Review

Seaweed Extracts as Bio-stimulants for Rice Plant (*Oryza sativa*) – a Review

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Abstract: Rice is considered as the most important crop for most of the world population. Utilization of seaweed as bio-stimulant can be an alternative way to enhance rice plant growth and productivity, as well as a strategic move to reduce the use of inorganic fertilizer that is harmful to the environment. Seaweed and its derivative products have been widely used as bio-stimulant in the agricultural industry because of their potential use in increasing plant growth and productivity. Auxins, gibberellins, and cytokinin are some of growth regulators found in seaweed extract, as well as macro and micronutrients required for plant growth and development. Several studies have found that seaweed extract has a variety of favorable effects as a plant growth promoter, including early seed germination and establishment, improved nutritional quality, increased yield and crop performance, and increased tolerance to environmental stress. The purpose of this paper is to give a comprehensive overview of the impacts of several seaweed species on seed germination, crop development and production, enhancement of rice plants (*Oryza sativa*) nutritional quality and the modes of action of seaweed extract includes the chemical components that might be causing plant physiological changes.

Keywords: bio-stimulant; seaweed extract; rice; Oryza sativa; plant growth promoter

1. Introduction

Rice (*Oryza sativa*) is the world's second most important crop next to wheat, which is consumed as a staple food, with Asia being the biggest producer and consumer. In major Asian countries, the rice consumption is expected to increase faster than the population growth. By the year 2025, rice consumption will increase more than 51% over the base year 1995 [1]. Asian countries produce almost 95% of the world's rice, and approximately half of the world's population consume it. Rice is the primary source of dietary energy in 17 Asian and Pacific countries, nine North and South American countries, and eight African countries [2].

The increase of food and fiber demand per unit area results in an increase of chemical fertilizer demand for producing more yields. The excessive use of chemical fertilizer will affect public health and cause environmental pollution. Therefore, every change in the agricultural system that leads to an increase in productivity should help to decrease the negative environmental impact and increase the system's sustainability. Promoting organic agricultural practices has become critical and important. One of the approaches to improve the agricultural system is the use of bio-stimulants, which are applied to plants to improve agricultural yields and nutrient efficiency, crop quality traits and abiotic stress tolerance.

Seaweed extracts have become popular as plant bio-stimulants in recent years. The use of seaweed extract in organic farming is one of the safest methods of conserving

natural resources, avoiding pollution, and obtaining food and agricultural products. Seaweed extracts account for more than 33% of the entire bio-stimulant industry and are expected to grow to a value of 894 million Euro by 2022 [3]. In fact, thousands of years have passed since seaweed was used in agriculture. Algae was used to cover plant seedlings in ancient Rome, which helped to promote their growth. Seaweed was put into the soil or utilized as compost by farmers around Europe's coasts [3]. Since 1948, 18 countries have improved their seaweed resources for fertilizers, and Milton had succeeded in producing a liquid seaweed product for use as a fertilizer by 1947 [4].

Seaweed extracts are utilized as liquid fertilizers and bio-stimulants because they contain a variety of macro and micronutrients as well as numerous growth regulators such as cytokinin [5], auxins [6], gibberellins [7], which are essential for plant growth and development. Additionally, seaweed extract aids in the establishment of beneficial soil bacteria [8], developing stress tolerance [9], increasing nutritional quality [4], enhancing antioxidant properties [10], and enhancing post-harvest quality [11]. Seaweed extracts are becoming more popular because of their biodegradable characteristics and potential for use in organic and sustainable agriculture, as an approach to avoid the use of excessive chemical fertilizer and to improve the absorption of minerals [6,12].

There are almost 10,000 species of seaweed, which are divided into four divisions based on pigmentation, surface and interior structure, and reproduction. These major classifications are Phaeophyceae (brown), Rhodophyceae (red), Chlorophyceae (green), and Cyanophyceae (blue) (Blue-green) [8,13,14]. Brown seaweeds (Phaeophyceae), which include *Fucus* sp., *Laminaria* sp., *Sargassum* sp., *Ecklonia* sp., *Durvillaea* sp., and *Turbinaria* sp., are the most widely utilized for agricultural and commercial bio-stimulant production because they can achieve large biomass levels and are widespread [8,15–17]. This paper is divided into eight main sections, including the importance of seaweed extract in agriculture, mechanism, and modes of action of seaweed extract in plants, chemical components in seaweed extracts, and the effects of seaweed extract on seed germination, growth, yield, nutritional properties, and tolerance to environmental stresses of rice plants.

2. Applications of Seaweed Extract in Agriculture

Nowadays, seaweed extracts are already commercialized and widely used in the agricultural industry, for example seaweed liquid fertilizers (SLF) are used as foliar spray, manure, and soil conditioners [18]. In particular, SLF has a particularly suitable content that helps to promote the growth and yield of plants such as macronutrients, organic substances, trace elements and plant growth regulators like cytokinin, auxin and gibberellins. Recently, the liquid extracts from seaweed are used as foliar sprays for many plants including various vegetable species, cereals, grasses, and flowers [6]. Foliar spray helps to promote a faster growth and increase yield of the crops like fruit plants, horticulture crops, cereals, and vegetables [19]. Foliar spray from seaweed extracts is commonly used in agricultural practice to enhance yield of many commercial crops [8]. According to previous studies, spraying seaweed extract on plants as a foliar spray provides a faster way of supplying nutrients to higher plants than conventional soil application methods [14,20]. This could be related to stomatal nutrient uptake being more active than cuticular uptake [21]. The research on the effects of seaweed extracts on rice plants are summarized in Table 1.

Among all the species of brown seaweed, Ascophyllum nodosum is the most used in research on plant biostimulant resources. Brown seaweed liquid extracts are commercialized under numerous brand names as biofertilizers or biostimulants. A variety of commercial seaweed extract products have been available in the agriculture and horticulture industries in recent years. Table 2 lists a variety of commercial seaweed products used in agriculture and horticulture industries.

Table 1. Effects of various seaweed extracts on rice plants.

