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Effect of foliar spray from seaweed liquid fertilizer of *Ulva reticulata* (Forsk.) on *Vigna mungo* L. and their elemental composition using SEM- energy dispersive spectroscopic analysis

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ABSTRACT

Objective: To identify the effect of seaweed liquied fertilizer (SLF) of *Ulva reticulata*, as biochemical characteristics of *Vigna mungo* as well as leaf morphometric analysis such as epidermal and stomata cell variation and distribution of minerals in the leaf. **Methods:** Experiments were conducted on black gram to study the potential green alga of *Ulva reticulata* as a biofertilizer. The seeds were sown in soil and SLF were added to soil bed in five different concentrations separately (1%, 2%, 4%, 6% and 8% w/v). **Results:** Seaweed extract was applied as a foliar spray, the SLF treated plants show maximum growth in 2% of SLF among the various experimental concentrations as well as control. Biochemical profiles like chlorophyll a and b, protein, sugar and starch were found to be higher at 2%. A significant increase in the number of epidermal and stomata cells were observed in 2% SLF treated plants. Whereas at higher concentrations of SLF such as 4%, 6%, and 8% the values of all the parameters were significantly decreased than in the control group. Further the leaf of 2% SLF treated *V. mungo* have subjected to Scanning Electron Microscopy with Energy Dispersive Spectroscopic analysis it reveals that thepresence of ten elements in the following order: Ca>P>N>Na>K>Mg>Mn>S>Fe>Zn in treated and Ca>N>P>Na>Mg>Mn>K>Zn>S>Fe in control plant. The data generated from study reveal that SLF of *U. reticulatea* could be used as foliar spray at low concentration of 2% to maximize the growth and yield of *V. mungo* and also increase the number of stomata in the leaf. **Conclusion:** The main objective of study result would be the manorial requirement for organic forming and serve as a cost effective ecofriendliness for sustainable agriculture and environment.

1. Introduction

Seaweed extract is a new generation of natural organic fertilizers containing highly effective nutritious and promotes faster germination of seeds and increase yield and resistant ability of many crops. Unlike, chemical fertilizers, extracts derived from seaweeds are biodegradable, non-toxic, nonpolluting and non-hazardous to humans, animals and birds [1]. 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, also rich in growth regulators [2]. The growth promoting effect of liquid extracts of seaweeds on seed germination [3, 4], vegetative growth [5] and biochemical characteristics [6] in agricultural crops have been reported. Much work has been carried out on determining the effect of SLF on black gram [5,6], *Dolichous* [7], cereals and millets [8]. But there was no report on analyzing distribution of elemental composition of plant body against application of SLF using Energy Dispersive Spectroscopic analysis (EDS). Seaweed extracts from *Sargassum wightii (S. wightii)* and *Kappaphycus alvarezii (K. alvarezii)* have been found to increase the yield of *Vigna sinensis* and *Phaseolus radiata (P. radiata)* [9]. The marine ecosystem is the treasure place for many natural resources [10].

Efforts have been made to enhance growth and yield of tomato plants and to improve lycopene and vitamin C content of fruits, by treatments with aqueous extract of *Sargassum johnstonii (S. johnstonii)* [11]. Seaweed manures have the advantage of being free from weeds and pathogenic fungi. Liquid extracts of brown algae are being sold as biostimulants or biofertilizers in various brand names.

