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Assessment the different extraction methods of SLF and SGLF on *Vigna mungo* seedlings

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ABSTRACT

This paper deals with the study of *Stoechospermum marginatum* SLF and *Halophila ovalis* SGLF obtained by four different methods of extraction (low temperature, boiling, autoclave, alcohol-aqueous) on the growth and photosynthetic pigments of *Vigna mungo* seedlings. Autoclave and alcoholic methods of extraction were found to be the most effective, inducing seedling growth and photosynthetic pigments to maximum level. Since the SLFs and SGLFs obtained by alcohol aqueous method impacted seedling growth parameters to a greater extent, it is suggested that the afore said method could be followed for SLF and SGLF preparation and its application either for soaking the seeds of land crops, prior to sowing or as foliar spray or even for drenching the soil or all these practices.

1. Introduction

In modern agriculture, chemical fertilizers have degraded the fertility of soil making it acidic and rendering it unsuitable for raising crop plants. Seaweeds are marine macro algae which form an important component of the marine living resources of the world. The plant growth hormone's effect of seaweed is advantageous to stimulate germination and growth, thereby increasing the yield and resistance ability of many crops. . In recent years, the use of natural seaweed as fertilizer has allowed for partial substitution of conventional synthetic fertilizer (Khan *et al.* 2009; Zodape *et al.* 2010). Sea grasses are only flowering plants living under water in the photic zone of coastal area. They form beds of monospecific or mixed communities and can support very high biodiversity. At present extracts from many species of seagrasses are being screened for their pharmaceutical properties. Research on the use of seagrasses as fertilizer are gaining momentum (Asir Selin Kumar *et al* 2006; Sobithabai et al, 2007, 2008 Asir selin Kumar, 2009, Asir Selin Kumar and Sobitha Bai, 2010 and Femila Jose *et al,* 2010). However comparative studies on the impact of SLF and SGLF are scanty (Asir Selin Kumar *et al* 2004 and Sobitha Bai *et* al 2007). Hence the present study was taken up to assess the effect *of Stoechospermum* SLF and *Syringodium* SGLF obtained by four methods of extractions on the seedling growth and photosynthetic pigments of *Vigna mungo.*

2. Materials and Methods

The sea weed *Stoechospermum marginatum* and sea grass *Halophila ovalis were* collected from Hare Island, Thoothukudi coast. The samples were handpicked and washed thoroughly with seawater to remove all the impurities, sand particles and epiphytes. Then extracts were prepared by using four different methods (low temperature heating method, boiling method, autoclave method, alcoholic extraction method) and then made into different concentrations (0.25%, 0.50%, 0.75%, 1.00%, 1.5%, 2.0%).

Heating at **60°C or low temperature method:** (Sylvia., 2005)

100 g of samples was weighed and cut into small pieces to which one liter of deionized water was added. It was heated to 60 "C and maintained in that temperature for 24 hours using hot air oven. The extract was filtered through a muslin cloth. Extraction by boiling method**:** (Bhosle *et al,* 1975) 100 g of samples suspended in one liter of deionized water was heated at 100°C in water bath for one hour. The extract was filtered through a muslin cloth**.**

Extraction by autoclave method: (Rama Rao,, 1990)

100 g of samples was weighed and cut into small pieces, to which deionized water was added in the ratio of 1:10 and was autoclaved at 15 lb pressure for 1 hour. Extract obtained was filtered through a muslin cloth.

Alcoholic extraction method: (Ramamoorthy and Sujatha, 2007)

100 g of powered samples were mixed with 100 ml of ethanol and stirred well and then filtered. The filtrate was considered as 100% concentration of SLF and SGLF.

Test crop plant

Test seedling plant selected for the present study was *Vigna mungo* (black gram). It is cultivated throughout Indian Sub-continent. Certified seeds of *Vigna mungo.* L. (Co.6) was purchased from Tamil Nadu Agriculture University (TNAU), Coimbatore.

Seed treatment

The seeds were sterilized using 1% mercuric chloride for 1-2 minutes and rinsed thoroughly with distilled water, and then soaked in different concentrations of SLF and SGLF (0.25 %, 0.50%, 0.75%, 1.0%, 1.5%, and 2.0%) for 12 hours. Control was maintained by soaking one set of seeds in distilled water.

