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# Utilization of seaweed *Sargassum myriocystum* extracts as a stimulant of seedlings of *Vigna mungo* (L.) Hepper

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## Abstract

The effect of seaweed liquid fertilizer (SLF) of *Sargassum myriocystum* on germination, growth and biochemical constituents of *Vigna mungo* was studied. Different concentrations (5%, 10%, 25%, 50% and 75%) of SLF were prepared using distilled water. A total of 100 seeds were soaked for each SLF concentration for a 24-h period and were later placed in various Petri plates and watered regularly. Water-soaked seeds were used as controls. Seed germination was 98% for the 10% SLF soaked seeds. Similarly, shoot length (21.13 cm seedling$^{-1}$), root length (12.19 cm seedling$^{-1}$), fresh weight and dry weight (3.910 and 0.320 g seedling$^{-1}$) recorded at 10% concentration of *S. myriocystum* treated seedlings were the highest. Besides, the chlorophyll (0.547 mg g$^{-1}$ fr. wt.) and carotenoid (0.359 mg g$^{-1}$ fr. wt.) contents in leaves recorded at 10% SLF soaked seedlings were the highest. The 10% SLF treated seedlings showed the highest content of protein (1.898 and 1.508 mg g$^{-1}$ fr. wt.), amino acid (0.370 and 0.306 mg g$^{-1}$ fr. wt.), reducing sugar (6.031 and 3.448 mg g$^{-1}$ fr. wt.), total sugar (8.414 and 7.055 mg g$^{-1}$ fr. wt.), α-amylase activity (1.403 and 1.380 µg min$^{-1}$ mg$^{-1}$ protein) and β-amylase activity (1.099 and 1.029 µg min$^{-1}$ mg$^{-1}$ protein) of shoots and roots respectively. The seaweed extract showed better response at lower concentration while higher concentrations of seaweed extract showed a decreasing trend.

**Additional key words:** biofertilizer; black gram; seaweed liquid fertilizer.

## Resumen

**Utilización de extractos del alga *Sargassum myriocystum* como estimulante de plántulas de *Vigna mungo* (L.) Hepper**

Se estudió el efecto de un fertilizante líquido de algas marinas (SLF) preparado con *Sargassum myriocystum* sobre la germinación, crecimiento y componentes bioquímicos de *Vigna mungo.* Se introdujeron 100 semillas durante 24 h en diferentes concentraciones (5%, 10%, 25%, 50% y 75%) de SLF (en agua el control), se colocaron en placas de Petri y se regaron con regularidad. La germinación fue del 98% para las semillas introducidas en SLF al 10%. La longitud de los brotes (21,13 cm plántula$^{-1}$), longitud de la raíz (12,19 cm plántula$^{-1}$), peso fresco y peso seco (3,910 y 0,320 g plántula$^{-1}$), clorofila (0,547 mg g$^{-1}$ peso fresco) y contenido en carotenoides (0,359 mg g$^{-1}$ peso fresco) fueron las mayores en esta concentración. Adicionalmente, esta concentración produjo el mayor contenido de proteínas (1,898 y 1,508 mg g$^{-1}$ peso fresco), aminoácidos (0,370 y 0,306 mg g$^{-1}$ peso fresco), azúcares reductores (6,031 y 3,448 mg g$^{-1}$ peso fresco), azúcares totales (8,414 y 7,055 mg g$^{-1}$ peso fresco), actividad α-amilasa (1,403 y 1,380 µg min$^{-1}$ mg$^{-1}$ proteína) y actividad β-amilasa (1,099 y 1,029 µg min$^{-1}$ mg$^{-1}$ proteína) de brotes y raíces, respectivamente. Por tanto, el SLF produjo una mejor respuesta a bajas concentraciones, mientras que con concentraciones más altas se obtuvo una respuesta decreciente.

