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Fish Emulsions, Cyano-Fertilizer, and Seaweed Extracts Affect Bell Pepper (*Capsicum annuum* L.) Plant Architecture, Yield, and Fruit Quality

Allison Wickham and Jessica G. Davis *

Department of Soil and Crop Sciences, Colorado State University, Fort Collins, CO 80523-1170, USA; wickham.allison@gmail.com
* Correspondence: jessica.davis@colostate.edu

Abstract: Bell peppers (*Capsicum annuum*) were grown in a greenhouse to evaluate organic fertilizer and foliar seaweed application effects on plant architecture, yield, and fruit quality. Many organic fertilizers contain phytohormones intrinsically. Hydrolyzed and non-hydrolyzed fish fertilizer and cyano-fertilizer treatments were applied in split applications every 7 days over a 135-day growing period. Control plants received no supplemental N. Each fertilizer treatment received applications of one of two different foliar seaweeds or no foliar seaweed in a 4 × 3 factorial design with three replications. Both hydrolyzed and non-hydrolyzed fish fertilizers and cyano-fertilizer increased the number of branches per plant compared to the N-deficient control. The plants receiving cyano-fertilizer or non-hydrolyzed fish fertilizer yielded more than the N-deficient control, and those treatments received 2–3 times the auxin application as the hydrolyzed fish fertilizer. In addition, the leaves from the plants treated with non-hydrolyzed fish fertilizer contained substantially higher levels of abscisic acid, although no abscisic acid was detected in the fertilizers. Both seaweed products decreased the number of fruits that were "bell"-shaped and increased the number of "long"-shaped fruits. Organic fertilizers are complex matrices of nutrients, phytohormones, and other metabolites, making it very challenging to determine the mechanisms behind the observations.

Keywords: organic fertilizer; *Capsicum annuum* L.; cyanobacteria; hydrolyzed fish emulsion; non-hydrolyzed fish emulsion; seaweed; phytohormones

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1. Introduction

Bell peppers (*Capsicum annuum* L.) are a warm-season annual in the Solanaceae family, originating from Central and South America and the Caribbean. There are numerous types within the species, including bell peppers. Bell peppers are green when immature and turn a variety of colors as they ripen, depending on plant variety [1].

Organically grown greenhouse bell peppers can be grown year-round and earn a higher price for producers than conventionally grown bell peppers [2]. Additionally, organically grown peppers may provide consumers with greater health benefits than conventionally grown bell peppers due to having greater levels of antioxidants, specifically, β-carotene [3]. While it is known that β-carotene acts as an antioxidant, it is also being explored as an anti-allergen and an anti-cancer agent [4,5].

Increasing yield in an organic system could be of great benefit to producers. The most common method for increased yield in agriculture is to provide plants with supplementary N. When N is deficient, pepper plants are often shorter, lower leaves may be chlorotic, and fruit number and size are below average. There have been many field and greenhouse studies conducted on the N supplementation of peppers, and in most experiments, supplying N to pepper plants increased the number of flowers, fruit set, and fruit yield [6,7]. Nitrogen fertilizer applied in the form of urea also affects the height, branch number, and leaf chlorophyll content of peppers [8]. The optimum N concentrations for the maximum stem and leaf dry matter production of peppers in an aeroponic system were in the range of 56 to 64 mg L^{-1} [9]. In a study using a N-deficient fertilizer solution for bell peppers grown on perlite (35 mg L^{-1} solution of N twice weekly), this level of N was not found to significantly reduce pepper yields [10].

Nitrogen management on organic farms can be difficult due to the nature of organic fertilizers. Organic farmers use compost, manure, legume cover crops, dried organic meals such as feather and blood meal, or liquid fertilizers such as fish emulsion to increase crop productivity [11]. Additionally, there are many specialty products on the market containing plant growth regulators or phytohormones intended to impact plant growth characteristics, which could increase yield. Meals and liquid fertilizers are energy-intensive to produce and ship, and their yield impacts are often inconclusive [12–14]. Fish fertilizers are manufactured in different ways. Hydrolyzed fish fertilizer is generally produced from whole fish that are cold processed in water and broken down using naturally occurring enzymes, whereas non-hydrolyzed fish fertilizer is heat processed or cooked down to concentrate the nutrients. In organic farming systems, the most common forms of N are largely organic matter, such as composted manure. This makes N management difficult, as organic N mineralization rates are difficult to predict and control [15]. To improve the predictability of N input, organic farmers often turn to fertilizers such as liquid fish fertilizer to supply their crops with supplemental N mid-season.

Cyano-fertilizer is an on-farm, grower-grown N source that contains cyanobacteria [16,17]. Cyanobacteria are capable of photosynthesis and atmospheric N_2 fixation. Creating an environment that fosters cyanobacterial growth (such as shallow ponds) results in a liquid fertilizer (cyano-fertilizer) that can be used as an organic N source. Cyano-fertilizer can be applied to crops through fertigation, and its use has been demonstrated on a variety of crops including peppers, kale, peaches, lettuce, sweet corn, and carrot [11,18–22]. Once in the soil, microbes mineralize the organic N from the cyanobacteria for plant use. By growing cyano-fertilizer organically on-farm, organic farmers can harness the N fixing power of these prokaryotes and potentially decrease the cost and carbon footprint of purchasing and transporting traditional organic fertilizers. In addition, cyanobacteria can produce an elaborate array of secondary compounds, including phytohormones such as auxins, cytokinins, and abscisic acid [23,24]. While cyano-fertilizer has a lower N concentration than fish fertilizers, fish fertilizers are usually diluted prior to application, and both can be applied multiple times throughout the season. Composts and manures, on the other hand, are usually applied pre-plant only.

Phytohormones are marketed by manufacturers to stimulate plant growth when applied exogenously. Organic farmers can purchase products such as organic liquid seaweed extract to apply exogenous phytohormones to crop foliage or as a soil soak. Seaweed products are best known for their auxin and cytokinin contents, which are responsible for cell division and root and shoot elongation, respectively [13]. Salicylic acid, found in cyano-fertilizer, is known to play a role in plant response to abiotic stress [25]. A newly discovered plant hormone, strigolactone, interacts closely with auxin within plants and is related to plant branching [26]. Increasing branching in food crops could increase yield and provide better weed control for organic farmers. The potential impact of applying plant growth regulators for optimum pepper yield is unknown.

Few studies have observed the impacts of applied phytohormones on bell peppers, specifically. Auxin impacts fruit set in peppers, and gibberellin may play an important role in preventing flower and fruit abscission [27]. Interactions between hormones are important as well; auxin and gibberellin applied together seem to promote a balance between cell elongation and expansion during fruit growth [27].

