



REGULAR ARTICLE

EFFECT OF SEAWEED CONCENTRATES ON THE GROWTH AND BIOCHEMICAL CONSTITUENTS OF *TRIGONELLA FOENUM-GRÆCUM* L.

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SUMMARY

Effect of seaweed extract on growth and biochemical constituents of fenugreek was investigated using *Ulva fasciata*, *Sargassum ilicifolium* and *Gracilaria corticata* compared with the Hoagland nutrient medium. Fresh, dried and boiled extracts of *Ulva*, *Sargassum* and *Gracilaria* influenced photosynthetic pigments percentage in fenugreek in similar way as did Hoagland nutrition medium. Shoot growth and fresh biomass recorded at 50% SWC was comparable to that observed with the Hoagland solution and notably significant when compared to the control plants. Carbohydrate, proteins, free amino acids, polyphenols and nitrogen content also increased in seaweed treated plants. Extract of *Ulva* was found slightly more effective than that of *Sargassum* and *Gracilaria*.

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1. Introduction

Seaweed concentrates are known to cause many beneficial effects on plants as they contain growth promoting hormones (IAA and IBA, Cytokinins) trace elements (Fe, Cu, Zn, Co, Mo, Mn, and Ni), vitamins and amino acids (Challen and Hemingway, 1965). Liquid extracts obtained from seaweeds are successfully used as foliar sprays for several crops Bokil *et al.*, (1974). Booth (1966) 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 increased yield and quality of fruits (Rama Rao, 1991). Growth promoting effect of seaweed liquid fertilizer (*Enteromorpha intestinalis*) on the sesame crop plant (Gandhiyappan and Perumal, 2001). Seaweed foliar applications increased harvestable bean yields by an average of 25% (Temple, 1989), staked tomato yields by up to 99% (Csizinszky, 1984), early yield of one variety of greenhouse cucumber (Passam *et al.*, 1995), and greenhouse tomato total fruit

fresh weights by 17% (Crouch and Van standen, 1992). Moreover, they function as plant conditioners. These extracts when applied to seeds or when added to the soil, stimulate growth of the plants (Blunden, 1971).

Some commercial products available in market such as, Maxicrop, Algifert, Goemar, Kelpak, Seaspray, Seasol, SM3, Cytex and Seacrop. Seaweed fertilizers are better than other fertilizers and are very economical. The present study was undertaken to investigate the effect of seaweed liquid fertilizers (SLF) on the growth and biochemical characteristics of *Trigonella foenum-græcum* L.

2. Materials and Methods

Collection of seaweeds

In the present study effect of three common seaweeds viz. *U. fasciata*, *S. ilicifolium* and *Gracilaria corticata* occurring along the west coast of India was investigated and compared with Hoagland nutrient medium. Fresh thalli of *Ulva*, *Sargassum* and *Gracilaria* were collected from the coastal area around the Sindhudurg district of Maharashtra during Nov-Jan 2006.

The algal material was washed thoroughly with sea water to remove all the unwanted impurities, adhering sand particles and epiphytes. Morphologically distinct thalli of algae were placed separately in polythene bags and were kept in an ice box and brought to the laboratory. Samples were washed thoroughly using tap water to remove the surface salt and then blotted to remove excess water.

Preparation of seaweed liquid extract

Fresh material was cut into small pieces and weight it approximately one kg. Sample extracted using blender and then mortar-pestle. It was filtered through a double layered muslin cloth to remove debris. These filtrates were designated as 100% SWC and from these different concentrations (10%, 25%, and 50%) were prepared by adding distilled water. As the liquid fertilizer contained organic matter, it was refrigerated between 0 - 4°C until use.

Samplings of seaweeds were dried at 45°C in oven and ground to a fine powder. For the preparation of fertilizer, 50g powder was boiled in 50 ml distilled water for one hour and filtered through muslin cloth. The volume of the filtrate was made up to 50 ml (100% SWC).

Simultaneously, 50 g dried powder was soaked in 50 ml distilled water for two days and filtered through muslin cloth to obtain 100 % SWC. The three types of extracts were stored at 4°C and diluted at various concentrations whenever required.