Seaweed Species	Effects	References	
Sargassum crassifolium, Sargassum cristaefolium, Sargassum aquifolium, and Turbinaria murayana	folium, mineral nutrition absorption. um, and Increased growth and yield of rice plants.		
Hydroclathrus sp.	• 15% of seaweed extract enhanced both growth and yield of riceplants.	[23]	
S. cristafolium	 Application of solid extract of seaweed on the soil media hasshown an increase in rice plants growth (plant height, number of tillers, shootand root dry weight) and yield (grain weight and panicle number per plant). 	[24]	
K. alvarezii and Gracilaria edulis	 5% and above concentrations of seaweed extracts + 100% RDF (Recommended dose of fertilizer) significantly enhanced plant height, chlorophyll index, dry matter accumulation, yield attributes, yield of rice and crop growth rate. 10% concentrations of seaweed extracts + 100% RDF helped to increase micro-nutrient (Fe, Mn, Zn and Cu) and protein concentration in rice grains. 	[25]	
K. alvarezii	 Application of 2.5, 5.0 and 7.5% of K-sap foliar spray along with 100% RDF helped to develop positive nitrogen balance of the system. Enhanced the quality of rice plants. Increased crop yield and better system efficiency. 	[26]	
K. alvarezii	Application of 10% seaweed extract together with 100% RDF resulted in more productive tillers, straw yield and grains yield.	[27]	
S. cristaefolium	 Application of seaweed extract added with combination amino acidhas potential to increase the growth and yield of upland rice. 	[28]	

 Table 1. Cont.

Seaweed Species	Effects	References	
K. alvarezii	Seaweed extract showed higher growth and increased plant height, leaf area index, total tillers, relative growth rate, crop growth rate, dry matter accumulation, net assimilation rate which will result in higher straw and grain yield, benefit, and net return.	[29]	
Sargassum wightii	The use of seaweed extract on rice seeds improved germination percentage, germination speed, shoot length, root length, and seedling fresh and dry weight.	[30]	
T. murayana, S.cristaefo- lium, S.crassifolium, S.aqui- folium, Hydrochlarus sp.	Assisted in the growth and development of the rice-paddy plant.	[31]	

Table 2. The commercial seaweed products that are used in agriculture and horticulture industries as plant growth stimulant.

Product Name	Seaweed Name	Company	Country of Manufacturer
Seaweed Liquid Extract	A. nodosum, Sargassum sp., Laminaria japonica	Conzii Agricultural Technology Co., Ltd.	China
X [®] Seaweed Extract	A. nodosum	X-humate	China
Kelpak [®]	Ecklonia maxima	Kelp Products International	South Africa
Seamac [®]	A. nodosum	Headland Amenity Ltd.	United Kingdom
Stimplex [®]	A. nodosum	Acadian Seaplants	Canada
Maxicrop [®]	A. nodosum	Maxicrop USA, Inc.	United States
Seasol®	A. nodosum	Seasol International Pty Ltd.	Australia
Acadian [®]	A. nodosum	Acadian Seaplants	Canada
AlgaeGreen [®]	A. nodosum	Southern AG Inc.	United States
Super Fifty [®]	A. nodosum	BioAtlantis Ltd.	Ireland
Liquid Kelp	Unspecified	Nutri Grow LC.	Malaysia
Alg-A-Mic	Unspecified	Biobizz Products	Netherlands
Seaweed NPK	Unspecified	Nutri Grow LC.	Malaysia
KelpGreen	A. nodosum	Organic AG Products	United States
Seaweed Extract Liquid	Unspecified	Qingdao Hibong Fertilizer Co. Ltd	China
Terra Aquatica	A. nodosum	GHE	France

Table 2. Cont.

Product Name	Seaweed Name	Company	Country of Manufacturer
Bio-Power V	r V Unspecified	Dongyang Lianfeng Biological Technol-	China
	anopecyteu	ogy Co., Ltd.	
Algino K+	A. nodosum	Baba Mr Ganick	Malaysia
Marinure [®]	A. nodosum	The Glenside	United Kingdom
		Group Limited	Officed Kingdom
AlgaGrow	K. alvarezii	Rhodomaxx	Malaysia
Seaweed Ex	A. nodosum	Ken Microbes Biotech Sdn. Bhd.	Malaysia
Wuxal [®] Ascofol	A. nodosum	Aglukon	Germany
Kelp Meal	A. nodosum	Espoma	United States
Nitrozyme [®]	Unspecified	Growth Technology Ltd.	United Kingdom
YaraVita Croplift Pro	A. nodosum	Yara UK Ltd.	United Kingdom

3. Mechanism and Modes of Action of Seaweed Extracts in Promoting Plant Growth

The products of seaweed show growth-stimulating activities. The chemical components in seaweed such as microelement and macroelement nutrients, vitamins, amino acids, auxins, cytokinin and abscisic acid (ABA)-like growth substances influence the cellular metabolism in treated plants which results in the enhancement of crop growth and yield [8,32,33]. Different types of seaweed show different physiological responses in promoting plant growth as each species exhibits growth-stimulating activities based on the bioactive compounds present in the individual seaweed extracts.

The active compound of seaweed extract is effective in a very low concentration [32,34]. The existing evidence from previous studies have shown that chemical components in the seaweed extract demonstrate synergistic activity in enhancing plants growth performance, although numerous of the chemical components of seaweed extract and their modes of action remain undiscovered [8,35]. Table 3 summarizes different modes of action of different species of seaweed extracts in stimulating plant growth.

Seaweed extract treatment in plants can be applied in a few ways including foliar spray, seed treatment, added in a soil in liquid or solid form, soil drenching and added to hydroponic solution [36–39]. Figure 1 provides a schematic illustration of the physiological responses evoked by seaweed extracts on plants.

The positive effects of seaweed extracts, such as plant development and stress reduction, are due to the seaweed extracts' stimulatory potentials, which activate critical signaling pathways and initiate a series of physiological changes in plants. Plants treated with seaweed extracts demonstrated enhanced nutrient acquisition capabilities as well as increased growth and vigor. For example, the application of A. nodosum extract on B. napus plants exhibited an increase in nitrogen and sulfur acquisitions [40,41]. Transcription studies revealed that this was caused by an overexpression of the BnNRT1.1/BnNRT2.1 and BnSultr4.1/BnSultr4.2 genes, which encode root transporters involved in nitrate and sulphate uptake, respectively [3,42,43].

