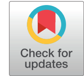
Biotechnology Reports 25 (2020) e00426

Contents lists available at ScienceDirect

Biotechnology Reports

journal homepage: www.elsevier.com/locate/btre

Microalgae polysaccharides bio-stimulating effect on tomato plants: Growth and metabolic distribution



Farid Rachidi[a,b], Redouane Benhima[a], Laila Sbabou[b], Hicham El Arroussi[a,*]

[a] Green Biotechnology Laboratory MAScIR (Moroccan Foundation for Advanced Science, Innovation & Research), Madinat Al Irfane, Rabat, Morocco
[b] Laboratory of Microbiology and Molecular Biology, Faculty of Sciences, University Mohammed V, Rabat, Morocco

ARTICLE INFO

Article history:
Received 1 June 2019
Received in revised form 24 January 2020
Accepted 26 January 2020

Keywords:
Microalgae
Polysaccharides
Biostimulant
Solanum lycopersicum
Metabolomic

ABSTRACT

Microalgae polysaccharides represent a potentially bioressource for the enhancement and the protection of agricultural crops. We investigate the possibility to use microalgae polysaccharides as a plant biostimulant. The crude polysaccharides extract (PS) from three microalgae strains were applied to *Solanum lycopersicum* plants by irrigation and compared basing on their effects on shoot and root length, nodes number and shoot and root dry weight. The application of 1 mg mL^{-1} PS from *A. platensis, D. salina* and *Porphorydium* sp. on tomato plants improved significantly the nodes number (NN), shoot dry weight (SDW), and shoot length (SS) by75 %, 46,6 %, 25,26 % compared to control respectively. Furthermore, crude PS treatment showed an improvement of carotenoid, chlorophyll and proteins content, and Nitrate Reductase (NR), NAD-Glutamate Dehydrogenase (NAD-GDH) activities in plants leaves compared to control. 1 mg mL^{-1} of *Porphorydium* sp. enhanced significantly the carotenoid content and NAD-GDH activity by 400 %, 200 % compared to control respectively. In the same way, *A. platensis* PS improved chl a, chl b and NR activity by 90.1 %, 102.7 % and 88.34 compared to control respectively. In addition, it is found that a PS treatment has affected the protein content, which reaches 88.3 % under 0.5 mg mL^{-1} of *D. salina* PS treatment. GC–MS metabolomics analysis also showed a change in lipids, sterol and alkanes profiles. Some sterols precursors were increased such as Cholesta-6,22,24-triene, which may indicate an enhancement of the biosynthesis of sterols and/or steroidal glycoalkaloids in treated plants.

Therefore, this is an evidence to use microalgae polysaccharides as a plant biostimulant.

© 2020 Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

Microalgae are a diverse group of eukaryotic and prokaryotic photosynthetic microorganisms that can grow rapidly and live in harsh conditions due to their unicellular structure [1]. They constitute a diverse group of organisms with a wide range of physiological and biochemical characteristics. Thus, they naturally produce many different bioactive compounds namely, proteins, lipids, carotenoids, vitamins and polysaccharides [2]. Polysaccharides produced mainly by seaweeds are widely used in different industries. Significant amounts of polysaccharides are used in food, pharmaceuticals and other products for human consumption. Microalgae and cyanobacteria have complex carbohydrate metabolic pathways encompassing the ability to synthesize intracellular monosaccharides, polymeric reserve glucans and structurally complex extracellular polysaccharides (EPSs) [3]. Several microalgae such as *C. reinhardtii*, *B. braunii*, *D. tertiolecta*, *P. cruentum, Spirulina* sp, *I. galbana* and *D. salina*, can be a source of exopolysaccharides [4,5].

Microalgae polysaccharides especially sulfated polysaccharides are exploited in different fields due to their biological properties including anticoagulant, anti-inflammatory, antiviral and antitumoral activities and also for their good biocompatibility, biodegradability, non-toxicity, low cost and abundance [6]. In the agricultural field, microalgae polysaccharides can be a new bioactive source of plant biostimulants for crop improvement and protection against biotic and abiotic stress [7–13]. Several studies have shown the potential of these molecules to stimulate different metabolic pathways of plants. In the same way, Guzmán-Murillo et al., [14] showed that exopolysaccharides extracted from *P. tricornutum* and *D. salina* stimulated the germination of pepper under salt stress conditions. In addition, *D. salina* exopolysaccharides have shown the potential to stimulate germination, growth and tolerance of tomato and wheat plants at salt stress [4–15]. On the other hand, the aqueous extract rich of *Scenedesmus* sp and *A. platensis* polysaccharides and oligosaccharides showed a good capacity for improving plant growth, development and leaf

* Corresponding author.
E-mail addresses: farid.rachidi.bio@gmail.com (F. Rachidi), h.elarroussi@mascir.com (H. El Arroussi).

https://doi.org/10.1016/j.btre.2020.e00426
2215-017X/© 2020 Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

nutrient status of *Petunia x hybrida* plant, offering an interesting potential use as biostimulants [16]. Another study showed that liquid extracts of *C. vulgaris* and *S. quadrica* induced stimulation of root development of *Beta vulgaris* L. plants [17].

The objective of this study was to study the biostimulatory effect of microalgal polysaccharides on the growth and metabolite distribution of tomato plants under controlled conditions.

2. Material and methods

2.1. Microalgae culture

Microalgae strains (*Arthrospira platensis MS001*, *Dunaleilla salina MS002*, *Porphyridium* sp. *MS099*) were isolated from Moroccan aquatic ecosystems and maintained in MAScIR's (Moroccan Foundation for Advanced Science, Innovation and Research) collection. The microalgae were cultivated in 500 mL Erlenmeyer containing Walne's medium with pH 8.2 at 25 °C for *D. salina*, and *Porphyridium.* sp. *A. platensis* was cultivated in Zarrouk medium in pH 9at 30 °C. All cultures were incubated by agitation in orbital shaker at 130 rpm under continuous illumination (150 ± 10 μmol m^{-2} s^{-1}). After 23 days, total biomass was harvested by centrifugation at 4200 *g* for 10 min and rinsed with water before lyophilizing.

2.2. Microalgae polysaccharides production and characterization

1g of dry microalgae biomass powder was suspended in 40 ml of distilled water and incubated at 90 °C for 2 h with stirring. The mixture was centrifuged at 4000 *g* for 15 min. After recovering the supernatant, the extraction process was repeated twice, and then the supernatants were mixed with two volumes of absolute ethanol, stirred vigorously and left overnight at 4 °C. The precipitated polysaccharides were recovered in pellet by centrifugation at 16800×g for 10 min. The PS pellet was washed three times with absolute ethanol, lyophilized and stored at −80 °C for future use.

The total neutral sugar and uronic acids contents of the crude PS were determined according to two methods [18,19]. Sulfate content in polysaccharides was determined by the barium chloride-gelatin method [20].

