SEAWEED LIQUID EXTRACT (SLE) PRIMING EFFECT ON GERMINATION AND GROWTH OF RICE, WHEAT, BLACK GRAM, GREEN GRAM, BENGAL GRAM & WATERMELON SEEDS

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Abstract

Seaweed Liquid Extracts (SLE) are the organic fertilizers widely used for improvement of the growth and productivity in plants. Five seaweed species were handpicked from the coastal areas of Visakhapatnam. They were thoroughly washed and prepared to make seaweed liquid extracts of different concentrations. Out of interest, seeds of six plants i.e. Wheat (*Triticum aestivum*), Rice (*Oryza sativa*), Black gram (*Vigna mungo*), Green gram (*Vigna radiata*), Bengal gram (*Cicer arietinum*), Watermelon (*Citrullus lanatus*) were selected and treated with the SLE of five seaweed species i.e. *Ulva intestinalia, Ulva lactuca*, *Gracilaria edulis, Audouinella.sp*.(black beard algae), *Jania rubens(*slender-beaded coral weed) and checked for various morphological and growth parameters. The data were analyzed and tabulated statistically using mean ± standard deviation and the comparisons were evaluated by ANOVA. All measurements were made in triplicate. By this study we conclude that, the seeds soaked in 20% seaweed liquid extracts showed better results in germination and growth, than the controls. Liquid extract of *Ulva lactuca* was reported to show more effect on seed germination, and on a whole among the five seaweed extracts, *Ulva intestinalia, Ulva lactuca*, *Gracilaria edulis* showed better effects, than *Jania rubens*, *Audouinella.sp.*

Keywords: Seaweed extract, Liquid fertilizer, Priming, Algae, Phytochemicals, Rice.

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1. Introduction:

The use of chemical fertilizers in agriculture for enhancing the yield has become a threat to the agricultural environment in today's scenario. These chemicals which are not taken up by the plants will accumulate in the soil in the form of salts and other harmful elements leading to an alteration in the pH of the soil and destroys the growth of favourable soil microbiome, therefore, cause poisoning of the soil, or leaching into nearby lakes and rivers which may pollute the water bodies and causes harm. They may also lead to plant damage when they come in contact with the leaves of the plant, causing leaf scorching. These chemical fertilizers may not show instant results on the growth of crops but destroys soil fertility. To avoid such problems many organic fertilizers are practiced in agriculture. Organic agricultural production is to provide good quality food without damaging the environment and maintaining soil fertility through optimal use of resources. Organic matter is a key for maintaining fertility in the soil-plant system [1], one among such organic fertilizers is seaweed fertilizers which are the extracts from the seaweeds. Seaweed extract has been utilized either directly or by mixing with soil as compost which increases soil fertility and crop production [2], [3]. Unlike chemical fertilizers, seaweed fertilizers are biodegradable, non-polluting, non-toxic, non-hazardous to the environment.

