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Mixotrophy of Algae: More Algal Biomass and More Biofertilization for Plants

Nermin El Semary [1,2,*], Amira Mohamed Abd El-Sattar [2,†], Eman Zakaria Ahmed [2,†] and Munirah Aldayel [1]

[1] Biological Sciences Department, College of Science, King Faisal University, Al-Ahsa 31982, Saudi Arabia
[2] Botany and Microbiology Department, Faculty of Science, Helwan University, Cairo 11790, Egypt
* Correspondence: nelsemary@kfu.edu.sa
† These authors contributed equally to this work.

Abstract: Background: The use of algae as biofertilizers is fast-spreading in order to meet the excessive demands for agricultural products. To achieve this, enough algal biomass needs to be supplied year-round. Hence, algal nutritional components must be optimized through mixotrophic conditions. Materials and methods: Two algal isolates, namely, *Phormidium* sp. and *Synechocystis* sp. were tested for their ability to produce mixotrophic growth using different supplementations including molasses, aqueous *Lepidium sativum*, *Trigonella foenum graecum* seed extract and *liquorice* root extract, as well as acetate salt solution. The algae that showed highest growth under optimized mixotrophic conditions was further used in cantaloupe seed growth experiments. GC-MS was also carried out on the biomass of *Phormidium* on one of the fractions of extract using solvent system to reveal some dominant novel bioactive compounds in algal biomass. Results: The sugarcane molasses significantly enhanced the growth of the two algal strains, followed by *Lepidium sativum* extract only in case of *Phormidium* sp. Therefore, it was used in subsequent experiments. All growth parameters for that algae were significantly enhanced by the addition of these nutritional sources with molasses being the best supplement. The *Phormidium* sp. was rich in its content of chlorophyll, proteins, sugars as well as some novel bioactive compounds as revealed by GC–MS. The germination percentage of seeds treated with *Phormidium* sp. showed a significant increase over that of control. The different growth-related metabolites of total soluble proteins, total soluble sugars and all photosynthetic pigment contents of the seedlings were all significantly increased using this algal treatment. Discussion: The sugarcane molasses was superior in enhancing the algal growth due to its rich content not only of sugars but also of minerals and nitrogenous compounds. The use of aqueous extracts of seeds of *Lepidium sativum* enhanced growth significantly more than that of the control set as seeds are rich in proteins, omega-3 fatty acids, phytochemicals and other essential nutrients. In growth experiments carried out on cantaloupe seeds, there was a significant increase in germination percentage as well as all growth parameters due to the rich nutritional content of *Phormidium* sp. Conclusion: Mixotrophic growth achieved better algal biomass production than autotrophy in the case of *Phormidium* sp. The use of cheap resources such as sugarcane molasses, which is the waste from the sugar industry, as well as the common herb extract of *Lepidium sativum*, is a cost-effective approach. The use of this mixotrophically grown blue-green alga as a biofertilizer significantly enhanced plant growth and seed germination, indicating the usefulness of this eco-friendly agricultural strategy for achieving both food security and environmental sustainability.

Keywords: biofertilizers; cyanobacteria; *Lepidium sativum* extract; mixotrophy; sugarcane molasses

Citation: El Semary, N.; Abd El-Sattar, A.M.; Ahmed, E.Z.; Aldayel, M. Mixotrophy of Algae: More Algal Biomass and More Biofertilization for Plants. *Sustainability* **2023**, *15*, 5815. https://doi.org/10.3390/su15075815

Academic Editor: José Carlos Magalhães Pires

Received: 25 February 2023
Revised: 21 March 2023
Accepted: 24 March 2023
Published: 27 March 2023

1. Introduction

Cyanobacteria (blue-green algae) are photosynthetic organisms that contain chlorophyll a. They are of ubiquitous distribution all over the world. Although being autotrophic, many species are capable of heterotrophy [1]. The latter enables algae to use organic

compounds as carbon and energy sources. In addition, many algae are able to grow in absence of light through utilizing organic compounds in their growth medium [2]. Many organic compounds can be used including sugars such as glucose and organic acids such as acetate [3]. Acetate is the most favorable as it easily enters the Krebs cycle leading to the high energy yield needed for the proliferation of algal cells [4,5]. However, volatile fatty acids such as acetate or butyrate can inhibit algal growth at concentrations above 0.5–1 g $C.L^{-1}$ [6]. Nonetheless, they found that decreasing undissociated acid levels at pH to 8.0 allowed growth without inhibition up to 5 g $C.L^{-1}$ VFAs. Interestingly, Ref. [7] reported that light is not crucial for mixotrophy as biomass can be produced using either light or organic carbon as an energy source and that organic carbon is the most important factor for mixotrophic growth. The mixotrophic growth rates, the algal biomass and products productivity are better than using solely autotrophic and heterotrophic conditions, as light energy and organic carbon substrate are provided concurrently [8]. Although heterotrophy can increase biomass, nonetheless, some algae cannot grow in complete darkness or use organic carbon without light [3]. Moreover, the synthesis of pigments can be inhibited in the dark. In addition, microorganisms such as bacteria and fungi can feed on the organic carbon in the medium thereby causing microbial contamination of cultures [9]. However, not all algae can utilize organic carbon substrates [10]. Therefore, screening for algae must take place in order to identify those that are capable of mixotrophic growth in order to provide algal supply that meets the increasing global demand. In relation to increasing algal biomass productivity, supplementation with natural growth stimulants can also lead to an increase in algal biomass.

Natural plant extracts are known to have multiple beneficial effects on humans. For example, extracts of *Lepidium sativum* contain many important phytochemicals (phenolic compounds, terpenoids, alkaloids and organosulfur compounds) are all found in *Lepidium sativum* seeds. *Lepidium sativum* also contains plant phytosterols and their derivatives, which have been shown to possess antioxidant potential. Phenolic compounds, most importantly the flavonoids, may protect against oxidative stress [11]. This cruciferous vegetable has high levels of organosulfur compounds [12], which exert diverse biological effects, including free radical scavenging, glucosinolates and a class of thioglycosides which are major secondary metabolites of *L. sativum* leaves and seeds [13]. Some researchers have shown that certain constituents of the *L. sativum* plant and the alcoholic extracts of its different parts have chemo-preventative and anti-cancer effects.

