

# A Comparative Study of Gracilaria Corticata SLF And Cymodocea Serrulata SGLF from Alcohol Aqueous Method of Extraction on the Drought Stressed Cowpea

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## Abstract

The present study aimed to compare the effectiveness of seaweed liquid fertilizer (SLF) prepared from *Gracilaria corticata* and seagrass liquid fertilizer (SGLF) prepared from *Cymodocea serrulata* using the alcohol–aqueous extraction method in alleviating water stress in cowpea (*Vigna unguiculata*). The seaweed and seagrass extracts were evaluated for their potential to improve the morphological, biochemical and physiological characteristics of drought-stressed seedlings. Cowpea seeds were grown under controlled pot culture conditions and subjected to different treatments, including SLF, SGLF, polyethylene glycol (PEG), water-stressed untreated plants and unstressed controls. The extracts were applied as a 1% soil drench, and the seedlings were analysed for growth parameters, photosynthetic pigments, biochemical constituents, enzyme activities and phytohormones. The results revealed that *Gracilaria* SLF-treated drought-stressed cowpea seedlings exhibited increased root length, lateral root development, photosynthetic pigments, sugar, amino acid and lipid contents, along with enhanced  $\alpha$ -amylase and nitrate reductase activities. Similarly, *Cymodocea* SGLF treatment improved root growth and lateral root formation and increased photosynthetic pigments, amino acids, proteins, lipids and nitrogen content under water stress. The accumulation of zeatin was also enhanced in SLF- and SGLF-treated seedlings compared with stressed untreated plants, whereas abscisic acid levels were highest in water-stressed untreated seedlings. Overall, the findings indicate that alcohol–aqueous extracts of *Gracilaria corticata* and *Cymodocea serrulata* have potential as natural biostimulants to improve selected growth and biochemical responses of cowpea under water stress. These findings support further investigation of seaweed- and seagrass-based liquid fertilizers as sustainable agricultural inputs for improving crop performance under drought conditions.

**Keywords:** Seaweed liquid fertilizer, seagrass liquid fertilizer, *Gracilaria corticata*, *Cymodocea serrulata*, cowpea, water stress, alcohol–aqueous extraction, plant biostimulants.

## INTRODUCTION

Seagrasses meadows play a significant role in the near shore dynamics, nutrient cycling, re-mineralization

and sinking the pollutants in the coastal areas. Seagrass ecosystems provide food and shelter to a variety of organisms and thus they act as “nursery grounds”. Some of the seagrasses, such as *Halophila ovalis*, are used by the fishing communities of Cuddalore and Nagapattinam districts of Tamilnadu, as medicine to treat various skin diseases, burns and boils (Kannan et al., 1999). The seeds (raw) and rhizomes (ground in to flour) of the seagrass *Enhalus acoroides* are also eaten (Banderira and Gell, 2003; Ochieng and Erftemeijer, 2003).

In recent years, liquid extracts of seaweeds known as Seaweed Liquid Fertilizers (SLF) and that of seagrasses termed as Seagrass Liquid Fertilizers (SGLF) are being used. There are a number of methods of extracting SLF and SGLF. The mode of application of SLF and SGLF could be either seed soaking or foliar spray or both. SLF can be used both for horticultural crops especially the pulses. All the three kinds of seaweeds (green, brown and red) could be employed for SLF preparation. Although a number of seaweed based commercial extracts have come out in the international market, our country is lagging behind, inspite of plethora of research work on the application of SLF (Venkataraman Kumar, 2010).

Water stress has become a universal problem prevalent in most parts of the world. In India, erratic monsoon rainfall and depleting ground water have made the soils devoid of sufficient growth water triggering another malady namely saline stress. Both water stress and saline stress are twin problems. So most of the crops are subjected to water stress and saline stress.

## REVIEW OF LITERATURE

Use of seaweeds is a common practice in coastal areas throughout the world. In India, it is used for coconut plantations especially in coastal Tamil Nadu and Kerala. The high amounts of water soluble potash, other minerals and trace elements present in seaweeds are readily absorbed by plants and they control deficiency diseases. The nitrifiability of organic nitrogen from *Ulva lactuca* and drift seaweeds from Veraval was studied and found to be high compared to farmyard or a few organic manures (Mehta et al., 1967). With a view to find out the effect of seaweed manure on the uptake of inorganic nutrients by the wheat plant, a pot culture experiment was conducted (Bokil et al., 1974). Aqueous seaweed sprays on the growth and yield of pigeon pea has been studied by The effect of commercial fertilizer and LSF of *Enteromorpha intestinalis* on the growth of *Capsicum annum* has been determined by Veeragurunathan, 2009. Effect of soaking and foliar spray with seaweed extract in Bhendi has been studied by Vethanayagi et al., 2009 a, b.