Jasmonic acid (JA) is a signaling hormone that is related to plant response to biotic and abiotic stressors. The addition of JA to crops is being explored as a method to inexpensively increase resistance to pests that would otherwise significantly reduce yield [28]. JA is required to signal a stress response in tomatoes, but a JA precursor, 12-oxophytodienoic acid (OPDA), can substitute for JA, as OPDA can readily be converted to JA in the plant [29]. To understand whether OPDA serves a specific role without being converted to JA, researchers found that several genes in *Arabidopsis* responded only to OPDA, and not to JA [30]. The signaling pathway also differed, showing that OPDA can readily be converted to JA if the plant requires it, but that OPDA acts independently in response to physical wounding in the plant.

This research investigates the effects of organic liquid N fertilizer type (applied at the same N rate) and foliar applications of liquid seaweed on plant architecture, including branching, flowering, leaf and fruit abscission, and their contributions to the overall yield and quality of bell pepper crops compared to a N-deficient control. Secondly, the phytohormone applications in the fertilizers are measured (specifically, auxin, cytokinin, and salicylic acid) to understand the potential mechanisms through which these organic fertilizers may impact plant architecture and fruit quality.

2. Materials and Methods

This study was carried out at the Colorado State University (CSU) Plant Growth Facility. The greenhouse allowed natural light in and provided supplemental light to achieve a 16 h daylength with 400 W high-pressure sodium lighting. Day and night temperatures averaged 24 °C and 18 °C, respectively, and relative humidity was ambient (~30%).

The bell pepper variety "Aristotle" was chosen for this study (Harris Seeds, Rochester, NY, USA); it is described as a 73-day, widely adaptable variety that does well all over the USA, and is a green to red pepper with a "blocky" shape. The vegetative part of the plant can be 50 to 61 cm tall. The "Aristotle" variety is described as having intermediate resistance to common diseases and is free of anthocyanins, which cause purpling during stress.

A randomized complete block design with a 4 × 3 factorial and three replications was utilized. Four soil N treatments (control, hydrolyzed fish fertilizer, non-hydrolyzed fish fertilizer, and cyano-fertilizer) each received three foliar seaweed treatments (none, Seacom PGR seaweed extract, and Neptune's Harvest seaweed extract). The control plants received no supplemental N, and the "no foliar" plants received no foliar seaweed applications. The pots were black 7.6 L plastic pots (21.5 cm tall and 22.8 cm diameter) with 5 drainage holes in the bottom. To keep the soil media in the pot, a 25 by 25 cm piece of fiberglass screen was fitted to the bottom of the inside of the pot. Each pot was placed in a black seeding tray that was tilted towards the pot to capture water and fertilizer applications that percolated through the pot, making it so that liquid was available for the soil to re-absorb.

Growing media were prepared using 60% sand, 20% vermiculite, and 20% perlite; this mixture was chosen to maximize water-holding capacity and minimize water loss. The soil mix was sampled and sent to the CSU Soil, Water, and Plant Testing Laboratory; following extraction with 2 M KCl, analysis was performed with a Lachat auto-analyzer. The soil mix contained, on average, 7.8 kg NH_4^+-N ha^{-1} and 0.90 kg NO_3^--N ha^{-1}.

A 72-cell Rockwool starter plug tray was watered with a dilute vinegar solution of pH 6.5 to provide a favorable pH for seed germination. On 6 February 2015, 150 pepper seeds were planted into the Rockwool tray, watered daily, and warmed with a heating pad below the tray. The seedlings emerged on 13 February 2015. Twelve days after emergence, the peppers were thinned and transplanted into pots for the remainder of the experiment. From transplant to harvest, the growing season lasted 135 days.

Half-strength N-free Hoagland's solution (Bio-World, Dublin, OH, USA) was mixed with the N fertilizers to provide the other plant-essential nutrients. The target total N rate for the peppers was 40 kg N ha^{-1}; however, the actual application rate was 31.8 kg N ha^{-1} (Table 1). The *Anabaena* spp. cyano-fertilizer was grown in a 75.7 L fish tank in the greenhouse following methods described previously [16,31]. Neptune's Harvest hydrolyzed fish fertilizer and Alaska non-hydrolyzed fish fertilizer were purchased from Neptune's Harvest (Gloucester, MA, USA) and Fort Collins Nursery (Fort Collins, CO, USA), respectively. The three fertilizers varied in N concentration and were applied at equal N rates (Table 1). N application rates were gradually increased throughout the season due to the low nutrient holding capacity of the soil mixture and the increasing nutrient demand of the growing plants.

Table 1. Nitrogen (N) fertilizer applications to potted bell peppers grown in a greenhouse. Dates, N application rate, and total diluted volume of fertilizer are shown.

Date	Soil N Fertilizer Applied	Volume of Fertilizer Mix
	kg N ha^{-1}	L pot^{-1}
28 February 2015	0.9	0.10
13 March 2015	1.7	0.10
23 March 2015	0.4	0.20
30 March 2015	3.7	0.20
8 April 2015	3.3	0.20
17 April 2015	4.0	0.30
28 April 2015	4.1	0.30
7 May 2015	1.2	0.30
14 May 2015	2.3	0.40
19 May 2015	1.6	0.40
26 May 2015	2.8	0.40
5 June 2015	1.6	0.40
12 June 2015	2.1	0.40
19 June 2015	2.1	0.15
Total	31.8	3.85

The N concentration of the cyano-fertilizer was measured on each application date using a Hach DR3900 Benchtop Spectrophotometer (Loveland, CO, USA) to measure the total Kjeldahl nitrogen. For each treatment, the fertilizer and half-strength Hoagland's solution were mixed thoroughly prior to application with a graduated cylinder. The fish fertilizers were mixed to match the N concentration of the cyano-fertilizer by diluting to volume with water (Table 1). Each pot received the same volume of water.

Seaweed extracts (Seacom PGR Organic seaweed extract, Johnny's Selected Seeds, Winslow, ME, USA; Neptune's Harvest organic seaweed extract, Neptune's Harvest, Gloucester, MA, USA) were applied to the leaves using a 900 mL spray bottle. The seaweed extracts were mixed according to the manufacturers' recommendations. An amount of 1.1 mL of PGR seaweed extract was diluted into 825 mL of water, and 3.2 mL of Neptune's Harvest seaweed extract was diluted into 825 mL of water, and equal volumes were applied to the leaves of the assigned pepper plants. The seaweed application rate was increased throughout the season following manufacturers' recommendations to cover each leaf adequately (Table 2).

