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Influence of seaweed extract as an organic fertilizer on the growth and yield of *Arachis hypogea* L. and their elemental composition using SEM-Energy Dispersive Spectroscopic analysis

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ABSTRACT

Objective: To investigate the effect of Seaweed Liquid Fertilizer (SLF) of the red seaweed *Hypnea musciformis* (Wulfen) Lamouroux (*H. musciformis*), on the growth, biochemical and pigment characteristics of *Arachis hypogea* (*A. hypogea*). **Methods:** Experiments were conducted on ground nut to study the potential red alga of *H. musciformis* 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:** The 2% concentration of water extract showed better results of growth parameters, biochemical and pigments constitutions. Among the different concentrations of SLF investigated, the plants that received with 2% SLF showed maximum germination percentage, fresh weight, dry weight, root and shoot length, number of branches, leaf area, root nodules and content of total chlorophyll, chlorophyll a and b, protein, carbohydrate and lipid were observed at 2% concentration of SLF. The leaf of 2% SLF treated *A. hypogea* has subjected to Scanning Electron Microscopy with Energy Dispersive spectroscopic analysis, it revealed that the presence 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 *H. musciformis* could be used as foliar spray at low concentration of 2% to maximize the growth and yield of *A. hypogea* and also increase the number of stomata in the leaf. **Conclusion:** It is suggested that there are considerable gains to be made in increasing yield and stabilizing the yield in environments characterized by terminal requirement for organic and by shortening crop duration nutrient management appear promising.

1. Introduction

The marine ecosystem is the treasure place for many natural resources^[1]. Seaweeds are among the important marine living resources with tremendous commercial application. In recent times, seaweed extract have been used as fertilizers. These extracts are commonly known as Seaweed Liquid Fertilizer (SLF). The *Vigna mungo* (*V. mungo*) seeds soaked with lower concentrations of the seaweed extracts showed higher rates of germination, while the higher concentrations of the extracts inhibited the germination^[2]. 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^[3].

Modern agriculture with use of chemical fertilizers and resulted in an increase of food production. Seaweed liquid fertilizer is widely used as foliar spray to increase yield in many commercial crops^[4]. 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^[5]. However, application of seaweed extract increased chlorophyll content^[6]. 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^[7]. Report on SLF using SEM-EDS technique is one among them which has not receive

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due attention. Hence, the present work is attempt to study the amount of different chemical elements present in the root, stem, and leaf of the *Arachis hypogea*.

2. Materials and methods

2.1. Collection of seaweeds

The seaweed used in the present study was *Hypnea musciformis* (*H. musciformis*) belonging to the Family Rhodophyceae. They were collected from the rocky coastal area of Kanyakumari (Lat.9° 11'N, Long.79° 24'E). The algal species were handpicked and washed thoroughly with seawater to remove all the unwanted impurities, adhering sand particles and epiphytes. The thallus of alga was placed separately in new polythene bags and were kept in an ice box containing slush ice and transported to the laboratory. Then seaweed was washed thoroughly using tap water and maximum care was taken to remove the salt and epiphytes on the surface of the sample. The water was drained off and the algae were spread on blotting paper to remove excess water.

2.2. Preparation of Seaweed Liquid Fertilizers

The seaweed was cut into small pieces separately, and boiled with 1 liter of distilled water for an hour and filtered. The filtrate was treated as 100% concentration of the seaweed extract and from this; different concentrations of the seaweed liquid fertilizer (SLF) (1%, 2%, 4%, 6%, and 8%) were prepared using distilled water[8]. As the seaweed liquid fertilizer contained organic matter, they were refrigerated between 0 °C and 40 °C.

2.3. Selection of crop plant

The crop plant, selected for the present study was *A. hypogea* belonging to the Family, Fabaceae. The seeds were collected from Regional Pulses Research station, Tamil Nadu Agricultural University, Coimbatore. The seeds with uniform size, colour and weight were chosen for the experimental purpose. The selected seeds were stored in a metal tin[9].

2.4. Experimental design and treatments

The certified seeds of *A. hypogea* 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 *A. hypogea* seeds were sown in soil field in 4 m × 3 m plot. One or two seeds 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 15th days each were given up to 60th 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.

The growth parameters such as shoot length, root length, total fresh weight and total dry weight were calculated. The biochemical parameters such as total chlorophyll and carotenoid were quantified by using the method of Wellburn and Lichtenthaler[10]. The total soluble protein content was analyzed by following the method of Lowry et al.[11].

