

# The potential using of three algal extracts as bio-stimulants to promote the growth and improvement of phytochemicals of *Allium sativum* L.

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## Abstract:

Garlic (*Allium sativum* L.) is ayurvedic herb that has been extensively used as medication and as the taste enhancer of food. The effects of foliar spraying of garlic at various concentrations (0.5, 1, 25 and 50%) derived from liquid extracts of *Spirulina platensis*, *Ulva fasciata*, and *Pterocladia capillacea* on growth, pigment, protein content, macronutrients, micronutrients, and phytochemical components were examined. The findings showed that spraying *Allium sativum* leaves with 25% *Spirulina* increased their protein content, chlorophyll content, and leaf length by 18, 20, and 49%, respectively. In addition, the total N and S increased by 1.2 and 5 folds, respectively. The examination of hexane and methanol extracts from control and treated plants with 25% *Spirulina* extract using GC-MS revealed a significant rise in the percentage of concentration of Diallyl disulphide, Trisulfide, methyl 2-propenyl, 2-Methoxy-4-vinylphenol and Phytol by 1.3, 1.9, 1.5, 2.7 in case of 25% *Spirulina* extract relative to the control plants. *Spirulina platensis* extract can be employed to improve the growth, minerals, organosulfur compounds of garlic.

**Keywords:** *Spirulina platensis*, *Ulva fasciata*, Gas Chromatography, foliar spraying, microelements, organic farming.

## Introduction

Organic farming is a comprehensive method of agricultural management that promotes and supports the health of the soil's biological activity, biological cycles, and biodiversity. In those days, synthetic fertilizers were frequently employed in agriculture, posing water logging, salinization, soil erosion, water contamination, pesticide poisoning, loss of biodiversity. For sustainable agriculture, growers are transitioning to organic fertilizers (Dhargalkar *et al.*, 2005; Vijayanand *et al.*, 2014).

In comparison to unsprayed plants, plants sprayed by algal extracts had higher N, P, K and dry weight contents, as well as higher yields, component yields, and bulb quality of garlic and onion (Shalaby and El-Ramady 2014;

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**Yassen et al., 2018**). The macroalgae extract has significant economic domination in agriculture as a seed germination enhancer, fertilizers, and improve vegetable output (**Hern'ez-Herrera et al., 2014**).

Amino acids and other organic nitrogenous substances are abundant in *Spirulina* microalga. These amino acids are essential for processes such as detoxification of heavy metal and toxin (Bashir et al., 2018; Hussain et al., 2018), protein structure preservation (**Kakkar et al., 2000**), chlorophyll synthesis (**Amin et al., 2011**), stress reduction, and biostimulation of growth (Souri & Hatamian, 2019), cell division, enlargement, and differentiation. In the tissues of the garlic plant, Amino acids enhance the levels of protein, N, Cu, and Mn (**Fawzy et al., 2012**).

Abdel-Latif et al., (2018) declared that foliar spraying of *Zea mays* seedlings with 0.5% or 1% *Ulva lactuca* aqueous extract sequel in a substantial boost in growth and biochemical components. Another study also reported that 8% of *Ulva fasciata* and *Padina gymnospora* aqueous extract enhanced the growth and phytochemical content in *Capsicum annum* (**Arokia rajan et al., 2020**). Also, Ashour et al., (2020) stated that growth, minerals, yield, and antioxidants of *Corchorus olitorius* were improved by using 10% of water extract of *Pterocladia capillacea* according to **Al Dayel and El Sherif (2022)**, foliar spraying with aqueous extract of *Spirulina platensis* (2 g/l) increased curcuminoid concentration and yield of *Curcuma longa*.

After the onion (*Allium cepa*), garlic (*Allium sativum L.*) is regarded as one of the most important vegetable crops (**Shafeek et al., 2015b**). It is frequently grown for both culinary and medicinal purposes. In Egypt, garlic has been grown for purpose of domestic use and export. In addition, Egypt produces 244.626 MT of garlic, ranking fourth in the world behind China, India, and Korea. (**FAO, 2011**). According to our knowledge it is the first time to inspect the influence of

aqueous extracts of *S. platensis*, *U. fasciata*, and *P. capillacea* on the growth of garlic so, the aim of this study was to probe, how foliar spraying of these extracts influenced on the growth and phytochemical components of garlic plant.