Seaweeds are macroalgae that occupy various ecological niches, they are attached to rocks, seafloor, and some are attached to substrate several meters deep, and some float freely. These seaweeds are used as manure in agricultural fields, as a feed for cattle. Macroalgae and microalgae have been applied as manure and which has good soil conditioning agents. The benefits of these macroalgae towards the growth and yields of plant crops were traditionally attributed to the supply of essential nutrients and improved soil texture and water holding capacity [4], [5], [6]. These seaweeds are composed of 80-90% of water and their dry weight basis contains 50% carbohydrates, 1-3% lipids, and 7-38% minerals. Seaweed extracts act as an organic fertilizer with high nutrient value, they also contain plant growth hormones i.e., auxins, cytokinins, gibberellins [7], [8], micronutrients i.e., vitamins (A, C, E, K), minerals (sodium, zinc, magnesium, calcium, etc) and macronutrients i.e., carbohydrates, protein, lipids, etc which enables proper intake of nutrients by plants, subsequently which leads to harvesting higher yields [9], [10], [11]. Seaweed contains electro-active compounds i.e., 3-hydroxyphenyl acetic acid, gibberellin, lysine, abscisic acid, indole-3-acetic acid, and zeatin. The dominant components of the seaweed fertilizers were lysine and gibberellin [12]. Seaweed grows in seawater, it contains not only the nutrients of terrestrial plants but also many trace elements such as iodine, potassium, magnesium, manganese, and titanium as well as polysaccharides [13], [14]. Seaweeds were the novel source of antioxidants, plant hormones, osmoprotectants, plant nutrients, and other novel bioactive metabolites of pharmaceutical and industrial applications [15]. Chlorophyll content in the plant significantly increased during early application of seaweed and lower concentrations of the seaweed liquid fertilizers promote the chlorophyll content. These seaweed extracts help in establishing a robust root system; enhance their absorption and utilization of soil nutrients, water, and gases; increase stem vascular cells and accelerate the transport of water, nutrients, and photosynthetic organic [16], [17]. In addition, alginic acid reduces soil compaction and delays salinization [18], [19]. Seaweed extracts enhance plant growth and quality and improve the resistance to climatic changes because they are rich in fatty acids, minerals, and polysaccharides [20]. Organic and inorganic fertilizers may play an important role in sustained soil fertility and crop productivity [21], [22]. The Metabolites from seaweeds are reported to protect plants against abiotic and biotic stresses and they also contain active biomolecules with antifungal, antiviral, antibacterial, and antiprotozoal activities. Polysaccharide or oligosaccharide extracted from seaweeds induces plant defense mechanism and offers promising protection strategy [23]. Seaweed treatment increases the activity of chalcone isomerase which is the key enzyme of flavanone precursors biosynthesis and induced the plant defense component phenylpropanoid [24], [25].

Gracilaria edulis is a Rhodophyta i.e., red algae, it has economic importance for agar production. The agar obtained from G. edulis has been reported to have a gel strength of 490 g cm2 with 8% alkali treatment [26]. Gracilaria contains 10-47% protein which can increase organic compounds to stimulate plant growth. It contains phytohormones, including auxins, cytokinins, and gibberellin [27]. It also possesses several biomedical properties such as antibacterial, antiviral, anti-fungal, antiprotozoal, anti-tumor, anti-inflammatory, anti-oxidant, cytotoxic, cardiovascular, hypoglycemia, anti-enzymes, spasmolytic, and allelopathic effects [28], [29]. Due to its rich mineral concentration, it's also used as fertilizer in agriculture [30], has reported a 37.8 % improvement in the yield of blackgram. FT-IR analysis of soluble polysaccharides revealed the presence of galactans, 3,6-anhydro-L-galactopyranose, sulfated galactose, and the gelling agent agar, with the sulfate content estimated as 51.01µg/mg of polysaccharide. Plant growth hormone is found in Gracilaria sp. including 144 ppm auxin, 1,552 ppm gibberellin, and cytokinins consisting of kinetin 65 ppm and zeatin 81 [31]. Results of physicochemical properties and nutritional profile reveal the presence of dietary fiber (8.9 ± 0.62 % DW), carbohydrate (101.61 ± 1.80 mg/g DW), crude protein (6.68 ± 0.94 mg/g DW), and lipid content (8.3 ± 1.03 mg/g DW). G. edulis contains biologically important fatty acids like palmitic acid (2.06%), linolenic acid (2.56%), and oleic acid (1.98%). The other nutritional components present in high amounts are proline, chlorophyll a & b, all the essential amino acids, and vitamin A, E, and C [32]. The combination of seaweed *Gracilaria* sp. through a fermentation process which is accelerated by bio-activators from Trichoderma sp. and *Azospirillium* sp. *Trichoderma* sp. is a group of fungi that acts as a decomposer of organic matter as well as controlling pests and plant diseases [33]. *Audouinella* belongs to the division Rhodophyta, also known as *black brush algae*, *Audouinella* thrives on dissolved phosphate and nitrates and they are tolerant of high levels of pollution, acidity [34]. Jania rubens, also known as corallina rubens, belong to rhodophyta. Jania rubens contains 4mg/g of nitrogen, 3.5mg/g of phosphorus, 1.6mg/g of potassium [35]. Lipid contents in Jania rubens is 1.88±0.26%, carbohydrate content is 30%, protein 9.9%, ash content 44% [36]. Laurencia obtusa + Jania rubens caused a 48.21% increase in plant length, 61.84% increase in the potassium content, and increase in leaves number in comparison to other treatments [35]. *Ulva intestinalia* is a Chlorophyta which contains of Zn (1.5± 0.2 mg/ 100g), Cu (0.9± 0.3 mg/ 100g), Mg (3098±1 157.2 mg/ 100g), K (2538.6 ±320.3 mg/ 100g), Na (1064.5 ±489.1 mg/ 100g) [37]. This genus of seaweed is widely used for animal feeds, fertilizers, and human foods [38]. *Ulva lactuca* is a Chlorophyta which contains 174.02 mg/g of nitrogen, 45.56 mg/g of phosphorus, 75.83 mg/g of potassium [39], 4.65± 0.41mg/100g of Fe, 1.87± 0.07 mg/100g of Zn, <0.55 mg/100g of Cu, 560± 4.85 mg/100g of Mg, 6026± 22.2 mg/100g of K, 3901± 71.6 mg/100g of Na [40]. Influence of the SLE of *K.alvareziion* species on the yield of rice crop (ADT53) was concluded that the application of seaweed extract at a ratio of 12.5 kg/ha along with the foliar spraying of SLE at a percentage of 0.5 twice at tillering and panicle initiation stage had higher crop growth parameters and also 18-20 % of yield growth was also reported [46]. The present study was aimed to investigate the effect of seaweeds fertilizers on seed germination and growth and development in plants.

