Impact of Different Types of Inert Substrates and Foliar Applications with Biostimulators on Some of Growth and Yield Characteristics of Lettuce Plants (*Lactuca sativa* L.) Under Soilless Culture Conditions

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ABSTRACT:

This factorial experiment was conducted according to randomized complete block design R.C.B.D. in a plastic green house of Horticulture Department, College of Agricultural Engineering Sciences, University of Sulaimani, Sulaymaniyah, Kurdistan region of Iraq, during the growing seasons of 2024-2025 to investigate the impact of different types of inert substrates which is the first factor and foliar application of biostimulators which is the second factor on some of growth and yield characteristics of lettuce plants under soilless culture conditions. The first factor included perlite (S1), cocopeat (S2), cocopeat-perlite 3:1 (S3), and cocopeat-perlite 1:3 (S4). The second factor consisted of vermi-tea (B1), amino acids with seaweed extract (B2), and a mixture of both (B3). Comparisons among the means were performed using Duncan multiple range test (α=0.05). Results showed that cocopeat substrate (S2) was significantly higher in mineral content in folded leaves; which included nitrogen, phosphorus, and potassium , by recording (2.87%), (0.28%), (2.86%) respectively, it also surpassed other substrates in relative chlorophyll content in leaves, plant yield, total marketable yield, concentration of carbohydrates in leaves, and concentration of carotenoids in leaves by recording (24.36 SPAD unit), (443.95 g), (48.40 Mg h^{-1}), (76.59 mg $100g^{-1}$ DW), and (553.09 mg $100g^{-1}$ DW) respectively. Foliar application of B3 significantly improved mineral content in leaves including nitrogen, phosphorus, potassium, magnesium, and iron by recording (2.86%), (0.29%), (2.81%), (0.36%), (149.28 mg kg^{-1} DW) respectively. Interaction treatment of S2B2 gave the best results in N, K, and total marketable yield by recording (3.30%), (3.10%), (55.90 Mg h^{-1}) respectively, while S2B3 gave the best results in nitrogen, phosphorous, and concentration of carbohydrates in leaves by recording (3.30%), (0.33%) ,(82.66 mg $100g^{-1}$ DW) respectively, S3B3 increased P (0.33%), Mg (0.42%), Fe (159.90 mg kg^{-1} DW), reduced nitrate accumulation in folded and unfolded leaves (1.91 and 1.10 mg $100g^{-1}$ DW respectively).

Keywords: Cocopeat, vermi-tea, perlite, substrate culture, amino acids with seaweed extract

INTRODUCTION

Lettuce is considered one of the important leafy vegetables due to its contents as it is rich in vitamin A, C, and K as well as various minerals such as C, Fe, and Mg, has a high water content (around 95%) and dietary fibers, it also contains a considerable amount of carotenoids, polyphenols, and flavonoids (Shi *et al.*, 2022). Lettuce also known for its low contents of calories, sodium and fats, which in turn makes it a good option for healthy diet (Ciriello *et al.*, 2021). According to Central Statistical Organization of Iraq (2023) lettuce production in Iraq achieved an average yield of 2.702 tons dunum^{1-} resulting in a total production of 36,326 tons in 2022. Soilless culture has expanded by using various types of inert media to eliminate soil problems and soil-borne diseases and reducing agricultural practices before sowing and transplanting such as plowing, weeding, preparing terraces and planting lines, additionally reducing water consumption, having complete control over environmental conditions, and reducing fertilizers waste and production costs (Fussy & Papenbrock, 2022). Soilless culture is known for its use of nutrient solutions that are prepared and delivered to different inert media, with constant monitoring of pH and EC to avoid the negative changes on plants growth, nutrient solution prepared according to the type of plant and its growth stage (Sambo *et al.*, 2019). pH ranging between 5.5 and 6.8 and EC ranging between 0.8-2.8 dS m^{-1} are preferable by many vegetables, higher or lower values will cause nutrients deficiency or toxicity (Al Meselmani, 2022; Fussy & Papenbrock, 2022). There are different types of standard nutrient solution, each has its own set of nutrients concentration such as Hoagland, Steiner, and Cooper standard nutrient solutions (Combrink & Kempen, 2019). Soilless culture divided into three types: Hydroponic, this type uses water as media to grow plants, as roots are submerged in water and exposed directly to the nutrient-rich solution (Rajendran *et al.*, 2024). Aeroponic, in this type plants grow without growing media as the roots remain suspended in the air while the nutrient-rich solution is delivered directly to the root system through a misting or fogging process (Kotzen *et al.*, 2019). Substrate culture, this type uses inert media or a mixture of medium as mechanical support and for the roots to grow (Monsalve Camacho *et al.*, 2021). Growing media used in soilless culture can be either organic and inorganic, each has its own physical and chemical properties and characteristics (Bihari *et al.*, 2023). Cocopeat substrate has good chemical and physical properties such as cation exchange capacity (CEC) ranging 39-60 meq 100g^{-1} (Agarwal *et al.*, 2023). pH ranging from 5.5 to 7.0, has 80 to 100 kg m^{-3} (Verasoundarapandian *et al.*, 2021; Singh *et al.*, 2024). According to Tuckeldoe *et al.* (2023), when using coconut peat as growing medium it improved growth and yield of sweet pepper (*Capsicum annum* L.). Perlite is an inert inorganic substrate that has a pH ranging from 6.5-8 and water retention capacity completely dependent on particle size (Bar-Tal *et al.*, 2019). Jordan *et al.* (2018) stated that lettuce had the most effective average weight when grown in coconut coir under both hydroponic and aquaponic systems with an average weight of 2.58 kg m-2 and 2.83 kg m-2, respectively. Raj *et al.* (2023) found that growing lettuce crops under Dutch buckets and NFT system with coconut coir as medium gave the highest result in terms of growth, yield, and quality compared to lightweight expanded clay aggregate (LECA) and rockwool. Perlite is chemically inert with no CEC and very low EC around 0.08 dS m^{-1}; therefore, it is unable to retain any nutrients (Alam *et al.*, 2024). Rahman *et al.* (2019) illustrated that when mixing different types of substrates in different ratios, it improved characteristics of the substrate and gave better results in term of growth and yield. Biostimulators are non-toxic compounds that improve plant metabolism when applied in a minor quantity without causing any changes in their natural pathways, and they are mostly of a natural source (Jindo *et al.*, 2022). Biostimulators can improve natural biological processes, such as increase tolerance to biotic and abiotic stresses, improve nutrient uptake and utilization, water use efficiency, plant yield and quality (Mounaimi *et al.*, 2024). There are many types of biostimulators and they are well known as eco-friendly substances, some groups contain different types of hormones such as seaweed extracts, and groups containing amino acids substances such as glutamate and histidine (Dasgan *et al.*, 2024). Vermicompost tea or vermi-tea is considered a biostimulator that contains organic substances such as humic and nutrients as well as microorganisms and plant growth regulators (Joshi *et al.*, 2020). due to the content of vermi-tea when applying it either as foliar application or drenching it improved root growth and nutrient uptake, and acted as biocontrol against many diseases (Aslam *et al.*, 2023). According to Arancon *et al.* (2019) using vermi tea as foliar application significantly improved the growth and yield of both lettuce and tomato even when the recommended nutrient ratio decreased to 25% and 50%. Spraying bak choy with vermi-tea improved plant growth with the highest leaf fresh weight (0.444 kg) when a concentration of 25% vermi-tea was used along with synthetic nutrient (Pearson *et al.*, 2023). Amino acids is another biostimulator and are the vital components of primary metabolism and serve as the building blocks in the synthesis of proteins, they have positive effects on plant growth, development and yield, also the ability to significantly reduce the impact of injuries and stresses from biotic and abiotic sources as they can enhance plant resistance to stresses (Kocira *et al.*, 2020). Seaweed extracts contain several complex biochemical compositions such as polysaccharides, hormones, as well as macro and micro nutrients, vitamins, amino acids, pigments, antioxidants, lipids and carbohydrates. However, their concentration and composition varies from species to another (Pereira *et al.*, 2020; El-Beltagi *et al.*, 2022). Furthermore, seaweed extracts can be used either as soil amendment or as foliar application, as it can improve plant tolerance to biotic and abiotic stress as well as enhancing nutrient uptake, root development, chlorophyll content, growth, yield and fruit quality (Mukherjee & Patel, 2020). Kocira *et al.* (2020) stated that amino acids and seaweed extracts both significantly increased yield, antioxidant levels, protein content, flavonoids, and phenolics in beans (*Phaseolus vulgaris* L.). Shafie *et al.* (2021) also stated that foliar application of seaweed extracts (2 ml L-1) and amino acids (3 ml L-1) on yarrow plants (*Achillea millefolium* L.) enhanced growth, antioxidant, and nutrient uptake.

