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Bio-fertilizer potential of seaweed liquid extracts of marine macro algae on growth and biochemical parameters of *Ocimum sanctum*

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Abstract
A pot experiment was conducted to identify the potential of *Sargassum wightii*, *Turbinaria ornata* and *Caulerpa racemosa* on growth and biochemical parameters of *Ocimum sanctum*. Seaweed liquid extracts were given to the tested plant in form of foliar spray. After 60 days, growth parameters such as shoot length, root length, total plant height, leaf area, number of the leaves, fresh and dried weight and biochemical parameters such as starch, glucose, protein, chlorophyll content were observed in the treated plants. Foliar application of individual seaweed treatments (T1, T2, T3) enhanced the overall growth and physiology of *Ocimum sanctum.* But, there was an appreciable increase in growth and biochemical parameters in the treated plants which received mixture of seaweeds (T4) when compared to individual treatment and control. This might may be due to synergistic and cumulative effect of qualitative and quantitative active ingredients such as micro and macroelements, vitamins and phytohormones present in the seaweed liquid extracts. Thus, seaweed liquid extract could serve as an promising effective organic biostimulant to replace the synthetic fertilizers for sustainable agriculture.

Keywords: seaweed liquid fertilizers, growth, biochemical, *Ocimum sanctum*

1. Introduction
Usage of different inorganic fertilizers, pesticides, insecticides has damaged the soil ecosystem extensively. This kind of practice makes the soil environment unsuitable for crop growth in future. Presently, the use of natural plant biostimulant is proposed as an innovative solution to address the challenges to sustainable agriculture, to ensure optimal nutrient uptake, crop yield and tolerance ot abiotic stress (Povero *et al*., 2016) [29].

Seaweeds are the macroscopic marine algae found attached to the bottom in relatively shallow coastal water. Seaweed extract is a new generation of natural organic fertilizers containing highly effective nutritious source and promotes faster germination of seeds, increase in yield and resistant ability of many crops (Ganapathy *et al*., 2013) [17]. Liquid fertilizers derived from natural sources like seaweeds are found to be viable alternatives to fertilizing input for agricultural crops due to its high level of organic matter, micro and macro elements, vitamins, fatty acids and also rich in growth regulators (Crouch and Vanstaden, 1993) [12]. Marine bioactive substances extracted from marine algae are used in agricultural and horticultural fields and many beneficial effects in the terms if enhancements of yield and quality have been reported (Blunden, 1991; Crouch and Van Staden, 1993) [7, 12]. The main objective of this study is to evaluate the effect of different concentrations of seaweed extracts on growth and biochemical parameters of *Ocimum sanctum.*

2. Material and Methods
2.1 Preparation of seaweed liquid extract
Brown algae (*Sargassum wightii and Turbinaria ornata*) and green alga (*Caulerpa racemosa*) used in the present investigation were collected from Pamban Coastal region of Gulf of Mannar located in the south east coast of Tamil Nadu, India, between longitude 70º15'E and latitude 9º45 N. Seaweeds were collected and washed with sea water to remove unwanted impurities and other debris. They were shade dried and brought to the laboratory and they were thoroughly washed with tap water for 3 to 4 times to remove all the epiphytes, sand particles and other fauna. After drying, it was cut into small pieces and kept in hot air oven for one day at 60 ºC. Then, it was made into coarse powder with help of mixer grinder. The powdered seaweeds were made into liquid extracts as per the method of Seaweed Liquid Fertilizer (SLF)

following the method of Bhosle *et al*. (1975) [4]. The obtained extract was designated as stock solution and it was considered as 100%. From the stock solution, 10% of concentration of liquid extracts was prepared by mixing of appropriate proportion of sterilized distilled water for all three extracts (T1, T2, and T3). For mixed treatment of liquid extracts (T4), 3% from *Sargassum wightii*, 3% from *Caulerpa racemosa* and 4% from *Turbinaria ornata* of liquid extracts from individual seaweed were taken and mixed together to make 10% of mixture SLE.