Selection of crop plant

The crop plant, selected for the present study was *Trigonella foenum-graecum* L. belonging to the family Fabaceae. The seeds were collected from Regional Agriculture College Kolhapur District, Maharashtra, India. The seeds with uniform size, colour and weight were chosen for the experimental purpose. The selected seeds were stored in a metal tin as suggested by Rao (1976).

Seaweed liquid fertilizer treatment

The selected concentrations of SWC [10, 25 and 50 %] were used to study the effect on growth of fenugreek, hundred seeds were soaked for each selected concentration aqueous extracts of seaweeds for 1 h and

Seeds were grown on sterilized coco peat and garden soil mixture (1:1) in plastic tray. After every five days, treatment of SWC was applied as a foliar spray and root dripped. Simultaneously one set of seeds was treated with Hoagland solution (full strength) protocol followed by (Epstein, 1972). Growth parameters including shoot length, fresh and dry weight, were analysed after 25 day.

The biochemical constituents pigments such as chlorophylls and carotenoids were estimated in control and treated plants using standard methods [Arnone, (1949); Kirk, (1965)]. Protein (Lowry et al., 1951), Amino acids (Moore and Stein, 1948), Total carbohydrate (Sadasivam and Manickam., 1992), Total Nitrogen (Hawk et al., 1948) and Total Polyphenols (Folin and Dennis., 1915) were estimated in *T. foenum-graecum* L..

Statistical analysis

Data were analyzed statistically for standard deviation. All the measurements were made in triplicate.

3. Results

Growth of shoot was influenced by all the concentrations of both the seaweed extracts and a maximum value was recorded for 50% SWC. These were very near to those exhibited by the Hoagland treatment in case of SWC of *Ulva*. *Sargassum* extract did not show any effect due to concentrations and growth was equally influenced at all the three concentration used.

Fresh and dry weights were found maximum with 50% treatment of *Ulva* and almost had similar values as obtained for the Hoagland nutrient solution (Table 4). *Sargassum* treatment of 50% concentration was also found effective and comparable to Hoagland solution.

Photosynthetic pigments were enhanced by both the treatments of SWC when compared to the control values (Table 5). *Sargassum* treatment (50%) was equally effective as that of Hoagland while that of *Ulva* was found less promotive.

The data of various biochemical studies are presented in Tables 4 and 5. There was a significant difference in biochemical status of different concentration levels. The biochemical constituents increased with

concentration levels upto 50% (Table 5). The highest values of total chlorophyll content (142.6 ± 0.12 mg/100g fr. wt.), carotenoid (13.16 ± 0.05 mg/100g fr. wt.), protein content of leaves (3.31 mg/100g fr. wt.), amino acid content of leaves (4.68 g 100^{-1} g dry wt.), total carbohydrate content of leaves (5.79 g 100^{-1} g dry wt.), total polyphenol

content of leaves (0.51 mg/100g fr. Wt), total nitrogen content in leaves (8.89 g 100^{-1} g dry wt) and total ash content of leaves (10.02 mg/100g dry. wt) activities were recorded at 50% *S. illicifolium* extract soaked seedlings (Table 2) All these values are very near to those exhibited by the Hoagland treatment in case of SWC of *Sargassum*.

Table 1: Effect of SWC on the Fenugreek growth.

A											
Days	<i>Ulva</i>			<i>Sargassum</i>			<i>Gracilaria</i>			Control	Hoagland
	10%	25%	50%	10%	25%	50%	10%	25%	50%		
5	2.4	2.8	2.8	2.2	2.8	2.7	2.3	2.6	2.8	1.69	1.74
10	3.7	3.8	3.9	3.8	3.9	4.1	3.7	4.1	4.1	3.31	4.54
15	7.6	7.9	8.2	7.8	7.9	8.5	7.7	8.4	8.3	4.23	8.75
20	10.1	10.4	10.4	10.2	10.5	10.5	10.1	10.2	10.5	4.62	10.82
25	12.4	12.6	12.7	12.5	12.4	12.8	12.1	12.4	12.9	9.25	13.60