Trigonella foenum-graecum of Leguminosae's Papilionacae family is a commonly cultivated herbaceous plant in the Mediterranean, Asia and Egypt [14,15]. Oil made up of linoleic, linolenic and oleic acids is found in the seeds in amounts ranging from 5 to 7 percent. It also serves as a diuretic and anti-cancer agent [16]. Furthermore, it exhibits antibacterial [17] and fungi activity [18]. In biological research, active chemicals from fenugreek seeds are used to treat several viral disorders [19]. They are additionally utilized as prospective antioxidant food supplements [20]. One of the most utilized and studied therapeutic plants in the world is licorice [21]. Glycyrrhizin, one of the key active components, has a cortisone-like action. Furthermore, 50 times sweeter than sucrose is glycyrrhizin [22]. The roots of Glycyrrhiza species have been reported to have antibacterial [23], antiviral [24], preliminary free radical scavenging [25], antioxidant effect [26], and anti-inflammatory activity [27].

Agricultural research always focuses on the improvement of plants in early growth stages to achieve good yield in terms of quantity and quality by using natural and low-cost ecofriendly methods through the use of natural biofertilizers to protect human health. One of the most important fruits in Egypt is cantaloupe (*Cucumis melo*). It is a cultivar of the muskmelon, a member of the Cucurbitaceae family. It is a warm-season annual vine that produces large fruit with high economic importance and nutritional value. Cantaloupe fruit is one of the most important and popular fruity vegetables grown in Egypt and it is used mainly as a refreshing fruit and desert. It is rich in bioactive compounds such as phenolics, flavonoids and vitamins as well as carbohydrates and minerals (especially

potassium). In addition, it is low in fat and calories (about 17 kcal/100 g). Furthermore, it has a large amount of dietary fibers as well as antioxidants which have the ability to protect body cells against cancer. Recently, cantaloupe growers in many areas of Egypt have to use protected cultivation to produce an off-season crop for exportation as well as local consumption, thereby emphasizing its economic importance [28].

Biofertilizer is well-known as a promising, low cost, eco-friendly, renewable source for plant nutrients compared to chemical fertilizers. Biofertilizers can be produced from algae as well as bacteria. Algae are considered rich source of nutrients, carbohydrates, proteins, vitamins and unique specific components for each group of algae. Cyanobacteria have great potential as a source of fine chemicals, and thereby they represent a plausible biofertilizer. They are also degraders of different kinds of environmental pollutants including metal ions and pesticides. The history of Cyanobacteria extends about 3.5 billion years back into the Precambrian era [29]. Cyano-bacteria can both photosynthesize and fix nitrogen, and therefore they are rich in carbohydrates and nitrogenous components.

A recent agronomic approach is to use algal homogenate and filtrate as bio-stimulants through seed soaking in addition to foliar spraying. The soaking of seeds prior to sowing has been used in arid areas of the world to give cereal crops a "head start" in germination [30]. This seed treatment enhances fresh and dry weight, leaf area, plant height and leaf development [30], as well as seedling vigor and chlorophyll content that reduce harmful seed microflora and increase the level of plant defense enzymes [31].

In the present study, we investigate the ability of some algae to utilize waste molasses which is economical in cost and contains many nutrients in addition to organic carbon. Indeed, Palmonari et al. [32] showed that there is a considerable amount of sugar and other nutrients in molasses which generally contains about 30–35% sucrose, 10–25% fructose and glucose and 2–3% non-sugar compounds in addition to minerals. In addition, the study also investigates the effects of plant extracts as source nutrients on algal growth in mixotrophic culture medium. The most algal growth-promoting treatments are to be added to culture media to increase algal biomass production. Algal extracts after an increase in biomass were used as a pretreatment for cantaloupe seeds before sowing.

2. Materials and Methods

2.1. Blue-Green Isolates and Mixotrophic Growth Experiment

Two cyanobacterial isolates *Phormidum* sp. and *Synechocystis* sp. previously isolated and identified by Prof. El Semary. They are all freshwater isolates initially kept at room temperature under 12/12, light/dark cycle. The growth medium used for culturing the algae is BG11.

BG11 media composition:

Stock 1: (1 L) › Na_2Mg EDTA 0.1 g/L › Ferric ammonium citrate 0.6 g › Citric acid.$1H_2O$ 0.6 g › $CaCl_2.2H_2O$ 3.6 g›.

Stock 2: (1 L) › $MgSO_4.H_2O$ 7.5 g›.

Stock 3: (1 L) › K_2HPO_4 3.05 g›.

Stock 4: Trace metal solution (1 L) › H_3BO_3 2.86 g › $MnCl_2.4H_2O$ 1.81 g › $ZnSO_4.7H_2O$ 0.222 g › $CuSO_4.5H_2O$ 0.079 g › $COCl_2.6H_2O$ 0.050 g › $Na_2MoO_4.2H_2O$ 0.391 g.

Prepare Stock 1–4 in 900 mL distilled H_2O, add components in the order specified on a magnetic stirrer. Bring the total volume to 1 L with dH_2O. Autoclave at 15 psi for 30 min at 121 °C.

Combine the following solutions and adjust pH to 7.5 (use 1.0 M HCl or NaOH). Stock per liter of medium: Stock 1: 10 mL; Stock 2: 10 mL; Stock 3: 10 mL Na_2CO_3 0.02 g; Stock 4: 1.0 mL and autoclave for 30 min at 121 °C.

2.2. Preparation of the Aqueous Extracts

The aqueous extracts of *Lepidium sativum*, *Trigonella foenum graecum* seeds and liquorice root were prepared by weighing 50 gm of dry powder of the seeds and put it in a conical flask, then 500 mL of hot distilled water was added on the powder which makes the ratio

(1/10) (*w*/*v*). After that, the mixture was shaken using a magnetic stirrer for (24 h), the mixture was filtered by using 4 layers of gauze, then centrifuged by a centrifuge (2000 rpm for 10 min), and the supernatant then was filtered again using filter paper wattman No. 4. The filtrated mixture was stored in a dark sterile screw bottle (4 °C) until use [33]. The sugarcane molasses was diluted before use to 2% using distilled water. Potassium acetate stock was 2 mM.

For each treatment, 5 replicate 250-mL conical flasks containing (100 mL BG11 media + 1 mL treatment) were inoculated by algal inoculum and incubated under 12/12, light/dark cycle for 20 days after that fresh and dry weight of algae were recorded in addition to chlorophyll at, soluble proteins and carbohydrates content analysis. The best treatment was selected for biomass production. Collected algal biomass were extracted for GC–MS analysis and seed treatment.

It is worth mentioning that preliminary experiments were also carried out using pomegranate syrup, but there was no growth enhancement of the algae tested; instead, there was slight growth reduction than that of the control. Therefore, it was not used in the experiments.