2.2 Elemental composition and hormone analyses of SLE

The composition of elements such as copper, manganese, iron, zinc, cobalt, potassium, magnesium and sodium were estimated using ICP-MS method (B'Hymer *et al*., 2000) [5]. Estimation of nitrogen was done as per Kjeldahl Method (Bremmer, 1960) [9]. In addition**,** liquid extracts were subjected for estimation of auxin (Gordon and Paleg, 1957) [19] gibberellin (Graham and Henderson, 1961) [20] and cytokinin (Syono and Torrey, 1976) [30].

2.3 Selected medicinal plant

Viable seeds of *Ocimum sanctum* were procured from seed storage bank of Agriculture College Madurai, Tamil Nadu. Healthy seeds free from visible infection, with uniform size were segregated. They were surface sterilized with 0.1% mercuric chloride and then sown in earthen ware pots (9 cm dia) filled with sterilized standard soil mix supplemented with sufficient quantity of NPK. The seed to seed distance in pot was maintained as 4 cm and the pots were irrigated regularly. After 30 days of germination, 10% foliar application of seaweed extracts were given in the form of foliar spray to potted plants. Separate set of potted plants were used for each seaweed extract treatment. The potted plants received 50 ml of *S. wightii* (T_1), *C. racemosa* (T_2), *T. ornata* (T_3) and mixture of SLE (T_4) as foliar spray. The extracts were given at interval of 5 days for a period of 60 days. Growth parameters viz., shoot and root length, total plant height, leaf area, number of leaves and branch, fresh a dry weight of whole plant and biochemical parameters such as starch (Rose *et al*., 1991) [30], glucose (Nelson, 1944) [26], protein (Lowry *et al.*, 1951) [25], chlorophyll content (Arnon, 1949) [2] were observed.

3. Result

3.1 Elemental composition and hormonal analysis of seaweed liquid extracts

Among the three seaweed liquid extracts, *Turbinaria ornata* exhibited highest amount of macro and minor nutrients such as Nitrogen, Potassium, Magnesium, Sodium, Iron and Molybdenum. This was followed by *Sargassum wightii* and *Caulerpa racemosa.* Similarly, highest amount of phytohormones such as auxin, gibberellin and cytokinin were recorded in *Turbinaria ornata* and *Sargassum wightii* when compared to *Caulerpa racemosa* (Table 1).

Table 1: Elemental Composition and hormonal analyze of seaweed liquid extracts

S. No	Analyte	Sargassum wightii	Caulerpa racemosa	Turbinaria ornata
***Elemental composition* (g/liter)**				
1	Sodium (Na)	72.47	69.72	98.59
2	Magnesium (Mg)	0.812	0.007	0.818
3	Potassium (K)	153.06	98.4	167.0
4	Iron (Fe)	0.85	0.85	0.89
5	Molybdenum (Mo)	0.03	0.0004	0.019
6	Nitrogen(N_2) %	1.93%	2.56%	2.83%
Hormone Analyses (mg/l)				
1	Auxin	3.0 mg/l	1.8 mg/l	3.7 mg/l
2	Cytokinin	3.9 mg/l	2.4 mg/l	4.9 mg/l
3	Gibberellin	2.2mg/l	2.0 mg/l	3.3 mg/l

3.2 Foliar application of seaweed liquid extract on growth parameters of *Ocimum sanctum*

Differential responses were observed in growth parameters when the plants were treated with 10% of (*Caulerpa racemosa* (T_1), *Sargassum wightii* (T_2), *Turbinaria ornata* (T_3) and mixture of liquid extracts (T4). Increase in shoot length (72%), root length (50%), total plant height (65%), leaf area (69%), number of the leaves (90%), and fresh (99%) and dried weight (145%) was observed in those plants which received 10% of *Turbinaria oranta* (T_3). This was followed by *Sargassum wightii* (T_2) and *Caulerpa racemosa* (T_1). Further, mixture of liquid extracts (T_4) increased the shoot length (97%), root length (61%), total plant height (85%), leaf area (95%), number of the leaves (134%), and fresh (123%) and dried weight (193%) than individual treatment of seaweeds (T_1, T_2, T_3) (Table 2).