**Palabras clave adicionales:** biofertilizantes; fertilizantes líquidos de algas marinas; lenteja negra.

# Introduction

Seaweeds are an integral part of the coastal ecosystem and they are known to aid and stimulate growth of vegetables, fruits and other crops (Blunden, 1991; Crouch & Van Staden, 1994; Washington *et al*., 1999). They contain all major and minor plant nutrients including bio-control properties; they also contain organic compounds such as auxins, gibberellins and precursors of ethylene and betaine that impact plant growth (Wu *et al.,* 1997). Beneficial effects from the use of seaweed extracts as natural regulators have included

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increased crop yield and plant vigor to withstand adverse environmental effects (Featonby-Smith & Van Staden, 1983). Significant increase in the yield of crops due to foliar application of seaweed extracts has been reported in the literature (Aurthur *et al*., 2003; Zodape *et al*., 2008; Rathore *et al*., 2009). Aqueous extract of *Sargassum wightii* when applied as foliar spray on *Zizyphus mauritiana* has shown an increased yield of fruits (Rama Rao, 1991). This study was undertaken to test the effect of seaweed liquid fertilizer of *Sargassum myriocystum* on germination, growth and biochemical constituents of the black gram or black lentil (*Vigna mungo* (L.) Hepper).

## Material and methods

The seaweed used in this study was *S. myriocystum* (class Phaeophyceae) and it was collected from the coastal area of Rameswaram in southern India (9°25' N and 79°15' E). The samples were washed thoroughly with seawater to remove all the unwanted impurities, adhering sand particles and epiphytes. Morphologically distinct *S. myriocystum* thalli were placed separately in polythene bags, kept inside an icebox and transported to the laboratory. Samples were washed thoroughly using tap water to remove surface salt and spread on blotting paper to remove excess water.

One kilogram of seaweed was cut into small pieces and boiled separately with 1-L distilled water for 1-h and filtered. The filtrates were considered as 100% seaweed extract and different concentrations viz., 5, 10, 25, 50 and 75% were prepared using distilled water (Bhosle *et al*., 1975). As the seaweed liquid fertilizer (SLF) contained organic and inorganic matter, they were kept in refrigerator between 0 and 4°C. The colour, pH, calcium, sodium, magnesium, potassium, iron, chloride, sulphate, silica, aluminum, zinc, copper and nitrate contents were analyzed following the methods of APHA (1995). The crop selected for the present study was the black gram or lentil (*Vigna mungo;* family Fabaceae), which is a common and highly priced pulse of India. The seeds were purchased from Regional Pulses Res. Stat., Tamilnadu Agric. Univ. (Tamil Nadu, India). Seeds with uniform size, colour and weight were chosen for experimental purposes and stored in a metal tin as suggested by Rao (1976).

A total of 100 seeds were soaked in SLF concentrations (5, 10, 25, 50, 75 and 100%) for 24-h and then the seeds were placed in various Petri plates and watered regularly with tap water. Water soaked seeds were used as controls. The germination percentage was recorded on the 3$^{rd}$ day after sowing. Samples soaked in each concentration were taken on 15$^{th}$ day after sowing for measuring growth variables (shoot length and root length, fresh and dry weight) and for analyses of biochemical constituents [chlorophyll content (Arnon, 1949), carotenoid (McKinney, 1941), protein (Lowry *et al.,* 1951), amino acid (Moore & Stein, 1948), reducing and total sugar (Nelson, 1944), α- and β-amylase activities (Bernfeld, 1955)]. Statistical analysis were carried out using Statistical Package of Social Sciences (Software,vers. 11.5, Chicago, IL, USA). ANOVA and Duncan multiple range test were carried out to determine significant differences ($p < 0.01$ and $p < 0.05$) between the means.

## Results

The physico-chemical properties of *S. myriocystum* SLFs were analyzed (Table 1). The SLFs contained higher levels of magnesium, sodium, potassium, chloride, sulphate and zinc than of other minerals. The effect of extracts of *S. myriocystum* on germination percentage and growth of *V. mungo* are shown in Table 2. The highest seed germination (98%) was found in the SLF 10% concentration. The germination percentage increased with the concentration levels up to 10% SLF but later declined. Highest records of shoot length (21.13 cm seedling$^{-1}$), root length (12.19 cm seedling$^{-1}$), fresh weight and dry weight (3.910 and 0.320 g seedling$^{-1}$) were found at 10% of *S. myriocystum*-treated seedlings.

