Advances in Bioresearch
Adv. Biores., Special Issue (1) 2024: 153-159
©2024 Society of Education, India
Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585. SPL1.153159

Advances in Bioresearch

Exploring *Sargassum tenerrimum* J. Agardh and *Sargassum swartzii* C Agardh.extracts and their potential effect in enhancing rate of germination, proteins and anthocyanins in red amaranth microgreens

Siddhi Tawade*, Sunil Shankhadarwar
Department of Botany, Ramnarain Ruia Autonomous College, Matunga(E), Mumbai – 400019, Maharashtra, India.
*Email id: -siddhitawade19@gmail.com

ABSTRACT
The present investigation aims to study the effect of Sargassum tenerrimum J. Agardh and Sargassum swartzii C Agardh.extracts in enhancing rate of germination, proteins and anthocyanins in red amaranth microgreens. The seaweed extracts were prepared by hot extraction method, different concentrations such as 0.2%,0.4%,0.6%,0.8% and 1% were used. The highest rate of germination was observed in 1% S. swartzii extract. All concentrations also showed increase in shoot length as compared to control NPK and distilled water. The protein content was highest in red amaranth microgreens sprayed with 0.8% of S. swartzii extract and highest anthocyanin content in microgreens sprayed with 1% S. swartzii extract followed by 1% S. tenerrimum extract. Estimation of minerals byICP-AES methodshowed presence of major mineral like K, Mg, Na, Ca and Zn, Cu, Fe and Ni were present in minor quantities in both extracts. LCMS analysis confirmed presence of D-Tryptophan a precursor of phytohormone auxin in S. swartzii extracts and trans-Zeatin-O-glucoside ribosidea cytokinin present in both extracts along other bioactive compounds responsible for enhancing cell growth, rate of germination and nutrient content in plants proving the algal extracts to be potential biostimulants.

Keywords: *Sargassum tenerrimum, Sargassum swartzii,* anthocyanins, red amaranth microgreens, HRLC-MS analysis.

Received 08.09.2024 Revised 27.10.2024 Accepted 07.12.2024

How to cite this article:
Siddhi T, Sunil S. Exploring *Sargassum tenerrimum* J. Agardh and *Sargassum swartzii* C Agardh.extracts and their potential effect in enhancing rate of germination, proteins and anthocyanins in red amaranth microgreens. Adv. Biores. Special Issue [1] 2024. 153-159

INTRODUCTION

The Indian coastline is about 8100 km long, and its diverse coastal environments enable the luxuriant growth of marine macroalgae. India imports seaweed biostimulants manufactured from Canada, Norway, Indonesia, the Philippines, and China worth US $25–30 million annually [1]. *Sargassum* species are among the most diverse members of class Phaeophyta in India, yet they remain underutilized and unexplored [2,3]. The accelerating global population encourages us to consider sustainability in agricultural practices. Several synthetic chemical fertilizers used to boost crop yields contain harmful substances such as inorganic pollutants, heavy metals. The persistent indiscriminate application of these fertilizers may cause the accumulation of hazardous pollutants in soil damaging its ecological environment and reducing soil fertility, making it unsuitable for growing crops [4]. Seaweed extracts are biodegradable, non-polluting, ecofriendly. They have been reported to improve seed germination, seedling growth, and plant resistance to environmental stress. The polysaccharides content and an adequate amount of potassium, nitrogen, growth promoting hormones and micronutrients present in seaweeds makes it an excellent alternative to chemically synthesized fertilizers [5].

MATERIAL AND METHODS

Collection and Sample preparation

In this investigation*, Sargassum tenerrimum and Sargassum swartzii* were collected from Kunkeshwar coast, Devgad, Maharashtra, India. The samples were authenticated by Botanical Survey of India,

Coimbatore. The collected seaweeds were thoroughly rinsed, blotted on blotting paper and then shade dried seaweeds were pulverised into a fine powder using a tissue blender. The powdered sample was then stored for further analysis.

Preparation of Seaweed Liquid extract

The seaweed extracts were prepared by adding one gram of dried seaweed powder in 100mL of distilled water and Autoclaved at15Psi for 30mins. The solution was allowed to cool and was further filtered using muslin cloth. The extract was then used to make various concentrations by dilution such as 0.2%,0.4%, 0.6%, 0.8% and 1% which was used for further analysis [6].