Table 2: Foliar application of seaweed liquid extracts on growth parameters of *Ocimum sanctum*

S. No	Treatment	Shoot Length (cm)	Root Length (cm)	Total plant Height (cm)	Leaf Area (cm^2)	Number of Leaves (n)	Number of branch (n)	Fresh Weight (g)	Dry Weight (g)
1	Control (T_0)	28.2±0.56	9.53±0.20	37.55±0.25	11.3±0.45	37.2±0.55	5.3±0.45	4.56±0.51	1.01±0.06
2	*Caulerpa racemosa* (T_1)	38.1±0.3 133	10.46±0.35 108	48.47±0.25 126	13.36±0.51 117	58.23±0.45 151	6.33±0.61 119	5.66±0.102 123	1.96±0.03 194
3	*Sargassum wightii* (T_2)	44.06±0.49 154	12.53±0.15 130	56.59±0.35 147	14.16±0.45 124	69.2±0.60 179	10.2±0.5 192	8.4±0.433 183	2.05±0.03 202
4	*Turbinaria ornata* (T_3)	49.3±0.62 172	14.5±0.3 150	63.6±0.3 165	19.3±0.7 169	73.16±0.49 190	14.33±0.7371 269	9.13±0.02 199	2.48±0.0602 245
5	Mixture of SLF (T_4)	56.53±0.28 197	15.53±0.40 161	71.5±0.25 185	22.23±0.40 195	90.2±0.556 234	16.3±0.793 306	10.2±0.045 223	2.96±0.07 293

3.3 Foliar application of seaweed liquid extract on growth parameters of *Ocimum sanctum*

In case of biochemical constituents, individual treatments of 10% concentration of *Turbinaria oranta* (T_3) enhanced the starch content , glucose , protein, chlorophyll a, chlorophyll b and total chlorophyll by 105 %, 79 %, 170%, 219%, 215% and 217% respectively. Individual treatment of liquid extracts (T_1 and T_2) also enhanced the biochemical constituents but it was found to be lesser than T_3 treatment. But, 10% concentration of mixed liquid extracts (T_4) exhibited maximum increase in starch (156%), glucose (102%), protein (208%), chlorophyll-a (266%), chlorophyll-b (254%) and total chlorophyll content (259%) than control and individual treatment of liquid extracts (T_1,T_2) (Table 3).

Table 3: Foliar application of seaweed liquid extracts on biochemical parameters of *Ocimum sanctum*

S. No	Treatments	Starch mg/g	Glucose mg/g	Protein mg/g	Chlorophyll a mg/g	Chlorophyll b mg/g	Total chlorophyll mg/g
1	Control (T_0)	31.3±1.24	31.23±0.81	26.3±2.05	0.53±0.29	0.61±0.04	1.16±0.01
2	*Caulerpa racemosa* (T_1)	57.2±0.81 177	44.3±2.05 141	59.3±1.69 225	0.99±0.22 186	1.71±0.03 277	2.73±0.05 234
3	*Sargassum wightii* (T_2)	60.3±1.24 186	51.6±1.24 165	59.6±2.05 226	1.54±0.28 288	1.72±0.14 278	3.29±0.16 283
4	*Turbinaria ornata* (T_3)	66.3±2.49 205	56.06±2.16 179	71.3±0.94 270	1.71±0.19 319	1.95±0.07 315	3.69±0.05 317
5	Mixture of SLF (T_4)	82.6±1.69 256	63.3±2.05 202	81.3±1.88 308	1.95±0.02 366	2.19±0.06 354	4.18±0.08 359

