

The Effect of Seaweed Extracts on Chemical Composition of Tomato Plant (*Solanum lycopersicum*)

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ABSTRACT

Seaweed extracts usually used in agriculture and horticulture as nutrient supplements, biostimulants, or biofertilizers to improve plant growth and yield. In this study, to explain these effects. GC-MS was used to measure the change in chemical composition of tomato plant that treated with liquid seaweed extracts (LSWE). The effect of LSWE isolated from *Ulva lactuca* (green algae) and *Grateloupia filicina* (red algae), on the chemical composition of tomato (*Solanum lycopersicum*) that ground under greenhouse condition. Were assessed LSWE was sprayed at concentration (1.5 %) on tomato seedlings seven times (one a week) at the end of seven weeks tomato leaves were collected from each treatment then extracted and subjected to GC-MS analysis. Our results indicate that seedlings treated with LSWE increased the percentage of terpenes in plant leaf extract compared to untreated plants that contained (17.15%), while in plants treated with *Ulva lactuca* and *Grateloupia filicina* extract it was (35.52%) and (35.87%) respectively. The terpenes content monoterpenes and sesquiterpenes. No pronounced differences were estimated in the aliphatic alcohols content between the treated and untreated plants. Moreover, treatment with *Ulva lactuca* extract was shown to have an effect in increasing the level of fatty acids 18% in tomato plant leaves, while there was no effect of treatment with *Grateloupia filicina* extract, (11%) compared with untreated plants (12%).

Key words: Seaweed, Chemical composition, Green algae, Red algae, Tomato.

INTRODUCTION

Seaweeds are rich in fiber, several minerals, including iodine, polyunsaturated fatty acids (PUFA), proteins, essential amino acids, polysaccharides, and other nutrients. They also contain various types of metabolites such as sesquiterpenes, phlorotannins, bromoditerpenes, halogenated furanones, lectins, and other compounds, which are released by the algae to protect them against various adverse conditions they face (El Arroussi 2019). Seaweeds, therefore, are promising source of diverse biological activities (Venugopal 2019). Seaweed has been used to improve plant growth and yield worldwide. Modern agriculture seeks new biotechnologies that would allow the use of chemical inputs to be reduced without adversely affecting crop yield or the income of farmers. In recent

years, for example, the use of natural seaweed products as fertilizer has Permission for partial replacement of traditional synthetic fertilizer (Dhargalkar and Pereira 2005; Hong *et al.* 2007; Khan *et al.* 2009; Zodape *et al.* 2010). These extracts are sold as biostimulants liquids because existence of a broad spectrum of plant growth-promoting substances such as auxins, cytokinins, and betaines has been seen by chemical analysis of seaweeds and their products. (Khan *et al.* 2009). Such substances can affect the development of the shoot and root system (Durand *et al.* 2003; Stirk *et al.* 2004). The use of seaweed extracts has been reported to have several beneficial effects. good responses Which include Enhanced germination, root improved, quality of leaves, vigor of plants, and pathogens resistance. The resistance of plants against pathogens is induced by the application of agents called elicitors and can contribute to disease prevention or decreased severity. The strength of elicitor extracted from the seaweed to elicit induced resistance in tomatoes were reported (Khan *et al.* 2009; Layth *et al.* 2015). In terms of human consumption, tomatoes are among the most valuable vegetable crops in the world., and They are the most popular garden vegetables as well. However, the intense application of chemical fertilizers and pesticides that affect soil ecology and agricultural systems is one of the key problems facing tomato production. (Villarreal-Sánchez *et al.* 2003). The aim of this study is to examine the effect of liquid seaweed extracts on the change in chemical composition of tomato plants.

MATERIAL AND METHODS

Chemicals: All chemicals and solvents were purchased from Merck, Germany.

Algae sampling and extraction: Algal materials were collected from the eastern port of Alexandria, Mediterranean Sea, during April 2019. The collected algal samples were stored in plastic bags and transported to the laboratory in ice box. The samples were initially washed thoroughly with sea water to remove sand and any adhering substance and then washed thoroughly with fresh water to remove salts. The algal species were identified based on the schemes reported in the literature (Bold, 1978; Aleem, 1993 and Coppejans *et al.*, 2009). Sample were oven-dried for 72

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h at 60°C, and then ground in an electric mill. This milled material 100 g was added to 1 L of distilled water with constant stirring for 15 min followed by sonication in water bath sonicator for 2 h at 40°C. The hot extract was filtered through a Whatman No. 40 filter paper and stored. The liquid seaweed extract (LSWE) stored at 4°C in the lab Phycology, Botany and Microbiology Department, national institute of oceanography and fishers (NIOF). Until use as foliar application spray.