## ***Materials and Methods***

### **1. Algal sampling**

*Spirulina platensis* (Nordstedt) Geitler (Oscillatoiales), *Ulva fasciata* (Linnaeus) and *Pterocladia capillacea* (S.G. Gmelin) Santelicas & Hommersand were the algal species used in this investigation. The algal laboratory's culture collection was used to obtain *S. platensis* at Faculty of Science, Alexandria University, Egypt. It was cultured in Zarrouk's medium (**Zarrouk, 1966**) at a temperature of  $30 \pm 2.0^\circ\text{C}$  and a pH range of 9–10 under continuous light ( $35 \mu\text{mol}/\text{m}^2/\text{s}$ ). Cells were carefully filtered out while in stationary phase later 24 days of incubation, cleaned with distilled water, and then dried at  $60^\circ\text{C}$  up to constant weight. *P. capillacea* and *U. fasciata* were harvested from the Abu-Qir coast near Alexandria, Egypt, in the summer of 2019. To get rid of any unwanted pollutants or sand fragments that had attached to the algae species, they were collected and delicately rinsed with seawater and brought to the lab in various polythene bags that contained morphologically diverse thallus of algae. In order to get rid of the salt that had been on the samples' surface, they were extensively rinsed with tap water. Seaweeds that were well cleaned and dried in the shade for three to five days were then desiccated in an oven for 72 hours at  $60^\circ\text{C}$  (**Hernández-Herrera et al., 2013**). For identification of both *Ulva fasciata* and *Pterocladia capillacea*, Aleem, (1993) was consulted as a reference, in addition to

herbarium specimens of late professor A. H. Nasr (Herb. Nasr) (Botany Department, Faculty of Science, Alexandria University).

## 2. Algal extract preparation

Algal extracts were made using the **Kannan *et al.* (2013)** method. 10 g of finely powdered dried *S. platensis*, *U. fasciata*, and *P. capillacea* was boiled at 60 °C for 15 minutes under vacuum with 100 ml of distilled water. Each extract was filtered using Millipore filter (0.22 µm), and then maintained at 4 °C. This extract was deemed as 100%. Various concentrations (0.5, 1, 25, and 50%) of the three algal extracts were produced by diluting these extracts with distilled water. Both macro- and microelement analysis was performed on each extract.

## 3. Experimental design and treatments

At Alexandria University, Faculty of science using a fully randomized methodology the experiment was carried out in three replicates. Garlic (*Allium sativum L.*) bulbs were received from Agriculture Research Center, Dokki, Giza, Egypt. Six bulbs were displaced in each pot. The pots were supplemented with three algal treatments under two low (0.5 and 1%) and two high (25 and 50%) concentrations i.e., C: control (no algal treatment), SP0.5, SP1, SP25, SP50 for *S. platensis* extract, UF0.5, UF1, UF25, UF50 for *U. fasciata* extract and PC0.5, PC1, PC25 and PC50 for *P. capillacea* extracts. All pots were watered with 200 ml of half strength modified Hogland nutrient solution as recommended by **Epstein, (1972)** twice a week until the 30<sup>th</sup> day of cultivation. After 30 days from the beginning of algal treatments, plants were taken carefully from the pots.

#### **4. Growth parameters, pigment and protein determination**

For each treatment, leaf length, shoot fresh weight, and shoot dry weight were measured. The N, N-dimethylformamide method was used to determine the amounts of chlorophyll a and chlorophyll b (Chl. a, Chl. b) (**Inskeep and Bloom, 1985**). Carotenoids (Carot.) were estimated according to the method of **Lichtenthaler, (1987)**. Total protein was estimated as described by **Hartree, (1972)**.

**5. Organic matter** estimated as described by **Walkley and Black (1934)**.

#### **6. Analysis of macro and micro-nutrients**

Oven dried garlic leaves of control and plant sprayed with SP25 were digested according to the methods of **Jones and Case (1990)**. According to **Ling (1963)** designation of the Kjeldahl method, the total nitrogen was approximated. A spectrophotometer was exploited to evaluate the amount of phosphorus using the vanadate-molybdate technique at 660 nm (**Chapman and Pratt, 1982**). A Perkin-Elmer Flame photometer was used to measure potassium and sodium (Page, 1982). Born was calculated using **Bingham's (1982)** methodology. Using a spectrophotometer, sulphur was examined at 420 nm (**Okalebo, 1993**). Following **Isaac (1980)**, Mg, Ca, Cu, Fe, Mn, and Zn were measured via an atomic absorption spectrophotometer (Perkin-Elmer 100B).

## **7. Phytochemical screening**

### **i- Extraction**

The fresh garlic leaves only from control and SP25 sprayed plants were shade dried After dryness, by using a mechanical blender, these leaves were ground totally into a fine powder. The plant material (10 g) was soaked in hexane, followed by methanol. Extraction with each solvent was done during a week period with occasional shaking, then dryness was completed using rotary evaporator at 40 °C.