2. Materials and Methods

Collection of Seaweeds:

The seaweeds used in the present study were *Gracilaria edulis, Audouinella.sp.(black beard algae), Jania rubens(*slender-beaded coral weed), which belong to class Florideophyceae and *Ulva intestinalia, Ulva lactuca* which belongs to class Ulvophyceae. They were collected from the coastal area of Visakhapatnam, Andhra Pradesh, India (17°44'N 83°21'E) and (17°46'N 83°23'E) during July 2022. The seaweeds were collected by handpicking and washed thoroughly with marine water to remove sand, dust particles, and epiphytes. Those were carefully packed in polythene bags and carried to the workplace. Those were washed under freshwater thoroughly to remove salts and other remained impurities. Those samples were placed on a blotting paper to remove excess water. The samples were dried completely under shade and stored.

Preparation of Seaweed Liquid Fertilizer:

Seaweeds of 100g were chopped into pieces and boiled in 100ml of distilled water. The extracts were allowed to cool, followed by filtering using whatman No: 1 Paper. This prepared extract was considered as 100 % concentration of seaweed fertilizers [41]. Required concentrations (10%,20%,30%, etc) were prepared by using distilled water. These extracts were stored at 0-4°C to avoid spoilage. Effective seed germination was found to be at 20% seaweed liquid fertilizer with or without chemical fertilizer [42]. Lower concentration (20%) of SLE enhanced the percentage of seed germination, growth, and yield [43]. Here 20% of the extract was prepared and used for the experiment.

Selection of the Seeds

We have chosen six diverse varieties of seeds. Namely, Rice (*Oryza sativa*) and Wheat (*Triticum aestivum*), belonging to the Poaceae family, Black gram (*Vigna mungo*), Green gram (*Vigna radiata*), Bengal gram (*Cicer arietinum*), belongs to Fabaceae family, Watermelon (*Citrullus lanatus*) belonging to Cucurbitaceae family. Viable seeds of uniform size and weight were selected and stored in airtight metal tin container [44].

Experimental Design

The viable seeds (i.e., 30/variety) of 6 varieties were taken and divided into 2 batches, each containing 15 seeds. Out of 30, 15 were used for control and the other 15 for each seaweed extract test sample. The batch of control seeds was soaked in water for 24 days and the other batch of seeds were soaked in seaweed extracts. The seeds were sowed in a container [11x8cms] filled with garden soil. Fifteen seeds were sowed in 1.5 cms depth of the container. Control batches were foliar sprayed with water and other batches with 20% seaweed extract for every 3 days. The samples were studied on the 10th day after sowing to evaluate the growth parameters like germination percentage, shoot length; root length, the number of leaves, and number of lateral roots.

Statistical Analysis

The data were analyzed statistically using mean ± standard deviation and the comparisons were evaluated by ANOVA. All measurements were made in triplicate.

