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Effect of *Ulva lactuca* extract on growth and proximate composition of *Vigna unguiculata* l. Walp.

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ABSTRACT:

As organic farming gains more attention, seaweed cultivation and its utilization may be an economical approach in agricultural production. The effect of seaweed *Ulva lactuca* extract on growth and proximate composition of *Vigna unguiculata* L. Walp was studied. Seedlings of *V. unguiculata* soaked with seaweed extract performed better when compared to seedlings with water soaked control. The low concentration (20%) of aqueous seaweed extract promoted seedling growth in terms of shoot length, root length, fresh and dry weight and chlorophyll and carotenoids content. The biochemical constitutents show similar patterns with protein content of shoot and root, amino acid of shoot and root, α-amylase and β-amylase activities being higher with seaweed extract in *V. unguiculata*. The seaweed extract in these experiments showed as biological fertilizer, which is a nontoxic and ecofriendly supplement to chemical fertilizer for many crops intended to attain higher yields.

Keywords:

Crop, seaweed extract, biochemical composition, fertilizer, organic farming

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INTRODUCTION

Seaweeds have been used as green manure, cattle feed, food for human consumption and as a source of phycocolloids such as sugar, alginic acid and carrageenan (Chapman, 1970). Besides their application as green manure, liquid extracts obtained from seaweeds (SLF) have gained importance as foliar sprays for several crops (Thivy, 1961, Bokil et al, 1974) because the extract contains growth promoting hormones auxins (IAA, IBA), cytokinins, trace elements, vitamins and amino acids (Challen and Hemingway 1965). Thus, these extract when applied to seeds or when added to the soil, can stimulate growth of plants (Blunden, 1971). Green manure was found to be better than chemical fertilizers because of the high level of organic matter and colloidal compounds that aids in retaining soil moisture and minerals in the upper soil level that are available to the roots (Wallen, 1955). Booth (1969) observed that the value of seaweeds as fertilizers was not only due to nitrogen, phosphorus and potash content, but also because of the presence of trace elements and metabolites. Aqueous extract of *Sargassum wightii* when applied as a foliar spray on *Zizyphus mauritiana* showed an increase yield and quality of fruits (Ramarao, 1991). Seaweed extracts are now available commercially as Maxicrop, Algifert Marinure, Goemar GA14, Kelpak 66, Seaspray, Cytex, Seasol and Seacrop 16. Recent, research demonstrated that seaweed fertilizers can compete with other fertilizers and are very economical (Gandhiyappan and Perumal, 2001). This study was undertaken to investigate the effect of seaweed liquid extract on the growth and biochemical characteristics of *Vigna unguiculata*.

MATERIALS AND METHODS

Seaweed *Ulva lactuca* (Chlorophyceae) used in the present study was collected from the coastal area of Rameswaram, India (9°25’ N and 79°15’ E). The algal species were washed thoroughly with seawater to remove all unwanted impurities, adhering sand particles and epiphytes. Morphologically distinct thallus of algae were placed separately in new polythene bags and were kept in an ice box containing slush ice and transported to the laboratory. Samples were washed thoroughly using tap water to remove the salt on thallus surface. The water was drained off and the algae were spread on blotting paper to remove excess water. One kg of seaweed was cut into small pieces, boiled with one litre of distilled water for an hour, and filtered through GF/C paper. The filtrate was taken as 100% concentration of the seaweed extract and from this stock solution, different concentrations (5%, 10%, 20%, 30%, 40% and 50%) were prepared using distilled water (Bhosle et al, 1975). As the seaweed liquid fertilizers contained organic matter, they were refrigerated between 0 and 4 °C. The colour, pH, calcium, magnesium, sodium, potassium, iron, chloride, sulphur, silicon, aluminium, zinc, copper and nitrate content were analyzed by the method described by American Public Health Association (APHA, 1995).

The crop plant selected for the present study was *Vigna unguiculata* belonging to the family Fabaceae. Seeds with uniform size, colour and weight obtained from Seed Bank of Kerala Agricultural University chosen for the experimental purpose.