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Promising increased crop yield, nutrient uptake, resistance to frost and stress, improved seed germination of reduced incidence of fungal and insect attack have been resulted by application of SLF. Seaweeds are known to contain appreciable quantities of plant growth regulators [12], cytokinin [13], IAA [14], gibberellins and gibberellin like substance [5,15, 16]. Hence marine algae, particularly seaweeds have a vital role to play in agriculture, especially in the third world country where irrational use of chemical fertilizer and pesticides is a cause of concern. Extensive regional tribals would need to be conducted with the product to determine the environmental limits on biological activity and monitor the survival and dispersal of the inocula [17]. Hence use of modern agriculture in conjunction with traditional farming practices is the sustainable solution for the future. Seaweed extracts are marketed as liquid fertilizers and biostimulants since they contain many growth regulators such as cytokinins [18], auxin [19]. The beneficial effect of seaweed extract application is as a result of many components that may work synergistically at different concentrations, although the mode of action still remains unknown [20]. However, application of seaweed extract increased chlorophyll content [21, 22]. Foliar spray application of mineral nutrients offers a quicker method of supplying nutrients to higher plants than methods involving root application. The preferential mode for foliar absorption of nutrient elements is still under debate. Recently, some authors pointed out the possibility of an active uptake through stomata pores instead of cuticular uptake [23, 24].

The main objective of the present study was to evaluate whether marine bioactive substances should be able to enhance mineral uptake by leaves when foliar fertilization in Blackgram (*Vigna mungo L.*). The effect of SLF of *Ulva reticulata (U. reticulata)*, as biochemical characteristics of *Vigna mungo (V. mungo)* as well as leaf morphometric analysis such as epidermal and stomata cell variation and distribution of minerals in the leaf.

2. Materials and methods

2.1. Collection of seaweeds

The marine green seaweed *U. reticulata* was collected during November 2011 from Tuticorin coastal region in Gulf of Mannar (Lat. 8° 45′N: Long. 78°10′E) located in the southeast coast of Tamil Nadu. The seaweeds were handpicked and washed thoroughly with seawater to remove all the unwanted impurities, epiphytes and adhering sand particles.

2.2. Preparation of seaweed liquid fertilizer

The washed and cleaned seaweeds were shade dried for (27 ℃ to 30 ℃) 10 days followed by oven drying for 48 h at 60 ℃. The oven dried material was then grounded with the help of Mixer grinder. The powdered seaweed samples were stored in the airtight container for the future use. To 500 g of powdered seaweeds 5 000 mL of water was added and contents were heated for 45 min at 60 ℃ in a plugged conical flask [25]. After cooling, the contents were filtered through four muslin cloth layers. The filtered recovered (3.075 mL) was used as 10% (*w/v*) aqueous seaweed extract, from which five different concentrations, 0% (control), 1% , 2%, 4%, 6%, and 8% (*w/v*) were prepared using double distilled water. The physical observations such as colour, pH and elements viz., calcium, magnesium, sodium, potassium, iron, phosphate, chloride, sulphate, zinc, copper and nitrate were estimated using atomic absorption spectrophotometer [26].

2.3. Experimental design and treatments

The certified seeds of *V. mungo* were procured from Regional Pulses Research Station, Tamil Nadu Agricultural University, Coimbatore. They were surface sterilized with 0.1 % mercuric chloride and then sown in soil field. Experimental trail was conducted at Botanical garden, Annamalai University, Annamalainagar on *V. mungo* seed were sown in earthenware in (4 m × 3 m) plot. One or two seed were sown along a side of the ridges at 30 cm spacing. For each experiment ten plants per row was taken. Five treatments were given to the field plants namely foliar spray of 1 %, 2%, 4%, 6%, and 8% of aqueous seaweed extract. In each of the foliar treatment, 100 mL aqueous extract was applied. The first spray treatment was given to 15–day–old seedlings. Thereafter, three sprays at interval of 15 days each were given up to 60 days. The control set was treated only with distilled water as foliar spray. Growths parameters like shoot length, root length and leaf area were recorded at 15th, 30th, 45th and 60th day.

2.4. Biochemical analysis

The biochemical parameters the amount of chlorophyll pigments [27], protein [28], reducing sugar [29], non–reducing sugar [28] and starch [30] were recorded at 45th day in treated and control of leaf.