Selection of seed and pre-treatment

The experiment was conducted on Red Amaranth (*Amaranthus cruentus L.*) seeds. The seeds were sterilized using distilled water and 70% alcohol two times and once again washed with distilled water to avoid contamination. The seeds were then blotted on filter paper and used for further analysis.

Effect of Seaweed Liquid extract on Germination, Root and shoot length of Red Amaranth

The rate of germination was determined by inoculating ten seeds of red amaranth per Petri plate with different concentrations of extract and NPK, distilled water as control. All the experiment were conducted in triplicates. Readings were taken at different time intervals such as 24hrs, 72hrs and 144hrs. At 144hrs root and shoot length was measured. The rate of germination was calculated using the formula:

$$\text{Rate of germination} = \frac{\text{No. of germinated seeds} \times 100}{\text{Total no. of seeds}}$$

Experimental design and treatment for growing red amaranth microgreens.

Seeds were sown in cocopeat to avoid soil mineral interactions. As the seeds are small and difficult to count one gram of seeds were sown in each pot. Total twelve pots of which five pots of different concentrations (0.2%,0.4%, 0.6%, 0.8% and 1%)of both extracts and two of control NPK and Distilled water.Foliar spray was applied 2 times per week of 10ml for each concentration. All the pots containing seaweed extracts were also sprayed with 1% NPK10ml for each concentration [6]. The microgreens were harvested after 2 weeks and were further used for estimation of proteins and anthocyanins.

Estimation of Proteins

Fresh plant material of harvested microgreens of different concentration of both algal extracts, control NPK and Distilled water were used to prepare ethanolic extracts by grinding 1g in 10ml ethanol which was further used to estimate protein content using Lowrys method [7].In a test tube 0.1ml of plant extract, 0.9ml of D/Wand 5ml of Reagent C was added.Finally,0.5ml of Folin's reagent was added in each tube mixed well and allowed to stand for 30min, O.D was taken at 660nm.Standard Bovine Serum Albumin(100 to 1000 µg/ml) dilutions similarly treated were used to construct a calibration curve.

Estimation of Anthocyanins

The anthocyanin content was estimated by Hillis et al., 1969 method [8]. Material harvested from each pot were ground in 10ml alcohol then filtered and centrifuged. In test tube add 1ml of alcohol extract and 3ml of HCL in aqueous methanol. Finally add 1ml of anthocyanin reagent. Blank was prepared similarly by adding 1ml of methanolic HCL instead of anthocyanin reagent. After 15mins of incubation in dark the absorbance was measured at 525nm against blank. A standard curve was prepared using cyanin hydrochloride(10-100µg/ml) dilutions.

Estimation of Minerals

A crucible containing one g of algal powder was placed in a Muffle furnace set to 800° to 900°C. The ash was then dissolved in aqua-regia (1:3 combination of nitric and hydrochloric acid) and diluted with distilled water[9,10]. The minerals were estimated by Inductively Coupled Plasma - Atomic Emission Spectrometry (ICP- AES) method carried out at SAIF-IIT Bombay. Analysis of minerals such as K, Mg, Ca, Na, Cu, Fe, Ni essential for plant growth and development was done using ARCOS, Simultaneous ICP Spectrometer. Lead and Mercury were also analysed.

Determination of Metabolites and Plant growth Regulators using HRLC-MS

Aqueous extracts were prepared by Hot extraction method and were further subjected to Liquid Chromatography Mass Spectroscopy Analysis carried out at SAIF-IIT Bombay. Agilent high resolution liquid chromatography and mass spectrometry model- G6550A, MS Q-TOF with Dual AJS ESI for metabolite screening. The acquisition method was set to be MS- minimum range 120(m/z) and maximum 1200 Dalton (m/z). Gas temperature at 250°C with gas flow 13 I/minute. HiP sampler with model-G4226A with auxiliary and ejection speed 100 µl/minute, with 3µl injection. Acquisition time35 minutes, Binary Pump Model: G4220B using solvent 100% Water and 100% Acetonitrile. Column Compartment Model: G1316C with temperature set initially at 40°C and finally at 80°C. Diode array detection model G4212B Spectrum Range 190.0 nm to 640.0 nm.

