




Article

Morphological and Biochemical Responses of *Glycine max* (L.) Merr. to the Use of Seaweed Extract

Sławomir Kocira ^{1,*}, Agnieszka Szparaga ², Maciej Kuboń ³, Ewa Czerwińska ⁴ and Tomasz Piskier ²

¹ Department of Machinery Exploitation and Management of Production Processes, University of Life Sciences in Lublin, Akademicka 13, 20-950 Lublin, Poland

² Department of Agrobiotechnology, Koszalin University of Technology, Raławicka 15–17, 75-620 Koszalin, Poland; agnieszka.szparaga@tu.koszalin.pl (A.S.); tomasz.piskier@tu.koszalin.pl (T.P.)

³ Institute of Agricultural Engineering and Informatics, Agricultural University Krakow, Balicka 116B, 30-149 Krakow, Poland; maciej.kubon@ur.krakow.pl

⁴ Department of Biomedical Engineering, Koszalin University of Technology, Śniadeckich 2, 75-453 Koszalin, Poland; ewa.czerwinska@tu.koszalin.pl

* Correspondence: slawomir.kocira@up.lublin.pl; Tel.: +48-815319735

Received: 21 December 2018; Accepted: 15 February 2019; Published: 18 February 2019



Abstract: Currently, modern agriculture aims to improve the quantity and quality of crop yield, while minimizing the negative impact of treatments on the natural environment. One of the methods to increase plant yield and quality, especially after the occurrence of both abiotic or biotic stress factors, is the application of biostimulants. The aim of the study was to determine the effect of *Ecklonia maxima* extract on plant growth, and the yield, nutritional, and nutraceutical properties of soybean seeds. A field experiment was conducted in three growing seasons (2014–2016). Soybean seeds of Atlanta cultivar were sown in the third 10-day period of April. *Ecklonia maxima* extract was applied in the form of single or double, spraying in the concentrations of 0.7% and 1.0%. Determinations were conducted for: biometric traits, seed yield, seed number, thousand seeds weight, contents of lipids, and proteins in seeds. Further analyses included the contents of total polyphenols, flavonoids, anthocyanins, and reducing power. The number of seaweed extract applications and its concentration modified biometric traits, yield, and quality of crop, while also also altering the nutraceutical and antioxidative potential of soybean. The application of this preparation improved the growth and yield of soybean without any negative effect on the nutritive value of seeds.

Keywords: antioxidant activity; growth; nutrients; nutraceutical potential; soybean; yield

1. Introduction

Soya (*Glycine max* (L.) Merrill.) is one of the most important leguminous plants that are cultivated around the world because it is a precious source of both protein and fat [1,2]. Its use for production of food, oil, and fodder means that the demand for this plant is continuously growing [3]. Due to its broad use, it is called a “wonderful crop” [4]. However, this plant is sensitive to unfavourable climatic conditions [5]. Thus, to ensure its effective protection against biotic and abiotic factors, it is recommended to use it in the cultivation of biostimulants, which may improve the biochemical, morphological, and physiological processes that take place in a plant [6–8].

Biostimulants as plant-growth promoters were defined for the first time in the world literature by Kaufman [9]. In turn, Du Jardin [10] claims that “a plant biostimulant is any substance or microorganism, in the form in which it is supplied to the user, applied to plants, seeds or the root environment with the intention to stimulate natural processes of plants to benefit their nutrient use

efficiency and/or their tolerance to abiotic stress, regardless of its nutrients content, or any combination of such substances and/or microorganisms intended for this use". The European Biostimulants Industry Council (EBIC) was established in order to develop legal regulations regarding the registration of biostimulants, according to the specificity of their action. However, currently, the registration of these preparations is still based on legal regulations that are set for fertilizers and plant protection products [11–13]. According to Colla et al. [14,15] and Battacharyya et al. [16], among the entire group of those preparations, extracts from seaweed and protein hydrolysates constitute the two most-important categories of substances of natural biostimulants. According to Aguilar, brown algae are the most often used in agriculture [17]. The most popular are, among others, *Ecklonia maxima* (Osbeck) Papenfuss and *Ascophyllum nodosum* (L.) Le Jolis. Brown-algae extracts include various phytohormones, such as auxins, gibberellins, cytokinins, abscisic acid, ethylene, betaine, and polyamines, and other growth promoters as well as trace elements and microelements [18]. Seaweeds also include a varied range of organic compounds, among others, aminoacids, such as asparaginic acid, glutamine acid, and alanine. While, alginic acid, laminarin, and mannitol constitute almost half of the total content of carbohydrates in such biostimulating preparations. Seaweeds also contain a wide range of vitamins that can be used by plants, such as C, B2, B12, D3, E, K, niacin, panthotenic acid, and folic acid. Although, vitamin A does not occur in algae extracts, the presence of its precursor—carotene and another possible precursor, fucoxanthin—was determined [18–20].

According to Ecoforce [21] and Van Oosten et al. [22], seaweed extracts that are used as biostimulants increase the yield and its quality in two ways: a. they stimulate hormone synthesis, influence absorption, and translocation of nutrients; b. they condition the soil, improving its ability to retain moisture and stimulate the activity of favourable microorganisms. Medjdoub estimates that the use of biostimulants, which include extracts from seaweeds, has greater meaning in agriculture. That is because plant growth and development is controlled by plant hormones, which directly or indirectly control the course of various physiological reactions and their integration with the total metabolism [23]. Many studies on seaweed extract indicate that they may increase: a. plant growth, b. activity of photosynthesis, c. resistance to fungi, bacteria and viruses, d. tolerance to ground frost, drought, and salt content, and e. yield and productivity of many cultivations [24–26], mainly by activation of protective mechanisms of plants [27]. Foliar application of biostimulants that are based on seaweeds is an agrotechnical treatment that brought many advantages in numerous cultivations, including grapevine, watermelon, strawberry, apple, tomato, spinach, onion, bean, pepper, carrot, potato, wheat, corn, barley, rice, and turf grass. The results show that plants treated with lower concentrations of extract indicated a stronger growth, higher yield, and higher mineral and nutritive elements content relative to the control [28–33]. Positive reactions also included an improved flowering and fructification ability, product quality and efficiency, and resistance to abiotic stress [31,34,35]. Studies that were performed on a wide group of crops proved that the application of sea-algae-based biostimulants stimulates the primary and secondary metabolisms in plants through the absorption and assimilation of nutrients [36–44]. Growth of productivity of crops induced by the use of such biostimulants in optimal and suboptimal conditions may be related to several direct and indirect mechanisms, including the stimulation of enzymatic activities that are related to carbon, nitrogen metabolism, Krebs cycle, and glycolysis. Such use may also induce activity similar to hormones, especially the one that is assigned to auxins and gibberellins, and improve the nutrition of treated plants by the modulation of the root system [14–16,45].

However, other results indicate that the application of such biostimulants, despite its numerous advantages, like faster germination and earlier growth [46,47], may inhibit the growth and development of many plants. This calls for greater care in the use of seaweeds extracts [48]. Therefore, the inhibition of plant growth after application of biostimulants is a potential problem in plant production. The concentration of these products is an important factor in this regard [49]. This issue may be caused directly by elements that are included in the extract [50], or it might be a consequence of modifications in the regular physiological growth of the plant [51,52]. Improvement of commercial-product formulas,

and knowing the mechanisms of active substances in plants and their persistence, should mitigate such negative effects. The exact effect of various elements (e.g., nutrients, betaines, oligomers, polymers) from seaweeds on improving plant growth, vigour, and fractioning of extracts is not fully known. Therefore, a detailed analysis of composition and the fractioning of elements on plant physiology, together with a better ability to monitor the impact of such extracts on those variables and on the expression of genes, would shed light on some of the performance mechanisms [53,54].

Among biostimulants that include seaweeds, Kelpak is particularly interesting. It is extracted from the species *Ecklonia maxima* Osbeck and then harvested along the shores of Africa. Kelpak contains phytohormones, such as auxins (11 mg dm^{-3}) and cytokinins (0.031 mg dm^{-3}), and also alginates (1.5 g L^{-1}), amino acids (total $441.3 \text{ mg } 100 \text{ g}^{-1}$), mannitol (2261 mg L^{-1}), neutral sugars (1.08 g L^{-1}), and small amounts of macro- (mean composition: N 0.09%, P 90.7 mg kg^{-1} , K $7163.3 \text{ mg kg}^{-1}$, Ca 190.4 mg kg^{-1} , Mg 337.2 mg kg^{-1} , Na $1623.7 \text{ mg kg}^{-1}$) and microelements (mean composition: Mn 17.3 mg kg^{-1} , Fe 40.7 mg kg^{-1} , Cu 13.5 mg kg^{-1} , Zn 17.0 mg kg^{-1} , B 33.0 mg kg^{-1}) [55,56]. Moreira Sisalema [57] indicates that Kelpak, due to the unique extraction process, contains a very-high auxins-to-cytokinins ratio. The dominance of auxins stimulates the dynamic growth of plant roots, which increases the absorption of indispensable nutrients and minerals, and consequently, of plant production. Activity of this biostimulant may also increase plant resistance to drought and enable faster plant regeneration after water stress. Cals [58] shows that Kelpak should be used in leguminous plants before flowering in the dose of 2.0 L ha^{-1} , in relation to the condition of plant nutrition. The concentration of these preparations in foliar applications is usually from 0.2% to 1% and it rarely exceeds these concentrations. Depending on the specific cultivation, cultivar, and climatic conditions, farmers are usually recommended to use these biostimulants in the form of two-week spraying in the stage of intense plant growth [28,59,60].

Because of the varied reactions of many plants to the application of biostimulants from seaweeds, and due to small number of studies on their influence in soya cultivation, a three-year log field study was carried out. Its main aim was to assess the impact of the application of *Ecklonia maxima* extract (Kelpak) on plant growth, yield size, and the quality and nutraceutical potential of genetically non-modified seeds of Atlanta cultivar. The initial hypothesis was that the introduction of agrotechnical treatment to soya cultivation in the form of plant spraying with Kelpak preparation would modify the plant growth, yield, and chemical composition of soya seeds. To test this hypothesis, the yield and structural elements of soya cropping were assessed, as well as the protein, fat, and anti-oxidant potential of seeds in relation with the applied doses and concentrations of the tested preparation. In order to know the morphological and biochemical plant reaction on seaweed extract performance, the responses of treated and untreated control plants in the same environmental conditions were compared. It was expected that the observation of plant reaction would considerably increase knowledge regarding the manner of seaweed extract performance, particularly in leguminous plants cultivation, which are sensitive to biotic and abiotic stresses. The present work is a concrete step towards broadening the understanding of the advantages of the application in agricultural practice of *Ecklonia maxima* seaweed extracts for the improvement of the size and quality of crops.

2. Materials and Methods

2.1. Plant Materials and Growth Conditions

The field experiment was carried out in 2014–2016 in Perespa ($50^{\circ}66' \text{ N}$; $23^{\circ}63' \text{ E}$, Poland). It was established in a randomized block design in four replications on experimental plots with an area of 10 m^2 . Soybean was cultivated on the soil belonging to the Gleyic Phaeozems, which was characterized by alkaline pH (pH in 1M KCl: 7.4–7.5). The soil content in the assimilable nutrients was at the medium level, as follows: P ($12.6\text{--}14.2 \text{ mg P}_2\text{O}_5$ in 100 g soil), K ($15.3\text{--}17.1 \text{ mg K}_2\text{O}$ in 100 g soil), and Mg ($6.2\text{--}6.8 \text{ mg Mg}$ in 100 g soil). Each year, winter wheat was used as a forecrop. Soybean seeds (*Glycine max* (L.) Merr.) of Atlanta cultivar (Agroyoumis, Poland) were sown on

the 25 of April in 2014 and 2015, and 23 of April in 2016 in rows every 30 cm at a row spacing of 3.5 cm. The weeds were mechanically and manually removed. No pesticides were used (pests did not exceed the thresholds of harmfulness). In the growing season, the plants were sprayed with biostimulant (water solutions) that was based on the *Ecklonia maxima* extract (Kelpak). Kelpak contains phytohormones (mostly auxins 11 mg kg⁻¹, cytokinins 0.03 mg kg⁻¹, and auxin: cytokinin ratio 367:1), carbohydrates (16.9 g kg⁻¹), amino acids (2.5 g kg⁻¹), vitamin B1 (0.9 mg kg⁻¹), B2 (0.1 mg kg⁻¹), C (20 mg kg⁻¹), and E (0.7 mg kg⁻¹). The elemental profile of the biostimulant is: N 3.6 g kg⁻¹, P 8.2 g kg⁻¹, K 7.2 g kg⁻¹, Ca 0.8 g kg⁻¹, Mg 0.2 g kg⁻¹, Fe 13.6 mg kg⁻¹, Mn 8.4 mg kg⁻¹, B 0.24 mg kg⁻¹, Zn 4.2 mg kg⁻¹, and Cu 0.2 mg kg⁻¹ [14,61]. The scheme of doses, developmental stages of plants, and terms of spraying are presented in Table 1.