### **ii- Gas Chromatography- mass spectrometry**

The following conditions were used when the gas chromatography-mass spectrometry analysis was executed, Instrument: a TRACE GC Ultra Gas Chromatographs (THERMO Scientific Corp., USA), coupled with a thermo mass spectrometer detector (ISQ Single Quadrupole Mass Spectrometer).The GC-MS system was set with a TR-5 MS column (30 m x 0.32 mm i.d., 0.25 µm film thickness) under the subsequent conditions: oven temperature was kept at 50 °C for 5 minutes and programmed to reach 250 °C at rate of 5 °C /min, then kept at 250 °C for 35 min; injector temperature, 250 °C; split ratio, 1:30; carrier gas, helium; flow rate, 1.8 ml /min. By means of AMDIS software, the detection of the compounds was de-convoluted and identified by its retention indices (relative to n-alkanes C8-C22), mass spectrum matching to Wiley spectral library collection, replib database and NSIT mainlib.

## 8. Statistical analysis

The results are denoted by the means of three replicates and the standard deviation (SD). One-way analysis of variance was used to statistically analyze the data for significance (ANOVA,  $P \leq 0.05$ ), and pairwise means comparisons were done at a 95% confidence level using Tukey's test. According to **Gunawan *et al.* (2018)**, statistical analysis was carried out using the statistical tool Minitab-17.

## Results

### 1. Chemical components of algal extracts

Table 1 shows some of the inorganic ingredients of *S. platensis*, *U. fasciata* and *P. capillacea* aqueous extracts. *S. platensis* extract had the highest percentages of total N (8.8%), P (1.65%), K (1.35%), Mg (2.03%), S (2.24%), B (46 mg/l), O.M (24%), O.C (13.92), and C/N ratio (1.58). *P. capillacea* extract, on the other hand, had the highest Co, Cd and Pb (0.121, 0.176 and 0.750 mg/l) respectively.

### 2. Growth parameters, pigments and total protein

Table 2 shows the influence of foliar spray of algal aqueous extracts on *A. sativum* growth characteristics. Except for PC50, all spraying treatments resulted in a substantial increase in leaf length when contrasted to the control (non-treated plants). The longest leaf length was reported with treatment SP25, which had a value of 33.53 cm, an increase of 49% above the control. Results also indicated that control and all treated plants except for SP0.5, SP1 and SP25 had similar values for shoot fresh and dry weight. The main effect of the different

concentrations of algal extracts on the growth parameters (leaf length, figure 1a), (shoot fresh weight, figure 1b), and (shoot dry weight, figure 1c) revealed that the three concentrations of *S. platensis* aqueous extracts (0.5, 1, and 25%) had a real influence on the mean values of all investigated growth limits. It is worth noting that 25% *S. platensis* aqueous extract had the most favorable effect on all growth limits when compared to the other concentrations of algal extracts examined. Data obtained from the effect of foliar spray of 25% of *S. platensis* aqueous extract on chlorophyll a, chlorophyll b, carotenoids and protein content were listed in Table 3. As compared with control values, chlorophyll a and protein content of *A. Sativum* plants sprayed with SP25 showed significant increases. The SP25 treatment led to an increase in chlorophyll a and protein content by 20 and 18%, respectively, contrasted to the control.

### **3. Macro and micro-nutrients**

The results in Table 4 revealed that SP25 treatment markedly caused in a boost in the percentage of total N, Mg, Ca and S. The increase of the total N & S was 1.2 and 5-fold, respectively over control plants in response to algal treatment. Whereas the concentration of Fe, Zn, Mn, Cu & B slightly increase in SP25 sprayed plants.

**Table 1. Chemical analysis of the studied algal extract.**

Parameters	Algal extracts		
	<i>S. platensis</i>	<i>U. fasciata</i>	<i>P. capillacea</i>
<b>pH</b>	5.49	5.39	5.94
<b>E.C dS/m</b>	3.51	9.19	6.9
<b>O.C (%)</b>	13.92	12.76	10.55
<b>O.M (%)</b>	24	22	18.2
<b>Total N (%)</b>	8.8	8.5	8.1
<b>C/N Ratio</b>	1.58	1.50	1.30
<b>P (%)</b>	1.65	1.64	1.57
<b>K (%)</b>	1.35	1.30	0.54
<b>Ca (%)</b>	2.30	3.71	3.40
<b>Mg (%)</b>	2.03	1.94	1.56
<b>S (%)</b>	2.24	1.63	1.30
<b>Fe (mg/L)</b>	9571	9571	7624
<b>Zn (mg/L)</b>	438	472	648
<b>Mn (mg/L)</b>	281	320	326
<b>Cu (mg/L)</b>	33	34	30
<b>B (mg/L)</b>	46	27	30
<b>Co (mg/l)</b>	Nd	0.115	0.121
<b>Pb(mg/l)</b>	Nd	0.519	0.750
<b>Cd (mg/l)</b>	Nd	0.167	0.176

O.C= organic carbon; O.M= organic matter; C/N= carbon nitrogen ratio.