Furthermore, seaweed extract treatments have been shown to enhance plant biomass and other essential bioactive molecular concentrations, such as phenolics and flavonoids. Previous study showed that the application of A. nodosum extract on spinach resulted in the enhancement of biomass, chlorophyll and carotenoid content, protein content, phenolics and flavonoids, and increased antioxidant activity. The rise in biomass was associated with an increase in the expression of the GS1 gene, which is involved in nitrogen integration.

Table 3. Different modes of action of different seaweed extract species in stimulating plant development

Types of Seaweed Extract	Types of Crops	Modes of Action	Reference
Laminaria sp. and A. nodosum	Maize (Zea mays)	 Promote the traits root morphology Phytohormones modulation Stimulate root elongation Accumulation of glucose Nutrient uptake Higher esterase enzyme activity 	[44]
K. alvarezii & G. edulis	Wheat (Triticum aestivum)	 Phytohormones modulation Nutrient uptake Enhance growth, yield, and quality of grains 	[20]
Sargassum sp.	Chilli (Capsicum annum) & To- mato (Lycopersi- con esculentum)	 Promote hypocotyl and root growth Increase seedling developments Vegetative growth Growth, yield, and quality of fruits 	[45]
A. nodosum	Rapeseed (Brassica napus)	 Phytohormones modulation N, C and S metabolism Increase in the number of chloroplasts 	[41]
Ulva lactuca & Gracilaria den- droides	Sunflower plant (Helianthus an- nuus)	 Growth and yield of plants Nutrient uptake Increase chlorophyll and carotenoids	[46]
S. wightii, Turbinaria ornata & Caulerpa racemosa	Holy Basil (Ocimum sanctum)	 Phytohormones modulation Increase in chlorophyll synthesis Nutrient uptake Growth and yield of plant 	[47]

An increase in chlorophyll content was linked to the increased expression of choline monooxygenase and betaine aldehyde dehydrogenase. While the increase of phenolic and flavonoid compounds was related to the upregulation of ascorbate peroxidase (APX), monodehydroascorbate reductase and glutathione reductase. These enzymes were discovered to be connected to the flavonoid and phenylpropanoid pathways, which are known to promote growth and improve overall nutrition [40,42,48].

In addition, seaweed extracts also help plants survive extreme climatic conditions including salinity, drought and cold. For example, under salinity conditions, the application of A. nodosum on Arabidopsis thaliana revealed the upregulation of 184 and 257 genes on the 1st and 5th days, respectively, and downregulation of 91 and 262 genes on day one and five, respectively. Abiotic stress-related genes accounted for 2.2% of all upregulated genes on day 1 and rose to 6% on day 5 [3,49]. Furthermore, the weekly application of an A. nodosum extract to asparagus under salinity conditions significantly increased the upregulation of aquaporin and water management related genes (ANN1, ANN2, and PIP1), as well as P5CS1 and CHS, two biologically active molecule metabolism-related genes [50].

Moreover, the treatment of A. nodosum extracts on Arabidopsis protected the plants from induced cold stress by increasing chlorophyll content, which could be attributed to the downregulation of chlorophyll degradation genes (AtCLH1 and AtCLH2). Additionally, the transcription factor DREB1A and the COR78/RD29A genes, which encode cryoprotection of chloroplast stromal protein and are essential regulators of cold stress tolerance, were shown to be upregulated [42,51]. In addition, the enhancement of unsaturated fatty acid, soluble sugar and proline content was also related to increased tolerance to cold stress. The increase in soluble sugars was associated to the upregulation of carbohydrate

biosynthesis genes (GOLS2 and GOLS3), the overexpression of polysaccharide degradation genes (9SEX1 and SEX4), and the downregulation of sucrose degradation genes.

While the accumulation of proline was aided by the overexpression of the proline biosynthesis genes (5CS1 and P5CS2), as well as the downregulation of ProDH, a gene involved in proline breakdown. Furthermore, the same study also observed the upregulation of gene DGD1, which is involved in the production of galactolipid, which is known to have a role in cold stress tolerance [52].

Under drought condition, the application of Gracilaria dura extract on wheat showed an alteration of wheat ABA homeostasis by the upregulation of several NCED (9-cisepoxycarotenoid dioxygenase) genes such as TaNCED3.1 and TaNCED3.2. This was related with a significant increase in ABA content as compared to the control [53]. Moreover, after the application of K. alvarezii sap, the transcript expression of an abiotic stress-responsive transcription factor TaWRKY10, the ROS scavenging genes TdCAT, TdSOD, and a stress signalling cascade gene WCK-1 of wheat plants under drought conditions was increased. As a result, the authors hypothesised that the production of phytohormones after the treatment of K sap might be the cause of the overexpression of genes and transcription factors under stress conditions [54]. The seaweed extract applications also improved the photochemistry of the photosystem II (PS-II) and enhanced non-photochemical quenching. Synergistic upregulation of antioxidant coding genes aids in the protection of PS-II againstoxidative damage during drought stress [40,55].

Besides, the involvement of GmCYP707A1a and GmCYP707A3b (engaged in ABA catabolism) was detected in the soy plants under drought stress that were treated with seaweed extracts. In these plants, there was an increase observed in the transcription of GmDREB1B (ABA inducible) and GmRD22 (encoding the BURP domain protein), as well as ROS scavenging genes such as GmGST, GmBIP, and GmTP55 [56].

The application of seaweed extracts also reduced numerous biotic stresses in plants. The primary metabolites in seaweed extracts activate plant defense pathways by causing an oxidative burst and channeling several phytohormonal signaling molecules such as salicylic acid (SA), jasmonic acid (JA), and ethylene (ET). These pathways drive downstream defense-related players, such as various pathogenesis-related (PR) protein classes. Furthermore, seaweed extracts can activate important secondary metabolites in plants by upregulating biosynthetic enzymes including chalcone synthase (CHS), phenylalanine ammonia lyase (PAL), and isoflavone reductase (IFR). Seaweed polysaccharides such as laminarins, carrageenans, and ulvans, as well as other derived oligosaccharides, induced protective reactions and conferred resistance towards pathogens in the treated plants [40,57].