Table 1. Plant developmental stages and dates of biostimulants application.

Biostimulant	Number of Sprays and Plant Developmental Stages in Which the Biostimulants were Applied	Concentration	Volume of Working Solution/Working Pressure	Date of Spraying		
				2014	2015	2016
Kelpak SL	Single spraying BBCH 13-15 (LSS)	0.7%	300 l·ha ⁻¹ / 0.30 MPa	June 21	June 20	June 7
	Double spraying BBCH 13-15, BBCH 61 (LDS)	0.7%		June 21, July 5	June 20, July 3	June 7, June 23
	Single spraying BBCH 13-15 (HSS)	1.0%		June 21	June 20	June 7
	Double spraying BBCH 13-15, BBCH 61 (HDS)	1.0%		June 21, July 5	June 20, July 3	June 7, June 23

Plants sprayed with water served as the control. The biostimulant (or water) was sprayed with a GARLAND FUM 12B battery field sprayer (Lechler LU 120-03) at a pressure of 0.30 MPa, using 300 l liquid per hectare. The average temperature and rainfalls in the soybean growing season are shown in Table 2.

Table 2. Temperature (T) and rainfalls during the soybean growing season 2014–2016.

Month	Year						Average from 2002 to 2013	
	2014		2015		2016		T (°C)	Rainfall (mm)
	T (°C) Average (min/max)	Rainfall (mm)	T (°C) Average (min/max)	Rainfall (mm)	T (°C) Average (min/max)	Rainfall (mm)		
IV	9.4 (−6.0/22.7)	36.5	8.2 (−1.7/24.3)	30.1	9.2 (−1.2/22.6)	68.4	8.5	41.2
V	13.7 (0.5/27.7)	208.3	12.7 (1.5/24.9)	108.6	13.8 (2.6/26.7)	61.3	12.7	63.4
VI	16.1 (6.7/28.9)	67.1	17.4 (6.6/30.5)	14.1	18.1 (4.2/31.5)	97.1	17.7	68.6
VII	20.3 (10.0/31.0)	104.2	19.6 (8.4/33.4)	59.2	19.5 (8.8/31.2)	107.6	18.9	79.1
VIII	18.2 (6.3/34.0)	115.4	21.6 (5.6/35.5)	23.4	18.2 (7.1/30.7)	95.3	19.4	71.8
IX	13.7 (3.7/25.8)	89.4	15.1 (4.2/34.5)	137.6	15.2 (1.6/28.7)	41.2	14.1	69.2
Average/Total	15.1	620.9	15.8	373.0	17.1	470.9	15.2	393.3

2.2. Plant Growth, Yield, and Nutritional Value Determination

After the pods have matured, when the seeds have obtained a typical color and hardness (BBCH 89), the plant height, the internode number on the main shoot, and the first pod height were recorded.

In addition, after harvesting, the number of pods per plant, the number of seeds per 1 m², the weight of seeds, and the weight of thousand seeds were determined. Subsequently, the seeds were dried and then grinded. The flour was used for further analysis.

Protein content was determined with the Kjeldahl method, whereas the content of lipids was based on the acid hydrolysis method [62].

2.3. Nutraceutical Potential

Seed extract was prepared following the methodology that was proposed by Świeca et al. [63]. The ground soybean seeds were extracted with a mixture of acetone, water, and hydrochloric acid (70:29:1; v/v/v). Afterwards, the samples were centrifuged for 10 min (6800 × g) and the resulting supernatant was collected and then used for further analyses.

2.3.1. Phenolics Determination

Determination of Total Phenolic Compounds (TPC)

The content of total phenolic compounds (TPC) was determined with the method of Singleton and Rossi using the Folin–Ciocalteu reagent [64]. Absorbance of the samples was measured with a UV-vis spectrophotometer at a wavelength of 725 nm, then TPC was computed and expressed as gallic acid equivalents (GAE) in mg per g of dry matter (DM).

Determination of Flavonoid Content (TFC)

The total content of flavonoids was determined acc. to the method that was presented by Lamaison and Carnet [65]. The prepared soybean extract was mixed with a methanolic solution of AlCl₃ × 6H₂O. After incubation, the absorbance was measured with a UV-vis spectrophotometer at the wavelength of 430 nm. The total flavonoid content was expressed as quercetin equivalents (QE) in mg per g DM.

Determination of Anthocyanins (TAC)

Using the method that was proposed by Fuleki and Francis using potassium chloride and sodium acetate buffer at two pH values (1.0 and 4.5), the content of anthocyanins was assayed [66]. After 15 min, absorbance of each sample was measured at wavelengths of 520 nm and 700 nm. Subsequently, anthocyanin content was calculated as cyanidin-3-glucoside equivalents (Cy3-GE) in mg per g DM.

2.3.2. Reducing Power

Reducing power was measured following the method that was provided by Pulido et al. [67]. The soybean extract was mixed with a phosphate buffer (200 mM, pH 6.6) and 1% solution of K₃[Fe(CN)₆]. Next, the samples were incubated at 50 °C for 20 min. The reaction was stopped with trichloroacetic acid and the samples were centrifuged (6800 × g, 10 min). The resulting supernatant was mixed with distilled water and FeCl₃. Afterwards, absorbance was measured at the wavelength of 700 nm. Reducing power was expressed as Trolox equivalents in mg per g DM.

2.4. The Index of Biostimulant Effect

The index of biostimulant effect (ABT-C) was determined as the difference between the mean result that was obtained after biostimulant application (ABT) and the control (C), which enabled the evaluation of the effect of biostimulant type on the analyzed traits. The mean value for each treatment has been obtained clustering the means of lower concentration single spraying (LSS), lower concentration double spraying (LDS), single application of the higher concentration (HSS), and higher concentration double spraying (HDS) from different years all together. The standard deviation value (SD) was determined for all reported mean values of ABT-C [5].

2.5. Statistical Analysis

The obtained results were statistically elaborated with Statistica 13 software (StatSoft, Inc.). The materials were collected over three seasons (2014–2016). Laboratory analyses were performed in triplicate. Normality of data distribution was assessed with the Shapiro–Wilk test. The significance of differences between the evaluated mean values was estimated with the Tukey test at a significance level of $p < 0.05$.

3. Results

3.1. Effect of Biostimulants on Biometric Traits

3.1.1. Plant Height

The single application of the higher concentration (HSS) of Kelpak biostimulant ensured better effects in increasing soybean plant height (increased by 35% as compared to the control) (Table 3). The highest plants were obtained in the growing season 2016 after their single spraying with the higher concentration of Kelpak. In contrast, the smallest plants were produced in the 2015 season and their height differed significantly from the values noted in seasons 2014 and 2016. The biostimulant increased the height of plants, which was indicated by a value of the Kelpak effect index (ABT-C) of 28.2 cm for this trait (Table 4).

Table 3. Effect of *Ecklonia maxima* extract (Kelpak) treatment on biometric traits of soybean (average from 2014–2016).

Parameters	<i>Ecklonia maxima</i> Treatment	Season			Average from 2014 to 2016
		2014	2015	2016	
Plant height (cm)	C	85.4 ^a	81.9 ^a	88.1 ^a	85.1 ^a
	LSS	114.9 ^b	107.7 ^b	112.6 ^b	111.7 ^b
	LDS	117.5 ^b	108.0 ^b	117.0 ^b	114.1 ^b
	HSS	118.8 ^b	106.4 ^b	120.0 ^b	115.0 ^b
	HDS	114.9 ^b	108.0 ^b	114.4 ^b	112.4 ^b
	AS	110.3 ^b	102.4 ^a	110.3 ^b	
Number of internodes in the main shoot	C	11.2 ^a	10.1 ^a	9.6 ^a	10.3 ^a
	LSS	10.4 ^a	8.6 ^a	10.2 ^a	9.7 ^a
	LDS	9.9 ^a	9.3 ^a	9.0 ^a	9.4 ^a
	HSS	10.0 ^a	9.8 ^a	10.3 ^a	10.0 ^a
	HDS	9.9 ^a	8.8 ^a	11.1 ^a	9.9 ^a
	AS	10.3 ^b	9.3 ^a	10.0 ^{ab}	
Location height of the first pod (cm)	C	12.5 ^a	11.1 ^a	11.7 ^a	11.7 ^a
	LSS	13.0 ^a	14.2 ^a	12.2 ^a	13.2 ^{ab}
	LDS	13.8 ^a	14.0 ^a	13.3 ^a	13.7 ^b
	HSS	12.0 ^a	12.5 ^a	12.2 ^a	12.2 ^{ab}
	HDS	13.0 ^a	12.7 ^a	13.3 ^a	13.0 ^{ab}
	AS	12.8 ^a	12.9 ^a	12.5 ^a	
Number of pods (per plant)	C	15.2 ^a	14.7 ^a	16.3 ^a	15.4 ^a
	LSS	20.9 ^b	22.5 ^{cd}	21.0 ^b	21.5 ^{bc}
	LDS	22.4 ^b	23.4 ^d	21.5 ^b	22.4 ^c
	HSS	19.9 ^b	21.4 ^{bc}	21.1 ^b	20.8 ^b
	HDS	20.4 ^b	20.3 ^b	20.8 ^b	20.5 ^b
	AS	19.8 ^a	20.4 ^a	20.1 ^a	

Abbreviations: C, control; LSS, lower concentration single spraying; LDS, lower concentration double spraying; HSS higher concentration single spraying; HDS, higher concentration double spraying. Means in the columns, concerning the selected traits, followed by different small letters are significantly different at $p < 0.05$.

Table 4. The index of biostimulant effect (ABT-C).

Parameters	Kelpak
Plant height (cm)	28.2
Number of nodes in the main shoot	−0.5
Location height of the first pod (cm)	1.3
Number of pods (per plant)	5.9
Number of seeds (per m ^{−2})	622
Seed yield (t ha ^{−1})	0.824
1000 seed weight (g 1000 ^{−1})	−10.7
Total protein (% DM)	0.35
Total fat (% DM)	−1.56
Total phenols (mg g ^{−1} DM)	2.53
Total flavonoids (mg g ^{−1} DM)	1.23
Anthocyanins (mg g ^{−1} DM)	0.01
Reducing power (mg TE g ^{−1} DM)	0.15

3.1.2. Number of Internodes in the Main Shoot

Internode number decreased regardless of the Kelpak concentration and the number of its applications, although the differences were insignificant (Table 3). The highest number of internodes on the main shoot was obtained in the first and third season. The highest number of internodes on the main shoot was obtained in the first season and it differed significantly from the number that was determined in 2015. The value of the ABT-C index computed for Kelpak was negative (Table 4).

3.1.3. Location Height of the First Pod

Biostimulant treatment increased the height of the first pod as compared to the control. Significant differences were observed between the double application of the lower concentration of Kelpak and the control (increased by 17%) (Table 3). The tallest heights of the first pods were observed in the 2015 season, however they did not significantly differ from the values that were reported in the two other seasons. Values of the ABT-C index demonstrate that the height of the first pod was larger with the application of Kelpak preparation (Table 4).

3.1.4. Number of Pods per Plant

Double foliar application of the lower concentration of Kelpak permitted achieving the highest number of pods per plant (increased by 45% as compared to the control) (Table 3). The study demonstrated that the mean number of pods determined in particular growing seasons was at a similar level and did not significantly differ among seasons. In turn, biostimulant increased the pod number per plant because the value of Kelpak effect index was 5.9 pods/plant after spraying with this preparation (Table 4).