4. Discussion

In our present study, presence of phytohormones such as auxin, cytokinin and gibberellins and macro and micro nutrients such as copper, manganese, iron, zinc, cobalt, potassium, magnesium and sodium were detected in the crude extracts of *Sargassum wightii, Caulerpa racemosa and Turbinaria ornata* and Among the three seaweed extracts, *Turbinaria ornata* was found to contain maximum amount of phytohormones and nutrient content. Many types of plant growth regulators that have been identified in seaweed extracts, such as auxins, cytokinins, gibberellins, abscisic acid and more (Khan *et al*., 2009; Kurepin *et al*., 2014) [24, 25]. The presence of phytohormones is in agreement with the earlier findings that reported auxins in the extracts of *Ascophyllum nodosum* (Sanderson and Jameson,1986) [32], cytokinins in the extracts of *Ulva* (Sekar, 1995) [33], *Durvillaea potatorum* and *Ascophyllum nodosum* (Craft *et al*., 2007) [11] which stimulates early seedling growth in the plants.

Further, foliar application of 10% of *Sargassum wightii, Caulerpa racemosa and Turbinaria ornata* and mixed seaweed liquid extract significantly enhanced the overall growth and physiology of *Ocimum sanctum.* Wide range of plant responses to seaweed extracts has been well documented in number of reviews. Similar reports regarding seaweed as biostimulants has been reported in *Calibrachoa* (Elansary *et al*., 2016) [15], *Lablab purpurens* (Vishnupriya and Flora, 2017) [39], *Capsicum annum* and *Lycopersicum esculentum* (Divya and Niranjana, 2017) [13], *Allium cepa* (Akash and Richa, 2017) [1] and *Abelmoschus esculentus* (Tensingh Baliah *et al*., 2017) [37]. Moreover, Zheng *et al*. (2016) [41] observed that kelp waste extracts (KWE) significantly increased the growth parameters viz., shoot length, root length, leaf length, fresh and dry weight of *Brassica chinensis plants that* received 10% of kelp waste extracts and also concluded that higher concentrations of Kelp waste extracts (20–100%) showed inhibitory effect. Suganthi and Sujatha (2014) [34] reported that foliar application of 5% *Sargasum myricosysutum, Gracilaria edulis* and *Caulerpa racemosa* enhanced the growth attributes *viz.,* plant height, dry matter production, leaf area index, crop growth rate of sunflower hybrid plant and also concluded that 5% of *Sargassum myricosysutum* was found to be effective when compared to other two seaweed extracts. In our study also, 10% of *Turbinaria ornata* was found to be effective than *Caulerpa racemosa and Sargassum wightii.* This might be due to the presence of nutrient and hormonal level present in the brown seaweed extract (Table 1). In another study, growth characters such as seed germination, seedling vigor index, shoot length, root length, plant fresh weight and plant dry weight were significantly higher in plants treated which received 5.0% of SLF of *Turbinaria ornata* over the control plants. But in our study, 10% of *Turbinaria ornata* enhanced the overall growth of *Ocimum sanctum.* Similarly, Tensingh Baliah *et al*. (2017) [38] reported that increased seedling growth in *Abelmoschus esculentus* may be due to the presence of phenyl acetic acid and other micro nutrients and trace elements in the seaweed liquid fertilizers as well as the presence of other growth promoting substances in *Sargassum wightii, Padina boergesenii* and *Ulva fasciata.* On the contrary, liquid extracts of *Sargassum wightii* and *Ulva fasciata* was found in effective in increasing the growth and yield in the low level (1.5%) But in our study, 10% of *Turbinaria ornata* increased the growth of the plant. Positive enhancement in growth parameters on crop plants may be due to the presence of quantitative amount of growth hormones, macro- and microelement, amino acids, vitamins present in the seaweed extracts (Blunden *et al*., 1997) [7].