3. Seed germination and seedling growth studies

The soaked seeds were sown in growth towels (old news paper sheets). The rolled growth towels were placed in plastic trough filled with water. The seed germination percentage and seedling growth parameters (shoot length, root length, no of

lateral roots, fresh weight, and dry weight) on 7 days old seedling were estimated. Pigment analysis Chlorophylls and Caroteins were estimated by the method of Amon (1949) as modified by Harborne, **1**973

Table 1-Effect of Stoechospermum marginatum SLF obtained by low boiling method on *Vigna mungo* seedlings

Parameters	Control	SLF concentration (%)					
		0.25	0.50	0.75	1.00	1.5	2.0
Germination (%)	80	100	100	98	98	98	98
Root length (cm)	10.7±1.14	8.0±1.1610	7.07±2.470	10.35±1.25	9.0±1.714	9.81±1.248	6.35±0.199
Shoot length (cm)	12.6±0.526	14.9±0.689	18.89±0.785	15.8±0.401	16.7±0.361	19.95±0.732	8.9±0.292
No of lateral roots	4.3±0.629	8.3±0.923	8.9±3.230	13.0±1.882	11.7±0.104	12.0±2.082	8.6±0.817
Fresh weight (g)	2.2±0.137	2.1±0.132	2.4±0.149	2.0±0.077	2.1±0.177	1.8±0.112	1.9±0.116
Dry weight (g)	0.16±0.013	0.16±0.001	0.19±0.027	0.18±0.023	0.19±0.001	0.1±0.001	0.1±0.001

N=15,Means±SE

Table 2- Effect of *Stoechospermum marginatum* SLF obtained by boiling method on *Vigna mungo* seedlings

parameters	Control	SLF concentration (%)					
		0.25	0.50	0.75	1.00	1.5	2.0
Germination (%)	90	100	90	90	95	95	100
Root length (cm)	13.3±2.27	14.0±0.427	10.6±1.261	9.9±1.181	10.7±0.751	8.4±3.097	10.4±1.496
Shoot length (cm)	18.5±2.530	18.6±3.023	15.4±1.927	12.8±2.232	11.5±0.030	9.2±0.441	14.2±1.057
No of lateral roots	8.9±1.354	14.1±3.608	10.8±2.031	10.5±2.408	9.1±2.562	6.7±2.102	8.7±1.415
Fresh weight (g)	2.14±0.160	1.19±0.072	2.24±0.137	1.20±0.073	1.00±0.021	1.13±0.063	1.29±0.078
Dry weight (g)	0.15±0.012	0.1±0.001	0.17±0.016	0.09±0.003	0.007±0.001	0.1±0.002	0.15±0.013

N=15,Means±SE

Table 3-Effect of Stoechospermum *marginatum* SLF obtained autoclave method on *Vigna mungo* seedlings

Parameters	Control	SLF concentration (%)					
		0.25	0.50	0.75	1.00	1.5	2.0
Germination (%)	97	100	100	98	100	96	97
Root length (cm)	14.5±0.845	14.55±1.024	18.45±1.995	14.0±0.579	16.51±0.815	10.56±2.406	12.6±1.478
Shoot length (cm)	24.15±0.176	23.75±0.909	26.24±4.484	26.3±1.288	25.7±2.310	24.3±0.445	24.5±0.779
No of lateral roots	3.24±3.231	20.5±4.845	27.0±2.162	22.2±4.902	23.0±2.180	18.7±4.249	23.5±1.121
Fresh weight (g)	2.08±0.014	2.15±0.132	2.70±0.165	2.39±0.138	2.50±2.227	2.29±0.137	2.40±0.201
Dry weight (g)	0.35±0.022	0.24±0.16	0.39±0.024	0.35±0.021	0.31±0.001	0.28±0.018	0.34±0.022

N=15,Means±SE

Table 4- Effect of Stoechospermum *marginatum* SLF obtained by alcoholic extraction method on *Vigna mungo* seedlings

parameters	Control	SLF concentration (%)					
		0.25	0.50	0.75	1.00	1.5	2.0
Germination (%)	90	90	100	100	100	100	98
Root length (cm)	14.5±5.295	16.2±0.591	18.05±0.45	16.84±0.92	15.91±0.850	17.37±2.027	15.8±0.305
Shoot length (cm)	25.4±1.700	28.2±0.700	22.67±0.938	24.95±0.857	25.01±.257	26.56±0.367	24.29±0.712
No of lateral roots	18.8±3.54	26.6±4.441	22.3±7.537	25.0±7.047	22.46±0.473	21.5±4.047	20.9±4.402
Fresh weight (g)	2.1±0.131	3.0±0.205	2.11±0.132	2.16±0.134	2.17±0.310	2.15±0.133	1.20±0.074
Dry weight (g)	0.19±0.016	0.34±0.021	0.18±0.014	0.23±0.015	0.23±0.025	0.22±0.012	0.21±0.012