3.2. Effect of Biostimulants on Soybean Yield

3.2.1. Number of Seeds

Double spraying soybean plants with the higher concentrations (HDS) of Kelpak had the largest effect on the increase in seed number per m² (increased by 43% as compared to the control) (Table 5). The analysis of growing seasons demonstrated the largest value of this trait in 2016 and the smallest one in 2015 (lower by 5% than that noted in 2016). The application of seaweed extract increased this number, which was indicated by values of the ABT-C index that were calculated for this trait (Table 4).

Table 5. Effect of *Ecklonia maxima* extract (Kelpak) treatment on yield and nutritional properties of soybean (average from 2014–2016).

Parameters	<i>Ecklonia maxima</i> Treatment	Season			Average from 2014 to 2016
		2014	2015	2016	
Number of seeds (per m ⁻²)	C	1793 ^a	1581 ^a	1907 ^a	1760 ^a
	LSS	2255 ^b	2210 ^b	2337 ^b	2267 ^b
	LDS	2340 ^b	2376 ^{bc}	2406 ^{bc}	2374 ^b
	HSS	2344 ^b	2350 ^b	2401 ^{bc}	2365 ^b
	HDS	2466 ^c	2528 ^c	2576 ^c	2524 ^c
	AS	2240 ^a	2209 ^a	2326 ^b	
Seed yield (t ha ⁻¹)	C	3.267 ^a	2.664 ^a	3.262 ^a	3.064 ^a
	LSS	3.677 ^b	3.636 ^b	3.767 ^b	3.693 ^b
	LDS	3.805 ^b	3.876 ^b	3.852 ^{bc}	3.844 ^b
	HSS	3.758 ^b	3.874 ^b	3.907 ^{bc}	3.846 ^b
	HDS	4.137 ^c	4.171 ^c	4.198 ^c	4.169 ^c
	AS	3.729 ^a	3.644 ^a	3.797 ^b	
1000 seed weight (g)	C	182.2 ^b	168.5 ^a	171.0 ^b	173.9 ^b
	LSS	163.1 ^a	164.6 ^a	161.2 ^a	162.9 ^a
	LDS	162.6 ^a	163.2 ^a	160.1 ^a	161.9 ^a
	HSS	160.3 ^a	164.9 ^a	162.7 ^a	162.3 ^a
	HDS	167.8 ^a	165.0 ^a	163.1 ^a	165.3 ^a
	AS	167.2 ^b	165.2 ^{ab}	163.6 ^a	
Total protein (% DM)	C	36.8 ^a	46.5 ^d	35.9 ^a	39.7 ^a
	LSS	37.7 ^b	45.7 ^c	38.6 ^d	40.7 ^a
	LDS	37.7 ^b	47.4 ^e	36.3 ^b	40.5 ^a
	HSS	38.0 ^b	42.7 ^b	38.9 ^d	39.9 ^a
	HDS	39.1 ^c	40.9 ^a	38.1 ^c	39.4 ^a
	AS	37.9 ^b	44.6 ^c	37.6 ^a	
Total fat (% DM)	C	17.5 ^d	15.0 ^d	16.6 ^c	16.4 ^b
	LSS	14.5 ^a	15.5 ^e	14.5 ^a	14.8 ^a
	LDS	15.4 ^b	12.8 ^a	15.1 ^b	14.4 ^a
	HSS	15.5 ^b	13.8 ^c	15.1 ^b	14.8 ^a
	HDS	15.7 ^c	13.3 ^b	16.4 ^c	15.2 ^{ab}
	AS	15.7 ^c	14.1 ^a	15.5 ^b	

Abbreviations: C, control; LSS, lower concentration single spraying; LDS, lower concentration double spraying; HSS higher concentration single spraying; HDS, higher concentration double spraying. Means in the columns, concerning the selected traits, followed by different small letters are significantly different at $p < 0.05$.

3.2.2. Seed Yield

The most positive response of plants to the use of biostimulant was observed after double spraying with the higher concentration of Kelpak preparation, as indicated by their seed yield increase by 36% when compared to the control (Table 5). The highest mean seed yield for Atlanta cv. was obtained in 2016. In contrast, the seed yield of 2015 season turned out to be the lowest among the studied seasons (lower by 4% than that noted in 2016). Foliar application of Kelpak increased the seed yield of soybean of Atlanta cv., which was indicated by positive values of the ABT-C index that were calculated for this trait (Table 4).

3.2.3. Thousand Seed Weight

Foliar application of Kelpak decreased 1000 seed weight. Its lowest value was determined after double application of Kelpak in the lower concentration (decrease by 7% as compared to the control) (Table 5). The least decrease of 1000 seed weight was achieved after double plant spraying with the

higher concentration of Kelpak biostimulant. The highest mean 1000 seed weight was reported in the 2014 growing season. The values of the biostimulant effect index calculated for this trait were negative, which points to the negative impact of Kelpak preparation on 1000 seed weight (Table 4).

3.3. Effect of Biostimulant on the Nutritional Properties

3.3.1. Total Protein in Soybean Seeds

Depending on concentration and number of applications, Kelpak increased or decreased the protein content in a dry matter of seeds. However, the statistical analysis demonstrated that differences in the effects of biostimulant on this trait were insignificant. Increased protein content was determined in seeds of plants single-sprayed with the lower concentrations of Kelpak (Table 5). Concerning growing seasons, the highest protein content of seeds was noted in 2015. Values of the ABT-C index that were calculated for this trait were positive for this preparations (Table 4).

3.3.2. Total Fat in Soybean Seeds

Regardless of the number of sprayings and concentration of biostimulant, its use decreased the fat content in dry matter of soybean seeds, with the greatest decrease (by 14% as compared to the control) being noted after double spraying the plants with the lower concentration of Kelpak (Table 5). In contrast, the smallest decrease in fat content of the seeds as compared to the control was determined after double spraying with the higher concentrations of Kelpak. The highest fat content of soybean seeds was noted in season 2014 and the lowest in 2015. The values of the ABT-C index that were calculated for this preparation were negative (Table 4), which is indicative of its negative effect on fat content in of Atlanta cv. soybean seeds.

3.4. Effect of Biostimulants on the Antioxidant Potential in Soybean Seeds

3.4.1. Total Phenolic Content

The use of Kelpak in soybean cultivation caused changes in contents of total polyphenols (TPC) in seeds (Table 6), which varied depending on both the number of applications and the concentration of this preparation. The use of the biostimulant based on *Ecklonia maxima* extract caused an increase in phenolics compounds content in soybean seeds. However, significant differences were only demonstrated in plants that were single-sprayed with 1% Kelpak (HSS). The TPC content that was determined for this combination was over twofold higher, when compared to the control combination. This nutraceutical property of soybean was influenced by meteorological conditions that occurred in a given growing season. The highest significant differences were observed in 2014 and 2016. A positive value of the difference between contents of phenolics in combinations that were treated with Kelpak biostimulant and the control samples (ABT-C) was calculated for soybean seeds (Table 4).

3.4.2. Total Anthocyanins Content

The presence of anthocyanins was detected in seven out of the 15 analyzed combinations of Kelpak biostimulant use in soybean cultivation. These compounds were not detected in the control samples in any of the growing seasons studied.

The use of Kelpak affected the content of anthocyanins in soybean seeds. However, their presence was only detected in 17% of the analyzed combinations. The number of applications and concentration of the biostimulant were the factors that determined anthocyanins content. The highest value of which was noted after plants spraying with the higher concentration of Kelpak. In this case, significant differences were also observed as influenced by conditions that occurred during the plant growth stage (Table 6). The values of biostimulant effect ABT-C index calculated for this trait were positive (Table 4).

Table 6. Effect of *Ecklonia maxima* extract (Kelpak) treatment on the antioxidant potential in soybean seeds (average from 2014–2016).

Parameters	<i>Ecklonia maxima</i> Treatment	Season			AA
		2014	2015	2016	
Total phenols (mg g ⁻¹ DM)	C	5.77 ^a	4.50 ^a	5.77 ^b	5.35 ^a
	LSS	7.36 ^b	5.85 ^e	7.74 ^c	6.98 ^a
	LDS	8.56 ^c	4.70 ^b	8.40 ^d	7.22 ^a
	HSS	15.02 ^d	5.05 ^c	15.20 ^e	11.76 ^b
	HDS	5.78 ^a	5.26 ^d	5.54 ^a	5.53 ^a
	AS	8.50 ^b	5.07 ^a	8.53 ^b	
Total flavonoids (mg g ⁻¹ DM)	C	1.99 ^a	1.44 ^a	1.99 ^a	1.81 ^a
	LSS	1.87 ^a	1.92 ^c	1.92 ^a	1.90 ^a
	LDS	2.64 ^b	1.84 ^b	2.59 ^b	2.36 ^a
	HSS	5.10 ^d	2.93 ^e	5.15 ^d	4.39 ^b
	HDS	4.18 ^c	2.08 ^d	4.21 ^c	3.49 ^b
	AS	3.16 ^b	2.04 ^a	3.17 ^b	
Anthocyanins (mg g ⁻¹ DM)	C	0.00 ^a	0.00 ^a	0.00 ^a	0.000 ^a
	LSS	0.00 ^a	0.00 ^a	0.00 ^a	0.000 ^a
	LDS	0.00 ^a	0.02 ^b	0.00 ^a	0.007 ^a
	HSS	0.00 ^a	0.04 ^c	0.00 ^a	0.013 ^{ab}
	HDS	0.04 ^b	0.00 ^a	0.05 ^b	0.030 ^b
	AS	0.008 ^a	0.012 ^b	0.010 ^{ab}	
Reducing power (mg TE g ⁻¹ DM)	C	0.15 ^a	0.10	0.15 ^a	0.13 ^a
	LSS	0.30 ^{bc}	0.22	0.33 ^c	0.28 ^b
	LDS	0.21 ^{ab}	0.14	0.28 ^b	0.21 ^{ab}
	HSS	0.45 ^d	0.08	0.42 ^e	0.31 ^b
	HDS	0.38 ^{cd}	0.16	0.37 ^d	0.30 ^b
	AS	0.30 ^b	0.14 ^a	0.31 ^b	

Abbreviations: C, control; LSS, lower concentration single spraying; LDS, lower concentration double spraying; HSS higher concentration single spraying; HDS, higher concentration double spraying. Means in the columns, concerning the selected traits, followed by different small letters are significantly different at $p < 0.05$.

3.4.3. Total Flavonoid Content

Flavonoid content analysis showed a significant effect of the application of biostimulant on its values. The foliar application of Kelpak resulted in the increased content of flavonoids in seeds. Significantly, the highest content of these compounds was noted after plant spraying with 1% solution of this preparation, regardless of the number of applications.

The analysis of the effect of biostimulants with different composition revealed that their foliar application resulted in an increased content of flavonoids when compared to the control samples (a positive value of the ABT-C difference) (Table 4).

3.4.4. Reducing Power

The evaluation of the effect of applying biostimulants with different compositions on the antioxidant activity of soybean included the determination of the reducing power, the value of which was increased by almost all combinations of this biostimulant.

Significant differences in reducing power values were observed upon the application of Kelpak biostimulant (Table 6). A tendency for an increase of reducing potential was noted after the application of this preparation in the higher concentration and after single spraying the plants with its 0.7% solution. In the second study year, the value of reducing power was the lowest when compared to the other analyzed years (over twofold decrease of RP value). Foliar application of *Ecklonia maxima* extract

increased its reducing power values, which was indicated by positive values of the ABT-C index that were calculated for this trait (Table 4).

4. Discussion

Biostimulants induce the growth and development of plants, from seed germination throughout the entire ontogenesis. They affect the metabolic processes that occur in the plant by enhanced activity and synthesis of phytohormones, by stimulating the growth of the root system, and by improving the uptake, translocation, and retention of nutrients, which determines quantity and quality of crop yield [6,68].

