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Influence of Seaweed Liquid Fertilizer on Growth, Biochemical and Yield Parameters of Cluster Bean Plant

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Abstract

Biofertilizing efficiency of seaweed liquid extracts of brown alga Turbinaria ornata on growth, biochemical and yield parameters of Cyamopsis tetragonoloba was studied. Seaweed Liquid extract (SLE) at low concentration (1.5%) was found to have maximum influence on growth parameters viz., shoot length, root length, fresh weight, dry weight, leaf area and moisture content. In case of biochemical parameters, differential responses in the content of photosynthetic pigments, protein, reducing sugar, ascorbic acid and in the activity of nitrate reductase were observed in the leaves of SLE treated seedlings when compared to untreated seedlings. Decline in growth and biochemical parameters was observed in higher concentrations (above 1.5%) of SLE. It may be concluded that liquid seaweed extracts could serve as cost effective eco-friendly product for sustainable agriculture.

Keywords: *Seaweed extract; Turbinaria ornata; growth, biochemical, yield, cluster bean.*

1. Introduction

Seaweeds are the macroscopic marine algae found attached to the bottom in relatively shallow coastal water. Seaweed liquid extracts (SLE) contains macro nutrients, trace elements, organic substances like amino acids and appreciable quantities of plant growth regulator such as auxin, cytokinin and gibberellins (Mooney and Van Staden, 1985). Seaweed extract is a new generation of natural organic fertilizers containing highly effective nutritious and promotes faster germination of seeds and increase yield and extracts resistant ability of many crops (Ganapathy selvam and Sivakumar, 2013). The organic matter of seaweeds increases humus content of the soil, hereby ameliorating the soil texture and preservation of its moisture. Marine algae not only increases the soil fertility, it also enhances the moisture holding capacity and supplies adequate trace metals thereby improving the overall soil structure. Chemical analysis of seaweeds and their extracts have revealed the presence of a wide variety of plant growth regulators such as auxins and cytokinins in varying amounts (Karthikeyan and Shanmugam, 2016). It has been speculated that seaweed liquid extracts can partially substitute for the requirement of chemical fertilizers (Hernandez-Herrera et al. 2014) when applied concomitantly. Seaweed liquid fertilizer is a blend of both plant growth regulators and organic nutrient input is eco-friendly promoting sustainable productivity and maintaining soil health. In recent years, the use of natural seaweed products as substitutes to the conventional synthetic fertilizers has assumed importance [Eman *et al.,* 2008; Erulan *et al.,* 2009; Sangeetha and Thevanathan, 2010]. Thus, the extracts are applied to improve nutritional status, vegetative growth, yield and fruit quality in crop plants [Abd EL-Migeed *et al.,* 2004; Eman *et al.,* 2008; Spinelli *et al.,* 2009; Jannin et al. 2013; Mancuso et al. 2006). Liquid crude extracts derived from marine algae such as *Ecklonia maxima* [Sławomir et al. 2019], Kappaphycus alvarezii (K sap) and Gracilaria edulis (Basavaraja et al. 2018) ; *Padina* and *Sargassum* [Hiral et al ., 2019) ; Gracilaria parvispora [Dania et al. 2018] and *Ulva* [Laura et al. 2017;] Laminaria and Ascophyllum nodosum (Andrea et al. 2018) were reported to have manorial efficiency. The present study was undertaken to evaluate the fertilizing efficiency of seaweed liquid extracts of *Turbinaria ornata* on cluster bean.

2. Materials and Methods

2.1 Preparation of algal extracts

The marine alga *Turbinaria ornata* which belongs to Phaeophyceae was collected from Mandapam (Lat 9°45'N; Long79°15'E) located in South East Coast of Tamilnadu. The alga was brought to the laboratory and washed thoroughly in tap water for 3 or 4 times to remove all epiphytes, sand particles and associated fauna. The wet weight of sample of collected algal samples was taken, shade dried and then the sample dry weight was determined. Different concentrations of boiled extracts were prepared by mixing appropriate level of liquid extracts with distilled water (Bhosle *et al.,* 1975). The SLE concentrations used in this experiment were ranged from 0.5 to 5.0%.