**Table 2. Effect of foliar spray of algal aqueous extracts on leaf length (cm), Shoot fresh weight (g) and shoot dry weight (mg) of *A. sativum*.**

Algal extract (%)	Parameters		
	Leaf length	Shoot fresh weight	Shoot dry Weight
Control	22.47 <sup>i</sup> ±9	1.28 <sup>c</sup> ±0.11	207.63 <sup>c</sup> ±20.94
SP0.5	27.50 <sup>c</sup> ±9	1.68 <sup>b</sup> ±0.16	269.13 <sup>b</sup> ±25.97
SP1	30.36 <sup>b</sup> ±7.5	1.84 <sup>b</sup> ±0.12	291.7 <sup>b</sup> 4±3.74
SP25	33.53 <sup>a</sup> ±5.7	2.10 <sup>a</sup> ±0.11	335.41 <sup>a</sup> ±22.58
SP50	26.50 <sup>c</sup> ±5.9	1.24 <sup>c</sup> ±0.09	201.02 <sup>c</sup> ±7.09
UF0.5	24.30 <sup>f</sup> ±4.37	1.265 <sup>c</sup> ±0.09	202.04 <sup>c</sup> ±14.68
UF1	24.50 <sup>ef</sup> ±3.4	1.30 <sup>c</sup> ±0.15	217.79 <sup>c</sup> ±7.58
UF25	25.80 <sup>de</sup> ±4.9	1.36 <sup>c</sup> ±0.04	218.81 <sup>c</sup> ±9.02
UF50	23.80 <sup>fg</sup> ±4.7	1.29 <sup>c</sup> ±0.05	216.08 <sup>c</sup> ±8.59
PC0.5	25.00 <sup>gh</sup> ±2.6	1.26 <sup>c</sup> ±0.08	202.04 <sup>c</sup> ±5.64
PC1	24.50 <sup>ef</sup> ±1.9	1.27 <sup>c</sup> ±0.08	202.04 <sup>c</sup> ±16.94
PC25	23.96 <sup>fg</sup> ±2.4	1.26 <sup>c</sup> ±0.07	201.24 <sup>c</sup> ±4.79
PC50	22.75 <sup>hi</sup> ±1.2	1.25 <sup>c</sup> ±0.06	200.44 <sup>c</sup> ±12.42

SP=*S. platensis*, UF= *U. fasciata*, PT=*P. capillacea*. by Tukey's test Means ± SD that do not share letters are considerably different at  $p < 0.05$ .

**Table 3. Effect of foliar spray of *A. sativum* with 25% *S. platensis* aqueous extract on pigment and protein content (mg /g dry matter).**

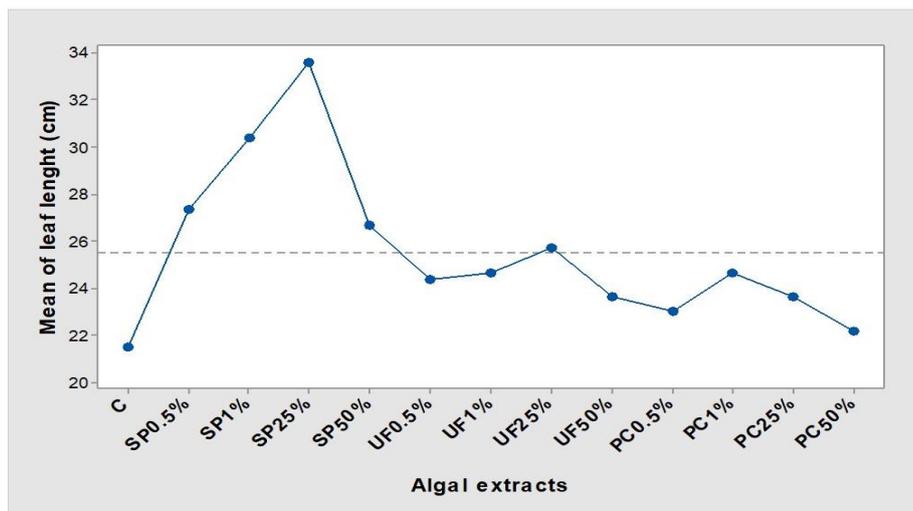
	Chl a	Chl b	Carotenoids	Protein
Control	11.95 <sup>b</sup> ±0.01	5.97 <sup>a</sup> ±0.0	3.50 <sup>a</sup> ±0.01	200 <sup>b</sup> ±0.29
25% <i>S. platensis</i>	14.34 <sup>a</sup> ±0.01	5.62 <sup>a</sup> ±0.01	3.68 <sup>a</sup> ±0.01	237 <sup>a</sup> ±0.01