Proteins content were determined according to the Bradford method using crystalline bovine serum albumin (BSA) as standard.

2.3. Plant growth and crude polysaccharides extracts treatment

In this set of experiments, we used *Solanum lycopersicum* L. var. Jana F1 (tomato) bayer nunhems netherlands bv. Seeds. Wide adaptable variety for main season (long production period). Plant is vigourous, good cover and short inter nodes, very high yield. Fruit is high quality with round shape and nice red color (purchased from "Syngenta Morocco") were surface-disinfected using sodium hypochlorite solution (1 %) containing 10 μL of Tween-20 for 20 min and then rinsed with sterile water.

Sterile seeds were kept in darkness in magenta boxes with MS/4 (Murashige and Skoog)-agar medium for 7 days at 25 °C and the seedlings were transplanted into 12 cm diameter pots containing a mixture of sandy soil and peat (60:40) sterilized twice in autoclave (121 °C,20 min). The young tomato plants were grown in a phytotron at 26 °C, 16 h/8 h Light/dark cycle and 240 μmol $m^{-2} s^{-1}$ illumination intensity and 68 % humidity. Plants were irrigated with 10 ml/plant of PSs solution (0.25, 0.5 and 1 mg/mL distilled water, pH 6.8) five time (one time a week).

Plants were irrigated every day with 20 mL of water, alternated twice a week with a nutrient solution containing: 8 μM $MnCl_2$, 0.5 μM $CuSO_4.5H_2O$, 1.4 μM $ZnSO_4$, 46 μM H_3BO_3, 0.25 μM $Na_2MoO_4.2H_2O$, 0.6 μM Fe-EDTA, 4.1 mM KNO_3, 0.9 mM K_2SO_4, 1 mM $MgSO_4.7H_2O$ and 1.5 mM KH_2PO_4 [21].

35 days after transplanting, the plants were harvested to measure growth parameters: shoot lenght, shoot and root dry weight (after an incubated at70 °C for 72 h) and nodes number. Five independent replicates for each treatment were performed.

2.4. Plant growth biochemical parameter analysis

Biochemical parameters such as pigments concentration (chlorophyll ***a***, chlorophyll ***b*** and carotenoids), protein and nitrogen reduction and assimilation-related enzymatic activities: Nitrate Reductase (NR) and NAD-Glutamate Dehydrogenase (NAD-GDH) were analyzed according to the following methods:

For the Chlorophylls and carotenoid concentration, we used the [22] equations to determine the content of pigments in which the ethanol (95 %) was used as the extraction solvent. The pigment was measured by spectrophotometer for chlorophyll *a*, chlorophyll b and carotenoid as follow:

Chl a=(13,36xA664)- (5,19xA648).

Chl b=(27,43xA648) – (8,12xA664).

Carotenoid = (1000xA470)–(2.270xChl a)–(81.4 Chl b/227)

The NR and NAD-GDH activities were measured according to the methods of Allegre [23] and Singh & Srivastava [24] respectively.

Simultaneously, an absorbance standard curve was prepared as a function of the concentrations of 0, 1, 2, 3, 4 and 5 μmol $L^{-1}$of NO_2^- in the reading solution. Thus, NR was estimated in μmol NO_2^- g^{-1} min^{-1}. Glutamate dehydrogenase activity is expressed in terms of μmol of NAD (H) oxidation/redaction (g FW) $^{-1}$ min^{-1}.

2.5. GC–MS metabolomics analysis of tomato plants treated with microalgae polysaccharides

The chloroform (5 mL) was added to 0.4 g of fresh tomato leaves ground treated with liquid nitrogen, the reaction was assisted with ultrasonic (Branson Sonifier 450): 40 KHz at room temperature for 1 h. Then the tubes were incubated in 85 °C for 4 h.

The chloroform extract was analyzed with GC–MS according to the method of EL Arroussi et al. [4]. GC MS analysis was performed after acid transesterification. The reaction was catalyzed by 6 % H_2SO_4 (w:w) in methanol and assisted by ultrasonic (Branson Sonifier 450) : 40 KHz at room temperature for 1 h. Volatile metabolites profile was characterized by gas chromatography (GC) (Agilent 7890A Series GC) coupled to mass spectrometry (MS) equipped with a multimode injector and 123-BD11 column with a dimension of 15 m x 320 μm x 0.1 μm and electron impact ionization. Detection was done using full scan mode between 30–1000 m/z, with gain factor of 5 and the identification was performed using NIST 2014 MS Library.

2.6. Statistical analysis

Statistical analysis was performed using SPSS 13.0 and R programs. Multivariate Analysis of Variance (MANOVA) and Tukey test perform Significant of difference analysis insured by SPSS. For the study of the correlation between the variables and parameters, we used the test of Pearson via the R program.

3. Results

3.1. Growth microalgae and polysaccharides production

The growth rate and biomass production of the three microalgae strains after 23 days of culture in laboratory conditions were (1.28 g L^{-1}; 0.13) (0.026, 0.95 g L^{-1}) and (0.03, 0.67 g L^{-1}) for *A. platensis*, *D. salina* and *Porphoridium* sp. respectively (Figs. 1a and b).

The Table 1 shows that the content of crude polysaccharides extracted from *A. platensis, D. salina* and *Porphyridium* sp. were 2.59 %, 4.1 % and 5.53 % respectively.

The microalgae crude PS was analyzed, and their neutral sugars, sulfate content, and uronic acid contents were measured by colorimetric analyses (Table 1). We note that PS are contain 17.27 %, 33.05 % and 35.35 % of neutral sugars; 3.6 %, 23.9 % and 13.8 % of uronic acids, and 5.3 %, 11.54 % and 10.4 % of sulfate group in *A. platensis, D. salina* and *Porphyridium* sp. respectively.

3.2. Effect of crude microalgae polysaccharides on growth of tomato plants

3.2.1. Effect on morphological parameters

The results on tomato plants growth irrigated with three concentrations (0.25 mg mL^{-1}, 0.5 mg mL^{-1} and 1 mg mL^{-1}) of crude PS from three microalgae species are presented in Table 2. All PS treatments significantly ($P < 0.05$) stimulated the tomato plant growth parameters compared to control. Treatment, 1 mg mL^{-1} of crude PS showed the greatest increase highly significant in shoot size (SS), numbers of nodes (NN), root dry weight (RDW) and shoot dry weight (SDW). The polysaccharide treatments of *D. salina, Porphorydium* sp. and *A. platensis,* at a concentration of 1 mg mL^{-1} induced a maximum improvement percentage highly significant of 46.61 %, 25.26 % and 12.12 % for SDW, SS, and RDW respectively. As the percentage of improvement of number of nodes induced by this concentration for all the strains is reached 75 % and it is highly significant in comparison with the control (Table 2).