**Table 1.** Physico-chemical properties of seaweed liquid fertilizer (SLF) of *Sargassum myriocystum*

| Variables | *S. myriocystum* |
|---|---|
| Colour | Brownish red |
| pH | 6.68 |
| Calcium (mg L$^{-1}$) | 32.00 |
| Magnesium (mg L$^{-1}$) | 115.20 |
| Sodium (mg L$^{-1}$) | 68.00 |
| Potassium (mg L$^{-1}$) | 200.00 |
| Iron (mg L$^{-1}$) | 0.52 |
| Chloride (mg L$^{-1}$) | 620.00 |
| Sulphate (mg L$^{-1}$) | 492.00 |
| Silica (mg L$^{-1}$) | 9.20 |
| Aluminium (mg L$^{-1}$) | 0.148 |
| Zinc (mg L$^{-1}$) | 0.218 |
| Copper (mg L$^{-1}$) | Nil |
| Nitrate (mg L$^{-1}$) | 0.45 |

**Table 2.** Effect of seaweed liquid fertilizer (SLF) of *Sargassum myriocystum* on germination and growth of *Vigna mungo*

| Concentration (%) | Germination (%) | Shoot length (cm seedling$^{-1}$) | Root length (cm seedling$^{-1}$) | Seedling fresh weight (g seedling$^{-1}$) | Seedling dry weight (g seedling$^{-1}$) |
|---|---|---|---|---|---|
| Control | 94 ± 0.94 | 15.40 ± 0.924 | 6.78 ± 0.407 | 2.590 ± 0.155 | 0.240 ± 0.014 |
| 5 | 96* ± 0.96 | 19.80* ± 1.188 | 9.99* ± 0.599 | 3.260* ± 0.196 | 0.260* ± 0.016 |
| 10 | 98* ± 0.98 | 21.13* ±1.268 | 12.19* ± 0.791 | 3.910* ± 0.235 | 0.320* ± 0.019 |
| 25 | 82* ± 1.64 | 16.06 * ± 0.964 | 7.05* ± 0.423 | 2.600* ± 0.156 | 0.300* ± 0.018 |
| 50 | 70** ± 1.40 | 13.27 ** ± 0.796 | 6.88** ± 0.413 | 2.260** ± 0.136 | 0.260** ± 0.016 |
| 75 | 42 ± 0.84 | 6.57 ± 0.394 | 3.96 ± 0.278 | 2.090 ± 0.125 | 0.185 ± 0.011 |
| 100 | 35 ± 0.70 | 3.40 ± 0.204 | 2.25 ± 0.135 | 1.900 ± 0.114 | 0.075 ± 0.005 |

* Significant at $p \leq 0.01$ level; ** Significant at $p \leq 0.05$ level.

In contrast, the germination percentage (35%), shoot length (3.40 cm seedling$^{-1}$), root length (2.25 cm seedling$^{-1}$), fresh weight (1.900 g seedling$^{-1}$) and dry weight (0.075 g seedling$^{-1}$) were lowest at 100% SLF of *S. myriocystum*. Data on biochemical studies are shown in Table 3, which shows that there were significant differences in biochemical status at different concentration levels. The biochemical constituents increased with the concentration level up to 10% SLF of *S. myriocystum* and it declined afterwards. The lowest value was observed at 100% SLF and the highest value of chlorophyll content (0.547 mg g$^{-1}$ fr. wt.), carotenoid content (0.359 mg g$^{-1}$ fr. wt.), shoot and root protein content (1.898 and 1.508 mg g$^{-1}$ fr. wt.), shoot and root amino acid content (0.370 and 0.306 mg g$^{-1}$ fr. wt.), shoot and root reducing sugar content (6.031 and 3.448 mg g$^{-1}$ fr. wt.), shoot and root total sugar content (8.414 and 7.055 mg g$^{-1}$ fr. wt.), shoot and root α-amylase activities (1.403 and 1.380 µg min$^{-1}$ mg$^{-1}$ protein) and shoot and root β-amylase activities (1.099 and 1.029 µg min$^{-1}$ mg$^{-1}$ protein) were recorded at 10% SLF of *S. myriocystum*-treated seedlings.

## Discussion

*Vigna mungo* seeds soaked with low concentrations of seaweed extracts showed higher rates of germination, while increasing concentrations of the extract inhibited the germination. The increased germination