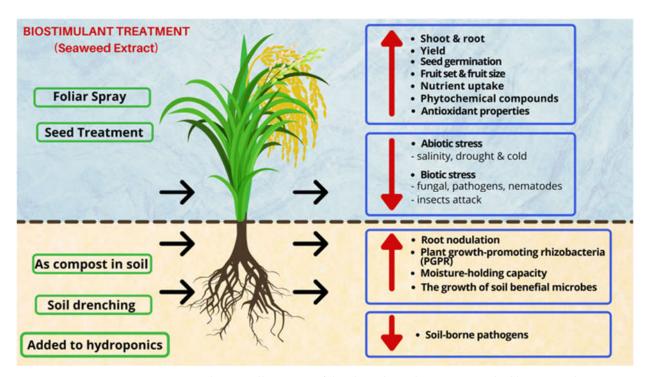


Figure 1. Schematic illustration of the physiological responses evoked by seaweed extract treatments on plants [8,13,39].

4. Chemical Components of Seaweed Extract that Stimulate Plant Growth

The potential of seaweed extract as a plant growth stimulant depends on the chemical components present in the extract. The presence of chemical compositions in seaweed extract is highly dependent on the extraction methods and the chemicals used during the production process. Therefore, the same seaweed species that are extracted by different methods will exhibit different biological activity on the treated plants [13,34]. The variety of seaweed-based biostimulants consist of a diverse range of organic and inorganic components that directly enhance plant growth and defense mechanisms. Figure 2 shows some chemical contents in seaweed extract and their roles in plant growth.

4.1. Polysaccharide

Polysaccharide is one of the major chemical components that present in seaweed extract. Seaweed contains a variety of polysaccharide, which the chemical structure is dependent on the species and class of the seaweed. For example, the common polysaccharides that present in brown seaweed extract (A. nodosum, Saccharina longicruris, and Fucus vesiculosus) includes fucoidan, alginate and laminaran (Figure 3) [8,58]. Fucoidan is a sulphated polysaccharide that is contained in the cell walls of brown seaweed. It is well known for its ability in antibacterial and antiviral actions [58]. Previous study has suggested that fucoidan may play a role in cell wall architecture and help in promoting plants desiccation tolerance [59-60].

Alginate can help in improving soil conditions by promoting the aggregates formation between soil particles, which results in the increase of the translocation and absorption of nutrients, microbial activity of soil and root growth [61]. Alginate is also responsible for activating some symbiotic fungi growth in the rhizosphere [58,62]. It has been reported that alginate derived oligosaccharides enhanced root growth of carrot and rice plants, and increased seed germination of maize plants [61,63,64]. While laminaran plays a role in stimulating genes to produce special proteins that participate in microbial activity [58]. This component can modulate the chloroplasts antioxidant system in abiotic stress situations [61].

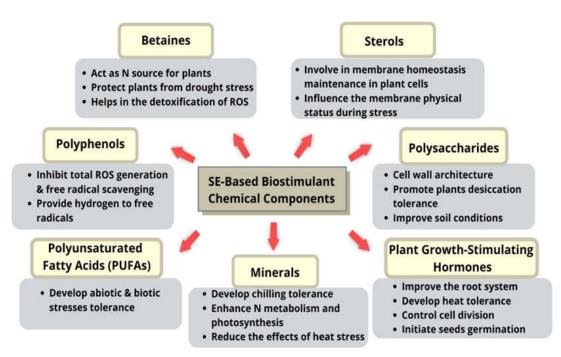
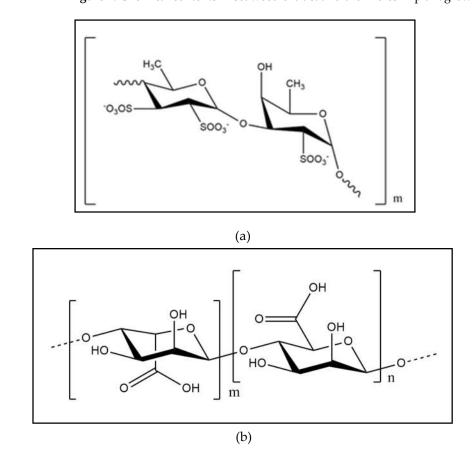


Figure 2. Chemical contents in seaweed extract and their roles in plant growth.



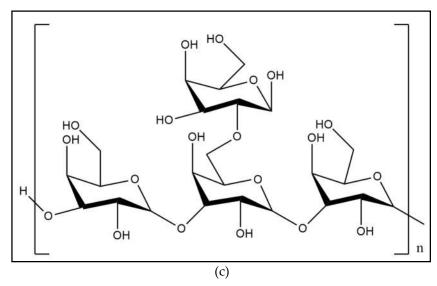


Figure 3. The chemical structures of fucoidan (a), alginate (b), and laminarin (c)

4.2. Plant Growth-Stimulating Hormones

Plant growth hormones, which may be found in seaweed extract, are bioactive chemicals that control plant growth and production. The activity of phytohormones determines the physiological effects of seaweed extract on treated plants [13]. Phytohormones affect plant growth at low concentrations and show inhibitory effects at higher concentrations [4]. It has been observed that the presence of many types of plant growth hormones regulates a wide variety of growth responses in plants treated with seaweed extracts [8,65]. Some phytohormones that are detected in seaweed extract include auxins, cytokinins and gibberellins (Figure 4) [8,42,66].

Auxin is of the plant growth-promoting hormones that present in seaweed extract, which plays a role in improving the root system of a plant by enhancing root elongation and formation [8,67]. The concentration of auxin in seaweed is different and highly dependent on the species. Auxins involved in promoting lateral root primordia initiation, formed lateral roots development and primary roots elongation [68,69]. It also has been reported that auxin can help in regulating plant defense [70].