Our study demonstrates a significant increase in the growth of soybean plants after the foliar application of biostimulant that is based on *Ecklonia maxima* extract. An earlier study also showed the growth stimulation of soybean treated with a biostimulant (Fylloton) containing *Ascophyllum nodosum* extract and free amino acids [8]. The marked growth responses in soybean plants are possibly due to *Ecklonia maxima* extract (Kelpak) composition, especially the PGRs (Plant Growth Regulator) that were identified (cytokinins, auxins, polyamines, gibberellins, brassinosteroids) and the mineral content in this biostimulant [55,69,70]. Additionally, the stimulatory role of Kelpak in the production of phytohormones has been demonstrated. For example, it increased the content of cytokinins in *Eucomis autumnalis* [70].

However, despite the observed favourable effects related to the application of biostimulants, including seaweeds, the precise mechanism of their activity still remains mostly unknown [26]. It should be emphasised that a full explanation of the principle or principles of their operation may cause a potential increase in the use of these preparations. According to Crouch and Van Staden [71] and Craigie [72], a wide scope of reported physiological responses of plants in cultivations where seaweed extracts were used is related to the fact that those products include numerous active compounds. Cytokinins, auxins, gibberellins, brassinosteroids, and other activating particles, like, for example, oligomers and polysaccharides are included [73]. According to Depuydt and Hardtke [74], cytokinins, together with auxins, function as regulators of various physiological processes, including those that are related to plant growth and development [75–79]. Thus, each change in the concentration of endogenous cytokinins influences the regulation of many physiological processes and as a result impacts the growth of the entire plant [80,81]. Studies by Aremu et al. [70] proved that the total content of cytokinins increases in plants after the application of the Kelpak preparation. The qualitative composition of the listed compounds is changed, which is related to their functional and physiological role, in particular, during plant morphogenesis. According to Strnad [76], isoprenic cytokinins determine the growth processes that include a continuation of the cell cycle. On the other hand, aromatic cytokinins model growth processes, such as morphogenesis and ageing. Aremu et al. [70] even assumed that the quantification of the endogenous content of cytokinins might provide information regarding possible physiological mechanisms that are related to the application of Kelpak biostimulant. However, researchers stress that, due to numerous active substances and compounds that are contained in Kelpak, the observed, favourable impact on the growth and development of plants may not only be assigned to cytokinins, but instead be the result of possible cross reactions of those compounds with other biologically active particles that are included in seaweed biostimulants. Therefore, further research concerning those fields is indispensable in order to obtain a full explanation for the Kelpak performance [70].

Still, in the literature, the prevailing hypothesis is that the majority of responses of plants that were cultivated with biostimulants' application, including seaweeds, results from the presence of compounds from the group of plant hormones, namely cytokinins [18]. The assumption stems from the fact that these compounds, isolated from seaweed extracts and individually tested in cultivations, mitigates the stress that is caused by free radicals through direct capturing and the prevention of reactive oxygen forms (ROS). This is done through the inhibition of xanthine oxidation [18,34,82–84].

Khan et al. [85] and Panda et al. [18] additionally indicate that extracts from seaweeds, such as Kelpak, support plant tolerance to stress, influencing the increase of K^+ capture in plants.

Additionally, in the literature, we may find hypotheses regarding modelling the growth and development of plants through the application of biostimulants from seaweeds as an effect of the presence of substances that are similar to gibberellins [86]. Research by Stephenson [19] indicates that these extracts include at least two compounds that behave like gibberellins (GA3 and GA7). However, they also show the presence of terpenoids and α -tokopherol, the performance of which may imitate gibberellins' activity on plants [18,87,88].

Recent theories indicate that the activity of seaweed extracts may be the result of their content of betaines. Panda et al. shows that these compounds have a similar impact on plants as the aforementioned cytokinins [18]. It was proved that seaweed extracts include, among others, gamma aminobutyric acid betaine, 6-amino valeric acid betaine, and glyco betaine. Mancuso et al. [89] suggested that, due to the presence of those active compounds, extracts influence the mitigation of the osmotic and oxidization stress in plants, which may lead to the damage of DNA lipids, carbohydrates, and proteins, and also disturb correct cell signalling. Genard et al. [90] and Blunden et al. [91] even assigned an improvement of plant yield to the presence of betaines in extracts, since that led to the increased concentration of chlorophyll. According to Naidu et al. [92], betaines constitute a source of nitrogen when they are provided in low doses, or act as osmolites in higher concentrations. Many studies also show that betaines play a role in the correct formation of somatic germs from cotyledons tissues and mature seeds [18,93,94].

The stimulation of plant growth and development may also result from the occurrence of polyamines in biostimulants based on seaweed extracts, since these compounds may act as plant growth regulators. However, it should be emphasised that they are not classified as plant hormones. Several amino groups that usually replace hydrogen in the alkaline chain (putrescine, spermidine, and spermine) are characteristic of polyamines' structure. Research by Haman et al. [95] proves that polyamines determine the stability of various RNA and DNA conformation states. These compounds are often related to important stages of the cell division cycle. They also ensure the stability of a membrane to various cell membranes. Thus, due to the fact that polyamines affect a wide scope of physiological growth processes, their occurrence in biostimulating products that are made of seaweed may influence plant growth [18].

In our study, the use of biostimulant increased the fat and protein content in soybean seeds. The stimulating effect of biostimulants on the nutritional composition of various plants is mainly due to the number of PGR contained in the solutions [89]. In addition, the increased nutritional content in *C. triloba* is probably related to the ability of biostimulators to improve the slow release of nutrients and their uptake by plants [68,96]. A stimulating activity of seaweeds extract is also found in the presence of abscisic acid (ABA) [97,98]. However, the ABA function remains not fully characterized. Nevertheless, it is known that this acid induces protein synthesis, which are needed by plants in dealing with stress factors during water deficiencies [99,100]. Davies [101] shows that, during drought, this compound in plants caused numerous physiological reactions, including the closing of stoma, increase of a trend for accumulation of protein in seeds, gene transcription for proteinase inhibitors, as well as inhibition of sprouts growth or initiation of some states of seeds dormancy.

Unfortunately, the precise mechanisms that are activated by those biostimulants are still difficult to be identified, despite even greater knowledge on the composition of extracts from seaweeds. This is also due to the fact that these preparations constitute an abundance of many biologically active chemical compounds. They also include bioactive secondary metabolites, vitamins, and vitamin precursors [102,103]. Many authors underline the meaning of their synergetic cooperation, which stimulates that growth of plants assuming a mechanism that has not been fully known yet [24,72,104]. One of the main components of seaweed extracts are polysaccharides, including alginians, fucoidans, and laminarans [85,105]. Fucoidans have various structures due to a varied degree of methylation, sulphurization, and branching [72]. Alginians are polymers of mannuronic and

galuronic acid, with a confirmed activity of plant-growth promotion [106]. Finally, laminarins that are included in extracts are registered compounds that increase plant resistance to fungi and bacteria pathogens [107].

Improvement of a nutritive value of soya seeds observed in our research, as expressed in protein and fat content, could also be caused by the fact that seaweeds are rich in phenolic compounds (complex chlorocluinol, eckol, and dieckol polymers). Phenols belong to secondary metabolites synthesized in plants under the influence of stress. Their task is to protect cells and the components of cell nuclei [108,109]. The ability to chelate metal ions [110] is a significant role of these compounds, besides their antioxidant activity. Research by Raj et al. [60] confirms that phenolic compounds with dihydroxybenzene or trihydroxybenzene groups show strong chelating activity. Rengasamy et al. [111] also shows that eckol belonging to phenolic compounds proves to have a strong auxinosimilar activity. According to the authors, the impact of these polyphenols, included in seaweed extracts, on the endogenic content of auxins is a key element that is necessary for understanding basic mechanisms of these preparations. Korasick et al. [112] reached similar conclusions. They conclude that auxins have a strong impact on many important stages of physiological growth in the life cycle of a plant. Researchers emphasise that maintaining proper concentration of active auxin in cells is of key significance for controlling almost all aspects that are related to the plant growth. The concentration of cell auxin is affected by the speed of anabolism, catabolism, transport, and conjugation [113]. In relation to the type of concentration, polyphenols may inhibit or stimulate the development of vegetative plants. It mainly takes place due to their abilities to modulate the metabolism and concentration of active auxin forms in plants [114–117]. Gaspar et al., [118] proves that the phenolic inhibitors of oxidase IAA, such as chlorogenic acid, influence the activity of auxins. Some of the mentioned compounds even constitute alternative substrates for the oxidizing enzyme, which in turn is related to the protection of auxins before oxygen decomposition. Wilson and Van Staden [119] prove that some of the phenolic acids that protect auxins before decarboxylation increase the concentration of active forms of auxins, which are indispensable for the stimulation of growth and development of crop roots. However, according to Arem et al., attempts to explain mechanisms that are responsible for a positive response of plants to application of extracts from seaweeds should take into account the possible cross reactions between phytohormones included therein and the quantitative concentration of auxins, which may justify the observed morphological differences [120].

In the literature, one may find hypotheses that assume that the increased growth and yield of plants that are treated with seaweed extracts resulted from a positive impact of those preparations on the activity of esterase enzymes. This enzyme is considered to be a marker of plant growth processes due to its role in organogenesis. It also works as an index of somatic embryogenesis [121–124]. According to Aremu et al., a higher activity of esterase in plants that were treated with seaweed extracts indicated their stimulating impact on the increase of plant biomass production [120].

Plant metabolism may be modelled through the use of biostimulants. According to Nardi et al., this group of active preparations affects most of all carbon and nitrogen metabolism, which is associated with an enhanced activity of enzymes participating in, among others, the process of glycolysis, Krebs cycle, or nitrogen assimilation [125]. Oboh et al. and Ertani et al. demonstrated that biostimulants application yielded metabolic pathways that are linked with secondary metabolites, like e.g. phenolic compounds [126,127]. It should also be emphasized that the synthesis of secondary metabolites proceeds as an element of chemical defense [128]. Already, in 1959, these compounds were no longer treated as ballast substances [129]. Today, they are believed to play a significant role in plant protection against adverse factors [130]. The most common indicator of plants resistance to biotic factors is the content of phenolic compounds [131], which are precursors of more complex phenolic structures, like flavonoids or lignins [132].

In our study, the foliar application of *Ecklonia maxima* extract (Kelpak) caused a significant increase in polyphenols content. Ertani et al. and Lakhdar et al. showed that the application of biostimulants in plant cultivation enhanced the synthesis of antioxidative compounds, which are

indicators of increased plant resistance to biotic and abiotic stress factors [38,133]. The physiological response of plants to the use of biostimulants results from the presence of active substances in them, such as phytohormones, amino acids, proteins, phenols, or triacontanol [38,127,134].

A positive impact of compounds that are included in seaweed extracts on the total content of phenolic compounds in soya seeds has a significant meaning in the attempt to explain the mechanisms of operation of those biostimulants. The antioxidant potential of plants is inseparable from the amount and quality of phenolic compounds [135]. In real environmental conditions, the regulation of phytochemical synthesis includes a range of advanced mechanisms that enable a precise control of production of specific particles in a suitable place and time, and also in response to outside signals [136]. Such compounds include those in seaweed extracts that may activate specific biochemical pathways that are responsible for the synthesis of secondary metabolites in plants [136–138]. According to Cheynier et al., eckol that is contained in biostimulants influences the phenylpropanoid pathway in the biochemical synthesis of phenolic acids [136]. Researchers assume that this impact is caused by the regulation of the enzymatic activity of ammonia lyase of phenylalanine and chalconic synthase. However, only the approach that is based on the genetic analysis will enable the observation of gene regulation engaged in those pathways [16,120]. Research results that were carried out by Jannin et al. proved that cysteine protease, related to the process of synthesis of phenolic compounds, were regulated downwards, while the expression of genes that are related to photosynthesis, cell metabolism, response to stress, and nitrogen metabolism were significantly raised in the case of plants treated with seaweed extracts [139]. Roupahel et al. observed an increased concentration of phenolic compounds after the application of such biostimulants assigned to their main components, such as polysaccharides (alginates, fucoidans, and laminarins) [140]. These compounds influence endogenous hormonal homeostasis [63,64]. Additionally, processes of synthesis and accumulation of secondary metabolites may be related to the activity of enzymatic groups that are engaged in phytochemical homeostasis (the so-called direct effect) [127,140]. They also depend on the plant nutrition condition and potassium and magnesium concentration (direct effect) [41]. Roupahel et al. [140] also search for the growth of the concentration of bioactive compounds in the activation of key enzymes, such as chalcone isomerase, which is engaged in the biosynthesis of flavon precursors [141].