N=15,Means±SE

Table 5-Effect of *Halophila ovalis* SLF obtained by low boiling method on *Vigna mungo* seedlings

Parameters	Control	SLF concentration (%)					
		0.25	0.50	0.75	1.00	1.5	2.0
Germination (%)	80	85	89	100	100	100	100
Root length (cm)	12.0±0.173	11.5±2.929	18.0±0.581	12.3±2.0.7	11.16±3.032	9.53±0.785	9.0±1.154
Shoot length (cm)	15.3±3.179	20.0±0.577	1616±2.587	19.0±0.154	24.6±3.711	20.0±4.041	16.0±2.516
No of lateral roots	13.33±4.333	21.0±2	18.3±2.185	18.3±0.666	24.83±1.589	21.0±2.516	20.0±1
Fresh weight (g)	0.36±0.035	0.313±0.115	0.320±0.047	0.3±0.017	0.4±0.040	0.346±0.076	0.31±0.045
Dry weight (g)	0.038±0.001	0.030±0.000	0.029±0.000	0.030±0.000	0.039±0.001	0.031±0.003	0.026±0.003

N=15,Means±SE

Table 6- Effect of *Halophila ovalis* SLF obtained by boiling method on *Vigna mungo* seedlings

parameters	Control	SLF concentration (%)					
		0.25	0.50	0.75	1.00	1.5	2.0
Germination (%)	98	97	98	100	100	100	99
Root length (cm)	12.0±2.516	12.3±0.210	15.0±0.309	15.3±2.848	10.8±0.440	12.3±0.906	11.3±2.333
Shoot length (cm)	25.66±2.4888	27.6±2.962	22.3±1.333	11.6±1.763	17.66±4.0555	23.66±1.666	16.83±2.920
No of lateral roots	19.33±6.888	15.66±3.844	20.66±2.728	21.1±1.527	16.33±1.763	18.3±0.881	19.0±1.732
Fresh weight (g)	0.31±0.624	0.353±0.052	0.253±0.026	0.343±0.061	0.35±0.047	0.393±0.026	0.36±0.066
Dry weight (g)	0.027±0.001	0.030±0.001	0.022±0.007	0.039±0.08	0.034±0.000	0.038±0.000	0.030±0.001

N=15,Means±SE

Table 7-Effect of *Halophila ovalis* SLF obtained autoclave method on *Vigna mungo* seedlings

parameters	Control	SLF concentration (%)					
		0.25	0.50	0.75	1.00	1.5	2.0
Germination (%)	90	98	100	100	100	100	100
Root length (cm)	10.8±3.881	13.9±0.024	10.5±3.417	9.85±4.295	8.6±4.452	12.39±5.223	10.1±4.965
Shoot length (cm)	13.8±0.293	18.9±5.665	11.7±4.498	13.2±3.207	12.7±4.808	22.5±4.881	20.1±3.891
No of lateral roots	9.8±6.160	19.1±8.887	19.7±7.616	12.3±6.395	9.2±5.553	18.8±6.373	15.6±2.160
Fresh weight (g)	0.20±0.001	0.241±0.001	0.263±0.00	0.249±0.003	0.210±0.003	0.340±0.000	0.30±0.004
Dry weight (g)	0.015±0.002	0.016±0.000	0.018±0.001	0.015±0.006	0.015±0.000	0.022±0.001	0.17±0.001

N=15,Means±SE

Table 8- Effect of *Halophila ovalis* SLF obtained by alcoholic extraction method on *Vigna mungo* seedlings

parameters	Control	SLF concentration (%)					
		0.25	0.50	0.75	1.00	1.5	2.0
Germination (%)	100	100	100	100	100	100	100
Root length (cm)	15.33±1.452	19.33±0.201	20.3±1.452	21.1±3.412	15.4±2.986	9.0±1.00	11.0±1.154
Shoot length (cm)	21.6±1.402	25.7±0.865	22.0±1.00	21.3±5.666	26.826±1.867	26.4±1.433	20.0±5.196
No of lateral roots	26.33±3.527	27.0±5.50	37.3±3.177	16.2±0.986	25.3±4.17	21.6±1.666	25.33±5.840
Fresh weight (g)	0.413±0.046	0.43±0.044	0.46±0.049	0.406±0.029	0.383±0.032	0.43±0.005	0.40±0.0251
Dry weight (g)	0.037±0.0001	0.031±0.003	0.036±0.003	0.038±0.002	0.031±0.001	0.030±0.000	0.031±0.000

