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ORIGINAL ARTICLE

Hydrodictyon reticulatum (L.) Lagerheim as A Renewable Source of Plant Biostimulants

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ABSTRACT
Bio-augmenting the water bodies and utilization of algal weeds as renewable source for agriculture purpose in which the algal extract (Hydrodictyon reticulatum) to be efficient plant biostimulant. The effect of algal extract on the growth performance of mung bean, Vigna radiata was studied and the algae were collected from Vellar river near Chidambaram for the study. Different concentration of the algal liquid extract (2.5%, 5% and 10%) and distilled water as control used for the experiment. Presence of sufficient micro and macronutrients of the algal extracts were analyzed by AAS. At 5% concentration, the algal extract shows maximum germination percentage, growth and yield parameters, biochemical constituents and pigments were observed. Overall present study revealed that the fresh water algae, H. reticulatum can be used as potential fertilizer and serve as a cost effective eco-friendliness for sustainable agriculture and environment.
Keywords: Water net, Biostimulant, Vigna radiata, Chlorophyll content

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INTRODUCTION

Freshwater algae commonly water net (*Hydrodictyon reticulatum*) is a filamentous green alga provides innovative opportunity to multiple industries due to their ability to grow in a wide range of wastewaters and the suitability of resultant biomass for a variety of applications. *H. reticulatum* is one such algal protein source which grows extensively in freshwater ponds. It is distinctive cylindrical colonies with six-sided to form mesh like structure (usually visible to naked eye) [19-24].

A vision of industrial ecology is to develop processes and practices that reduce environmental impacts through the worth wile use of waste products as raw material [28] and is an unconventional model for economic growth, social development and environmental management [34].

Recently, there is increased interest in natural products that stimulate the growth of plants. Recent attention on the raw material – the algal biomass which is useful in the production of plant growth biostimulants [3]. The natural products obtained from algae constitute interest in agriculture with emphasis on its application in sustainable agriculture [17].

An innovative initiative in developing industrial ecological model is to cultivate algae in wastewater as a bioremediation technology, with the biomass then serving as an input for algal-based end-products such as food, feed and bio-energy [28]. India being one of the largest producer and consumer of pulses which requires abundant amount of pulse production to fulfill the demands of ever growing populations. The present study conducted to evaluate the growth of mung bean (*Vigna radiata*) at different concentration of algal extracts *Hydrodictyon reticulatum*.

MATERIAL AND METHODS

Preparation of the extract

Algae (*Hydrodictyon reticulatum)* sample was collected from the local pond at Thanjavur. Subsequently, the algal material were rinsed with water to clean the sand and other impurities. Algae sample was allowed to dry upto 15% of moisture and finally it was ground to particle size < 0.5 mm and further the algae was extracted with water in the ratio 1:10 at 120°C. Then added 5 gm of yeast after cooling and 0.5 kg of jaggery as additional carbon source for fermentation. The sample was incubated at room temperature (27 ± 20°C) for 3 days. The sample was centrifuged at 4000 rpm for 5 min and filtered. The supernatant that acquired was taken as 100% algal liquid extract.