2.2 Physico-chemical and hormone analysis of SLE of Turbinaria ornata

The physical characteristics such as colour and pH were observed using standard method. The composition of elements such as copper, manganese, iron, zinc, cobalt, potassium, magnesium and sodium were estimated using Atomic Absorption Spectrophotometer (Humpshires *et al.,* 1956). Further, the liquid extracts of marine algae was also subjected for estimation of auxin (Gordon and Paleg, 1957), gibberellin (Graham and Henderson, 1961) and cytokinin (Syono and Torrey, 1976).

2.3 Growth promoting efficiency of SLE effects on cluster bean seedlings

Seeds of cluster bean were purchased from Agriculture College and Research Institute, Madurai. 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 (9cm dia) filled with sterilized standard soil mix supplemented with sufficient quantity of NPK. The seed to seed distance in pot was maintained as 3-5 cm and the pots were irrigated regularly. Foliar application of different concentrations of liquid extracts was given to potted plants after 20 days. About 50 ml of different concentrations of extracts was given at interval of 3 days. Growth parameters viz., shoot length, root length, total height, total fresh and dry weight, leaf area and moisture content were determined. Biochemical profiles such as photosynthetic pigments (Arnon, 1949), protein (Lowry *et al.,* 1951), reducing sugar (Nelson, 1944), ascorbic acid (Roe, 1954) and nitrate reductase activity [Jaworski, 1971) were assessed in the leaves of treated plants. Growth and biochemical parameters were observed in 6 weeks old treated and control plants. All pot experiments were done in four replicates each under natural uniform conditions.

2.4. Statistical Analysis

Data were subjected to one-way ANOVA and means were separated by Duncan's test ($P<0.05$, $n=5$). Statistical analysis was carried out using IRRISTAT ver. 4.0 (IRRI, Manila, Phillipines) (Duncan, 1965).

3. Results and Discussions

The manurial analyses of liquid extract of our experimental algae revealed the presence of potassium, copper, manganese, zinc, iron, cobalt, sodium, potassium and magnesium in appreciable level (Table 1). Further, magnesium was found to be abundant in the extract (Table 1). In addition, Cytokinin was found to be present more when compared to auxin and cytokinin. Seaweed liquid extracts have been used as plant growth promoter (Craigie 2011), because they contribute with essential nutrients such as minerals, amino acids, oligosaccharides, and phytohormones to the crops (Nabti, et al. 2017). The presence of phytohormones is in agreement with the earlier findings that reported auxins in the extracts of *Ascophyllum nodosum* (Sanderson et al., 1987), cytokinins in the extracts of *Ulva* (Sekar, 1995), *Durvillaria potatorum* and *Ascophyllum nodosum* (Tay et al. 1987) stimulates early seedling growth in the plants.

Table 1: Physio-chemical and hormone analyses of liquid extract of T.oranta

Physical parameters	Turbinaria ornata
Colour	Brown
pH	6.5
Chemical parameters	
Copper	2.2
Manganese	1.53
Zinc	1.8
Iron	0.88
Potassium	1.37
Magnesium	16.31
Cobalt	1.103
Sodium	5.3
Phytohormones	
Auxin	2.5
Cytokinin	5.5
Gibberellin	2.8