MATERIALS AND METHODES

The factorial experiment was conducted in a plastic greenhouse of Horticulture Department, College of Agricultural Engineering Sciences, University of Sulaimani, Sulaymaniyah, Kurdistan Region of Iraq during 2024-2025 growing season. The site of the experiment is situated between 35°32'11.0" N latitude and 45°21'55.0" E longitude. The design of the experiment was randomized complete block design (R.C.B.D.) with forty-eight treatments divided on three replicates (sixteen treatments per replicate), and each treatment consisted of nine lettuce plants. The first factor (S) was inert substrates included perlite (S1), cocopeat (S2), cocopeat-perlite 3:1 (S3) and cocopeat-perlite 1:3 (S4). The second factor (B) was foliar application of biostimulators, which consisted of treatment without biostimulators (B0) vermi-tea (B1), amino acids with seaweed extract (B2) and the mixture of both types (B3). The experiment was conducted on two A-frame stands supported by twelve channels, and these channels are divided into 48 treatments that arranged vertically so that all treatments will be exposed to the same environment, each treatment separated by a plastic wall to avoid substrates and roots from mixing with each other. The shape of the channels was trapezoidal, and the area of each treatment was 80cm x 60cm. Each treatment consisted of three separated trapezoidal channels with the following dimensions: 80 cm x (top width 16 cm and bottom width 13.5 cm) x 14 cm. Pieces of filter were placed at the bottom of each channel to prevent the substrates from clogging drainage channels.

Experiment Materials

Romaine lettuce Farid F1 plants were used in this experiment, seedlings transplanted to the growing channels when four true leaves formed on 16th of November 2024. Tap water was used in this experiment, and analyzed by Lab. Food and Water, Directorate of Preventive Health, Directorate of Health - Sulaymaniyah before preparing the nutrient solution (Table 1).

Table 1: Some chemical properties of the tap water used to prepare nutrient solution.

Result	Name
7.90	pH
0.30 dS m^{-1}	EC
5.30 ppm	Na
1.20 ppm	K
5. ppm	NO_3
47 ppm	Ca
50 ppm	Mg

Nutrient solution used in this experiment was based on Cooper's standard nutrient solution with some adjustments made to it. Two ratios of nutrient solution were used for two different growth stages (Table 2).

Table 2: Types of salts and their weights used during the experiment to prepare the nutrient solution.

1st Ratio (22/11/2024 to 31/12/2024)	
Amount (g L^{-1})	Fertilizer name
0.75	NPK (30-10-10 + TE)
0.15	NPK (20-20-20 + TE)
0.25	Trace Elements
0.34	Mg
1 (foliar application)	Ca
2nd ratio (1/1/2025 – 25/3/2025)	
0.80	NPK (20-20-20 + TE)
0.25	Trace Elements
0.34	Mg
1 (foliar application)	Ca

pH, EC and total dissolved solids (TDS) of the prepared nutrient solution were checked with EC, TDS and pH meters after preparing the solution and before fertigation to ensure they are within the required range. During the experiment the pH ranged from 6.5-6.85, EC ranged from 1.4-1.8 dS m^{-1} and TDS ranged from 808-892ppm. Foliar application of Ca was carried out once every two weeks, and the first application was carried out after two weeks from transplanting. The experiment used an open system, and fertigation process was carried out by using drip irrigation system. The water pump attached to three tanks (200L each), these tanks are filled with the nutrient solution. Before fertigating the plants, the nutrient solution was properly mixed for five minutes by using a mixer installed within each tank, after ensuring that pH, EC and TDS were within the required range, the nutrient solution was pumped to the two A-frame stands. The flow rate of the pump is set on 0.9 L h^{-1}, and fertigation time and space between fertigation varied according to the surrounding environment, moisture content of the substrates and growth stage of the plants (Figure 1).

Figure 1: Fertigation system used during the experiment period.

Biostimulators preparation and application

Vermi-tea was prepared from vermicompost two days prior to foliar application process by soaking 2kg of vermicompost wrapped in a cloth sack in 5L of deionized water for 48 hours and supplied with oxygen. Then, 20 ml L^{-1} of brewed vermi-tea was diluted with deionized water and a drop of sodium sulfonate was added before foliar application process to reduce water surface tension (Cerna & Radores, 2022). Amino acids with seaweed extract solution was prepared for foliar application by diluting 2 ml in 1 liter of deionized water and a drop of sodium sulfonate was added to reduce water surface tension, then mixed properly in the spraying pump before foliar application. Foliar application of the biostimulators was conducted once every 14 days during the morning by wetting the plant completely, and the period between each type of foliar application of biostimulator was two days to avoid stressing out the plants. The first foliar application was conducted after two weeks from transplanting the seedlings to the growing channels, the process continued until the 7th application.

Head formation and data collection

Lettuce grown in S2 and S3 initiated head formation on 6th of January 2025, lettuce grown in S4 initiated head formation on 25th of February 2025, and lettuce grown in S1 substrate failed to form heads. Three plants selected randomly from each treatment to find the followings: Mineral content in folded leaves (including N, P, K, Mg, and Fe) which are found by following (Cresser & Parsons, 1979) procedure. Relative chlorophyll content in leaves found by using SPAD device. Plant yield, found by weighing the entire plant then the average mean was recorded. Total marketable yield was found by using equation given below:

$$\text{Total marketable yield } (\text{Mg h}^{-1}) = \frac{\text{N} \times \text{W}}{\text{A}} \times 10$$

- N= number of marketable plants per treatment.
- W= average fresh weight of a treatment (kg).
- A= area of the treatment (m^2).
- Factor 10=Megagram hectar^{-1}.

Nitrate concentration in folded and unfolded leaves, found by following the procedure of (Cataldo *et al.*, 1975). Concentration of carbohydrates was estimated by using (Joslyn, 1970) procedure. concentration of carotenoids was estimated by using (Choi *et al.*, 2023) procedure. Data were analyzed statically using a computer application XLSTAT v21.2.59614 (Addinsoft, 2021). Duncan multiple test range (α=0.05) was used to compare the means of the findings.

RESULTS AND DISCUSSION

Results shown in Table (3) indicates the significant effect of substrate factor on the content of N in the leaves as the maximum record was obtained from S2 substrate with a value of 2.87% and it was significantly higher than S1 treatment which gave 1.93%. While the highest record obtained from biostimulator factor was from B3 treatment (2.86%) compared to B0 treatment (2.09%) which gave the least significant difference than B3 by 26.31%. Results also showed that the interaction between study factors significantly affected leaf content of N in lettuce as treatment combination of S2B2 and S2B3 improved N content by 3.30 % with an increase ratio of 91.90% compared to S1B0 which gave less result to this treatment (1.72%).

Table (4) indicates the impact of different types of inert substrates and foliar applications with biostimulators on unfolded leaf content of lettuce plants under soilless culture conditions. Results shows that S2 contents of P in leaves was significantly higher than S1 as it recorded 0.28% in treatment S3 compared to S1 treatment which gave the least P content 0.21%. In biostimulator treatments, B3 treatment was significantly higher in P leaf content bye 0.29% compared to B0 which it gave a minimum result of 0.23% and was 23.33% less than B3. A significant increase in P leaf content was successful achieved from combination of S2B3 and S3B3 as they gave the highest result ratio of 0.33%, the increase was 73.70% higher than S1B1 as it gave the least P leaf content percentage (0.19%) as showed in Table (4).

Table 3: Impact of different types of inert substrates and foliar applications with biostimulators on Folded leaf content of N in lettuce plant (*Lactuca sativa* L.) under soilless culture conditions.

Mean of Substrate	Folded leaf content of N (%)				Biostimulator / Substrate
	Vermi-tea + amino acids with seaweeds extract (B3)	Amino acids with seaweeds extract (B2)	Vermi-tea (B1)	Without biostimulators (B0)	
1.93 c	2.12 d	1.83 ef	2.05 de	1.72 f*	Perlite (S1)
2.87 a	3.30 a	3.30 a	2.80 c	2.10 d e	Cocopeat (S2)
2.69 b	3.24 ab	2.23 d	2.97 bc	2.32 d	Cocopeat 3: Perlite 1 (S3)
2.77 ab	2.80 c	3.24 ab	2.83 c	2.23 d	Cocopeat 1: Perlite 3 (S4)
CV*=5.20	2.86 a	2.65 b	2.66 b	2.09 c	Mean of biostimulator

*Different letters indicate there is a significant difference between the means according to Duncan's multiple range test (α=0.05).
*CV stands for coefficient of variation

Table 4: Impact of different types of inert substrates and foliar applications with biostimulators on Folded leaf content of P in lettuce plant (*Lactuca sativa* L.) under soilless culture conditions.