2.5. Preparation of SEM studies in leaf, stem and root of groundnut

Scanning electron microscope with energy dispersive spectroscopy (EDS): The scanning electron microscopy of red alga treated groundnut was done by using the method of Hayat. Reagents included glutaraldehyde, phosphate buffer (pH 6.8), Alcohol, for SEM study the groundnut (root, stem and leaf) 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 °C 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 electronmicrograph were taken selectively from the computer screen[12].

3. Results

3.1. Germination and growth studies

The SLF of seaweed at 2% concentration are the most optimum for high growth germination (Table 1). The physical

Table 1

Effect of *H. musciformis* SLF on the growth of *A. hypogea* under field trial on 60th day and germination.

Parameters	Control	SLF Concentrations				
		1%	2%	4%	6%	8%
Germination (%)	78.00±3.75	93.00±2.10	98.00±1.20	94.00±5.15	88.00±4.75	77.00±3.65
Total plant height (cm)	18.50±1.80	19.80±2.56	22.20±2.42	21.00±0.80	20.79±0.78	18.00±0.68
Shoot height (cm)	12.30±2.09	13.50±2.56	16.20±2.05	15.00±0.65	14.60±0.65	12.00±0.50
Root height (cm)	5.20±0.42	5.80±0.25	7.25±0.79	6.30±0.84	6.00±0.73	4.80±0.65
Total fresh weight (g)	19.30±0.80	22.45±2.14	28.09±0.96	25.78±3.78	24.56±0.80	18.65±0.50
Shoot fresh weight (g)	18.25±0.95	22.10±2.10	26.41±0.87	25.54±2.85	22.70±0.48	18.00±0.68
Root fresh weight (g)	1.15±0.08	1.36±0.09	1.65±0.11	1.51±0.12	1.40±0.10	1.10±0.07
Total dry weight (g)	3.71±0.07	4.71±0.13	5.40±0.16	4.97±0.08	4.28±0.05	3.65±0.03
Shoot dry weight (g)	3.49±0.08	4.35±0.10	5.10±0.14	4.78±0.09	4.29±0.04	3.00±0.04
Root dry weight (g)	0.22±0.04	0.25±0.05	0.30±0.03	0.27±0.03	0.23±0.02	0.20±0.02
Number of branches	3.00±1.22	4.00±0.70	3.80±1.30	3.45±1.22	3.00±0.04	2.78±0.05
Leaf area (cm ²)	10.2	12.0	15.8	13.3	14.1	10.0

Values are mean ± SD; Simple (n) = 6

parameters like total plant shoot height and root height (cm), total fresh weight, shoot and root fresh weight, total dry weight, shoot and root dry weight (g), number of branches and leaf area (cm²) were also recorded a maximum 60th day plants received with 2% *H. musciformis* SLF (Table 1).

The plant received with 2% SLF contained a maximum of 1.75 mg/g fresh weight of Chlorophyll a on 30th day old plants. Further, the concentration of Chlorophyll b was 0.46 mg/g fresh weight, when compared to control. The application of 2% SLF increased the Chl 'a', 'b' and total chlorophyll

in most concentrations which were generally higher respectively, when compared to control. The accumulation of total carbohydrate, total protein and total lipid content also increased due to the SLF treatment. A maximum accumulation of the above parameters was recorded when the plant applied with 2% SLF on 30th day. At this condition, the 30th day old plants showed an increment of more than 2.8 mg/g, 1.5 mg/g, 0.45 mg/g towards the accumulation of total carbohydrate, total protein and total lipid content, respectively, when compared to control (Table 2).

Table 2

Effects of different concentrations of *H. musciformis* SLF on the pigments of *A. hypogea* on 30th day.

Biochemical constituents	Control	1%	2%	4%	6%	8%
Chlorophyll a (mg/g Fw)	1.200±0.025	1.510±0.030	1.750±0.035	1.550±0.028	1.350±0.025	1.170±0.020
Chlorophyll b (mg/g Fw)	0.300±0.005	0.390±0.006	0.460±0.009	0.410±0.007	0.370±0.003	0.280±0.002
Total chlorophyll (mg/g Fw)	1.500±0.030	1.900±0.036	2.210±0.041	1.960±0.028	1.720±0.028	1.450±0.022
Total Carbohydrates (mg/g/Fw)	1.800±0.351	1.900±0.235	2.800±0.257	2.500±0.318	2.100±0.278	1.600±0.535
Total Protein (mg/g/Dw)	1.100±0.125	1.200±0.115	1.500±0.185	1.400±0.212	1.200±0.282	0.900±0.370
Lipid (mg/g/Dw)	0.280±0.028	0.350±0.018	0.450±0.183	0.410±0.052	0.380±0.071	0.250±0.65

Values are mean ± SD; Sample (n) = 6

It is evident from the results that the increased growth (shoots and root length, leaf area, fresh weight of shoot and root) and biochemical constituents (Chlorophyll 'a', 'b', Total chlorophyll, carbohydrates, protein and lipid) are possible due to the SLF 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 ecofriendly seaweed resources available in the east coast of Tamil Nadu.