3.2.2. Microalgae polysaccharides effect on pigments, protein concentration and enzymatic activities

The assessment of Chlorophyll, carotenoid, proteins concentration and enzymatic activity of NAD-GDH and NR as markers used in this study to assess the effect of microalgae crude PS on tomato plant growth. All microalgae treatment studied had a significant effect on carotenoid, Chlorophyll and protein content, as well as enzymatic activities of NAD-GDH and NR in tomato plant leaves compared to the control (Table 3). *A. platensis* crude polysaccharide treatments improved significantly chl **a** by 90.08 % in 0.25 mg mL^{-1}, chl **b** by 102.71 % in 0.5 mg mL^{-1} and nitrate reductase activity by 124 % in 1 mg mL^{-1} of PS compared to control plant. As we see that, the 1 mg mL^{-1} treatment of *Porphorydium* sp. improved highly significant the carotenoids content and NAD-Glutamate dehydrogenase activity with 468 % and 124 % respectively compared to control. Finally, the results show that the 0.5 mg mL^{-1} of *D. salina PS* induced a protein biosynthesis with a very significant percentage improvement of 88 % (Table 3).

3.2.3. GC–MS metabolomics analysis of tomato plants responses to microalgae crude polysaccharides

3.2.3.1. Lipid profile of tomato leaves. Very long chain fatty acids (VLCFAs) are essential to plants. They are involved as membrane constituents and signaling molecules in sphingolipids and phospholipids and are necessary for the production of cuticular waxes phospholipids and complex sphingolipids have, collectively, profound effects on embryo, leaf, root and flower development. In the same way, unsaturated fatty acids (UFAs) play key roles in membrane structure and function. We therefore observed the effect of PS crude extracts on Fatty acids using GC–MS. The results (Table 4) showed that the all-crude PS treatments allowed a modification of the Fatty Acid profiles in tomato leaves (Table 4). The highest improvement of VLCFA concentration was obtained by 0.5 mg mL^{-1} and 1 mg mL^{-1} treatment of *Porphyridium* sp and *D. salina*, reaching up to 70 % and 42 % respectively (Table 4). On the other hand, 0.25 mg mL^{-1} crude PS of *A. platensis* generally increased the UFA content by 41.03 % compared to the control, and particularly improvement of linolenic acid (C18:3) by 48.43 %. While, 0.5 mg mL^{-1} crude PS of *A. platensis* increased SFA content with 46.06 % and decreased UFA with 68.5 % compared to control (Table 4).

3.2.3.2. Sterol and Alkanes profile. Phytosterols and Alkanes are another's categories of biochemical markers evaluated in this study. They play an important role in plant growth and development, including cell division, cell elongation, cellulose biosynthesis, and cell wall formation, while very long chain alkanes (VLCA) are the predominant wax components of plant cuticle. In order to elucidate the phytosterol and alkane profile in tomato leaves, we measured their content using GC–MS. Fig. 2 shows that phytosterols content increased after treatment with crude polysaccharides extracted from *A. platensis* and *D. salina*. Contrarily, *Porphyridium* sp. crude PS significantly decreased the phytosterols content in tomato plant leaves. The highest phytosterol enhancement was observed in plants treated with 0.25 mg mL^{-1} crude PS of *A. platensis* reaching up to 113 % compared to the control. While 1 mg mL^{-1} of crude PS extracted

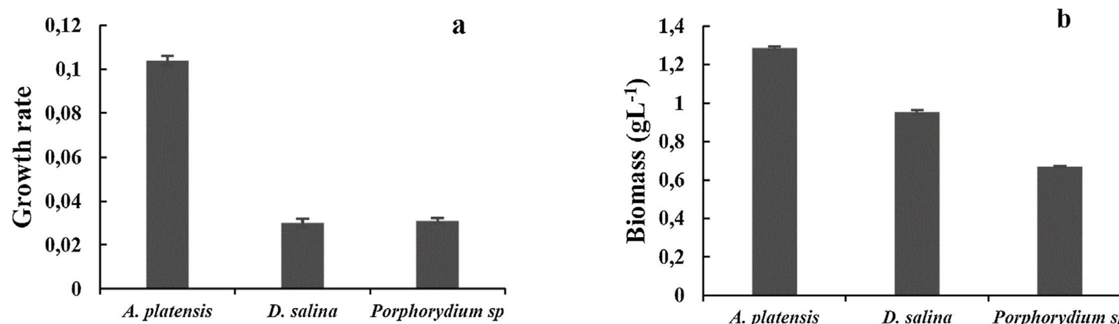


Fig. 1. Growth of microalgae strains: Growth rate (a), Biomass production (b).
Strains microalgae were cultivated in 150 ml Erlenmeyer at 25 °C during 23 days under continuous illumination (150 ± 10 µmol m^{-2} s^{-1}). Data represents average of 3 replicates ± standard error. µ = ln (C2/C1) / t2 − t1 of the exponential phase, C: cellule concentration, t: time.

Table 1
Production and composition of three microalgae polysaccharides.

Strains	Polysaccharide (%/DB)	Total sugars (%/PS)	Sulfate (%/PS)	Uronic acid (%/PS)	Protein (%/PS)	Amino acid(%/PS)
Arthrospira platensis	2.590 ± 0.408	17.275 ± 1.05	5.306 ± 0.37	3.602 ± 1.581	2.746 ± 0.211	0.206
Dunaleila salina	4.103 ± 0.134	33.050 ± 0.925	11.541 ± 0.924	23.951 ± 2.622	4.202 ± 0.021	0.083
Porphorydium sp.	5.529 ± 0.158	35.350 ± 0.025	10.427 ± 0.542	13.808 ± 5.452	2.701 ± 0.008	0.017

Data represents average of 3 replicates ± standard error.

Table 2
Effect of the microalgae crudes polysaccharides extract (PS) on the tomato plant growth.