According to Azcona et al. [142] and Ertani et al. [127], the high effectiveness of biostimulants in plant crops is also influenced by the number of treatments at the appropriate stages of plant development. The first treatment of plant with these preparations resulted most of all in the increased number and weight of leaves, which is referred to as “short-time effect”. Another dose of biostimulants, applied at the plant blooming stage, led to the long-term effect, which was manifested by changes in crop size and quality. In the case of fruits, it results in, among others, an increase in their number and weight when compared to control samples that were not treated with biostimulants [125,127]. The increased content of polyphenols in the crop may indeed result from the use of biostimulants at the appropriate growth stages of plants. Experiments that were conducted by Oboh et al. [126] and by Zhang and Hamauzu [143] confirmed that the first application of these preparations led to an increased content of phenolic compounds in leaves, and that this increase was smaller after the second application of biostimulants. According to Ertani et al. [127], changes in the total polyphenolics content resulting from different numbers of applications of biostimulants are also linked with changes in contents of individual phenolic acids.

This was since the increasing total content of phenolic acids led to an increased number of their functional groups, which are sequesters of free radicals [144]. It must be emphasized that the increased content of polyphenols in plant tissues, as evoked by the action of biostimulants, is a beneficial phenomenon, not only because of the increased plant resistance to stress factors, but also because of significant importance to consumers, since such plant products are rich sources of antioxidative compounds being valuable to the human body [145,146]. Phenolic acids, such as caffeic, gallic, and ferulic, are claimed to exhibit anticarcinogenic and antimicrobial activities [147,148].

To sum up, biostimulants that contain seaweed extracts enable many opportunities to improve plant growth. Their use in agriculture is considered to be favourable for cropping. However, the operation mechanism of such products is not completely described [149]. That is because the impact of the application of growth regulators on plants is not only a consequence of their direct ability to control metabolic pathways, since their activity may be multidirectional. Limited knowledge on the mechanisms of the preparations' activity are still mainly based on assumptions and hypotheses highlights the need of further research within this scope [5]. So far it has been proved that seaweed extracts influence the plant physiology through changes in their general profile of transcriptome, and also in metabolism [139,150]. Research by Fan et al., concerning the analysis of gene expression, expanded the understanding of the possible mechanisms that regulate the activity of these preparations [141]. Researchers indicate that, after the application of extracts from seaweed, increases in the amount of transcripts of regulatory enzymes that are related to the nitrogen metabolism (cytolase glutamine synthesis), antioxidant ability (glutathione reductase), and glycine betaine synthesis (betaine aldehyde dehydrogenesis and choline monooxygenesis) were observed [16,18,22,72].

Although biostimulants are extensively used in agricultural practice, presently the most significant research on those preparations requires a better understanding of the mechanism of their influence [22,151]. According to Van Osten et al. [22] and Povero et al. [152], only after obtaining a complete explanation of those mechanisms, can the design and production of new generation biostimulants can take place. Due to their complex composition and interactions between particular compounds, mechanisms of operation of preparations based on seaweed extracts are slowly and successively discovered, with applications of molecular biology, metabolomics, and genomics techniques. However, according to many researchers, observed favourable biological effects of extracts activity is caused by the activity of small organic particles, as well as polymers that are included in products that have an ability to regulate genes' operation responsible for ensuring and modelling plant resistance systems [16,71,139].

5. Conclusions

The number of biostimulant applications and its concentration modified the biometric traits, crop size, and yield, as well as the nutraceutical and antioxidative potential of soybean seeds. The study demonstrated that the foliar application of *Ecklonia maxima* extract improved the growth and yield of soybean without any negative effect on the nutritive value of its seeds. Our experiment showed a positive effect of double foliar application of the higher concentrations of this biostimulant on soybean seed number and yield. The application of *Ecklonia maxima* extract increased the antioxidative activity of soybean seeds, and content of total phenolic compounds, flavonoids, and anthocyanins. The results of our study indicate the need for continuing investigations and extending their scope with the aim to identify responses of different cultivable plants on the use of biostimulants that are based on various biologically-active compounds.

Author Contributions: S.K. and A.S. conceived and designed the research. S.K., A.S. and E.C. performed the experiments. S.K., A.S., E.C., M.K. and T.P. prepared the materials. S.K., A.S., and M.K. analyzed the data. S.K. and A.S. wrote the paper. M.K. and T.P. revised the manuscript. All authors read and approved the final manuscript.

Funding: This research received no external funding.

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

References

1. Bezerra, A.R.G.; Sedyama, T.; Borém, A.; Soares, M.M. Importância econômica. In *Soja: Do plantio à colheita*; Sedyama, T., Silva, F., Borém, A., Eds.; UFV: Viçosa, Brazil, 2015; pp. 9–26.
2. Kocira, S. Effect of amino acid biostimulant on the yield and nutraceutical potential of soybean. *Chil. J. Agric. Res.* **2019**, *79*, 17–25. [CrossRef]

3. Stein, H.H.; Berger, L.L.; Drackley, J.K.; Fahey, G.C., Jr.; Hernot, D.C.; Parsons, C.M. Nutritional properties and feeding values of soybeans and their coproducts. In *Soybeans, Chemistry, Production, Processing, and Utilization*; Johnson, L.A., White, P.J., Galloway, R., Eds.; AOCS Press: Urbana, IL, USA, 2008; pp. 613–660.
4. Lodhi, K.K.; Choubey, N.K.; Dwivedi, S.K.; Pal, A.; Kanwar, P.C. Impact of seaweed saps on growth, flowering behavior and yield of soybean [*Glycine max* (L.) Merrill.]. *Bioscan* **2015**, *10*, 479–483.
5. Szparaga, A.; Kocira, S.; Kocira, A.; Czerwińska, E.; Świeca, M.; Lorencowicz, E.; Kornas, R.; Koszel, M.; Oniszczyk, T. Modification of growth, yield, and the nutraceutical and antioxidative potential of soybean through the use of synthetic biostimulants. *Front. Plant Sci.* **2018**, *9*. [[CrossRef](#)] [[PubMed](#)]
6. Basak, A. Biostimulators. Definitions, classification and legislation. In *Biostimulators in Modern Agriculture, General Aspects*; Gawronska, H., Ed.; Editorial Housen Wies Jutra: Warszawa, Poland, 2008; pp. 7–17.
7. Paradiković, N.; Vinkovic, T.; Vinkovic, V.I.; Zuntar, I.; Bojic, M.; Medic, M. Effect of natural biostimulants on yield and nutritional quality: An example of sweet yellow pepper (*Capsicum annuum* L.) plants. *Sci. Food Agric.* **2011**, *91*, 2146–2152. [[CrossRef](#)] [[PubMed](#)]
8. Kocira, S.; Szparaga, A.; Kocira, A.; Czerwińska, E.; Wójtowicz, A.; Bronowicka-Mielniczuk, U.; Koszel, M.; Findura, P. Modeling biometric traits, yield and nutritional and antioxidant properties of seeds of three soybean cultivars through the application of biostimulant containing seaweed and amino acids. *Front. Plant Sci.* **2018**, *9*, 388. [[CrossRef](#)] [[PubMed](#)]
9. Kaufmann, G.L., III; Kneivel, D.P.; Watschke, T.L. Effects of a biostimulant on the heat tolerance associated with photosynthetic capacity, membrane thermostability, and polyphenol production of perennial ryegrass. *Crop Sci.* **2007**, *47*, 261–267. [[CrossRef](#)]
10. Du Jardin, P. The Science of Plant Biostimulants—A bibliographic analysis. Ad hoc Study Report to the European Commission DGENTR. 2012. Available online: https://orbi.uliege.be/bitstream/2268/169257/1/Plant_Biostimulants_final_report_bio_2012_en.pdf (accessed on 1 February 2019).
11. Traon, D.; Amat, L.; Zotz, F.; Du Jardin, P. *A Legal Framework for Plant Biostimulants and Agronomic Fertiliser Additives in the EU. Report for the European Commission Enterprise & Industry Directorate—General*; Publications Office of the European Union: Luxembourg, 2014.
12. Matyjaszyk, E. The introduction of biostimulants on the Polish market. The present situation and legal requirements. *Przem. Chem.* **2015**, *10*, 1841–1844. [[CrossRef](#)]
13. Du Jardin, P. Plant biostimulants: Definition, concept, main categories and regulation. *Sci. Hortic.* **2015**, *196*, 3–14. [[CrossRef](#)]
14. Colla, G.; Cardarelli, M.; Bonini, P.; Roupheal, Y. Foliar applications of protein hydrolysate, plant and seaweed extracts increase yield but differentially modulate fruit quality of greenhouse tomato. *HortScience* **2017**, *52*, 1214–1220. [[CrossRef](#)]
15. Colla, G.; Hoagland, L.; Ruzzi, M.; Cardarelli, M.; Bonini, P.; Canaguier, R.; Roupheal, Y. Biostimulant action of protein hydrolysates: Unraveling their effects on plant physiology and microbiome. *Front. Plant Sci.* **2017**, *8*, 2202. [[CrossRef](#)] [[PubMed](#)]
16. Battacharyya, D.; Babgohari, M.Z.; Rathor, P.; Prithiviraj, B. Seaweed extracts as biostimulants in horticulture. *Sci. Hortic.* **2015**, *196*, 39–48. [[CrossRef](#)]
17. Aguilar, J. Algas marinas para la agricultura de alto rendimiento. Available online: <http://www.interempresas.net/Horticola/Articulos/136576-Algas-marinas-para-la-agricultura-de-alto-rendimiento.html> (accessed on 1 February 2019).
18. Panda, D.; Pramanik, K.; Nayak, B.R. Use of sea weed extracts as plant growth regulators for sustainable agriculture. *Int. J. Bioresour. Stress Manag.* **2012**, *3*, 404–411.
19. Stephenson, W.A. *Seaweed in Agriculture and Horticulture*; Faber and Faber: London, UK, 1968.
20. Crouch, I.J.; van Staden, J. Evidence for the presence of plant growth regulators in commercial seaweed products. *Plant Growth Regul.* **1993**, *13*, 21–29. [[CrossRef](#)]
21. Ecoforce, A. Extracto de algas para agricultura ecológica—Fertilizantes ecoforce. Available online: <http://fertilizantesecoforce.es/es/blog/index/list/cat/agricultura-ecologica/?p=19> (accessed on 1 February 2019).
22. Van Oosten, M.J.; Pepe, O.; De Pascale, S.; Silletti, S.; Maggio, A. The role of biostimulants and bioeffectors as alleviators of abiotic stress in crop plants. *Technol. Agric.* **2017**, *4*, 5. [[CrossRef](#)]
23. Medjdoub, R. *La algas marinas y la agricultura*; Adiego: Zaragoza, Spain; pp. 1–3. Available online: http://catsaigner.adiego.com/sites/default/files/las_algas_marinas.pdf (accessed on 1 February 2019).