Greenhouse growth: Tomato plants were grown in a growth chamber under controlled condition 16 hours of light at 25°C and 8 hours of darkness at 18°C in sterilized soil peatmoss. One hundred and fifty 15 days old plants were selected and randomly allocated to various treatment groups and transplanted into pots containing a sand and peatmoss (2:1 w/w) soil mix. Tomato plants were fertilized after one week of transplanting and treated with 50 mL of 20:20:20 (N-P-K) soil drench solution. Irrigation was done with water (50 mL every three days), Potted plants were grown in a greenhouse at 25±2°C and 85% relative humidity. Plants sprayed with 50 ml of (LSWE 1.5%) and water for untreated for each treatment every week for 7 weeks. The design and treatment for the experiment were similar to those reported by Crouch and van Staden (1992). The experiment was designed into three different treatments. Each treatment is Five replicates each one consisting of ten plants. The control was the first treatment which plants were grown without LSWE. The other two treatment treated with the two type of seaweed species used to produce the LSWE.

Extraction methods: After 7 weeks spray tomato plant leaves from each treatment were extracted. Harvest the leaves (1.0 g) from tomato immediately freeze at -80 °C and Frozen tissues dried using Lyophilizer then tissue Transferred in a glass vial with cap and 10 ml of methanol was added and 1 g sodium sulfate anhydrous then sonication was done using water bath sonicator at 37 °C for 2 h. Result and extract was filtered through a syringe filter 0.2 µm, evaporated under vacuum at 45 °C. Organic extracts results were dissolved in methanol and used for injection in GC-MS.

Chemical analysis: GC/MS analyses were conducted in Central Lab of national institute of oceanography and fishers (NIOF). The GC-MS analysis was done using A Thermo Scientific gas chromatograph GC TRACE ULTRA 1300 coupled with an EI Mass spectrometer ISQ 7000 model (Thermo Scientific USA) equipped with Thermo TR-50 MS capillary column (30 m in length × 250 µm in diameter × 0.25 µm film thickness).

Spectroscopic detection by GC-MS involved an electron ionization system which utilized high energy electrons (70 eV), MS transfer line temperature 300 °C and ion source temperature 300 °C. Pure helium gas (99.995%) was used as the carrier gas with flow rate of 1 mL/min. The initial temperature was set at 60°C for 2 min, then increased to 100 °C at a rate of 10 °C/min kept for 5 min, then with 10 °C/min to 150 °C and kept for 5 min, then with 10 °C/min to 200 °C and kept for 5 min, then with 10 °C/min to 250 °C and kept for 20 min. One microliter of the prepared extracts was injected in a splitless mode. Wiley and Wiley NIST mass spectral data base were used in the identification of the separated peaks.

RESULTS AND DISCUSSION

The results obtained from analyzing tomato leaf extract using mass GCMS are shown in (Figure 1). The emergence of a variety of compounds and active groups are shown in (Table 1).

The results are expressed in percentage of the methanolic extract of tomato leaves *Solanum lycopersicum*

The data showed that most important of existence compounds are the terpenes (Figure 2), where 6 compounds of terpenes appeared, 4 compounds belonging to monoterpene. α -Terpinene, Phellandrene, Z-Citral and 2-carene and two compounds of sesquiterpenes, Caryophyllene and α -Elemene. The results indicated that treatment with LSWE had an effect on terpenes levels in tomato plants *S. lycopersicum*, where the percentage of terpenes results of treatment with LSWE isolated from *U. lactuca* (green algae) was 35.87%, while LSWE made from *G. filicina* (red algae) was 35.52%, while the untreated plant values were 17.15%. The results showed a pronounced difference were estimated of treatment with LSWE. There was No differences between treatment with LSWE made from red or green algae, as the results obtained proved the presence of terpenes in the extract, and these results are compatible with (Ghada *et al.* 2017 and María *et al.* 2017). As has been proven in previous studies, terpenes are considered compounds that have a strong effect on plant pathogens, especially bacteria and fungi, and some of them have an effect on some types of insects and worms. Riju *et al.* (2019). These results may explain how the algae effect increases the resistance of plants treated with algae, which naturally leads to the good growth of the plant and the plant becomes healthier.