Lower concentrations (10%) of *Sargassum wightii, Caulerpa racemosa and Turbinaria ornata* and mixed seaweed liquid extract (10%) enhanced the biochemical characters of *Ocimum sanctum.* This is in accordance with earlier reports that lower concentrations of seaweed liquid extracts increased the biochemical constituents in *Allium cepa* (Akash and Richa, 2017) [1], *Solanum melongena* (Ramya *et al*., 2015) [30], *Vigna unguiculata* (EL- Kazen, 2011) [16], *Solanum melongena and Capsicum annum* (Divya and kalyani, 2016) [13], *Vigna mungo* (Sujatha and Vijayalakshmi, 2013) [15]. *Lepidium sativum* (Godlewska *et al*., 2016) [23]. The increase in the protein content at lower concentrations of SLF confirmed the efficiency of foliar spray of SLE as it enhanced the absorption of most of the necessary elements by the seedlings (Anantharaj and Venkatesalu, 2002) [2]. In *Zea mays,* 10% of *Sargassum linearifolium* showed best positive results on total carbohydrates, total proteins, total lipids, total phenols and pigments namely total chlorophylls, total carotenoids (John Peter Paul *et al*., 2017) [21].

In our studies, individual application of seaweed extracts and mixed liquid extract enhanced the protein content significantly than control. Photosynthetic pigments such as chlorophyll-a, b and total chlorophyll were also found to be

increased in the plants that received 10% of individual seaweed extracts and mixed seaweed liquid extract. The increase in chlorophyll content was a result of reduction in chlorophyll degradation, which might be caused in part by betaines in the seaweed liquid extract (Whapman *et al*., 1993) [39]. Moreover, glycinebetaine delays the loss of photosynthesis activity by inhibiting chlorophyll degradation during storage conditions in isolated chloroplasts (Genard *et al*., 1991) [17]. Vishnupriya and Flora (2017) [38] observed that seaweed liquid concentrate *Padina tetrastromatica* enhanced the photosynthetic pigments, carotenoids significantly than *Ulva fasciculata*. Further, they also concluded that the presence of Magnesium (Mg) and Iron (Fe) could have influenced the chlorophyll synthesis. In our chemical analysis of seaweed extracts, magnesium and iron content was found to be present in *Turbinaria, Sargassum wightii ornate* and *Caulerpa racemosa* liquid extracts.

It was also observed that the seaweed liquid fertilizers prepared from the red algae, *Gracilaria corticata* when applied to crop plants gave better results in soil nutrient contents when compared to the seaweed liquid fertilizer prepared from the green algae *Caulerpa peltata* (Chitra and Sreeja, 2017) [9]. Similarly, seaweed liquid fertilizer prepared from the brown alga, *Rosenvingea intricata*, applied to crop plant gave better results in soil nutrient content when compared to the seaweed fertilizer of green alga. It is probably due to the presence nutrients in more quantities in the brown alga than in other groups of algae (Thirumaran *et al*., 2009) [37].

5. Conclusions

In our study, growth and biochemical parameters of *Ocimum sanctum* exhibited significant increase by all the seaweed liquid extracts. Among the three seaweed liquid extracts, better results exhibited *in Turbinaria ornata* when compared to *Sargassum wightii* and *Caulerpa racemosa.* Apart from individual applications, mixed seaweed liquid extract showed positive results when compared to individual and control treatment. In general, magnesium and iron plays a pivotal role in synthesis of chlorophyll in the plant metabolism. Presence of magnesium and iron in seaweed extracts could have induced the metabolism involved in chlorophyll synthesis (Table 1). In addition, presence of phycocolloids in brown algae might also be responsible for enhancement in growth and physiology of *Ocimum sanctum.* Increase in growth and biochemical parameters on our experimental plant might be due to the cumulative action of the phytohormones and macro and micronutrients present in mixed seaweed liquid extract (T4). Further studies are in underway to examine the optimal concentration and best combination of seaweeds on different crop plants.

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