Table 2. Foliar seaweed applications to potted bell peppers grown in a greenhouse. Dates and total volume of Seacom PGR seaweed and Neptune's Harvest seaweed are shown.

Date	Volume of Seaweed Solution Applied
	mL
3 March 2015	5
20 March 2015	10
16 April 2015	20
7 May 2015	20
28 May 2015	25
19 June 2015	25
Total	105

Phytohormone analyses of the fertilizer and seaweed treatments were conducted at the Proteomics and Metabolomics Facility, CSU, following a gas chromatography–mass spectrometry procedure described previously [22,32]. The amounts of phytohormone applied over the growing season were calculated from the measured concentrations (Table 3).

Although phytohormones in cyano-fertilizer are relatively diluted in comparison to fish fertilizers, fish fertilizers are diluted when applied, while the cyano-fertilizer is applied at full strength. The N rate used to calculate phytohormone applications for fertilizers in kg ha^{-1} was 31.8 kg N ha^{-1}, to match the N application rate (Table 1). Since the N content of the seaweeds was zero, the phytohormone applications in kg ha^{-1} for seaweeds were based upon manufacturers' recommendations.

Table 3. Phytohormone concentrations as measured in the fertilizers and foliar seaweeds used in the greenhouse study in Fort Collins, CO, USA. The N rate used to calculate phytohormone applications for fertilizers in kg ha^{-1} was 31.8 kg N ha^{-1}, to match the N application rate. Since N content of seaweeds was zero, the phytohormone applications in kg ha^{-1} for seaweeds were based upon manufacturers' recommendations. No cytokinin isomers were detected in any of the fertilizers or seaweed products. The Seacom PGR seaweed label stated that it contained 400 mg cytokinin kg^{-1}. n/d = not detected.

Treatment	Auxin	Salicylic Acid	Auxin	Salicylic Acid
	mg kg^{-1}		kg ha^{-1}	
Fertilizers				
Cyano-fertilizer	6.50×10^{-5}	5.92×10^{-3}	9.73×10^{-5}	0.01
Hydrolyzed fish fertilizer	3.97×10^{-4}	0.018	6.30×10^{-7}	1.22×10^{-4}
Non-hydrolyzed fish fertilizer	1.436	0.077	9.26×10^{-4}	1.16×10^{-5}
Foliar Seaweeds				
Seacom PGR seaweed	0.802	48.17	4.52×10^{-7}	2.72×10^{-5}
Neptune's Harvest seaweed	n/d	n/d	n/d	n/d

Each pot received 1 L of water every other day after transplanting. On the days when fertilization occurred (Table 1), the water supplied in the fertilizer mix was the only water applied on that day. The total irrigation supplied to the peppers over the growing season was 57.4 L pot^{-1}.

Individual 25 cm × 25 cm squares of aluminum foil were folded over the exposed soil to eliminate soil surface algal growth 49 days after transplanting (DAT). The pepper plants were treated with Entrust® SC Naturalyte®, a fermented spinosad product, to control thrips 50 DAT.

Plant height, leaf number, and flower number measurements were taken each week. Plant height was measured to the tip of the tallest leaf. Flowers were separated into five categories: green flowers, dead flowers or abscissions, white flowers, finished flowers, and peppers (Figure 1).

The peppers were harvested, measured, and weighed 135 DAT. The peppers had developed noticeably different shapes: traditional blocky, bell-shaped peppers (bell), elongated peppers (long), and peppers that curved dramatically (curved). The dominating color of each fruit was also recorded. Examples of the shape and color differences can be seen in Figure 1. After physical measurements were taken, the peppers were sent to the Proteomics and Metabolomics Facility at CSU and were analyzed for β-carotene concentration via LC-MS.

The pepper plants were cut 1 cm from the soil surface and removed from the pots. The stem and the branches were carefully measured to determine the number and location of branches, as well as the location of every leaf and flower. Additionally, distances between branches were measured, and locations where leaves, flowers, and fruits were lost were noted. The average number of branches, the distance between branches, the number of abscissions that had occurred, and the final leaf and flower counts were recorded. Additionally, the ratio of the height of the first branch to the total height of the plant was calculated. The plants were then separated into leaves and stems and weighed. Several leaves from each plant were sent to the CSU Proteomics and Metabolomics Facility. Utilizing UPLC-MS, phytohormones were extracted and measured. The assay is designed to measure cytokinins, auxins, gibberellins, brassinosteroids, jasmonates, and salicylates.

The remainder of the plant material was dried at 49 °C for 5 days. The dried leaves and stems were re-weighed to obtain the dry matter content of each.

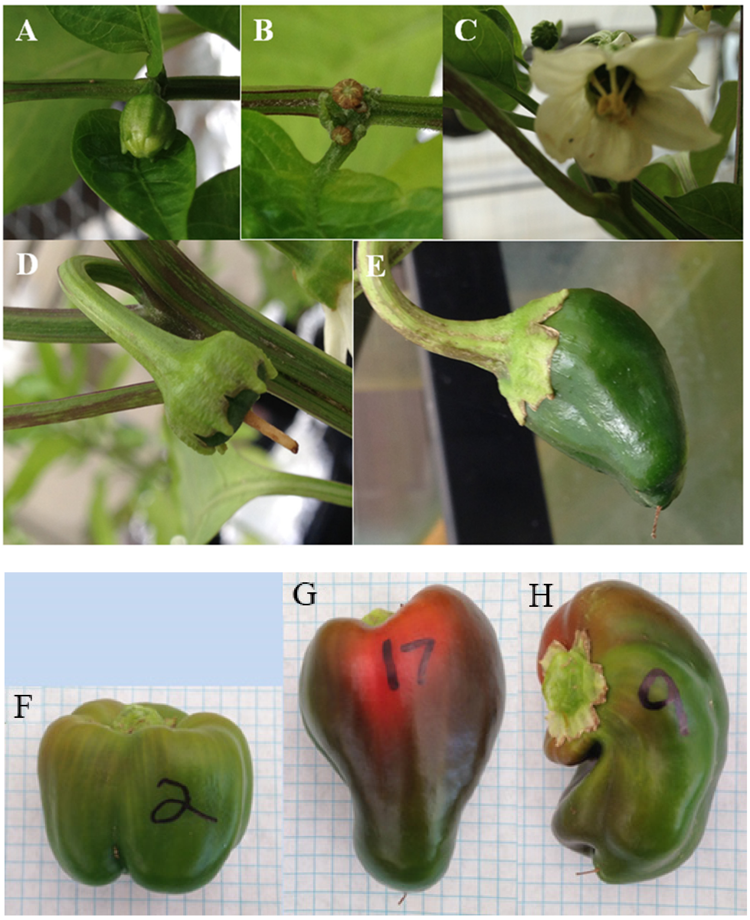

Figure 1. Pepper flower classifications, fruit shape, and colors. The types of flowers present on bell pepper plants were counted each week and compared among treatments. The flower types fell into five categories (**top**): green flowers (**A**), dead flowers (**B**), open flowers (**C**), finished flowers (**D**), and peppers (**E**). Three main shapes and two main colors of pepper fruit were observed in the greenhouse study (**bottom**). These peppers were classified as "green, bell shaped" (**F**), "red, long shaped" (**G**), or "green, curved" (**H**). The photographs above were taken from the bell pepper plants grown in a greenhouse in Fort Collins, CO, USA, in 2015.