By Tukey's test, means ± SD that do not share letters are significantly different at  $p < 0.05$ .

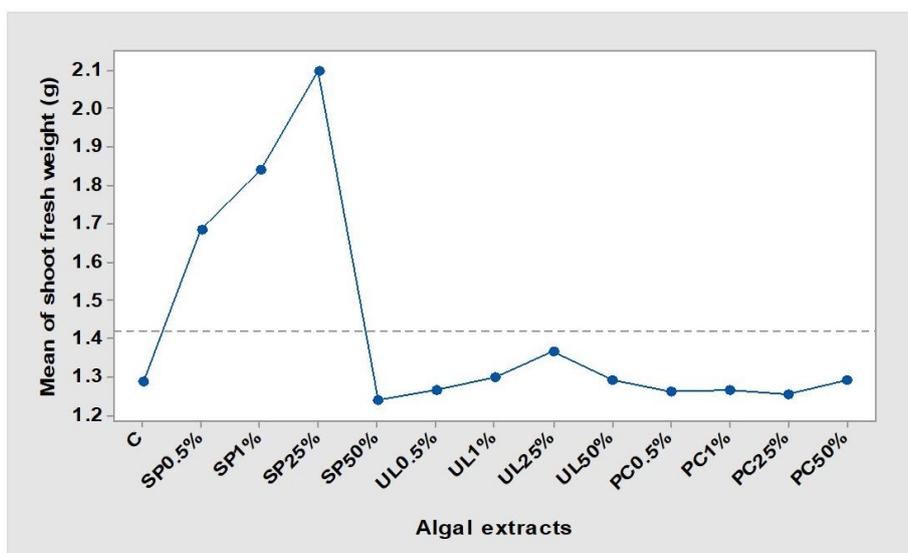
**Table 4. Impact of foliar spray of *A. sativum* with 25% *S. platensis* aqueous extract on macro (%) and micro-nutrients (mg/L).**

	N	P	K	Ca	Mg	S	Fe	Zn	Mn	Cu	B
<b>Control</b>	3.8	2.9	2.1	11	22	0.46	95	16	62	12	3.50
<b>SP25</b>	4.8	3	2	14	28	2.60	105	20	64	11	3.06

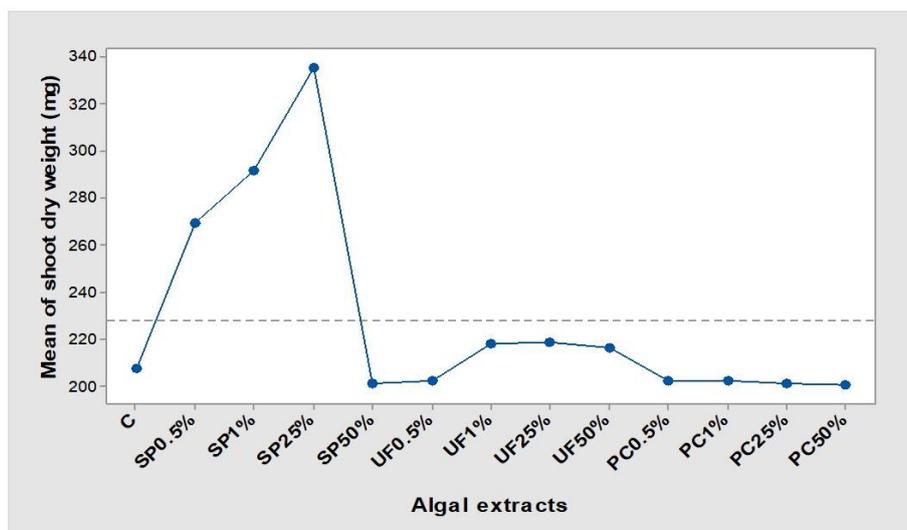
SP25: leaves spray with 25% *S. platensis*.