Statistical analysis:
All the assays were carried out in triplicate and the results were expressed as mean ± standard errors. Statistical Analysis was performed in SPSS (Version 18). Comparison in rate of germination with respect to time and treatments was done using Two-way ANOVA with Tukey's multiple comparison test. One way ANOVA with Tukey's multiple comparison test was used to compare protein, anthocyanin, root and shoot length for all treatments.

RESULTS

Effect of Seaweed Liquid extract on Germination, Root and shoot length of Red Amaranth.

The comparison of treatments with control NPK and distilled water at different time intervals are shown in Table1.The significance was tested with in treatment showing p-value 0.00, F value 17.32and in between different time intervals p-value 0.005, F value 2.8 was obtained indicating significance as $p \leq 0.05$. Total mean rate of germination was highest in 1 % *S. swartzii* extract but showed a significant difference only with control NPK and 0.2%*S. swartzii* extract. There was no difference of treatments on root length as compared to NPK and distilled water. Highest shoot length was observed in microgreens treated with 0.8% *S. swartzii* extracts. All extracts showed increase in shoot length compared to control NPK and distilled water. The values are represented in Table2.

Estimation of proteins

There was a significant difference in the treatments showing p-value 0.00 and F value 55.61 at 0.05% significance level. The protein content was highest 7.1±0.002mg/g in red amaranth microgreens sprayed with 0.8% of *S. swartzii* extract and 1% *S. tenerrimum* extract which was 6.7±0.001mg/g being most significant as compared to other treatments, NPK and distilled water.

Estimation of Anthocyanins

High significant difference in the treatments was observed with p-value 0.00 and F value 61.96 with $p \leq 0.05$. The highest anthocyanin content 0.145±0.003mg/g was observedin microgreens sprayed with 1% *S. swartzii* extract followed by 1% *S. tenerrimum* extract which was 0.113±0.004mg/g and was most significant as compared to other treatments, controls NPK and distilled water.

Estimation of Minerals

The algal extracts showed presence of potassium in highest amounts. Sodium and Magnesium was high in *S. tenerrimum* while Calcium was highest in *S. swartzii* extract. Zinc, Copper, Iron and Nickel were present in minor quantities in both extracts, toxic heavy metals like lead and mercury were absent.

Determination of Metabolites and Plant growth Regulators using LCMS

LCMS analysis in positive ion mode separation confirmed the presence of D-Tryptophan a precursor of phytohormone auxin [11] in *S. swartzii* extracts and trans-Zeatin-O-glucoside riboside, Isopentenyladenine, 8-Hydroxy Adenine which are cytokinin [12,13,14]in both extracts. In addition to that *S. swartzii* extracts also showed presence of 2-Hexylbenzothiazole which exhibits plant growth regulatory activities [15] and Sphinganine which has significance in cell growth and differentiation [16]. Many bioactive compounds, nitrogenous compounds and amino acid derivatives were also detected in both extracts which are listed in Table 4 and 5.

Table 1: Effect of treatments on rate of germination in red amaranth seeds at different time intervals.

Extracts	Conc.	Rate of Germination in percentage (%)			
		At 24hrs	72hrs	144hrs	Mean
Sargassum tenerrimum	0.2%	60 ± 17.32	60 ± 17.32	76.66 ± 5.77	65.56± 4.11[a]
	0.4%	50 ± 10.00	70 ± 10.00	80 ± 10.00	66.67± 4.11[a]
	0.6%	60 ± 10.00	70± 0.00	73.33 ± 5.77	67.78± 4.11[a]
	0.8%	53.33 ± 15.27	66 ± 11.54	73.33 ± 5.77	62.22± 4.11[a]
	1%	50 ± 17.32	56.66 ± 15.27	80 ± 10.00	62.22± 4.11[a]
Sargassum swartzii	0.2%	53.33 ± 11.54	53.33 ± 11.54	63.33 ± 5.77	56.67± 4.11[b]
	0.4%	70 ± 17.32	73.33 ± 11.54	80 ± 10.00	74.44± 4.11[a]
	0.6%	63.33 ±5.77	70 ± 10.00	80 ± 20.00	71.11± 4.11[a]
	0.8%	63.33 ± 15.27	66 ± 10.00	76.66 ± 11.54	68.89± 4.11[a]
	1%	70 ± 0.00	76.66 ± 0.577	90 ± 0.00	78.89± 4.11[a]
NPK	1%	53.33 ± 11.54	53.33 ± 11.54	63.33 ± 20.81	56.67± 4.11[b]
Distilled water	-	53.33 ± 20.81	73.33 ± 10.00	76.66 ± 11.54	66.67± 4.11[a]

All readings were taken in triplicates and expressed as mean ± standard errors. Total Means of all treatments showing different letters in superscript denotes statistical significance. Readings sharing similar letters shows no significant difference while different letter indicates significance $p \leq 0.05$.