		Length (cm)		Number	Fresh. Weight (g)		Dry. weight (g)	
		Root	Shoot	Nodes	Root	Shoot	Root	Shoot
Control	**water**	15.17 ± 2.11	33.53 ± 0.69	04.00 ± 0.00	2.61 ± 0.20	8.79 ± 0.30	0.33 ± 10^{-3}	1.33 ± 0.03
A. Platensis	025 **mg mL^{-1}**	15.02 ± 1.52	37.90 ± 1.25***	05.00 ± 0.00***	2.61 ± 0.26	9.70 ± 0.05***	0.29 ± 0.01	1.64 ± 0.02***
	0,5 mg mL^{-1}	12.46 ± 0.75	36.80 ± 1.00***	06.00 ± 0.00***	3.32 ± 0.14***	10.71 ± 0.32***	0.33 ± 0.02	1.49 ± 0.03***
	1 mg mL^{-1}	14,00 ± 0,33	34,87 ± 0,22	07.00 ± 0.00***	2.96 ± 0.15***	8.57 ± 0.27	0.37 ± 0.02***	1.43 ± 0.02**
D. salina	025 **mg mL^{-1}**	15.50 ± 0.33	39.1 ± 0.45***	05.00 ± 0.00***	2.61 ± 0.19	9.75 ± 0.21***	0.31 ± 0.01	1.65 ± 0.05***
	0,5 mg mL^{-1}	13.56 ± 0.08	37.87 ± 0.74***	06.00 ± 0.00***	3.42 ± 0.15***	10.62 ± 0.21	0.35 ± 0.02	1.73 ± 0.04***
	1 mg mL^{-1}	14.67 ± 0.56	40.20 ± 1.15***	07.00 ± 0.00***	3.26 ± 0.12***	11.38 ± 0.03***	0.32 ± 0.01	1.95 ± 0.01***
Porphorydium sp.	025 **mg mL^{-1}**	15.27 ± 0.81	35.14 ± 0.77	06.00 ± 0.00***	2.44 ± 0.21	9.73 ± 0.15***	0.29 ± 0.01	1.58 ± 0.23***
	0,5 mg mL^{-1}	15.27 ± 0.84	39.03 ± 0.69***	07.00 ± 0.00***	2.95 ± 0.25	11.41 ± 0.36***	0.34 ± 0.02	1.93 ± 0.07***
	1 mg mL^{-1}	15.00 ± 1.33	42.00 ± 0.67***	07.00 ± 0.00***	3.40 ± 0.16***	11.58 ± 0.37***	0.32 ± 004	1.91 ± 0.03***

Data represents average of 5 replicates ± standard error; asterix represents a significant difference compared to the control treatment using MANOVA analysis ($p \leq 0.05$) and Tuky test ±: refers to standard deviation with n = 5 replicas. *5 %; **1 %; ***0.1 %.

Table 3
Microalgae crudes polysaccharides effect on biochemical and enzymatic parameters of tomato plant.

		Carotenoid (µg ml^{-1})	Chl a (µg ml^{-1})	Chl b (µg ml^{-1})	Proteins (mg ml^{-1})	NAD-GDH (mmNADH min^{-1} g^{-1} MF)	NR (µM NO2 min^{-1} mg^{-1} Protein)
Control	**water**	0.16 ± 0.05	1.21 ± 0.07	1.84 ± 0.14	0.283 ± 0.007	0.0066 ± 0.001	0.80 ± 0.03
A. Platensis	0,25 **mgmL^{-1}**	0.21 ± 0.03	2.30 ± 0.11***	2.99 ± 0.15**	0.507 ± 0.020***	0.0062 ± 0.001	1.66 ± 0.11***
	0,5 mgmL^{-1}	0.28 ± 0.03**	2.24 ± 0.28***	3.73 ± 0.86***	0.364 ± 0.017**	0.0074 ± 0.002	1.45 ± 0.24***
	1 mgmL^{-1}	0.33 ± 0.01***	1.21 ± 0.34	1.63 ± 0.24	0.441 ± 0.034***	0.0080 ± 0.001	2.52 ± 0.26***
D. salina	0,25 **mgmL^{-1}**	0.47 ± 0.05***	2.03 ± 0.82*	1.61 ± 0.19	0.354 ± 0.040*	0.0103 ± 0.0002***	2.07 ± 0.05***
	0,5 mgmL^{-1}	0.42 ± 0.06***	1.09 ± 0.15	1.14 ± 0.21	0.533 ± 0.047***	0.0120 ± 0.001***	0.75 ± 0.06
	1 mgmL^{-1}	0.43 ± 0.07***	1.70 ± 0.43	1.83 ± 0.46	0.519 ± 0.011***	0.0119 ± 0.0006***	1.04 ± 0.19
Porphorydium sp.	0,25 **mgmL^{-1}**	0.55 ± 0.05***	0.76 ± 0.09	1.32 ± 0.13	0.446 ± 0.003***	0.0110 ± 0.0004***	1.31 ± 0.13***
	0,5 mgmL^{-1}	0.81 ± 0.05***	1.66 ± 0.02	2.96 ± 0.32**	0.341 ± 0.023	0.0140 ± 0.0008***	1.18 ± 0.10*
	1 mgmL^{-1}	0.91 ± 0.06***	1.67 ± 0.32	2.18 ± 0.51	0.413 ± 0.018***	0.0148 ± 0.001***	1.40 ± 0.26***

Data represents average of 5 replicates ± stantard error; asterix represents a significant difference compared to the control treatment using MANOVA analysis ($p \leq 0.05$) and Tuky test ±: refers to standard deviation with n = 5 replicas.*5 %; **1 %; ***0.1 %.

from *Porphyridium* sp. showed the most significant decrease in phytosterol in tomato leaves (Fig. 2a).

The profile of sterols showed an important change after treatment with crude PS of *D. salina* and *Porphyridium* sp. (Fig. 2b). All *D. salina* crude PS treatments decreased the content of the different sterols detected in this study. However, there Cholesta-6,22,24-triene appeared in plants treated with 1 mg mL^{-1} of crude PS which is not present in the control (Fig. 2b).

Table 5 shows that the crude PS of three microalgae decreased the total alkane content in tomato plant leaves. Alkane profile underwent a redistribution after treatment by the various microalgae crude PS (Table 5). For exemple, tomato plants treated with 0.5 mgmL^{-1}of crude PS extracted from *A. platensis* showed a disappearance of all alkanes detect in the control and which were replaced with 100 % Tetracosane. The appearance of three new alkanes (Nanacosane, tricosane and docosane) and the disappearance of five alkanes (Octadecane, triacontane, heptacosane, 1-chloro, heneicosane, hentriacontane) in tomato plants leaves after treatment by 0.25 mg mL^{-1} of crude PS from *D. salina* (Table 5). The treatment by 1 mg mL^{-1} of crude PS from *Porphyridium* sp. allowed for the disappearance of most of the alkanes, thus increasing the percentage of Eicosane and Octacosane (Table 5).

4. Discussion

Three microalgae *A. platensis*, *D. salin*a and *Porphorydium* sp. belonging to 3 distinct classes Cyanophyceae, Chlorophyceae and Porphyridiophyceae were investigated for their prospective biostimulant activities in tomato plant. Indeed, an increase of the leaves, nodes and consequently shoot dry weight was revealed in tomato plant treated by crude polysaccharide extracts of these microalgae (Table 2). PCA analysis showed that *Porphorydium* sp. and *D. salina* crude polysaccharides had greater effect on growth parameters compared (Fig. 3) to PS of *A. platensis* previously studied [25].