3. Results

The effect of seaweed extracts on germination and growth in Bengal gram (*Cicer arietinum*) the highest shoot length is 29.05 cms observed in *Gracilaria edulis*, germination is 86.66 % and root length is 12.53 cms which are observed in *Ulva lactuca*, no of lateral roots is 17 observed in *Gracilaria edulis* which is presented in table-1.

Table-1: - Effect of seaweed liquid fertilizers on Bengal gram (*Cicer arietinum*)

Growth parameters	**Control**	***Ulva lactuca* 20%**	***Ulva intestinalis* 20%**	***Gracilaria edulis* 20%**	***Jania rubens* 20%**	***Audouinella .sp.* 20%**
Germination (%)	73.33±0.00	86.66±0.00	80.00±0.00	80.00±0.00	80.00±0.00	73.3±0.00
Shoot length(cm)	24.38±0.62	25.01±0.67	27.03±0.54	29.05±0.75	27.43±1.32	26.25±0.36
Root length(cm)	8.97±0.70	12.53±0.89	9.46±0.70	10.02±0.39	10.62±0.74	9.61±0.19
No of leaves	60	70	70	70	70	70
No of lateral roots	12	16	15	17	16	14

In Wheat, the highest shoot & root lengths of 16.40 cms & 8.72 cms were observed respectively under the treatment with *Jania rubens*, germination of 93.33% and the number of lateral roots of 5 were observed in *Ulva lactuca*, which is presented in table-2.

Table-2: Effect of seaweed liquid fertilizers on Wheat (*Triticum aestivum*)

Growth parameters	**Control**	***Ulva lactuca* 20%**	***Ulva intestinalis* 20%**	***Gracilaria edulis* 20%**	***Jania rubens* 20%**	***Audouinella .sp.* 20%**
Germination (%)	71.03±3.85	93.33±0.00	86.66±0.00	86.66±0.00	80.00±0.00	80.00±0.00
Shoot length(cm)	10.40±0.97	14.22±0.78	11.41±1.22	13.34±0.40	16.40±0.92	14.25±0.18
Root length(cm)	7.72±0.10	8.10±0.53	8.02±0.40	8.33±0.24	8.72±0.42	7.74±0.14
No of leaves	2	2	2	2	2	2
No of lateral roots	3	5	4	4	5	4

In Rice, the highest shoot length of 20.92 cms, germination of 91.05% and a root length of 9.41cms were observed under the treatment with *Ulva intestinalis,* and 16 lateral roots were observed under the treatment with *Gracilaria edulis*, which is shown in table-3.

Table-3: - Effect of seaweed liquid fertilizers on Rice (*Oryza sativa*)

Growth parameters	**Control**	***Ulva lactuca* 20%**	***Ulva intestinalis* 20%**	***Gracillaria edulis* 20%**	***Jania rubens* 20%**	***Audouinella. sp.* 20%**
Germination (%)	75.48±3.85	84.38±3.84	91.05±3.85	77.71±3.85	82.16±3.94	80.00±0.00
Shoot length(cm)	17.15±0.91	20.83±0.22	20.92±1.99	19.35±0.70	20.48 ±0.90	20.35±1.16
Root length(cm)	5.24 ± 0.69	9.08 ± 0.60	9.41± 0.52	6.25 ± 0.12	6.41 ± 0.11	7.33 ±0.86
No of leaves	2	2	2	2	2	2

No of lateral roots	13	15	14	16	15	15

In Green gram, it was reported that the highest shoot length is 18.66 cms, the number of lateral roots are 9 observed under the treatment with *Ulva intestinalis,* root length of 7.48 cms, and 97.72% germination were observed in *Ulva lactuca,* which is presented in table -4, and was also reported in Figure B.