Experiment I (water soaked)

Eight hundred uniform sized seeds were soaked in water for 24 hours. After soaking, they were divided into batches of 100 seeds each and were placed in 8 Petri dishes with filter paper. One batch of seeds was considered as the control and they were watered with 10 ml of tap water every 24 hours. The reminder of batches were treated once with 10 ml/Petri dish of 5%, 10%, 20%, 30%, 40% and 50% of aqueous seaweed extract every 24 hours.

Experiment II (seaweed extract soaked)

One hundred seeds were soaked for each concentration of aqueous seaweed extracts for 24 hours and then were placed in various Petri dish plates with filter paper and sprayed water regularly every 24 hours. Water soaked seeds were used as controls. Samples were taken from each set 15 days after sowing. The growth parameters including germination percentage, fresh and dry weight and shoot and root length was calculated. Seven biochemical constituents, chlorophyll content (Arnon, 1949), carotenoid (Mackinney, 1941), protein (Lowry et al, 1951), amino acids (Moore and Stein 1948), total sugar content (Nelson, 1944) and α-amylase and β-amylase activities (Bernfeld, 1955) were estimated from *V. unguiculata* seedlings. Data were statistically analyzed using correlation of coefficient method and all the measurements were made triplicate.

RESULTS

The physico-chemical properties of seaweed *Ulva lactuca* extract was analyzed (**Table I**). The

Table I. Physico-chemical properties of seaweed Ulva lactuca extract

Parameters	Seaweed extract
Physical parameters	
Colour	Yellow
pH	7.3
Chemical parameters (mg.l)	
Sodium	185.00
Potassium	113.00
Magnesium	108.30
Calcium	195.26
Phosphorous	51.35
Iron	0.37
Chloride	415.55
Sulphate	16.84
Silica	38.12
Copper	0.38
Zinc	1.01
Nitrate	19.05

pH of the yellow coloured extract was 7.3 and had high levels of calcium, sodium, potassium, magnesium, phosphorous, iron and chloride. The effect of extract on germination percentage and growth of *V. unguiculata* are presented in **Tables IIa and IIb.** Maximum seed germination (99%) was found at 20% concentration in both water soaked and seaweed extracts soaked seeds. The germination percentage increased with concentration up to 20% and thereafter it declined. No germination was seen at concentrations above 50%. The lowest germination percentage (19%) was found in water soaked seeds at 50% concentration. The highest shoot length (15.7 cm/ seedling), root length (5.41 cm/seedling), fresh and dry weight (3.970, 0.807 g/seedling) was observed at 20% concentration of seaweed extract soaked plants. The lowest shoot length (8.20 cm/seedling), fresh and dry weight (1.980, 0.701 g/seedling) were found at 50% concentration of seaweed extract soaked seeds. The biochemical constituents increased with concentration levels up to 20% and thereafter declined (**Tables III a-c**). The highest values of chlorophyll content (2.268 mg/g fr. Wt.), carotenoid (0.852 mg/g fr. wt.), amino acid content of shoot (1.343 mg/g fr. wt.), α and β amylase (1.639, 1.613 µg/min/mg protein respectively), total sugar content of shoot and root (11.207, 8.602 mg/g fr. wt.) were recorded at 20% seaweed extract soaked seedlings. The lowest values were observed at 50% *U. lactuca* extract soaked seedlings (Tables III a-c). There was a significant difference in growth and biochemical status at different

Table IIa. Effects of seaweed extract on germination and growth of *V.* unguiculata seedlings

	Germination (%)		Shoot length (cm/seedling)		Root length (cm/seedling)	
	I	II	I	II	I	II
Control	85 ± 2.65	85 ± 2.65	7.0 ± 0.20	7.0 ± 0.20	2.5 ± 0.06	2.5 ± 0.06
5%	93 ± 0.58	97 ± 0.58	10.7 ± 0.20	11.7 ± 0.06	2.6 ± 0.12	2.6 ± 0.06
10%	94 ± 1.0	97 ± 1.0	13.3 ± 0.15	13.9 ± 0.06	3.0 ± 0.12	3.5 ± 0.12
20%	99 ± 0.58	99 ± 0.58	14.2 ± 0.06	15.7 ± 0.21	4.2 ± 0.06	5.4 ± 0.06
30%	91 ± 1.7	93 ± 0.58	12.4 ± 0.06	13.1 ± 0.20	4.4 ± 0.10	4.8 ± 0.06
40%	65 ± 0.58	78 ± 1.00	8.60 ± 0.10	9.3 ± 0.20	3.0 ± 0.12	4.6 ± 0.07
50%	19 ± 1.15	37 ± 11.0	8.20 ± 1.15	8.7 ± 0.06	2.9 ± 0.05	2.9 ± 0.20

