Assessment of Four White Sea Brown Seaweed Extracts as Biostimulants of Plant Growth

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Abstract. The study was aimed on the assessment of extracts from four arctic brown seaweeds (*Ascophyllum nodosum, Fucus vesiculosus, Laminaria digitata* and *Saccharina latissimi)* as stimulators of plant growth. Seaweeds were collected from the Rebalda Bay of the White Sea in August 2021. Extracts were obtained by isopropanol maceration method, then vacuum-dried at 40°C and stored at -4°C. Protein, polyphenols, chlorophyll, carotenoids, alginates, mannitol and polysaccharides contents of extracts were determined. Wheat and cucumber seedlings were used as test plants. Extracts from all four brown seaweeds enhanced growth, accelerated development of plants and increased chlorophyll content in leaves. Plant responses to seaweed extracts were concentration-dependent. Higher concentration of *A. nodosum* and *F. vesiculosus* extracts inhibited plant growth possibly due to higher phenolic content. The results show that investigated seaweed extracts obtained by isopropanol maceration method with subsequent drying and freezing have proven to be effective in plant growth stimulation.

1 Introduction

Brown seaweeds contain many components such as phytohormones (auxins and auxin-like compounds, cytokinins, abscisic acid), polyphenols, amino acids, betaines, sterols, polysaccharides, minerals and trace elements which can stimulate plant growth [1-3]. Liquid extracts are proven to be the most effective form of algal based products for agriculture [1-2;4]. Low concentrations of seaweed extracts are used in agriculture as biostimulants as they induce a positive response in plant growth and increase plant tolerance to various stress factors [5]. The beneficial effects of seaweed extracts on plant growth can be attributed to direct and indirect stimulation mechanisms [5]. Seaweed extracts are able not only to improve crop growth, but also to increase nutrient uptake, photosynthesis, yield, quality and plant tolerance to abiotic and biotic stress [1-2;6].

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There is a number of commercially available algal based products used in modern agriculture [7]. Russia has large reserves of different seaweeds, which are traditionally harvested in the Far East (Japanese Sea), on the Kuril Islands, Sakhalin, in Primorye, on the mainland coast of the Sea of Okhotsk and in the White Sea. Studies of kelp and fucus from the White Sea, Japanese Sea and Yellow Sea confirmed the general similarity of their chemical composition with that characteristic of these taxonomic groups: polysaccharides, vitamins, fatty polyenoic acids, auxins and gibberellins, almost the same primary and secondary metabolites, a rich composition of micro- and macroelements [8-10]. Therefore, brown seaweeds from the White Sea can be considered as suitable resource for the production of seaweed extract-based biostimulants. The extracts from the White Sear brown seaweeds are already commercially exploited and getting increasing attention in food, cosmetics and pharmaceutical industries. So far, seaweed based agricultural products from the White Sea are fertilizers and soil amendment products, but not bioactive extracts.

For the elaboration of innovative products for agriculture the information about bioactive compounds in seaweed extracts and their effects on plant growth and biomass accumulation is essential Currently, commercially available algal extracts are liquids and most of them were produced by conventional extraction techniques. The composition and concentration of the bioactive compounds in seaweed extracts depend on their structure and content in seaweed cells, but a key role plays the method of their extraction [11].

The seaweed extract formulations are available as liquid concentrates which limits their shelf life. Efforts are needed to produce user-friendly and stable solid formulations that can have improved shelf-life [2]. In this study we assessed biostumulatory properties of the seaweed extracts obtained by isopropanol maceration method with subsequent drying and freezing. Effects of for extracts from the White Sea brown seaweed *Ascophyllum nodosum, Fucus vesiculosus, Laminaria digitata* and *Saccharina latissima* on plant growth, development and chlorophyll content were studied.

2 Materials and methods

2.1 Collection of seaweeds and preparation of extracts

Four arctic brown seaweeds *Ascophyllum nodosum* (L.) Le Jolis*, Fucus vesiculosus* L.*, Laminaria digitata* (Huds.) Lamouroux and *Saccharina latissima* (L.) C.E. Lane, C. Mayes, Druehl, et G. W. Saunders were freshly collected from the Rebalda Bay near the Solovetsky Islands in the White Sea in August 2021 by the Arkhangelsk Seaweed Factory LLC. The collected seaweeds were brought to the laboratory, cleaned and were given a quick freshwater rinse to remove surface salts. Subsequently the seaweeds were air dried at 40°C. The dried samples were finely powdered and sieved. The material obtained was stored in sealed vessels in the dark at 20°C until use. The fraction with the particle size of 0.25–0.50 mm was used for further study. Seaweed samples were macerated with 40 % isopropanol at 60°C for 1 h under constant stirring. Then they were centrifuged at 10595 g for 5 min and supernatant (extract) was collected. Obtained extracts were vacuum-dried at 40°C and stored at -4°C.