**Fig. 1a. Plot of main impacts (tested by ANOVA) for leaf length of *Allium sativum* sprayed with different liquid algal extracts (C= Control, SP= *Spirulina platensis*, UF= *Ulva fasciata* and PT= *Pterocladia capillaceae*).**



**Fig. 1b.** Plot of main impacts (tested by ANOVA) for shoot fresh weight of *Allium sativum* sprayed with different liquid algal extracts (C= Control, SP= *Spirulina platensis*, UF= *Ulva fasciata* and PT= *Pterocladia capillaceae*).



**Fig. 1c.** Plot of main impacts (tested by ANOVA) for shoot dry weight of *Allium sativum* sprayed with different liquid algal extracts (C= Control, SP= *Spirulina platensis*, UF= *Ulva fasciata* and PT= *Pterocladia capillaceae*).

#### 4. Phytochemicals

Table 5 shows the GC-MS analysis data of hexane and methanol extracts from control and SP25-treated plants. The phytochemicals quantities that identified in these extracts have been articulated as ratios from the total extract (Area %). Comparison of the analytical data of the hexane and methanol extracts revealed differences quantitative composition. There was a marked increase in the

percentage concentration of Diallyl disulphide, Trisulfide, methyl 2-propenyl, 2-Methoxy-4-vinylphenol and Phytol by 1.3, 1.9, 1.5, 2.7 under the influence of SP25 treatment relative to the control plants. However, the percentage concentration of Pentadecanoic acid, n-Hexadecanoic acid, 9,12-Octadecadienoic acid (Z,Z)-methyl ester decreased by 3, 1.7, 2.4 in SP25 treated plants in comparison with untreated plants.

**Table 5. Phytochemical compounds identified in hexane and methanolic extracts of control and *A. sativum* leaves sprayed with SP25.**

Retention Time (minutes)	Compound	Molecular formula	Molecular weight	Area %		Library
				C	SP25	
<b>Hexane Extract</b>						
6.03	Nonane	C <sub>9</sub> H <sub>20</sub>	128	0.84	0.12	Replib
12.24	Octanoic Acid	C <sub>8</sub> H <sub>16</sub> O <sub>2</sub>	144	0.94	0.26	Mainlib
16.21	Tetradecane	C <sub>14</sub> H <sub>30</sub>	198	0.09	0.53	Replib
17.99	Pentadecane	C <sub>15</sub> H <sub>32</sub>	212	0.51	0.66	Replib
19.68	Hexadecane	C <sub>16</sub> H <sub>34</sub>	226	1.10	0.38	Replib
21.47	Heneicosane	C <sub>21</sub> H <sub>44</sub>	296	2.05	2.76	Replib
25.58	Pentadecanoic acid	C <sub>15</sub> H <sub>30</sub> O <sub>2</sub>	242	15.0	12.08	mainlib
29.43	Tetracosane	C <sub>24</sub> H <sub>50</sub>	338	3.25	1.31	Replib
<b>Methanol Extract</b>						
5.11	2-Methylpyrazine	C <sub>5</sub> H <sub>6</sub> N <sub>2</sub>	94	1.38	0.79	Replib
5.70	2-Furanmethanol	C <sub>5</sub> H <sub>6</sub> O <sub>2</sub>	98	2.28	5.87	Replib
7.79	Dimethyl trisulfide	C <sub>2</sub> H <sub>6</sub> S <sub>3</sub>	126	2.79	2.81	mainlib
10.07	Diallyl disulphide	C <sub>6</sub> H <sub>10</sub> S <sub>2</sub>	146	4.94	6.25	Replib
11.40	Trisulfide, methyl 2-propenyl	C <sub>4</sub> H <sub>8</sub> S <sub>3</sub>	152	8.98	10.90	mainlib

14.68	Trisulfide, di-2-propenyl	C6H10S3	178	2.46	3.07	mainlib
15.03	2-Methoxy-4-vinylphenol	C9H10O	150	1.27	2.74	mainlib
17.85	4-Phenylpyridinium bromide, 1-allylidene	C14H14BrN	275	1.16	1.53	mainlib
21.37	6-(3-Hydroxy-but-1-enyl)-1,5,5-trimethyl-7-oxabicyclo[4.1.0]heptan-2-ol	C13H22O3	226	0.74	1.38	mainlib
23.45	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	C20H40O	296	0.55	0.46	mainlib
24.73	Hexadecanoic acid, methyl ester	C17H34O2	270	2.05	1.30	Replib
25.56	n-Hexadecanoic acid	C16H32O2	256	12.76	11.08	Replib
27.06	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	C19H34O2	294	4.15	1.68	Replib
27.32	Phytol	C20H40O	296	7.78	10.45	Replib

C= control plants; SP25= 25% *S. platensis* aqueous extract.