I – Water soaked; II- Seaweed extract soaked

Table IIb. Effects of seaweed extract on germination and growth of *V.* unguiculata seedlings

Seedling (g/seedling)				
	Fresh weight		Dry weight	
	I	II	I	II
Control	1.82 ± 0.06	1.82 ± 0.06	0.679 ± 0.00	0.679 ± 0.00
5%	1.99 ± 0.58	2.06 ± 0.12	0.719 ± 0.01	0.731 ± 0.00
10%	2.07 ± 0.6	3.93 ± 0.05	0.782 ± 0.00	0.873 ± 0.01
20%	2.52 ± 0.03	3.97 ± 0.04	0.807 ± 0.01	0.882 ± 0.01
30%	2.12 ± 0.01	3.73 ± 0.36	0.800 ± 0.00	0.875 ± 0.01
40%	1.78 ± 0.02	1.98 ± 0.01	0.701 ± 0.15	0. 790 ± 0.00
50%	1.85 ± 0.05	1.98 ± 0.01	0.688 ± 0.01	0.701 ± 0.01

I – Water soaked; II- Seaweed extract soaked

Table IIIa. Effects of seaweed extract on proximate composition of *V.* unguiculata leaf

Leaf (mg/g fr. wt.)				
	Chlorophyll		**Carotenoids**	
	I	II	I	II
Control	1.253 ± 0.11	1.253 ± 0.11	0.667 ± 0.01	0.667 ± 0.01
5%	1.293 ± 0.02	1.793 ± 0.02	0.790± 0.00	0.826 ± 0.01
10%	1.370 ± 1.01	1.960 ± 0.00	0.826 ± 0.00	0.840 ± 0.01
20%	1.427 ± 0.01	2.268 ± 0.02	0.850 ± 0.00	0.852 ± 0.00
30%	1.110 ± 0.00	1.226 ± 0.00	0.604 ± 0.00	0.647 ± 0.02
40%	0.980 ± 0.01	1.000 ± 0.00	0.355 ± 0.01	0. 422 ± 0.01
50%	0.595 ± 0.00	0.624 ± 0.01	0.310 ± 0.01	0.711 ± 0.01
Leaf (mg/g fr. wt.)				
	α - amylase		**β - amylase**	
	I	II	I	II
Control	1.388 ± 0.003	1.388 ± 0.003	1.354 ± 0.000	1.354 ± 0.000
5%	1.402 ± 0.001	1.522 ± 0.020	0.378 ± 0.001	1.404 ± 0.010
10%	1.477 ± 1.064	1.530 ± 0.001	0.383 ± 0.001	1.466 ± 0.010
20%	1.636 ± 0.005	1.639 ± 0.010	1.566 ± 0.005	1.613 ± 0.031
30%	0.852 ± 0.045	0.896 ± 0.010	1.125 ± 0.576	0.864 ± 0.010
40%	0.396 ± 0.003	0.509 ± 0.031	0.668 ± 0.005	0.765± 0.011
50%	0.289 ± 0.003	0.288 ± 0.001	0.606 ± 0.005	0.734 ± 0.030

I – Water soaked; II- Seaweed extract soaked

Table IIIb. Effects of seaweed extract on proximate composition of *V.* unguiculata shoot