Blunden et al., (1974) studied the effects of aqueous seaweed extract on sugar beet. Williams et al., 1974 reported the presence of gibberellin in the seaweed extract for a short time after preparation and of cytokinin over a longer period. Stephenson, 1974 confirmed that pasture treated with liquid seaweed extract contained a higher protein content at harvest time (3.8 to 5.0%) than pasture treated with the usual fertilizers. Blunden and Wildgoose, 1977 reported a significant increase in the yield of potatoes of the variety king Edwards by foliar application of an aqueous seaweed extract of known cytokinin activity. Liquid seaweed fertilizers are easily absorbed through roots and leaves, besides releasing trace elements bound to the soil (Chapman and Chapman, 1980). Asir Selin Kumar, 2009 has studied the influence of seagrass *Syringodium isoetifolium* extract on germination and growth of *Oryza sativa*. Sobithabai et al., 2010 carried out studies on the impact of seagrass *Syringodium isoetifolium* extract on germination and linear growth of *Sorghum bicolor*. The impact of SGLF on photosynthetic pigments and chlorophyllase activity in *Sorghum* and *Vigna* has been assessed by Asir Selin Kumar and Sobitha Bai, 2010 and Femila Jose et al., 2010.

Seaweed extracts have gained increasing attention as natural plant biostimulants because they contain minerals, amino acids, polysaccharides, vitamins, and other biologically active compounds. These constituents may influence plant growth, nutrient uptake, photosynthetic activity, and tolerance to environmental stress. Kumar et al. (2024) reviewed the role of seaweed extracts in enhancing plant resilience to biotic and abiotic stresses and discussed their application in sustainable crop production. Similarly, Supriya et al. (2023) highlighted the potential of seaweed-derived products to improve plant growth, soil fertility, and stress resistance.

Drought stress affects plant growth by reducing water availability, disturbing photosynthesis, and altering physiological and biochemical processes. Seaweed-based biostimulants have been investigated as potential tools to alleviate these effects by supporting root development, photosynthetic function, osmotic adjustment, and stress-response mechanisms. Mughunth et al. (2024) reviewed the use of seaweed extracts in horticultural crops and described their potential to improve growth and mitigate environmental stress. Their review also emphasized the importance of selecting appropriate seaweed species, concentrations, and application methods.

## MATERIALS AND METHODS

### Collections of seaweeds and seagrasses

The red seaweed *Gracilaria corticata* (J. Agardh) J. Agardh was collected from the Red Gate end of Hare Island, Tuticorin. The seagrass *Cymodocea serrulata* (R. Brown) Ascherson et magnus was collected from the Light House end of Hare Island, Tuticorin.

### Alcohol–Aqueous method (Ramamoorthy and Sujatha, 2007b)

In this method, the seaweed/seagrass after thorough washing was sundried followed by oven drying at 40°C for 36 hours and then powdered. 500g of the dry powder was soaked in 200ml alcohol for 12 hours and shaken vigorously to dissolve the alcohol soluble constituents and the supernatant saved. The residue was boiled in 300ml of distilled water for 30 minutes, cooled and filtered. Alcohol and water-soluble supernatants were mixed and the volume was made up to 500ml with distilled water to get 100% extract.

### Water Stress Study

*Gracilaria* SLF, and *Cymodocea* SGLF obtained by Alcohol-Aqueous method were assessed for their possible role in water stress alleviation/water stress recovery in the test plant, a pulse *Vigna unguiculata* (L.) Walp. Ankur-Gomathi -V.U.-89 (cow pea).

### Experimental Design for water stress study

Certified seeds of *Vigna unguiculata* (L.) Walp. Ankur-Gomathi-V.U.-89 were purchased from Tamil Nadu Agricultural University. The seeds were surface sterilized in 0.1% mercuric chloride for 1 minute followed by thorough washing to remove traces of mercuric chloride. Then the seeds were soaked in distilled water for a period of 12 hours.

Earthen pots were filled up with 500g of red soil: clay soil: composite (1.00: 0.50:0.25). Then the sterilized seeds were sown in the filled up earthen pots. The seedlings were regularly irrigated using tap water of uniform quantity. After 5 days of germination, only 5 uniformly healthy seedlings were retained in each pot.

### Treatment

Four sets of experiments were carried out with the potted seedlings. In first set of the experiment at every 5 days interval after germination, 60ml of 1% SLF/SGLF was added as soil drench up to 20 days, ie, 4 applications were made. In the second set of experiment, 1% polyethylene glycol (PEG) was added as

mentioned in the first set (PEG stabilize the cell osmotica). In the third set of experiment, seedlings were grown in tap water without any treatment for 20 days. After 20 days, watering was stopped in order to study the effect of water stress. In the fourth set of experiment, seedlings were grown in tap water without any treatment up to the end of the experiment. This was treated as control. The experimental seedlings treated with 1% SLF/SGLF (I set of experiment) and 1% PEG (II set of experiment) were subjected to water stress after 20 days of germination. In each set of experiments, 6 replicates were maintained. Sampling was done on the 5<sup>th</sup> day after water stress. The parameters analysed in the samples (25 days old seedlings) were as follows;

Morphological characters; shoot length, root length, number of lateral roots, fresh weight and dry weight (Venkataraman Kumar et al., 1993). Biochemical parameters; Chlorophyll a, Chlorophyll b, Total Chlorophyll, Chlorophyll a/b, Carotenoids (Arnon (1949) as modified by Harborne (1973) Sugar (Sheifter et al, 1950), Aminoacid (Rosen, 1957) Protein (Lowry et al, 1951), Lipid (Bligh and Dyer, 1959) Proline (Bates et al., 1973) Nitrogen (Humphires, 1956), Nitrate reductase (Showe and Demoss, 1968),  $\alpha$ -amylase (Vivekanandan, 1991), Abcissic Acid (ABA) and cytokinin (Zhang et al., 1993; Zheng et al., 2008).