Table 2: Effect of treatments on root length, shoot length in germinated seeds at 144hrs time interval

Extracts	Concentration	Root Length in cm	Shoot Length in cm
Sargassum tenerrimum	0.2%	2.6±1.17[a]	5.8±0.67[a]
	0.4%	1.9±0.89[ab]	4.6±0.67[a]
	0.6%	2.1±0.74[a]	5±1.01[a]
	0.8%	1.3±0.45[b]	6.1±1.02[a]
	1%	1.1±0.09[b]	4.9±0.89[a]
Sargassum swartzii	0.2%	2.9±0.22[a]	5.9±1.34[a]
	0.4%	3.2±0.91[a]	5.8±1.03[a]
	0.6%	2±0.35[a]	5.8±0.84[a]
	0.8%	2.8±1.30[a]	6.2±0.76[a]
	1%	3.1±0.65[a]	5.7±0.45[a]
NPK	1%	2.24±1.61[a]	2.2±1.22[b]
Distilled water	-	3.66±1.52[a]	3.1±1.06[b]

All readings were taken in triplicates and expressed as mean ± standard errors. The different alphabets in superscript denotes statistical significance. Readings sharing similar letters shows no significant difference while different letter indicates significance p≤ 0.05.

Table 3: Effect of treatments on proteins, anthocyanin content in red amaranth microgreens.

Extracts	Concentration	Anthocyanin Content(mg/g)	Protein Content(mg/g)
Sargassum tenerrimum + NPK	0.2%	0.088±0.006[c]	4.5±0.001[e]
	0.4%	0.061±0.003[d]	6.5±0.002[b]
	0.6%	0.056±0.002[d]	5.6±0.001[c]
	0.8%	0.099±0.006[c]	6.4±0.001[b]
	1%	0.113±0.004[b]	6.7±0.001[ab]
Sargassumswartzii +NPK	0.2%	0.067±0.001[d]	4.3±0.004[e]
	0.4%	0.062±0.003[d]	5.8±0.004[c]
	0.6%	0.090±0.003[c]	5.7±0.001[c]
	0.8%	0.088±0.003[c]	7.1±0.002[a]
	1%	0.145±0.002[a]	6.4±0.002[b]
NPK	1%	0.088±0.004[c]	5.1±0.0015[d]
Distilled water	-	0.051±0.015[d]	5.2±0.003[c]

All readings were taken in triplicates and expressed as mean ± standard errors. The different alphabets in superscript denotes statistical significance. Readings sharing similar letters shows no significant difference while different letter indicates significance p≤ 0.05.

Table 4: Compounds detected in *Sargassum tenerrimum* extracts by HR-LCMS analysis

Peak	Name of compound	Molecular Formula	Molecular weight	Retention Time
1	Capillene	$C_{12}H_{10}$	154.0782	0.985
6	Chinomethionate	$C_{10}H_6N_2OS_2$	233.9901	1.088
11	1-Nitroheptane	$C_7H_{15}NO_2$	145.1091	1.379
13	Valyl-Isoleucine	$C_{11}H_{22}N_2O_3$	230.1597	1.467
14	Retronecine	$C_8H_{13}NO_2$	155.0946	1.47
15	Isopentenyladenine	$C_{10}H_{13}N_5$	203.1136	1.544
16	trans-Zeatin-O-glucoside riboside	$C_{21}H_{31}N_5O_{10}$	513.2134	1.682
19	gamma-L-Glutamyl-gamma-L-glutamyl-L-methionine	$C_{15}H_{25}N_3O_8S$	407.1377	1.713
22	N-Nitrosopyrrolidine	$C_4H_8N_2O$	100.0645	1.74
23	Isonoruron	$C_{13}H_{22}N_2O$	222.1691	1.793
30	Medicanine	$C_7H_{13}NO_3$	159.0897	1.958
31	Neuraminic acid	$C_9H_{17}NO_8$	267.0941	2.014
33	Muramic acid	$C_9H_{17}NO_7$	251.0989	2.132
39	8-Hydroxy Adenine	C5 H5 N5 O	151.0476	2.773
41	L-2-Amino-3-methylenehexanoic acid	C7 H13 N O2	143.0952	3.375
47	2,4,6-Triethyl-1,3,5-trioxane	C9 H18 O3	174.1254	7.519