## Discussion

The extensive utilize of chemical fertilizers to augment crop quality and production has major consequences for the health of human and the environment (**Win et al., 2018**). Agriculture is the second-largest contributor to global climate change, and chemical fertilizer production and application both produce significant amounts of emissions (**CAIT, 2022**). As a result, it is strongly

encouraged to look for environmentally friendly, practical alternatives to current crop improvement techniques.

Algal biofertilizers are inexpensive, efficient natural nutrient recyclers and reservoirs that improve plant protection, increase plant growth, crop output, and reduce additional environmental damage (**Tilman *et al.*, 2002; Bhardwaj *et al.*, 2014; Iqbal *et al.*, 2021**). Previous research has demonstrated that foliar spraying of algal extracts promotes the development of a variety of plants, including mango (**El-Sharony *et al.*, 2015**), tomato (**Garcia-Gonzalez *et al.*, 2016**), cardoon (**Amer *et al.*, 2019**), broccoli (**Shams *et al.*, 2019**), and wheat (**Mansour *et al.*, 2019**). The recent study found that foliar spraying of aqueous extracts from algal species improved plant growth when compared to controls, with the SP25 extract producing the best effects. However, additional spray doses above 25% did not significantly improve aspects of garlic growth. In accordance with these findings, applying seaweed extract to bean plants with *Fucus spiralis* (25%) or *Ulva rigida* (25%) concentrations increased vegetative growth (**Latiq *et al.*, 2013**). **Garcia-Gonzalez *et al.* (2016)** found that foliar application of *Acutodesmus dimorphus* aqueous extract up to 50% increased tomato plant growth parameters. On the contrary, **Hernández-Herrera *et al.* (2013)** noticed that seaweed extract foliar sprays at concentrations more than (0.4%) shorten the shoot lengths of tomato plants. The effectiveness of foliar applications of algal extract for plant growth promotion may be influenced by a variety of parameters, such as light intensity, temperature, humidity, application rate, nutrient concentration, and surfactants. (**Fernández and Eichert, 2009**).

According to this study, the improvement in growth parameters in algal treated garlic plants might be attributable to the presence of organic acids, hormones, amino acids, and minerals in algal extracts, that can affect and improve agricultural crop production (**Iqbal *et al.*, 2021**). It was shown that auxin in algal extracts plays fundamental functions in cell proliferation and cell expansion,

resulting in an expansion in dry matter and varied chemical compositions. (Gollan and Wright, 2006; Chu *et al.*, 2010; Zarezadeh *et al.*, 2020). Furthermore, Tarakhovskaya *et al.* (2007) shown that specific microalgal extracts promote crop development, that have been interacted to plant growth regulators (cytokinins, auxins and gibberellins) and high amounts of macro- and micronutrients. Additionally, Elarroussi *et al.* (2016) claimed that algal extract includes polysaccharides stimulated the physiological actions of the developed plants, favourably reflecting on their growth features. Pigment and protein content increased in treated plants with SP25. This agrees with the earlier reports that, Foliar application of liquid biofertilizer increases the proportion of chlorophyll, proteins of *Solanum melongena* (Sivasangari *et al.*, 2015)

Several elements (both micro & macro) including Cu, Fe, Mg, Mn, Zn N, P, K and Ca, are abundant in *S. platensis*, according to our investigation. Also, further elemental analysis of SP25-treated garlic leaves showed enhancements in N, S, Mg and Ca. Therefore, it can be concluded that the application of algal treatment was responsible for the increase in the amounts of these elements. This finding is consistent with that of Marrez *et al.* (2014), who noted that *S. platensis* extract contains a variety of macro- and micro-elements, which play a variety of roles in physiological processes in various crops such as cell divisions, cell elongation and photosynthesis that reflect healthy growth of plant, dry matter contents, various chemical constituents and yield.

The SP25 algae treatment had an impact on the phytochemical components of garlic leaves, according to the GC-MS analysis. This finding is consistent with that of Amer *et al.* (2019), who found that a very significant alternations has been occurred in the main and minor fractions of phenolic compounds in cardoon plant after applying the foliar application of extracts from both *Chlorella vulgaris* and *Amphora coffeaeformis*. Also, Abd El-Aleem *et al.*

(2017), shown that fennel oil concentration intensified after algal treatment with *S. platensis*.