Shoot (mg/g fr. wt.)						
	Total sugar		**Protein**		**Amino acid**	
	I	II	I	II	I	II
Control	8.340 ± 0.003	8.340 ± 0.003	1.548 ± 0.001	1.548 ± 0.001	0.434 ± 0.001	0.434 ± 0.005
5%	10.004 ± 0.01	10.280 ± 0.02	2.513 ± 0.002	2.632 ± 0.004	0.627 ± 0.003	0.646 ± 0.003
10%	10.632 ± 0.003	10.670 ± 0.004	2.915 ± 0.004	3.084 ± 0.004	0.847 ± 0.003	0.864 ± 0.005
20%	11.270 ± 0.003	11.290 ± 0.016	3.156 ± 0.005	3.338 ± 0.005	1.279 ± 0.002	1.343 ± 0.002
30%	8.276 ± 0.002	8.340 ± 0.002	1.315 ± 0.004	1.503 ± 0.012	0.787 ± 0.002	0.858 ± 0.001
40%	6.369 ± 0.010	6.391 ± 0.006	0.983 ± 0.001	1.114 ± 0.040	0.691 ± 0.001	0.752 ± 0.001
50%	0.509 ± 0.008	0.553 ± 0.003	0.863 ± 0.006	0.983 ± 0.007	0.602 ± 0.002	0.616 ± 0.001

I – Water soaked; II- Seaweed extract soaked

Table IIIc. Effects of seaweed extract on proximate composition of *V.* unguiculata root

Root (mg/g fr. wt.)						
	Total sugar		**Protein**		**Amino acid**	
	I	II	I	II	I	II
Control	5.803 ± 0.003	8.340 ± 0.003	1.124 ± 0.005	1.124 ± 0.005	0.407 ± 0.003	0.407 ± 0.003
5%	7.481 ± 0.002	7.533 ± 0.010	2.124 ± 0.004	2.120 ± 0.056	0.430 ± 0.002	0.480 ± 0.010
10%	7.816 ± 0.003	7.906 ± 0.005	2.131 ± 0.002	2.142 ± 0.002	0.493 ± 0.006	0.554 ± 0.004
20%	8.496 ± 0.001	8.602 ± 0.001	2.542 ± 0.008	2.721 ± 0.008	0.554 ± 0.003	0.684 ± 0.010
30%	6.433 ± 0.003	6.627 ± 0.037	0.551 ± 0.026	0.555 ± 0.003	0.506 ± 0.006	0.633 ± 0.010
40%	4.394 ± 0.007	4.620 ± 0.011	0.429 ± 0.001	0.452 ± 0.016	0.451 ± 0.002	0.595 ± 0.008
50%	3.492 ± 0.003	3.001 ± 0.021	0.400 ± 0.003	0.413 ± 0.003	0.408 ± 0.002	0.440 ± 0.002

I – Water soaked; II- Seaweed extract soaked

concentration levels. Correlation of coefficient was carried out to find the significance level (*p<0.05 and ** p<0.01). High significance was observed in 20% concentration (**Table IV**).

DISCUSSION

Vigna unguiculata seeds soaked with lower concentration (5-40%) of the seaweed extract showed high germination rates (85-95%), while higher concentrations (≥ 40%) have inhibited germination. The increased seedling growth may be due to the presence of phenyl acetic acid and other closely related compounds in the extract (Taylor and Wilkinson, 1977) as well as the presence of growth promoting hormones like auxins, gibberellins, cytokinins, trace elements, vitamins and amino acids (Challen and Hemingway, 1965). The present findings agree with field trials in other crops such as in *Cajanus cajan* (Mohan *et al*., 1994), maize, ragi and kambu (Rajkumar and Subramanian, 1999) and *Dolichos buflorus* (Anantharaj and Venkatesalu, 2002). Statistically significant differences were observed for leaf pigments and total sugar contents in both shoot and root. A positive response was observed for shoot length at 10-20% seaweed extract soaked seedlings. Trace elements, especially calcium that exists in this seaweed extract are in a naturally chelated form, which can absorb more readily than from soil. The higher concentrations (≥ 40%) showed a decreasing for *V. unguiculata*. Similar results were recorded in *C. cajan* (Mohan *et al*., 1994) and *Vigna radiata* (Venkataraman *et al*., 1993) where maximum seedling growth occurred at lower concentrations of *Padina* extracts. Dhargalkar and Untawale (1983) also reported comparable results with red and brown algal extracts on the growth of chillies, turnips and pineapple.