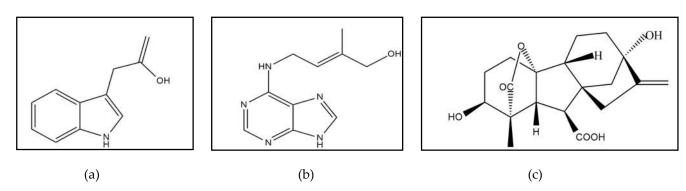


Figure 4. The chemical structures of auxin (a), cytokinin (b), and gibberellin (c).

4.3. Minerals

Seaweed is one of marine species that is rich in minerals due to the bioaccumulation of metal ions found in the sea water and concentrates the accumulated components as carbonate salts [71]. Seaweed, especially brown and green seaweeds, have been shown to be a significant source of essential nutrients for plant development in previous studies [72]. The common macronutrients that present in seaweed extracts include calcium (Ca), magnesium (Mg), phosphorus (P), sodium (Na), potassium (K) and sulphur (S). While

copper (Cu), zinc (Zn), boron (B), manganese (Mn), iron (Fe), cobalt (Co), and molybdate (Mo) were among the micronutrients found in the extract [42].

All mineral nutrients in seaweed extract play a different role in promoting the growth and productivity of treated plants as well as developing environmental stress tolerance of the plant. Ca, for example, helps in the regulation of plant development, response, and environmental stress at various levels. Ca also involved in developing the chilling tolerance of a plant through a stomatal closure [73]. Mg is involved in various physiological and biochemical processes, which facilitate the plant growth and development. Mg also helps in increasing the surface area and growth of roots to improve the photosynthetic rate by maintaining the structure of chloroplast under heat stress [73,74]. P is an essential nutrient for the regulation of protein activity in plants and development of roots in highly weathered tropical soils [75,76].

Na is not essential for most plants, but it can be beneficial and nutritious in other species. It has been reported that many (glycophytic) plants give positive response to Na fertilization, during K deficiency [77]. While K plays an essential role in the development and growth of plants, including the root system. K helps in activating many enzymes in plants, and it is also necessary for photosynthesis, protein synthesis, and transportation of photosynthate. S is also an important nutrient for the activation of enzymes in plants. S is required for the chlorophyll, nitrogenase, and vitamins formations, and it is also involved in the reactions of oxidation-reduction [76].

While Mn is involved in enhancing the nitrogen (N) metabolism and photosynthesis to reduce the consequences of heat stress on plants. Besides, Mn also shows antioxidative effects in the tissue of plants [74,78]. Cu is a redox-active elements which plays important roles in respiration, photosynthesis, N and C metabolisms, and protection against oxidative stress [75]. While Zn helps in maintaining the membrane turgidity, to enhance the defense system of plants against heat stress [74]. Fe is an essential nutrient for maintaining the structure and function of chloroplast, and it is also involved in chlorophyll synthesis [79].

In plants, B plays important roles for the growth of pollen tube and pollen germination [76]. Co also plays a critical role in the overall plant growth process. Co is required for the growth of stem, coleoptiles elongation, and expanding leaf discs. It is necessary for a plant to reach maturity and for the development of healthy bud [80]. Mo is also important for plants, as some of enzymes in plants use it to catalyze most essential reactions in the acclimatization of N, phytohormone synthesis, purine degradation, and sulfite detoxification [81].

4.4. Polyphenols

Polyphenol is a secondary metabolite synthesized by plants including marine algae. It is produced under stress for the protection of cells and cellular components [13]. Among the seaweed species, brown seaweed has the highest polyphenol content [58,82]. Polyphenols play essential roles in scavenging radicals like superoxide, hydroxyl, single oxygen, and antioxidant activity [83].

It has been reported that polyphenols have strong antioxidant properties due to their unique structure [84]. One of the unique types of phenolic compounds discovered in seaweed extracts is phlorotannin. It is a complex polymer consisting of phloroglucinol as its structural unit, which is involved in the inhibition of total ROS generation and free radical scavenging [13,85]. ROS is produced in organisms as a metabolism integral part. It is very reactive and can lead to cytotoxicity and cellular dysfunction. In this condition, polyphenols can provide hydrogen to free radicals and create non-reactive radicals [58,86].

4.5. Polyunsaturated Fatty Acids (PUFAs)

Polyunsaturated fatty acids (PUFAs) are the other essential bioactive compounds that present in seaweed extract. Due to their metabolic connections, PUFAs may be divided into two families: α -linolenic acid (n-3 fatty acid) and linoleic acid (n-6 fatty acid) [87].

PUFAs are involved in developing environmental stress tolerance. They act as general defenders for plants against various abiotic and biotic stresses including infection of pathogens, cold, drought and salinity stresses [88]. For example, the accumulation of PUFAs is induced by the seaweed when a decrease in temperature occurs, as a reaction to develop cold stress tolerance. Hence, PUFAs content in the species that lives in cold areas is higher than the species that live in a high environmental temperature [58,87].

4.6. Betaines

Betaine is an unconventional plant hormone that also presents in seaweed species. Previous study has shown that seaweed extract, particularly brown seaweed extract contains a variety of betaines [8]. Types of betaines that were detected in the extract of brown seaweed (A. nodosum) include γ-amino butyric acid betaine, glycine betaine, laminine and δ-aminovaleric acid betaine (Figure 5) [89]. Betaines are N-methylated compounds which act as nitrogen source for plants, when applied in low concentration and work as an osmolyte at higher temperature. They are also involved in protecting plants from drought stress, glycine betaine which is an osmoprotectant compound will accumulate in plants under the drought stress [8,90].

Furthermore, it has been observed that glycine betaine helps in the detoxification of reactive oxygen species (ROS), allowing photosynthesis to recover and reducing oxidative damage. Glycine betaine alleviates the oxidative stress damaging effects by the stabilization or activation of ROS-scavenging enzymes and/or the repression of ROS production by other mechanisms [91]. This compound also helps in increasing the content of chlorophyll in leaves by reducing its degradation, hence results in the enhancement of plant yield [8,58].