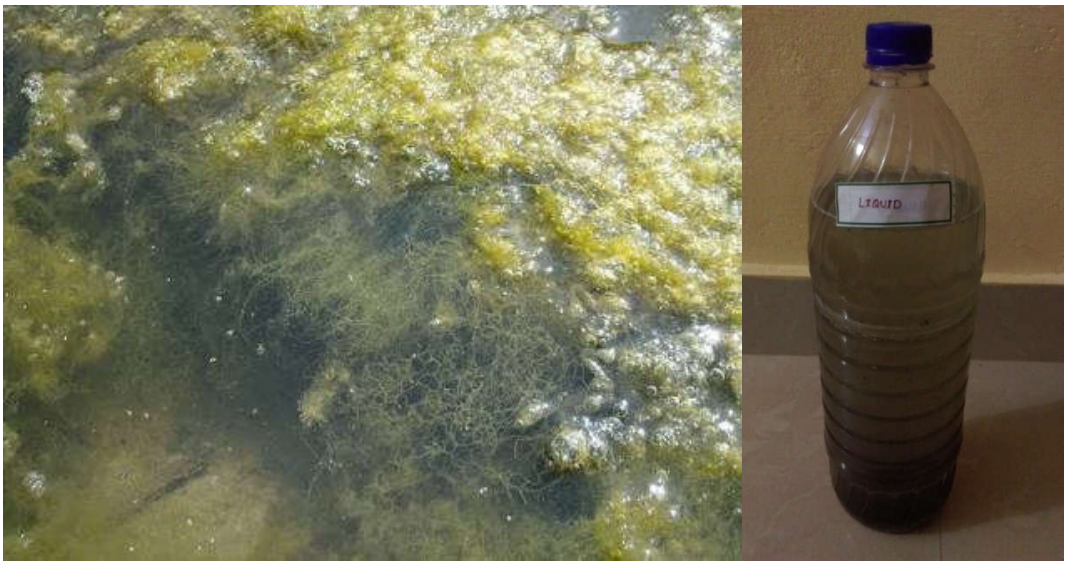

Fig 1: ***(a) Hydrodictyon reticulatum*** **(b)Algal Liquid extract**

Multielimental composition of Algal extracts - (AAS)

Firstly, the samples of the algal biomass (0.5g) was acid treated followed by demineralized water to 50 g. Flame atomic absorption is a widely used technique that offers many advantages. However, there are analytical situations in which flame methodology is limited in sensitivity or sampling flexibility. The HGA graphite furnace, which requires only a few microliters of sample, is ideal for ultra-trace determination of more than 60 elements. It can provide detection limits 1000 times lower than those of conventional flame techniques. The graphite furnace can be used with all current Perkin-Elmer atomic absorption instruments.

Germination Tests: Petri Dish Tests.

The useful properties of algal extracts were evaluated and we performed the germination tests (three replicates on Petri dishes (8.9 cm), 30 seeds each) with mung bean (*Vigna radiata*). Experiments were conducted in standardized conditions with 12 light/dark . Then each dish was treated with appropriate algal extract (5 mL). The control group (C) was treated with distilled water (5 mL). After three days, all dishes were treated with the same subsequent doses of extract/water. The tests were performed for 7 days, after weighted the plants and measured the height of shoot length [27].

Plant Growth under Pot Techniques

Four pots measuring 17 cm height, 18 cm width and 5 cm depth were taken for the study. The pots were filled with equal amount of (1 : 1 : 1) clay, soil and sand then the seeds were sown in 1cm depth. After seed emergence, seeding was thinned to 10 plants/pot. Different concentration viz. 2.5%, 5% and 10% of the algal extracts was used in each pot. After irrigation event, sufficient water poured in the soil in each pot. Plant growth stage 7 to 29 days and flowering stage of 30 to 35 days was observed. Plants were harvested after 60th day after planting and recorded various morphological parameters *viz*. shoot, leaf, and root length were recorded and yield parameters were observed.

Estimation of Chlorophyll

Chlorophyll was estimated at regular intervals followed by Holden,[15]. The leaves were weighed (100 mg) and cut into small pieces, homogenized with 80% acetone and $CaCO_3$ added to prevent pheophytin formation. The homogenized materials were centrifuged at 5000 rpm for 10 minutes at room temperature. The supernatant was collected and then made up to 10 ml with distilled water. The test tube

wrapped with black paper to protect chlorophyll degradation. The colorimeter was adjusted at wave length of 663 nm for chlorophyll 'a' and 645 nm for chlorophyll 'b' set at 100% transmittance using 80% acetone as blank before taking the readings of the samples. Optical density was measured and the chlorophyll content in the original extract was estimated.

