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RESEARCH ARTICLE

RESPONSE OF *VIGNA UNGUICULATA* ON LIQUID SEAWEED FERTILIZER

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ABSTRACT

In the present investigation, an attempt has been made to study the effect of different concentrations of liquid seaweed fertilizer of *Ulva lactuca* on morphological and biochemical changes of *Vigna unguiculata L.* was assessed. The liquid seaweed fertilizer of *Ulva lactuca* is having a higher amount of organic and inorganic elements. The physic-chemical analysis of aqueous extracts is light brown in colour and it was rich in calcium, magnesium, sodium, potassium, chloride, sulphate, copper, phosphorus, nitrate and lead. Different concentration of Seaweed Liquid Fertilizer was used and the morphological parameters like root and shoot length, fresh weight showed a decreasing trend with increase in seaweed liquid fertilizer concentration with compare to control plants. Chlorophyll a, b and total sugars, protein and amino acids were decreased at higher concentrations. The study reverals that lower concentration liquid seaweed fertilizer was beneficial for the overall growth of the crop plant.

INTRODUCTION

Seaweeds are one of the commercially important marine living renewable resources. From the time immemorial the macroscopic marine algae have been closely associated with human life and are being exhaustively used in numerous ways as a source of food, feed, fertilizer, medicine and chiefly for economically important phycocolloids. Seaweeds occur in the intertidal and deep shallow water of the sea and also in estuaries and back water. Several species of green, brown and red algae with luxuriant growth occur along the Southern Tamil Nadu Coast use of seaweeds as manure is a common practice in coastal areas throughout the world. They are rich in potassium but poor in nitrogen and phosphorus them the farm manure (Kingman and Moore, 1982). Seaweeds have been used as manure in agricultural field particularly for paddy and coconut plantations. The highest amount of water soluble potash, other minerals and trace elements are present in seaweeds and are readily absorbed by plants and they control deficiency diseases (Crough and Van Staden, 1993). The carbohydrate ad other organic matter present in seaweeds alter the nature of soil and improve its moisture retaining capacity. Recent researchers proved that seaweed fertilizer are better than other fertilizer the natural seaweed products as substitutes to the conventional inorganic fertilizers assumed importance (Gangatharan 1998).

They have proved effective in enhancing yield, pest and frost resistance in vegetables, fruits, flowers, cereals and pulses. Seaweed extracts had beneficial effect on seed germination and plant growth (Anandharaj and Venkatesalu, 2002). The liquid extract from green seaweeds contains cytokinins (Beckett and Van Staden, 1990). In the present investigation, the effect of Liquid Seaweed Fertilizer from *Ulva lactuca* on growth of *Vigna unguiculata L*. was studied.

MATERIALS AND METHODS

The seaweed used in the present study was collected from Sunnambar Estuary, Pondicherry, and South East Coast of India (Lat. 11 ° 45N; Long 76° 50E). They were hand picked and washed thoroughly with estuarine water and finally with fresh water to remove all the unwanted impurities, epiphytes and adhering sand particles. They were shade dried for 4 days followed by oven drying for 24 hours at 60°C. The oven dried material of Liquid Seaweed Fertilizer [LSF] as per the method of Rama Rao (1990).

Preparation of plant materials

The Liquid Seaweed Fertilizer were analysed for various physico- chemical properties as per the method mentioned in America Public Health Association [APHA, 1992] are shown in (Table 1). Healthy selected *Vigna unguiculata* L. seeds were surface sterilized with 0.1% mercuric chloride ($Hg Cl_2$) for 1 minute and then thoroughly washed with distilled water. Twenty seeds were placed in each petriplate and irrigated with equal volume of different concentrations of LSF (0.25, 0.50,

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0.75, 1.00, 1.50 and 2.00 percent) prepared using in distilled water. Distilled water was used as control. The experiment was run in triplicate for 10 days. At the end of this period root length, shoot length and fresh weight of root and shoot were recorded.

Chlorophyll Determination: Plant material from harvested seedlings was homogenized with the addition of 10 mg CaCO3 and 3 ml 80% acetone (Merck) after they were measured for their fresh weights. The volume of the supernatant was measured after 15- minute centrifugation at 3000 g. The absorption values of raw chlorophyll extract at wavelengths of 645 and 663 nm were used to determine the total chlorophyll amount was calculated as mg/g fresh weight Arnon (1949). as follows:

Total Chlorophyll Amount = (20.2 x D645) + (8.02 x D663)

Total Protein Determination: Dye-binding method was employed in the determination of total proteins (Bradford, 1976). The experimental materials were homogenized in 0.1M phosphate buffer (pH 7.0) with the proportion of 100 mg fresh weight/ml, and then the extracts were centrifugated for 45 minutes at 13000 rpm. 0.1 ml of supernatants was added to 5 ml of ¼ diluted Coomassie Brillant Blue G-250 (Merck) (Bio-Rad) and vigorously mixed. After keeping it in the dark for 15 minutes, the absorption of the protein in the extract against blank at 595 nm was spectrophotometrically measured and calculated as µg protein/ml. Bovine Serum Albumin (BSA) was used as standard.