2.3. GC–MS of Phormidium sp. *Biomass*

One gram of the air-dried *Phormidium* sp. biomass was added to a 28-milliliter stoppered culture tube and mixed with 30 mL mixture of chloroform: methanol: water, 1:1:1, *v*/*v*/*v* for one day with shaking at 100 rpm on a rotary shaker. The extract was filtered through a 0.2-micrometer syringe filter, and 2 µL was injected into the GC–MS system. Gas chromatography–mass spectrometry (GC–MS) was performed using a GC 1310-ISQ MS (Thermo Scientific, Austin, TX, USA). The separation conditions and method for identifying the separated components were as described by [34].

2.4. Preparation of the Methanolic Extracts of Phormidium sp.

Five grams *Phormidium* sp. were extracted by methanol: chloroform 80:20 for 24 h at 60 °C with continuous stirring. The filtrate was evaporated until complete dryness, then dissolved in distilled water for application on cantaloupe seeds.

2.5. Plant and Growth Conditions

Seed germination

Before the pots experiment, the establishment a wide range of *Phormidium* sp. extract concentrations 0, 0.25, 0.5, 1, 1.5 and 2% were tested on cantaloupe seed germination as a preliminary experiment. Five replicates were performed for each concentration, with each replicate represented by a Petri dish containing 20 seeds of cantaloupe on a filter paper wetted by 20 mL solution, with distilled water applied for control. Germination percentage was recorded after 4 days. From the results, the most promotive concentrations were used in the pretreatment of seeds planted in pots.

Seedling pot experiment

Healthy uniform cantaloupe seeds were presoaked for 6 h in *Phormidium* sp. extract after preparation of different concentrations from stock using distilled water. Five replicates of loamy clay filled 20 cm pots were performed for each concentration including control seeds which were soaked in distilled water. Seeds were planted below the soil's surface about 1 cm depth and completely covered with soil with 5 cantaloupe seeds per pot and irrigated regularly with tap water to maintain 70% of field capacity to the end of the experiment.

2.5.1. Growth Metrics

Five randomly chosen plants from each treatment were measured for their shoot height, root length (in cm), fresh and dried weights (g/plant) and shoot and root weights (in g/plant) after 21 days of planting. Dry weights were calculated after 48 h of oven drying at 70 °C.

2.5.2. Physiological and Biochemical Studies

Chlorophyll *a*

Chlorophyll *a* was extracted using acetone (90%) as solvent. Then, supernatant was measured at the absorbance 665 nm by spectrophotometer according to [35]. Chlorophyll *a* content was calculated according to Ritchie [36] by the following equation:

$$\text{Chlorophyll } a = (11.9035 \times \text{A665} \times \text{V}) \times (\text{g soil}) \times \text{L}$$

where V is the volume of solvent (mL) and L is the path length.

Photosynthetic Pigments Content

Photosynthetic pigments content in leaves of cantaloupe was measured according to the Metzener et al. [37] method, and the following equations were used to determine the concentrations of chlorophyll *a*, *b*, total chlorophyll and carotenoids:

$$\text{Chl. } a = 10.3\text{E}_{664} - 0.918\text{E}_{645}$$

$$\text{Chl. } b = 19.7\text{E}_{645} - 3.87\text{E}_{664}$$

$$\text{Total chlorophyll } (a + b) = \text{Chl. } a + \text{Chl. } b$$

$$\text{Carotenoids} = 4.3\text{E}_{452}\ (0.0265\text{Chl. } a + 0.426\text{Chl. } b)$$

Total Soluble Sugars and Proteins Contents

In order to calculate total soluble sugars using the anthrone reagent [38] and total soluble proteins [39], a known weight of germinated seeds was extracted in 5 mL of 70% ethanol and completed to a specified volume with distilled water following filtration.

Total Soluble Sugars

Total sugars were determined using the anthrone technique; about 6 mL of anthrone solution (2 g/L H_2SO_4 95%) were added to 3 mL sample plant extract and maintained on a boiling water bath for 3 min. After cooling, the developed color was measured spectrophotometrically at 620 nm.

Total Soluble Proteins

For soluble protein estimation 1 mL extract was mixed with 5 mL freshly mixed solution (50:1 *v*/*v*) of 2% sodium carbonate in 4% sodium hydroxide and 0.5% copper sulphate in 1% sodium tartarate. The mixture stood 10 min before the addition of 0.5 mL Folin phenol reagent (1:3) and made up to a definite volume. The optical density of the mixture was measured after 30 min at 750 nm.

2.6. Statistical Analysis

Snedecor and Cochran's method [40] of complete randomized blocks design (CCRBSE. Bas) utilizing the analysis of variance was used to determine the significance of the data using LSD values at $p = 0.05$. The various treatments were compared using a Duncan's multiple range test after this study. Different alphabetical letters represent significant variance between treatments.

A Principal component analysis was also performed in order to help identify the interrelationships among the variables.

3. Results

3.1. GC–MSs of Biomass Phormidium sp. *Using Multisolvent System*

The biomass of *Phormidium* extracted by the specified solvent system showed the presence of some dominant bioactive compounds, mostly novel, including: Isocyanic acid, methyl ester; 2,2-Dimethylbutane; Hexane, 2,2,5,5-tetramethyl; and n-Heptadecane in the first fraction of the extract (Figure 1). These compounds are novel except n-Heptadecane and are believed to indicate unique metabolism in this exotic strain. The nutritional values of these compounds await future investigations.

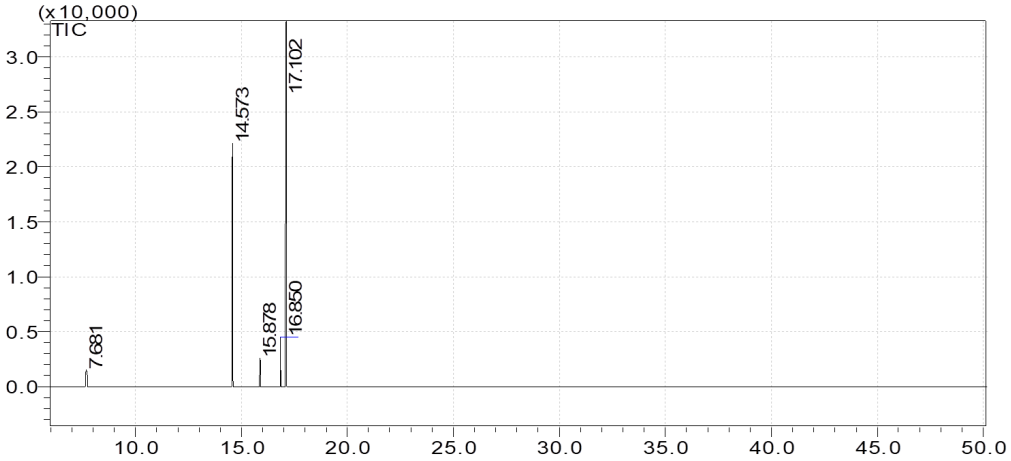


Figure 1. GC–MS spectra of *Phormidium* sp. extract.