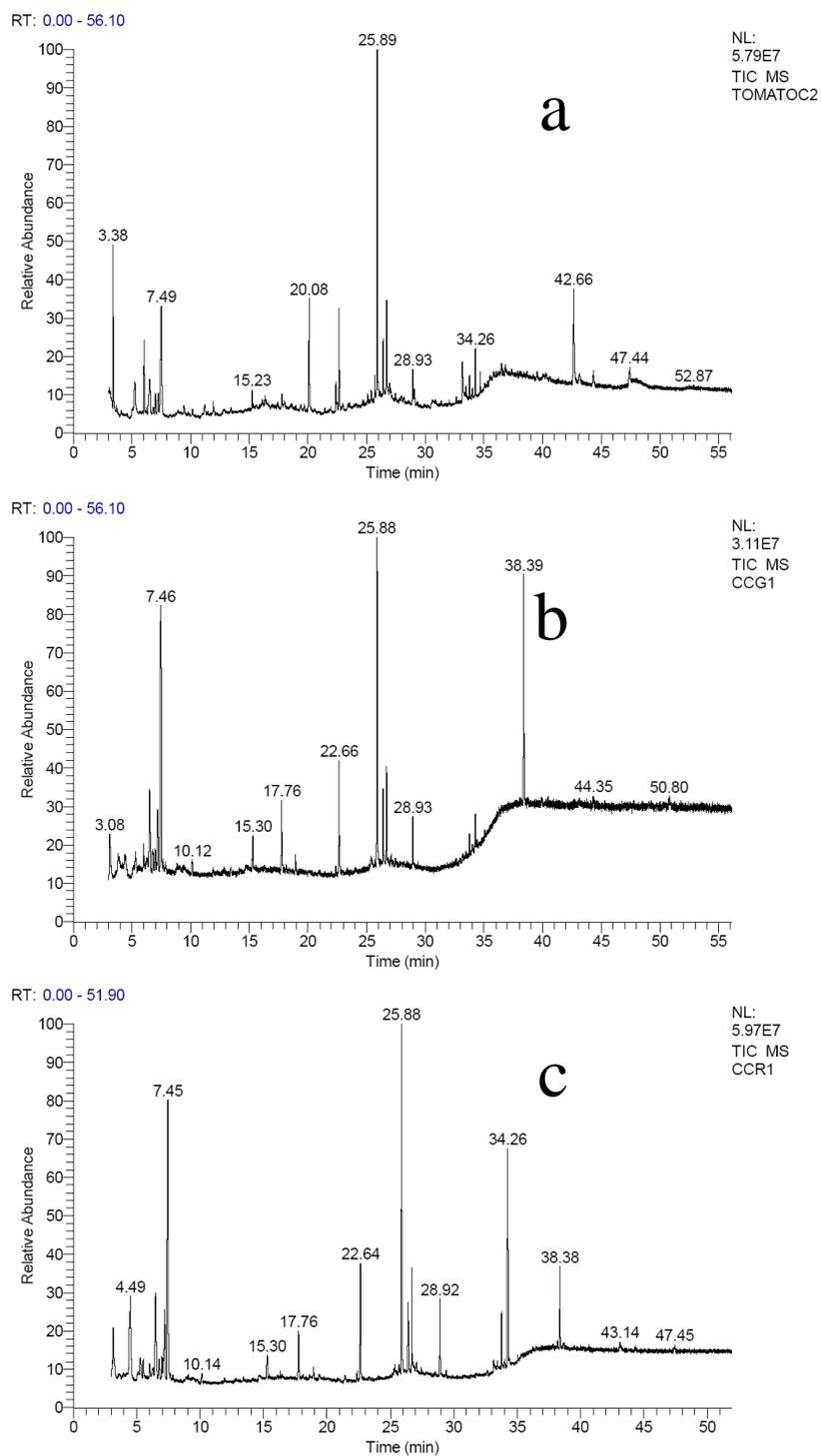


Fig. 1. GCMS analysis of tomato (*Solanum lycopersicum*) leaf methanolic extract, untreated tomato (a), treated with *Ulva lactuca* (green algae) extract (b) and treated with *Grateloupia filicina* (red algae) extract (c)