3.2. Scanning electron microscopy study

The scanning electron microscopic image of leaf epidermal portion of *A. hypogea* treated with 2% *H. musciformis* (SLF) results obtained from the EDS analysis of different chemical elements present in the cell wall of leaf of 2% SLF treated and control of *A. hypogea* (Figure 1 and 2). 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 *A. hypogea* 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. The high value of calcium obtained in the leaf is understandable since its involvement in the formation of cell wall layer. Magnesium (Mg) present in the leaf because it forms nucleus for the porphyrin ring and hence its presence in the photosynthetic mechanism is understandable. The scanning electron micrographic images of stem and root portion of *A. hypogea* treated with 2% *H. musciformis*, thickness of epidermal cells, cortex, medullary cells, primary and secondary phloem and xylem structures clearly present in these treatments (Figure 3 and 4).

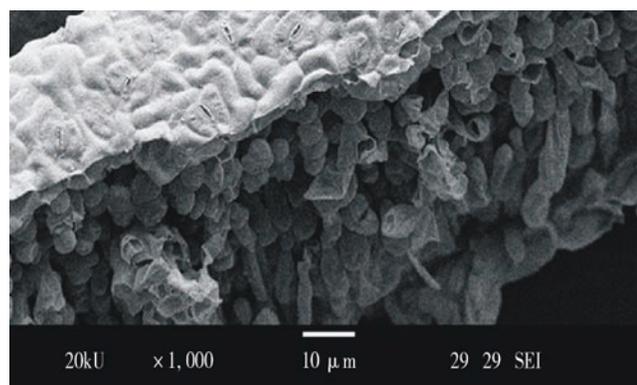


Figure 1. SEM image of leaf epidermal portion of *A. hypogea* treated with 2% *H. musciformis* SLF with EDS.

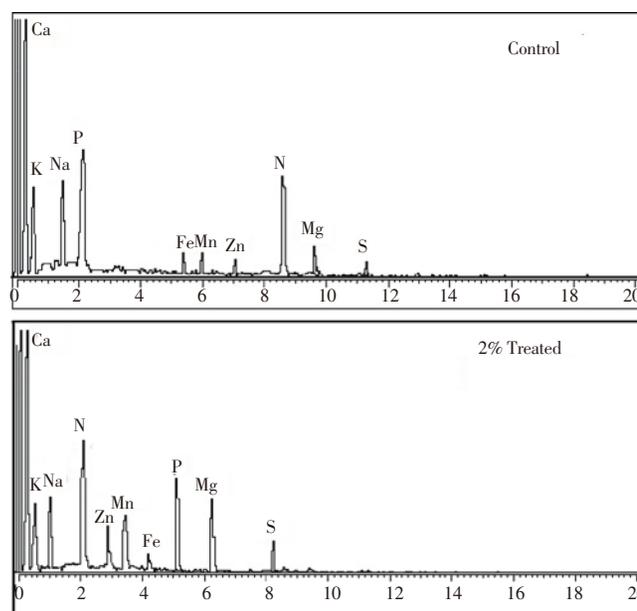


Figure 2. The Energy Dispersive Spectroscopic (EDS) analysis of leaf of *A. hypogea* treated with SLF of *H. musciformis* at 45th day of control and 2% SLF treated plants.

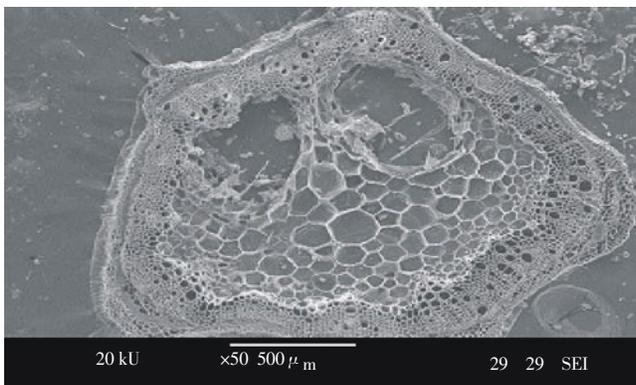


Figure 3. SEM image of stem portion of *A. hypogaea* treated with 2% *H. musciformis* SLF.