Chlorophyll content

Chlorophyll a = Absorbance663nm * (0.058) –(Absorbance 645nm) * 0.032)
Chlorophyll b = Absorbance 645nm* (0.096) –(Absorbance 663nm* 0.01872)
Total chlorophyll content = (Absorbance 645nm*20.2)+ (Absorbance 663nm*8.3)* (V/100 *W) where W= Dry weight of plant material;
V= Final volume of chlorophyll extraction in 80% acetone.

Estimation of Protein

Total protein estimation was performed by Lowry *et.al.,* [18] 5g of plant leaves were weighed 5g and grinded well with 10 ml of distilled water in mortar and pestle. After vortex for 2 minutes, all the tubes were centrifuged for 10 minutes at 5000 rpm. Supernatant was taken and made up the volume to 1 ml with distilled water and 3 ml of reagent (50 ml of reagent 'A' (2% sodium carbonate in 0.1 N sodium hydroxide) and 1 ml of reagent 'B' (0.5 % copper sulfate in 1% potassium sodium tartarate)) were added ten 0.2 ml of Folin Cio-calteau reagent tube was incubated for 30 minutes at room temperature . Bovine serum albumin was used as standard in a range of 20 – 100 mg/ml. All the samples along with standards were prepared in triplicates and absorbance was measured at 750 nm. Statistical analysis and mean values of three measurements with standard deviation (SD) were taken.

Estimation of Total Carbohydrate

The carbohydrate content was estimated by the method of Dubois *et al*.1956. An aliquot (100µl) of the supernatant diluted to 1ml with extraction buffer was mixed with 1ml of 5% phenol (aqueous w/v) and 5ml of concentrated sulphuric acid was added rapidly and mixed through and the tubes were incubated for 10 min. at 37°C. The colour development was read at 490 nm using Spectrophotometer. The reagent without the sample served as blank. The amount of sugar was estimated using a standard graph prepared using D-glucose at the range of 10-100µl.

$$\text{Carbohydrate} = \frac{\text{Standard value} \times \text{OD of the sample}}{\text{Weight of the sample taken}} \times 100$$

Estimation of Total Lipid [42]

Typically cells were harvested by centrifugation at 8500 rpm for 5 min and washed once with distilled water. After drying the samples using freeze drier, the samples were pulverized in the mortar and extracted using mixture of chloroform: methanol (2:1,v/v). About 50 ml solvents were used for every gram of dried sample in each extraction step. After stirring the sample using magnetic stirrer bar for 5 hrs and ultrasonicated for 30 min, the sample were centrifuged 3000 rpm for 10 min. The solid phase was separated carefully using filter paper (Advantech filter paper, no.1) in which two pieces of filter papers were applied twice to provide complete separation using rotary evaporator at 40-45°C.

$$\text{Lipid} = \frac{\text{Amount of sample in the sample}}{\text{Weight of the sample taken}} \times 100$$

RESULTS AND DISCUSSION

Atomic Absorption Spectroscopy For Analysis: The pH of the algal extract is neutral (pH 6.4). All essential major and micronutrients were present in the prepared Algal extract (Table-1).

Major nutrients such as Nitrogen (N), Phosphorus (P) and Potassium (K) followed by secondary nutrients namely Calcium (Ca), Magnesium (Mg), Sulfur (S) and micronutrients or trace elements: Boron (B), Chlorine (Cl), Copper (Cu), Iron (Fe), Manganese (Mn), Molybdenum (Mo) and Zinc (Zn). High concentration of N, P and K were found to be essential source for the growth of plants presented in the algal extract. Presence of these nutrients in algal extract fulfill the need of plants to grow through the growth period.

Intensive crop cultivation mostly leads to nutrient depletion of the soil reservoir all over the world. Unfavorable soil conditions such as high pH, salinity and $CaCO_3$content [35-37] and the antagonism of soil macro and micronutrients, can also affect the availability of some nutrients for plant roots.