Table 1. Main components of tomato leaves extracts in control, *Ulva lactuca* (green algae) extract and *Grateloupia filicina* (red algae) extract and identified by GC/MS

R. Time	Compounds	Control	<i>U. lactuca</i> (green algae)	<i>G. filicina</i> (red algae)
		%	%	%
3.09	Tetraacetyl-d-xylonic nitriled-	N.D.	3.29	N.D.
315	Cyclododecanone, thiosemicarbazone	N.D.	N.D.	3.28
3.19	2-methyl-1,3-Butanediol,	0.76	N.D.	N.D.
3.81	2H-Pyran,tetrahydro-2-(2,5-undecadiynyloxy)-	N.D.	2.06	N.D.
4.40	5,7-Dodecadiyn-1,12-diol	N.D.	1.41	N.D.
4.49	3-Hexenal	N.D.	0.67	6.52
5.25	n-Decane	2.98	1.35	1.28
5.51	Hexadecane, 1,1-bis(dodecyloxy)-	N.D.	N.D.	1.11
6.04	2-hydroxy-2-methyl-Propanoic acid,	3.70	1.15	0.71
6.50	α -TERPINEN)	3.92	5.94	6.26
6.77	8,11-Octadecadiynoic acid, methyl ester	N.D.	1.58	1.37
7.01	Undecane	1.54	1.16	0.90
7.24	2-Carene	1.80	3.96	5.18
7.49	α -Phellandrene	9.47	18.90	19.15
10.16	3-hydroxy-2-Dodecanoic acid,	0.53	0.93	0.60
11.20	9-Octadecen-12-ynoic acid, methyl ester	0.97	N.D.	N.D.
11.94	α -methyl-Benzeneethanamine,	0.93	0.46	N.D.
15.23	2-methyl-1-Hexadecanol,	1.01	N.D.	N.D.
15.30	α -Elemene	N.D.	2.17	1.44
16.32	2-Hexadecanol	0.46	N.D.	N.D.
17.79	Caryophyllene	1.02	4.26	2.75
18.94	2,5-Octadecadiynoic acid, methyl ester	N.D.	1.05	0.60
20.09	Fumaric acid, ethyl2-(2-methylenecyclopropyl) propyl ester	7.76	0.48	N.D.
22.38	2H-Indeno[1,2-b] furan-2-one-octahydro-8,8- dimethyl	1.90	0.54	0.60
22.65	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	6.29	6.13	6.06
25.07	12-Methyl-E, E-2,13-octadecadien-1-ol	0.48	N.D.	N.D.
25.39	2-Hexadecanol	0.61	0.68	0.58
25.68	3,7,11-trimethyl-1-Dodecanol,	1.08	0.29	0.36
25.89	Phytol	13.93	12.34	12.61
26.41	13-Heptadecyn-1-ol	2.42	3.10	2.81
26.69	1-Octadecyne	4.33	4.39	3.85
28.93	Hexadecanoic acid, methyl ester	2.18	2.75	3.56
33.16	(3,5 α)-Cholestan-3-ol, 2-methylene-,	2.69	N.D.	0.63
33.46	Ethyl iso-allocholate	0.67	N.D.	0.39
33.77	(Z,Z,Z)-8,11,14-Eicosatrienoic acid,	1.09	1.08	2.30
34.03	(E)-10-Heptadecen-8-ynoic acid, methyl ester,	0.59	0.67	0.52
34.26	Linolenic acid, methyl ester	2.11	1.59	8.40
34.67	9-Hexadecenoic acid	1.09	N.D.	N.D.
35.46	1-Heptatriacotanol	0.44	N.D.	N.D.
43.13	Z-Citral	0.94	0.64	0.74