2.5. Epidermal study

Epidermal peels were obtained uniformly from the fourth leaf of the treated and control of field experiment plants by mechanical means and were stained with aqueous safranin (1%). They were mounted in 50% glycerine and sealed with DPX. The variations in epidermal cells and stomata cells among the treated and control was observed under light microscope as well as Scanning Electron Microscope (SEM).

2.6. Yield parameters

The yield parameters such as number of days for flowering, number of pods per plant, length of pods, number of seeds/ pod and number of days for maturity of SLF treated V. mungo were recorded at 60th day. The results were statistically analyzed using ANOVA.

2.7. Scanning electron microscope with EDS studies

The scanning electron microscopy of seaweed was done[31]. Reagents inoluded Glutaraldehyde Phophatate buffer (pH 6.8) Alcohol. Leaves of SLF treated V. mungo were fixed in primary fixative 3% glutaraldehyde. The fixed sample were given 3 washes thoroughly in 0.1 M phosphate buffer (pH 6.8) they were dehydrated through a graded series of alcohol 10–15 minutes interval at 4 ℃ upto 70%. Then 90% and 100% alcohol were kept in room temperature at 2–3 hr interval. Then dehydrated samples treated with critical point drier (CPD) were on a stub and the specimens were examined with joel JSM–56010 with INSA–EDS and electromicrograph were taken selectively from the computer screen [31]. Electron micrographs were taken selectively from the computer screen. Simultaneously, the selected electron micrographs of leaf portion of adaxial layer from 2% SLF treated and control plants were subjected to Energy Dispersive Spectroscopic analysis. This was conducted with an EDS 700 series interfaced with a data general NOVA2 computer and a Texas

instrument silent 700 ASR. The EDS X-ray spectrometer was interfaced with a scanning electron microscope (20 KV) stage. The area of different components such as epidermal portion and cellular inclusion were analyzed. To find out the fluxes, both the count per second (S-1 or CPS) value and theapparent relative atomic percentage of weight particular mineral in different composition details was documented.

3. Results

Physico-chemical properties of SLF of *U. reticulata* before preparation of different concentrations were shown in Table 1. In SLF treated plants, the growth and biochemical responses were found in accordance with concentrations of SLF sprayed on *V. mungo*. The growth parameters such as shoot length, root length, and leaf area were increased up to 2% SLF treatments thereafter decreased up to 8% SLF treatments, whereas control plants showed lowest value in all growth parameters comparatively (Table 2).

Table 1
Physico-chemical analysis of *U. reticulata* SLF.

Parameters	Values
Colour	Green
pH	7.02
Calcium (mg/L)	158.52
Magnesium (mg/L)	108.25
Sodium (mg/L)	295.08
Potassium (mg/L)	175.20
Iron (mg/L)	5.22
Phosphate (mg/L)	45.05
Chloride (mg/L)	2 240.56
Sulphate (mg/L)	55.42
Zinc (mg/L)	1.25
Copper (mg/L)	1.15
Nitrate (mg/L)	124.85

The SLF of *U. reticulata* enhanced the amount of chlorophyll a and b and total chlorophyll content in the SLF concentrations below 4% as compared to control and other treatments. Significantly there is increase in the levels of reducing and non reducing sugars, starch and protein contents in 2% SLF treated plants comparing with other treatments and control (Table 3).

Epidermal cells (Non-costal) of both surfaces were large, sometimes small, irregularly distributed, anisodiametric, sometime tending to become isodiametric; walls thin and arched or slightly sinuous, stomata are sub-circular to oval, irregularly distributed, found on both surface (epiamphistomatic) (Figure 1 & 2). The adaxial leaf epidermal cell showed increase in stomal size, frequency of stomata and epidermal cells. Similarly, the abaxial surface also showed an increase in the size and frequency of epidermal cells and frequency of stomata. The paracytic types of stomata were found as predominant irrespective to treatments and control. But the size and frequency of stomata was higher on abaxial side than adaxial side of the leaf (Table 4). The maximum number of stomata was recorded in 2% SLF treated plants and the lowest value in control. Even though a decreasing trend was observed above 2% SLF treated plants but treated plants exhibited higher number of stomata than control (Figure 1-6).