Mean of Substrate	Folded leaf content of P (%)				Biostimulator / Substrate
	Vermi-tea + amino acids with seaweeds extract (B3)	Amino acids with seaweeds extract (B2)	Vermi-tea (B1)	Without biostimulators (B0)	
0.21 b	0.27 bc	0.21 de	0.19 e	0.20 e*	Perlite (S1)
0.28 a	0.33 a	0.3 abc	0.26 bcd	0.23 cde	Cocopeat (S2)
0.28 a	0.33 a	0.27 bc	0.27 bc	0.26 bcd	Cocopeat 3: Perlite 1 (S3)
0.25 a	0.26 bcd	0.29 ab	0.24 bcde	0.23 bcde	Cocopeat 1: Perlite 3 (S4)
CV=8.10	0.29 a	0.26 b	0.24 bc	0.23 c	Mean of biostimulator

*Different letters indicate there is a significant difference between the means according to Duncan's multiple range test (α=0.05).
*CV stands for coefficient of variation

Table (5) shows the impact of different types of inert substrates and foliar applications with biostimulators on unfolded leaf content of K in lettuce plant under soilless culture conditions. There was no significant difference between S2, S3, and S4, however they gave higher percentage of K content than S1 treatment as it recorded 2.29%. Similarly, B1, B2, and B3 shown no significant difference between them but they were higher than B0 as it recorded the least value (2.32%). A significant increase was achieved with a value of 63.20% from the interaction between cocopeat and amino acids with seaweed extract (S2B2) as it recorded the highest K leaf content (3.10%) compared to S1B0 treatment which gave the least value (1.90%) among the interaction treatments.

Table 5: Impact of different types of inert substrates and foliar applications with biostimulators on Folded leaf content of K in lettuce plant (*Lactuca sativa* L.) under soilless culture conditions.

Mean of Substrate	Folded leaf content of K (%)				Biostimulator / Substrate
	Vermi-tea + amino acids with seaweeds extract (B3)	Amino acids with seaweeds extract (B2)	Vermi-tea (B1)	Without biostimulators (B0)	
2.29 b	2.40 d	2.55 cd	2.32 d	1.90 e*	Perlite (S1)
2.86 a	3.02 a	3.10 a	2.81 abc	2.54 d	Cocopeat (S2)
2.75 a	2.96 a	2.61 bcd	2.92 a	2.52 cd	Cocopeat 3: Perlite 1 (S3)
2.75 a	2.86 ab	2.98 a	2.85 ab	2.33 cd	Cocopeat 1: Perlite 3 (S4)
CV=5.40	2.81 a	2.81 a	2.72 a	2.32 b	Mean of biostimulator

*Different letters indicate there is a significant difference between the means according to Duncan's multiple range test (α=0.05).
*CV stands for coefficient of variation

Results shown in Table (6) indicates that S2, S3, and S4 treatments shown no significant difference between each other. While S1 treatment shown the least significant difference (0.24%). Mg leaf content percentage was significantly affected by biostimulator application as B3 treatment gave the best result (0.36%) while the least percentage was recorded from B0 which gave 0.23%, while B1 and B2 treatments shown no significant difference between them. As showed in the table, interaction between inert substrates and biostimulators further improved Mg leaf content as combination treatment of S3B3 gave the best value (0.42%) this value was higher than that of S1B0 by 75% as S1B0 gave only 0.21%.

Result provided in Table (7) explains the significant effect of inert substrate on concentration of Fe in leaves as S3 substrate treatment gave the highest value which is 129.97 mg kg^{-1} dry weight, this value was higher than that obtained from S1 treatment by 35.65% as S1 treatment recorded 95.69 mg kg^{-1} dry weight. Applying biostimulators such as B3 further improved Fe concentration as it recorded the highest vale among other treatments (149.80 mg kg^{-1} dry weight) while the least value obtained from biostimulator treatments was from B0 (87.14 mg kg^{-1} dry weight). Same table shows that interaction between inert substrates and biostimulators significantly affected Fe concentration, the highest result recorded was from S3B3 combination as they gave 159.90 mg kg^{-1} dry weight of Fe concentration compared to S1B0 combination which gave the least result 71.70 mg kg^{-1} dry weight.

Table 6: Impact of different types of inert substrates and foliar applications with biostimulators on Folded leaf content of Mg in lettuce plant (*Lactuca sativa* L.) under soilless culture conditions.

Mean of Substrate	Folded leaf content of Mg (%)				Biostimulator Substrate
	Vermi-tea + amino acids with seaweeds extract (B3)	Amino acids with seaweeds extract (B2)	Vermi-tea (B1)	Without biostimulators (B0)	
0.24 b	0.28 def	0.24 fg	0.25 efg	0.21 g*	Perlite (S1)
0.30 a	0.36 bc	0.31 cd	0.32 cd	0.22 g	Cocopeat (S2)
0.31 a	0.42 a	0.27 def	0.31 cd	0.25 efg	Cocopeat 3: Perlite 1 (S3)
0.31 a	0.38 ab	0.30 de	0.29 def	0.27 def	Cocopeat 1: Perlite 3 (S4)
CV=8.10	0.36 a	0.28 b	0.29 b	0.23 c	Mean of biostimulator

*Different letters indicate there is a significant difference between the means according to Duncan's multiple range test (α=0.05).
*CV stands for coefficient of variation

Table 7: Impact of different types of inert substrates and foliar applications with biostimulators on concentration of Fe in folded leaves in lettuce plant (*Lactuca sativa* L.) under soilless culture conditions.

Mean of Substrate	Folded leaf content of Fe (mg kg^{-1} dry weight)				Biostimulator Substrate
	Vermi-tea + amino acids with seaweeds extract	Amino acids with seaweeds extract	Vermi-tea	Without biostimulators	
95.69 c	127.83 bc	94.90 def	88.34 efg	71.70 g*	Perlite (S1)
118.32 b	157.00 a	128.80 bc	109.35 cde	78.13 fg	Cocopeat (S2)
129.97 a	159.90 a	139.23 ab	118.20 bcd	102.55 de	Cocopeat 3: Perlite 1 (S3)
118.00 b	154.50 a	114.72 cd	106.60 cde	96.20 def	Cocopeat 1: Perlite 3 (S4)
CV=6.90	149.80 a	119.41 b	105.62 c	87.14 d	Mean of biostimulator

*Different letters indicate there is a significant difference between the means according to Duncan's multiple range test (α=0.05).
*CV stands for coefficient of variation

Table (8) indicates the influence of inert substrates and foliar application of biostimulators on relative chlorophyll content in lettuce leaves under soilless culture. According to the results, S2, S3, and S4 had no significant difference between them, but they were higher than S1 treatment as it recorded the least significant difference

(19.48 SPAD unit). While biostimulator treatments did not show any significant influence on relative chlorophyll content in leaves. Interaction between study factors affected relative chlorophyll content, as the highest result achieved from interaction between S4 and B2 (25.94) while the least chlorophyll content observed from (S1B2) treatment as it recorded 18.34 only as showed in the table below.

Table 8: Impact of different types of inert substrates and foliar applications with biostimulators on relative chlorophyll content in leaves of lettuce plant (*Lactuca sativa* L.) under soilless culture conditions.

Mean of Substrate	Relative chlorophyll content in leaves (SPAD unit)				Biostimulator / Substrate
	Vermi-tea + amino acids with seaweeds extract (B3)	Amino acids with seaweeds extract (B2)	Vermi-tea (B1)	Without biostimulators (B0)	
19.48 b	19.11 d	18.34 d	21.10 cd	19.40 d*	Perlite (S1)
24.36 a	24.70 ab	24.70 ab	23.74 abc	24.30 ab	Cocopeat (S2)
23.16 a	23.55 abc	22.11 bc	23.10 abc	23.90 abc	Cocopeat 3: Perlite 1 (S3)
24.16 a	23.99 abc	25.94 a	23.74 abc	22.98 abc	Cocopeat 1: Perlite 3 (S4)
CV=6.30	22.83 a	22.77 a	22.92 a	22.64 a	Mean of biostimulator

*Different letters indicate there is a significant difference between the means according to Duncan's multiple range test (α=0.05).
*CV stands for coefficient of variation

Results in Tables (3-7) show the significant impact S2 and S3 substrates on the concentration of the nutrients in lettuce leaves, and this might be due to the chemical and physical characteristics of cocopeat substrate, as it is characterized by its good porosity levels which in turn gave a good balance between water and air around the root system; thus, improved water and nutrients uptake (Mariotti *et al.*, 2020). Cocopeat substrate is also known for its ability to retain water, and have chelating properties as it can hold the nutrients and prevent them from leaching due to the presence of carboxyl and phenol groups, which has the ability to chelate K, Mg, Ca and NH_4 ions. This in turn allows the root system to absorb them easily (Agarwal *et al.*, 2023; Stelte *et al.*, 2023). Because of such properties, concentration of these minerals increased in plant tissues. Therefore, such increase in these minerals is beneficial to the plants. These results are in agreement with the results found by (Sahin, 2023; Kim & Yoo, 2024).