**Table-1: Effect of Gracilaria SLF (Alcohol-Aqueous) on morphological parameters in Cowpea**

| Parameter           | Unstress          | Stress Untreated              | Stress PEG Treated             | Stress Gracilaria Treated       |
|---------------------|-------------------|-------------------------------|--------------------------------|---------------------------------|
| Shoot length (cm)   | 35.1 $\pm$ 0.72   | 30.3 $\pm$ 1.48               | 26.1 $\pm$ 0.73 <sup>a</sup>   | 30.6 $\pm$ 0.44                 |
| Root length (cm)    | 19.6 $\pm$ 1.20   | 17 $\pm$ 0.58                 | 16.8 $\pm$ 1.01                | 24 $\pm$ 2.08                   |
| No. of Lateral root | 305 $\pm$ 12.58   | 281 $\pm$ 4.41                | 298 $\pm$ 4.41 <sup>ab</sup>   | 333 $\pm$ 14.52                 |
| Fresh weight (g)    | 4.057 $\pm$ 0.05  | 0.954 $\pm$ 0.03 <sup>a</sup> | 2.753 $\pm$ 0.04 <sup>a</sup>  | 2.449 $\pm$ 0.41                |
| Dry weight (g)      | 0.339 $\pm$ 0.004 | 0.094 $\pm$ 0.002             | 0.264 $\pm$ 0.007 <sup>a</sup> | 0.193 $\pm$ 0.009 <sup>ac</sup> |

Mean  $\pm$  SE (n=3)

<sup>a</sup> Significance at (P< 0.01) between treated and untreated.

<sup>bc</sup> Significance at (P< 0.01) within treated.

**Table- 2 : Effect of Cymodocea SGLF (Alcohol-Aqueous) on morphological parameters in Cowpea**

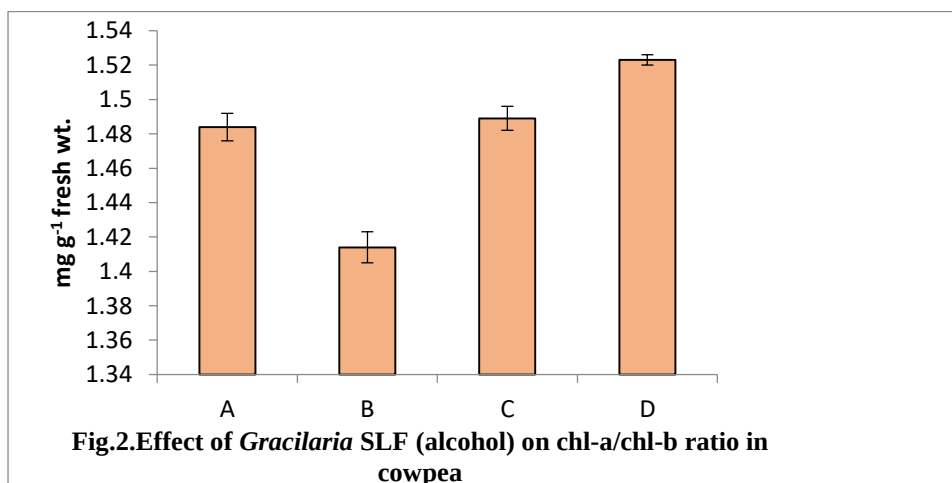
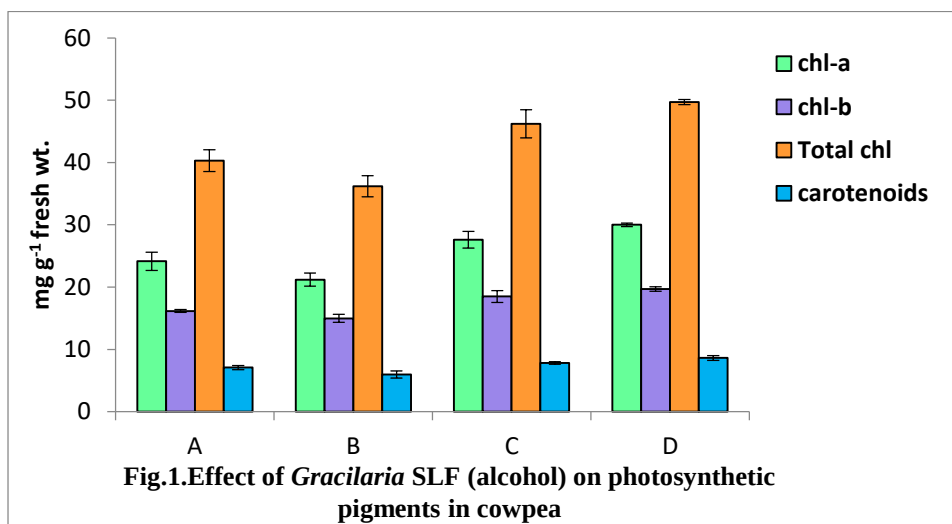
| Parameter           | Unstress         | Stress Untreated            | Stress PEG Treated           | Stress Cymodocea Treated |
|---------------------|------------------|-----------------------------|------------------------------|--------------------------|
| Shoot length (cm)   | 33 $\pm$ 1.73    | 27.6 $\pm$ 1.763            | 26 $\pm$ 3.05                | 30.1 $\pm$ 3.67          |
| Root length (cm)    | 18.66 $\pm$ 0.88 | 15.6 $\pm$ 0.88             | 14.66 $\pm$ 1.20             | 21 $\pm$ 2.08            |
| No. of Lateral root | 310 $\pm$ 10.40  | 223 $\pm$ 6.12 <sup>a</sup> | 223 $\pm$ 14.10 <sup>a</sup> | 313 $\pm$ 28.40          |