N=15,Means±SE

***Stoechospermum marginatum* SLF obtained by low temperature heating method (Table-1; Fig-1)**

Compared to control, shoot length exceeded at certain concentrations of SLF (0.25-1.00%o), root length only at 0.75%, number of lateral roots at all the concentrations of SLF (0.25-2.00%), fresh weight at 0.50% and dry weight at 0.50, 0.75 and 1.00%o SLF (Table-1). The amount of biochemicals exceeded over the control at almost all the concentrations of SLF (Fig-1).

***Stoechospermum marginatum* SLF obtained by boiling method (Table- 2; Fig-2)**

Shoot **length** and root length were found to be more than that of control at 0.25%) SLF. Number of lateral roots was more than the control at concentrations ranging from 0.25 to 1.00%). Fresh weight and dry weight exceeded over the control at 0.50%) SLF (Table-2). The amount of chlorophyll a and chlorophyll b was more than the control only at certain concentrations of SLF while the amount of total chlorophyll and carotenoids exceeded over the control at all the concentrations (Fig-2).

***Stoechospermum marginatum* SLF obtained by autoclave method (Table-3; Fig-3)**

At SLF concentration from 0.25 to 2.00%, the shoot length exceeded over that of control. Root length showed enhancement over the control at some of the concentrations. Number of lateral roots and fresh weight were found to be more than the control at all the concentrations. Dry weight exceeded than the control at 0.50% SLF (Table-3). The amount of chlorophyll a was more than the control at all the concentrations, chlorophyll b at certain concentrations, total chlorophyll at all the concentrations except 1.00% SLF and carotenoids at all the concentrations except at 0.75% (Fig-3).

***Stoechospermum marginatum* SLF obtained by aqueous-alcohol extraction method (Table-4; Fig-4)**

At 0.25 and 0.50%) SLF, the shoot length got enhanced over the control, whereas root length and number of lateral roots exceeded than the control at all the concentrations. At almost all the concentrations of SLF, fresh weight and dry weight were found to be more than the control (Table-4). Chlorophyll a content showed increment over the control at most of the concentrations while chlorophyll b at all the concentrations. Amount of total chlorophyll exceeded over the

control at 0.75%) SLF whereas carotenoids at almost all the concentrations (Fig-4).

SGLF obtained by *Halophila ovalis* low temperature heating method (Table-5; Fig-5)

Shoot length showed enhancement over the control at all the concentrations of SGLF, root length at certain concentrations only (0.50, 0.75%)), number of lateral roots at all the concentrations of SGLF, fresh weight and dry weight at 1.00%) SGLF (Table-5). The amount of chlorophyll a and chlorophyll b was more than the control at all the concentrations except 0.25%) SGLF. Whereas total chlorophyll content exceeded over that of control at all the concentrations of SGLF (0.25 to 2.00%)). At almost all the concentrations, the amount of carotenoid was more than the control (Fig-5).

***Halophila ovalis* SGLF obtained by boiling method (Table-6; Fig-6)**

When compared to the control, shoot length was more at 0.25% SGLF, root length at 0.50, 0.75 and 1.50%, number of lateral roots at 0.50 and 0.75%, fresh weight and dry weight at all the concentrations except at 0.50%) SGLF (Table-6). Only at some of the concentrations only chlorophyll a, b and total chlorophyll content exceeded over the control. Whereas the arnount of carotenoid was more than the control at all the concentrations of SGLF expect at 2.00% (Fig-6).

4.1.2.7. *Halophila ovalis* SGLF obtained by autoclave method (Table-7; Fig-7)

Compared to control, shoot length was more at 0.25, 1.50 and 2.00% SGLF, root length at 0.25 and 1.50%), number of lateral roots at all the concentrations except at 1.00%, fresh weight at all the concentrations and dry weight at 0.25, 0.50, 1.50 and 2.00% SGLF (Table-7). The amount of chlorophyll a, b and total chlorophyll exceeded over the control at all the concentrations of SGLF while carotenoid at some of the concentrations (0.25, 0.50, and 1.00%) (Fig-7).