Results given in the same tables show the significant effect of biostimulator B2 treatment on concentration of minerals in lettuce leaves. This is due to the role of amino acids with seaweed extract in improving plant growth as amino acids are considered the primary building blocks of proteins in plants, is also involved in the pathways of creating enzymes and plant hormones which improve plant growth by enhancing carbon construction efficiency and ease nutrients absorption and transportation in the plant (Colla *et al.*, 2017). Treating plants with amino acids with seaweed extract can reduce energy consumption. Plants consume large amounts of energy in order to absorb N in the form of NO_3^- and NH_4^+ and convert it into amino acids. Thus, foliar application of amino acids with seaweed extract might have reduced energy that the plants used in other metabolic activities, and some of these amino acids are considered prefixes in the construction of some plant hormones (Baqir *et al.*, 2019; Altai *et al.*, 2020; Heidarzadeh, 2025). For instance, the amino acid tryptophan contributes as a precursor in the creation of IAA which promotes cell elongation and root growth, while the amino acid methionine contributes in the synthesis of ethelyn and polyamines which affect cell division, and the amino acid arginine involved in the production of nitric oxide (NO), which plays an essential role in regulating the opening and closing of the stomata (Bajguz & Piotrowska-Niczyporuk, 2023; Graska *et al.*, 2023). The increase in the concentration of Fe in the amino acids with seaweed extract treatment as showed in (Table 7) can be due to some amino acids such as glycine, as it can bind to with other trace elements such as Fe to form complexes that prevent the loss of these trace elements, and in turn it can be easily absorbed by the leaves or the root system; thus, improve plant growth due to the various roles of these trace elements in different metabolic activities (Trevisan *et al.*, 2024). Amino acids with seaweed extract had an essential role due to their content of phytohormones and hormone-like compounds, such as Glycosides which promotes cell division and elongation, Cytokinesis which delays plant aging by delaying chlorophyll degradation and Gibberellin (Ali *et al.*, 2021).

The increase in concentration of nutrients in the interaction between study factors might be due to the synergistic effect between the components, physical and chemical characteristics of cocopeat substrate and the essential contents of biostimulators and specifically amino acids with seaweed extract and their primary roles in plant metabolic activity (Colla *et al.*, 2017; Mariotti *et al.*, 2020). Vermi-tea is distinguished as a biostimulator as it contains high concentration of nutrient minerals (N, P, K, Mg and Ca) in an available form that can be absorbed easily by the plants (Aslam *et al.*, 2020). It also contains plant growth promoting rhizobacteria (PGPR), which can improve and enhance plants growth and yield (Musa *et al.*, 2018). Vermi-tea also known for its ability to improve tolerance toward biotic and abiotic stresses (Jiang *et al.*, 2023). The results obtained from this experiment are in agreement with the results found by (Khan *et al.*, 2015; Alkobaisy *et al.*, 2021). Furthermore, vermi-tea contains amino acids which are organic nitrogenous compounds contain high amount of N, when the plant absorbs these compounds, the root system releases bicarbonate (HCO_3^-) and hydroxide (OH^-) into the nutrient solution which in turn inhibited nitrate absorption (NO_3^-) and other anions (Ali *et al.*, 2022). Moreover, vermi-tea also contains natural chelated compounds which has a role in improving permeability of protective cell membranes in the roots, and this in turn enhances nutrients uptake (Arancon *et al.*, 2006). Therefore, an increase in Fe^+ in the plant can be due to the presences of chelated compounds and various acids in vermi-tea that might contributed in reducing Fe^{+3} to Fe^{+2} and chelating it which in turn increased its concentration in the plant as indicated in Table (7).

Results of B3 showed a significant impact on the concentration of nutrients in the leaves. This can be due to their contents of organic compounds and growth hormones contributed in increasing the concentration of these nutrients in the plant through improving root system growth and nutrient uptake (Battacharyya *et al.*, 2015; Elansary *et al.*, 2016). Furthermore, seaweed extracts contain polysaccharides, such as alginates and fucoidan which improved plant's ability to store water, and contain compounds similar to elicitors and betaine compounds that have an essential physiological role in plant as well as promoting defense mechanism against biotic and abiotic stresses (Kumar *et al.*, 2024). Thus, from the results obtained and showed in the tables mentioned above, increasing in nutrient concentration in plant contributed in promoting strong root system, which in turn improved nutrients and water uptake as well as improved vegetative growth characteristics, and this affected the quality and yield of lettuce heads.

Results showed in (Table 9) indicates the impact of inert substrates on the yield of lettuce plants under soilless culture conditions. The result of plant yield of lettuce plants grown in S2 treatment was significantly higher (443.95g) than those grown in S1 treatment (95.21g), while S3 and S4 had no significant difference between them. On the other hand, biostimulator treatments did not show any significant effect on plant yield. While an interaction between study factors (S2B0) obtained a significant record (526.41g) compared to S1B2 as it gave a minimum record of 50.10g which it was significantly lower than S2B0 by 90.50% as showed in the table below.

Table 9: Impact of different types of inert substrates and foliar applications with biostimulators on the yield of lettuce plant (*Lactuca sativa* L.) under soilless culture conditions.

Mean of Substrate	Plant yield (g)				Biostimulator / Substrate
	Vermi-tea + amino acids with seaweeds extract (B3)	Amino acids with seaweeds extract (B2)	Vermi-tea (B1)	Without biostimulators (B0)	
95.06 c	165.00 d	49.50 d	106.00 d	59.75 d*	Perlite (S1)
443.95 a	392.91 bc	487.25 ab	369.25 bc	526.41 a	Cocopeat (S2)
322.17 b	379.50 bc	278.10 c	316.00 c	315.20 c	Cocopeat 3: Perlite 1 (S3)
375.69 b	378.25 bc	397.12 bc	324.70 c	402.70 bc	Cocopeat 1: Perlite 3 (S4)
CV=22.30	328.91 a	303.11 a	278.98 a	326.01 a	Mean of biostimulator

*Different letters indicate there is a significant difference between the means according to Duncan's multiple range test (α=0.05).
*CV stands for coefficient of variation

Table (10) shows impact of different types of inert substrates and foliar applications with biostimulators on total marketable yield of lettuce plant under soilless culture conditions. Results shows that S2 treatment was significantly higher than other treatments by given total marketable yield of 48.40 Mg h^{-1} , S3 and S4 shown no significant difference between them, while lettuce grown in S1 treatment was unable to form any heads, thus gave 0 Mg h^{-1}. Treating lettuce with biostimulators did not give any significant effect on total marketable yield. However, when it comes to the interaction between study factors, there was a significant increase in S2B2 treatment, as it gave more yield compared to other treatments (55.90 Mg h^{-1}), while interaction treatments of perlite and biostimulators gave no yield due to lack of marketable heads (0 Mg h^{-1}) as showed in Table (10).

Table (11) explains the influence of inert substrate and biostimulators on the concentration of nitrate in folded leaves, inert substrates significantly affected nitrate concentration as the lowest value recorded from S4 treatment (2.40 mg g^{-1} dry weight), while there was no significant difference between S2 and S4 treatments. While the highest concentration was found in S1 treatment (2.47 mg g^{-1} dry weight). On the other hand, B3 treatment recorded a significant lower nitrate concentration (2.09 mg g^{-1} dry weight) compared to B0 and B1 treatments as they gave the highest nitrate concentration in leaves (2.48 and 2.57 mg g^{-1} dry weight, respectively) among the biostimulators which was 28.60% higher than B3 treatment. The interaction between inert substrate and biostimulators specially treatment (S3B0) recorded the highest nitrate concentration in leaves (2.74 mg g^{-1} dry weight) while S3B3 was significantly lower as it recorded 1.91 mg g^{-1} dry weight of nitrate concentration in folded leaves.

Table 10: Impact of different types of inert substrates and foliar applications with biostimulators on total marketable yield of lettuce plant (*Lactuca sativa* L.) under soilless culture conditions.