|                  |              |                            |                          |             |
|------------------|--------------|----------------------------|--------------------------|-------------|
| Fresh weight (g) | 3.98 ±0.02   | 0.878 ±0.006 <sup>a</sup>  | 2.607 ±0.08 <sup>a</sup> | 3.23 ±0.58  |
| Dry weight (g)   | 0.391 ±0.001 | 0.087 ±0.0004 <sup>a</sup> | 0.293 ±0.04              | 0.297 ±0.04 |

Mean ±SE (n=3)

<sup>a</sup> Significance at (P< 0.01) between treated and untreated.

<sup>bc</sup> Significance at (P< 0.01) within treated.

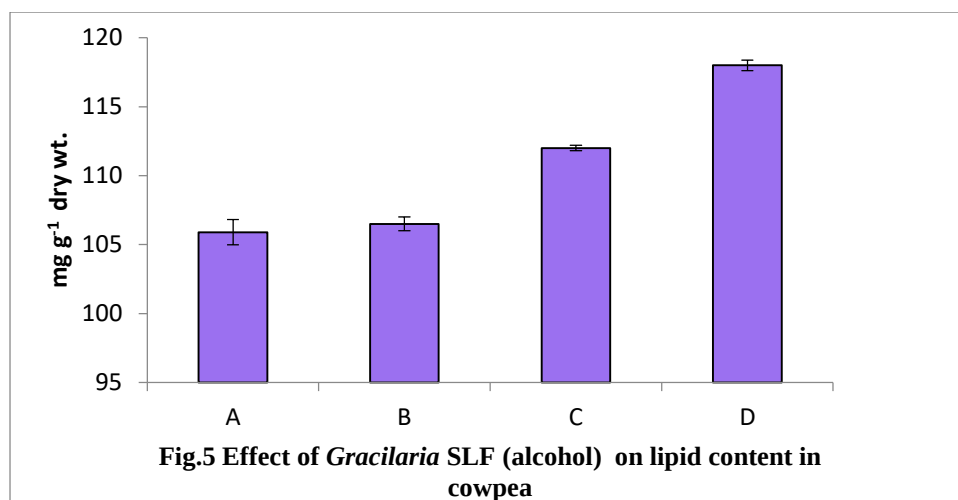
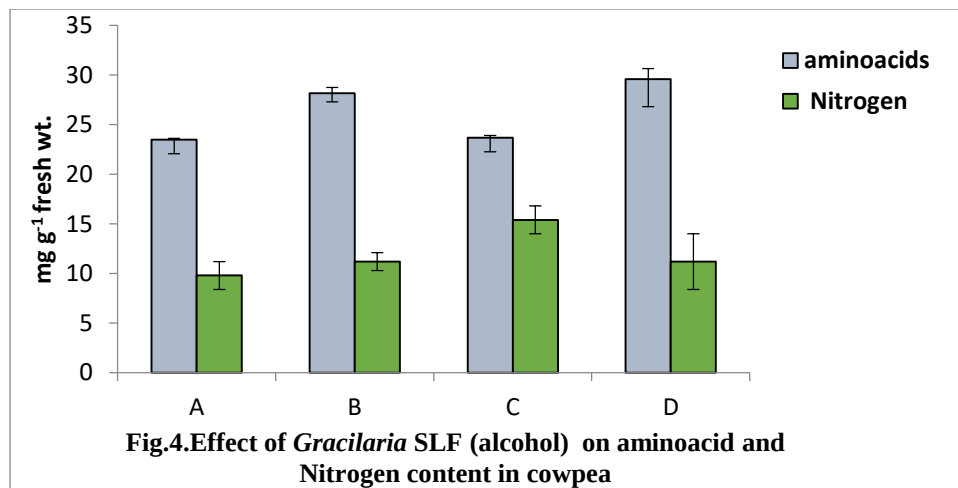
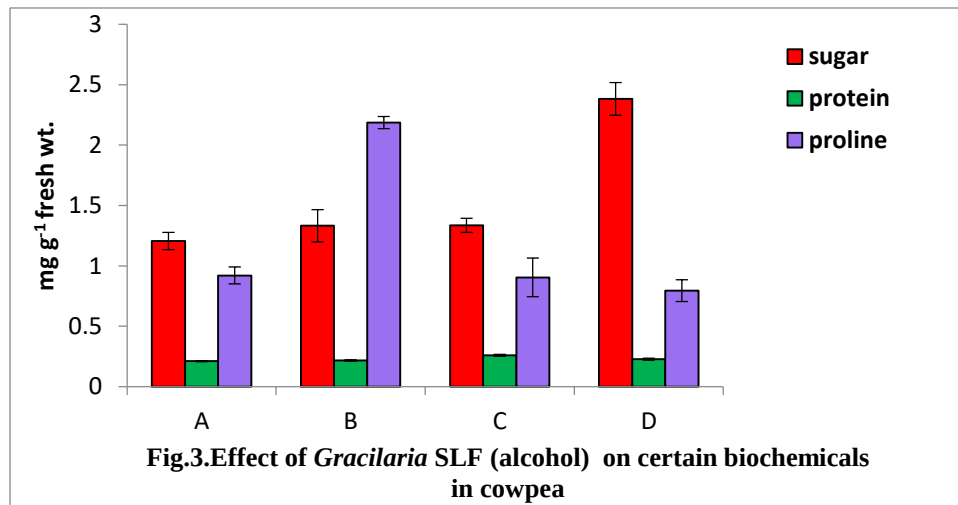