Table IV. Coefficient values of seaweed extract with respect to different parameters

			5%	10%	20%	30%	40%	50%
Seed	Germination	I	0.655	0.866*	1.000*	0.500	-1.000	-0.500
		II	0.655	0.866*	0.500	0.500	-0.866	0.419
Seedling	Fresh weight	I	-0.904	-0.999	-0.533	-0.999	-0.914	-0.983
		II	-0.604	-0.785	-0.997	-1.000	-0.821	-0.569
Seedling	Dry weight	I	-0.329	0.245	-0.456	-0.381	0.381	-0.381
		II	-0.520	-0.381	0.536	0.610	-0.893	-0.810
Shoot	Length	I	0.500	-0.327	-0.866	-0.866	0.500	-
		II	-0.866	0.866*	0.961**	0.500	0.500	-0.866
Root	Length	I	-0.500	0.500	-0.500	-0.500	-1.000	-0.500
		II	-0.866	-0.500	-0.500	-1.000	-0.500	-
Leaf	Chlorophyll	I	-0.987	-0.172	-0.294	-0.939	-0.980	-0.985
		II	-0.987	-0.857	0.965**	0.284	-1.000	0.054
	Carotenoids	I	0.189	-0.500	0.933*	-0.156	-	-0.286
		II	0.292	0.768*	0.933*	0.115	0.143	0.069
	α - amylase	I	-0.993	-0.915	0.999**	-0.803	-0.300	-0.845
		II	0.999**	0.981**	0.995**	-0.826	-0.767	-0.596
	β - amylase	I	-0.189	-0.655	0.945*	-0.945	-0.968	-0.866
		II	-0.945	-0.904	0.811*	-0.918	-0.945	-0.951
Shoot	Total sugar	I	-0.746	0.700	0.700	-0.803	0.684	0.545
		II	-0.797	0.717	0.765*	-0.127	0.115	0.500
	Protein	I	0.655	0.500	0.101	0.500	0.500	0.545
		II	0.427	0.277	0.819	0.126	0.062	0.352
	Amino acid	I	-0.882	-	-1.000	-0.891	-0.327	-0.756
		II	0.945	0.945*	-0.786	-0.945	-0.655	-0.189
Root	Total sugar	I	-0.169	0.300	0.945*	0.655	0.655	-
		II	0.933*	0.929*	0.982**	-0.644	-0.810	-0.304
	Protein	I	-0.349	-0.023	0.928	0.658	-0.600	-0.911
		II	-0.994	0.877*	-0.890	-0.120	-0.970	0.392
	Amino acid	I	-0.091	-0.583	0.529	-0.137	0.500	0.371
		II	-0.374	0.327	-0.693	-0.176	0.047	-0.619

I – Water soaked; II- Seaweed extract soaked
Significant at * p<0.05 level; Significant **p< 0.01 level

The lower concentrations of the extract also promoted the chlorophyll content of *V. unguiculata* up to 20% when compared to the control while higher concentrations (> 20%) decreased the chlorophyll content. A similar observation was made in *Vigna mungo* (Venkataraman and Mohan, 1997) and when seaweed extract (15-20%) applied as foliar spray enhanced the leaf chlorophyll level in plants (Blunden *et al*., 1996).

The highest total sugar content was recorded at 20% concentration of seaweed extract soaked treatment in *V. unguiculata*. The sugar content increased up to 20% concentration of seaweed extract and decreased at higher concentrations. The increase in the total sugar content at lower concentration of seaweed extract might be due to absorption of most of the necessary elements (Kannan and Tamilselvan, 1990). The same trend was observed in the *H. musciformis* with NPK application in black gram (Tamilselvan and Kannan, 1994), and *D. biflorus* (Anantharaj and Venkatesalu, 2002). It has been observed that α – amylase activity was higher than the β – amylase activity. Both α – amylase and β -amylase activity increased at lower concentrations and decreased in higher concentrations. In conclusion, *Ulva lactuca* extract was found to be a promising fertilizer. The extract act as a biological fertilizer for organic farming, which is non-toxic, non-flammable for attaining better seed germination and growth.

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