Mean of Substrate	Total marketable yield (Mg h^{-1})				Biostimulator / Substrate
	Vermi-tea + amino acids with seaweeds extract (B3)	Amino acids with seaweeds extract (B2)	Vermi-tea (B1)	Without biostimulators (B0)	
0 c	0 f	0 f	0 f	0 f*	Perlite (S1)
48.40 a	44.10 abc	55.90 a	39.40 bcde	54.22 ab	Cocopeat (S2)
36.63 b	42.62 abcd	35.60 cde	34.00 cde	34.33 cde	Cocopeat 3: Perlite 1 (S3)
29.57 b	38.23 bcde	24.30 e	26.25 de	29.50 cde	Cocopeat 1: Perlite 3 (S4)
CV=31.00	31.23 a	28.95 a	24.91 a	29.51 a	Mean of biostimulator

*Different letters indicate there is a significant difference between the means according to Duncan's multiple range test (α=0.05).
*CV stands for coefficient of variation

Table 11: Impact of different types of inert substrates and foliar applications with biostimulators on concentration of nitrate in folded leaves in lettuce plant (Lactuca sativa L.) under soilless culture conditions.

Mean of Substrate	Concentration of nitrate in folded leaves (mg g^{-1} dry weight)				Biostimulator Substrate
	Vermi-tea + amino acids with seaweeds extract (B3)	Amino acids with seaweeds extract (B2)	Vermi-tea (B1)	Without biostimulators (B0)	
2.47 a	2.30 cd	2.40 bcd	2.60 ab	2.60 ab*	Perlite (S1)
2.37 ab	2.20 def	2.25 cd	2.64 ab	2.40 bcd	Cocopeat (S2)
2.36 ab	1.91 f	2.30 cd	2.50 abcd	2.74 a	Cocopeat 3: Perlite 1 (S3)
2.26 b	1.97 ef	2.35 bcd	2.54 abc	2.20 de	Cocopeat 1: Perlite 3 (S4)
CV=6.00	2.09 c	2.32 b	2.57 a	2.48 a	Mean of biostimulator

*Different letters indicate there is a significant difference between the means according to Duncan's multiple range test (α=0.05).
*CV stands for coefficient of variation

According to the results showed in (Table 12) there was no significant impact on nitrate concentration in unfolded leaves of lettuce plants between substrate treatments. While (B3) recorded the least nitrate concentration in unfolded leaves (1.21 mg g^{-1} dry weight) which was significantly lower than B1 treatment as it gave 1.68 mg g^{-1} dry weight. Combining cocopeat and vermi-teat (S2B1) resulted in an increase in the nitrate accumulation by 66.40% as it recorded 1.83 mg g^{-1} dry weight compared to S3B3 treatment which yielded the least nitrate accumulation (1.1 mg g^{-1} dry weight) as showed in (Table 12).

Table 12: Impact of different types of inert substrates and foliar applications with biostimulators on concentration of nitrate in unfolded leaves of lettuce plant (Lactuca sativa L.) under soilless culture conditions.

Mean of Substrate	Concentration of nitrate in unfolded leaves (mg g^{-1} dry weight)				Biostimulator / Substrate
	Vermi-tea + amino acids with seaweeds extract (B3)	Amino acids with seaweeds extract (B2)	Vermi-tea (B1)	Without biostimulators (B0)	
1.50 a	1.40 def	1.44 cde	1.74 ab	1.43 cdef*	Perlite (S1)
1.46 a	1.21 fg	1.30 efg	1.83 a	1.53 bcd	Cocopeat (S2)
1.42 a	1.10 g	1.30 efg	1.64 abc	1.64 abc	Cocopeat 3: Perlite 1 (S3)
1.42 a	1.14 g	1.30 efg	1.54 bcd	1.72 ab	Cocopeat 1: Perlite 3 (S4)
CV=7.90	1.21 d	1.33 c	1.68 a	1.65 b	Mean of biostimulator

*Different letters indicate there is a significant difference between the means according to Duncan's multiple range test (α=0.05).
*CV stands for coefficient of variation

Table (13) illustrate the effect of different types of inert substrates and foliar applications with biostimulators on concentration of carbohydrates in leaves of lettuce plants under soilless culture conditions. Results given in Table (13) indicates the significant effect of inert substrate on carbohydrates in leaves as treatment with S2 substrate achieved the highest value for this trait (76.59 mg g^{-1} dry weight) while S1 and S4 substrate gave the least carbohydrates value 63.66 and 65.56 mg g^{-1} dry weight, respectively. Applying biostimulators improved carbohydrates concentration in leaves, as B3 treatment obtained the highest value 76.55 mg g^{-1} dry weight which was higher than B1 treatment by 16.90% as B1 treatment gave 65.5 mg g^{-1} dry weight of carbohydrates in leaves, however B0, B1, and B2 shown no significant difference between them. On the other hand, when S2 and B3 combined they gave the highest result possible among other treatment combinations (82.66 mg g^{-1} dry weight) while the least combination affected carbohydrates concentration was S1B1 treatment which recorded 60 mg g^{-1} dry weight.

Results showed in Table (14) significant success of S2 substrate treatment in concentration of carotenoids in leaves which recorded 553.09 mg $100g^{-1}$ dry weight compared to S1 substrate treatment (364.90 mg $100g^{-1}$ dry weight) as it was less than S2 by 51.60%. While B2 and B3 treatment among other biostimulators was able to achieve the highest value by recording 528.08 and 494.55 mg $100g^{-1}$ dry weight, respectively compared to B0 treatment which recorded the least (380.16 mg $100g^{-1}$ dry weight), B2 and B3 had no significant difference between each other. Combing S2 and B1 significantly improved concentration of carotenoids in lettuce leaves by recording 603.40 mg $100g^{-1}$ dry weight while the least result obtained from combination of S1B0 which recorded only 259.60 mg $100g^{-1}$ dry weight as showed in the Table (14) given below.

Table 13: Impact of different types of inert substrates and foliar applications with biostimulators on concentration of carbohydrates in leaves of lettuce plant (*Lactuca sativa* L.) under soilless culture conditions.

Mean of Substrate	Concentration of carbohydrates in leaves (mg g^{-1} dry weight)				Biostimulator / Substrate
	Vermi-tea + amino acids with seaweeds extract (B3)	Amino acids with seaweeds extract (B2)	Vermi-tea (B1)	Without biostimulators (B0)	
63.66 c	70.70 bcde	63.10 ef	60.00 f	60.85 f*	Perlite (S1)
76.59 a	82.66 a	75.08 abc	74.83 abc	73.79 bcd	Cocopeat (S2)
70.84 b	78.70 ac	72.31 bcd	65.60 def	66.76 cdef	Cocopeat 3: Perlite 1 (S3)
65.56 c	74.20 bc	65.55 def	61.51 f	60.98 f	Cocopeat 1: Perlite 3 (S4)
CV=7.70	76.56 a	69.01 b	65.48 b	65.59 b	Mean of biostimulator
*Different letters indicate there is a significant difference between the means according to Duncan's multiple range test (α=0.05). *CV stands for coefficient of variation					

Table 14: Impact of different types of inert substrates and foliar applications with biostimulators on concentration of carotenoids in leaves of lettuce plant (*Lactuca sativa* L.) under soilless culture conditions.

Mean of Substrate	Concentration of carotenoids in leaves (mg $100g^{-1}$ dry weight)				Biostimulator / Substrate
	Vermi-tea + amino acids with seaweeds extract (B3)	Amino acids with seaweeds extract (B2)	Vermi-tea (B1)	Without biostimulators (B0)	
364.91 d	429.02 fg	375.06 h	395.97 gh	259.60 i*	Perlite (S1)
553.09 a	581.21 a	579.40 a	603.40 a	448.35 f	Cocopeat (S2)
524.13 b	566.59 abc	530.16 cde	570.53 ab	429.27 fg	Cocopeat 3: Perlite 1 (S3)
484.15 c	535.53 bcd	493.61 e	524.05 de	383.43 h	Cocopeat 1: Perlite 3 (S4)
CV=4.80	528.08 a	494.55 a	523.48 b	380.16 c	Mean of biostimulator
*Different letters indicate there is a significant difference between the means according to Duncan's multiple range test (α=0.05). *CV stands for coefficient of variation					

According to the result given in Table (6) and (8), there was an increase in leaves content of Mg in S2 and S3 substrates and biostimulator mixture B3 treatment, which in turn increased the intensity of chlorophyll in leaves, an increase in chlorophyll content resulted in an increase in photosynthesis. Concentration of N nutrient in treatment S2 (Table 3) led to an increase in relative chlorophyll content in leaves (8), this led to an increase in plant metabolism by accumulation of carbohydrates and saccharase (Noor et al., 2023).

Table (10) indicates the positive impact of cocopeat substrate (S2) as well as the interaction treatment of S2B2 on total marketable yield of lettuce plant, such superiority compared to other treatments can be due to the various beneficial contents in biostimulator (B2), such as plant growth hormones and chelating compounds, which had a positive role in increasing plant growth and metabolism, this in turn positively affected total marketable yield of lettuce plant (İkiz *et al.*, 2024).