A- Unstress

B- Stress untreated

C- Stress PEG treated

D- Stress SLF treated

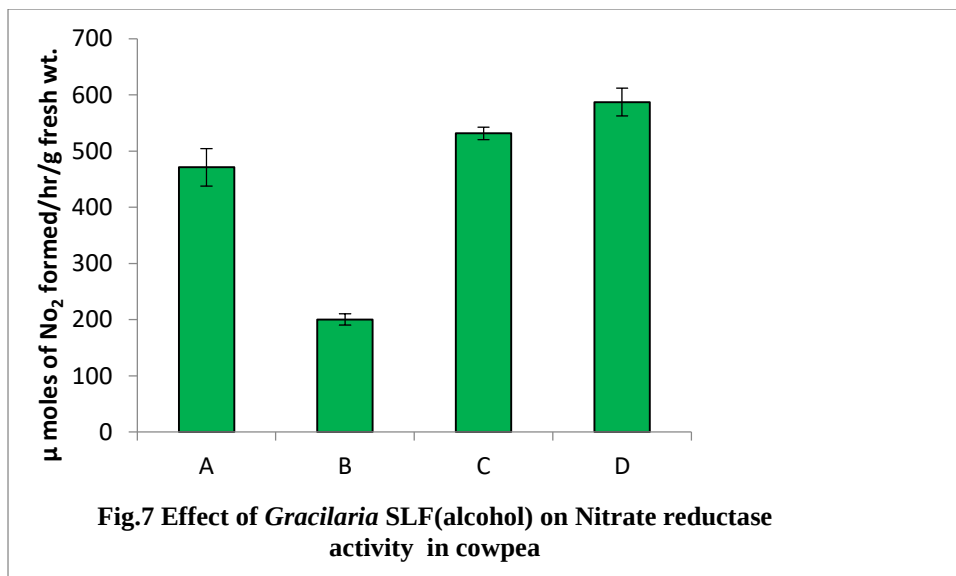
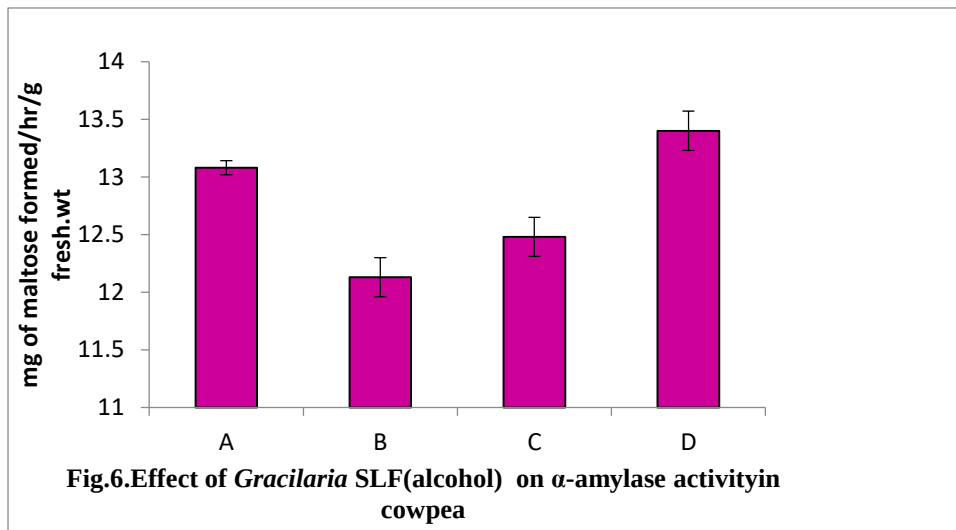