B											
Days	<i>Ulva</i>			<i>Sargassum</i>			<i>Gracilaria</i>			Control	Hoagland
	10%	25%	50%	10%	25%	50%	10%	25%	50%		
5	1.59	1.68	1.83	1.68	1.94	2.70	1.7	2.2	2.8	1.69	1.74
10	3.39	3.47	3.68	3.30	3.87	3.80	3.9	3.5	3.9	3.31	4.54
15	5.53	5.73	6.65	4.10	4.70	4.90	4.5	7.2	7.8	4.23	8.75
20	6.41	7.39	8.02	3.18	3.90	8.30	5.1	9.9	8.8	4.62	10.82
25	10.92	10.92	13.10	9.54	9.75	10.25	10.7	11.8	12.1	9.25	13.60

C											
Days	<i>Ulva</i>			<i>Sargassum</i>			<i>Gracilaria</i>			Control	Hoagland
	10%	25%	50%	10%	25%	50%	10%	25%	50%		
5	1.55	2.1	2.7	1.9	2.2	2.1	1.4	2.2	1.22	1.69	1.74
10	3.22	3.4	3.9	3.51	3.7	4.1	3.7	4.0	2.87	3.31	4.54
15	5.00	6.9	7.7	4.8	4.7	7.5	4.7	5.3	4.03	4.23	8.75
20	5.73	8.2	8.5	5.8	6.1	9.8	5.5	7.9	7.74	4.62	10.82
25	10.28	11.9	12.7	10.12	10.2	12.6	11.2	12.1	12.40	9.25	13.60

A- Fresh B- Boiled C- Soaked (Height of the plant in cm.)

Table 2: Effect of SWC on Fresh, Dry and Moisture percentage of fenugreek

Parameter	Control	Hoagland	Fresh extract			Boiled extract			Soaked extract		
			10%	25%	50%	10%	25%	50%	10%	25%	50%
Fresh weight	0.711	1.078	0.775	0.935	1.020	0.875	0.944	1.025	0.799	0.890	1.018
Dry weight	0.234	0.340	0.237	0.272	0.342	0.244	0.269	0.336	0.229	0.240	0.335
Moisture	65.82	68.46	69.03	70.90	66.47	72.11	71.50	67.21	71.33	73.03	67.09

B

Parameter	Control	Hoagland	Fresh extract			Boiled extract			Soaked extract		
			10%	25%	50%	10%	25%	50%	10%	25%	50%
Fresh weight	0.711	1.078	0.775	0.935	1.015	0.757	0.816	1.040	0.810	0.880	1.019
Dry weight	0.234	0.340	0.240	0.272	0.334	0.231	0.234	0.332	0.212	0.248	0.331
Moisture	65.82	68.46	69.03	70.90	67.70	69.48	70.58	68.07	73.82	71.81	67.51

C

Parameter	Control	Hoagland	Fresh extract			Boiled extract			Soaked extract		
			10%	25%	50%	10%	25%	50%	10%	25%	50%
*Fresh wt.	0.711	1.078	0.794	0.918	1.009	0.799	0.889	1.027	0.789	0.877	1.020
*Dry wt.	0.234	0.340	0.244	0.267	0.337	0.254	0.289	0.388	0.252	0.277	0.382
@Moisture	65.82	68.46	69.26	70.91	66.50	68.21	67.49	62.22	68.06	68.41	62.54

A- Ulva, B- Sargassum C- Gracilaria * - g 100⁻¹g @ - Percentage

Table 3: Effect of SLF of *U. fasciata* on biochemical composition of the seedling of *T. foenum-graecum* L.