24. Norrie, J.; Keathley, J.P. Benefits of *Ascophyllum nodosum* marine-plant extract applications to 'Thompson seedless' grape production. *Acta Hortic.* **2006**, *727*, 243–247. [CrossRef]
25. Gajc-Wolska, J.; Spizewski, T.; Grabowska, A. The effect of seaweed extracts on the yield and quality parameters of broccoli (*Brassica oleracea* var. *cymosa* L.) in open field production. *Acta Hortic.* **2013**, *1009*, 83–89. [CrossRef]
26. Sharma, H.S.S.; Fleming, C.; Selby, C.; Rao, J.R.; Martin, T. Plant biostimulants: A review on the processing of microalgae and use of extracts for crop management to reduce abiotic and biotic stresses. *J. Appl. Phycol.* **2014**, *26*, 465–490. [CrossRef]
27. Guerrero, J. La aplicación de las algas marinas para la fertilización Hortalizas. Available online: <http://www.hortalizas.com/nutricion-vegetal/la-aplicacion-de-las-algas-marinas-para-la-fertilizacion/> (accessed on 1 February 2019).
28. Jayaraj, J.; Norrie, J.; Punja, Z.K. Commercial extract from the brown seaweed *Ascophyllum nodosum* reduces fungal diseases in greenhouse cucumber. *J. Appl. Phycol.* **2011**, *23*, 353–361.
29. Araujo, I.B.; Peruch, L.A.M.; Stadnik, M.J. Efeito do extrato de alga e da argila silicatada na severidade da alternariose e na produtividade da cebolinha comum (*Allium fistulosum* L.). *Trop. Plant Pathol.* **2012**, *37*, 363–367. [CrossRef]
30. Uppal, A.K.; El Hadrami, A.; Adam, L.R.; Tenuta, M.; Daayf, F. Biological control of potato *Verticillium* wilt under controlled and field conditions using selected bacterial antagonists and plant extracts. *Biol. Control* **2008**, *44*, 90–100. [CrossRef]
31. Zodape, S.T.; Gupta, A.; Bhandari, S.C.; Rawat, U.S.; Cahudhary, D.R.; Eswara, K.; Chikara, J. Foliar application of seaweed sap as biostimulant for enhancement of yield and field quality of tomato (*Lycopersicon esculentum* Mill.). *J. Sci. Ind. Res.* **2011**, *70*, 215–219.
32. El Modafar, C.; Elgadda, M.; El Boutachfaiti, R.; Abouraicha, E.; Zehhar, N.; Petit, E.; El Alaoui-Talibi, Z.; Courtois, B.; Courtois, J. Induction of natural defence accompanied by salicylic acid-dependant systemic acquired resistance in tomato seedlings in response to bioelicitors isolated from green algae. *Sci. Hortic.* **2012**, *138*, 55–63. [CrossRef]
33. Shah, M.T.; Zodape, S.T.; Chaudhary, D.R.; Eswaran, K.; Chikara, J. Seaweed sap as an alternative to liquid fertilizer for yield and quality improvement of wheat. *J. Plant Nutr.* **2013**, *36*, 192–200. [CrossRef]
34. Zhang, X.Z.; Ervin, E.H. Impact of seaweed extract-based cytokinins and zeatin riboside on creeping bentgrass heat tolerance. *Crop Sci.* **2008**, *48*, 364–370. [CrossRef]
35. Jayaraj, J.; Wan, A.; Rahman, M.; Punja, Z.K. Seaweed extract reduces foliar fungal diseases on carrot. *Crop Prot.* **2008**, *10*, 1360–1366. [CrossRef]
36. Botta, A. Enhancing plant tolerance to temperature stress with amino acids: An approach to their mode of action. *Acta Hortic.* **2013**, *1009*, 29–35. [CrossRef]
37. Colla, G.; Svecova, E.; Roupael, Y.; Cardarelli, M.; Reynaud, H.; Canaguier, R.; Roupael, Y. Effectiveness of a plant-derived protein hydrolysate to improve crop performances under different growing conditions. *Acta Hortic.* **2013**, *1009*, 175–179. [CrossRef]
38. Ertani, A.; Schiavon, M.; Muscolo, A.; Nardi, S. Alfalfa plant-derived biostimulant stimulate short-term growth of salt stressed *Zea mays* L. plants. *Plant Soil* **2013**, *364*, 145–158. [CrossRef]
39. Lucini, L.; Roupael, Y.; Cardarelli, M.; Canaguier, R.; Kumar, P.; Colla, G. The effect of a plant-derived protein hydrolysate on metabolic profiling and crop performance of lettuce grown under saline conditions. *Sci. Hortic.* **2015**, *182*, 124–133. [CrossRef]
40. Xu, C.; Leskovar, D. Effects of *A. nodosum* seaweed extracts on spinach growth, physiology and nutrition valued under drought stress. *Sci. Hortic.* **2015**, *183*, 39–47. [CrossRef]
41. Roupael, Y.; Colla, G.; Giordano, M.; El-Nakhel, C.; Kyriacou, M.C.; De Pascale, S. Foliar applications of a legume-derived protein hydrolysate elicit dose-dependent increases of growth, leaf mineral composition, yield and fruit quality in two greenhouse tomato cultivars. *Sci. Hortic.* **2017**, *226*, 353–360. [CrossRef]
42. Roupael, Y.; Cardarelli, M.; Bonini, P.; Colla, G. Synergistic action of a microbial-based biostimulant and a plant derived-protein hydrolysate enhances lettuce tolerance to alkalinity and salinity. *Front. Plant Sci.* **2017**, *8*, 131. [CrossRef] [PubMed]
43. Roupael, Y.; De Micco, V.; Arena, C.; Raimondi, G.; Colla, G.; De Pascale, S. Effect of *Ecklonia maxima* seaweed extract on yield, mineral composition, gas exchange and leaf anatomy of zucchini squash grown under saline conditions. *J. Appl. Phycol.* **2017**, *29*, 459–470. [CrossRef]

44. Di Stasio, E.; Roupahel, Y.; Colla, G.; Raimondi, G.; Giordano, M.; Pannico, A.; El-Nakhel, C.; De Pascale, S. The influence of *Ecklonia maxima* seaweed extract on growth, photosynthetic activity and mineral composition of *Brassica rapa* L. subsp. *sylvestris* under nutrient stress conditions. *Eur. J. Hortic. Sci.* **2017**, *82*, 286–293. [CrossRef]
45. Colla, G.; Nardi, S.; Cardarelli, M.; Ertani, A.; Lucini, L.; Canaguiera, R.; Roupahel, J. Protein hydrolysates as biostimulants in horticulture. *Sci. Hortic.* **2015**, *196*, 28–38. [CrossRef]
46. Möller, M.; Smith, M.L. The significance of the mineral component of seaweed suspension on lettuce (*Lactuca sativa* L.) seedling growth. *J. Plant Physiol.* **1998**, *153*, 658–663. [CrossRef]
47. Farooq, M.; Aziz, T.; Basra, S.M.A.; Cheema, M.A.; Rehman, H. Chilling tolerance in hybrid maize induced by seed treatments with salicylic acid. *J. Agron. Crop Sci.* **2008**, *194*, 161–168. [CrossRef]
48. Sivritepe, N.; Sivritepe, H.Ö.; Türkan, I.; Bor, M.; Özdemir, F. NaCl pre-treatments mediate salt adaptation in melon plants through antioxidative system. *Seed Sci. Technol.* **2008**, *36*, 360–370. [CrossRef]
49. Zodape, S.T.; Kawarkhe, V.J.; Patolia, J.S.; Warade, A.D. Effect of liquid seaweed fertilizer on yield and quality of okra (*Abelmoschus esculentus* L.). *J. Sci. Ind. Res. India* **2008**, *67*, 1115–1117.
50. Aitken, J.E.; Senn, J.I. Seaweed products as fertilizers and soil conditioners. *Bot. Mar.* **1965**, *8*, 144–147. [CrossRef]
51. Rayorath, P.; Narayanan, J.M.; Farid, A.; Khan, W.; Palanisamy, R.; Hankins, S.; Critchley, A.T.; Prithiviraj, B. Rapid bioassays to evaluate the plant growth promoting activity of *Ascophyllum nodosum* (L.) Le Jol. using a model plant, *Arabidopsis thaliana* (L.) Heynh. *J. Appl. Phycol.* **2008**, *20*, 423–429. [CrossRef]
52. Subramanian, S.; Sangha, J.S.; Gray, B.A.; Singh, R.P.; Hiltz, D.; Critchley, A.T.; Prithiviraj, B. Extracts of the marine brown macroalga, *Ascophyllum nodosum*, induce jasmonic acid dependent systemic resistance in *Arabidopsis thaliana* against *Pseudomonas syringae* pv. *Tomato* DC3000 and *Sclerotinia sclerotiorum*. *Eur. J. Plant Pathol.* **2011**, *131*, 237–248. [CrossRef]
53. Pereira, L.; Gheda, S.F.; Ribeiro-Claro, P.J.A. Analysis by vibrational spectroscopy of seaweed polysaccharides with potential use in food, pharmaceutical, and cosmetic industries. *Int. J. Carbohydr. Chem.* **2013**, *7*. [CrossRef]
54. Ordog, V.; Stirk, W.A.; van Staden, J.; Novak, O.; Strand, M. Endogenous cytokinins in the three genera of microalgae from the Chlorophyta. *J. Phycol.* **2004**, *40*, 88–95. [CrossRef]
55. Stirk, W.A.; Tarkowska, D.; Turečová, V.; Strnad, M.; van Staden, J. Absciscic acid, gibberellins and brassinosteroids in Kelpak[®], a commercial seaweed extract made from *Ecklonia maxima*. *J. Appl. Phycol.* **2014**, *26*, 561–567. [CrossRef]
56. Lötze, E.; Hoffman, E.W. Nutrient composition and content of various biological active compounds of three south African-based commercial seaweed biostimulants. *J. Appl. Phycol.* **2016**, *28*, 1379–1386. [CrossRef]
57. Moreira Sisalema, J.M. Comportamiento agronómico del cultivo de soya (*Glycine max* L.), a la aplicación de tres extractos de algas marinas, en la zona de Pueblviejo. Available online: <http://dspace.utb.edu.ec/bitstream/49000/5048/1/TE-UTB-FACIAG-ING%20AGROP-000018.pdf> (accessed on 1 February 2019).
58. Ficha Técnica. Available online: <https://www.calstiendavirtual.cl/html5/Fichas/6CASA%20JARD%C3%8DN/0126756%20KELPAK.pdf> (accessed on 1 February 2019).
59. Ali, N.; Farrell, A.; Ramsubhag, A.; Jayaraman, J. The effect of *Ascophyllum nodosum* extract on the growth, yield and fruit quality of tomato under tropical conditions. *J. Appl. Phycol.* **2016**, *28*, 1353–1362. [CrossRef]
60. Raj, T.; Nishanthi, P.; Hane Graff, K.; Ann Suji, H. Seaweed extract as a biostimulant and a pathogen controlling agent in plants. *Int. J. Trop. Agric.* **2018**, *36*, 563–580.
61. Roupahel, Y.; Giordano, M.; Cardarelli, M.; Cozzolino, E.; Mori, M.; Kyriacou, M.C.; Bonini, P.; Colla, G. Plant- and seaweed-based extracts increase yield but differentially modulate nutritional quality of greenhouse spinach through biostimulant action. *Agronomy* **2018**, *8*, 126. [CrossRef]
62. AOAC. *Official Methods of Analysis of AOAC International*, 17th ed.; Horwitz, W., Ed.; AOAC International: Gaithersburg, MD, USA, 2000.
63. Świeca, M.; Gawlik-Dziki, U.; Kowalczyk, D.; Złotek, U. Impact of germination time and type of illumination on the antioxidant compounds and antioxidant capacity of *Lens culinaris* sprouts. *Sci. Hortic.* **2012**, *140*, 87–95. [CrossRef]
64. Singleton, V.; Rossi, J. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am. J. Enol. Viticult.* **1965**, *16*, 144–158.