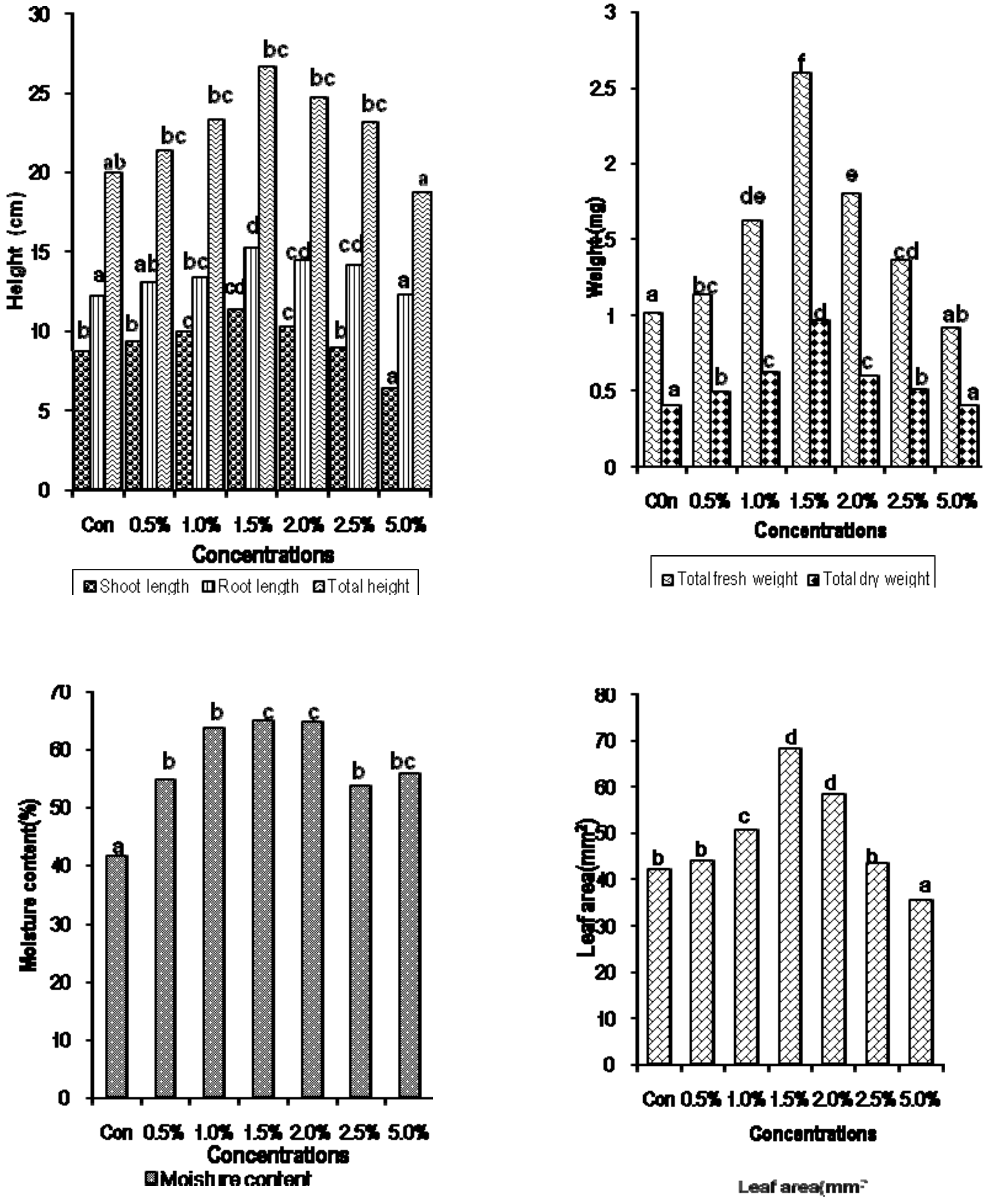
All the parameters given are in mg/L except colour and pH.

In our experiments, use of seaweed liquid extracts of *Turbinaria ornata* significantly promoted the rate of growth and physiology of cluster bean. There was a noticeable increase in growth and biochemical parameters when 1.5% of seaweed liquid extracts of *Turbinaria ornata* applied to cluster bean plant. Higher concentrations (2.0% and above) were found to show inhibiting effect on all the above parameters studied. Total plant height, total fresh and dry weight, leaf area and moisture content were enchanced when 1.5% concentrations of liquid extracts was applied. Further, the retarding effect in growth parameters was corresponding to increase in the concentrations (2.0%, 2.5% and 5.0%) (Fig.1). Statistically significant differences were noticed in total plant height fresh and dry weight, leaf area and moisture content.