Fresh water green microalgal extracts appeared to be promising natural fertilizers. They contain high macro and micronutrient concentrations in addition to the natural enzymes and hormones [35]. Micronutrients are essential for growth and development of all higher plants [25]. They serve in the redox systems and as co-enzymes for numerous fundamental processes in the plant cell activities [13,

33]. Thus, micronutrients deficiency causes severe problems in plant cell metabolism which finally lead to growth retardation and less yields [9, 37].
Micronutrients as soil application may not meet the crop requirements for growth and nutrient balance within the plant tissues. Further studies claimed that algal extract promoting growth as it contains a series of plant growth promoters.

TABLE 1: Multelemental Composition of Algal Extracts

S.NO	PARAMETER	LIQUID
1	pH	6.4
2	EC(dsm-1)	-
3	Nitrogen(mg/1)	7
4	Calcium(mg/1)	4.8
5	Potassium(mg/1)	266
6	Phosphorus(mg/1)	474
7	Magnesium(mg/1)	38
8	Sulfur(mg/1)	46
9	Boron(mg/1)	32
10	Chlorine(mg/1)	29
11	Copper(mg/1)	41
12	Ion(mg/1)	56
13	Manganese(mg/1)	87
14	Molybdenum(mg/1)	35
15	Zinc(mg/1)	45
16	Sodium(mg/1)	40

Seed Germination Percentage

The table-2 shows seed germination in terms of shoots, root length and Number of leaves at varying concentration (2.5%, 5% and 10%). In the present study, no mortality observed in all the treatments and the seeds were exhibited successful germination.

The Table 2 shows the difference in the growth of root, shoot length and number of leaves ratio which was significantly influenced by the alga extract than the control. Plant shoot length was recorded high in 10% concentration as 9.1 cm , the root length was 3.44 cm per plant, and the number of leaves per plant is 3, whereas in the treatments of 2.5% concentration, the shoot length is 5.9 cm, root length is 1.14 cm and the number of leaves per plant is 2 followed by 5 % concentration were recorded as shoot length of 7.1cm, root length of 2.26 cm and the number of leaves per plant is 3 per plant respectively. The study indicates that the number of leaves, root and shoot length was increased significantly under nutrient conditions than that of the control and the similar results have been reported by Burun *et al*. [2] and Paliwal *et al*. [29].

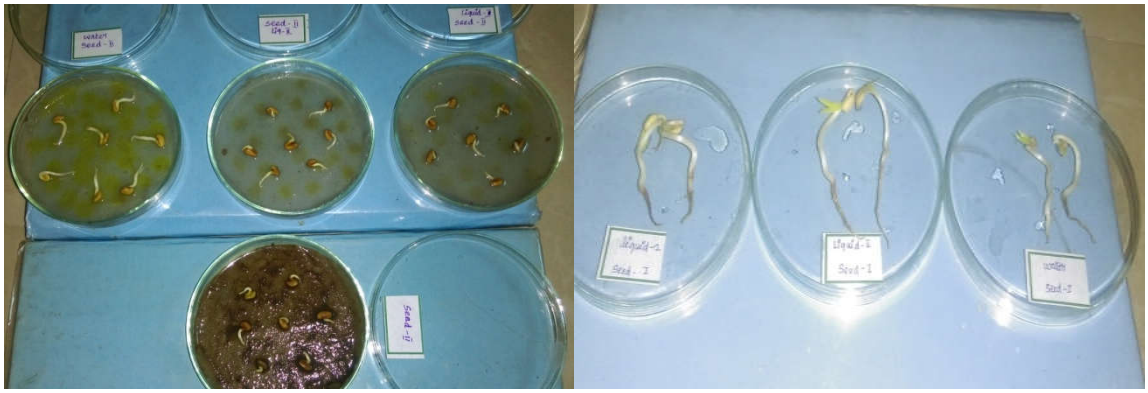

Fig 2: Seed germination traits

TABLE 2: Effect Of Algal Extract Concentration On *Mung Bean* Seed Germination Traits

S.No	Parameters	Treatment	% germination	Shoot Length(cm)	Root Length (cm)
1	Control		90%	7.4±1.9	2.54±
2	Liquid	2.5%	70%	6.2 ±1.9	4.8 ±1.3
		5%	100%	12.8 ±1.4	8.2 ±1.3
		10%	80%	10.6 ±1.3	6.2 ±2.16

Shoot and Root length -Pot method

The algal extract of *Hydrodictyon reticulatum* at 5% concentration is the most optimum for high growth germination (Table. 2 and fig. 1). The physical 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 (cm^2) were also recorded on 15th, 30th, 45th and 60th day plants received with 5% *H. reticulum* extracts (Table 2 and 3). Overall, the nutrient availability in 100% has positive influence on the plant survival, height, number of seeds and leaf area on mung bean plants.