Table (13) refers to the significant impact of S2 and biostimulator mixture B3 as well as the combination of S2B3 on concentration of carbohydrates in lettuce leaves, such result can be due to the role of various content in biostimulator mixture which reduced or delayed chlorophyll degradation process and prolonged green pigment age as well as its content of different types of plant growth hormones such as auxins, gibberellins and cytokinins which improved photosynthesis efficiency. Therefore, such process led to an increase in carbohydrates accumulation in leaves (Pasković *et al.*, 2024). The Delay in chlorophyll degradation led to continuous photosynthesis process. This in turn led to accumulation of carbohydrates, which also affected concentration of carotenoids in leaves by increasing its concentration as indicated in Table (14). Increase in concentration of carotenoids is due to the content of biostimulator mixture B2 and B3 of phosphorus and potassium that promotes pigment formation and enzymes that are connected to photosynthesis process (Ashour *et al.*, 2024; Dasgan *et al.*, 2024).

Results from Table (12) show the significant decreases in nitrate accumulation in unfolded leaves of lettuce in S3 substrate and in biostimulator mixture (B3), this might be due to the aging process of old leaves which in turn decreased nitrate absorption and increased reduction activities due to high nitrate reductase enzyme activity which is responsible for converting nitrate to ammonium then to amino acids (Nieves-Silva *et al.*, 2024). On the other hand, accumulation of proteins and other organic compounds took place in unfolded leaves due to reducing nitrate to amino acids and proteins, and since unfolded leaves are exposed to light more than folded leaves, there will be higher rates of photosynthesis and metabolism activities which in turn increase N reductase enzyme activity, and this will further decreases nitrate accumulation; therefore, decreases its concentration in unfolded leaves (Zayed *et al.*, 2023).

Similarly, a significant decrease in nitrate accumulation in folded leaves in combination treatment of S3B3 as indicated in Table (11) can be related to the content of vermi-tea of various dissolved organic compounds, amino acids and plant hormones which promoted nitrate reductase enzyme and increased its activity. With such an increase in the enzyme activity, it led to an increase in nitrate conversion to NH_4^+ and amino acids. Vermi-

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tea is known for its content of fulvic acid and humic acid along with various essential nutrients (mostly N), which contributed in increasing photosynthesis process (Kocaman *et al.*, 2024). Since cocopeat is known for its ability to store high amounts of water and nutrients (Stelte *et al.*, 2023). Therefore, there was an increase in photosynthesis, and this increased the joining process of N with amino acids to form proteins, in turn reduced nitrate accumulation in folded leaves (Ruiz *et al.*, 2000). Vermi tea also known for its content of amines and peptides which might inhibit nitrate accumulation due to the presence of these compounds for the plants, as well as the presences of various beneficial microorganisms that contributed in nitrate reduction in leaves to different forms; thus, might be resulted in a decrease in nitrate concentration in folded leaves (Teixeira *et al.*, 2018).

Conclusion

Based on the results obtained from this experiment, we can conclude that Cocopeat-based substrate (S2 and S3) consistently outperformed perlite based substrates (S1 and S4). Foliar application of amino acids with seaweed extracts (B2) and biostimulator mixture of vermi-tea and amino acids with seaweed extract (B3) gave the best improvement in terms of yield quality characteristics and nutrients uptake. Interaction between substrates and biostimulators gave the best result in yield and yield quality. Lettuce grown in S2 and S3 substrates formed heads approximately 50 days earlier than S1 and S4 substrates. Conversely, lettuce grown in S1 only failed to form marketable heads and overall performed poorly.

Recommendations

Based on the results achieved from this experiment, the following recommendations are given: It is recommended using cocopeat alone or mixed with other inert substrates to grow vegetables. Foliar application with biostimulators to improve growth, yield, and yield quality characteristics of vegetable crops. Preparing modified cooper's standard nutrient solution from locally available nutrients. It is recommended using channels with wider spaces and depth to produce better yield and quality of vegetables. It is recommended using other organic inert substrates such as corn husks, rice husks, and wheat straws with biostimulators in different types of vegetables.

References

Addinsoft. (2021). *XLSTAT*. In (Version v21.2.59614) [Computer software]. https://www.xlstat.com/

Agarwal, P., Saha, S., & Hariprasad, P. (2023). Agro-industrial-residues as potting media: Physicochemical and biological characters and their influence on plant growth. *Biomass conversion and biorefinery*, *13*(11), 9601–9624. https://doi.org/10.1007/s13399-021-01998-6

Al Meselmani, M. A. (2022). Nutrient solution for hydroponics. In *Recent Research and Advances in Soilless Culture*. IntechOpen. https://doi.org/10.5772/intechopen.101604

Alam, T., Ikram, M., Chaudhry, A. N., Subhan, C. M., Alotaibi, K. D., -Haq, Z.-U., Yousaf, M. S., Ahmed, H. P., Fatima, N., & Jilani, G. (2024). Utilization of organic-residues as potting media: Physico-chemical characteristics and their influence on vegetable production. *PLoS One*, *19*(6), 0302135. https://doi.org/10.1371/journal.pone.0302135

Ali, N. S., Allawi, M. M., & Majeed, N. H. (2022). Rhizosphere management and agricultural sustainability (Vol. 324). Ministry of Higher Education and Scientific Research.

Ali, O., Ramsubhag, A., & Jayaraman, J. (2021). Biostimulant properties of seaweed extracts in plants: Implications towards sustainable crop production. *Plants*, *10*(3), 531. https://doi.org/10.3390/plants10030531

Alkobaisy, J., Abdel Ghani, E., Mutlag, N., & Lafi, A. S. A. (2021). Effect of vermicompost and vermicompost tea on the growth and yield of broccoli and some soil properties. IOP Conference Series: Earth and Environmental Science,

Altai, D. S., Alhasany, A. R., & Al Tameemi, K. A. (2020). Role of Humic Acid and Amino Acids in Increasing Growth and Productivity of Mungbean Varieties Grown under Newly Reclaimed Soil. *Indian Journal of Ecology*, *47*(10), 11–16. https://systems.uomisan.edu.iq/cv/uploads/files/05jq2sibkm3dpga.pdf

Arancon, N. Q., Edwards, C. A., Lee, S., & Byrne, R. (2006). Effects of humic acids from vermicomposts on plant growth. *European journal of soil biology*, *42*, S65–S69. https://doi.org/10.1016/j.ejsobi.2006.06.004

Arancon, N. Q., Owens, J. D., & Converse, C. (2019). The effects of vermicompost tea on the growth and yield of lettuce and tomato in a non-circulating hydroponics system. *Journal of Plant nutrition*, *42*(19), 2447–2458. https://doi.org/10.1080/01904167.2019.1655049

Ashour, M., Khairy, H. M., Bakr, A., Matter, M., & Alprol, A. E. (2024). Seaweed liquid extract AS novel sustainable solutions for phycobioremediation plant germination, and feed additive for marine invertebrate copepod. *Scientific reports*, *14*(1), 29553. https://doi.org/10.1038/s41598-024-80389-z

Aslam, Z., Ahmad, A., Bellitürk, K., Iqbal, N., Idrees, M., Rehman, W. U., Akbar, G., Tariq, M., Raza, M., & Riasat, S. (2020). Effects of vermicompost, vermi-tea and chemical fertilizer on morpho-physiological characteristics of tomato (Solanum lycopersicum) in Suleymanpasa District, Tekirdag of Turkey. *Pure and Applied Biology (PAB)*, *9*(3), 1920–1931. http://dx.doi.org/10.17582/journal.jis/2020/6.1.41.46

Aslam, Z., Ahmad, A., Bellitürk, K., Kanwal, H., Asif, M., & Ullah, E. (2023). Integrated use of simple compost, vermicompost, vermi-tea and chemical fertilizers NP on the morpho-physiological, yield and yield related traits of tomato (Solanum lycopersicum L.). *J. Innov. Sci*, *9*(1), 1–12. https://dx.doi.org/10.17582/journal.jis/2023/9.1.1.12

Bajguz, A., & Piotrowska-Niczyporuk, A. (2023). Biosynthetic pathways of hormones in plants. *Metabolites*, *13*(8), 884. https://doi.org/10.3390/metabo13080884

Baqir, H., Zeboon, N., & Al-Behadili, A. (2019). The role and importance of amino acids within plants: A review. *Plant Archives*, *19*(2), 1402–1410. https://www.researchgate.net/publication/349966775_the_role_and_importance_of_amino_acids_within_plants_a_review