Estimation of Total Sugar: Reducing sugars and non-reducing sugars were estimated by Nelson-Somoyogi method (Nelson, 1944) Plant samples were treated with 80 per cent boiling ethanol for taking extractions (5ml extract representing 1g of tissue). Five readings for each sample were taken. One ml of ethanol extract taken in the test tubes was evaporated in a water bath. To the residue, 1 ml of distilled water and 1 ml of 1N sulphuric acid were added and incubated at 49˚C for 30 minutes. The solution was neutralized with 1N sodium hydroxide using methyl red indicator. One ml of Nelson's reagent was added to each test tube prepared by mixing reagent A and reagent B in 25:1 ration (Reagent A: 25g sodium carbonate, 25g sodium potassium tartarate, 20g sodium bicarbonate and 200g anhydrous sodium sulphate in 1000ml: Reagent B: 15g cupric sulphate in 100ml of distilled water with e drops of concentrated sulphuric acid) was added. The test tubes were heated for 20 minutes in a boiling water bath, cooled and 1ml of arsenomolybdate reagent (25g ammonium molybdate, 21ml concentrated sulphuric acid, 5g sodium arsenate dissolved in 475 ml of distilled water and incubated at 37˚C in a water bath for 48 hours) was added. The solution was thoroughly mixed and diluted to 25 ml and measured at 495 nm in a spectrophotometer. The reducing sugar contents of unknown samples were calculated from glucose standard.

Estimation of Total Free Amino Acids: The Free Amino Acids was determined by the method of Moore and Stein, 1948. One ml ethanol extract was taken in 25 ml test tubes and neutralized with 0.1N sodium hydroxide using methyl red indicator. Oned ml of ninhydrin reagent was added (800mg stannous chloride in 500 ml citrate buffer, pH 5.0, 20 g ninhydrin in 500 ml methyl cellosolve; both solutions were mixed). The contents were boiled in a water bath for 20 minutes, 5 ml of diluents solution (distilled water and n-propanol mixed in equal volum) was added, cooled and diluted to 25 ml with distrilled water. The absorbance was measured at 570 nm in a spectrophotometer. The standard graph was prepared using leucine.

RESULTS

The liquid seaweed fertilizer was brown in colour and *Ulva lactuca* was light green the P^H value of the aqueous extracts was 6.39 (*Ulva lactuca*). The aqueous extracts contained high amount of calcium, potassium, phosphorus, magnesium, sodium, chloride, sulphate, copper, nitrate and lead. The growth as reflected in root and shoot length, fresh weight of root and shoot of cowpea plant *vigna unguiculata* increased with the 0.75 percentage of liquid seaweed fertilizer furnished in (Table 1 and 2).

Table 1. Physico - Chemical properties of liquid seaweed fertilizer of Ulva lactuca

S.No.	Parameters	*Ulva lactuca*
1	P^H	Light brown
2	Colour	6.39
3	Calcium	135.0
4	Magnesium	318.10
5	Sodium	325.0
6	Potassium	102.18
7	Chloride	1020.0
8	Sulphate	312.21
9	Lead	1.3
10	Copper	2829.08
11	Phosphorus	101.0
12	Nitrate	204.68

All the parameters are expressed in mg/l except colour and P^H.

The highest values of shoot and root length, and the fresh weight of root and shoot were observed at 0.75% of the LSF. The application of LSF increased the chlorophyll 'a', 'b' and total chlorophyll in most concentrations, which were generally higher than the control and the highest sugar, protein and free amino acid were observed in plants treated with 0.75% LSF (Table 3). It is evident from the result that the increased growth (root and shoot length, fresh weight of root and shoot) and biochemical constituents (Chlorophyll 'a', 'b', total chlorophyll, total suger, protein, free amino acids) are possible due to the LSF which induced absorption of essential nutrients and the related increased enzyme activity. The present finding will be useful to the agriculturalists for utilizing seaweeds as fertilizer and making use of the rich natural seaweed resources available in our coast for this purpose.

DISCUSSION

Similar results were recorded with LSF of *Sargassum plagiophyllum* which enhanced maximum seedling growth in greengram and blackgram at 0.75% and 1.50% concentrations respectively (Venkataraman Kumar et al, 1993) and black gram showed maximum seedling growth at 1.0% concentration (Venkataraman kumar and Mohan, 1997) and in groundnut maximum seedling growth at 1.0% concentration (Nedumaran, 2002).