Statistics were performed using Statistical Analysis System 9.4 (Cary, NC, USA). The PROC MIXED statement was used, and the experimental design was run as a 4 × 3 factorial. Fertilizer treatment and foliar seaweed applications were treated as fixed effects, and block (or replicate) was treated as a random effect. The slice (F-test) statement was used to analyze the effects of the foliar seaweed extract. An adjusted F-test of fixed effects was performed using the REML method. Least square means were estimated with the lsmeans statement, compared with the pdiff statement, and p-values < 0.05 were considered significant.

3. Results

We tested the main effects (the fertilizer treatments and the foliar seaweed treatments) and their interactions. Since the interactions were never significant ($p < 0.05$), we present only the significant main effects.

3.1. Plant Architecture

The N fertilizer treatments impacted the total number of branches, with the control plants having significantly fewer branches than all other fertilizer treatments (Figure 2).

Foliar seaweed treatments also presented significant differences in the total number of branches on each plant. Seacom PGR seaweed produced plants with a greater number of branches than no seaweed at all, and Neptune's Harvest seaweed was statistically similar to the control and the PGR seaweed treatments. While there were no significant differences in the height to the first branch, meaning that branching started at approximately the same height in all treatments, Neptune's Harvest foliar seaweed, averaged across N treatments, produced pepper plants with differing branching height (distance containing branches, measured as the height above the first branch) to total height ratios. The Neptune's Harvest seaweed resulted in plants with shorter branching sections compared to the PGR seaweed and the control. These plants were described as "tree-like" as they had more stem height than branches, whereas the control and PGR seaweed treatment produced plants with more proportionate branch and stem sections. In addition, all treatments produced pepper plants with similar heights, leaf and stem weights, leaf-to-stem-weight ratios, and water contents.

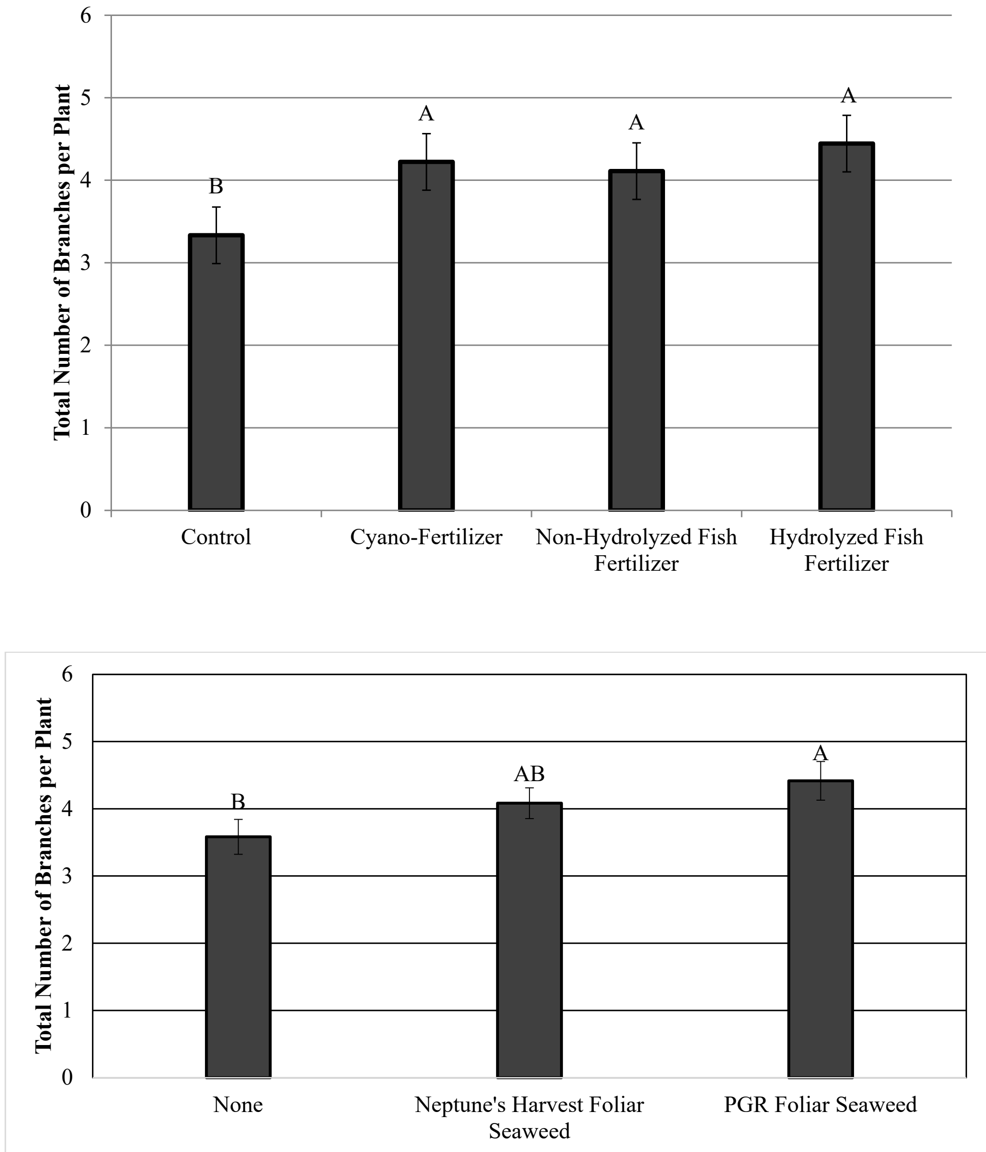

Figure 2. Average total number of branches in pepper plants at harvest as affected by fertilizer treatment (**top**) and foliar seaweed application (**bottom**). Bell peppers were grown in a greenhouse experiment in Fort Collins, CO. Treatments that share a common capital letter are statistically similar based on the least square means test ($p < 0.05$). Error bars represent the standard error of each mean. There was no significant interaction between fertilizer treatments and seaweed applications.