Our findings also corroborated with the previous reports made on *Vigna mungo* (Ganapathy selvam and Sivakumar, 2013), *Cajanus cajan* (Mohan *et al.,* 1994; Erulan *et al.,* 2009), *Vigna radiata* (Kumar *et al.,* 1993), *Zea mays, Eleusine coracana* and *Pennisetum typhoides* (Rajkumar Immanuel and Subramanian, 1999), *Dolichos biflorus* (Anantharaj and Venkatesalu, 2002), *Solanum melongena* (Thirumalthangam *et al.,* 2003), *Triticum aestivum* (Zodape *et al.,* 2009), *Abelmoschus esculentus* (Zodape *et al.,* 2008; Sasikumar et al., 2011), *Brassica nigra* (Kalidass *et al.,* 2010), *Lycopersicon esculentum* (Zodape, 2011), *Cyamopsis tetragonoloba* (Thambiraj et al, 2012). Moreover, lower concentration of 1% *Padina boergesenii* extract significantly increased the shoot length, leaf breadth, leaf length, root length and number of roots in *Rhizophora mucronata* (Pise and Sabale, 2010). *Arachis hypogea* plants which received 1.0% SLF of *Ulva lactuca* showed maximum fresh weight, dry weight, root and shoot length, number of branches and leaf area (Sridhar and Rengasamy *et al.,* 2010b). On the contrary, Jothinayagi and Anbazhagan (2009) reported that concentration at 20% of *Turbinaria ornata* promoted shoot length, root length, fresh and dry weight of *Abelmoschus esculentus.* Further, Thirumaran *et al.* (2009) also observed that 20% of *Rosenvigea intricata* extract increased the shoot length, root length, number of lateral root and number of leaves of *Cyamopsis tetragonolaba.*

It has been reported that the growth enhancing potential of seaweed might be attributed to the presence of carbohydrate (Booth, 1965), Phenyl acetic acid (Taylor and Wilkinson, 1977), micro and macro elements (Challen and Hemingway. 1965). The increased growth of these crops may be due to the presence of some growth promoting substances present in the seaweed extract (Mooney and Van staden, 1986; Blunden, 1991). These hormones play an important role in enhancement of cell size and cell division and together they complement each other as cytokinin are effective in shoot formation and auxin in root development, while micronutrient improve soil health. (Liu and Liju, 2011). Several studies have shown that kelp extracts increase nutrient uptake to plants by chelating nutrients due to presence of some organic acids (Jannin et al. 2013; Crouch et al. 1990). Therefore, the higher growth rate in lower concentration of extracts may be directly attributed by the presence of optimum level of essential nutrients and phytohormones as observed during chemical analysis of SLE of *Turbinaria ornata.*

Fig. 1 : Influence of liquid extract of Turbinaria ornata on growth characteristics of cluster bean.



Different letters above each bar indicate a statistically significant difference based on DMRT(p<0.05)

Cluster bean plants when treated with different concentrations of *T.ornata* extracts showed remarkable increase in biochemical parameters also. The amount of photosynthetic pigments, protein, sugar and the activity of nitrate reductase were found to be enhanced in cluster bean plants when 1.5% concentration of extract was given. Other treatments such as 2.0%, 2.5% and 5.0% showed declined trend on the treated plants (Table. 2). This is in accordance with the earlier reports that lower concentrations of seaweed extracts enhanced the biochemical constituents in *Cyamopsis tetragonoloba* (Thirumalthangam *et al.,* 2003), *Citrullus lanatus* (Abdel-Mawgoud *et al.,* 2010), *Abelmoscus esculentus* (Jothinayagi and Anbazhagan, 2009; Sasikumar et al., 2012), *Cajanus cajan* (Erulan *et al.,* 2009), *Brassica nigra* (Kalidass et al. 2010), *Solanum melongena* (Bozorgi et al., 2012) and *Vigna mungo* (Kalaivannan et al., 2012), *Trigonella foenum-graecum* (Pise and Sabale, 2010). On the contrary, seaweed liquid fertilizer at 10% extracted from brown alga *Turbinaria ornata* increased the content of chlorophyll-a, chlorophyll-b, total chlorophyll, protein and total sugars in *Vigna radiata* (Sivasankari *et al.,* 2006). Similarly, Thirumaran *et al.,* (2009) also reported that seaweed liquid fertilizer at 20% enhanced the photosynthetic pigments and carotenoids in *Cyamopsis tetragonoloba*. But, Erulan *et al.* (2009) has observed that lower concentration (0.5%) enhanced the protein content, total chlorophyll content and carotenoids than that of higher concentration (1.5%) in *Cajanus cajan.* Moreover, 1% *Ulva lactuca* extract along with 50% recommended rate of chemical fertilizers enhanced the content of protein, carbohydrate and lipid in *Tagetus erecta.* (Sridhar and Rengasamy, 2010b). But in our case, lower concentrations increased all the biochemical parameters when compared to higher concentration.