In the present study, algal liquid extract has no negative effect on the plant growth and also has the ability to increase shoot and root length at different concentration. Significant increases were recorded in survival rate of plants under various percentage concentration of irrigation between test and control.

The modern concept of plant hormones suggests that bioactive compounds can influence physiological process in plants at low concentrations and can inhibit at higher concentration [7].

The increased growth and yield at low concentrations may be due to the presence of some growth regulators, as well as micro- and macronutrients [6] and vitamins Shaaban [35]. They may improve a nutrient assimilation and solute translocation from leaves to grains which led to significant increase in yield and grain weight [35].

Two nutrient sources might be considered in the case of algal extract treatments. One source is the algal extract itself and the second is its positive effect on the nutrients uptake by the plant roots [36]. However, micronutrients fertilizer has only the effect of encouraging roots to absorb more nutrients from the soil medium [37].

Fig 3 (a) Germination (b) Mature Plant

Table .3 Effect of different concentration of *Hydrodictyon reticulatum* on shoot and root length (cm) and leaf area (cm^2) in Vigna radiata 15th, 30th, 45th and 60th day

Days	Parameters	Control	2.5%	5%	10%
15th	**Shoot length**	12.72±0.17	13.20±0.09	15.45±0.10	12.86±0.28
	Root length	8.05±0.14	9.06±0.16	11.26±0.35	8.05±0.15
	Leaf area	18.84±029	26.85±0.17	35.45±0.18	27.21±0.51
30th	**Shoot length**	16.02±0.25	23.13±0.28	26.05±0.10	18.86±0.28
	Root length	13.15±0.04	14.06±0.16	16.86±0.25	13.85±0.23
	Leaf area	25.84±0.19	27.18±0.17	39.45±0.18	29.21±0.51
45 th	**Shoot length**	25.72±0.07	28.20±0.19	35.05±0.10	25.86±0.08
	Root length	15.05±0.24	16.06±0.36	20.26±0.15	15.85±0.45
	Leaf area	34.14±0.19	36.85±0.17	40.45±0.18	32.21±0.5
60 th	**Shoot length**	31.12±0.27	33.20±0.19	38.05±0.10	31.86±0.08
	Root length	18.05±0.24	20.06±0.36	25.26±0.15	18.85±0.45
	Leaf area	40.14±0.19	44.85±0.17	4.45±0.18	40.21±0.51

Table 4.Effect Of Algal Extract With Different Concentration On Fresh And Dry Weight of *Mung Bean* Morphological Traits - Pot Method

Parameters	Control	2.5%	5%	10%
No.of branches	7-10±2	10-15±3	16-20±2	10-13±3
No.of nodules	32-45±5	45-50±4	60-70±3	45-48±3
Freshweight of shoot mg/g/FW	90.195±7.651	110.130±6.710	125.173±8.152	103.101±3.120
Fresh weight of Root mg/g/FW	1.105±1.41	1.205±1.413	17.011±1.213	11.100±1.110
Dry weight of shoot mg/g/FW	0.040±0.003	0.049±0.004	0.058±0.003	0.040±0.003
Dry weight of Root mg/g/FW	0.038±0.003	0.046±0.003	0.051±0.002	0.034±0.002

Algal extracts contain plant hormones, amino acids, fatty acids and trace elements responsible for controlling plant growth and development and for improving the resistance to pathogens [31]. In the literature, there are data supporting the positive effects of algae and algal extracts on the growth of vegetables, fruits and other crops. Algal extracts are used both for conditioning seeds or as fertilizers for soil or foliar application during the growing season and flowering. They stimulate seed germination, growth and yield of different crops [16, 17, 26].