Concentration	mg 100 ⁻¹ g fresh wt.						g 100 ⁻¹ g dry wt.			g 100 ⁻¹ g fresh wt.		
	Total chlorophyll			Carotenoids			Total carbohydrate			Protein		
	I	II	III	I	II	III	I	II	III	I	II	III
CONTROL	125.2±0.5	117.3±0.5	117.3±0.4	11.88±0.06	12.44±0.03	12.44±0.1	3.71±0.01	4.19±0.01	4.25±0.03	0.91±0.01	0.99±0.01	0.99±0.01
10%	133.1±0.1	127.8±0.1	123.9±0.7	12.69±0.04	12.84±0.03	12.60±0.02	3.93±0.03	4.21±0.01	4.8±0.03	0.92±0.00	1.01±0.03	1.01±0.02
25%	133.8±0.2	134.9±0.9	130.4±0.8	12.96±0.4	13.22±0.02	12.60±0.02	4.13±0.01	4.26±0.00	4.39±0.00	1.80±0.03	1.42±0.03	1.94±0.02
50%	138.9±0.8	142.2±0.3	135.5±0.4	13.12±0.01	13.36±0.02	13.12±0.01	5.91±0.02	4.42±0.02	4.57±0.02	3.21±0.01	2.91±0.02	3.18±0.02
Hoagland	144.0±0.3	147.7±0.2	144±0.1	13.16±0.01	13.19±0.01	13.12±0.01	6.51±0.01	5.09±0.03	5.64±0.00	3.36±0.02	3.26±0.02	3.36±0.01

Concentration	g 100 ⁻¹ g dry wt.			g 100 ⁻¹ g fresh wt.			g 100 ⁻¹ g fresh wt.			g 100 ⁻¹ g dry wt.		
	Total Ash			Amino acid			Polyphenols			Total nitrogen		
	I	II	III	I	II	III	I	II	III	I	II	III
CONTROL	7.2±0.3	6.2±0.3	6.9±0.3	0.89±0.01	1.29±0.01	1.28±0.02	0.33±0.04	0.31±0.02	0.23±0.02	7.71±0.03	7.29±0.03	7.38±0.02
10%	7.1±0.2	6.5±0.4	7.3±0.2	1.21±0.02	1.25±0.03	1.31±0.04	0.22±0.03	0.31±0.03	0.23±0.05	7.82±0.02	7.33±0.02	7.45±0.01
25%	8±0.1	6.6±0.2	7.8±0.3	1.94±0.03	1.31±0.02	1.41±0.02	0.37±0.01	0.34±0.01	0.30±0.03	8.39±0.03	7.53±0.04	7.72±0.03
50%	10.2±0.1	7.8±0.6	8.8±0.05	4.63±0.02	2.83±0.06	2.21±0.03	0.50±0.3	0.36±0.03	0.39±0.42	8.96±0.02	7.93±0.01	8.28±0.04
Hoagland	10.8±0.1	8.9±0.2	9.6±0.11	4.75±0.04	3.97±0.02	3.36±0.02	0.53±0.02	0.46±0.02	0.45±0.01	9.14±0.01	8.66±0.02	8.83±0.01

I— Aqueous extract soaked; II— Water soaked; III— Boiled extract; ± Standard deviation. * Significant at p = 0.05 level.

Table 4: Effect of SLF of *S. ilicifolium* on biochemical composition of *T. foenum-graecum* L.

Concentration	mg 100 ⁻¹ g fresh wt.						g 100 ⁻¹ g dry wt.			g 100 ⁻¹ g fresh wt.		
	Total chlorophyll			Carotenoids			Total carbohydrate			Protein		
	I	II	III	I	II	III	I	II	III	I	II	III
CONTROL	117.3±0.7	117.3±0.1	117.3±0.4	12.44±0.4	12.44±0.5	12.44±0.3	3.71±0.1	4.19±0.06	4.25±0.04	0.91±0.06	0.99±0.01	0.99±0.02
10%	128.3±0.04	123.9±0.5	123.9±0.4	12.88±0.2	12.64±0.03	12.64±0.04	3.87±0.01	4.20±0.02	4.35±0.03	0.94±0.02	0.98±0.03	0.99±0.03
25%	139.1±0.3	132.0±0.7	132.0±0.5	13.08±0.02	12.76±0.2	12.76±0.1	5.16±0.27	4.27±0.02	4.48±0.01	1.87±0.3	1.52±0.03	1.51±0.00
50%	142.6±0.2	142.5±0.3	142.5±0.2	13.16±0.02	13.28±0.1	13.28±0.2	5.79±0.05	4.46±0.05	5.59±0.04	3.31±0.03	2.97±0.01	3.09±0.09
Hoagland	147.8±0.00	147.8±0.2	147.8±0.1	13.60±0.1	13.60±0.2	13.60±0.6	6.51±0.06	5.09±0.00	5.64±0.06	3.36±0.01	3.26±0.00	3.36±0.02

