Micromorphological study of *Vigna mungo* L. using Seaweed liquid fertilizer from *Hypnea musciformis* (Wulf.) Lamouroux.

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The effect of seaweed liquid fertilizer from the red seaweed *Hypnea musciformis*, on the micromorphology of *Vigna mungo* was studied. *H. musciformis* was collected from Kanyakumari (Lat.9º11’N,’Long.79º24’E) rocky coast for the present study. Different concentration (1%, 2%, 4%, 6% and 8%) of *H. musciformis* liquid fertilizer and for the control experiment distilled water was used. Maximum germination percentage, growth and yield parameters, pigments, number of branches, root nodules and biochemical constituents were observed at 2% concentration of SLF. Present study revealed that the seaweed *H. musciformis* can be used as potential fertilizer and serve as a cost effective ecofriendliness for sustainable agriculture and environment.

[Keywords: *Hypnea musciformis*; seaweed liquid fertilizer; *Vigna mungo*; light microscopy; scanning electron microscopy]

**Introduction**

Owing to excessive usage of inorganics in agriculture, the soil fertility and physical property have been altered at the cost of productivity. Organic manures can be integrated in present day agriculture of the several organic components, the performance of seaweed manure is effective. Seaweeds are now commonly used as bio-fertilizer because of their abundance in marine habitat and can be safely used to increase soil fertility. Indian coastal areas are rich in algal diversity, harboring about 844 marine algal species belonging to different families and genera. Coastal farmers applied seaweed manure to many crops. Seaweeds contain good amount of nitrogen, potassium and other minerals and trace elements and also the carbohydrates and other organic matters present in seaweeds helps in altering the nature of soil and improving its moisture retaining capacity.

Booth observed that the value of seaweeds as fertilizers was not only due to nitrogen, phosphorus and potash content, but also the presence of trace elements and metabolites. Modern screening programs are motivated by the chemical ecology of marine organisms. The selection of samples for assays of biological activities useable in drug development is often based on ecological observations and includes specimens with unique (usually chemical) mechanisms for coping with environmental pressures. Seaweed liquid fertilizers prepared from *S. myriocystum* showed biostimulating effect on *V. mungo*. Seed pelleting commonly applicable technique in direct sown crops, also provides an opportunity to package effective quantities of material (inputs) which supplies not only micro and macro nutrients but also protects the crop from pests and disease during the earlier stages. Density and operation (opening) of stomatal pores on leaf surfaces are both heavily influenced by environmental cues. Together, they control leaf stomatal conductance to water vapour over short (minute to hour) and long (seasonal to lifetime) timescales and enable the plant to balance the conflicting needs to capture atmospheric carbon dioxide for photosynthesis and to minimize water loss through transpiration. Plants maintain plasticity in their capacity to moderate stomatal density during leaf growth and, although stomatal density correlates with the macro-environment over geological timescales, there is also a strong inverse correlation with water use efficiency (WUE) during growth and development.
The frequency of stomata on the leaf epidermis (Stomatal Index, SI: stomata as a percentage of epidermal cells) responds to light, CO₂ concentration, drought, and evaporative demand - relative - humidity. In recent years, liquid extracts prepared from different seaweeds have started gaining importance as foliar sprayers or soil conditioners for several important crops. Seaweed extract can be used as a growth enhancer for variety of plants at a lower concentration without any harmful effects. Plants sprayed with seaweed extract showed healthy growth with bright green and larger leaves, early flowering and fruit bearing as compared to the group where no seaweed extract was used. Ultrastructure of blackgram root nodule meristem with special reference to mitochondrial activity was noticed. Energy dispersive spectroscopic analysis (EDS) provides a unique approach for obtaining quantitative compositional analysis of individual cell and intra cellular compartments. Elemental quantification of semi-thin sections with electron probe X-ray microanalysis (EPMA) in generally based on the linear relationship between elemental concentration and the ratio of number of characteristic/continues like X-ray photons. Macro and micro elements are generally regarded as being cell wall associated and has been detected by XRMA in a range of algal cells including blue green algae. But still there is no report on ultrastructural studies of root nodules; root, stem and leaf of SLF treated blackgram. Present work is an attempt to study the effect of seaweed extracts of Hypnea musciformis on the growth, micromorphological, morphoanatomical and biochemical evaluation of the pulse crop V. mungo.

Materials and Methods
The crop plant, selected for the present study was V. mungo L. Pusa belonging to the family Fabaceae. Seeds were procured from Regional Pulses Research Station, Tamil Nadu Agricultural University, Coimbatore. Seeds with uniform size, colour and weight were chosen for the experimental purpose. Selected seeds were stored in a metal tin as suggested by Rao. Healthy selected black gram seeds (V. mungo L.) were soaked in beakers containing equal volume of different concentrations (1%, 2%, 4%, 6%, and 8%) of seaweed extracts. One batch of seeds was kept as control by soaking them in distilled water. Solutions were decanted after 24 hours and the imbibed seeds were placed in trays with wet filter paper for 10 days. At the end of this period percentage of seed germination, growth and yield parameters, pigments, number of branches, and root nodules were recorded. Estimation of Chl. ‘a’, ‘b’ and Xanthophylls + Carotenes, total Carbohydrate, total protein and lipid was made. Employing ‘t’ test the differences in parameters were analyzed by Zar.