The significant increase in sulphur, which led to an increase in sulfur-containing amino acids necessary for the biosynthesis of organosulfur compounds, may explain the higher percentage of diallyl disulphide, trisulfide, methyl 2-propenyl, trisulfide, and di-2-propenyl in SP25-treated plants compared to control plants in this study. Our observations are in line with **Jones *et al.* (2004)** who declared that the availability of Sulphur controls the synthesis of the precursor of organosulfur compounds in *Allium* species. Moreover, **Khalid, (2013)** stated that plant nutrition was the most important aspects influencing the development, production, and chemical components of medicinal plants. Algae extracts, on the other hand, are thought to be a good source of protein that separates into natural amino acids that are crucial for metabolism (**Marrez *et al.*, 2014**). This may explain the higher fraction of various organosulfur compounds in this study.

There are two proposed pathways for the synthesis of methyl cysteine sulphoxide (precursor of organosulfur compounds in *Allium* species). The first pathway in which methyl cysteine sulphoxide is synthesized *via* glutathione (**Granroth, 1970**), while, in the alternative route *via* O-acetyl serine (**Lancaster and Shaw, 1989**). Depending on the physiological state of the plant, either process may occur.

A significant bioactive component of garlic called diallyl disulfide (DADS) has been shown to have various pharmacological properties, comprising, antioxidant, anti-inflammatory, antimicrobial, neuroprotective, cardiovascular protective, and anticancer activities (**Zhang *et al.*, 2020; Song *et al.*, 2021**). Methyl 2-propenyl, Trisulfide and di-2-propenyl (diallyl trisulfide) are members of the class of organic compounds known as trisulfides, which have been shown

to have antimicrobial and anticancer properties (**Rattanachaikunsopan and Phumkhachorn, 2008; Kaschula et al., 2010; Chen et al., 2012**). It is possible that the treatment of the algae increased the amount of these chemicals, which in turn improved the plant's medicinal capabilities.

### **Conclusion**

This study shown that a liquid algal extract from *S. platensis* efficiently induced the development of garlic (*Allium sativum L.*) and enhanced several organosulfur components (Diallyl disulphide and Trisulfide), which boosted the nutritional and therapeutic value of garlic. Consequently, the extract of *S. platensis* may be a good choice for making powerful bio stimulants. *S. platensis* aqueous extract is a superb option for an organic fertilizer because it contains inorganic minerals. The use of eco-friendly algal extract might thus be recommended to agronomists in order to improve the growth of other significant crops.

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إمكانية استخدام ثلاثة مستخلصات طحلبية كمنشطات حيوية لتعزيز نمو وتحسين المواد  
***Allium sativum* L.** الفيتوكيميائية لنبات الثوم

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الثوم (*Allium sativum* L) هو عشب أيورفيدي تم استخدامه على نطاق واسع كدواء وكمحسن لمذاق الطعام. تم تحديد تأثيرات الرش الورقي للثوم بتراكيز مختلفة (0.5، 1، 25 و 50%) المشتقة من المستخلصات السائلة لثلاثة طحالب: *Spirulina platensis*، *Ulva fasciata* و *Pterocladia capillacea* على النمو، الأصباغ، محتوى البروتين، كما تم دراسة المغذيات الكبيرة والذقية، والمكونات الفيتوكيميائية. أظهرت النتائج أن رش أوراق نبات الثوم بـ 25% *Spirulina* أدى إلى زيادة محتواها من البروتين ومحتوى الكلوروفيل وطول الورقة بنسبة 18 و 20 و 49% على التوالي. بالإضافة إلى ذلك، زاد المحتوى الكلي للنيتروجين والكبريت بمقدار 1.2 و 5 أضعاف على التوالي. وقد أظهرت الدراسة أن استخدام مستخلصات الهكسان والميثانول على النباتات الغير معاملة والمعاملة بمستخلص 25% *Spirulina* باستخدام GC-MS ارتفاعاً في نسبة تركيز ثنائي كبريتيد الديليل، ثلاثي كبريتيد، 2-ميثيل بروبيثيل، 2-ميثوكسي-4-فينيلفينول وفينول. بنسبة 1.3، 1.9، 1.5، 2.7 في النباتات المعاملة بالمستخلص الطحلي عنها في النباتات الغير معاملة بالمستخلص. وخلصت الدراسة أنه يمكن استخدام مستخلص *Spirulina platensis* لتحسين النمو والمعادن ومركبات الكبريت العضوية لنبات الثوم.