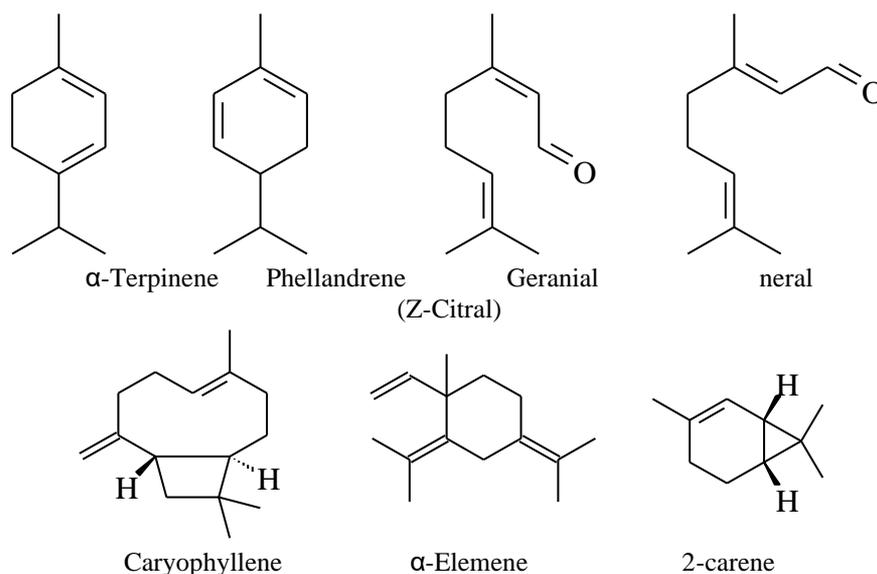


Fig. 2. Chemical structure of monoterpenes and sesquiterpenes in tomato (*Solanum lycopersicum*) leaf methanolic extract

Fatty acids are also among the important groups that are present in the tomato plant, as shown in (Table 1), which has a very important role in biological processes and increase plant resistance to pathogens and unfavorable conditions. The results showed that the treatment with algae extract affected the fatty acid content. The effect was greater in the case of treatment with green algae extract, where the percentage of fatty acids was 18%, while the treatment with red algae

extract was 11% compared to the non-treatment was 12%. A pronounced differences were estimated in the case of treatment with green algae extract in one site and red algae extract or control in another site. Similar results were obtained by Hansson *et al.* (2012), they proved the presence of ethyl ester in tomato extract. Tikunov *et al.* (2005), also, recognized hexadecanoic acid, ethyl ester in tomato plant extract (*Lycopersicon esculentum*) genotype.

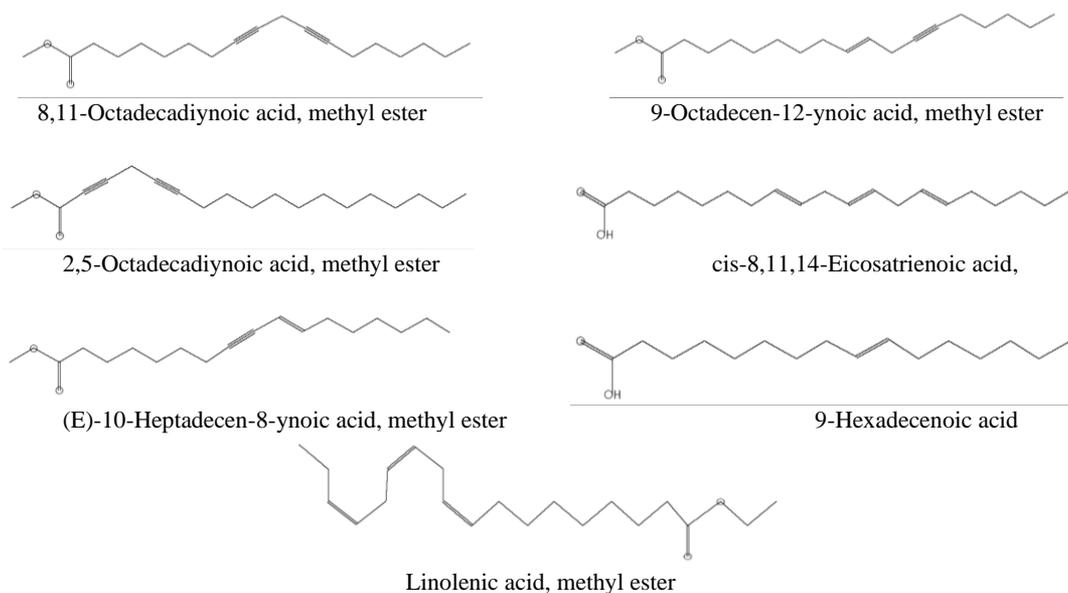


Fig. 3. Chemical structure of unsaturated fatty acid (USFA) in tomato (*Solanum lycopersicum*) leaf methanolic extract

The unsaturated fatty acids were also considered due to their great importance, as shown in (Figure 3). The results showed that most of the fatty acids are unsaturated fatty acids in the case of treatment with green algae extract, where it was 13.19%. While in the case of treatment with red algae extract, it was similar to the non-treatment where the percentage of unsaturated fatty acids was 5.97% and 5.85%, respectively, which shows the decrease in saturated fatty acids, unlike in the case of treatment with green algae extract, that showed increase.