Crop cultivation using organic fertilizers has contributed for deposition of residues, improving physical and chemical properties of soil that is important for biological development [11]. This enhanced growth is thought to be due to various organic compounds present in the algal extract.

Effects of Biochemical Content

The leaf area was maximum in 5% and minimum in 2.5% giving more area for photosynthetic activity as shown in Table 3. The chlorophyll (total a, b), total protein, total carbohydrate and total lipid content was observed in the leaves varied in the order 5% >2.5% > 10% concentration.

Table.5. Effect of algal extract in biochemical constituent of *Vigna radiata* (*Hydrodictyon reticulatum*).

Biochemical constituent	Control	2.5%	5%	10%
Chlorophyll 'a' µg/g/Fw	290±5.270	300±6.177	350±7.132	282±3.768
Chlorophyll 'b' 'µg/g/Fw	188±5.787	210±7.813	246±5.236	178±4.670
Total Carbohydrate Mg/g/fw	1.8±0.351	1.9±0.235	2.8±0257	1.6±0.535
Total protein Mg/g/Dw	1.1±0.125	1.2±0.115	1.5±0.185	0.9±0.370
Lipid Mg/g/Dw	0.28±0.28	0.35±0.018	0.45±0.183	0.25±0.65

Chlorophyll percentage increased in algal extract treated plants relative to control was significantly higher and were much greener and also the senescence started delayed. Table 4 also shows that the amount of chlorophyll (chl. *a* and *b*) was high in plants from treatments followed by successive significant in plants of increasing algal extract levels as in5% algal extract contained a maximum of 350 µg/g fresh weight of Chlorophyll 'a' on 30th day old plants. Further, the concentration of Chlorophyll 'b' was 246 µg/g fresh weight, when compared to control. The reduction in photosynthetic material in plants grown in high concentrations (10%) produced comparatively less productivity than the plants grown in ordinary habitat. Chlorophyll content was enhanced in the prepared algal liquid extract of 5% which may be due to higher availability of magnesium [29].

Accumulation of total carbohydrate, total protein and total lipid content also increased due to the algal extract treatment. A maximum accumulation of the above parameters was recorded when the plant foliar spray of 5% 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 5). It is evident from the results that the increased growths (shoot and root length, leaf area, fresh weight of shoot and root) and biochemical constituents (Chlorophyll 'a', 'b', carbohydrates, protein and lipid) are possible due to the algal extract induced absorption of essential nutrients and the related increased enzyme activity.

The effect of algal extract on the plant growth depends on many factors, for example: dose, method and time of application, [5]. In the work of Anisimov and Chaikina [1], it was also confirmed that extracts obtained from various algae have a different effectiveness. Increases in phenolic components, flavonoids

and antioxidant levels were reported in leaves from spinach plants watered with soluble *A. nodosum* extract [10]. SLF treatment increased the number of branches and concentration of photosynthetic pigments [39]. More specifically it is understandable to be due to the presence of phytohormones, mainly cytokinins in the seaweed extracts [41]. From the previous report it is clear that the algae are rich in plant growth regulators and the composition varies in different algae.

CONCLUSION

Algal extracts were tested in assays of *Vigna radiata* germination at doses of 2.5%, 5% and 10 % per Petri dish. The utility of seed coating was verified by measurements of fresh weight of plants and root length of sprouts, as compared to untreated crops. Each of the applied doses of the formulation proved to affect both sprout and root development. The field studies showed good bio-stimulatory effect, have resulted in elongation of root, shoot length with number of leaves and hence overall growth of the plant has been increased markedly. So this algal liquid extract can be regarded as the new alternative renewable energy source for coming generations, substituting the needs of chemical fertilizers and promoting sustainable agriculture and bio-augmenting the water contaminants from algal weeds.

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