A- Unstress

B- Stress untreated

C- Stress PEG treated

D- Stress SLF treated

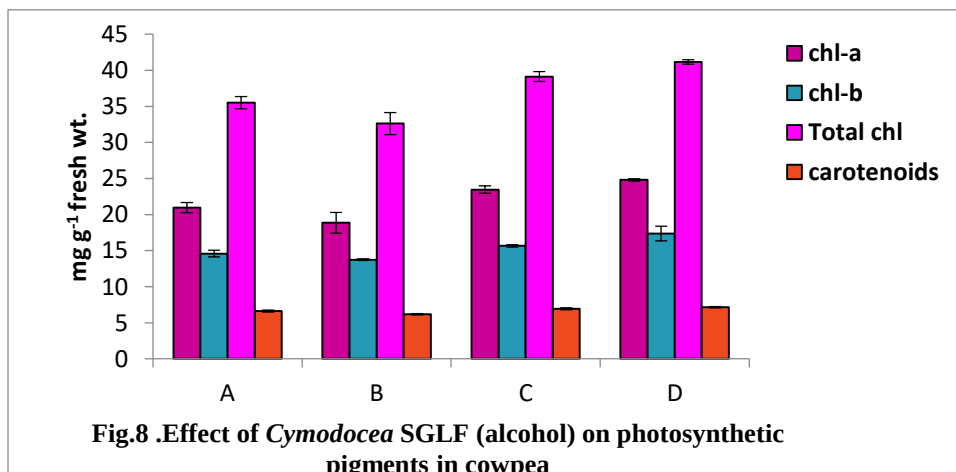


A- Unstress

B- Stress untreated

C- Stress PEG treated

D- Stress SLF treated



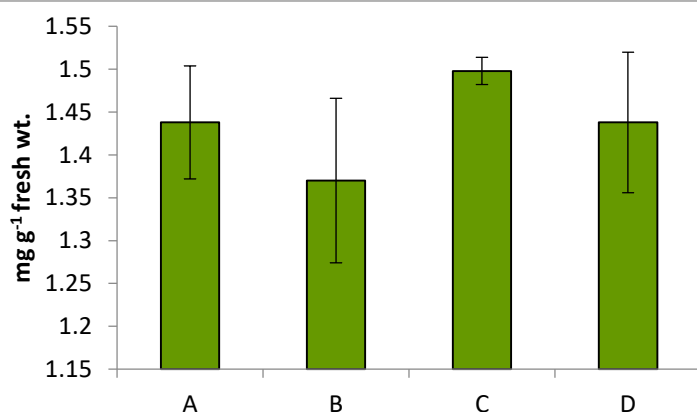


**Fig.9.Effect of *Cymodocea* SGLF (alcohol) on chl-a/chl-b ratio in cowpea**

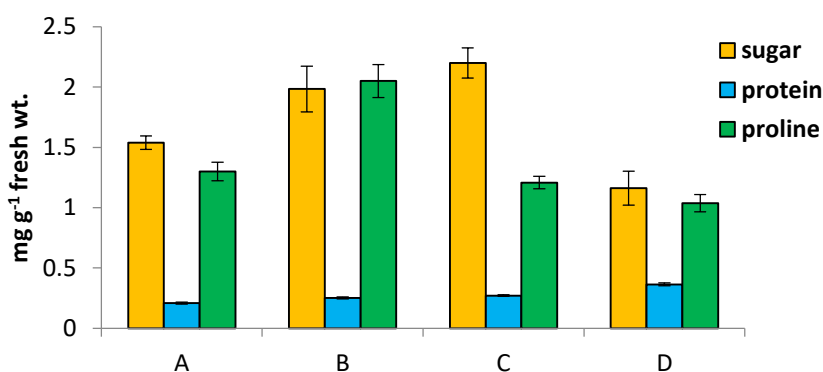


**Fig. 10 Effect of *Cymodocea* SGLF (alcohol) on certain biochemicals in cowpea**

A- Unstress

B- Stress untreated

C- Stress PEG treated

D- Stress SLF treated

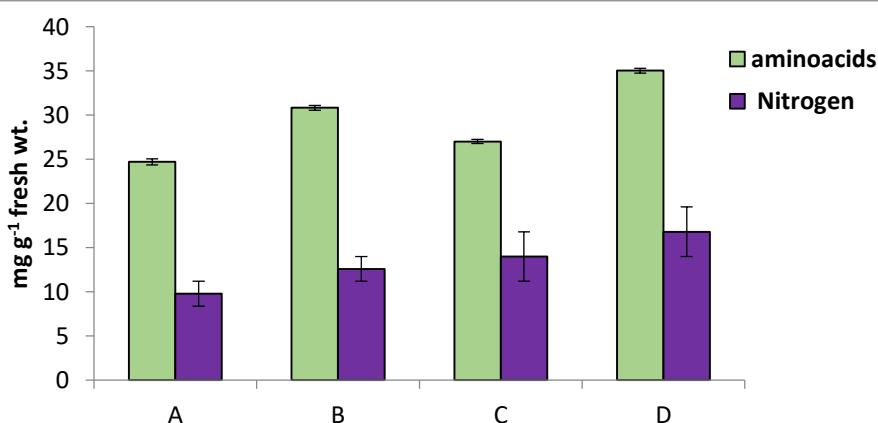
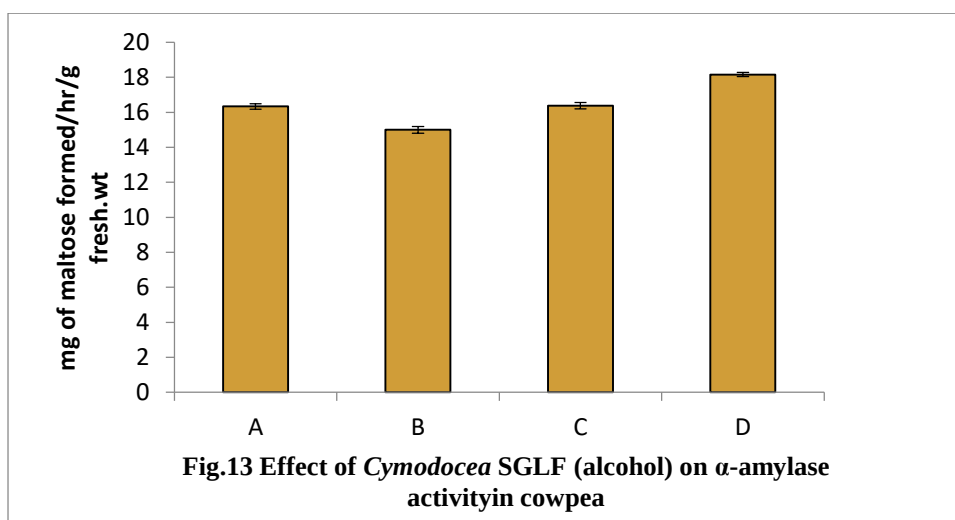
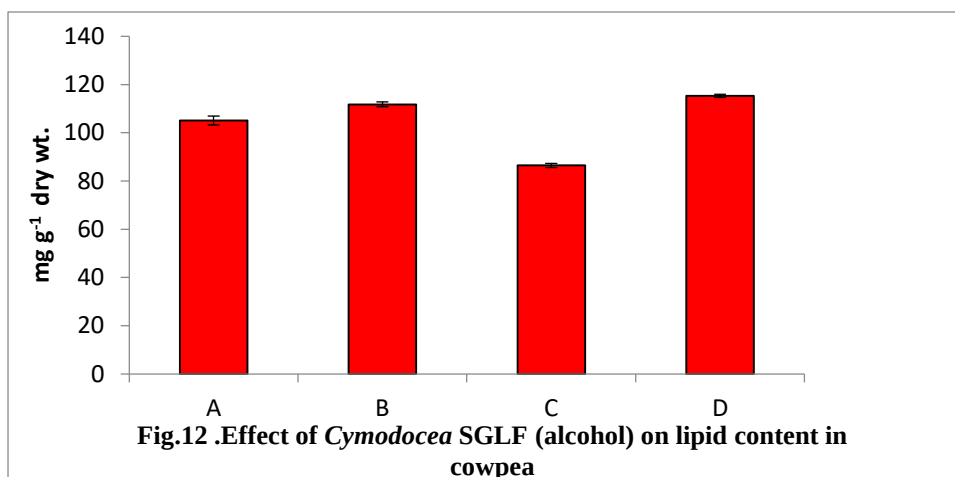


**Fig.11 .Effect of *Cymodocea* SGLF (alcohol) on aminoacid and Nitrogen content in cowpea**

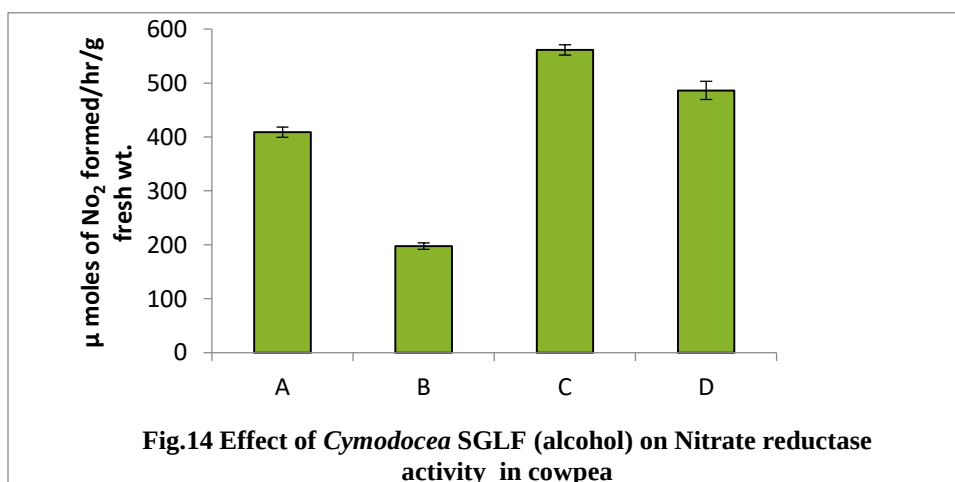


A- Unstress

B- Stress untreated

C- Stress PEG treated

D- Stress SLF treated



A- Unstress

B- Stress untreated

C- Stress PEG treated

D- Stress SLF treated

**Table-3: Effect of Gracilaria SLF from Alcohol-Aqueous method of extraction on Zeatin and Absciscic acid (ABA)**

| Parameter                                         | Unstress | Stress Untreated | Stress Treated PEG | Stress Gracilaria Treated |
|---------------------------------------------------|----------|------------------|--------------------|---------------------------|
| Amount of Zeatin ( $\mu\text{g g}^{-1}$ )         | 340.40   | 200.80           | 204.64             | 314.50                    |
| Amount of Absciscic acid ( $\mu\text{g g}^{-1}$ ) | 140.10   | 188.92           | 142.70             | 141.65                    |