Bar-Tal, A., Saha, U. K., Raviv, M., & Tuller, M. (2019). Inorganic and synthetic organic components of soilless culture and potting mixtures. In M. Raviv, J. H. Lieth, & A. Bar-Tal (Eds.), *Soilless culture: Theory and practice* (2nd ed., pp. 259–301). Elsevier. https://shop.elsevier.com/books/soilless-culture-theory-and-practice/raviv/978-0-444-63696-6

Battacharyya, D., Babgohari, M. Z., Rathor, P., & Prithiviraj, B. (2015). Seaweed extracts as biostimulants in horticulture. *Scientia Horticulturae*, *196*, 39–48. https://doi.org/10.1016/j.scienta.2015.09.012

Bihari, C., Ahamad, S., Kumar, M., Kumar, A., Kamboj, A. D., Singh, S., Srivastava, V., & Gautam, P. (2023). Innovative Soilless Culture Techniques for Horticultural Crops: A Comprehensive Review. *International Journal of Environment and Climate Change*, *13*(10), 4071–4084. https://doi.org/10.9734/IJECC/2023/v13i103084

Cataldo, D. A., Maroon, M., Schrader, L. E., & Youngs, V. L. (1975). Rapid colorimetric determination of nitrate in plant tissue by nitration of salicylic acid. *Communications in soil science and plant analysis*, *6*(1), 71–80. https://doi.org/10.1080/00103627509366547

Central Statistical Organization of Iraq. (2023). *Secondary Crops and Vegetables Report by Governorate for the Year 2022*. C. S. Organization. https://cosit.gov.iq/documents/agriculture/agri_other/full%20reports/تقرير%20المحاصيل%20الثانوية%20والخضروات%20حسب%20المحافظات%202022.pdf

Cerna, G. M. Z. D., & Radores, L. C. (2022). Response of Cucumber (Cucumis sativus L.) Plants to Vermi Tea as Organic Fertilizer Supplementation. *Asian Journal of Advances in Agricultural Research*, *18*(1), 22–29, Article AJAAR.84241. https://doi.org/10.9734/AJAAR/2022/v18i130209

Choi, J., An, J., Lee, H.-D., Kim, W. J., Lee, S., & Lee, S. (2023). Comprehensive analysis of phenolic compounds, carotenoids, and antioxidant activities in Lactuca sativa var. longifolia cultivated in a smart farm system. *Processes*, *11*(10), 2993. https://doi.org/10.3390/pr11102993

Ciriello, M., Formisano, L., Pannico, A., El-Nakhel, C., Fascella, G., Duri, L. G., Cristofano, F., Gentile, B. R., Giordano, M., & Rouphael, Y. (2021). Nutrient solution deprivation as a tool to improve hydroponics sustainability: Yield, physiological, and qualitative response of lettuce. *Agronomy*, *11*(8), 1469. https://doi.org/10.3390/agronomy11081469

Colla, G., Hoagland, L., Ruzzi, M., Cardarelli, M., Bonini, P., Canaguier, R., & Rouphael, Y. (2017). Biostimulant action of protein hydrolysates: Unraveling their effects on plant physiology and microbiome. *Frontiers in Plant Science*, *8*, 1–22. https://doi.org/10.3389/fpls.2017.02202

Combrink, N. J. J., & Kempen, E. (2019). Nutrient solution management.

Department of Agronomy Stellenbosch University. https://www.researchgate.net/publication/339447423_Nutrienet_solution_management

Cresser, M., & Parsons, J. (1979). Sulphuric—Perchloric acid digestion of plant material for the determination of nitrogen, phosphorus, potassium, calcium and magnesium. *Analytica Chimica Acta*, *109*(2), 431–436. https://doi.org/10.1016/S0003-2670(01)84273-2

Dasgan, H. Y., Aksu, K. S., Zikaria, K., & Gruda, N. S. (2024). Biostimulants enhance the nutritional quality of soilless greenhouse tomatoes. *Plants*, *13*(18), 2587. https://doi.org/10.3390/plants13182587

El-Beltagi, H. S., Mohamed, A. A., Mohamed, H. I., Ramadan, K. M., Barqawi, A. A., & Mansour, A. T. (2022). Phytochemical and potential properties of seaweeds and their recent applications: A review. *Marine drugs*, *20*(6), 342. https://doi.org/10.3390/md20060342

Elansary, H. O., Skalicka-Woźniak, K., & King, I. W. (2016). Enhancing stress growth traits as well as phytochemical and antioxidant contents of Spiraea and Pittosporum under seaweed extract treatments. *Plant Physiology and Biochemistry*, *105*, 310–320. https://doi.org/10.1016/j.plaphy.2016.05.024

Fussy, A., & Papenbrock, J. (2022). An overview of soil and soilless cultivation techniques—chances, challenges and the neglected question of sustainability. *Plants*, *11*(9), 1153. https://doi.org/10.3390/plants11091153

Graska, J., Fidler, J., Gietler, M., Prabucka, B., Nykiel, M., & Labudda, M. (2023). Nitric oxide in plant functioning: metabolism, signaling, and responses to infestation with Ecdysozoa parasites. *Biology*, *12*(7), 927. https://doi.org/10.3390/biology12070927

Heidarzadeh, A. (2025). Role of amino acids in plant growth, development, and stress responses: A comprehensive review. *Discover Plants*, *2*(1), 1–31. https://doi.org/10.1007/s44372-025-00322-0

İkiz, B., Dasgan, H. Y., Balik, S., Kusvuran, S., & Gruda, N. S. (2024). The use of biostimulants as a key to sustainable hydroponic lettuce farming under saline water stress. *BMC Plant Biology*, *24*(1), 808. https://doi.org/10.1186/s12870-024-05520-8

Jiang, X., Lu, C., Hu, R., Shi, W., Zhou, L., Wen, P., Jiang, Y., & Lo, Y. M. (2023). Nutritional and microbiological effects of vermicompost tea in hydroponic cultivation of maple peas (Pisum sativum var. arvense L.). *Food Science and Nutrition*, *11*(6), 3184–3202. https://doi.org/10.1002/fsn3.3299

Jindo, K., Goron, T. L., Pizarro-Tobías, P., Sánchez-Monedero, M. Á., Audette, Y., Deolu-Ajayi, A. O., van der Werf, A., Goitom Teklu, M., Shenker, M., & Pombo Sudré, C. (2022). Application of biostimulant products and biological control agents in sustainable viticulture: A review. *Frontiers in Plant Science*, *13*, 932311. https://doi.org/10.3389/fpls.2022.932311

Jordan, R. A., Ribeiro, E. F., Oliveira, F. C. d., Geisenhoff, L. O., & Martins, E. A. (2018). Yield of lettuce grown in hydroponic and aquaponic systems using different substrates. *Revista Brasileira de Engenharia Agrícola e Ambiental*, *22*(8), 525–529. https://doi.org/10.1590/1807-1929/agriambi.v22n8p525-529

Joshi, T. N., Nepali, D. B., Sah, R., Bhattarai, T., & Midmore, D. J. (2020). A comparison of composting and vermicomposting for the disposal of poultry waste. *Animal Production Science*, *60*(7), 986–992. https://doi.org/10.1071/AN17177

Joslyn, M. A. (1970). Methods in food analysis: physical, chemical, and instrumental methods of analysis. Academic Press. https://openlibrary.org/books/OL5073765M/Methods_in_food_analysis_physical_chemical_and_instrumental_methods_of_analysis

Khan, F., Eudoxie, G., & Martin, M. (2015). Effects of compost tea source and application method on lettuce (Lactuca sativa) yield and nutrient content, grown in a vermicompost amended medium. International Symposium on Growing Media, Composting and Substrate Analysis-SusGro2015 1168,

Kim, S. K., & Yoo, Y. K. (2024). Mixing ratio of peat moss, coco peat, and perlite affects the physicochemical properties of media and growth of Tetragonia tetragonioides. *Horticultural Science and Technology*, *42*(1), 15–27. https://doi.org/10.7235/HORT.20240002

Kocaman, A., İnci, Y., Kıtır, N., Turan, M., Argın, S., Yıldırım, E., Giray, G., Ersoy, N., Güneş, A., & Katırcıoğlu, H. (2024). The effect of novel biotechnological vermicompost on tea yield, plant nutrient content, antioxidants, amino acids, and organic acids as an alternative to chemical fertilizers for sustainability. *BMC Plant Biology*, *24*(1), 868. https://doi.org/10.1186/s12870-024-05504-8

Kocira, S., Szparaga, A., Hara, P., Treder, K., Findura, P., Bartoš, P., & Filip, M. (2020). Biochemical and economical effect of application biostimulants containing seaweed extracts and amino acids as an element of agroecological management of bean cultivation. *Scientific reports*, *10*(1). https://doi.org/10.1038/s41598-020-74959-0