Figure 5. The chemical structures γ -amino butyric acid betaine (a), glycine betaine (b), laminine (c), and δ -aminovaleric acid betaine (d).

4.7. Sterols

Sterols are known as an important group of lipids. Seaweed is a rich source of sterols which are involved in numerous processes in plant metabolism, ranging from the development and growth regulations to the environmental stress tolerance [92]. Some sterol

compounds that can be found in seaweed extract include stigmasterol, campesterol, b-sitosterol, cholesterol and 24-methylenecholesterol [8,93]. Previous studies have reported that fucosterol and its derivatives are the dominant sterol present in brown seaweed [94]. The variety of sterols and their derivatives may influence sessile plants adaptation to environmental stresses [92].

Sterols are necessary for the control of cell membranes [95-96]. They are involved in membrane homeostasis maintenance in plant cells, in which they are expected to contribute to developing plant stress tolerances, particularly for abiotic stresses such as cold, heat, radiation of UV, drought and salinity [92]. Sterols can influence the membrane physical status during the stress conditions occur, by changing the total content of sterols and the profile variations, specifically the composition ratios of sterols molecular species such as stigmasterol and sitosterol [92,97].

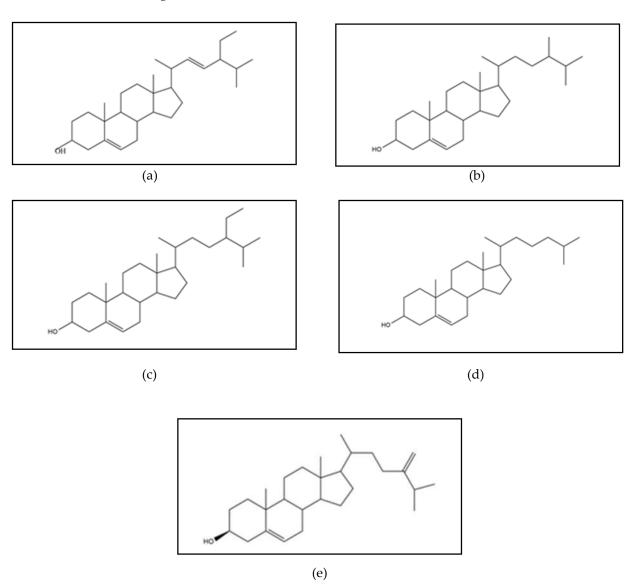


Figure 6. The chemical structures of stigmasterol (a), campesterol (b), b-sitosterol (c), cholesterol (d), and 24-methylenecholesterol (e).

5. Effect of Seaweed Extract on the Seeds Germination of Rice Plant

Seed germination is an essential stage in the rice life cycle and seed damage is easy to occur by salinity stress due to a low defense system during the stage [98]. Earlier studies have found that seaweed extracts are very effective in enhancing plant germination and seedling vigour. It has been reported that the seed germination is concentration

dependent, most studies have shown that seaweed extract work effectively at lower concentration and they showed inhibitory effect at higher concentration [99–103].

Application of 10% Seaweed Liquid Extract (SLE) of Caulerpa racemosa on O. sativa significantly increased the percentage of germination by 6.5% over the control, while higher concentration of SLE (75%) significantly reduced the germination rate [104]. Besides, it has been reported that the application of 0.01% of Seaweed Liquid Fertilizer (SLF) of low molecular weight Grateloupia filicina polysaccharide on rice seeds recorded the increase in germination potential by 26.67% and germination index by 14.27% [105]. Previous work also recorded that the treatments of 2.5 and 5.0% of K. alvarezii and G. edulis saps on the rice seeds, showed maximum germination percentages and seedling vigour index as compared to control. While the extracts at higher concentrations (7.5% and above) showed inhibitory effect on the percentage of germination and seedling vigour index [25]. Moreover, the rice seeds that are treated with 1% of S. wightii seaweed extract, recorded higher germination speed (34.69) and germination percentage (91%) as compared to the untreated seeds [30].

The presence of growth-promoting substances such as gibberellins (A & B), cytokinins, indole-3-butyric acid (IBA) and indole-3-acetic acid (IAA), amino acids, vitamins, micronutrients (Zn, Fe, Cu, Mn, Ni, Mo, Co), and phenyl acetic acid (PAA) may explain the increase in germination rate and seedling vigour at low concentrations of seaweed liquid extract [103,106-107]. While the rate of germination was inhibited at higher doses, this might be due to the high concentration of salt in the seaweed extracts. Salt stress leads to the inhibition of seed germination, growth of seedling and metabolic processes of plants [108,109]. The retardation of plant growth at higher concentration of seaweed extract may be due to the excess of hormones or minerals [6,106]. However, it has been reported in a previous study that the seed germination of plants was found maximum at the highest concentration of Enteromorpha intestinalis seaweed liquid fertilizer in the study (60%) [110].

6. Effect of Seaweed Extract on the Growth of Rice Plant

Many researchers have reported the benefit of seaweed extract application on plants growth like wheat (Triticum aestivum L.) [20], sesame [111], brinjal [102], tomato (Solanum lycopersicum) [112], soybean (Glycine max) [38], and black gram (Vigna mungo L.) [113]. Seaweed liquid extract (SLE) was found to be very effective in increasing plant growth at lower concentration. It has been reported that the application of 5.0% K. alvarezii and G. edulis saps recorded an increase in the rate of crop growth of rice plants as compared to the water spray. While the maximum height is observed on the rice plant that is treated with 15% G. edulis sap [25].

Besides, the foliar application of 10% K. alvarezii sap recorded a significant increase in plant height, total tillers, leaf area index, an accumulation of dry matter, crop growth rate, crop relative growth rate and a net assimilation rate of rice plants [29]. Moreover, previous work has reported that the rice plants that were treated with S. cristaefolium seaweed extract added with some combinations of amino acids, recorded a significant increase in dry and fresh weight of roots, total chlorophyll, tillers number and productive tillers number [28]. Next, the foliar applications of S. cristaefolium, S. crassifolium, S. aquifolium, and Hydrochlarus sp. seaweed extracts showed an enhancement in tillage number, spikelet number and seeds number of rice plants [31]. It also has been reported that the application of 10% K. alvarezii sap + RDF, showed an increase in plant height (103.33 cm) and tillers number per hill (10.09 tillers/hill) of rice plants. While the highest value of paniclelength was observed on the rice plants that were treated with 10 % G. edulis sap + RDF (27.41 cm) [114].