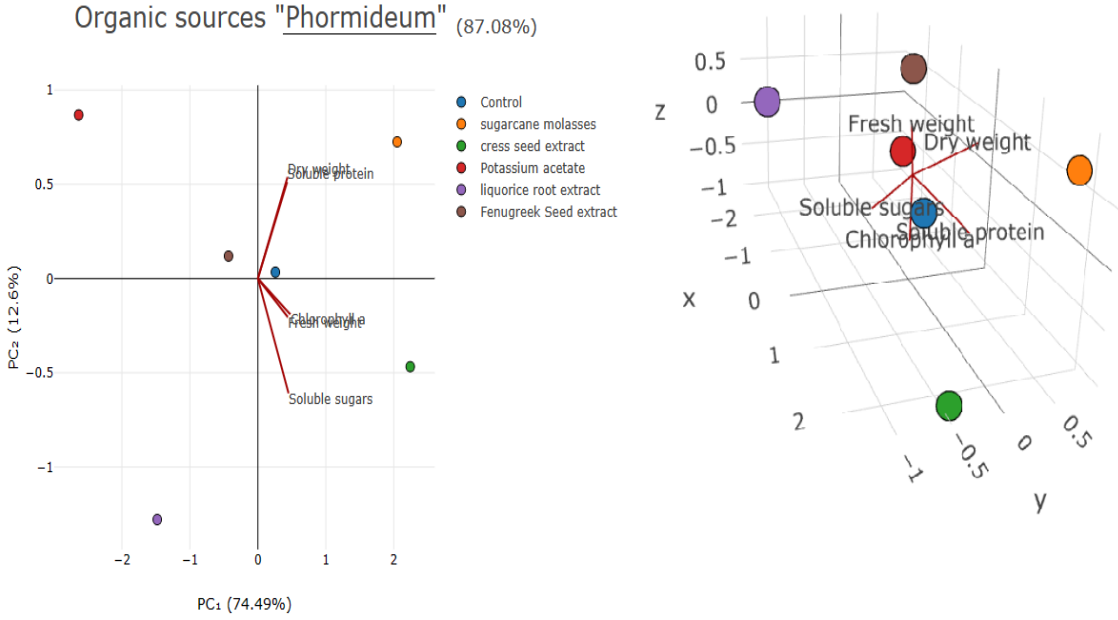
3.2. Effect of Different Extracts on Growth Metrics of Algal Strains

The results from (Table 1; Figure 2) show that *Lepidium sativum* extract and sugarcane molasses both considerably improved the growth of *Phormidium* sp. All growth metrics for *Phormidium* sp. were significantly improved by the addition of these nutritional sources, with molasses being the best supplement. However, the results from Table 2 demonstrated that the addition of these dietary sources drastically decreased all growth indices for *Synechocystis* sp.

Table 1. Effect of different extracts on fresh weight, dry weight (mg/100 mL), soluble sugars, proteins (mg g^{-1} f.m) and chlorophyll *a* content (µg g^{-1} f.m) in *Phormidium* sp. Data shown in the table represent the mean ± standard error, followed by a small letter; similar letters indicate that means were not different significantly at 5%, probability based on Duncan's test, f.wt (fresh weight), d.wt (dry weight) and chl *a* chlorophyll *a*.

Treatments		f.wt (mg)	d.wt (mg)	Total Soluble Protein	Total Soluble Sugars	Chl. *a*
Phormidium sp.	0	420 ± 11.11 c	29 ± 0.76 b	92.20 ± 2.4 c	12.06 ± 0.32 c	1.19 ± 0.03 b
	sugarcane molasses	640 ± 16.9 a	43 ± 1.13 a	100.52 ± 2.7 b	16.02 ± 0.42 b	1.24 ± 0.04 ab
	cress seed extract	520 ± 13.75 b	30 ± 0.79 b	130.09 ± 3.4 a	17.5 ± 0.46 a	1.72 ± 0.05 a
	potassium acetate	180 ± 4.76 d	17 ± 0.44 c	44.67 ± 1.18 e	10.32 ± 0.27 d	0.419 ± 0.01 c
	liquorice root extract	430 ± 11.36 c	15 ± 0.39 c	91.40 ± 2.41 c	5.2 ± 0.13 e	0.677 ± 0.02 c
	fenugreek seed extract	500 ± 13.22 b	30 ± 0.79 b	82.2 ± 2.17 d	9.43 ± 0.25 d	0.629 ± 0.02 c
L.S.D at 5%		70	12	8.3	1.48	0.51

(**a**)



(**b**)

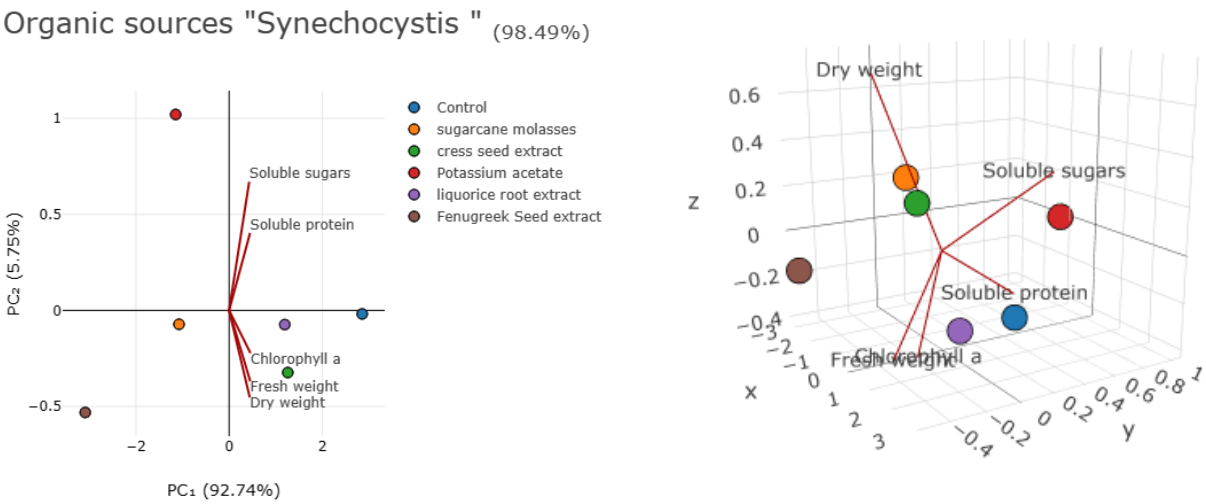


Figure 2. Principal component analysis illustrating the effect of different extracts on fresh weight, dry weight (mg/100 mL), soluble sugars, proteins (mg g^{-1} f.m) and chlorophyll a content (µg g^{-1} f.m) in (**a**) *Phormidium* sp. And (**b**) *Synechocystis* sp.