The results obtained from the EDS analysis of *V. mungo*, different chemical elements present in the cell wall of 2% SLF treated leaf and control were shown in figure. 7. The number of days for maturity was longer in the SLF treated plants than control. But the lowest value (56 days) was recorded in 8% SLF treated plant when compared to 1%, 2%, 6% and 4% SLF treated plants. Among the treated plants, the 2% SLF treated plants showed earlier flowering followed by 4%, 6% and 8%. But the longer duration (29 days) of number of days for flowering was recorded in control as well as 8% SLF treated plants. The values of number of pods per plant, length of pods and number of seeds were higher in 2% SLF treated and lower value in control. But SLF treated plants showed higher value than control irrespective to their concentration were shown in Table 5. Totally ten elements namely N, P, K, Ca, S, Na, Mg, Mn, Zn and Fe were observed. Among the elements, maximum value of Ca was detected followed by P and N both in control and 2% SLF treated plants. But the higher value of N, lower value of P and almost similar value of Ca were recorded in 2% SLF than control. The order of chemical elements from epidermal portion of the leaf of SLF treated *V. mungo* and control was as follows; Ca>P>N>Na>K>Mg>Mn>S>Fe>Zn, Ca>N>P>Na>Mg>Mn>K>Zn>S>Fe respectively.

Table 2
Effect of different concentration of *U. reticulata* seaweed liquid fertilizer on shoot, root length (cm) and leaf area (mm^2) in *V. mungo*.

SLF Con.	15th day			30th day			45th day			60th day		
	Shoot length	Root length	Leaf area	Shoot length	Root length	Leaf area	Shoot length	Root length	Leaf area	Shoot length	Root length	Leaf area
Control	12.72±0.07	8.05±0.04	18.84±0.09	16.22±0.08	13.54±0.06	25.65±0.15	25.12±0.15	16.34±0.08	37.25±0.22	31.62±0.15	20.14±0.12	40.24±0.24
1%	13.20±0.09	9.06±0.06	26.95±0.16	23.05±0.13	15.96±0.95	34.25±0.20	28.05±0.19	20.28±0.12	44.85±0.26	38.23±0.22	23.69±0.14	46.63±0.27
2%	15.35±0.07	11.15±0.05	37.45±0.18	25.53±0.12	16.20±0.09	46.34±0.23	30.35±0.15	20.85±0.12	52.38±0.36	41.25±0.28	25.45±0.17	55.98±0.33
4%	15.05±0.10	10.26±0.06	35.34±0.21	23.25±0.13	16.08±0.09	43.48±0.26	29.72±0.17	18.95±0.11	50.64±0.30	37.28±0.26	25.05±0.17	52.65±0.36
6%	14.86±0.08	9.85±0.05	23.76±0.11	20.68±0.12	14.85±0.07	32.52±0.22	28.25±0.14	18.22±0.09	44.47±0.26	32.86±0.18	22.85±0.13	45.55±0.27
8%	13.95±0.06	7.98±0.04	20.25±0.10	17.15±0.08	13.98±0.06	28.15±0.19	26.45±0.13	17.65±0.08	39.35±0.23	32.12±0.16	21.89±0.10	42.38±0.25

Values are mean ± SD; Sample (n) =6

Table 3

Effect of *U. reticulata* seaweed liquid fertilizers (SLF) on biochemical parameters of leaf of *V. mungo* (mg/g fr. wt.) at 45th day.