Table – 4: -Effect of seaweed liquid fertilizers on Green gram (*Vigna radiata*)

Growth parameters	**Control**	***Ulva lactuca* 20%**	***Ulva intestinalis* 20%**	***Gracilaria edulis* 20%**	***Jania rubens* 20%**	***Audouinella. sp.* 20%**
Germination (%)	82.16±3.84	97.72±3.85	86.66±0.00	84.38±3.84	91.10±3.85	82.16±3.84
Shoot length(cm)	15.27±0.94	18.47±0.17	18.66±0.35	17.10±0.72	17.26 ±0.75	17.44 ±0.55
Root length(cm)	6.61 ± 0.69	7.48 ± 0.36	6.68 ± 0.55	7.31 ± 0.12	7.32 ± 0.04	7.27 ±0.28
No of leaves	2	2	2	2	2	2
No of lateral roots	4	6	9	8	8	8

In Black gram the highest shoot length is 25.52 cms was observed under the treatment with *Ulva intestinalis*, germination of 88.82% and root length of 9.29 cms were observed under the treatment with *Ulva lactuca*, the number of lateral roots is 13 which was observed in both *Ulva lactuca* and *Jania rubens.* which is presented in table-5 and also the shown in Figure- A.

Table-5: - Effect of seaweed liquid fertilizers on Black gram (*Vigna mungo*)

Growth parameters	**Control**	***Ulva lactuca* 20%**	***Ulva intestinalis* 20%**	***Gracilaria edulis* 20%**	***Jania rubens* 20%**	***Audouinella. sp.* 20%**
Germination (%)	68.81±3.85	88.82±3.85	86.66±0.00	77.71±3.85	77.71±3.85	73.33±0.00
Shoot length(cm)	17.14±1.49	24.46±1.03	25.52±0.66	20.81±0.52	18.57±0.30	21.08±1.29
Root length(cm)	4.71±0.37	9.29± 0.55	8.45± 0.33	6.95 ± 0.37	8.49± 0.08	8.15±0.14
No of leaves	2	2	2	2	2	2
No of lateral roots	9	13	10	12	13	12

In Watermelon the highest shoot length of 24.75 cms was observed under the treatment with Gracilaria edulis, 93.33% germination was observed under the treatment with *Ulva lactuca,* a root length of 8.45 cms, and number of lateral roots of 10 were observed under the treatment with *Ulva intestinalis,* which is presented in table-6.

Table-6: - Effect of seaweed liquid fertilizers on Watermelon (*Citrullus lanatus*)

Growth parameters	**Control**	***Ulva lactuca* 20%**	***Ulva intestinalis* 20%**	***Gracilaria edulis* 20%**	***Jania rubens* 20%**	***Audouinella. sp.* 20%**
Germination (%)	79.24±1.28	91.05±3.85	86.66±0.00	86.66±0.00	82.16±3.84	80.00±0.00

Shoot length(cm)	16.98±0.68a	22.69±1.12	22.23±0.58	24.75±0.08	23.86 ±0.13	22.47 ±0.33
Root length(cm)	6.52±0.90	7.09±0.07	8.45±0.40	7.80±0.43	7.42±0.25	7.42±0.30
No of leaves	2	2	2	2	2	2
No of lateral roots	6	8	10	9	7	8

On a whole various parameters like rate of germination, Lengths of roots and shoots and Number of lateral roots were calculated and the results were presented graphically as follows.