Table 2. Effect of different extracts on fresh weight, dry weight (mg/100 mL), soluble sugars, proteins (mg g^{-1} f.m) and chlorophyll a content (µg g^{-1} f.m) in *Synechocystis* sp. Data shown in the table represent the mean ± standard error, followed by a small letter; similar letters indicate that means were not different significantly at 5%, probability based on Duncan's test, f.wt (fresh weight), d.wt (dry weight) and chl *a* chlorophyll *a*.

	Treatments	f.wt (mg)	d.wt (mg)	Total Soluble Protein	Total Soluble Sugars	Chl *a*
Synechocystis sp.	0	90 ± 3.45 a	5 ± 0.13 a	100.9 ± 2.6 a	15.06 ± 0.4 a	3.22 ± 0.08 a
	sugarcane molasses	31 ± 0.82 c	2.5 ± 0.06 d	77.25 ± 2 d	9.11 ± 0.24 d	0.91 ± 0.02 c
	cress seed extract	70 ± 1.85 b	4.4 ± 0.11 b	92.24 ± 2.4 b	11.20 ± 0.29 c	2.29 ± 0.06 b
	potassium acetate	23 ± 0.60 d	1.1 ± 0.02 e	88.12 ± 2.4 b	9.93 ± 0.26 d	0.79 ± 0.02 c
	liquorice root extract	72 ± 1.90 b	3.5 ± 0.09 c	90.51 ± 2.1 b	12.36 ± 0.32 b	2.36 ± 0.06 b
	fenugreek seed extract	15 ± 0.39 e	0.6 ± 0.02 f	60.85 ± 1.6 e	5.1 ± 0.13 e	0.25 ± 0.007 d
L.S.D at 5%		8	0.6	8.6	1.1	0.54

3.3. Seed Germination

Results presented in (Figure 3) showed a stimulating effect of *Phormidium* sp. methanolic extract on seed germination at different concentrations, notably at 1%, which improved seed germination from 82 to 96%.

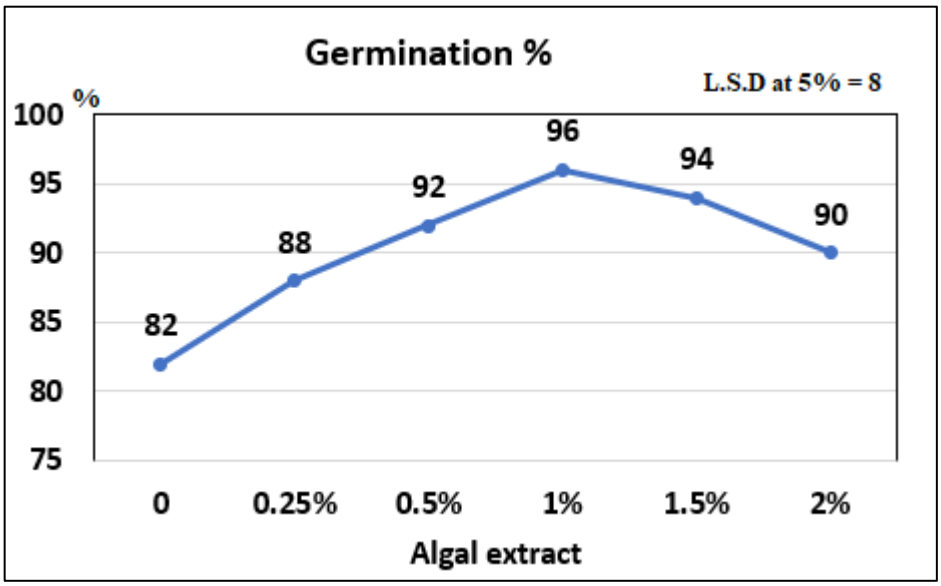


Figure 3. Effect of different concentrations of algal extract of *Phormidium* sp. on the germination of cantaloupe seeds for 4 days. Values represent the mean of five replicates.

3.4. Vegetative Growth

Pre-soaking cantaloupe (*Cucumis melo*) seeds in *Phormidium* sp. methanolic extract at concentrations of up to 2% had a pronounced and significant effect on all measured growth parameters including shoot length, fresh and dry weight of shoots by root length, as well as the fresh and dry weights of roots compared to the corresponding control plants especially at 0.5% (Figures 4 and 5).

(**A**)