Table 5: Compounds detected in *Sargassum swartzii* extracts by HR-LCMS analysis

Peak	Name of compound	Molecular Formula	Molecular weight	Retention Time
3	trans-Zeatin-O-glucoside riboside	$C_{21}H_{31}N_5O_{10}$	513.2134	1.03
6	Chinomethionate	$C_{10}H_6N_2OS_2$	233.9901	1.071
11	Medicanine	$C_7H_{13}NO_3$	159.0897	1.34
12	1-Nitroheptane	$C_7H_{15}NO_2$	145.1091	1.381
14	Isopentenyladenine	$C_{10}H_{13}N_5$	203.1136	1.557
19	2S-amino-3-oxo-butanoic acid	$C_4H_7NO_3$	117.0433	1.734
21	Hydroxypropyl-. Alanine	$C_8H_{14}N_2O_4$	202.0928	1.793
26	Neuraminic acid	$C_9H_{17}NO_8$	267.0941	2.033
29	Muramic acid	$C_9H_{17}NO_7$	251.0989	2.242
31	8-Hydroxy Adenine	$C_5H_5N_5O$	151.0476	2.346
38	L-2-Amino-3-methylenehexanoic acid	$C_7H_{13}NO_2$	143.0952	3.269
43	2-Hexylbenzothiazole	$C_{13}H_{17}NS$	219.1079	3.718
47	D- Tryptophan	$C_{11}H_{12}N_2O_2$	204.0874	4.658
50	Sphinganine	$C_{18}H_{39}NO_2$	301.2943	14.408