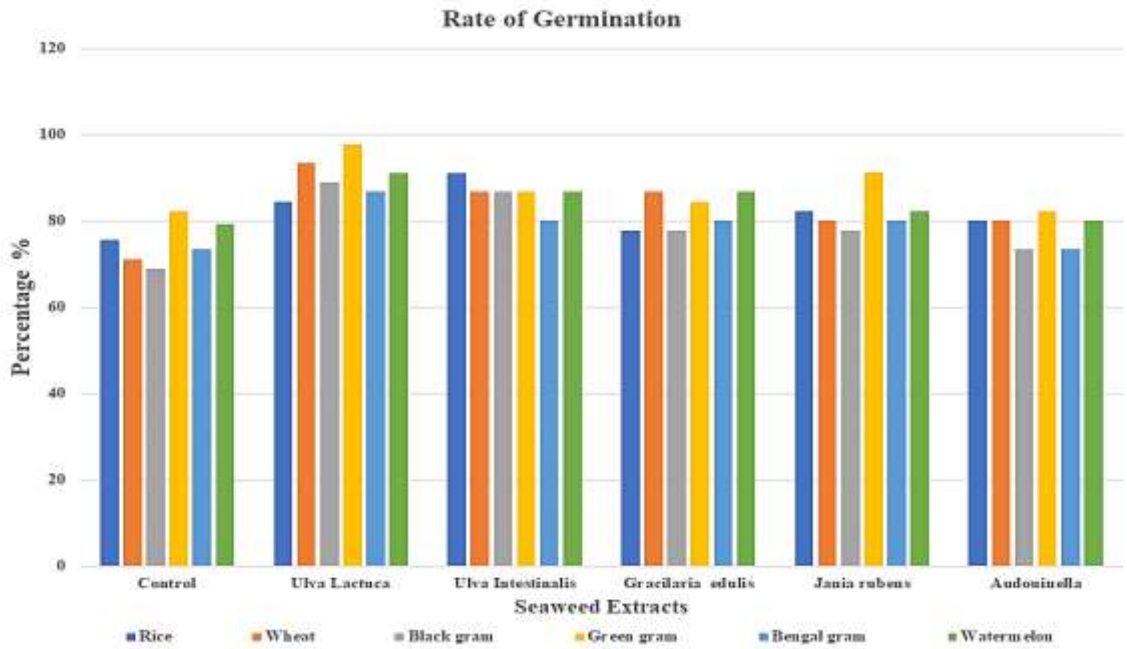

Figure-1: Percentage of rate of germination in the seeds of rice, wheat, black gram, green gram, Bengal gram and watermelon.

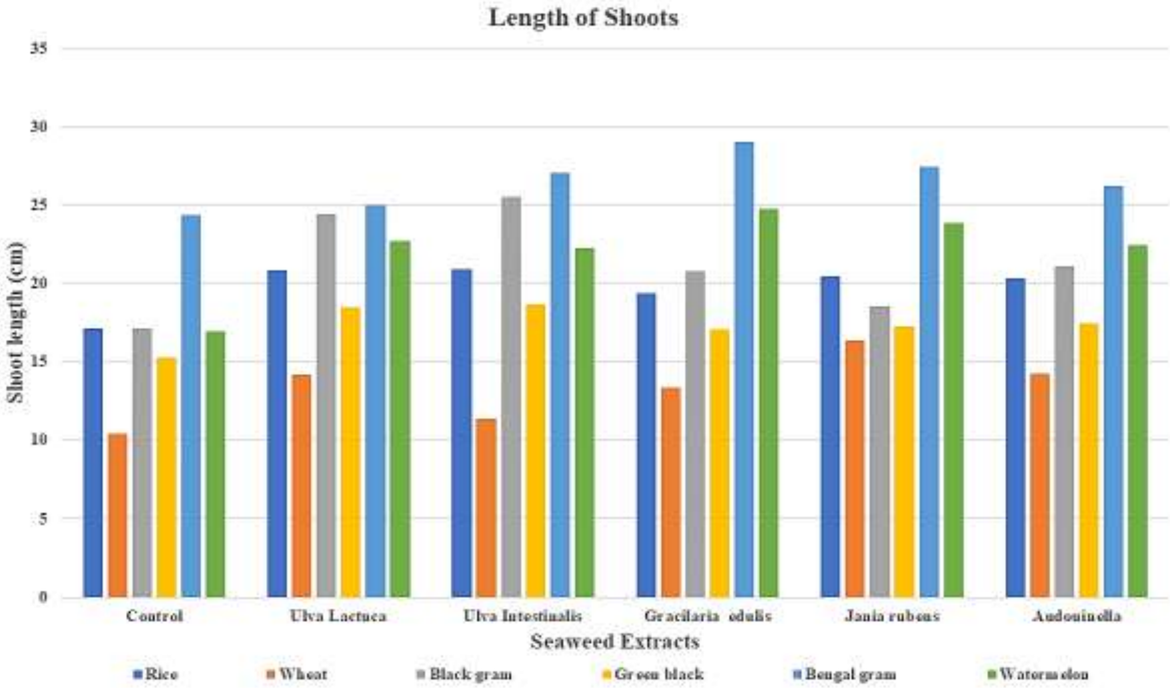

Figure-2: Shoot Length (in cm) in the germinated seeds of rice, wheat, black gram, green gram, Bengal gram and watermelon.