4.1.2.8. *Halophila ovalis* SGLF obtained by alcohol-aqueous extraction method (Table-8; Fig-8)

Shoot length exceeded over that of control at all the concentrations of SGLF except at 0.75 and 2.00%, root length at all the concentrations except at 1.50 and 2.00%, number of lateral roots at 0.25 and 0.50%, fresh weight at 0.25, 0.50 and 1.50%) and dry weight at 0.75% of SGLF (Table-8). The amount of chlorophyll a, b total chlorophyll was more than that of control at 0.25 and 1.00 to 2.00% SGLF. Carotenoid content exceeded over the control at most of the concentrations of SGLF (Fig-8).

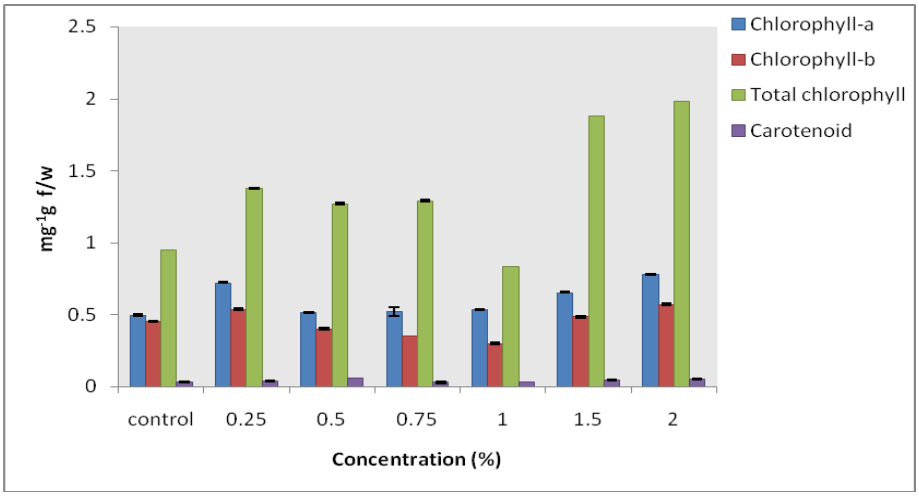

Fig-1. Effect of *Stoechospermum* SLF obtained by (Low temperature heating) on chlorophyll and carotenoids

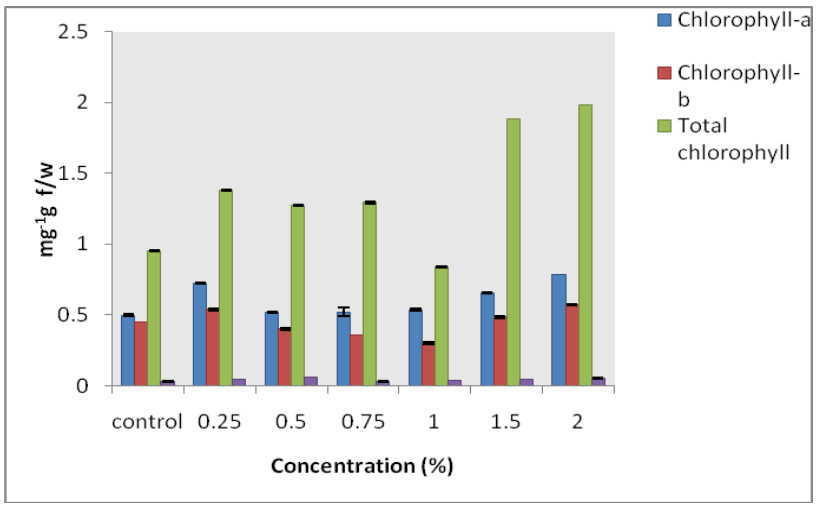

Fig-2. Effect of *Stoechospermum* SLF obtained by (Boiling) on chlorophyll and carotenoids