PCA analysis (Fig. 3), showed that the stimulatory effect of PS crude extracts increased with concentration. Remarkably, growth parameters induced depend on the concentration of polysaccharides as well as microalgae strains. Shoot length and weight, carotenoid content and NAD_GDH activity were highly stimulated by treatment with 1 mg mL^{-1} crude *Porphorydium* sp. PS, followed by 0,5 mg mL^{-1} crude of *Porphorydium* sp. PS and lastly 1 mg mL^{-1} crude of *D. salina* PS treatment. These growth parameters show a positive correlation between them. Contrariwise, other parameters

Table 4
The effect of microalgae crude polysaccharides on the distribution of fatty acid in tomato plants.

	Faty acid	Control	*A. platensis*			*D. salina*			*Porphorydium* sp.		
		Water	0,25 mgmL^{-1}	0,5 mgmL^{-1}	1 mgmL^{-1}	0,25 mgmL^{-1}	0,5 mgmL^{-1}	1 mgmL^{-1}	0,25 mgmL^{-1}	0,5 mgmL^{-1}	1 mgmL^{-1}
SFA	C8:0	0.40	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	C9:0	0.71	0.30	0.00	0.24	0.55	0.48	0.23	0.18	0.33	0.34
	C12:0	0.59	0.22	0.00	0.00	0.24	0.36	0.00	0.18	0.29	0.31
	C14:0	1.14	0.77	0.81	0.80	0.84	0,97	0,97	0,80	1,03	1,07
	C15:0	0.00	0.00	1.18	0.00	0.96	0.00	0.00	0.00	0.00	0.00
	C16:0	41.03	30.32	44.81	35.78	43.47	38.07	42.09	32.65	40.93	38.86
	C17:0	1.14	1.86	1.84	2.55	0.97	3.02	2.32	2.41	1.53	1.96
	C18:0	11.59	7.29	35.77	11.40	26.31	10.43	11.58	23.46	9.48	9.14
	C20:0	0.99	0.74	1.05	1.01	0.97	1.02	1.63	0.99	1.19	1.14
UFA	C16:1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.35
	C16:3	4.58	11.44	0.00	5.28	0.00	4.61	3.30	3.69	3.03	3.37
	MDD*	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.11	0.00	0.00
	C18:2	12.92	11.55	4.07	12.81	6.77	9.89	9.31	9.73	9.11	9.98
	C18:3	22.71	33.71	8.60	27.77	17.20	28.46	25.48	22.61	29.34	30.82
	C21:0	0.00	0.00	0.00	0.00	0.00	0.56	0.00	0.00	0.00	0.00
VLCFA	C22:0	0.97	0.62	0.71	0.85	0.77	1.10	1.07	0.68	1.16	1.15
	C23:0	0.00	0.23	0.00	0.45	0.00	0.00	0.57	0.30	0.41	0.44
	C24:0	1.22	0.93	1.16	1.07	0.96	1.03	1.47	1.03	1.18	1.09
	C25:0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.19	0.00	0.00
	C26:0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.98	0.00
Total	VLCFA	2.19	1.79	1.87	2.36	1.73	2.13	3.11	2.20	3.73	2.69
	UFA	40.21	56.71	12.67	45.87	23.97	43.52	38.09	37.14	41.48	44.52
	SFA	59.79	43.29	87.33	54.13	76.03	56.48	61.91	62.86	58.52	55.48

C8:0, Sbericacid; C9:0, Azelaicacid; C12:0, Lauricacid; C14:0, Myristicacid, C15:0, Pentadecyclicacid; C16:0, Palmiticacid; C17:0, Margaricacid; C18:0, Stearicacid; C20:0, Arachidicacid; C16:1, Palmitoleicacid; C16:3, Roughanicacid ; Methyl 6,10-octadecad; C18:2, linoleicacid; C18:3, Linolenicacid ;C21:0, Henicosanoicacid ; C22:0, Behenicacid ; C23:0, Tricosylicacid ; C24:0,Lignocericacid ; C25:0, Hyenicacid ; C26:0,Ceroticacid; SFA, saturatedfattyacid ; UFA, unsaturatedfattyacid ; VLCFA, verry long chainfattyacid.

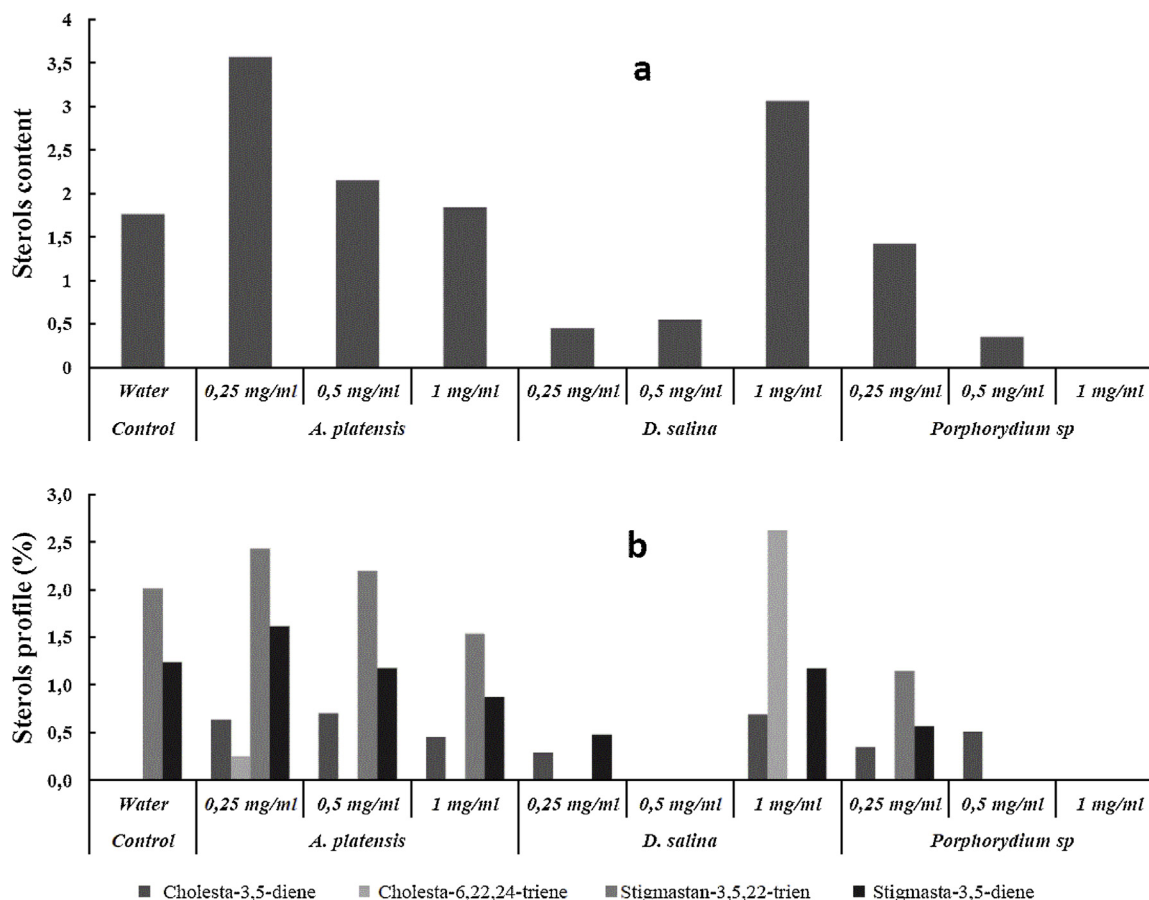


Fig. 2. Microalgae crude polysaccharides effect on the distribution of Sterols in tomato plants.