The N fertilizer treatments influenced flower numbers on several measurement days (Table 4). On 109 DAT, hydrolyzed fish fertilizer had significantly more living flowers than non-hydrolyzed fish fertilizer. On the other hand, cyano-fertilizer and non-hydrolyzed fish fertilizer had more dead flowers than either the control or the hydrolyzed fish fertilizer.

Table 4. Pepper plant flower count. The number of flowers alive or dead 109 days after transplanting and the % of abscissions on 135 days after transplanting are shown. Bell peppers were grown in a greenhouse in Fort Collins, CO, USA. Treatments that share a common capital letter within a column are statistically similar based on the least square means test ($p < 0.05$). If all means within a column were statistically similar, no letter follows the mean.

Treatment	Flowers Alive or Dead		
	Day 109		Harvest (Day 135)
	Alive	Dead	% Abscissions
Control	2.3 AB	3.0 B	65.4 A
Cyano-fertilizer	2.2 AB	4.4 A	54.8 AB
Hydrolyzed fish fertilizer	3.7 A	2.6 B	51.8 B
Non-hydrolyzed fish fertilizer	1.8 B	5.0 A	55.6 AB

All leaf abscissions and dead flowers were summed on the day that the pepper plants were harvested for a total percentage of abscissions and abortions compared to the percentage of leaves and flowers that remained vital at the end of the experiment (Table 4). The percentage of abscissions and dead flowers compared to the total number of leaves and flowers was deemed as "abscission events". In this metric, hydrolyzed fish fertilizer presented with a 13.6% lower number of abscission events than the control.

Foliar seaweed did not impact flowers on most measurement days or at harvest, but 87 DAT Seacom PGR seaweed increased the number of dead flowers (2.3) compared to Neptune's Harvest seaweed (0.67) and the control (0.50) ($p < 0.05$). On 97 DAT, Seacom PGR seaweed increased the number of dead flowers (3.2) compared to the control (1.5).

3.2. Pepper Yield

Plants treated with cyano-fertilizer and non-hydrolyzed fish fertilizer produced 12.2 g plant^{-1} and 13.3 g plant^{-1} more fruit yield than the control plants (18.0 g plant^{-1}), respectively (Figure 3). The yield under the hydrolyzed fish fertilizer was not different from the control or the higher-yielding treatments, and the foliar seaweed treatments had no impact on pepper yield.

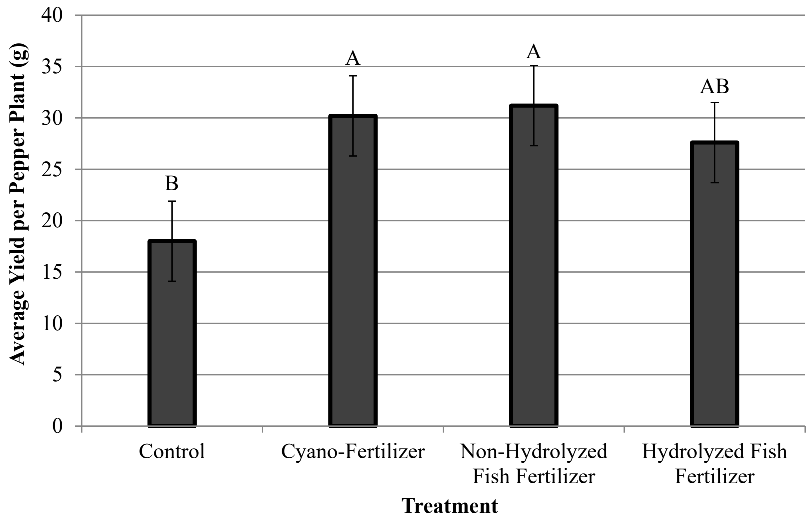

Figure 3. Average fruit yield per pepper plant as affected by fertilizer treatment. Bell peppers were grown in a greenhouse experiment in Fort Collins, CO, USA, in 2015. Treatments that share a common

capital letter are statistically similar based on the least square means test ($p < 0.05$). Error bars represent the standard error of each mean. Yield was not significantly affected by foliar seaweed treatments.

3.3. Fruit Quality

Although the fertilizer treatments had no impact on fruit quality, the foliar seaweed treatments did significantly impact pepper fruit color and shape (Figure 4). The Seacom PGR foliar seaweed treatment increased the number of green peppers harvested by 57% and reduced the number of red peppers harvested by 75%. Neptune's Harvest seaweed had similar numbers of green and red fruits as the control. Both foliar seaweed treatments reduced the number of "bell"-shaped peppers by 50–67% and increased the number of "long"-shaped peppers by 4–5× compared to the control. All seaweed treatments had similar numbers of "curved"-shaped peppers.