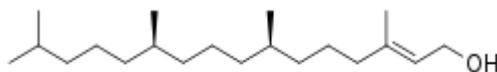


Fig. 4. Phytol

While there were no pronounced differences were estimated in the percentage of aliphatic alcohols as a result of the different treatments. The most important compounds are phytol and 3,7,11,15-Tetramethyl-2-hexadecen-1-ol, as shown in (Figure 4), where 13% and 6% were present, respectively, as shown in (Table 1). Similar results were obtained by Juliana et al. (2016) they recognized phytol as bioactive compound of tomato extract. Also, Hansson et al. (2012) reported that treatment with green algae (*Ulva lactuca*) extract led to an increase in the concentration of some biologically important compounds such as Tetraacetyl-d-xylic nitriled - as in Figure 5. This compound have bio-efficiency as stated in the study at Imad et al. (2015). that proved the presence of the compound in the methanolic extract and has efficacy against bacteria and fungi. This finding may ex. Tetraacetyl-d-xylic nitriled might be an indication of plant tolerant or resistant against microbiological pathogens explain the improvement of the plant's condition and increase its resistance against infection with fungal and bacterial pathogens. Generally of some important compounds such as terpenes, phenols, fatty acids, especially unsaturated and other compounds.

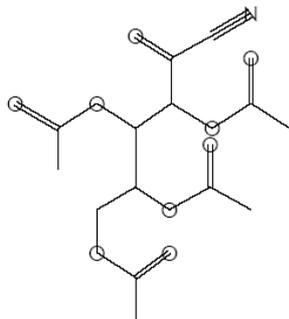


Fig. 5. Tetraacetyl-d-xylic nitriled

CONCLUSION

The results show that the treatment with LSWE had a great effect on increasing the levels of very important compounds, especially green algae. Therefore, by looking at the change in the chemical composition as a result of treatment with algae extract explains the improvements that might occur to the plant from that treatment.

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الملخص العربي

تأثير مستخلصات الطحالب البحرية على التركيب الكيميائي لنبات الطماطم (*Solanum lycopersicum*)

محمد احمد الشيخ، سامية نعيم سليم وحزمة سمير أبوالنصر

باستخدام كروماتوجراف الغاز - مطياف الكتلة GC-MS حيث أظهرت النتائج ان المعاملة بمستخلص الطحالب ادي لزيادة نسبة التريينات في أوراق النبات حيث كانت نسبة التريينات في مستخلص أوراق النبات ٣٥.٨٧ و ٣٥.٥٢ للمعاملة بمستخلص الطحالب الخضراء والحمراء على الترتيب مقارنة بالكنترول ١٧.١٥٪. اما بالنسبة للكحولات الأليفاتية لم يكون هناك فرق يذكر بين المعاملات المختلفة. بينما أدت المعاملة بمستخلص الطحالب الخضراء لزيادة مستويات الاحماض الدهنية في مستخلص أوراق نبات الطماطم حيث كانت ١٨٪ بينما الطحالب الحمراء كانت ١١٪ والكنترول ١٢٪.

الطحالب البحرية أصبح لها دور هام في مجالات عديدة خاصة في مجال الزراعة حيث تستخدم مستخلصاتها كمخصبات طبيعية للنبات حيث تعمل علي تحسين النمو الخضري والجذري وتحسين مواصفات المنتج ورفع المناعة المستحثة في النبات. في هذه الدراسة تم دراسة تأثير المعاملة بمستخلص نوعين من الطحالب البحرية الطحالب الخضراء *Ulva lactuca* والطحالب الحمراء *Grateloupia filicina* على التغير في التركيب الكيماوي في نبات الطماطم تحت ظروف متحكم بها بالصوبة حيث تم معاملة النبات رشاً بمستخلص الطحالب بتركيز ١.٥٪ عدد سبع مرات لبادرة الطماطم بمعدل معاملة أسبوعيا لمدة سبع أسابيع. تم جمع أوراق الطماطم للمعاملات والكنترول وتم استخلاصها للتحليل