Figure 1: Estimation of Minerals by ICP-AES analysis

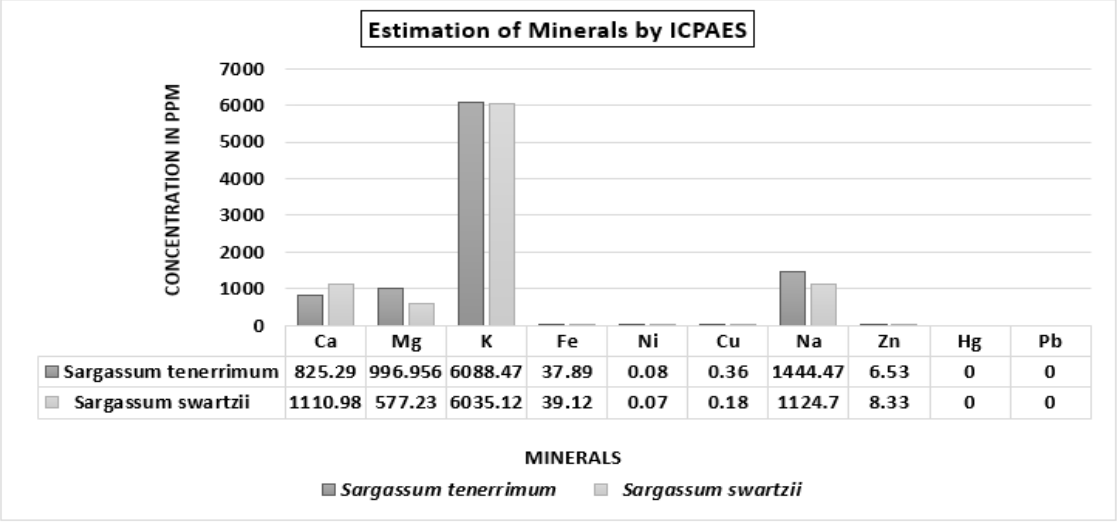


Figure 2: HR-LCMS chromatogram of *Sargassum tenerrimum* extracts

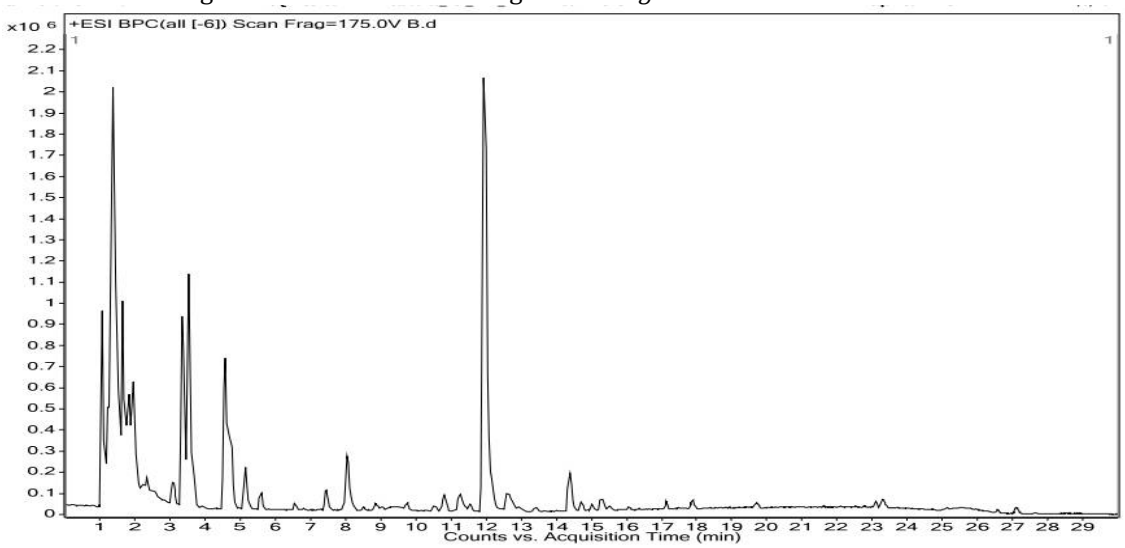
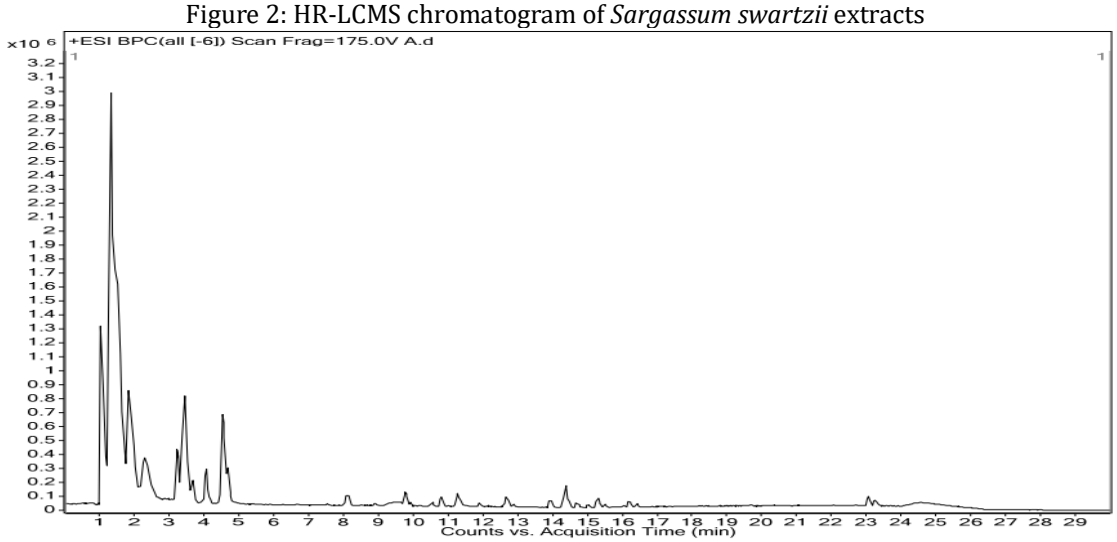


