growth-promoting bacteria, including *Bacillus subtilis*, *Bacillus pumilus*, and *Bacillus amyloliquefaciens*. Root inoculation of *Bacillus amyloliquefaciens* was found to increase yield, and mineral nutrients (including calcium and iron), vitamin C, and antioxidant capacity of bell pepper cultivars [31]. The three biostimulants used in this study did not affect the marketable yield of any cultivar at any harvest during 2020 or 2021 but showed beneficial effects on fruit quality.

When considering the effects of biostimulants, plant stage, application dosage, and frequency all affect treatment outcome. The seaweed extract C-Bio CPS increased fruit length and diameter compared with Vitazyme in August 2020, July, and September 2021 in our study. The number and size of marketable fruit of three bell pepper varieties produced in a greenhouse were improved by the treatment of transplant soaking for two hours plus three times of foliar spray at 21-day intervals with 0.4% Kelpak (a commercial seaweed extract product from *Ecklonia maxima*) compared with control [32]. When pepper seedlings were just soaked with the 0.4% Kelpak solution or sprayed three times, both treatments resulted in similar fruit numbers and sizes of marketable fruit of test sweet pepper cultivars to control. In our study, the three biostimulant treatments were applied three times during a growing season at 2-to-3-week intervals approximately three weeks after transplanting of 8-week-old seedlings. It is possible that biostimulant treatments may show a stronger effect when applied to seedlings at a younger age and/or combined with another application method, e.g., root dip.

Another important benefit of biostimulants is that they promote plant performance under abiotic stresses, including drought, chilling, heat, and salinity [12,16,19,25]. When a seaweed extract was applied to spinach every 4 days through a foliar spray, drench, or a combination of both, it increased leaf area, and fresh and dry leaf weights under drought stress conditions [19]. However, the seaweed treatment did not affect spinach plant growth, physiology, or nutrient concentrations under full irrigation. Biological treatments with *Bacillus* species were found to improve fresh plant weight under salt stress provided by 50 mM and 100 mM NaCl when directly seeded or when using transplants [33]. Beneficial effects of plant growth-promoting bacterias, including *Bacillus*, *Pseudomonas*, *Actinobacterial*, and *Lactobacillus,* were attributed to several mechanisms, e.g., inducing the production of phytohormones and bioactive compounds, fixing nitrogen, and enhancing mineral nutrient uptake by plants [34,35].

In our study, Tribus increased yellow coloration and increased lightness of 'Cayenne Purple' compared with C-Bio CPS in July 2020 and 2021, respectively. Vitazyme increased the yellow coloration of 'Chile de Arbol' compared with the control in August 2020. Vitazyme and C-Bio CPS also increased fruit lightness of 'Chile de Arbol' compared with control in September 2021. Such results agree with trials conducted by Vitazyme showing increased fruit size and marketable color of several fruit crops, including cherry, apple, and blueberries [36]. The development of natural pigments (including green, red, and yellow) not only contributes to the antioxidant compounds but also enhances fruit attractiveness to customers [31]. Barrajón-Catalán et al. [11] also reported that biostimulants might increase some metabolites related to fruit maturity and coloration in peppers. The precise mechanisms of action require further investigation.

5. Conclusions

Heirloom pepper cultivars varied as to their marketable yield and fruit quality, including single fruit weight, fruit length, diameter, and color. 'Anaheim', 'Big Jim', and 'Jamaica Hot Red' were the most productive among tested cultivars due to both fruit size and number. Biostimulant application did not affect marketable yield throughout the two crop-growing seasons but enhanced fruit quality, including fruit length, diameter, and green coloration, likely resulting from a single application method (fertigation or foliar spray) during the later stage of seedling growth. Biostimulant treatments increased fruit quality, including fruit length, diameter, and coloration at some harvests, the effects of which were cultivar dependent.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae9010012/s1, Table S1: Fruit diameter of chile peppers affected by the interaction between cultivar and biostimulant treatment in 2020 and 2021; Table S2: Fruit color of chile peppers affected by the interaction between cultivar and biostimulant treatment in 2020; Table S3: Fruit color of chile peppers affected by the interaction between cultivar and biostimulant treatment in 2021; Figure S1: Daily average outdoor air temperatures within the experiment duration in 2020 (A) and 2021 (B).

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