**Table- 4: Effect of Cymodocea SGLF from Alcohol-Aqueous method of extraction on Zeatin and Absciscic acid (ABA)**

| Parameter                                         | Unstress | Stress Untreated | Stress Treated PEG | Stress Gracilaria Treated |
|---------------------------------------------------|----------|------------------|--------------------|---------------------------|
| Amount of Zeatin ( $\mu\text{g g}^{-1}$ )         | 340.40   | 200.80           | 204.64             | 287.35                    |
| Amount of Absciscic acid ( $\mu\text{g g}^{-1}$ ) | 140.10   | 188.92           | 142.70             | 152.45                    |

## RESULT

### **Effect of Gracilaria SLF from alcohol-aqueous method on morphological parameters (Table 1) and biochemical parameters (Fig. 1-7)**

Enhanced shoot length to the maximum extent was observed in unstressed untreated control seedlings of cowpea followed by Gracilaria SLF treated drought stressed seedlings. The Gracilaria SLF treated stressed seedlings showed maximum number of lateral roots and root length. Fresh weight and dry weight were more in unstressed control seedlings compared to that of the other three treatments. Gracilaria SLF treated and PEG treated drought stressed 25 days old cowpea seedling exhibited amount of photosynthetic pigments (chlorophyll a, b, total chlorophyll and carotenoids) more than that of unstressed and stressed untreated seedlings (Fig. 1). Chlorophyll a/ chlorophyll b ratio was found to be the highest in Gracilaria SLF treated drought stressed cowpea seedlings (Fig. 2). The amount of sugar, amino acid and lipid was maximum in the Gracilaria SLF treated stressed seedlings (Fig.3,4,5) whereas maximum amount of protein and nitrogen was prevalent in PEG treated drought stressed cowpea seedlings (Fig.3,4). Activity of  $\alpha$ -amylase and Nitrate reductase was at the peak in Gracilaria SLF treated drought stressed 25days old cowpea seedlings (Fig. 6,7).

### **Effect of Cymodocea SGLF from alcohol-aqueous method on morphological parameters (Table- 2) and biochemical parameters (Fig. 8-14)**

Shoot length was enhanced to maximum extent in unstressed seedling whereas root length and number of lateral roots in Cymodocea SGLF treated drought stressed 25-day old cowpea seedlings. Maximum fresh

weight was observed in unstressed seedling closely followed by that of Cymodocea SGLF treated seedlings. Dry weight was more in unstressed cowpea seedling.

Highest amount of photosynthetic pigments (chlorophyll a, b, total chlorophyll and carotenoids) (Fig. 8) and chlorophyll a/chlorophyll b ratio was registered in Cymodocea SGLF treated water stressed seedlings and PEG treated seedling respectively (Fig. 9). Maximum amount of sugar was recorded in PEG treated water stressed seedlings (Fig. 10). The amount of amino acids, protein, lipid and nitrogen was highest in Cymodocea SGLF treated drought stressed 25 days old seedlings (Fig. 10,11,12). Maximum proline content was recorded in stressed untreated seedlings (Fig. 10). Highest activity of  $\alpha$ -amylase and nitrate reductase activity was observed in Cymodocea SGLF treated and PEG treated drought stressed seedlings respectively (Fig.13,14)

Gracilaria SLF, Cymodocea SGLF soil drenched water stressed seedlings of cowpea exhibited enhanced amount of zeatin (cytokinin) compared to that of the stressed untreated seedlings. In PEG treated water stressed seedlings, the amount of zeatin slightly exceeded over the stressed untreated in cowpea (Table3,4). The amount of phytohormone Abcissic acid (ABA) was found to be maximum in water stressed untreated seedlings of cowpea and paddy. The amount of ABA detected in the water stressed untreated (SLFs, SGLFs and PEG) seedlings of cowpea exceeded over that of the unstressed seedlings (Table3,4).

## DISCUSSION

### Comparative performance of Gracilaria SLF and Cymodocea SGLF

The comparative findings demonstrate that both extracts improved selected growth and biochemical parameters in drought-stressed cowpea. Gracilaria SLF produced a greater root length and lateral root number than Cymodocea SGLF in the reported experiments and was associated with higher  $\alpha$ -amylase activity and zeatin content. Cymodocea SGLF also improved root development, photosynthetic pigment content and several biochemical constituents, particularly protein and nitrogen.

These differences indicate that the effectiveness of a liquid fertilizer should be evaluated in relation to the specific plant response being considered rather than by relying on a single parameter. The beneficial effects may be associated with minerals, amino acids, polysaccharides and other biologically active constituents present in seaweed and seagrass extracts.

### Agricultural significance

The results highlight the potential of seaweed- and seagrass-derived liquid fertilizers as supplementary biostimulants for improving selected growth responses of cowpea under water stress. Their application may offer an alternative approach to supporting crop performance under drought-prone agricultural conditions and could contribute to the development of more sustainable crop management practices. These findings are consistent with the broader potential of seaweed-based products in sustainable agriculture discussed by Supriya et al. (2023).

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