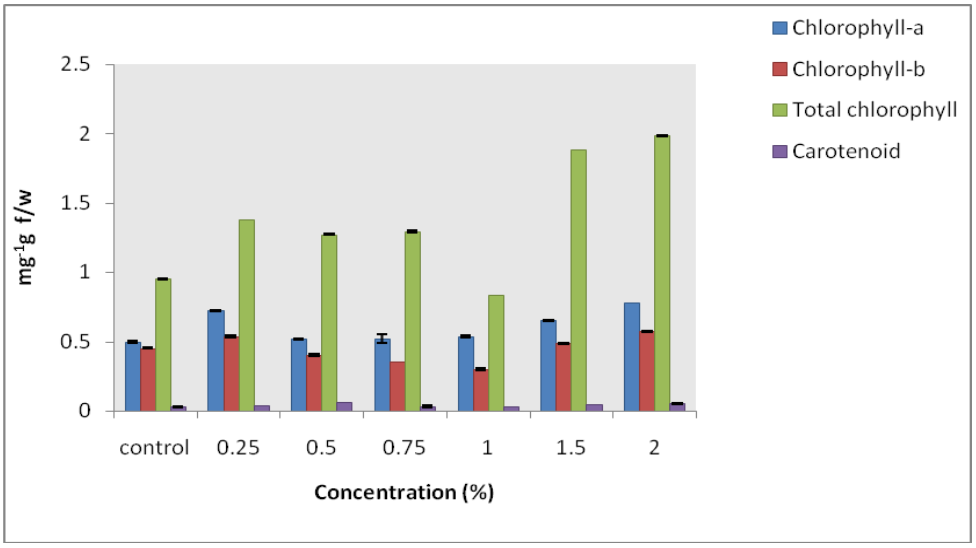

Fig-3. Effect of *Stoechospermum* SLF obtained by (Autoclave) on chlorophyll and carotenoids

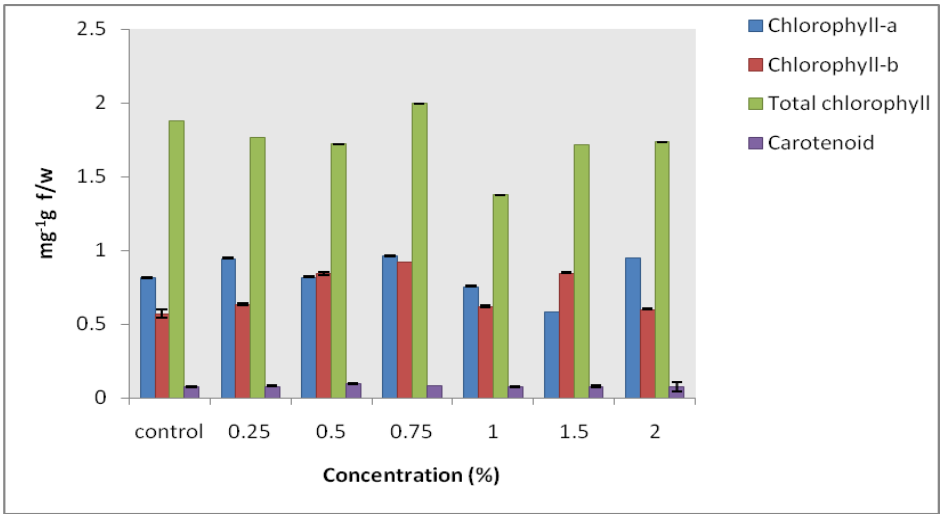

Fig-4. Effect of *Stoechospermum* SLF obtained by (Alcohol-aqueous) on chlorophyll and carotenoids.