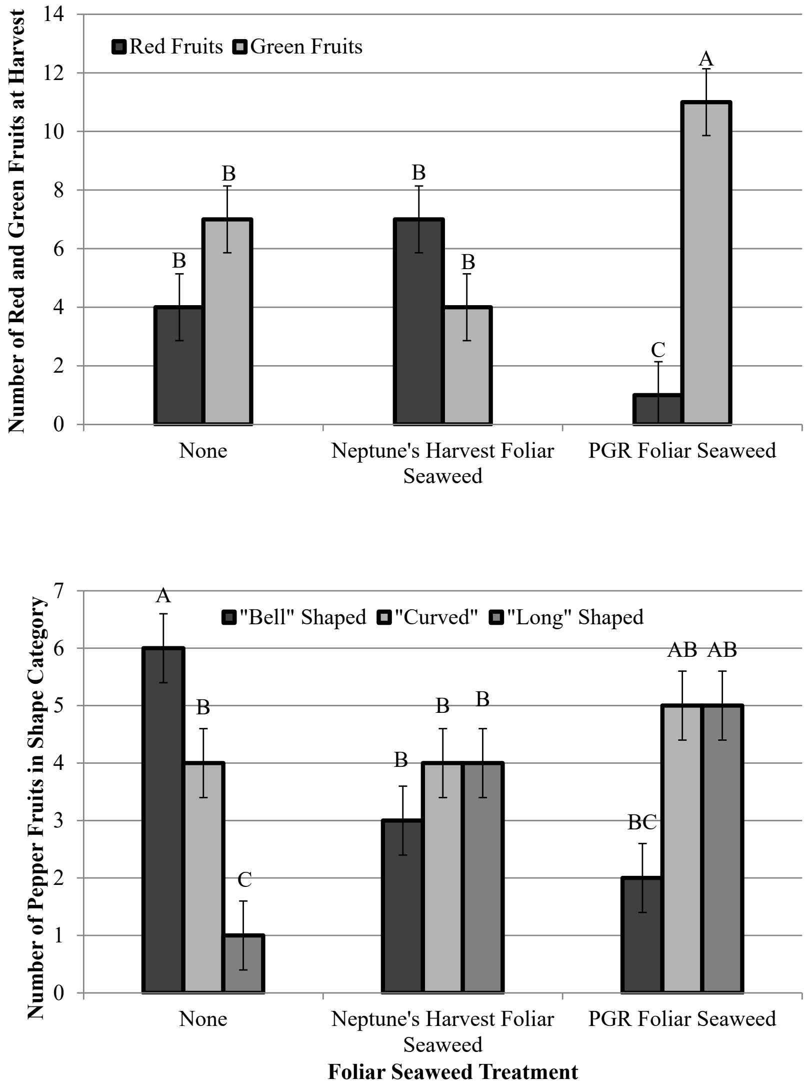

Figure 4. Differences in bell pepper fruit colors (**top**) and shapes (**bottom**) among foliar seaweed treatments. Bell peppers were grown in a greenhouse experiment in Fort Collins, CO, USA, in 2015. The totals are given for number of "bell"-shaped, "long"-shaped, and "curved"-shaped peppers produced (see Figure 1). Treatments that share a common capital letter are statistically similar based on the least square means test ($p < 0.05$). Error bars represent the standard error of each mean. Fruit color and shape were not significantly affected by fertilizer treatments.

3.4. Foliar Phytohormone Concentrations

The leaves from the non-hydrolyzed fish-fertilizer-treated pepper plants contained more than six times higher levels of abscisic acid (ABA), a stress and dormancy signaling phytohormone, than the control (Table 5); the leaves from all other treatments were not different from the control or the non-hydrolyzed fish fertilizer. The leaves from the non-hydrolyzed fish fertilizer treatment also contained a greater concentration of 12-oxo-phytodienoic acid (OPDA), a wound-stress signal and a cyclopentenone precursor of jasmonic acid (JA). However, JA concentration was not different among treatments. Many phytohormones that were expected to be detected were not found in this assay, including cytokinins, auxins, phaseic acids, benzoic acid, salicylic acids, brassinosteroids, and gibberellins.

Table 5. Phytohormone concentrations in pepper leaves from greenhouse plants grown using different N fertilizers in Fort Collins, CO, USA. Treatments that share a common capital letter within a row are statistically similar based on the least square means test ($p < 0.05$). If all means within a row were statistically similar, no letter follows the mean. There were no significant differences in phytohormone concentrations in pepper leaves among the foliar seaweed treatments.

Phytohormone	N Fertilizer			
	Control	Cyano-Fertilizer	Hydrolyzed Fish Fertilizer	Non-Hydrolyzed Fish Fertilizer
Abscisic acid (pg/mg)	21.48 B	41.04 AB	76.27 AB	131.8 A
Jasmonic acid (pg/mg)	271.3	266.7	418.0	302.4
12-oxo-phytodienoic acid (relative abundance)	294.6 AB	189.2 B	299.9 AB	350.0 A

4. Discussion

Both hydrolyzed and non-hydrolyzed fish fertilizers and cyano-fertilizer increased the number of branches per plant compared to the N-deficient control (Figure 2). Previous greenhouse research has documented that cyano-fertilizer application increases pepper branching [18]. There were also significant effects of the fertilizer treatment on flowering (Table 4). On the other hand, the plants receiving cyano-fertilizer or non-hydrolyzed fish fertilizer yielded more than the N-deficient control (Figure 3), in contrast to previously published results in which pepper yield was not significantly decreased by N-deficient fertilizer treatments [10]. Having more dead flowers in the cyano-fertilizer and non-hydrolyzed fish fertilizer treatments could be a sign that these treatments contributed to faster maturity and, therefore, resulted in greater yield.

It is common for N fertilizer to increase the yield of many crops, but in addition to the N itself, the cyano-fertilizer and non-hydrolyzed fish fertilizer resulted in 2–3 times higher auxin application than the hydrolyzed fish fertilizer (Table 3). Auxin is known to play many important roles in plant growth and development in sweet peppers, including flowering, fruiting, and adaptation to environmental stressors [33]. However, auxins have a greater effect on flowering and fruit set when pepper plants are under stress [34].

Based on the ABA content in the leaves (Table 5), it would be expected that the non-hydrolyzed fish fertilizer would have the greatest number of abortions and abscissions upon harvest. This was not the case, as the non-hydrolyzed fish fertilizer had a similar abscission percentage compared to all other fertilizer treatments on harvest day (Table 4). In this study, leaves were sampled for phytohormone analysis at the final harvest; although it is known that endogenous ABA generally increases during fruit ripening, it is not clear whether those ABA levels remain high through harvest [35]. The absence of gibberellins in the leaf tissue is interesting in the context of flower and leaf abscission. Gibberellins play a role in preventing flower abortion; although auxin is the primary inducer of fruit set, auxin also induces gibberellin biosynthesis [27]. It is possible that the treatments impacted the maturation rate of the plants, and that this is reflected in the flower numbers, making the data difficult to interpret. At the end of the experiment, however, the control plants had the fewest living leaves and flowers, suggesting that N deficiency limited the growth and flowering of the control plants.

The foliar seaweed treatments had varying effects on branching (Figure 2), no effect on flowering or yield, and significant effects on fruit color and shape (Figure 4). Other studies have evaluated bell pepper quality characteristics related to flavor (e.g., capsaicin or total soluble solids) and antioxidant activity [36,37], but no other published studies were found related to fruit shape. PGR seaweed increased the total number of branches compared to plants not receiving any foliar seaweed, while Neptune's Harvest foliar seaweed reduced the ratio of branching length to the total height of plants. However, applying PGR foliar seaweed increased the number of green peppers and decreased the number of red peppers compared to Neptune's Harvest seaweed and the control, possibly due to delayed maturity. Despite differences in fruit color, there were no significant differences in pepper fruit β-carotene levels among the fertilizer or seaweed treatments, although previous research has shown an impact of organic fertilizer selection on β-carotene levels in lettuce [20], where indole-3-acetic acid application in the fertilizers was positively correlated with β-carotene concentration in lettuce.

In addition, both seaweed products decreased the number of fruits that were "bell"-shaped and increased the number of "long"-shaped fruits (Figure 4). As the "bell" shape is the marketable shape of bell peppers, it may be advantageous to growers to avoid these two foliar seaweed products on peppers. It is difficult, however, to determine the mechanism by which Neptune's Harvest foliar seaweed treatment impacted the plant growth characteristics, since no phytohormones were detected in the product.