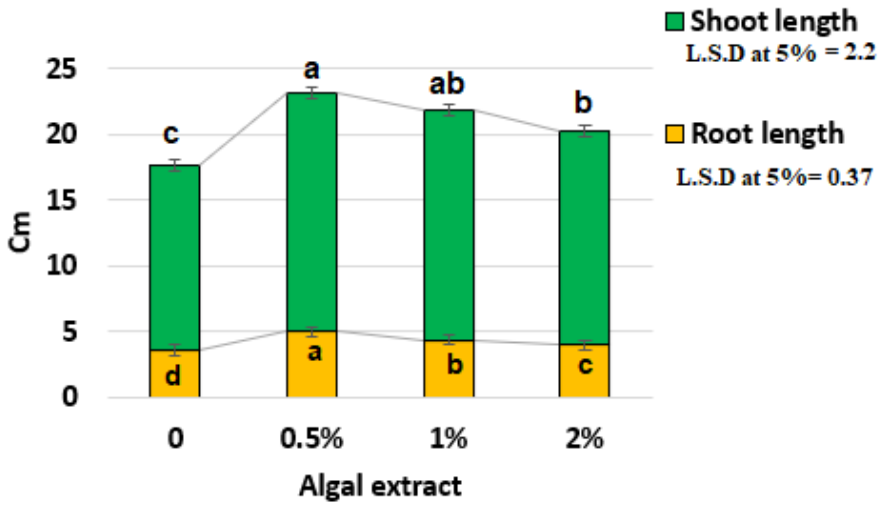


Figure 4. *Cont.*

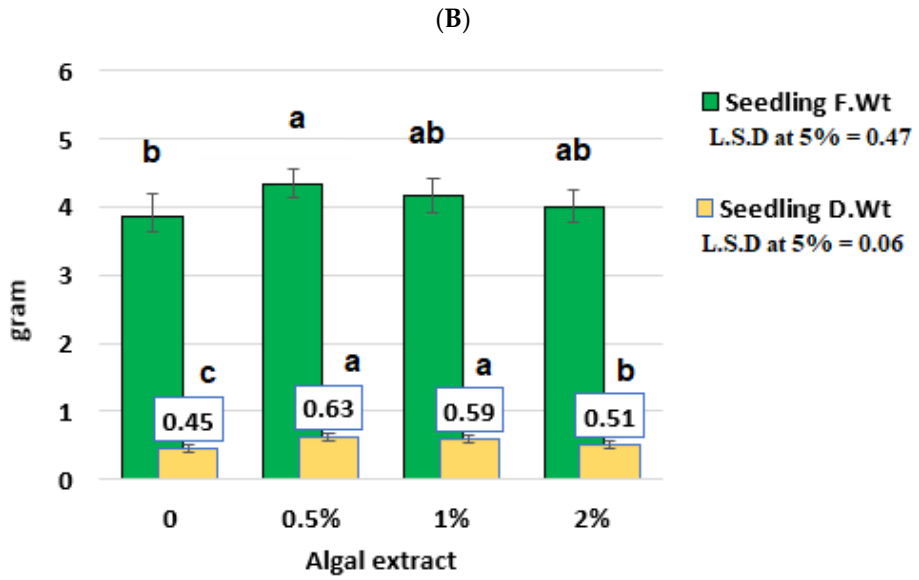


Figure 4. Effect of different concentrations of algal extract of *Phormidium* sp. on (**A**) shoot length, root length and (**B**) seedling fresh wt., seedling dry wt. of cantaloupe. Values represent the mean of five replicates. Different letters (a, b, c and d) indicate statistical differences at 5% probability according to Duncan's test. Error bars are standard errors of the mean, f.wt (fresh weight) and d.wt (dry weight).

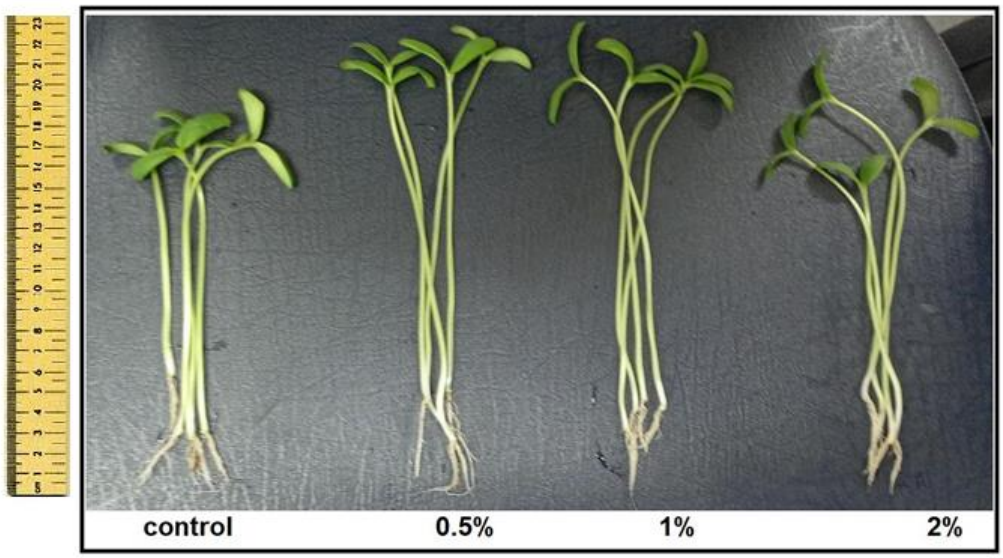


Figure 5. Effect of different concentrations of algal extract of *Phormidium* sp. on cantaloupe seedling growth.

3.5. Metabolic Activity

a. Photosynthetic Pigments

The present data (Figure 6) shows a significant increase in photosynthetic pigments chl. *a*. and carotenoids in cantaloupe pretreated by soaking in *Phormidium* sp. at 0.5 and 1% concentrations recording (0.661, 599 mg/g chl. *a* and 0.70, 0.63 mg/g for carotenoids, respectively), compared to the high extract concentration of 2% and the corresponding control plants, while there was no significant change in chl. *b* content.

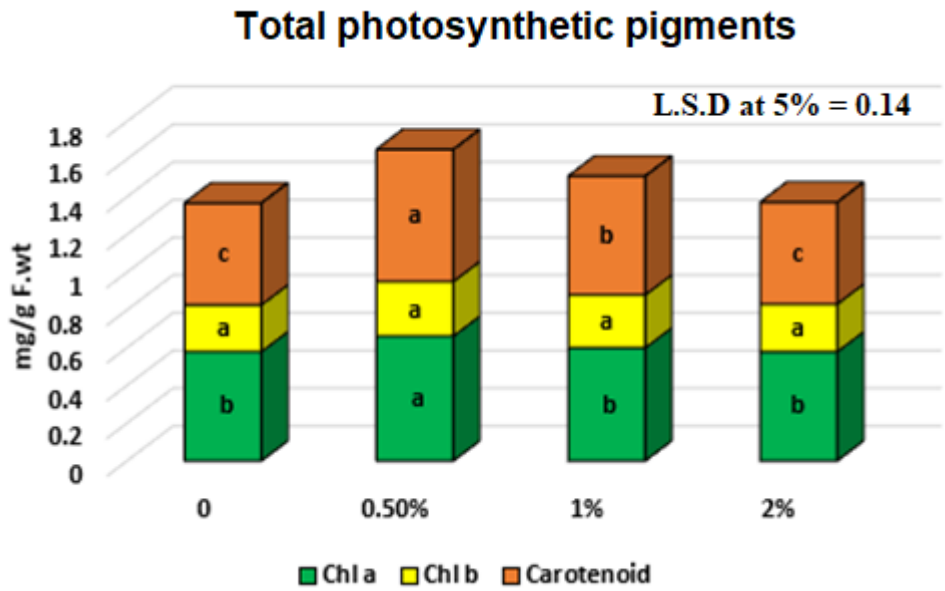


Figure 6. Effect of different concentrations of algal extract of *Phormidium* sp. on photosynthetic pigments content (mg g^{-1} f.m) of cantaloupe leaves (mg g^{-1} f.m). Values represent the mean of three replicates. Different letters (a, b and c indicate statistical differences at 5% probability according to Duncan's test. Error bars are standard errors of the mean. Chl. *a*—chlorophyll *a* and chl. *b*—chlorophyll *b*.

b. Carbohydrates and Proteins

The data presented in (Figure 7) shows that the total soluble protein and sugar contents significantly increased after being pretreated with cyanobacterium *Phormidium* sp. extract especially at 0.5% by (9.96 and 278.19 mg/g), respectively, compared to (7.49 and 240.35 mg/g) for the control plants.