Table 5
The effect of microalgae crudes polysaccharides on the distribution of alkanes in tomato plants.

Alkane	Control	*A. platensis*			*D. salina*			*Porphorydium* sp.		
	Water	0,25 mgmL^{-1}	0,5 mgmL^{-1}	1 mgmL^{-1}	0,25 mgmL^{-1}	0,5 mgmL^{-1}	1 mgmL^{-1}	0,25 mgmL^{-1}	0,5 mgmL^{-1}	1 mgmL^{-1}
Eicosane	23.05	53.96	0.00	35.25	24.01	7.24	2.39	56.77	4.03	69.60
Heptadecane	6.07	0.00	0.00	0.00	0.00	40.70	35.95	0.00	0.00	0.00
Octacosane	7.13	3.83	0.00	0.00	34.17	39.28	0.00	0.00	0.00	30.40
Octadecane	8.30	0.00	0.00	10.13	0.00	12.79	20.85	16.86	68.37	0.00
Triacontane	4.21	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Heptacosane	2.42	0.00	0.00	0.00	0.00	0.00	0.00	0.00	27.60	0.00
Heneicosane	44.36	0.00	0.00	0.00	0.00	0.00	0.00	3.00	0.00	0.00
Hentriacontane,	4.46	7.52	0.00	0.00	0.00	0.00	7.70	8.17	0.00	0.00
Tetracosane	0.00	8.71	100.00	25.90	0.00	0.00	28.92	0.00	0.00	0.00
Octadecane	0.00	25.99	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2-Methyltriacontane	0.00	0.00	0.00	11.59	7.92	0.00	0.00	9.20	0.00	0.00
Hexacosane	0.00	0.00	0.00	17.14	0.00	0.00	0.00	0.00	0.00	0.00
Nonacosane	0.00	0.00	0.00	0.00	2.07	0.00	0.00	0.00	0.00	0.00
Tricosane	0.00	0.00	0.00	0.00	4.65	0.00	0.00	0.00	0.00	0.00
Docosane	0.00	0.00	0.00	0.00	27.19	0.00	0.00	0.00	0.00	0.00
Heptadecane	0.00	0.00	0.00	0.00	0.00	0.00	4.19	0.00	0.00	0.00
Cyclotetradecane	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.65	0.00	0.00
Octadecane	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.00	0.00	0.00
Dotriacontane	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.34	0.00	0.00
Total of alkanes	**16.14**	**7.58**	**2.44**	**10.27**	**11.62**	**7.74**	**13.38**	**9.67**	**9.42**	**9.54**

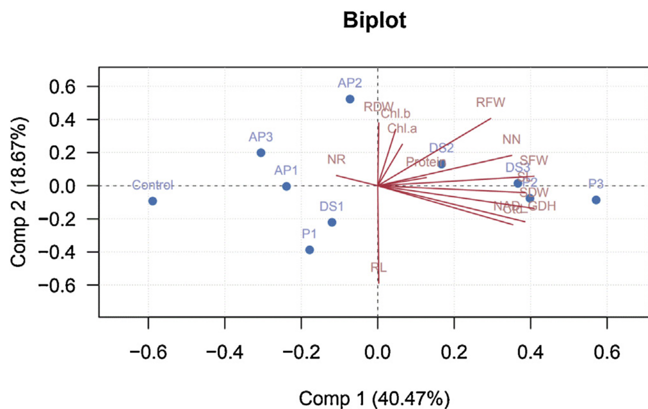


Fig. 3. Principal component analysis (PCA) biplot for investigated growth parameters.
RL, Root length; RFW, Root fresh weight; RDW, Root dry weight; SL, Shoot length; SFW, Shoot fresh weight; SDW, Shoot dry weight; NN, Nodes number; Chl a; Chl b; Cad, Carotenoid; Protein; Nitrate reductase, NAD_GDH, NAD-Glutamate dehydrogenase. Crud polysaccharide of (AP, *Arthrospira platensis*; D.S, *Dunaleila salina*; P, *Porphorydium* sp.) with tree concentrations (1 : 0.25 mg mL^{-1}, 2 : 0.5 mg mL^{-1}and 3 : 1 mg mL^{-1}

such as chlorophyll content, root weight and NR activity seem to be slightly influenced by two *A. platensis* PS extracts concentrations (0.5 mg mL^{-1} and 1 mg mL^{-1}).

The enhancement of tomato growth after PS treatment was accompanied by an increase of its major element such as nitrogen enzymes activities and protein content. Photosynthesis and nitrate assimilation and basal metabolism were enhanced by carboxyled or sulfated polysaccharides in similar studies [26,27]. Moreover, it was shown that basal metabolism and cycle regulatory cyclins were enhanced in treated plants with oligo-carrageenan which caused an increase in leaf biomass by the stimulation of cell division [27]. In *Pinus radiate*, Oligosaccharide kappa increased the level of IAA and GA displaying “a reciprocal positive interaction” and their effects overlapped regarding cell division and expansion, and tissue differentiation [28].

Biological activity of PS extracts results from its composition [29–32]. Crude PS extract contained essentially carbohydrates, sulfate content and uronic acids. These elements could be the origin of the stimulation. Overall, correlation test showed that growth stimulation was mainly related to sugars content, sulfated and carboxylated groups (uronic acid) of polysaccharides (Table 6).

These results confirm previous studies. Carrageenan as seaweeds sulfated oligosaccharides showed the plant growth biostimulant activity [33] or carboxylated PSs such as alginate [26] was demonstrated. Polysaccharides from *Spirulina platensis* increased the length of tomato and pepper plants by 20 % and 30 % respectively [15]. While *Dunaliella salina* PS were used to enhance tomato tolerance to salt stress [4]

Lipid profiling by GC–MS showed that all plants treated with crude microalgae polysaccharides displayed Lipid profile rearrangements. A decrease in stigmasterols and an accumulation of cholesta was observed (Fig. 2). Some sterols in minute amounts, such as campesterol in *Arabidopsis thaliana*, are precursors of oxidized steroids acting as growth hormones collectively named brassinosteroids. BRs have several functions in plant metabolism. BRs regulate photosynthesis by inducing rubisco and carbonic anhydrase [34]. Cell division and cell expansion were also positively regulates by BRs [35]. There are so far no studies done on the impact of biostimulants on plant phytosterols.