The increase in photosynthetic pigments may be due to the presence of betaines (Blunden *et al.,* 1997), increase in number and size of the chloroplast and better grana development (Atzmon and Van Staden, 1994). 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). 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). Moreover, glycinebetaine delays the loss of photosynthesis activity by inhibiting chlorophyll degradation during storage conditions in isolated chloroplasts (Genard et al. 1991).

Table 2: Effect of liquid extracts of T.ornata on biochemical characteristics of cluster bean.

Seaweed extract Treatments	Chl-a (mg./g/fr.wt)	Chl-b (mg./g/fr. wt)	Total chlorophyll (mg./g/fr.wt)	Protein (mg./g/fr.wt)	Reducing sugar (mg./g/fr.wt)	Ascorbic acid (mg./g/fr.wt)	NRA (µ moles No-2/gm.fr.wt/hr)
Con	0.5225 a	0.4200 a	0.9425 a	19.200 b	44.650 a	0.568 a	0.900 a
0.5%	0.6500 a	0.4450 b	1.195 a	24.550 b	57.850 b	0.656 ab	1.090 b
1.0%	0.6650 ab	0.515 c	1.280 c	28.250 c	68.640 c	0.700 c	1.435 d
1.5%	0.975 d	0.710 d	1.685 d	33.30 d	84.10 d	0.775 bc	3.125 e
2.0%	0.805 c	0.530 b	1.335 b	26.625 bc	66.80 c	0.637 ab	1.725 d
2.5%	0.650 c	0.442 b	1.092 a	23.350 a	51.50 b	0.593 a	1.345 c
5.0%	0.590 b	0.3 15 a	0.905 a	20.90 a	39.10 a	0.562 a	0.970 ab

Means sharing within the rows are significantly different ($P \leq 0.05$ level).

Different letters above each bar indicate a statistically significant difference based on DMRT($p<0.05$)

In case of yield attributes, seaweed extracts showed differential responses when applied to cluster bean plants. *Turbinaria ornata* at 1.5% concentration enhanced the fruit weight and fruit number when compared to untreated plants. Inhibitive effect was observed when the plants were treated with higher concentrations (2.0%, 2.5% and 5.0%) (Table 3). Similarly, seaweed extract increased fruit yield when sprayed on tomato plants during the vegetative stage, producing large sized fruits (30% increase in fresh fruit weight over the controls) with superior quality (Crouch and Van Staden, 1992). The number of flowers and seeds per flower head increased (as much as 50% over the control) when marigold seedlings were treated with SWC Kelpak immediately after transplanting (Aldworth and Van Staden, 1987).

Zodape et al.2008 reported that seaweed liquid extract increased length (31.7%) diameter (18.2%) and yield (37.4%) of *Abelsmoshus esculentus* than control. Similarly, foliar application of aqueous extract of *Ulva lactuca,* Turbinaria conoides and *Sargassum polycystum* on cowpea (Ramamoorthy et al. 2006; 2007), *Ulva lactuca* on *Vigna mungo* (Ganapathy selvam & Sivakumar, 2013), *Ascophyllum nodosum* on mango (Ahmed et al. 2013), *Spatoglossum asperum* on *Vigna radiate* (Parthiban et al. 2013), *Saragassum tennerium* on *Dolichos biflorus* (Renuka bai et al. 2013), *Kappaphycus* and *Gracilaria* on *Vigna radiata* (Pramancik et al. 2013); *Kappaphycus alvarezii* on *Glycine max* (Rathore et al. 2009).

Table 3: Effect of liquid extracts of Turbinaria ornata on yield parameters of cluster bean.

Seaweed treatments	Number of clusters/plant	Number of flowers/plant	Number of Pods/plant	Pod length (cm)	Pod weight (g)
Control	3.200 b	4.000 b	2.050 a	6.250 b	3.075 sb
0.5%	4.750 cd	5.000 d	2.500 ab	6.750 cd	3.350 ab
1.0%	5.000 d	5.500 de	3.250 bc	8.250 e	3.750 bc
1.5%	6.250 e	8.750 e	4.500 c	9.650 de	5.725 c
2.0%	5.000 cd	4.750 cd	2.500 ab	6.750 cd	4.125 bc
2.5%	3.500 b	3.500 ab	2.500 ab	5.500 b	3.500 ab
5.0%	2.500 a	3.000 a	1.750 a	3.750 a	2.600 a

Means sharing within the rows are significantly different ($P \leq 0.05$ level).