Increases in plant growth may be owing to the availability of natural growth-promoting chemicals in seaweed extracts, such as macro and micronutrients, auxins, betaines, gibberellins, and cytokinins, which serve to promote the photosynthetic process, hence increasing plant vegetative growth of the plants [103,115]. In a previous study, seaweed

extract treatment improved not just crop growth but also the number of functional nodules as compared to a control group. This is because of the presence of cytokinins that are contained in brown seaweed extracts, including trans-Zeatin-riboside and their dihydro derivatives [6]. Furthermore, it was shown that the presence of bioactive chemicals in A. nodosum (brown seaweed) extract altered the legume-rhizobia signaling processes in plants [116].

7. Effect of Seaweed Extract on the Yield of Rice Plant

Previous research has shown the potential of organic seaweed extract to replace the use of synthetic chemical fertilizer for sustainable agriculture. The application of seaweed liquid extracts to a variety of crops resulted in a substantial increase in production, including Thompson seedless grape [117], brinjal [102], potato [118], sesame [111], rice [25], wheat [20], and cluster bean [119]. It has been reported that the foliar applications of 5% G. edulis sap + RDF and 5% K. alvarezii sap + RDF seaweed extracts have significantly increased the rice grain yield by 10.3% and 13.8%, respectively as compared to water spray + RDF. These studies have shown that crop yield is highly concentration dependent [120].

Moreover, a recent study has reported that the combination treatment of 10% K. alvarezii sap + RDF is the optimum concentration of seaweed sap which showed the maximum value of rice grains yield (6484.7 kg/ha) and straw yield (6376.8 kg/ha) of paddy crop. This study also showed that even a lower level of seaweed extracts, 2.5% G. edulis sap + RDF and 2.5% K. alvarezii sap have increased the value of rice grains yield by 25.33% and 18.15%, respectively, as compared to the control treatment. This proves the potential of both seaweed extracts (K. alvarezii and G. edulis) as biostimulants for improving rice productivity [114]. Similar results were also reported for the applications of S. cristaefolium, S. crassifolium, S. aquifolium, and Hydrochlarus sp. seaweed extracts on paddy plants, which showed an increase of seed number per spikelet of the crop [31].

Next, another study also showed that the application of 15% K. alvarezii sap obtained the highest value of rice yield, as compared to the control treatment. However, the G. edulis sap only showed an increase in rice yield at the concentration up to 10%. The treatment of 15% K. alvarezii sap for year 2012 and 2013 has recorded an increase in rice yield, including the number of panicles (12.9/hill) and (12.9/hill), effective grains (134.8/panicle) and (153.0/panicle), grain yield (5.36 t/ha) and (5.07 t/ha), straw yield (7.34 t/ha) and (7.26 t/ha), respectively, as compared to G. edulis sap and control treatments [25].

Like the growth and germination of crops, in which the enhancement in plant yield may be caused by the presence of plant growth promoting constituents in seaweed extracts such as vitamins, amino acids, IAA and IBA, cytokinins, gibberellins, and microelements in the seaweed extract [25,106,121–123].

8. Effect of Seaweed Extract on the Nutritional Properties of Rice Plant

The application of 15% K. alvarezii (K sap) and G. edulis (G sap) extracts has increased the protein content in rice grain. The application of seaweed extract also showed an increase in micro-nutrient content like copper (Cu), zinc (Zn), iron (Fe) and manganese (Mn) in rice grains. The concentration of Cu and Zn are increased up to 10% while the concentration of Fe and Mn are increased up to 5% [25]. According to previous studies, the use of seaweed extract on rice plants has recorded the maximum potassium (K) (27.12 kg/ha) and nitrogen (N) uptakes (54.12 kg/ha) of rice grain, and K (163.62 kg/ha), N (37.33 kg/ha) and P (4.78 kg/ha) uptakes of rice straw. While the highest total nutrients uptake of rice plants that were recorded include total K (190.74 kg/ha), N (91.45 kg/ha) and P uptake (12.52 kg/ha). The chelating compounds (i.e., mannitol in seaweeds) that are contained in seaweed extract may help to increase the availability of some micronutrients to crops [27,124].

Moreover, the previous study in rice plants (O. sativa) also showed a significant increase in nutrient uptake of P (12.06 kg/ha), N (89 kg/ha) and K (185.4 kg/ha) with the foliar application of A. nodosum seaweed extract as compared to the control [125].

Besides, the foliar application of K. alvarezii sap also increased the nutrients content in rice grain as compared to the treatment of G. edulis sap. The total nutrient content increased with the increase of seaweed saps concentrations up to 10%. The highest total nutrient content of rice grains that were recorded in the study are K (59.22 kg/ha), N (77.18 kg/ha), S (10.40 kg/ha) and P (16.97 kg/ha) [27]. Similar outcomes were also recorded on the rice plants that were treated with the combined application of 15% K. alvarezii sap + 75% RDF, which showed the highest nutrient uptake by grain and straw of rice plants, as compared to the application of 15% G. edulis sap + 75% RDF. The maximum value of nutrient uptake by grain and straw of rice that were recorded in the study, are N (64.23 kg/ha) and (49.12 kg/ha), K (65.67 kg/ha) and (209.22 kg/ha), P (13.68 kg/ha) and (17.05 kg/ha), respectively [121].

Seaweed extract contains marine bioactive chemicals that help to improve stomata uptake efficiency in treated crops as compared to untreated crops [126]. The presence of plant growth-promoting chemicals such as gibberellins, cytokinins, minerals, vitamins, and amino acids may explain the beneficial effects of seaweed extract on plants [42,58,119].