**Table 3.** Effect of seaweed liquid fertilizers of *Sargassum myriocystum* on the biochemical composition of shoots and roots of *Vigna mungo* seedlings

| | Concentration of SLF (%) | | | | | | |
|---|---|---|---|---|---|---|---|
| | Control | 5 | 10 | 25 | 50 | 75 | 100 |
| *Vigna mungo* shoots | | | | | | | |
| Total chlorophyll (mg g$^{-1}$ fr wt) | 0.422 ± 0.026 | 0.459* ± 0.028 | 0.547* ± 0.034 | 0.383* ± 0.023 | 0.285** ± 0.016 | 0.056 ± 0.010 | 0.030 ± 0.008 |
| Carotenoids (mg g$^{-1}$ fr wt) | 0.250 ± 0.015 | 0.348* ± 0.021 | 0.359* ± 0.022 | 0.343* ± 0.020 | 0.333** ± 0.019 | 0.304 ± 0.018 | 0.206 ± 0.012 |
| Protein (mg g$^{-1}$ fr wt) | 0.663 ± 0.398 | 1.170* ± 0.070 | 1.898* ± 0.114 | 1.146* ± 0.069 | 0.986** ± 0.059 | 0.596 ± 0.036 | 0.475 ± 0.029 |
| Amino acid (mg g$^{-1}$ fr wt) | 0.290 ± 0.017 | 0.328* ± 0.019 | 0.370* ± 0.018 | 0.292* ± 0.017 | 0.246** ± 0.015 | 0.210 ± 0.014 | 0.145 ± 0.009 |
| Reducing sugars (mg g$^{-1}$ fr wt) | 2.327 ± 0.140 | 4.013* ± 0.241 | 6.031* ± 0.362 | 5.424* ± 0.325 | 4.935** ± 0.296 | 4.469 ± 0.268 | 3.516 ± 0.210 |
| Total sugars (mg g$^{-1}$ fr wt) | 5.125 ± 0.263 | 7.604* ± 0.456 | 8.414* ± 0.505 | 8.201* ± 0.552 | 7.957** ± 0.477 | 7.595 ± 0.456 | 6.544 ± 0.393 |
| α-amylase (µg min$^{-1}$ mg$^{-1}$ protein) | 0.620 ± 0.037 | 1.122* ± 0.090 | 1.403* ± 0.112 | 0.842* ± 0.067 | 0.561** ± 0.045 | 0.311 ± 0.025 | 0.290 ± 0.017 |
| β-amylase (µg min$^{-1}$ mg$^{-1}$ protein) | 0.490 ± 0.029 | 0.842* ± 0.067 | 1.099* ± 0.088 | 0.701* ± 0.056 | 0.580* * ± 0.046 | 0.233 ± 0.018 | 0.200 ± 0.012 |
| *Vigna mungo* roots | | | | | | | |
| Protein (mg g$^{-1}$ fr wt) | 0.468 ± 0.028 | 0.780* ± 0.046 | 1.508* ± 0.090 | 0.936* ± 0.056 | 0.416** ± 0.025 | 0.312 ± 0.018 | 0.290 ± 0.017 |
| Amino acid (mg g$^{-1}$ fr wt) | 0.252 ± 0.015 | 0.277* ± 0.017 | 0.306* ± 0.018 | 0.258* ± 0.016 | 0.239** ± 0.014 | 0.211 ± 0.013 | 0.191 ± 0.011 |
| Reducing sugars (mg g$^{-1}$ fr wt) | 1.320 ± 0.079 | 3.256* ± 0.195 | 3.448* ± 0.207 | 3.444* ± 0.201 | 3.152** ± 0.189 | 2.960 ± 0.178 | 2.300 ± 0.138 |
| Total sugars (mg g$^{-1}$ fr wt) | 3.210 ± 0.192 | 6.772* ± 0.406 | 7.055* ± 0.423 | 4.583* ± 0.275 | 4.394** ± 0.264 | 4.110 ± 0.246 | 3.920 ± 0.235 |
| α-amylase (µg min$^{-1}$ mg$^{-1}$ protein) | 0.620 ± 0.037 | 0.982* ± 0.079 | 1.380* ± 0.110 | 0.655* ± 0.052 | 0.491** ± 0.039 | 0.201 ± 0.017 | 0.187 ± 0.011 |
| β-amylase (µg min$^{-1}$ mg$^{-1}$ protein) | 0.410 ± 0.025 | 0.795* ± 0.064 | 1.029* ± 0.082 | 0.678* ± 0.054 | 0.467** ± 0.037 | 0.187 ± 0.015 | 0.098 ± 0.006 |

* Significant at $p \leq 0.01$ level; ** Significant at $p \leq 0.05$ level.