Concentration	g 100 ⁻¹ g fresh wt.			g 100 ⁻¹ g fresh wt.			g 100 ⁻¹ g dry wt.			g 100 ⁻¹ g dry wt.		
	Amino acid			Total polyphenols			Total nitrogen			Total Ash		
	I	II	III	I	II	III	I	II	III	I	II	III
CONTROL	0.89±0.03	1.29±0.01	1.28±0.03	0.33±0.02	0.31±0.02	0.23±0.01	7.2±0.1	6.2±0.1	6.9±0.1	7.71±0.08	7.29±0.02	7.38±0.06
10%	1.10±0.1	1.31±0.03	1.42±0.03	0.34±0.01	0.31±0.02	0.34±0.01	7.3±0.2	6.6±0.2	7±0.2	7.74±0.04	7.39±0.01	7.48±0.03
25%	1.98±0.04	1.75±0.06	1.79±0.03	0.44±0.01	0.33±0.01	0.35±0.01	8.4±0.03	6.8±0.2	7±0.1	8.49±0.01	7.63±0.02	8.24±0.05
50%	4.68±0.02	3.89±0.02	4.01±0.3	0.52±0.00	0.35±0.01	0.47±0.01	10.2±0.1	7.9±0.01	8.9±0.1	8.89±0.08	8.23±0.02	8.62±0.02
Hoagland	4.75±0.02	3.97±0.03	3.36±0.03	0.53±0.00	0.46±0.01	0.43±0.01	10.8±0.2	8.9±0.1	9.6±0.2	10.09±0.01	10.12±0.01	9.8±0.03

I— Aqueous extract soaked; II— Water soaked; III— Boiled extract; ± Standard deviation. * Significant at p = 0.05 level.

Table 5: Effect of SLF of *G. corticata* on biochemical composition of the seedling of *T. foenum-graecum* L.

Concentration	mg 100 ⁻¹ g fresh wt.						g 100 ⁻¹ g dry wt.			g 100 ⁻¹ g fresh wt.		
	Total chlorophyll			Carotenoids			Total carbohydrate			Protein		
	I	II	III	I	II	III	I	II	III	I	II	III
CONTROL	117.3±0.8	117.3±0.2	117.3±0.6	12.44±0.01	12.44±0.02	12.44±0.05	3.71±0.02	4.19±0.01	4.25±0.03	0.91±0.01	0.99±0.03	0.99±0.02
10%	136.2±0.4	122.0±0.8	126.3±0.5	12.60±0.4	12.76±0.7	12.28±0.1	3.91±0.07	4.22±0.01	4.30±0.2	0.92±0.00	0.99±0.01	0.98±0.03
25%	136.9±0.5	129.5±	135.0±0.1	12.72±0.4	12.80±0.05	12.76±0.1	4.92±0.07	4.25±0.05	4.39±0.02	1.77±0.08	1.20±0.02	1.33±0.04
50%	140.6±0.2	139.6±0.7	139.9±0.3	12.80±0.5	13.40±0.5	13.12±0.2	5.41±0.1	4.37±0.02	5.13±0.15	3.28±0.02	2.51±0.06	2.41±0.06
Hoagland	147.8±0.2	147.8±0.4	147.8±0.6	13.68±0.2	13.62±0.1	13.60±0.8	6.51±0.03	5.09±0.11	5.64±0.09	3.36±0.08	3.26±0.03	3.36±0.04