The Seacom PGR seaweed contained measurable levels of auxin and salicylic acid (SA), while Neptune's Harvest Seaweed contained no measurable phytohormones (Table 3). Foliar SA application has been reported to increase fruit number and the weight of greenhouse-grown peppers, but no evaluation of its effect on pepper shape or color has been found [38]. The interplay of phytohormones can influence fruit set [27]. In addition, the source of phytohormones also plays an important role; for example, exogenous auxin can set bell pepper fruits, but it cannot replace endogenously produced hormones to enhance fruit growth [39]. It may be that in the case of the "long"-shaped peppers, the cell elongation rate exceeded the cell division rate. This may be explained by unbalanced levels of auxin and gibberellin [27]. If the auxins and gibberellins in the PGR seaweed (no auxin was detected in Neptune's Harvest, and no gibberellins were detected in either seaweed product; see Table 3) disrupted the balance of these two important phytohormones, the effect may be the opposite of the intent of the product. This theory, however, does not explain the occurrence of "curved" peppers, unless they are the result of a different process, such as improper flower development or an environmental stressor.

To fully elucidate the interactions between N and phytohormones and their impact on bell peppers and the mechanisms through which they affect yield, the authors suggest future researchers should study N and phytohormone applications themselves, rather than organic fertilizers that contain N, phytohormones, and many other plant nutrients in a complex matrix.

5. Conclusions

Fertilizer treatments influenced the branching, flowering, and yield of bell peppers. Plants receiving cyano-fertilizer or non-hydrolyzed fish fertilizer yielded more than the N-deficient control. The foliar seaweed treatments had no effect on flowering or yield but significantly impacted fruit color and shape. Applying PGR foliar seaweed enhanced the number of green peppers and reduced the number of red peppers compared to Neptune's Harvest seaweed and the control. In addition, both seaweed products decreased the number of fruits that were "bell"-shaped and increased the number of fruits with other shapes, potentially reducing the marketability of the fruit. The evaluation of phytohormones in the treatments and in the leaves of the treated plants did not provide a definitive explanation for these effects.

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References

1. United States Department of Agriculture. Natural Resource Conservation Service, Plants, *Capsicum annuum* L. Available online: https://plants.usda.gov/home/plantProfile?symbol=CAAN4 (accessed on 23 June 2016).
2. United States Department of Agriculture. Agricultural Marketing Service, Market News. Available online: https://www.marketnews.usda.gov (accessed on 23 June 2016).
3. Hallmann, E.; Rembialkowska, E. Characterization of antioxidant compounds in sweet bell pepper (*Capsicum annuum*) under organic and conventional growing systems. *J. Sci. Food. Agric.* **2012**, *92*, 2409–2415. [CrossRef]
4. Teng, Y.; Sheu, M.; Hsish, Y.; Wang, R.; Chiang, Y.; Hung, C. B-carotene reverses multidrug resistance cancer cells by selectively modulating human P-glycoprotein function. *Phytomedicine* **2016**, *23*, 316–323. [CrossRef] [PubMed]
5. Hiragun, M.; Hiragun, T.; Oseto, I.; Ochida, K.; Yanase, Y.; Tanaka, A.; Okame, T.; Ishikawa, S.; Mihara, S.; Hide, M. Oral administration of β-carotene or lycopene prevents atopic dermatitis-like dermatitis in HR-1 mice. *J. Dermatol.* **2016**, *43*, 1188–1192. [CrossRef]
6. Maynard, D.N.; Lachman, W.H.; Check, R.M.; Vernell, H.F. The influence of nitrogen levels on flowering and fruit set of peppers. *Proc. Amer. Soc. Hort. Sci.* **1969**, *81*, 385–389.
7. Dai, Z.; Zhao, X.; Yan, H.; Qin, L.; Niu, X.; Zhao, L.; Cai, Y. Optimizing water and nitrogen management for green pepper (*Capsicum annuum* L.) under drip irrigation in sub-tropical monsoon climate regions. *Agronomy* **2023**, *13*, 34. [CrossRef]
8. Aminifard, M.H.; Aroiee, H.; Ameri, A.; Fatemi, H. Effect of plant density and nitrogen fertilizer on growth, yield and fruit quality of sweet pepper (*Capsicum annum* L.). *Afr. J. Agric. Res.* **2012**, *7*, 859–866.
9. Bar-Tal, A.; Aloni, B.; Karni, L.; Rosenberg, R. Nitrogen nutrition of greenhouse pepper. ii. Effects of nitrogen concentration and NO_3:NH_4 ratio on growth, transpiration, and nutrient uptake. *HortScience* **2001**, *36*, 1252–1259. [CrossRef]
10. Urrea-Lopez, R.; Diaz de la Garza, R.I.; Valiente-Banuet, I.I. Effects of substrate salinity and nutrient levels on physiological response, yield, and fruit quality of Habanero pepper. *HortScience* **2014**, *49*, 812–814. [CrossRef]
11. Yoder, N.; Davis, J.G. Organic fertilizer comparison on growth and nutrient content of three kale cultivars. *HortTechnology* **2020**, *30*, 176–184. [CrossRef]
12. Hemphill, D. *Bulletin: Response of Vegetables to Cytex, a Cytokinin Preparation*; Oregon State University Dept. of Horticulture: Corvallis, OR, USA, 1981.
13. Hamza, B.; Suggars, A. Biostimulants: Myths and realities. *Turfgrass Trends* **2001**, *10*, 6–10.
14. Aliyu, O.M.; Adeigbe, O.O.; Awopetu, J.A. Foliar application of the exogenous plant hormones at pre-blooming stage improves flowering and fruiting in cashew (*Anacardium occidentale* L.). *J. Crop Sci. Biotechnol.* **2011**, *14*, 143–150. [CrossRef]
15. Sierra, C.; Trumbare, S.E.; Davidson, E.A.; Vicca, S.; Janssens, I. Sensitivity of decomposition rates of soil organic matter with respect to simultaneous changes in temperature and moisture. *J. Adv. Model. Earth Syst.* **2015**, *7*, 335–356. [CrossRef]
16. Barminski, R.; Storteboom, H.N.; Davis, J.G. Development and evaluation of an organically-certifiable growth medium for cultivation of cyanobacteria. *J. Appl. Phycol.* **2016**, *28*, 2623–2630. [CrossRef]
17. Wolde, G.; Asmamaw, M.; Sido, M.Y.; Yigrem, S.; Wolde-meskel, E.; Chala, A.; Storteboom, H.; Davis, J.G. Optimizing a cyanobacterial biofertilizer manufacturing system for village-level production in Ethiopia. *J. Appl. Phycol.* **2020**, *32*, 3983–3994. [CrossRef]
18. Asmamaw, M.; Wolde, G.; Yohannes, M.; Yigrem, S.; Woldemeskel, E.; Chala, A.; Davis, J.G. Comparison of cyanobacterial bio-fertilizer with urea on three crops and two soils of Ethiopia. *Afr. J. Agric. Res.* **2019**, *14*, 588–596.
19. Sterle, D.G.; Stonaker, F.; Ela, S.; Davis, J.G. Cyanobacterial biofertilizer as a supplemental fertilizer for peaches: Yield, trunk growth, leaf nutrients and chlorosis. *J. Am. Pomol. Soc.* **2021**, *75*, 165–175. Available online: http://www.pubhort.org/aps/75/v75_n3_a5.htm (accessed on 22 March 2024).
20. Sukor, A.; Amer, F.S.M.; Vanamala, J.; Davis, J.G. Phytohormones in organic fertilizers influence β-carotene concentration and marketable yield of lettuce (*Lactuca sativa*). *Acta Hortic.* **2022**, *1348*, 15–22. [CrossRef]
21. Sukor, A.; Qian, Y.; Davis, J.G. Organic nitrogen fertilizer selection influences water use efficiency in drip-irrigated sweet corn. *Agriculture* **2023**, *13*, 923. [CrossRef]
22. Wickham, A.; Davis, J.G. Optimizing organic carrot (*Daucus carota* var. sativus) yield and quality using fish emulsions, cyanobacterial fertilizer, and seaweed extracts. *Agronomy* **2023**, *13*, 1329. [CrossRef]
23. Yadav, S.; Sinha, R.P.; Yyagi, M.B.; Kumar, A. Cyanobacterial secondary metabolites. *Int. J. Pharma Biosci.* **2011**, *2*, 144–167.