Different letters followed in each row statistically significant based on DMRT

In many crops, yield is associated with the number of flowers at maturity. As the onset and development of flowering and the number of flowers produced are linked to the developmental stages of plants, seaweed extracts, probably encourage flowers by initiating robust plant growth. Yield increases in seaweed extract treated plants are thought to be associated with hormonal substances present in the extracts, especially cytokinins (Featon-by Smith and Van Staden, 1984). Cytokinins have been implicated in nutrient mobilization in vegetative plant organs (Gersani and Kende, 1982) as well as reproductive organs (Davey and Vanstaden, 1978). Such a response indicates the seaweed extracts are involved either in enhancing the mobilization of cytokinins from the roots to the developing fruit or more likely by improving the amount or synthesis of endogenous fruit cytokinin (Hahn et al. 1974). This increase in cytokinin availability will eventually result in a greater supply of cytokinins to the maturing growth. We can conclude that cytokinins present in *Turbinaria ornata* extracts could have triggered the mobilization of nutrients from other vegetative parts and induced the synthesis of endogenous cytokinin for fruit setting when applied as foliar spary..

In general, low concentrations of liquid extracts of seaweed extracts had maximum positive influence on growth, biochemical and yield characteristics of cluster bean and cluster bean plants as reported in previous studies (Van Staden *et al.,*1994; Zahid, 1999; Nedumaran and Perumal, 2005; Sylvia *et al*., 2005; Xavier and Jesudass,, 2007; Ramamoorthy *et al.,* 2007 and Sethi and Adhikary, 2008 ; Ganapathy selvam &

Sivakumar, 2013; Ahmed et al. 2013; Parthiban et al. 2013; Bai et al. 2013; Pramancik et al. 2013) due to the presence of micro and macro elements, growth hormones and vitamins. It is in agreement that the liquid extracts of brown algae may yield better promising results than green algae (Subramanian and Kannathasan, 1987). Further, presence of phycocolloids and other ingredients in brown algae may be responsible for better enhancement in growth and yield production. It has also been reported that the brown algal extracts were more effective in enhancing the growth of *Cyamopsis tetragonoloba, Lablab purpuriensis* and *Arachis hypogea* (Thevanathan *et al.,* 2005).

Brown macroalgae, with Ascophyllum, Ecklonia, Fucus, Laminaria, and Sargassum as main genera, are widely used in crops as PBs for their plant-growth promoting benefits, abiotic stress resistance, and improved postharvest quality and shelf-life (Vernieri et al., 2006; Khan et al., 2009; Craigie, 2011; Rouphael et al., 2017b). The beneficial effects of SWE may be attributed to several growth enhancing mechanisms like (i) physiological (delayed senescence) and biochemical changes (increased micronutrients), (ii) improved WUE (improved stomatal conductance and rootto- shoot ratio), (iii) differential regulation of genes (CBF3, SOS, RD22), and (iv) rhizosphere effects (increased activity of rhizobacteria and mycorrhizae) (Battacharyya et al., 2015).

Magnesium is one of the main constituent in chlorophyll molecule structure. It plays a vital role in synthesis of chlorophyll molecule. In our chemical analysis of seaweed extract, magnesium was found to be abundant when compared to other elements. It may be concluded that presence of magnesium might have triggered the chlorophyll synthesis in the plant and subsequently better growth have been observed. Dhargalkar and Untawale (1983) observed that out of several seaweed extracts, *Turbinaria ornata* extract gave maximum growth, mean weight, girth of bulb, mean length and breadth of leaves in *Vicia faba* and also concluded that brown algae can be used in preparation of seaweed liquid fertilizers better than any other algae.

Conclusions

It may be concluded that the growth and biochemical characteristics of vegetable crop *Cyamopsis tetragonoloba* could be promoted by the presence of micro and macro elements, growth hormones, vitamins etc. in the SLE of *Turbinaria ornata.* Cytokinin and magnesium, which are considered as essential growth promoting constituents in chlorophyll biosynthesis might have played a vital role in enhancement in growth and physiology of cluster bean. However, optimum concentration of seaweed liquid extracts is necessary to increase crop growth and productivity are yet to be examined. The study also emphasizes that seaweed extracts can be effectively used as organic biostimulants to the plants and also an eco-friendly approach towards organic farming.

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