Figure 2: HR-LCMS chromatogram of *Sargassum swartzii* extracts



DISCUSSION

The utilisation of seaweeds in modern agriculture has been widely investigated [4]. The current investigation focuses on the application of seaweed extracts to enhance nutrients, plant growth and development. Similar work was done to determine the effect of *Sargassum* extracts used on growth and germination of *Capsicum annum* and *Lycopersicon Esculentum*. The results showed optimum concentration of 2.50 mg/L for seedling germination and development [17]. *Sargassum sp.* extract was also tested to determine growth and yield in mustard greens [18]. In an earlier study carried out using *S. wightii* extracts also shows enhancement in protein content [19]. Significance of seaweed extracts on increasing anthocyanin content is also revealed [20]. Seaweed extracts have also been shown to boost tolerance to abiotic stresses, drought and toxicity. Macroalgal extracts consist of phytohormones such as Abscisic acid, Auxins, Betaine, Gibberellins, and Polyamines, as well as micronutrients and trace elements, which can stimulate plant development and increase crop yield when administered exogenously [4]. The *S. tenerrimumand S. swartzii* extracts showed presence of macro and micro minerals which are essential in plant growth and metabolism. In a study conducted using *S.johnstonii* and *S.wightii* liquid extracts reveals that foliar spray helps in better absorption of minerals which are found in adequate amounts in seaweeds [21]. Many studies reveal the presence of bioactive compounds and plant growth hormones in seaweed extracts [6, 22, 23, 24, 25]. Both the extracts used in this study showed the presence of plant growth hormones, bioactive compounds and amino acid derivative which could play a vital role in substantial increase in biochemical compounds and enhanced yield of crops.

CONCLUSION

The results in the investigation suggest that seaweed extracts enhance germination, shoot length, proteins and anthocyanins in red amaranth. The abundant macronutrients, micronutrients, bioactive and plant growth stimulating compounds present in both the extracts could play a significant role in enhancing cell growth, rate of germination and nutrient content in plants proving the algal extracts to be potential biostimulants.

ACKNOWLEDGEMENT

The author would like to express sincere gratitude to the Principal and Botany Department of Ramnarain Ruia Autonomous College, Matunga (E), Mumbai, Maharashtra for the support and providing lab facility for research work, Botanical Survey of India, Coimbatore -641003, Tamil Nadu for authentication of the algal species and SAIF- IIT Bombay, Mumbai for ICP-AES and HRLC-MS analysis.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest. The research carried out received no grant or funding from any public or private sectors.

AUTHORS CONTRIBUTION

The study was conceptualized by SS and ST. ST carried out collection, experimental work, data analysis and wrote the manuscript. SS guided the entire research work.