SLF Con.	Chlorophyll 'a'	Chlorophyll 'b'	Total chlorophyll	Starch	Reducing sugar	Non-Reducing sugar	Total sugar	Protein
Control	0.872±0.003	0.623±0.003	1.495±0.009	11.53±0.07	4.23±0.02	19.40±0.01	23.63±0.14	14.12±0.08
1%	0.925±0.004	0.675±0.004	1.600±0.009	14.34±0.08	4.84±0.02	21.26±0.10	26.10±0.15	20.68±0.10
2%	1.145±0.006	0.695±0.004	1.840±0.009	17.99±0.10	5.28±0.02	23.52±0.14	28.80±0.16	28.95±0.17
4%	1.069±0.006	0.675±0.003	1.744±0.008	16.86±0.06	5.07±0.02	21.07±0.10	26.14±0.10	25.57±0.12
6%	0.985±0.005	0.643±0.002	1.628±0.010	16.05±0.06	4.95±0.01	20.96±0.10	25.91±0.09	21.37±0.10
8%	0.920±0.005	0.638±0.002	1.528±0.008	13.45±0.05	4.54±0 .01	20.78±0.08	25.32±0.09	16.57±0.06

Values are mean ± SD; Sample (n) = 6.

Table 4

Effect of different concentration of *U. reticulata* seaweed liquid fertilizers (SLF) on epidermal and stomata cells variation in *V. mungo* (45th day).

SLF Con.	Epidermal cells				Stomata cells			
	size (μm)		Frequency		Size (μm)		Frequency	
	AD	AB	AD	AB	AD	AB	AD	AB
Control	(40.00 ± 0.25) ×(19.00 ± 0.11)	(40.00 ± 0.28) ×(16.00 ± 0.11)	760.00 ± 4.42	640.00 ± 5.21	(20.00 ± 0.12) ×(12.00 ± 0.07)	(20.00 ± 0.12) ×(11.00 ± 0.07)	240.00 ±0.99	220.00 ± 0.56
1%	(42.00 ±0.22) ×(21.00 ±0.10)	(39.00 ± 0.23)× (20.00 ± 0.12)	882.00 ± 5.53	780.00 ± 4.60	(21.00 ± 0.10) ×(12.00 ±0.09)	(20.00 ± 0.16) × 12.00 ± 0.12)	252.00 ± 0.84	240.00 ± 0.69
2%	(44.00 ± 0.36) ×(22.00 ± 0.16)	(42.00± 0.21) ×(21.00 ± 0.12)	968.00 ± 5.80	882.00 ± 4.14	(22.00 ± 0.14) ×(14.00 ±0.10)	(21.00 ± 0.14) ×(12.00 ± 0.10)	308.00 ± 1.30	252.00 ± 0.81
4%	(44.00 ± 0.31) ×(19.00 ± 0.11)	(41.00 ± 0.24) ×(21.00 ± 0.12)	836.00 ± 4.66	861.00 ± 4.02	(21.00 ± 0.13) ×(14.00 ± 0.10)	(21.00 ± 0.15) ×(11.00 ± 0.10)	294.00 ± 1.06	231.00 ± 0.64
6%	(42.00 ±0.26) ×(20.00 ± 0.12)	(39.00 ± 0.19) ×(19.00 ± 0.11)	798.00 ± 4.59	741.00 ± 3.93	(19.00 ± 0.12) ×(13.00 ± 0.08)	(19.00 ±0.15) ×(11.00 ± 0.09)	247.00 ± 0.84	209.00 ± 0.84
8%	(39.00 ± 0.24) ×(19.00 ± 0.11)	(38.00 ± 0.19) ×(18.00 ± 0.09)	741.00 ± 4.45	684.00 ± 3.78	(18.00 ± 0.11) ×(13.00 ± 0.08)	(18.00 ± 0.11) ×(10.00 ± 0.08)	234.00 ± 0.79	180.00 ± 0.59

AD-Adaxial side, AB-Abaxialside.

Table 5

Effect of different concentration of *U. reticulata* SLF on yield parameters of *V. mungo*.