(**A**)

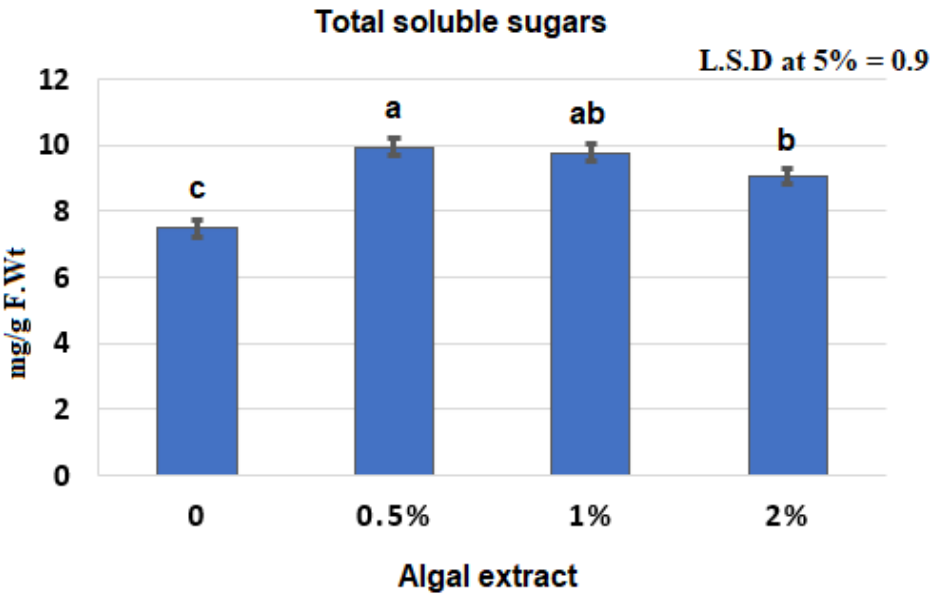


Figure 7. *Cont*.

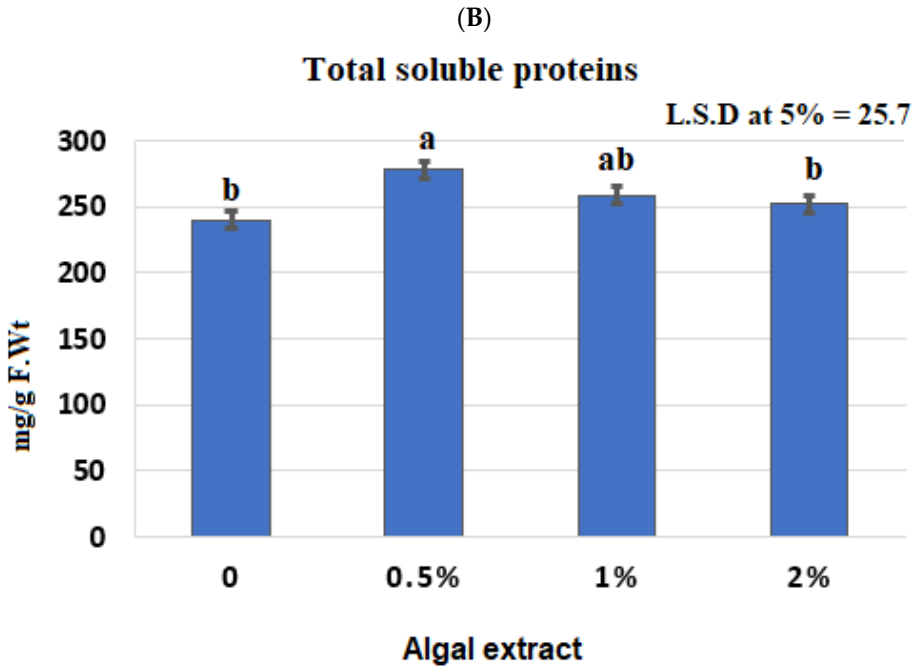


Figure 7. Effect of different concentrations of algal extract of *Phormidium* sp. on (**A**) total soluble sugars and (**B**) total soluble protein of cantaloupe. Values represent the mean of three replicates. Different letters (a, b and c) indicate statistical differences at 5% probability according to Duncan's test. Error bars are standard errors of the mean.

4. Discussion

Abundant natural organic chemical substances are present in both soil and aquatic environments. They may promote mixotrophic nutrition in different algal species. Mixotrophy combines autotrophic and heterotrophic production of microalgae. In our experiments, mixotrophy was valid only for enhancing the growth of *Phormdium* sp. but not *Synechocystis* sp. This is consistent with the fact that some algae are capable of mixotrophy while others are not. Using Mixotrophy to increase algal growth has been reported before, however, using plant extracts for this purpose has never been reported before. Plant extracts are known to have multiple beneficial effects, for example, Fenugreek seeds are distinguished by being abundant in phytochemical components with antioxidant effects [41].

Fenugreek seeds contain volatile oils, vitamins, and minerals in addition to 45–60% carbohydrates (primarily mucilaginous fiber: galactomannans), 20–30% proteins (primarily lysine and tryptophan), 5–10% fixed oils (lipids), flavonoids (apigenin, luteolin, and quercetin), pyridine-type alkaloids (mainly trigonelline), steroidal saponins (trigoneoside and fur [42]. *Lepidium sativa*. or garden-cress rich in vitamins A and C, iron, and calcium and contains arachidic, ascorbic, behenic, erucic, gadoleic, linoleic, miacin, palmitic, stearic, and uric acids, carbohydrates, cellulose, fiber, riboflavin, beta-carotene, beta-sitosterol, thiamine, alpha-tocopherol, and d-xylose [43].

Different research reported that the application of microalgae and cyanobacteria either as biomass or extract contributes immensely to the nitrogen composition of plants and soil. Moreover, growth-enhancing substances such as phytohormones, amino acids, phenolic compounds, and important trace elements are supplied which are essential for plant health, development, and ion distribution [44]. Several authors have also observed that cyanobacteria (blue-green algae) have a stimulatory effect on seed germination. e.g., [45] on *Vicia faba* [46] on *Lactuca sativa*; [47] on *Vinca* and [48] on *Triticum aestivun*. According to Christopher et al. [49], pre-soaking of rice seeds had direct evidence for culture filtrate hormonal effects, which improved germination and sped up seedling growth. According to Palaniappan et al. [50], the aqueous extract of the cyanobacterium *Phormidium* sp. significantly improved the germination of cowpea seeds under in vitro conditions. As a result, it

was suggested that the cyanobacterium's extracellular organic bioactive compounds might be beneficial for plant growth, a rich source of lipids, vitamins, minerals, and antibacterial substances, according to Yadav [51], who also noted that algae are known to be stimulating for seed germination and plant growth. The effect of the algal extract may also contribute to the increase in the activity of the main hydrolytic enzymes necessary for seed germination. Similar findings were attained by Moorthy and Malliga, and [52] on *Aloe barbadensis*. In this regard, Gupta and Shukla [53] indicated that an increase in leaf area ratio and net assimilation rate may be responsible for the stimulatory effect of cyanobacterial biofertilizers on relative growth rate. They asserted that an increase in leaf area ratio showed a steady rise in plant photosynthetic productivity. The effect of growth-promoting substances in extracts of *Phormidium foveolarum*, *P. tenue*, and *P. frigidum* on rice seedlings has been studied by presoaking seed treatment increased significantly the growth and development of roots and shoots [54].