REFERENCES

1. Ganesan, M., Trivedi, N., Gupta, V., Madhav, S. V., Radhakrishna Reddy, C., & Levine, I. A. (2019). Seaweed resources in India–current status of diversity and cultivation: prospects and challenges. *Botanica Marina*, *62*(5): 463-482.
2. Marimuthu, J., Essakimuthu, P., Narayanan, J., Anantham, B., Tharmaraj, R. J. J. M., & Arumugam, S. (2012). Phytochemical characterization of brown seaweed *Sargassum wightii*. *Asian Pacific Journal of Tropical Disease*, *2*: S109-S113.
3. Abdi, G., Karande, V. C., Mohammed, A., Abbasi Tarighat, M., Wen Goh, K., Abdul Kari, Z.,& Barwant, M. M. (2022). Pharmacological potential of Sargassum sp. of west coast of Maharashtra Kunkeshwar, India. *Frontiers in Marine Science*, *9*: 1011218.
4. Zafar, A., Ali, I., & Rahayu, F. (2022). Marine seaweeds (biofertilizer) significance in sustainable agricultural activities: A review. In *IOP Conference Series: Earth and Environmental Science* (Vol. 974, No. 1, p. 012080). IOP Publishing.
5. MM, E. S., Ismail, M. M., & Hamouda, M. M. (2016). Influence of some brown seaweed extracts on germination and cytological responses of Trigonella foenum-graecum L. *L. BioTechnology: An Indian Journal*, *12*: 1-12.
6. Parab, A., & Shankhadarwar, S. (2022). Growth enhancement of agricultural crops using seaweed liquid fertilizer. *Plant Science Today*, *9*(2): 322-330.
7. Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. *J biol Chem*, *193*(1): 265-275.
8. Hillis, W. E., & Swain, T. (1959). The phenolic constituents of Prunus domestica. II.—The analysis of tissues of the Victoria plum tree. *Journal of the Science of Food and Agriculture*, *10*(2): 135-144.
9. Thompson, M., Walsh, J. N., Thompson, M., & Walsh, J. N. (1989). Inductively coupled plasma mass spectrometry. *Handbook of Inductively Coupled Plasma Spectrometry*, 238-269.
10. Moore, G. L. (2012). *Introduction to inductively coupled plasma atomic emission spectrometry*. Elsevier.
11. Zhao, Y. (2014). Auxin biosynthesis. *The Arabidopsis Book/American Society of Plant Biologists*, *12*.
12. Veach, Y. K., Martin, R. C., Mok, D. W., Malbeck, J., Vankova, R., & Mok, M. C. (2003). O-glucosylation of cis-zeatin in maize. Characterization of genes, enzymes, and endogenous cytokinins. *Plant physiology*, *131*(3): 1374-1380.
13. Hallmark, H. T., & Rashotte, A. M. (2020). Cytokinin isopentenyladenine and its glucoside isopentenyladenine-9G delay leaf senescence through activation of cytokinin-associated genes. *Plant Direct*, *4*(12): e00292.
14. Chen, C. M., Smith, O. B. C., & McChesney, J. D. (1975). Biosynthesis and cytokinin activity of 8-hydroxy and 2, 8-dihydroxy derivatives of zeatin and N6-(Δ2-isopentenyl) adenine. *Biochemistry*, *14*(14): 3088-3093.
15. Simonova, E., Henselová, M., & Zahradník, P. (2005). Benzothiazole derivatives substituted in position 2 as biologically active substances with plant growth regulation activity. *Plant Soil and Environment*, *51*(11): 496.
16. Spiegel, S., Cuvillier, O., Edsall, L., Kohama, T., Menzeleev, R., Olivera, A., ... & Wang, F. (1998). Roles of sphingosine-1-phosphate in cell growth, differentiation, and death. *Biochemistry-New York-English Translation of Biokhimiya*, *63*(1): 69-73.
17. Fatimah, S., & Daud, N. (2018). The effect of seaweed extract (Sargassum Sp) used as fertilizer on plant growth of capsicum annum (Chilli) and *Lycopersicon Esculentum* (Tomato). *Indonesian Journal of Science and Technology*, *3*(2): 115-123.
18. Yusuf, R., Laude, S., Syakur, A., & Kalaba, Y. (2021). The Effect of Seaweed Extract (Sargassum sp.) on Growth and Yield Enhancement of Mustard Greens (Brassica juncea L.). In *IOP Conference Series: Earth and Environmental Science*,828(1): p. 012011). IOP Publishing.
19. Vijayanand, N., Ramya, S. S., & Rathinavel, S. (2014). Potential of liquid extracts of *Sargassum wightii* on growth, biochemical and yield parameters of cluster bean plant. *Asian Pacific Journal of Reproduction*, *3*(2): 150-155.
20. Taha, S. S., & Abdelaziz, M. E. (2015). Effect of different concentrations of seaweed extract on growth, yield and quality of two carrot (Daucus carota L.) cultivars. *Curr Sci Int*, *4*: 750-9.
21. Kumari, R., Kaur, I., & Bhatnagar, A. K. (2011). Effect of aqueous extract of *Sargassum johnstonii* Setchell & Gardner on growth, yield and quality of Lycopersicon esculentum Mill. *Journal of Applied Phycology*, *23*: 623-633.
22. Omar, H. H., Abdullatif, B. M., Al-Kazan, M. M., & El-Gendy, A. M. (2015). Various applications of seaweed improve growth and biochemical constituents of Zea mays L. and Helianthus annuus L. *Journal of plant nutrition*, *38*(1): 28-40.
23. Sujatha, R., Siva, D., & Nawas, P. M. A. (2019). Screening of phytochemical profile and antibacterial activity of various solvent extracts of marine algae *Sargassum swartzii*. *World Scientific News*, (115): 27-40.
24. Albratty, M., Alhazmi, H. A., Meraya, A. M., Najmi, A., Alam, M. S., Rehman, Z., & Moni, S. S. (2021). Spectral analysis and Antibacterial activity of the bioactive principles of *Sargassum tenerrimum* J. Agardh collected from the red sea, Jazan, Kingdom of Saudi Arabia. *Brazilian Journal of Biology*, *83*.
25. Barot, M., Kumar, N. J. I., & Kumar, R. N. (2016). Bioactive compounds and antifungal activity of three different seaweed species *Ulva lactuca*, *Sargassum tenerrimum* and *Laurencia obtusa* collected from Okha coast, Western India. *Journal of Coastal Life Medicine*, *4*(4): 284-289.