S. No	Number of days for flowering	Number of pods/plant	Length of pods (cm)	Number of seeds/pod	No. of days for maturity
Control	29	32	5.25	6	53
1% SLF	28	33	5.50	9	57
2% SLF	26	35	7.00	10	57
4% SLF	27	34	6.00	9	60
6% SLF	28	32	5.00	8	58
8% SLF	29	30	5.00	7	56

Values are average of 50 plants.

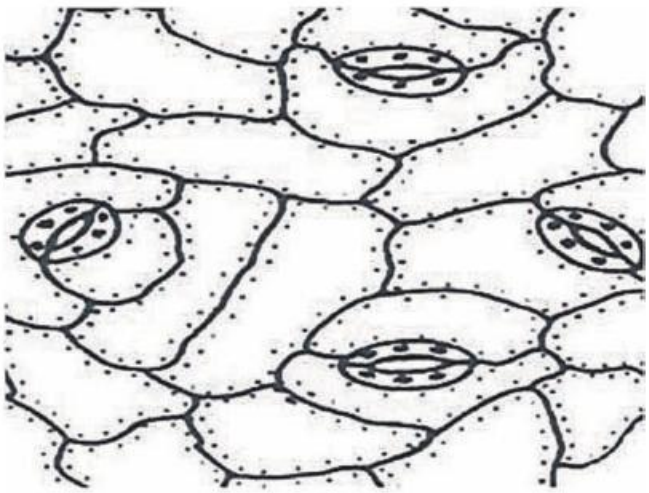

Figure 1. Leaf epidermis of *V. mungo* control.

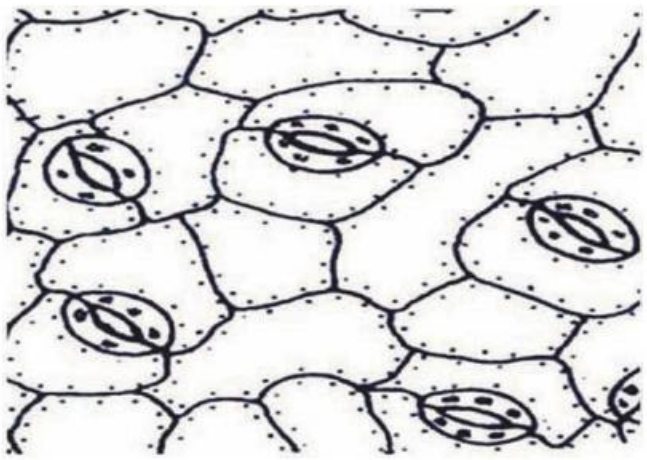

Figure 2. Leaf epidermis of *V. mungo* treated with 2% SLF.

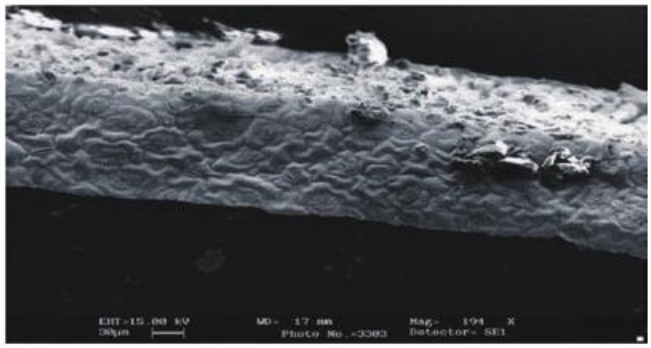

Figure 3. SEM image of leaf epidermal layer showing stomata of *V. mungo* treated with 2% SLF.

Figure 4. A portion enlarged view of paracytic stomata.