On the other hand, we revealed that very long chain alkanes dropped under PS treatment (Table 5). VLCA constitute with very long chain fatty acids and theirs derivate a cuticles wax in epidermal leaf. This have a protective function against abiotic and biotic stresses. This cuticle however is a hydrophobic barrier so the

Table 6
Correlation between tomato shoot dry weight (g) and polysaccharides treatment concentration (1 mg mL^{-1} PS) and composition.

Correlation coefficient					
PS (%)	Total sugars (%)	Sulfate(%)	Uronic acid(%)	Protein (%)	Amino acid(%)
0.839	0.983	0.995	0.899	0.536	−0.913

decrease of VLCA under PS treatment could be explained by the humectant function of PS ensured by its strong hydrophobicity. Nevertheless, no significant variation was detected in fatty acid profile (Table 4).

More investigations are needed to confirm our finding about the role of BRs function and to elucidate and involved pathways. The biostimulatory effect of PS from a many algae species has been proven. PS have proven their promoting activity to herbaceous plants such as tobacco, pepper [25], chickpea, maize [33], opium poppy [26], *Catharan thusroseus* [36] as on trees such as Monterey pine [28] and *Eucalystus* [37]. During last few years, PS have found its path to the market. We promote PS from microalgae. This is a renewable source, easy to cultivate, with a good PS productivity and a high plant promoting activity.

Funding

The authors would link to give special thanks for the financial support of MESRSFC and CNRST Morocco, for the realization of this project under the best conditions (Grant number: PPR2).

Author contributions statement

E.A.H. and L.S conceived the experiments, R.F. and R.B conducted the experiments, E.A.H., R.F and R.B analyzed the results. All authors reviewed the manuscript. The authors declare that they have no conflict of interest.

Declaration of Competing Interest

The authors declare no competing interests.

Acknowledgments

The authors would like to acknowledgement Chanda Mutale Joan for the revision of the quality of the English language of this article. We also acknowledgement Mr. Rida RABI who has been involved in the statistical analysis of this work.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.btre.2020.e00426.