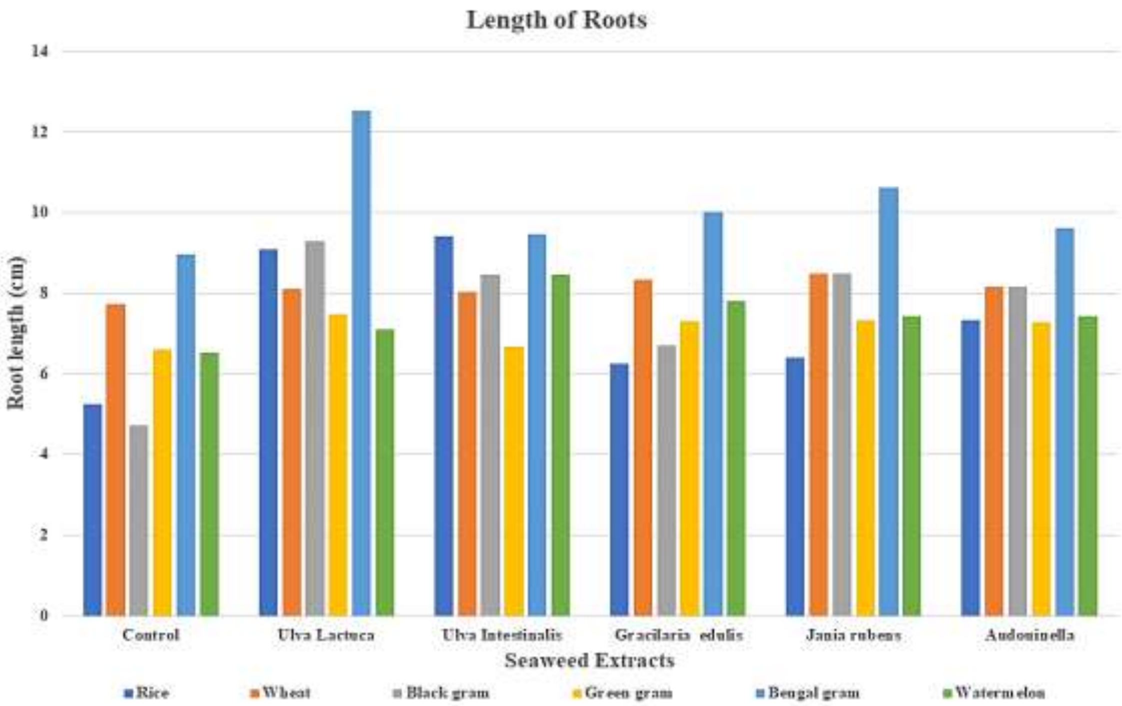

Figure-3: Root Length (in cm) in the germinated seeds of rice, wheat, black gram, green gram, Bengal gram and watermelon.