65. Lamaison, J.L.C.; Carnet, A. Teneurs en principaux flavonoids des fleurs de *Crataegus monogyna* Jacq et de *Crataegus laevigata* (Poiret D. C) en fonction de la vegetation. *Pharm. Acta Helv.* **1990**, *65*, 315–320. [[CrossRef](#)]
66. Fuleki, T.; Francis, F.J. Quantitative methods for anthocyanins. 1. Extraction and determination of total anthocyanin in cranberries. *J. Food Sci.* **1968**, *33*, 72–77. [[CrossRef](#)]
67. Pulido, R.; Bravo, L.; Saura-Calixto, F. Antioxidant activity of dietary polyphenols as determined by a modified ferric reducing/antioxidant power assay. *J. Agric. Food Chem.* **2000**, *48*, 3396–3402. [[CrossRef](#)] [[PubMed](#)]
68. Calvo, P.; Nelson, L.; Kloepper, J.W. Agricultural uses of plant biostimulants. *Plant Soil* **2014**, *383*, 3–41. [[CrossRef](#)]
69. Papenfus, H.B.; Stirk, W.A.; Finnie, J.F.; Van Staden, J. Seasonal variation in the polyamines of *Ecklonia maxima*. *Bot. Mar.* **2012**, *55*, 539–546. [[CrossRef](#)]
70. Aremu, A.O.; Plačková, L.; Gruz, J.; Biba, O.; Novák, O.; Stirk, W.A.; Doležal, K.; Van Staden, J. Seaweed-derived biostimulant (Kelpak®) influences endogenous cytokinins and bioactive compounds in hydroponically grown *Eucomis autumnalis*. *J. Plant Growth Regul.* **2016**, *35*, 151–162. [[CrossRef](#)]
71. Crouch, I.J.; van Staden, J. Commercial seaweed products as biostimulants in horticulture. *J. Home Consum. Hortic.* **1994**, *1*, 19–76. [[CrossRef](#)]
72. Craigie, J.S. Seaweed extract stimuli in plant science and agriculture. *J. Appl. Phycol.* **2011**, *23*, 371–393. [[CrossRef](#)]
73. Stadnik, M.J.; de Freitas, M.B. Algal polysaccharides as source of plant resistance inducers. *Trop. Plant Pathol.* **2014**, *39*, 111–118. [[CrossRef](#)]
74. Depuydt, S.; Hardtke, C.S. Hormone signalling crosstalk in plant growth regulation. *Curr. Biol.* **2011**, *21*, 365–373. [[CrossRef](#)] [[PubMed](#)]
75. Horgan, R. Present and future prospects for cytokinin research. In *Physiology and Biochemistry of Cytokinins in Plants*; Kamínek, M., Mok, D.W.S., Eds.; SPB Academic Publishing: Hague, The Netherlands, 1992; pp. 3–13.
76. Strnad, M. The aromatic cytokinins. *Physiol. Plant* **1997**, *101*, 674–688. [[CrossRef](#)]
77. Sakakibara, H. Cytokinins: Activity, biosynthesis, and translocation. *Ann. Rev. Plant Biol.* **2006**, *57*, 431–449. [[CrossRef](#)] [[PubMed](#)]
78. Santner, A.; Calderon-Villalobos, L.I.A.; Estelle, M. Plant hormones are versatile chemical regulators of plant growth. *Nat. Chem. Biol.* **2009**, *5*, 301–307. [[CrossRef](#)] [[PubMed](#)]
79. Frébort, I.; Kowalska, M.; Hluska, T.; Frébortová, J.; Galuszka, P. Evolution of cytokinin biosynthesis and degradation. *J. Exp. Bot.* **2011**, *62*, 2431–2452. [[CrossRef](#)] [[PubMed](#)]
80. Stirk, W.A.; Van Staden, J. Flow of cytokinins through the environment. *Plant Growth Regul.* **2010**, *62*, 101–116. [[CrossRef](#)]
81. Zalabák, D.; Pospíšilová, H.; Šmehilová, M.; Mrázová, K.; Frébort, I.; Galuszka, P. Genetic engineering of cytokinin metabolism: Prospective way to improve agricultural traits of crop plants. *Biotechnol. Adv.* **2013**, *31*, 97–117. [[CrossRef](#)] [[PubMed](#)]
82. McKersie, B.D.; Leshem, Y.Y. *Stress and Stress Coping in Cultivated Plants*; Kluwer Academic Publishers: Dordrecht, The Netherlands, 1994.
83. Fike, J.H.; Allen, V.G.; Schmidt, R.E.; Zhang, X.; Fontenot, J.P.; Bagley, C.P.; Ivy, R.L.; Evans, R.R.; Coelho, R.W.; Wester, D.B. Tasco-Forage: I. Influence of a seaweed extract on antioxidant activity in tall fescue and in ruminants. *J. Anim. Sci.* **2001**, *79*, 1011–1021. [[CrossRef](#)] [[PubMed](#)]
84. Ervin, E.H.; Zhang, X.; Fike, J. Ultraviolet-B Radiation Damage on Kentucky Bluegrass II: Hormone Supplement Effects. *HortScience* **2004**, *39*, 1471–1474. [[CrossRef](#)]
85. Khan, W.; Rayirath, U.P.; Subramanian, S.; Jithesh, M.N.; Rayorath, P.; Hodges, D.M.; Critchley, A.T.; Craigie, J.S.; Norrie, J.; Prithiviraj, B. Seaweed extracts as biostimulants of plant growth and development. *J. Plant Growth Regul.* **2009**, *28*, 386–399. [[CrossRef](#)]
86. Jennings, R.C. Gibberellins as endogenous growth regulators in green and brown algae. *Planta* **1968**, *80*, 34–42. [[CrossRef](#)]
87. Gopala, R.P. Gibberellin-like behaviour of α -tocopherol in green gram *Vigna radiata*. *Geobios* **1984**, *11*, 21–25.
88. Jensen, A. Tocopherol content of seaweed and seaweed meal. I. Analytical methods and distribution of tocopherols in benthic algae. *J. Sci. Food Agric.* **1969**, *20*, 449–453. [[CrossRef](#)]
89. Mancuso, S.; Azzarello, E.; Mugnai, S.; Briand, X. Marine bioactive substances (IPA extract) improve foliar iron uptake and water tolerance in potted *Vitis vinifera* plants. *Adv. Hort. Sci.* **2006**, *20*, 156–161.

90. Genard, H.; Le Saos, J.; Billard, J.P.; Tremolieres, A.; Boucaud, J. Effect of salinity on lipid composition, glycine betaine content and photosynthetic activity in chloroplasts of *Suaeda maritima*. *Plant Physiol. Biochem.* **1991**, *29*, 421–427.
91. Blunden, G.; Jenkins, T.; Liu, Y. Enhanced leaf chlorophyll levels in plants treated with seaweed extract. *J. Appl. Physiol.* **1997**, *8*, 535–543. [[CrossRef](#)]
92. Naidu, B.P.; Jones, G.P.; Paleg, L.G.; Poljakoff-Mayber, A. Proline analogues in *Melaleuca* species: Response of *Melaleuca lanceolata* and *M. uncinata* to water stress and salinity. *Aust. J. Plant Physiol.* **1987**, *14*, 669–677. [[CrossRef](#)]
93. Wachira, F.; Ogada, J. In vitro regeneration of *Camellia sinensis* (L.) O. Kuntze by somatic embryogenesis. *Plant Cell Rep.* **1995**, *14*, 463–466. [[CrossRef](#)] [[PubMed](#)]
94. Akula, A.; Akula, C.; Bateson, M. Betaine: A novel candidate for rapid induction of somatic embryogenesis in tea (*Camellia sinensis* [L.] O. Kuntze). *Plant Growth Regul.* **2000**, *30*, 241–246. [[CrossRef](#)]
95. Hamana, K.; Matsuzaki, S.; Niitsu, M.; Samejima, K.; Nagashima, H. Polyamines of unicellular thermoacidophilic red alga, *Cyanidium caldarium*. *Phytochemistry* **1990**, *29*, 377–380. [[CrossRef](#)]
96. Rathore, S.S.; Chaudhary, D.R.; Boricha, G.N.; Ghosh, A.; Bhatt, B.P.; Zodape, S.T.; Patolia, J.S. Effect of seaweed extract on the growth, yield and nutrient uptake of soybean (*Glycine max*) under rainfed conditions. *S. Afr. J. Bot.* **2009**, *75*, 351–355. [[CrossRef](#)]
97. Hirsch, R.; Hartung, W.; Gimmler, H. Absciscic acid content of algae under stress. *Bot. Acta* **1989**, *102*, 326–334. [[CrossRef](#)]
98. Boyer, G.L.; Dougherty, S.S. Identification of abscisic acid in the seaweed *Ascophyllum nodosum*. *Phytochemistry* **1988**, *27*, 1521–1522. [[CrossRef](#)]
99. Rensing, S.A.; Lang, D.; Zimmer, A.D.; Terry, A.; Salamov, A.; Shapiro, H.; Nishiyama, T.; Perroud, P.F.; Lindquist, E.A.; Kamisugi, Y.; et al. The *Physcomitrella* genome reveals evolutionary insight into the conquest of land by plants. *Science* **2008**, *319*, 64–69. [[CrossRef](#)] [[PubMed](#)]
100. Verslues, P.E.; Agarwal, M.; Katiyar-Agarwal, S.; Zhu, J.; Zhu, J.K. Methods and concepts in quantifying resistance to drought, salt and freezing, abiotic stresses that affect water plant status. *Plant J.* **2006**, *45*, 523–539. [[CrossRef](#)] [[PubMed](#)]
101. Davies, P.J. *Plant Hormones. Biosynthesis, Signal Transduction, Action!* 3rd ed.; Kluwer Academic Publishers: Dordrecht, The Netherlands, 2004; p. 750.
102. Berlyn, G.P.; Russo, R.O. The use of organic biostimulants to promote root growth. *Belowground Ecol.* **1990**, *2*, 12–13.
103. Blunden, G.; Currie, M.; Mathe, J.; Hohmann, J.; Critchley, A.T. Betaine yields from marine algal species utilized in the preparation of seaweed extracts used in agriculture. *Nat. Prod. Commun.* **2010**, *5*, 581–585. [[PubMed](#)]
104. Craigie, J.; MacKinnon, S.; Walter, J. Liquid seaweed extracts identified using ¹H NMR profiles. *J. Appl. Phycol.* **2008**, *20*, 665–671. [[CrossRef](#)]
105. Rayirath, P.; Benkel, B.; Hodges, D.M.; Allan-Wojtas, P.; MacKinnon, S.; Critchley, A.; Prithiviraj, B. Lipophilic components of the brown seaweed, *Ascophyllum nodosum*, enhance freezing tolerance in *Arabidopsis thaliana*. *Planta* **2009**, *230*, 135–147. [[CrossRef](#)] [[PubMed](#)]
106. Yabur, R.; Bashan, Y.; Hernandez-Carmona, G. Alginate from the macroalgae *Sargassum sinicola* as a novel source for microbial immobilization material in waste water treatment and plant growth promotion. *J. Appl. Phycol.* **2007**, *19*, 43–53. [[CrossRef](#)]
107. Mercier, L.; Laffite, C.; Borderies, G.; Briand, X.; Esquerre-Tugaye, M.T.; Fournier, J. The algal polysaccharide carrageenans can act as an elicitor of plant defence. *New Phytol.* **2011**, *149*, 43–51. [[CrossRef](#)]
108. Nakamura, T.; Nagayama, K.; Uchida, K.; Tanaka, R. Antioxidant activity of phlorotannins isolated from the brown alga *Eisenia bicydis*. *Fish. Sci.* **1996**, *62*, 923–926. [[CrossRef](#)]
109. Wang, T.; Jonsdottir, R.; Olafsdottir, G. Total phenolic compounds, radical scavenging and metal chelation of extracts from Icelandic seaweeds. *Food Chem.* **2009**, *116*, 240–248. [[CrossRef](#)]
110. Andjelkovic, M.; Van Camp, J.; De Meulenaer, B.; Depaemelaere, G.; Socaciu, C.; Verloo, M.; Verhe, R. Iron-chelation properties of phenolic acids bearing catechol and galloyl groups. *Food Chem.* **2006**, *98*, 23–31. [[CrossRef](#)]