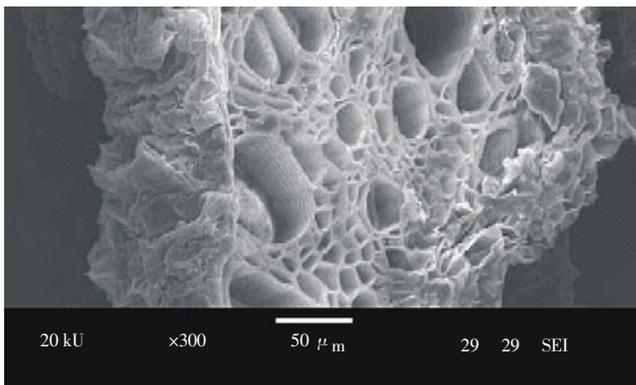


Figure 4. SEM image of root portion of *A. hypogaea* treated with 2% *H. musciformis* SLF.

4. Discussion

Seaweed treatment of crops has grown in popularity and led to development of a large number of processed seaweed products. *Vigna catajung* (*V. catajung*) when treated with 10% *Caulerpa racemosa* (*C. racemosa*) extract contained highest protein and amino acid contents^[13]. Effect of seaweed liquid fertilizer (SLF) prepared from *Sargassum wightii* (*S. wightii*) and *H. musciformis* on growth and biochemical constituents of the pulse, *Cyamopsis tetragonoloba* (*C. tetragonoloba*)^[14]. Growth promoting effect of seaweed liquid fertilizer [*Enteromorpha intestinalis* (*E. intestinalis*)] on the sesame crop plant^[15]. Similar observations were also reported in earlier studies on proteins, total sugars and amino acids^[16, 17]. The increase in starch and sugars showed their close relationship and their accumulation due to SLF application^[17]. *Sorghum vulgare* (*S. vulgare*) with 1.5% seaweed extract prepared from *Hydroclathrus clathratus* (*H. clathratus*)^[18]. Effect of Seaweed Liquid Extract (SLE) of *Caulerpa scalpelliformis* (*C. scalpelliformis*) on growth and biochemical constituents of *V. mungo* was studied. The lower concentration of SLE of *C. scalpelliformis* (25%) enhanced the percentage of germination, shoot length, root length

and biochemical constituent's viz., chlorophyll, carotenoid, amino acid, reducing sugar and total sugar contents and α -amylase and β -amylase activities of shoot and root^[19]. *Arabidopsis thaliana* (*A. thaliana*)^[20], *Cajanus cajan* (*C. cajan*)^[21], *Brassica nigra* (*B. nigra*)^[22], *Lycopersicon esculentum* (*L. esculentum*)^[23], *Vigna radiata* (*V. radiata*)^[24] and *Triticum aestivum* (*T. aestivum*)^[25]. 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*. Sivakumar and Gandhi reported similar effect of SLF prepared from *S. wightii* on *V. mungo*^[26]. Significance of seaweed liquid fertilizers for minimizing chemical fertilizers and improving yield of *Arachis hypogaea* (*A. hypogaea*) under field trial^[27]. The extract of *Ulva reticulata* (*U. reticulata*) found to have promising result by possessing fertilizer activity to enhance the germination and growth of *V. mungo*^[2].

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. Sundari and Selvaraj using X-ray microanalysis EDAX. Electron microscopic studies and x-ray microanalysis on *S. wightii*^[28]. Ganapathy selvam and Sivakumar reported seaweed extract 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^[29].

In conclusion, the observations on SLF treated *A. hypogaea* 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 *H. musciformis*. Decomposition of the soil organic matter is apparently a function of the available energy material added to the soil. This observation is further supported by the fact that the performance of *H. musciformis* seaweed liquid fertilizer. *H. musciformis* is eco-friendly, easily manageable input farming and a self-regenerating system which provide nutrients and maintains health status of soil. Energy dispersive spectroscopic analysis (EDS) study elucidates the various constituents within a cell which differed in their chemical composition, certain elements being specific to individual cell component. Hence use of modern agriculture in conjunction with traditional farming practices is the sustainable solution for the future.

Conflict of interest statement

We declare that we have no conflict of interest.

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