Kotzen, B., Emerenciano, M. G. C., Moheimani, N., & Burnell, G. M. (2019). Aquaponics: Alternative types and approaches. In *Aquaponics food production systems: Combined aquaculture and hydroponic production technologies for the future* (pp. 301–330). Springer Nature. https://doi.org/10.1007/978-3-030-15943-6

Kumar, G., Nanda, S., Singh, S. K., Kumar, S., Singh, D., Singh, B. N., & Mukherjee, A. (2024). Seaweed extracts: enhancing plant resilience to biotic and abiotic stresses. *Frontiers in Marine Science*, *11*, 1457500. https://doi.org/10.3389/fmars.2024.1457500

Mariotti, B., Martini, S., Raddi, S., Tani, A., Jacobs, D. F., Oliet, J. A., & Maltoni, A. (2020). Coconut coir as a sustainable nursery growing media for seedling production of the ecologically diverse Quercus species. *Forests*, *11*(5), 522. https://doi.org/10.3390/f11050522

Monsalve Camacho, O. I., Henao Toro, M. C., & Gutiérrez Díaz, J. S. (2021). Characterizing potential substrate materials in soilless culture systems. *Ciencia y Tecnología Agropecuaria*, *22*(1), 1977. https://doi.org/10.21930/rcta.vol22_num1_art:1977

Mounaimi, S., Hamim, A., El Boukhari, M. E. M., Elarroussi, H., & Lyamlouli, K. (2024). A comprehensive review of integrating biostimulants and biopesticides for organic berry farming: exploring challenges and opportunities for Africa. *Frontiers in Sustainable Food Systems*, *8*, 1–24. https://doi.org/10.3389/fsufs.2024.1452823

Mukherjee, A., & Patel, J. (2020). Seaweed extract: biostimulator of plant defense and plant productivity. *International Journal of Environmental Science and Technology*, *17*(1), 553–558. https://doi.org/10.1007/s13762-019-02442-z

Musa, S., Njoku, L., & Ndiribe, C. (2018). The effect of vermi tea on the growth parameters of Spinacia oleracea L.(Spinach). *Journal of Environmental Science and Pollution Research*, *3*(4), 236–238. https://doi.org/10.13140/RG.2.2.25268.71041

Nieves-Silva, E., Sandoval-Castro, E., Delgado-Alvarado, A., Castañeda-Antonio, M. D., & Huerta-De la Peña, A. (2024). Nitrate Reductase and Glutamine Synthetase Enzyme Activities and Chlorophyll in Sorghum Leaves (Sorghum bicolor) in Response to Organic Fertilization. *International Journal of Plant Biology*, *15*(3), 827–836. https://doi.org/10.3390/ijpb15030059

Pasković, I., Popović, L., Pongrac, P., Polić Pasković, M., Kos, T., Jovanov, P., & Franić, M. (2024). Protein hydrolysates—Production, effects on plant metabolism, and use in agriculture. *Horticulturae*, *10*(10), 1041. https://doi.org/10.3390/horticulturae10101041

Pearson, C., Gawel, R., & Maki, D. (2023). Effects of vermicompost and beneficial microbes on biomass and nutrient density in purple lady bok choy (Brassica rapa var. chinensis) in a vertical hydroponic grow tower system. *Fine Focus*, *9*(1), 46–64. https://doi.org/10.33043/FF.9.1.46-64

Pereira, L., Bahcevandziev, K., & Joshi, N. H. (2020). Seaweeds as plant fertilizer, agricultural biostimulants and animal fodder. CRC Press Boca Raton, FL, USA. https://doi.org/10.1201/9780429487156-1

Rahman, M. J., Chawdhery, M. R. A., Begum, P., Quamruzzaman, M., Zakia, M. Z., & Raihan, A. (2019). Growth and yield of hydroponic lettuce as influenced by different growing substrates. https://doi.org/10.29252/azarinj.003

Raj, A. K., Vijayalatha, K., Jegadeeswari, V., Ahamed, A. S., & Pandian, P. S. (2023). Effect of media on growth and yield of lettuce under different hydroponic system. *The Pharma Innovation*, *12*(8), 1748–1753. https://doi.org/10.26832/24566632.2022.070103

Rajendran, S., Domalachenpa, T., Arora, H., Li, P., Sharma, A., & Rajauria, G. (2024). Hydroponics: Exploring innovative sustainable technologies and applications across crop production, with Emphasis on potato mini-tuber cultivation. *Heliyon*, *10*(5), e26823.

ISSN 2072-3857

https://doi.org/10.1016/j.heliyon.2024.e26823

Ruiz, J. M., Castilla, N., & Romero, L. (2000). Nitrogen metabolism in pepper plants applied with different bioregulators. *Journal of Agricultural and Food Chemistry*, *48*(7), 2925–2929. https://doi.org/10.1021/jf990394h

Sahin, O. (2023). Growing Media Effect on Essential and Non-essential mineral composition of Lettuce. *Journal of Plant nutrition*, *46*(5), 665–674. https://doi.org/10.1080/01904167.2022.2087087

Sambo, P., Nicoletto, C., Giro, A., Pii, Y., Valentinuzzi, F., Mimmo, T., Lugli, P., Orzes, G., Mazzetto, F., & Astolfi, S. (2019). Hydroponic solutions for soilless production systems: issues and opportunities in a smart agriculture perspective. *Frontiers in Plant Science*, *10*, 923. https://doi.org/10.3389/fpls.2019.00923

Shafie, F., Bayat, H., Aminifard, M. H., & Daghighi, S. (2021). Biostimulant effects of seaweed extract and amino acids on growth, antioxidants, and nutrient content of yarrow (Achillea millefolium L.) in the field and greenhouse conditions. *Communications in soil science and plant analysis*, *52*(9), 964–975. https://doi.org/10.1080/00103624.2021.1872596

Shi, M., Gu, J., Wu, H., Rauf, A., Emran, T. B., Khan, Z., Mitra, S., Aljohani, A. S., Alhumaydhi, F. A., & Al-Awthan, Y. S. (2022). Phytochemicals, nutrition, metabolism, bioavailability, and health benefits in lettuce—A comprehensive review. *Antioxidants*, *11*(6), 1158. https://doi.org/10.3390/antiox11061158

Singh, A., Singh, J., Kaur, S., Gunjal, M., Kaur, J., Nanda, V., Ullah, R., Ercisli, S., & Rasane, P. (2024). Emergence of microgreens as a valuable food, current understanding of their market and consumer perception: A review. *Food Chemistry: X*, *23*, 101527. https://doi.org/10.1016/j.fochx.2024.101527

Stelte, W., Reddy, N., Barsberg, S., & Sanadi, A. R. (2023). Coir from Coconut Processing Waste as a Raw Material for Applications Beyond Traditional Uses. *BioResources*, *18*(1), 2187–2212. https://doi.org/10.15376/biores.18.1.Stelte

Teixeira, W. F., Fagan, E. B., Soares, L. H., Soares, J. N., Reichardt, K., & Neto, D. D. (2018). Seed and foliar application of amino acids improve variables of nitrogen metabolism and productivity in soybean crop. *Frontiers in Plant Science*, *9*, 396. https://doi.org/10.3389/fpls.2018.00396

Trevisan, F., Waschgler, F., Tiziani, R., Cesco, S., & Mimmo, T. (2024). Exploring glycine root uptake dynamics in phosphorus and iron deficient tomato plants during the initial stages of plant development. *BMC Plant Biology*, *24*(1), 495. https://doi.org/10.1186/s12870-024-05120-6

Tuckeldoe, R. B., Maluleke, M. K., & Adriaanse, P. (2023). The effect of coconut coir substrate on the yield and nutritional quality of sweet peppers (Capsicum annuum) varieties. *Scientific reports*, *13*(1), 2742. https://doi.org/10.1038/s41598-023-29914-0

Verasoundarapandian, G., Zakaria, N. N., Shaharuddin, N. A., Khalil, K. A., Puasa, N. A., Azmi, A. A., Gomez-Fuentes, C., Zulkharnain, A., Wong, C. Y., & Rahman, M. F. (2021). Coco peat as agricultural waste sorbent for sustainable diesel-filter system. *Plants*, *10*(11), 2468. https://doi.org/10.3390/plants10112468

Zayed, O., Hewedy, O. A., Abdelmoteleb, A., Ali, M., Youssef, M. S., Roumia, A. F., Seymour, D., & Yuan, Z.-C. (2023). Nitrogen journey in plants: From uptake to metabolism, stress response, and microbe interaction. *Biomolecules*, *13*(10), 1443. https://doi.org/10.3390/biom13101443