24. Wenz, J.; Davis, J.G.; Storteboom, H. Influence of light on endogenous phytohormone concentrations of a nitrogen-fixing *Anabaena* sp. cyanobacterium culture in open raceways for use as fertilizer for horticultural crops. *J. Appl. Phycol.* **2019**, *31*, 3371–3384. [CrossRef]
25. Kumar, D. Salicylic acid signaling in disease resistance. *Plant Sci.* **2014**, *228*, 127–134. [CrossRef] [PubMed]
26. Gomez-Roldan, V.; Fermas, S.; Brewer, P.B.; Puech-Pagès, V.; Dun, E.A.; Pillot, J.P.; Letisse, F.; Matusova, R.; Danoun, S.; Portais, J.C.; et al. Strigolactone inhibition of shoot branching. *Nature* **2008**, *455*, 189–194. [CrossRef] [PubMed]
27. Tiwari, A.; Offringa, R.; Heuvelink, E. Auxin-induced fruit set in *Capsicum annuum* L. requires downstream gibberellin biosynthesis. *J. Plant Growth Regul.* **2012**, *31*, 570–578. [CrossRef]
28. Thaler, J.S. Jasmonate-inducible plant defenses cause increase parasitism of herbivores. *Nature* **1999**, *399*, 686–688. [CrossRef]
29. Bosch, M.; Wright, L.P.; Gershenzon, J.; Wasternack, C.; Hause, B.; Schaller, A.; Stintizi, A. Jasmonic acid and its precursor 12-oxophtodienoic acid control different aspects of constitutive and induced herbivore defenses in tomato. *Plant Physiol.* **2014**, *166*, 396–410. [CrossRef]
30. Taki, N.; Sasaki-Sekimoto, Y.; Obayashi, T.; Kikuta, A.; Kobayashi, K.; Ainai, T.; Yagi, K.; Sakura, N.; Suzuki, H.; Masuda, T.; et al. 12-Oxo-Phytodienoic acid triggers expression of a distinct set of genes and plays a role in wound-induced gene expression in Arabidopsis. *Plant Physiol.* **2005**, *139*, 1268–1283. [CrossRef] [PubMed]
31. Massey, M.S.; Davis, J.G. Beyond soil inoculation: Cyanobacteria as a fertilizer replacement. *Nitrogen* **2023**, *4*, 253–262. [CrossRef]
32. Edlund, A.; Eklof, S.; Sundberg, B.; Moritz, T.; Sandberg, G. A microscale technique for gas chromatography-mass spectrometry measurements of pictogram amounts of indole-3 acetic acid in plant tissues. *Plant Physiol.* **1995**, *108*, 1043–1047. [CrossRef]
33. Yu, C.; Zhan, Y.; Feng, X.; Huang, Z.-A.; Sun, C. Identification and expression profiling of the auxin response factors in *Capsicum annuum* L. under abiotic stress and hormone treatments. *Int. J. Mol. Sci.* **2017**, *18*, 2719. [CrossRef]
34. Silveira, H.L.; Aguiar, L.; Leitão, A.; Lourdes Taborda, M. Effects of growth regulators for fruit setting on pepper (*Capsicum annuum* L.) production. *Acta Hortic.* **1986**, *191*, 189–198. [CrossRef]
35. Xiao, K.; Chen, J.; He, Q.; Wang, Y.; Shen, H.; Sun, L. DNA Methylation is involved in the regulation of pepper fruit ripening and interacts with phytohormones. *J. Exp. Bot.* **2020**, *71*, 1928–1942. [CrossRef]
36. Chatzistathis, T.; Tsaniklidis, G.; Papaioannou, A.; Giannakoula, A.; Koukounaras, A. Comparative approach on the effects of soil amendments and controlled-release fertilizer application on the growth, nutrient uptake, physiological performance and fruit quality of pepper (*Capsicum annuum* L.) plants. *Agronomy* **2022**, *12*, 1935. [CrossRef]
37. Wang, J.; Gao, Z.; Sun, T.; Huang, W.; Jia, Y.; Li, X.; Zhang, Z.; Hu, X. Preharvest reduction in nutrient solution supply of pepper (*Capsicum annuum* L.) contributes to improve fruit quality and fertilizer efficiency while stabilizing yields. *Agronomy* **2022**, *12*, 3004. [CrossRef]
38. Elwan, M.W.M.; El-Hamahwy, M.A.M. Improved productivity and quality associated with salicylic acid application in greenhouse pepper. *Sci. Hortic.* **2009**, *122*, 521–526. [CrossRef]
39. Thanopoulos, C.; Bouranis, D.; Passam, H.C. Comparative development, maturation, and ripening of seedless and seed-containing bell pepper fruits. *Sci. Hortic.* **2013**, *164*, 573–577. [CrossRef]

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