2.2. Chemical analyses of seaweed extracts

Protein content in extracts was measured by following the Bradford method using bovine serum albumin as a standard. Proteins were expressed as percentage of algal fresh weight.

The content of polyphenolic compounds was determined according to modified Folin-Ciocalteu colorimetric method with UV-spectrophotometer (Spekol UV 1300, Analytic Jena, Germany). Phloroglucinol (≥ 99,0 %, Sigma Aldrich, USA) was used as a standard for calibration curve.

Extraction and estimation of chlorophyll. The samples were centrifuged at 3270 *g* for 15 min. Absorbance of the extract was read at 663 and 645 nm for chlorophyll *a* and chlorophyll *b*, respectively using UV-spectrophotometer.

Carotenoids content of seaweeds was determined spectrophotometrically at 480 nm according using the same extract used for chlorophyll estimation.

Alginates content in extracts was measured by UV-spectroscopy after the reaction with Alcian blue dye (ApliChem, Italy) resulting in precipitation of alginates.

Mannitol content was measured according to the method based on the reaction between dissolved mannitol and added copper ions in strong alkaline medium which produce the insoluble residue.

Polysaccharide content was determined by a colorimetric reaction of reducing sugars with 3,5-dinitrosalicylic acid (98 %, Acros Organics, Belgium).

2.3.Plant material

Wheat (*Triticum aestivum* L. var. Zlata) and cucumber (*Cucumis sativus* L. var. Zozulya F1) were grown for two weeks in hydroponic vessels under controlled environmental conditions in a growth chamber at the average air temperature of 23°C and relative air humidity of 70%. Plants were grown under 14 h photoperiod with the PPFD of 150 μmol m^{-2} s^{-1}. Each vessel contained 16 wheat plants or 9 cucumber plants. Hydroponic vessels were filled with liquid extracts (dry extracts dissolved in distilled water) of four seaweeds in concentrations 30, 60, 120, 300 and 1200 mg/L. Distilled water was used as a control (0 mg/L). Two vessels per each treatment were used.

2.4. Biometric measurements

Ten seedlings of each species were randomly selected and measured to determine plant height, cotyledon (cucumber) and first leaf (wheat) area, length of the first true leaf (cucumber), root length and dry weight (DW) of shoots and roots. To determine DW samples were dried at 105°C in an oven until a constant DW was observed.

2.5.Chlorophyll content measurement

A SPAD-502 Plus chlorophyll meter was used to determine a chlorophyll content index (CCI). Five measurements were taken per leaf and averaged to provide a single CCI per leaf. The adaxial side of the leaves was always placed toward the emitting window of the instrument.

2.6.Statistical analysis

The experiment was carried out twice. The tables show mean values and their standard errors. The data were analyzed by one-way analysis of variance (ANOVA). Difference between the mean values was considered significant at $P \leq 0.05$.

3 Results

Microgreen The results of chemical analyses of four seaweed extracts are presented in table 1. The extracts of *A. nodosum* and *F. vesiculosus* had higher content of protein, polyphenols and carotenoids and lower content of polysaccharides compared to the other two seaweed extracts.

Table 1. Chemical composition of seaweed extracts, % of dry weight.

Compounds	***Ascophyllum nodosum***	***Fucus vesiculosus***	***Laminaria digitata***	***Saccharina latissima***
Protein	1.42±0.07	1.07±0.13	0.22±0.02	0.17±0.02
Polyphenols	5.89±0.15	4.71±0.17	0.12±0.01	0.20±0.02
Chlorophyll	0.008±0.001	0.007±0.001	0.015±0.001	0.005±0.001
Carotenoids	0.023±0.001	0.016±0.001	0.009±0.001	0.008±0.001
Alginates	1.58±0.01	1.47±0.01	1.15±0.02	0.97±0.01
Mannitol	8.03±0.34	10.92±0.05	8.93±0.31	5.22±0.24
Polysaccharides	5.84±0.03	5.37±0.37	8.6±0.40	7.21±1.15

The growth and productivity of wheat and cucumber seedlings were significantly affected by all four seaweed extracts. The effects were concentration-dependent. *A. nodosum* and *F. vesiculosus* extracts were more effective at medium concentrations (60, 120 and 300 mg/L) while higher concentration (1200 mg/L) decreased the growth rate and biomass accumulation compared to control (figures 1 and 2; tables 2 and 3). Cucumber plants treated by 1200 mg/L seaweed extracts in most cases outperformed control and other treated plants in terms of plant height, leaf area, root length, shoot DW and root DW.