In the current study, the use of cyanobacteria as biofertilizer boosted cell division and elongation without interfering with the process of nutrient uptake, leading to better outcomes because of better nutrition. Additionally, enhancing the growth parameter features lead to an increase in productivity [55]. According to several studies, cyanobacteria can enhance plant growth by enhancing soil structure since they have the ability to release extracellular polysaccharides that aid in soil aggregation and water retention [56]. Indeed, it has been demonstrated that inoculating plants with phosphate-solubilizing and nitrogen-fixing cyanobacteria improved plant growth by increasing the availability of phosphate and nitrogen content. Phosphorus and nitrogen are two highly important nutrients for plant growth [57]. The action of one or more growth-promoting compounds, particularly auxins and cytokinin's found in cyanobacteria, may be the cause of the increase in root and shoot lengths of wheat seedlings [58,59]. Increase in seedling growth is also well related to increase in photosynthetic pigments content thereby promoting photosynthesis which is responsible for production of primary metabolites needed for growth.

According to [60], fertilizers containing cyanopith and cyanospray increased the biochemical characteristics of *Aloe barbadensis*, including the amounts of total chlorophyll, chlorophyll a, chlorophyll b, carotenoids, sugars, and free amino acids, because more nutrients are present in the soil treated by cyanopith, the plant's nutrient status has improved. Similar outcomes were attained by Anandharaj [61] on *Alium cepa* and *Oryza sativa* and [62] on *Helianthus annus*. Results agree with Rajula and Padmadevi [63], on *Helianthus. annuus* L. and [64] on *pea*. The activities of nitrogen as well as nitrate reductases may be responsible for the improvement in growth and nitrogen contents in response to the application of cyanobacteria as bio-fertilizers on seed and related processes of wheat, sorghum, maize, and lentil [65]. From previous reports, the development of plants is known to be aided by cyanobacteria like *Arthrospira platensis* and *Nostoc muscorum*, which can increase plant growth, yield, protein and carbohydrate content [66]. According to [67,68], wheat plants treated with various cyanobacterial strains, such as *Nostoc* sp., *Anabaena* sp., *Chroococcidiopsis* sp., *Calothrix* sp., and *Phormidium* sp., showed improvements in shoot length, lateral root formation, dry weight, and leaf area. It appears that the extensive range of bio-active chemicals produced by cyanobacteria, which may play a direct or indirect role in cell division, cell expansion, and root initiation, have a good impact on seed germination and seedling growth indices [69].

Phormidium sp. has been shown to be able to synthesize almost all classes of phytohormones, including growth promotors auxins, cytokinins and gibbrelins. These phytohormones may stimulate the creation of biomass, promote growth and development, and improve the germination and viability of seeds [70]. According to [64], the content of secondary metabolites that promote growth varies depending on the cyanobacterial strain genotype and habitat distribution. Auxins are one of the several secondary metabolites that cyanobacteria secrete that may help in growth. Ref. [71] looked at how different cyanobacterial strains' water extracts affected the development of pumpkin, cucumber and tomato plants. They were able to demonstrate the auxins indole acetic acid (IAA) and

indole butyric acid (IBA) in the extracts. Increased root length, height and plant wet and dry mass measurements were seen after the extracts were applied. The cyanobacterial filtrates also included gibberellic acid and cytokinins, which are plant growth regulators in addition to auxins. The application of cyanobacterial filtrates increased the concentration of these secondary metabolites within the plants, as demonstrated by [72], who showed that how cyanobacterial filtrates from *Cylindrospermum muscicola* and *Anabaena oryzae* can promote plant growth in *Lupinus termis* leaves by increasing chlorophyll a and b concentration, photosynthetic activity and nitrogen and carbon content. Whereas *Oscillatoria angustissima* exhibited higher quantities of gibberellic acid, *Nostoc entophytum* had higher levels of auxins (IAA) and cytokinin. Different impacts on pea plant growth are also brought on by the varying constitution. While fertilization with *N. entophytum* resulted in higher contents of nitrogen, protein, exopolysaccharide and chlorophyll a compared to fertilization with *O. angustissima*, higher contents of carbohydrates and phosphate were obtained in the opposite direction.

5. Conclusions

A cost-effective strategy is to use low-cost resources such as sugarcane molasses, a waste product of the sugar industry, and the common herb extract of *Lepidium sativum*. For *Phormidium* sp., mixotrophic growth produced more algal biomass than autotrophy. Pre-soaking cantaloupe (*Cucumis melo*) seeds in *Phormidium* sp. methanolic extract before sowing led to a considerable increase in plant development and seed germination when this mixotrophically grown blue-green algae was used as a biofertilizer. All measured growth parameters, in addition to seed germination percent, were significantly affected by methanolic extract at concentrations up to 1%, specially at 0.5% significantly increased photosynthetic pigments and biochemical components in cantaloupe seedlings, demonstrating its value as an environmentally friendly agricultural strategy for achieving both food security and environmental sustainability. In conclusion, this study provides a novel application of unusual mixotrophic supplementation and opens the door for further investigations on the mixotrophic ability of other types of algae. The application of *Phormidium* sp. algal extract also needs to be applied on more and more economic plants from different categories in further studies.

Author Contributions: N.E.S. was responsible for obtaining funding, the administration of the project and providing algal cultures, as well as methodology, data validation and writing-up and response to reviewers; A.M.A.E.-S. was responsible for methodology, formal analysis, data evaluation, writing-up and response to reviewer; E.Z.A. was responsible for methodology, formal analysis, data evaluation, writing-up and response to reviewer; M.A. was responsible for GC–MS of *Phormidium* sp. All authors have read and agreed to the published version of the manuscript.

Funding: The authors extend their appreciation to the Deputyship for Research and Innovation, Ministry of Education in Saudi Arabia, for funding this research work (Project number INST081).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: The authors extend their appreciation to the Deputyship for Research and Innovation at the Ministry of Education in Saudi Arabia for funding this research work (Project number INST081). All authors have consented the acknowledgement.

Conflicts of Interest: The authors declare no conflict of interest.

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