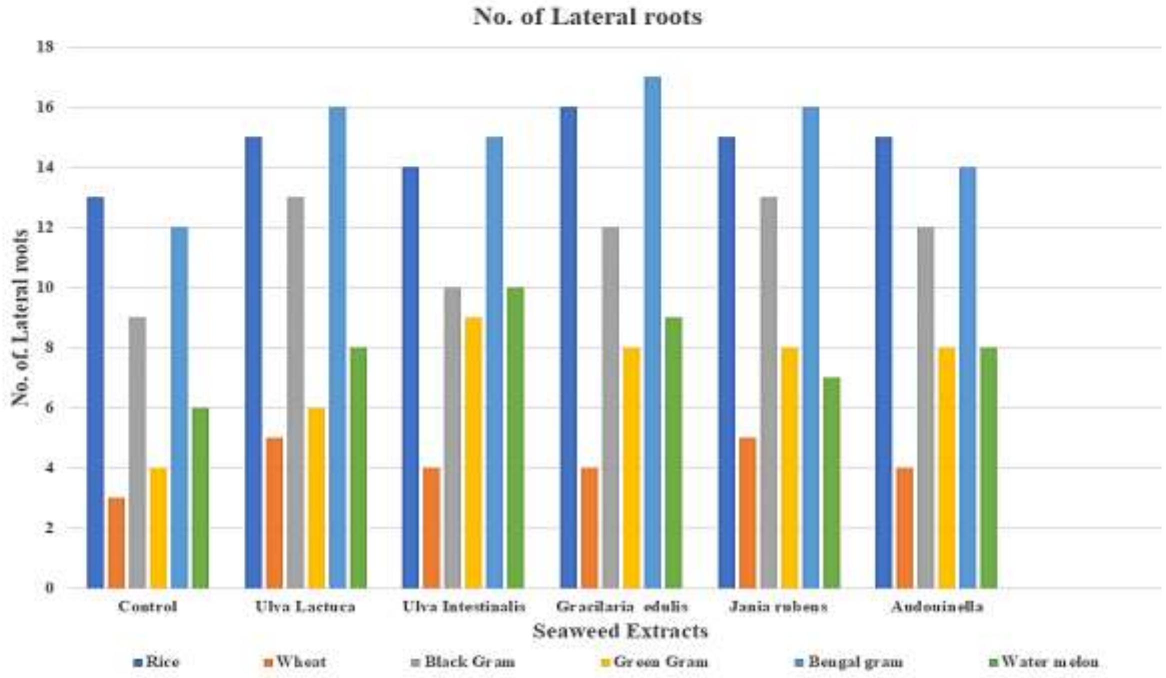

Figure-4: Number of lateral roots in the germinated seeds of rice, wheat, black gram, green gram, Bengal gram and watermelon.

Figure-5: The effect of seaweeds extract on Black gram (*Vigna mungo*) the highest shoot length is 25.52 cms is observed in *Ulva intestinalis,* germination is 88.82% and root length 9.29 cms is observed in *Ulva lactuca*, number of lateral roots is 13 which is observed in both *Ulva lactuca* and *Jania rubens.* Here, the control is treated with water and T1:20%Ulva lactuca,T2:20%Ulva intestinalis, T3:20%Gracilaria edulis, T4:20% Jania rubens, T5:20%Audouinella.sp.

Figure-6: The effect of seaweeds extract on green gram (*Vigna radiata*) the highest shoot length is 18.66 cms, number of lateral roots is 9 observed in *Ulva intestinalis*, root length is 7.48 cms and germination 97.72% is observed in *Ulva lactuca*. Here the control is treated with water and T1:20%Ulva lactuca, T2:20%Ulva intestinalis, T3:20%Gracilaria edulis, T4:20% Jania rubens, T5:20%Audouinella.sp.

4. Conclusion:

Application of organic seaweed fertilizers in agricultural fields, can increase the yield without using chemical fertilizers. Generally, different seaweeds have different production of growth regulators like Abscisic acid in (Ascophyllum, Laminaria), Auxins in (Ascophyllum, Fucus, Laminaria, Macrocystis, Undaria). Cytokinins in (Ascophyllum, Cystoseira, Ecklonia, Fucus, Macrocystis, Sargassum) [45]. Among the five different seaweed extracts *Ulva lactuca*, *Ulva intestinalia*, and *Gracilaria edulis* yielded better results when compared with others, but when compared with controls all the seaweed extracts showed better results. *Ulva lactuca* and *Ulva intestinalia* showed effective germination, *Ulva intestinalia*, and *Gracilaria edulis*,*Jania rubens* showed effective results in shoot growth and the increasing number of lateral roots of the plants. *Ulva lactuca*, *Ulva intestinalia*, and *Jania rubens* showed effective results in root growth. Slight growth is observed in the number of leaves in all plants when compared with controls. The present study shows that all five seaweed liquid fertilizers of *Ulva lactuca, Ulva intestinalia, Gracilaria edulis, Jania rubens,* and *Audouinella.* showed promising results, but *Ulva lactuca*, *Ulva intestinalia*, *Gracilaria edulis* showed effective germination and growth in plants.

Declarations:

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Conflict of Interest: The authors declare that they have no conflicts of interest.

Author Contributions: TSRSS has designed the study, and contributed in writing the manuscript and polished the manuscript along with reviewing. MS has contributed equally in the design of study and preparation of the manuscript, developing the idea and in the experimentation. VJ & KP were contributed in field work for sample collection. SG reviewed overall manuscript preparation and supported the study. All authors read and approved the final manuscript.

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