Chlorophyll contents measured as CCI was higher in plants treated by seaweed extracts except in wheat plants treated by *F. vesuculosus* extract (table 4).

Fig. 1. General overview of the effects of different concentrations of (a) *Fucus vesiculosus,* and (b) *Saccharina latissimi* extracts on wheat plants. From left to right: extract concentrations – 0, 30, 60, 120, 300, 1200 mg/L.

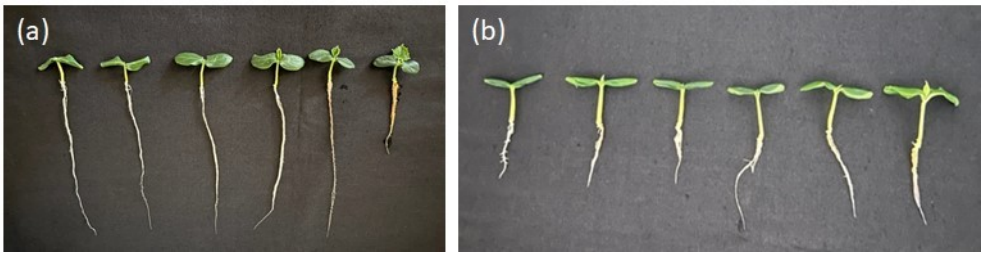

Fig. 2. General overview of the effects of different concentrations of (a) *Fucus vesiculosus,* and (b) *Saccharina latissimi* on cucumber plants. From left to right: extract concentrations – 0, 30, 60, 120, 300, 1200 mg/L.

Table 2. Growth characteristics of *Triticum aestivum* plants treated by four seaweed extracts (in percentage relative to control plants).

Parameter	Seaweed extract concentration, mg/L					
	0 (control)	30	60	120	300	1200
Ascophyllum nodosum						
Plant height, mm	100 C	103 C	109 B	111 B	119 A	83 D
First leaf area, mm^2	100 B	100 B	103 B	102 B	109 A	94 C
Root length, mm	100 D	110 C	140 B	153 A	149 A	60 E
Shoot DW, mg	100 B	103 B	104 B	104 B	108 A	79 C
Root DW, mg	100 D	107 C	105 C	107 C	116 B	135 A
Fucus vesiculosus						
Plant height, mm	100 C	109 B	118 A	123 A	122 A	99 C
First leaf area, mm^2	100 C	117 B	116 B	123 A	124 A	70 D
Root length, mm	100 B	130 A	131 A	86 C	30 D	18 E
Shoot DW, mg	100 C	107 B	116 B	124 A	132 A	83 D
Root DW, mg	100 B	146 A	144 A	145 A	107 B	94 C
Laminaria digitata						
Plant height, mm	100 D	100 D	107C	119 B	130 A	124 A
First leaf area, mm^2	100 C	103 C	111 B	113 B	121 A	106 C
Root length, mm	100 A	102 A	104 A	95 B	85 C	51 D
Shoot DW, mg	100 B	93 C	91 C	103 B	122 A	118 A
Root DW, mg	100 C	101 C	106 B	120 A	113 B	98 C
Saccharina latissima						
Plant height, mm	100 B	104 B	113 A	115 A	112 A	105 B
First leaf area, mm^2	100 D	101 D	120 C	139 B	169 A	167 A
Root length, mm	100 B	102 B	136 A	102 B	81 C	69 D
Shoot DW, mg	100 A	102 A	103 A	94 B	88B	91 B
Root DW, mg	100 C	123 A	125 A	115 B	95 D	95 D

Table 3. Growth characteristics of *Cucumis sativus* plants treated by four seaweed extracts (in percentage relative to control plants).