111. Rengasamy, K.R.R.; Kulkarni, M.G.; Stirk, W.A.; Van Staden, J. Eckol Improves Growth, Enzyme Activities, and Secondary Metabolite Content in Maize (*Zea mays* cv. Border King). *J. Plant Growth Regul.* **2015**, *34*, 410–416. [[CrossRef](#)]
112. Korasick, D.A.; Enders, T.A.; Strader, L.C. Auxin biosynthesis and storage forms. *J. Exp. Bot.* **2013**, *64*, 2541–2555. [[CrossRef](#)] [[PubMed](#)]
113. Ljung, K. Auxin metabolism and homeostasis during plant development. *Development* **2013**, *140*, 943–950. [[CrossRef](#)] [[PubMed](#)]
114. Peer, W.A.; Murphy, A.S. Flavonoids and auxin transport: Modulators or regulators? *Trends Plant Sci.* **2007**, *12*, 556–563. [[CrossRef](#)] [[PubMed](#)]
115. Wu, H.C.; Du Toit, E.S.; Reinhardt, C.F.; Rimando, A.M.; Van der Kooy, F.; Meyer, J.J.M. The phenolic, 3,4-dihydroxybenzoic acid, is an endogenous regulator of rooting in *Protea cynaroides*. *Plant Growth Regul.* **2007**, *52*, 207–215. [[CrossRef](#)]
116. De Klerk, G.J.; Guan, H.; Huisman, P.; Marinova, S. Effects of phenolic compounds on adventitious root formation and oxidative decarboxylation of applied indoleacetic acid in *Malus 'Jork 9'*. *Plant Growth Regul.* **2011**, *63*, 175–185. [[CrossRef](#)]
117. Teixeira da Silva, J.; Dobranszki, J.; Ross, S. Phloroglucinol in plant tissue culture. *In Vitro Cell. Dev. Biol. Plant* **2013**, *49*, 1–16. [[CrossRef](#)]
118. Gaspar, T.; Kevers, C.; Penel, C.; Greppin, H.; Reid, D.; Thorpe, T. Plant hormones and plant growth regulators in plant tissue culture. *In Vitro Cell. Dev. Biol. Plant* **1996**, *32*, 272–289. [[CrossRef](#)]
119. Wilson, P.J.; Van Staden, J. Rhizocaline, rooting co-factors, and the concept of promoters and inhibitors of adventitious rooting—A review. *Ann. Bot.* **1990**, *66*, 479–490. [[CrossRef](#)]
120. Aremu, A.O.; Nqobile, A.; Kannan, M.; Rengasamy, R.R.; Amoo, S.O.; Gruz, J.; Biba, O.; Šubrtová, M.; Pěnčík, A.; Novák, O.; et al. Physiological role of phenolic biostimulants isolated from Brown seaweed *Ecklonia maxima* on plant growth and development. *Planta* **2015**, *241*, 1313–1324. [[CrossRef](#)] [[PubMed](#)]
121. Chibbar, R.N.; Schyluk, J.; Georges, F.; Mallard, C.S.; Constabel, F. Esterase isozymes as markers of somatic embryogenesis in cultured carrot cells. *J. Plant Physiol.* **1988**, *133*, 367–370. [[CrossRef](#)]
122. Pedersen, S.; Andersen, S.B. Developmental expression of isoenzymes during embryogenesis in barley anther culture. *Plant Sci.* **1993**, *91*, 75–86. [[CrossRef](#)]
123. Krsnik-Rasol, M.; Cipicic, H.; Hagege, D. Isoesterases related to cell differentiation in plant tissue culture. *Chem. Biol. Interact.* **1999**, *120*, 587–592. [[CrossRef](#)]
124. Balen, B.; Krsnik-Rasol, M.; Simeon-Rudolf, V. Isoenzymes of peroxidase and esterase related to morphogenesis in *Mammillaria gracilis* Pfeiff. tissue culture. *J. Plant Physiol.* **2003**, *160*, 1401–1406. [[CrossRef](#)] [[PubMed](#)]
125. Nardi, S.; Carletti, P.; Pizzeghello, D.; Muscolo, A. Biological activities of humic substances, in biophysico-chemical processes involving natural nonliving organic matter in environmental systems. In *Fundamentals and Impact of Mineral-Organic-Biota Interactions on the Formation, Transformation, Turnover, and Storage of Natural Nonliving Organic Matter (NOM)*, 3rd ed.; John Wiley: Hoboken, NJ, USA, 2009; pp. 305–339.
126. Oboh, G.; Puntel, R.L.; Rocha, J.B.T. Hot pepper (*Capsicum annuum*, Tepin and *Capsicum chinense*, Habanero) prevents Fe²⁺-induced lipid peroxidation in brain-*in vitro*. *Food Chem.* **2007**, *102*, 178–185. [[CrossRef](#)]
127. Ertani, A.; Pizzeghello, D.; Francioso, O.; Sambo, P.; Sanchez-Cortes, S.; Nardi, S. *Capsicum chinensis* L. growth and nutraceutical properties are enhanced by biostimulants in a long-term period: Chemical and metabolomic approaches. *Front. Plant Sci.* **2014**, *5*, 375. [[CrossRef](#)] [[PubMed](#)]
128. Złotek, U.; Świeca, M.; Jakubczyk, A. Effect of abiotic elicitation on main health-promoting compounds, antioxidant activity and commercial quality of butter lettuce (*Lactuca sativa* L.). *Food Chem.* **2014**, *148*, 253–260. [[CrossRef](#)] [[PubMed](#)]
129. Karolewski, P.; Jagodziński, A.M. Udział węgla w związkach obronnych przed czynnikami biotycznymi u roślin drzewiastych. *Sylwan* **2013**, *157*, 831–841.
130. Czerwińska, E.; Szparaga, A. Antibacterial and antifungal activity of plant extracts. *Rocznik Ochrona Środowiska* **2015**, *17*, 209–229.
131. Stevens, M.T.; Lindroth, R.L. Induced resistance in the interminate growth of aspen (*Populus tremuloides*). *Oecologia* **2005**, *145*, 298–306. [[CrossRef](#)] [[PubMed](#)]

132. Kocira, S.; Szparaga, A.; Kocira, A.; Czerwińska, E.; Depo, K.; Erlichowska, B.; Deszcz, E. Effect of applying a biostimulant containing seaweed and amino acids on the content of fiber fractions in three soybean cultivars. *Legum. Res.* **2018**. [[CrossRef](#)]
133. Lakhdar, A.; Iannelli, M.A.; Debez, A.; Massacci, A.; Jedidi, N.; Abdelly, C. Effect of municipal solid waste compost and sewage sludge use on wheat (*Triticum durum*): Growth, heavy metal accumulation, and antioxidant activity. *J. Sci. Food Agric.* **2010**, *90*, 965–971. [[CrossRef](#)] [[PubMed](#)]
134. Jindo, K.; Martim, S.A.; Navarro, E.C.; Pérez-Alfocea, F.; Hernandez, T.; Garcia, C.; Aguiar, N.O.; Canellas, L.P. Root growth promoting by humic acids from composted and non-composted urban organic wastes. *Plant Soil* **2012**, *353*, 209–220. [[CrossRef](#)]
135. Gülçin, I. Antioxidant activity of food constituents: An overview. *Arch. Toxicol.* **2012**, *86*, 345–391. [[CrossRef](#)] [[PubMed](#)]
136. Cheynier, V.; Comte, G.; Davies, K.M.; Lattanzio, V.; Martens, S. Plant phenolics: Recent advances on their biosynthesis, genetics, and ecophysiology. *Plant Physiol. Biochem.* **2013**, *72*, 1–20. [[CrossRef](#)] [[PubMed](#)]
137. Pavarini, D.P.; Pavarini, S.P.; Niehues, M.; Lopes, N.P. Exogenous influences on plant secondary metabolite levels. *Anim. Feed Sci. Technol.* **2012**, *176*, 5–16. [[CrossRef](#)]
138. Moyo, M.; Amoo, S.O.; Aremu, A.O.; Gruz, J.; Šubrtová, M.; Doležal, K.; Van Staden, J. Plant regeneration and biochemical accumulation of hydroxybenzoic and hydroxycinnamic acid derivatives in *Hypoxis hemerocallidea* organ and callus cultures. *Plant Sci.* **2014**, *227*, 157–164. [[CrossRef](#)] [[PubMed](#)]
139. Jannin, L.; Arkoun, M.; Etienne, P.; Laîné, P.; Goux, D.; Garnica, M.; Fuentes, M.; San Francisco, S.; Baigorri, R.; Cruz, F.; et al. *Brassica napus* growth is promoted by *Ascophyllum nodosum* (L.) Le Jol. Seaweed extract: microarray analysis and physiological characterization of N, C, and S metabolisms. *J. Plant Growth Regul.* **2013**, *32*, 31–52. [[CrossRef](#)]
140. Rouphael, Y.; Kyriacou, M.C.; Petropoulos, S.A.; De Pascale, S.; Colla, G. Improving vegetable quality in controlled environments. *Sci. Hortic.* **2018**, *234*, 275–289. [[CrossRef](#)]
141. Fan, D.; Hodges, D.M.; Critchley, A.T.; Prithiviraj, B. A commercial extract of brown macroalga (*Ascophyllum nodosum*) affects yield and the nutritional quality of Spinach in vitro. *Commun. Soil Sci. Plant Anal.* **2013**, *44*, 1873–1884. [[CrossRef](#)]
142. Azcona, I.; Pascual, I.; Aguirreolea, J.; Fuentes, M.; García-Mina, J.M.; Sánchez-Díaz, M. Growth and development of pepper are affected by humic substances derived from composted sludge. *J. Plant Nutr. Soil Sci.* **2011**, *174*, 916–924. [[CrossRef](#)]
143. Zhang, D.; Hamazu, Y. Phenolic compounds, ascorbic acid, carotenoids and antioxidant properties of green, red and yellow bell peppers. *J. Food Agric. Environ.* **2003**, *1*, 22–27.
144. Pantelidis, G.E.; Vasilakakis, M.; Manganaris, G.A.; Diamantidis, G.R. Antioxidant capacity, phenol, anthocyanin and ascorbic acid contents in raspberries, blackberries, red currants, gooseberries and cornelian cherries. *Food Chem.* **2007**, *102*, 777–783. [[CrossRef](#)]
145. Świeca, M.; Sęczyk, Ł.; Gawlik-Dziki, U. Elicitation and precursor feeding as tools for the improvement of the phenolic content and antioxidant activity of lentil sprouts. *Food Chem.* **2014**, *161*, 288–295. [[CrossRef](#)] [[PubMed](#)]
146. Złotek, U.; Szychowski, K.A.; Świeca, M. Potential in vitro antioxidant, anti-inflammatory, antidiabetic, and anticancer effect of arachidonic acid-elicited basil leaves. *J. Funct. Foods* **2017**, *36*, 290–299. [[CrossRef](#)]
147. Raina, K.; Rajamanickam, S.; Deep, G.; Singh, M.; Agarwal, R.; Agarwal, C. Chemopreventive effects of oral gallic acid feeding on tumor growth and progression in TRAMP mice. *Mol. Cancer Ther.* **2008**, *7*, 1258–1267. [[CrossRef](#)] [[PubMed](#)]
148. Chen, J.Y.; Wen, P.F.; Kong, W.F.; Pan, Q.H.; Zhan, J.C.; Li, J.M.; Wan, S.B.; Huang, W.D. Effect of salicylic acid on phenylpropanoids and phenylalanine ammonia-lyase in harvested grape berries. *Postharvest Biol. Technol.* **2006**, *40*, 64–72. [[CrossRef](#)]
149. Polo, J.; Mata, P. Evaluation of a biostimulant (Pepton) based in enzymatic hydrolyzed animal protein in comparison to seaweed extracts on root development, vegetative growth, flowering, and yield of gold cherry tomatoes grown under low stress ambient field conditions. *Front. Plant Sci.* **2018**, *8*, 2261. [[CrossRef](#)] [[PubMed](#)]
150. Nair, P.; Saveetha, K.; Zhang, J.; Ji, X.; Chris, K.; Bernhard, B.; Mark, D.H.; Alan, T.C.; David, H.; Balakrishnan, P. Transcriptional and metabolomics analysis of *Ascophyllum nodosum* mediated freezing tolerance in *Arabidopsis thaliana*. *BMC Genom.* **2012**, *13*, 643. [[CrossRef](#)] [[PubMed](#)]

151. Biesaga-Kościelniak, J.; Kościelniak, J.; Filek, M.; Marcińska, I.; Krekule, J.; Machackova, I.; Kuboń, M. The effect of plant growth regulators and their interaction with electric current on winter wheat development. *Acta Physiol. Plant* **2010**, *32*, 987–995. [[CrossRef](#)]
152. Povero, G.; Mejia, J.F.; Tommaso, D.D.; Piaggese, A.; Warrior, P. A systematic approach to discover and characterize natural plant biostimulants. *Front. Plant Sci.* **2016**, *7*, 435. [[CrossRef](#)] [[PubMed](#)]



© 2019 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).