9. Effect of Seaweed Extract on Rice Plant's Tolerance to Environmental Stress

Rice (O. sativa) is a salt-sensitive crop that can be severely harmed by salt stress during the germination stage [105]. Its productivity could be seriously affected by the soluble salt accumulation in soil. It is easy for a salt-sensitive plant to be damaged by soil soluble salt, which is one of the leading causes of low productivity [127]. Salinity stress, which is mainly driven by sodium (Na⁺) and chloride (Cl⁻) ions, has an impact on rice growth and development by causing ionic, osmotic, and oxidative stresses [128,129]. Hence, protecting rice seeds from salt damage is essential for better development and higher yield.

Salinity stress has a number of growth-inhibiting effects on rice plants, including reduction of net carbon dioxide absorption rates, leaf development, enlargement of leaf cell, accumulation of dry matter, and relative growth [128,130,131]. When a high concentration of salt enters a rice plant, it will eventually increase the toxicity level in adult leaves, causing early senescence of leaves and reducing the photosynthetic leaf area of rice to a point where growth can no longer be sustained [128,132]. Salt stress induced panicle sterility in several rice cultivars, notably during pollination and fertilization phases, due to genetic processes and nutritional shortages caused by the salinity impact [133].

Moreover, salinity stress reduced rice development, caused metabolic disorder, and decreased the plant's ability to absorb water and nutrients [128,134]. Furthermore, salinity stress can lead to the reduction of rice grain yield. The primary reason for the decrease of grain yield under salt stress is a lack of carbohydrate transition to vegetative growth and spikelet development. Hence, results in poor rice spikelet growth, particularly inferior spikelets, and significantly lowered rice grain yield [128,135].

Salinity stress in soil may also lead to nutrient imbalances in rice plants. High concentration of Na⁺ ion in plants damages the cell membrane and organelles, resulting in a reduction in plant physiological systems and plant cell death [136]. These physiological disorders in plants include membrane disruption, inability of ROS detoxification, decreased photosynthetic rate, and antioxidant enzyme transformations [137]. The excessive amount of ROS from oxidative stress will lead to the damage of routine functions of numerous plant cellular components such as nucleic acids, proteins, and lipids, and interrupt membrane permeability [105,128].

Polysaccharide, which is made up of polymeric carbohydrate molecules with long monosaccharide units, is a key component of seaweed, and research has shown that it can boost seed germination, production, and disease resistance in a variety of plants [105,138]. The use of seaweed polysaccharide improved plant salinity stress tolerance by increasing the quantity of osmotic adjustment substances, antioxidant activity, and anti-salt gene expression [105,139]. The number of sulfated polysaccharides in halophyte was lowered when salinity was reduced in a prior study, indicating that polysaccharides played a role in plant salt stress tolerance [140]. It was revealed that seaweed polysaccharide may

protect plants from salinity stress, suggesting that plant salt resistance might be enhanced by polysaccharide contained in Grateloupia filicina. This study proved that polysaccharides from G. filicina could improve rice anti-salt resistance in the germination stage by increasing germination index, germination potential, length of root or shoot, and vigor index [105]. Figure 7 shows the effects of seaweed extract treatment on plants under salinity stress.

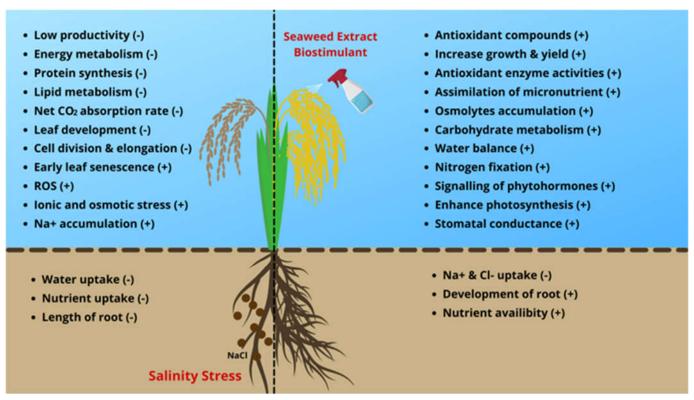


Figure 7. The effects of seaweed extract treatment of plants under salinity stress [105,128,130].

10. Conclusion and Future Prospectives

As shown in the current review, it is obvious that seaweed and its products are a promising option to enhance the growth, yield, and quality in rice plants (O. sativa). Seaweed extracts are made from a variety of starting materials and using a variety of methods and have been linked to a number of positive effects on plants, including increased crop growth and yield, increased nutrient uptake, improved product quality, and tolerance to biotic and abiotic stresses. Therefore, seaweed extract is highly recommended to be applied on plants due to their potential as a natural growth promoting agent for sustainable agriculture, and it is also environmentally friendly as compared to synthetic chemical fertilizers. However, more efforts are needed to improve the quality and potential of seaweed extracts for the better use of seaweed as a bioresource. The following suggestions would be useful to produce a highly potential biostimulant from seaweed resource:

- i. Study the best stage of the crop growth where the seaweed extracts should be applied to get the greatest benefits. The frequency and timing of the foliar application and the particular rate also must be considered to get a desired result. This would help to evaluate the seaweed application on net returns of investment. For example, how much the value of the plants increases for the seaweed application cost invested in every unit.
- ii. Find out the best combination of different sources of seaweed extract at different concentrations for synergistic benefits. This is because not all types of seaweed extract show the same effects on plants, even the same raw materials of seaweed that are processed by different extraction methods result in the different characteristics of

- seaweed extracts. Hence, combining different seaweed extracts might be a useful way for producing seaweed-based biostimulants with more advanced characteristics.
- iii. Find out the time duration for the effects of seaweed extract persist after being applied to the plants. The study of the longevity of the seaweed extract physiological effects must be carried out to help in the scheduling of the extract application frequency. Based on previous studies, most crop species showed different reactions to the extract, depending on the concentration level and the frequency of the applied extracts. Thus, more research on the effect of seaweed extract on specific crops is needed to optimize the utilization of seaweed extract in agricultural practice. Currently, most products of seaweed extracts in the market are extracts of the whole seaweeds [13]. It would be more interesting to undergo the study of the physiological effects on the specific chemical components of the seaweed extract to produce and develop the next generation of seaweed products with specific activities of plant biostimulants.

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