Parameter	Seaweed extract concentration, mg/L					
	0 (control)	30	60	120	300	1200
Ascophyllum nodosum						
Plant height, mm	100 C	106 B	107 B	106 B	105 B	112 A
Cotyledons area, mm^2	100 E	142 D	168 C	177 C	221 B	260 A
True leaf length, mm	100 C	100 C	100 C	105 C	224 B	586 A
Root length, mm	100 D	117 C	122 C	187 B	213 A	209 A
Shoot DW, mg	100 D	109 C	115 C	120 C	141 B	176 A
Root DW, mg	100 E	141 D	153 D	188 C	335 B	500 A
Fucus vesiculosus						
Plant height, mm	100 C	105 B	114 A	115 A	115 A	113 A
Cotyledons area, mm^2	100 D	144 B	152 A	164 A	114 C	116 C
True leaf length, mm	100 E	194 D	239 D	394 C	517 B	678 A
Root length, mm	100 C	119 B	119 B	119 B	136 A	133 A
Shoot DW, mg	100 D	117 C	173 A	179 A	174 A	154 B
Root DW, mg	100 D	150 C	200 B	232 A	234 A	203 B
Laminaria digitata						
Plant height, mm	100 B	100 B	106A	106 A	105 A	91 C
Cotyledons area, mm^2	100 E	107 D	130 C	141 C	181 A	155 B
True leaf length, mm	100 C	100 C	125 B	180 A	171 A	180 A
Root length, mm	100 C	149 B	151 B	153 B	167 A	146 B
Shoot DW, mg	100 D	103 D	118 C	128 B	148 A	153 A
Root DW, mg	100 E	128 D	133 D	156 C	194 B	228 A
Saccharina latissima						
Plant height, mm	100 C	102 C	117 B	117 B	119 B	127 A
Cotyledons area, mm^2	100 E	107 D	124 C	133 C	159 B	190 A
True leaf length, mm	100 D	175 C	188 C	200 C	288 B	400 A
Root length, mm	100 C	108 B	122 A	119 A	125 A	131 A
Shoot DW, mg	100 D	105 C	108 C	109 C	139 B	160 A
Root DW, mg	100 E	121 D	157 C	175 B	185 B	300 A

Table 4. CCI of wheat and cucumber plants treated by seaweed extracts.

Seaweed species	Seaweed extract concentration, mg/L					
	0	30	60	120	300	1200
	Triticum aestivum					
Ascophyllum nodosum	33.6±1.0	38.3±0.9*	37.4±1.0*	40.8±0.6*	39.6±1.1*	35.1±0.9*
Fucus vesiculosus	33.5±0.7	33.4±1.0	33.1±1.0	32.9±0.9	33.8±0.9	33.2±1.2
Laminaria digitata	33.4±1.3	34.1±1.0	36.6±0.7*	35.8±1.0*	35.0±1.0*	34.1±1.0
Saccharina latissimi	33.5±1.0	37.0±1.1*	38.1±1.1*	41.0±0.6*	40.3±1.1*	36.2±0.8*
	Cucumis sativus					
Ascophyllum nodosum	56.0±1.9	62.4±1.6*	62.5±2.0*	68.3±2.0*	68.9±2.3*	70.3±2.3*
Fucus vesiculosus	55.8±1.8	62.2±1.3*	62.0±2.5*	68.5±2.1*	69.4±1.5*	73.6±2.4*
Laminaria digitata	56.9±2.9	63.7±4.0*	62.5±1.7*	70.4±3.2*	68.0±1.6*	69.4±1.6*
Saccharina latissimi	57.2±2.0	65.0±1.6*	64.3±0.9*	68.2±2.5*	69.7±2.6*	66.1±3.1*

* - significant differences with control.

4 Discussion

The present results are in agreement with those who reported that the low concentrations of seaweed extracts promoted the seedling growth [1-7;11-12]. Sharp decrease in values of growth characteristics in wheat plants treated by high concentration *A. nodosum* and *F. vesiculosus* extracts can possibly be due to their much higher polyphenolic content (table 1). Phenolics synthesized by brown seaweeds are among the most numerous and important bioactive compounds. They have been shown to promote shoot and root lengthening and development of adventitious roots in plants by regulating the activity of auxins [12]. Therefore, it is possible that high concentration of phenolics may inhibit plant growth in sensitive plant species. Chlorophyl content increase in plants affected by seaweed extracts was also reported for different plant species [2].

5 Conclusion

This study investigated the growth responses of wheat and cucumber seedlings on brown seaweed *Ascophyllum nodosum, Fucus vesiculosus, Laminaria digitata* and *Saccharina latissimi* extracts (30-1200 mg/L) obtained by isopropanol maceration method. The obtained results suggest that investigated arctic seaweeds have biostimulatory properties that affect plant growth, biomass accumulation and chlorophyll content. It is important that extracts were stored being dried and frozen. Further research are needed to enhance the shelf life and stability of seaweed extracts.

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