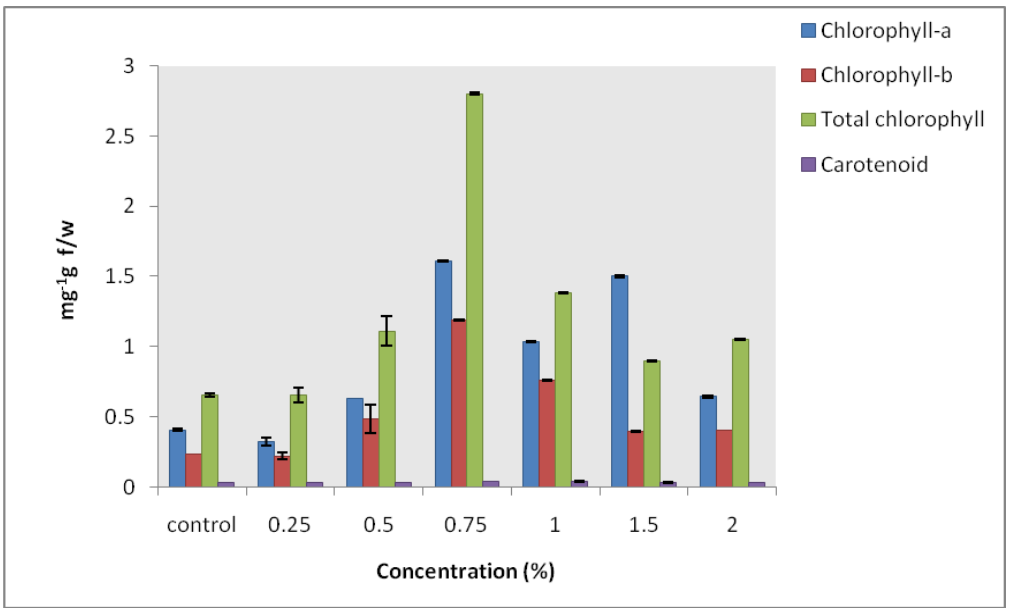

Fig-5. Effect of *Halophila* SGLF obtained by (Low temperature heating) on chlorophyll and carotenoids

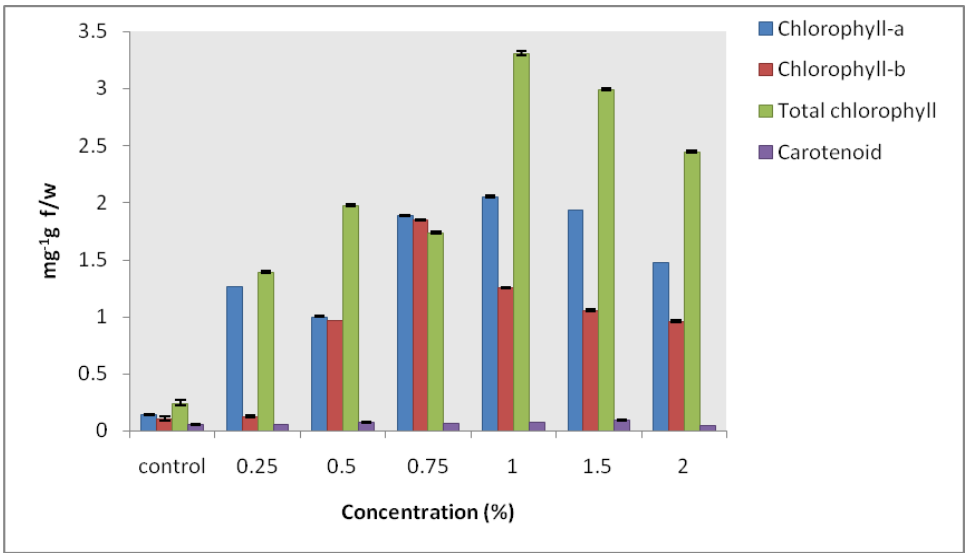

Fig-6. Effect of *Halophila* SGLF obtained by (Boiling) on chlorophyll and carotenoids

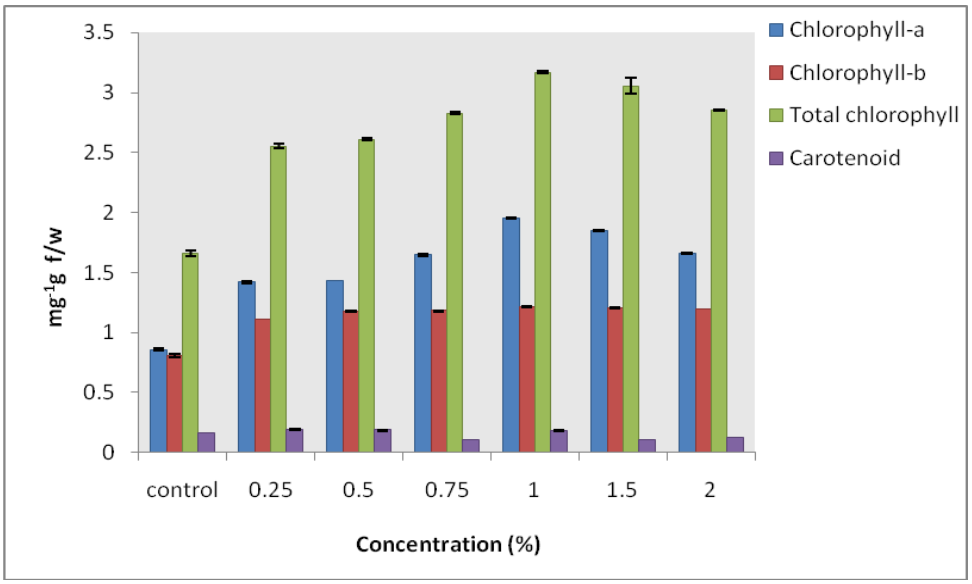

Fig-7.Effect of *Halophila* SGLF obtained by (Autoclave) on chlorophyll and carotenoids