References

[1] Y. Chisti, Biodiesel from microalgae, Biotechnol. Adv. 25 (3) (2007) 294–306, doi:http://dx.doi.org/10.1016/j.biotechadv.2007.02.001 PubMed PMID: 17350212.
[2] L. Gouveia, B. Nobre, F. Marcelo, S. Mrejen, M. Cardoso, A.F. Palavra, R.L. et Mendes, Functional food oil coloured by pigments extracted from microalgae with supercritical CO2, Food Chem. 101 (2) (2007) 717–723.
[3] F. Rossi, R. De Philippis, Exocellular Polysaccharides in Microalgae and Cyanobacteria: Chemical Features, Role and Enzymes and Genes Involved in Their Biosynthesis. The Physiology of Microalgae, Springer, 2016, pp. 565–590.
[4] H. El Arroussi, R. Benhima, A. Elbaouchi, B. Sijilmassi, N. El Mernissi, A. Aafsar, I. Meftah-Kadmiri, N. Bendou, A. Smouni, Dunaliella salina exopolysaccharides: a promising biostimulant for salt stress tolerance in tomato (Solanum lycopersicum), J. Appl. Phycol. (2018), doi:http://dx.doi.org/10.1007/s10811-017-1382-1.
[5] A. Mishra, K. Kavita, B. Jha, Characterization of extracellular polymeric substances produced by micro-algae Dunaliella salina, Carbohydr. Polym. 83 (2) (2011) 852–857, doi:http://dx.doi.org/10.1016/j.carbpol.2010.08.067.
[6] P. Manivasagan, J. Oh, Marine polysaccharide-based nanomaterials as a novel source of nanobiotechnological applications, Int. J. Biol. Macromol. 82 (2016) 315–327, doi:http://dx.doi.org/10.1016/j.ijbiomac.2015.10.081 PubMed PMID: 26523336.
[7] S.I. Abdel-Hafez, K.A. Abo-Elyousr, Ismail R. Abdel-Rahim, Fungicidal activity of extracellular products of cyanobacteria against, Alternaria porri 50 (2) (2015) 239–245.
[8] a.m. Abo-Shady, B.A. Al-ghaffar, M. Rahhal, H.A. Abd-El Monem, Biological control of faba bean pathogenic fungi by three cyanobacterial filtrates, Pak. J. Biol. 10 (18) (2007) 3029–3038.
[9] H.A. Alwathnani, K.J.A.Jo B. Perveen, Biological control of fusarium wilt of tomato by antagonist fungi and cyanobacteria, Afr. J. Biotechnol. 11 (5) (2012) 1100–1105.
[10] H. Righini, E. Baraldi, Y. García Fernández, A. Martel Quintana, Roberti RJMd, Different antifungal activity of Anabaena sp., Ecklonia sp., and Jania sp. against Botrytis cinerea, Mar. Drugs 17 (5) (2019) 299.
[11] R. Roberti, S. Galletti, P. Burzi, H. Righini, S. Cetrullo, C.J.B.C. Perez, Induction of defence responses in zucchini (Cucurbita pepo) by Anabaena sp. water extract, Biol. Control 82 (2015) 61–68.
[12] R. Prasanna, L. Nain, R. Tripathi, V. Gupta, V. Chaudhary, S. Middha, et al., Evaluation of fungicidal activity of extracellular filtrates of cyanobacteria–possible role of hydrolytic enzymes, J. Basic Microbiol. 48 (3) (2008) 186–194.
[13] J.-D.J.M. Kim, Screening of cyanobacteria (blue-green algae) from rice paddy soil for antifungal activity against plant pathogenic fungi, Mycobiology 34 (3) (2006) 138–142.
[14] M.A. Guzmán-Murillo, F. Ascencio, J.A. Larrinaga-Mayoral, Germination and ROS detoxification in bell pepper (Capsicum annuum L.) under NaCl stress and treatment with microalgae extracts, Protoplasma 250 (1) (2012) 33–42, doi: http://dx.doi.org/10.1007/s00709-011-0369-z.
[15] H. El Arroussi, A. Elbaouchi, R. Benhima, N. Bendaou, A. Smouni, I. Wahby, Halophilic microalgae Dunaliella salina extracts improve seed germination and seedling growth of Triticum aestivum L. Under salt stress, Acta Hortic. 1148 (2016) 13–26, doi:http://dx.doi.org/10.17660/ActaHortic.2016.1148.2.
[16] B.M. Plaza, C. Gómez-Serrano, F.G. Acién-Fernández, S. Jimenez-Becker, Effect of microalgae hydrolysate foliar application (Arthrospira platensis and Scenedesmus sp.) on Petunia x hybrida growth, J. Appl. Phycol. (2018), doi: http://dx.doi.org/10.1007/s10811-018-1427-0.
[17] V. Barone, A. Baglieri, P. Stevanato, C. Broccanello, G. Bertoldo, M. Bertaggia, M. Cagnin, D. Pizzeghello, V.M.C. Moliterni, G. Mandolino, F. Fornasier, A. Squartini, S. Nardi, G. Concheri, Root morphological and molecular responses induced by microalgae extracts in sugar beet (Beta vulgaris L.), J. Appl. Phycol. 30 (2) (2017) 1061–1071, doi:http://dx.doi.org/10.1007/s10811-017-1283-3.
[18] N. Blumenkrantz, G. Asboe-Hansen, New method for quantitative determination of uronic acids, Anal. Biochem. 54 (2) (1973) 484–489.
[19] M. DuBois, K.A. Gilles, J.K. Hamilton, P.A. Rebers, F. Smith, Colorimetric method for determination of sugars and related substances, Anal. Chem. 28 (3) (1956) 350–356, doi:http://dx.doi.org/10.1021/ac60111a017.
[20] A. Llyod, N. Tudball, K. Dodgson, Infrared studies on sulphate esters III. O-sulphate esters of alcohols, amino alcohols and hydroxylated amino acids, Biochim. Biophys. Acta 52 (3) (1961) 413–419.
[21] W. Claussen, Proline as a measure of stress in tomato plants, Plant Sci. 168 (1) (2005) 241–248, doi:http://dx.doi.org/10.1016/j.plantsci.2004.07.039.
[22] H.K. Lichtenthaler, C. Buschmann, Chlorophylls and carotenoids: Measurement and characterization by UV-VIS spectroscopy, Curr. Protocols Food Anal. Chem. (2001).
[23] A. Allegre, J. Silvestre, P. Morard, J. Kallerhoff, E. Pinelli, Nitrate reductase regulation in tomato roots by exogenous nitrate: a possible role in tolerance to long-term root anoxia, J. Exp. Bot. 55 (408) (2004) 2625–2634, doi:http://dx.doi.org/10.1093/jxb/erh258 PubMed PMID: 15475378.
[24] R.P. Singh, H. Srivastava, Regulation of glutamate dehydrogenase activity by amino acids in maize seedlings, Physiol. Plant. 57 (4) (1983) 549–554.
[25] H. Elarroussi, N. Elmernissi, R. Benhima, I. Meftah El Kadmiri, N. Bendaou, A. Smouni, I. Wahby, Microalgae polysaccharides a promising plant growth biostimulant, J. Algal Biomass Utln. (2016).
[26] Z.H. Khan, M.M.A. Khan, T. Aftab, M. Idrees, M. Naeem, Influence of alginate oligosaccharides on growth, yield and alkaloid production of opium poppy (Papaver somniferum L.), Front. Agric. China 5 (1) (2011) 122–127, doi:http://dx.doi.org/10.1007/s11703-010-1056-0.
[27] J. Castro, J. Vera, A. González, Moenne AJJopgr, Oligo-carrageenans stimulate growth by enhancing photosynthesis, basal metabolism, and cell cycle in tobacco plants (var. Burley), J. Plant Growth Regul. 31 (2) (2012) 173–185.
[28] S. Saucedo, R.A. Contreras, A. Moenne, Oligo-carrageenan kappa increases C, N and S assimilation, auxin and gibberellin contents, and growth in Pinus radiata trees, J. For. Res. 26 (3) (2015) 635–640, doi:http://dx.doi.org/10.1007/s11676-015-0061-9.
[29] J. Silva, N. Dantas-Santos, D.L. Gomes, L.S. Costa, S.L. Cordeiro, M.S. Costa, N.B. Silval, M.L. Freitasll, K.C. Scorteccill, E.L. Leitelv, H.A.O. Rochal, Biological activities of the sulfated polysaccharide from the vascular plant Halodule wrightii, Rev. Bras. Farmacogn. 22 (1) (2012) 94–101.
[30] K. Zhu, Y. Zhang, S. Nie, F. Xu, S. He, D. Gong, G. Wu, L. Tan, Physicochemical properties and in vitro antioxidant activities of polysaccharide from Artocarpus heterophyllus Lam. Pulp, Carbohydr. Polym. 155 (2017) 354–361, doi:http://dx.doi.org/10.1016/j.carbpol.2016.08.074 PubMed PMID: 27702522.
[31] R. Rodriguez-Jasso, S. Mussatto, L. Pastrana, C. Aguilar, J. Teixeira, Chemical composition and antioxidant activity of sulphated polysaccharides extracted from Fucus vesiculosus using different hydrothermal processes, Chem. Pap. 68 (2) (2014), doi:http://dx.doi.org/10.2478/s11696-013-0430-9.
[32] M.Fd J. Raposo, R.M.S.C. De Morais, B. de Morais, a.m.J.Md Miranda, Bioactivity and applications of sulphated polysaccharides from marine microalgae, Mar. Drugs 11 (1) (2013) 233–252.
[33] F. Bi, S. Iqbal, M. Arman, A. Ali, M.-u. Hassan, Carrageenan as an elicitor of induced secondary metabolites and its effects on various growth characters of

chickpea and maize plants, J. Saudi Chem. Soc. 15 (3) (2011) 269–273, doi: http://dx.doi.org/10.1016/j.jscs.2010.10.003.
[34] H. Siddiqui, S. Hayat, A. Bajguz, Regulation of photosynthesis by brassinosteroids in plants, Acta Physiol. Plant. 40 (3) (2018), doi:http://dx.doi.org/10.1007/s11738-018-2639-2.
[35] S.D. Clouse, J.M. Sasse, Brassinosteroids: essential regulators of plant growth and development, Annu. Rev. Plant Biol. 49 (1) (1998) 427–451.
[36] M. Idrees, M. Naeem, M. Alam, T. Aftab, N. Hashmi, Moinudin Khan MMA, L. Varshney, Utilizing the γ-irradiated sodium alginate as a plant growth promoter for enhancing the growth, physiological activities, and alkaloids production in Catharanthus roseus L, Agric. Sci. China 10 (8) (2011) 1213–1221, doi:http://dx.doi.org/10.1016/s1671-2927(11)60112-0.
[37] A. González, J. Castro, J. Vera, A.J.Jo P.G.R. Moenne, Seaweed oligosaccharides stimulate plant growth by enhancing carbon and nitrogen assimilation, basal metabolism, and cell division, J. Plant Growth Regul. 32 (2) (2013) 443–448.