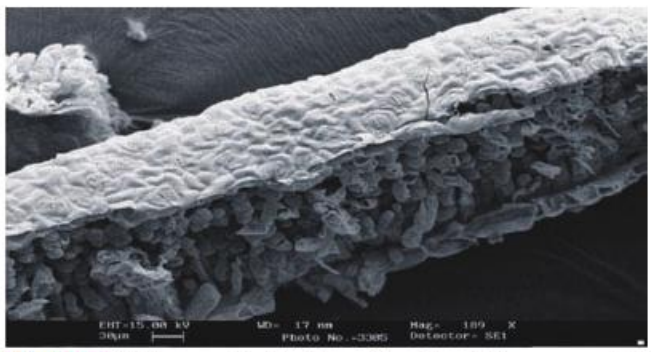

Figure 5. SEM image of leaf epidermal portion of *V. mungo* control subjected EDS analysis.

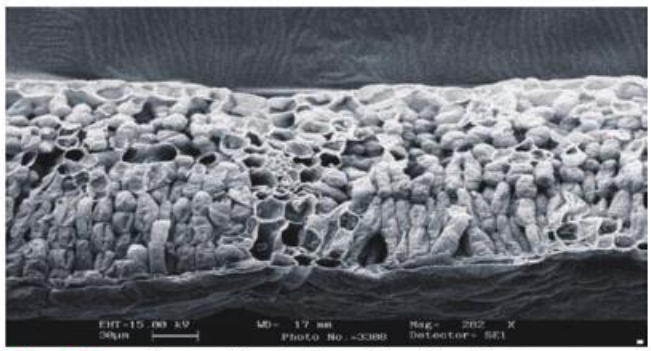

Figure 6. SEM image of leaf epidermal portion of *V. mungo* treated with 2% SLF subjected EDS analysis.

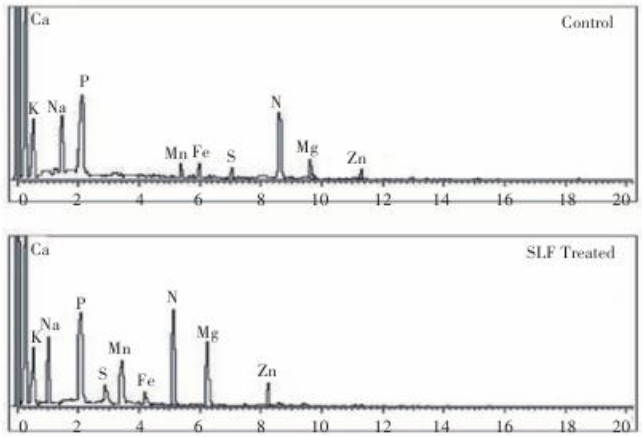

Figure 7. The energy dispersive spectroscopic (EDS) analysis of leaf of *V. mungo* treated with different concentration of *U. reticulata* SLF at 45th day of control and 2% SLF treated plants (Values are % in weight).

4. Discussion

The results were in agreement with the previous studies on growth stimulation of SLF prepared *Gracilaria*, *Sargassum* and *Caulerpa* on crops like cumbu by [32] and cluster bean [6]. The seaweed liquid extract of Sargassum also promoted the seedling growth of green gram and black gram at the concentrations below 1.5% [3]. The growth enhancing potential of seaweeds might be attributed to the presence of carbohydrates [33], phenylacetic acid [34], macro and micro elements [35], vitamins and plant grown regulators like cytokinin and gibberellin [4].