percentage at low concentrations could be due to the presence of growth promoting substances such as indole-3-acetic acid (IAA) and indole butyric acid (IBA), gibberellins A and B, cytokinins, micronutrients (Fe, Cu, Zn, Co, Mo, Mn and Ni), vitamins and amino acids as reported in the past (Challen & Hemingway, 1965). Our results coincide with those of earlier studies made in *Cajanus cajan* (Mohan *et al.,* 1994), *Zea mays*, *Elusine coracona* and *Pennisetum typhoides* (Rajkumar Immanual & Subramanian, 1999), *Vigna catajung* and *Dolichos biflorus* (Anantharaj & Venkatesalu, 2001; 2002), *Vigna sinensis* (Sivasankari *et al*., 2006) and *Abelmoschus esculentus* (Thirumaran *et al*., 2009).

Statistically significant differences were observed for shoot length, root length, fresh and dry weight. A positive response in growth variables was observed at 10% SLF of *S. myriocystum* soaked seedlings. The increased seedling growth may be due to the presence of some growth promoting substances. The growth enhancing potential of seaweeds might be attributed to the presence of macro and micronutrients (Challen & Hemingway, 1965). Higher concentrations showed a decreasing trend. Similar results were recorded in *Padina* which induced maximum seedling growth at lower concentration in *C. cajan* (Mohan *et al*., 1994), *Vigna radiata* (Venkataraman Kumar *et al*., 1993) and other similar observation was made in *Rosenvigea intricata* (Thirumaran *et al.,* 2009) and *Ascophyllum nodosum* (Abdel-Mawgoud *et al*., 2010). Cytokinins and gibberellins, growth hormones detected in many species of *Sargassum*, might be responsible for beneficial effects to black gram in the present study. The increase in the growth variables at lower concentration of the SLF treated plant may be due to the uptake of magnesium, phosphorus, potassium, nitrate and iron from the seaweed extract of *S. myriocystum.* Dhargalkar & Untawale (1983) also reported similar findings with *Hypnea musciformis*, *Spatoglossum asperum*, *Stoechospermum marginatum* and *Sargassum* on the growth of crops such as *Capsicum frutescens*, *Brassica rapa* and *Ananas comosus*.

Lower concentrations of *S. myriocystum*-SLFs also promoted the chlorophyll content of *V. mungo* up to 10% SLF when compared to control. Higher concentrations decreased the chlorophyll content. A similar observation was made in *Scytonema* sp. (Venkataraman Kumar & Mohan, 1997). The seaweed extract applied as foliar spray enhanced the leaf chlorophyll level in plants (Blunden *et al.,* 1996). Similar observations were reported for *Chaetomorpha antennina* and *Rosenvingea intricate* on the chlorophyll content of *Abelmoschus esculentus* and *Raphanus sativus* plants (Thirumaran *et al.,* 2006; 2007) where the application of *Ascophyllum nodosum*-SLFs increased the chlorophyll content of cucumber and tomato plants (Whapham *et al*., 1993) and *Gracillaria edulis* extract on *Vigna unguiculata* (Lingakumar *et al*., 2002). Our findings show that increased chlorophyll content at lower concentration could be due to the presence of high amount of magnesium in the *S. myriocystum*-SLFs.

The highest protein content was recorded at concentration 10% of *S. myriocystum* SLF. 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 & Tamilselvan, 1990; Anantharaj & Venkatesalu, 2001; 2002; Sivasankari *et al*., 2006).

The sugar content increased up to concentration 10% of *S. myriocystum* SLF. The same trend was observed in *V. catajung* and *Dolichos biflorus* (Anantharaj & Venkatesalu, 2001; 2002) and in *V. sinensis* (Sivasankari *et al*., 2006). It has been observed that the α-amylase activity was higher than the β-amylase activity in the SLF and similar observation was made in *V. sinensis* (Sivasankari *et al*., 2006). The results indicate that a higher dilution of SLF is necessary to promote the growth and biochemical constituents of black gram.

This study is an important step towards the utilization of the seaweed *S. myriocystum* to improve the growth and biochemical constituents of commercially viable *V. mungo* in South India. The presence of phosphorous, magnesium, potassium and some trace elements in seaweeds make an excellent choice as organic fertilizers. Thus, the practice of application of eco-friendly seaweed extract is recommended to growers for attaining better germination, growth and crop yield. This study concludes that the seaweed liquid fertilizer of *S. myriocystum* is an effective and low cost fertilizer that can be promoted as an eco-friendly bio-fertilizer across India.

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