Concentration	g 100 ⁻¹ g fresh wt.			g 100 ⁻¹ g fresh wt.			g 100 ⁻¹ g dry wt.			g 100 ⁻¹ g dry wt.		
	Amino acid			Total polyphenols			Total nitrogen			Total Ash		
	I	II	III	I	II	III	I	II	III	I	II	III
CONTROL	0.89±0.07	1.29±0.03	1.28±0.03	7.2±0.2	6.2±0.3	6.9±0.4	0.33±0.02	0.31±0.02	0.23±0.04	7.71±0.02	7.29±0.09	7.38±0.005
10%	1.09±0.1	1.21±0.07	1.36±0.03	6.9±0.7	6.2±0.3	7.1±0.2	0.28±0.03	0.31±0.03	0.23±0.05	7.71±0.07	7.29±0.05	7.45±0.06
25%	1.77±0.2	1.30±0.1	1.56±0.1	7.8±0.1	6.5±0.05	7.3±0.2	0.34±0.03	0.32±0.02	0.33±0.06	8.14±0.06	7.73±0.1	7.76±0.1
50%	4.65±0.3	2.41±0.03	3.64±0.01	9.8±0.2	7.1±0.2	8.1±0.2	0.44±0.03	0.35±0.02	0.47±0.03	8.57±0.04	8.03±0.05	8.07±0.1
Hoagland	4.75±0.01	3.97±0.02	3.36±0.07	10.8±0.3	8.9±0.5	9.6±0.4	0.53±0.04	0.46±0.00	0.45±0.04	9.14±0.06	8.66±0.00	8.83±0.06

I— Aqueous extract soaked; II— Water soaked; III— Boiled extract; ± Standard deviation. * Significant at p = 0.05 level.

4. Discussion

Fenugreek with lower concentrations of both the seaweed extracts showed higher growth (up to 50% conc.). Enhanced of the growth may be due to presence of growth promoting factor in SWC. Similar observations have been made in earlier studies. *Padina* which induced maximum seedling growth at lower concentrations in *C. cajan* (Mohan et al., 1994) and *Vigna radiata* (Venkataraman Kumar et al., 1993) of earlier studies in maize, ragi and kambu (Rajkumar Immanuel and Subramanian, 1999). Statistically significant differences were observed for shoot length and fresh and dry weight. The growth enhancing potential of seaweeds might be attributed to the presence of macro and micronutrients (Challen and Hemingway, 1965).

The highest protein content was recorded at 50% concentration of SLF soaked treatment in fenugreek. The increase in the protein content at lower concentration of SLF might be due to absorption of most of the necessary elements by the seedlings (Kannan and Tamilselvan, 1990; Anantharaj and Venkatesalu, 2001, 2002). The total carbohydrate content increased up to 50% concentration of SLF. The same trend was observed in the *H. musciformis* with NPK application in blackgram (Tamilselvan and Kannan, 1994), *V. catajung* and *D. biXorus* (Anantharaj and Venkatesalu, 2001, 2002). It has been observed that total nitrogen activity increased at lower to higher concentrations of both the treatments of seaweeds. All values are compared with Hoagland treated fenugreek plant and *Sargassum* SLF is very near to the Hoagland treatment.

Among the two seaweed liquid fertilizers, *S. illicifolium* liquid fertilizer yielded better results. The higher concentrations showed a decreasing effect. The lower concentrations of the two seaweed liquid fertilizers (*Sargassum* and *Ulva*) also promoted the chlorophyll content of fenugreek when compared to the control. Blunden *et al.*, (1996) reported that the seaweed extract applied as foliar spray enhanced the leaf chlorophyll level in plants. In the present study high Mg and Fe content in *Sargassum* might have

influenced the synthesis of chlorophyll. Both the seaweeds extract prepared from *Sargassum illicifolium* and *Ulva fasciata* were found to have fertilizing ability.

5. Conclusion

The present investigations revealed that seaweed species were observed to be a potential sources of fertilizer. Present findings encourage the application of such seaweeds as natural fertilizer in agricultural sector. Hence, this simple practice of application of ecofriendly seaweed liquid fertilizers to crops may be useful for the growers for attaining better germination, growth and yield. Findings of this work are useful to further research to evaluation by isolation, characterization and identifications of growth hormones.

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