Effect of seaweed liquid fertilizer (SLF) prepared from *S. wightii* and *Hypnea musciformis(H. musciformis)* on growth and biochemical constituents of the pulse, *Cyamposis tetragonoloba(C. tetragonoloba)* [36].The increase in photosynthetic pigments content might be due to the presence of betains [37], the increase in number and size of the chloroplasts and better grana development [38]. Growth promoting effect of seaweed liquid fertilizer (*Enteromorpha intestinalis*) on the sesame crop plant [39]. Similar Observations were also reported in earlier studies on proteins, total sugars and amino acids [5, 40]. The increase in starch and sugars showed their close relationship [41, 42] and their accumulation due to SLF application [6]. *Sorghum vulgare (S. vulgare)* with 1.5% seaweed extract prepared from *Hydroclathrus clathratus (H. clathratus)* [43]. Effect of Seaweed Liquid Extract (SLE) of *Caulerpa scalpelliformis (C. scalpelliformis)* on growth and biochemical constituents of *Vigna mungo* was studied. The lower concentration of SLE of *C. scalpelliformis* (25%) enhanced the percentage of germination, shoot length, root length and biochemical constituents viz., chlorophyll, carotenoid, amino acid, reducing sugar and total sugar contents and α-amylase and β-amylase activities of shoot and root [44]. *Arabidopsis thaliana (A. thaliana)*[45], *Cajanus cajan (C. cajan)* [46], *Brassica nigra (B. nigra)* [47], *Lycopersicon esculentum (L. esculentum)* [48], *Vigna radiata (V. radiata)* [49] and *Triticum aestivum (T. aestivum)* [50]. The increased growth parameters at lower concentration may be due to the presence of higher levels of N, P, K in the seaweed extract of *C. scalpelliformis*. Similar effect of SLF prepared from *S. wightii* on *V. mungo* was reported by [51]. Significance of seaweed liquid fertilizers for minimizing chemical fertilizers and improving yield of *Arachis hypogaea*

(A. hypogaea) under field trial [52].

Contrasting data on the improved foliar uptake efficacy by the application of biostimulants are, at the moment, available. Some authors suggest that the use of biostimulants could induce an increase in membrane permeability by an increment in permeases, specific enzyme protein carrier molecules in the membrane, or a higher and improved activity of existing carriers [53], as stomata penetration of molecules has been considered for a long time impossible to happen for physical reasons. On the contrary, clear evidence for a strong role of stomata in explaining the enhanced influx of K^+ and Ca^{2+} in the leaves treated with marine bioactive substances, as stomata seemed to be the preferential entry way for K^+ and Ca^{2+} for ion uptake [54]. The preferential uptake of externally applied substances by stomatal guard cells has previously been reported [55], and [56] showed penetrations of calcium salts via stomatous abaxial leaf cuticles in *Vicia faba (V. faba)*. Different influx patterns occurred in different regions of *Vitis* leaves, too, from a very low base influx at cuticular level to a very high stomata influx, providing for a leading role of stomata in foliar absorption of mineral elements. In addition, the presence of marine bioactive substances strongly improves stomata uptake efficacy compared to non treated plants, thus providing a direct result of the importance of marine bioactive substances [54].

Nevertheless, the great variability in the functionality of stomata, also in the same leaf sector, and the well-known phenomenon of the patchiness of the stomata [57] make it difficult to generalize the results. Unfortunately, the role of biostimulants in plant growth enhancement is still unclear, mainly due to the multitude of products and compositions and to the fact that the effects of biostimulant application seems to be species-related.

The energy dispersive X-ray microanalysis provides a unique approach for obtaining qualitative and quantitative compositional analysis of individual cell and intra cellular compartment to localize distribution of chemicals elements of leaf differed not only by quality but also in quantity. The cell structure and different cellular inclusions have been reported by [58] using X-ray microanalysis EDAX. Electron microscopic studies and x-ray microanalysis on *S. wightii* were made by [59].

In conclusion, the observations on SLF treated *V. mungo* plants suggested that growth and biochemical characteristics of pulse crop plants might be promoted by micro and macro elements and growth promoting hormones present in the extract of *U. reticulata*. Thus one can prefer the seaweed extracts of *U. reticulata* to increase crop productivity by spraying 2% concentration. This practice could meet the manurial requirement for organic farming and serve as a cost effective eco-friendly approach for sustainable agriculture and environment.

Conflict of interest statement

We declare that we have no conflict of interest

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