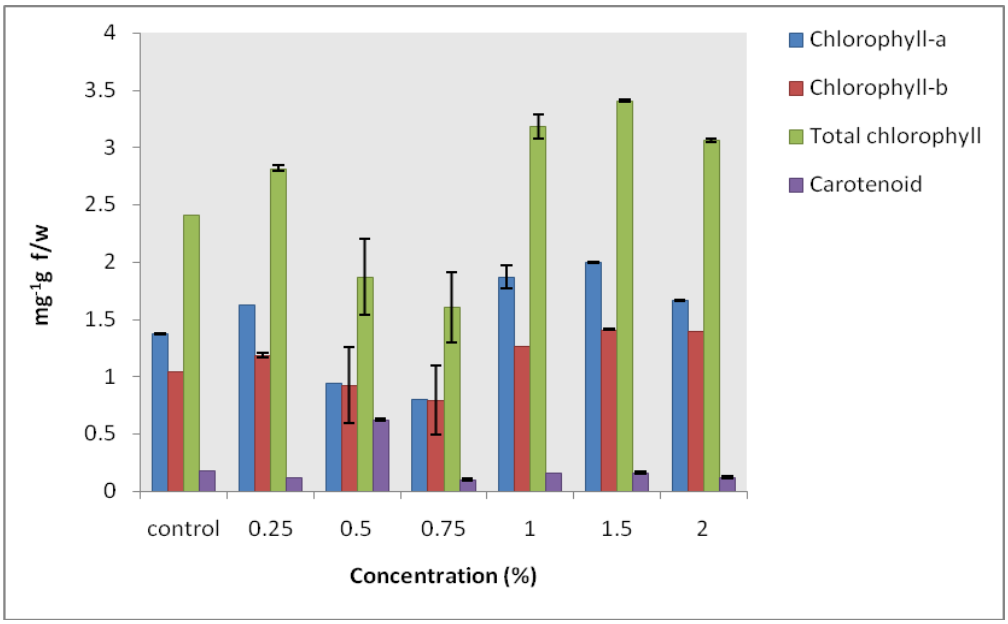

Fig-8. Effect of *Halophila* SGLF obtained by (Alcohol-aqueous) on chlorophyll and carotenoids

4. Discussion

In India, so far liquid seaweed fertilizer (SLF/LSF) was obtained either by boiling method or autoclave method. There are a only few reports on the liquid seaweed fertilizer (SLF) obtained by using low temperature and alcohol-aqueous method. Seagrass liquid fertilizer (SGLF) was extracted using Bhosle *et al,* (1975) method (Asir Selin Kumar and Sobitha Bai, 2010). Though the present study revealed that the Stoechospermum marginatum SLF *Halophila ovalis SGLF* from all the four methods of extraction (low temperature, boiling, autoclave, alcohol-aqueous) were capable of inducing increment of all the growth parameters (shoot length, root length, number of lateral roots, fresh weight and dry weight) and biochemical parameters (chlorophyll a, chlorophyll b, total chlorophyll and carotenoids) in black gram *(Vigna mungo)* seedlings, the promotional impact was *most* pronounced in the case of alcohol-aqueous method. Maximum *shoot* length (25.66cm±2.448) was triggered by 0.25% *Stoechospermum* SLF. Similarly, maximum number of lateral roots (30.0±0.550) was induced by 0.50% *Halophila* SGLF. Highest amount of chlorophyll a (1.373mg g"±0.001), chlorophyll b (1.042mg g"'±0.001) and total chlorophyll (2.416mg g"±0.002) was promoted by *Halophila* SGLF. Jeba Ananthi *et al,* 2010, also reported that the alcohol-aqueous method to be the most effective in promoting seedling growth to the maximum level.

The alcohol-aqueous method of extraction is more effective because all the growth promoting ingredients getting completely isolated in the SLF&SGLF. These ingredients could be gibberellic acid (Rabie, 1996), cytokines (Mooney and Vanstaden, 1986), auxin (Hart, 1982) and other organic and inorganic substances especially the mineral constitutions (Chennubhotla *et al,* 1987).

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