Table 2. Effect of different concentration of LSF [Liquid Seaweed Fertilizer] on root and shoot length ((cm), fresh weight root and shoot [mg/gf.wt] in *Vigna unguiculata* L

Parameters	Concentration in Percentage (%)						
	Control	0.25	0.50	0.75	1.00	1.50	2.00
Root length	1.3 ± 0.06	1.4 ± 0.07	1.8 ± 0.16	2.4 ± 0.12	1.8 ± 0.09	1.8 ± 0.09	1.6 ± 0.08
Shoot length	5.0 ± 0.25	5.5 ± 0.27	5.8 ± 0.29	6.6 ± 0.33	6.0 ± 0.30	5.8 ± 0.29	5.4 ± 0.27
Fresh weight of Root	10.3 ± 0.51	13.3 ± 0.66	15.7 ± 0.78	17.3 ± 0.86	16.9 ± 0.84	16.1 ± 0.80	11.1 ± 0.55
Fresh weight of Shoot	87.2 ± 4.36	89.1 ± 4.45	92.1 ± 4.60	98.1 ± 4.90	96.3 ± 4.81	91.9 ± 4.59	89.3 ± 4.46

Table 3. Effect of Liquid Seaweed Fertilizer [LSF] of Ulva lactuca on chlorophyll 'a' , 'b' and total chlorophyll [mg/g f.wt], total sugar [mg/g f.wt], protein [mg/g f.wt] and free amino acid [mg/g f.wt] of *Vigna unguiculata* L

Parameters	Concentration in Percentage (%)						
	Control	0.25	0.50	0.75	1.00	1.50	2.00
Chlorophyll 'a'	0.124 ± 0.006	0.136 ± 0.006	0.142 ± 0.007	0.168 ± 0.008	0.146 ± 0.007	0.138 ± 0.006	0.130 ± 0.006
Chlorophyll 'b'	0.225 ± 0.011	0.240 ± 0.012	0.269 ± 0.013	0.292 ± 0.014	0.280 ± 0.014	0.254 ± 0.012	0.239 ± 0.011
Total Chlorophyll	0.363 ± 0.018	0.389 ± 0.019	0.402 ± 0.020	0.428 ± 0.021	0.408 ± 0.020	0.392 ± 0.019	0.374 ± 0.018
Total Sugar	2.318 ± 0.015	3.561 ± 0.178	4.120 ± 0.206	5.007 ± 0.250	4.155 ± 0.207	3.743 ± 0.187	2.263 ± 0.113
Protein	13.163 ± 0.658	14.116 ± 0.705	15.210 ± 0.760	16.276 ± 0.813	15.337 ± 0.766	14.198 ± 0.709	13.212 ± 0.660
Free Amino Acids	9.593 ± 0.479	10.286 ± 0.514	11.120 ± 0.556	12.386 ± 0.619	11.232 ± 0.561	10.410 ± 0.520	9.780 ± 0.489

± Standard deviation

Plants treated with LSF showed difference in growth responses which is in agreement with the earlier observations of Bhosle et al. (1975) that different seaweed extracts induce different growth responses. The increased seedling growth may also be due to the presence of phenyl acetic acid [PAA] and other closely related compounds [P-CH-PAA] in the LSF (Taylor and Wilkinson, 1977). It has been suggested that the growth promoting activity of seaweed extracts was due to macro and micro elements as well as growth promoting substances like cytokinin [Sridhar and Rengasamy, 2002].

This is in accordance with the earlier reports that the application of *Enteromorpha intestinalis* extract increased the chlorophyll contents of *Sesamum indicum* (Gandhiyappan and Perumal, 2001) and *Caulerpa scalpelliformis* and *Gracilaria corticata* LSF increased the chlorophyll contents of *Cyamposis tetragonoloba* (Thirumal Thangam et al., 2003).The enhanced leaf chlorophyll concentration of plants treated with LSF may be due to the presence of betains (Whapham et al., 1993; Blunden et al., 1997). In *Vigna mungo* (Venkataraman Kumar and Mohan, 2000) however, plants treated with LSF showed low chlosophyll contents.

The total sugar and protein contents in *Vigna catajung* and *Dolichos biflorus* showed a significant increase due to seaweed application (Anantharaj and Venkatesalu, 2001), Carbohydrate, protein and lipid contents in *Cyamposis tetragonoloba* increased due to LSF application (Thirumal Thangam, 2003). The increase in the protein content was due to the absorption of most of the necessary elements by seedlings (Rajkumar Immanuel and Subramanian, 1999).

CONCLUSION

In the present study, the 0.75% concentration of Liquid Seaweed Fertilizer of *Ulva lactuca* showed best results in *Vigna unguiculata*. The seaweed extracts were found to be more effective than the chemical fertilizer in promoting the growth of seedlings.

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