The materials of leaf, stem, root and root nodules were preserved in 70% ethanol. Cross sections of leaf, stem, root and root nodules were prepared by hand from preserved material in chloral hydrate and sartur reagent. Sartur reagent contains KI-I, aniline, sudan III, lactic acid, alcohol and water. Illustrations were made using a Leitz drawing prism attached to a Leitz-Wetzlar (45°) microscope. Photomicrographs were taken with a camera adapted to Magnum MLX microscope. Preparation of SEM studies in leaf, stem, root and root nodules of blackgram
Scanning Electron Microscopy of blackgram was done by using the method of Hayat, 1970. Reagents included Glutaraldehyde, Phosphate buffer (pH 6.8), Alcohol. For SEM study the blackgram (leaf, stem, root and root nodules) were fixed in primary fixative 3% glutaraldehyde. The fixed sample were given 3 washes thoroughly in 0.1 M phosphate buffer (pH 6.8) they were dehydrated through a graded series of alcohol 10-15 minutes interval at 4°C upto 70%. Then 90% and 100% alcohol were kept in room temperature at 2-3 h. interval. Dehydrated samples treated with critical point drier (CPD) were on a stub and the specimens were examined with Joel JSM-56010 with INSA-EDS and electron
micrograph were taken selectively from the computer screen.

Results

The SLF of seaweed at 2% concentration are the most optimum for high growth germination (Tab. 1 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 2% H. musciformis SLF (Table 2 and 3). 2% SLF 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 application of 2% SLF increased the Chl ‘a’, ‘b’ and Xanthophyll + carotenes in most concentrations respectively higher, when compared to control. Accumulation of total carbohydrate, total protein and total lipid content also increased due to the SLF treatment. A maximum accumulation of the above parameters was recorded when the plant foliar spray of 2% 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 4). 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’, Xanthophyll + carotenes, carbohydrates, protein and lipid) are possible due to the SLF induced absorption of essential nutrients and the related increased enzyme activity.

<table>
<thead>
<tr>
<th>Concentrations (%)</th>
<th>H. musciformis Germination (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>78±3.75</td>
</tr>
<tr>
<td>1%</td>
<td>93±2.10</td>
</tr>
<tr>
<td>2%</td>
<td>98±1.20</td>
</tr>
<tr>
<td>4%</td>
<td>94±5.15</td>
</tr>
<tr>
<td>6%</td>
<td>88±4.75</td>
</tr>
<tr>
<td>8%</td>
<td>77±3.65</td>
</tr>
</tbody>
</table>

Fig. 2- Anatomical studies of Vigna mungo L. 2% H. musciformis (SLF) control and treated plant leaf, stem, root and root nodules

Light microscopic studies of leaf, stem, root and root nodules

The H. musciformis (SLF) 2% treated plant leaf, the cuticle is thickest layer. Cross section, measuring cuticle thickness is 10-15μm. Epidermis is usually made up of a single layer of cells that are closely packed. It is 8-13μm thickness. Cuticle on the upper epidermis is thicker than that of lower epidermis. Stoma was more in number on the lower epidermis than on the upper epidermis (fig. 2f). Palisade parenchyma cells are seen beneath the upper epidermis. It consists of vertically elongated cylindrical cells in one or
Table 2- Effect of different concentration of *Hypnea musciformis* seaweed liquid fertilizer (SLF) on shoot and root length (cm) and leaf area (cm²) in *Vigna mungo* 15<sup>th</sup>, 30<sup>th</sup>, 45<sup>th</sup> and 60<sup>th</sup> day.

<table>
<thead>
<tr>
<th>Days</th>
<th>Parameters</th>
<th>Control</th>
<th>1%</th>
<th>2%</th>
<th>4%</th>
<th>6%</th>
<th>8%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Shoot length</td>
<td>12.72 ± 0.17</td>
<td>13.20 ± 0.09</td>
<td>15.45 ± 0.10</td>
<td>15.05 ± 0.17</td>
<td>14.10 ± 0.14</td>
<td>12.86 ± 0.28</td>
</tr>
<tr>
<td>15&lt;sup&gt;th&lt;/sup&gt; Day</td>
<td>Root length</td>
<td>8.05 ± 0.14</td>
<td>9.06 ± 0.16</td>
<td>11.26 ± 0.35</td>
<td>10.55 ± 0.14</td>
<td>9.85 ± 0.25</td>
<td>8.05 ± 0.15</td>
</tr>
<tr>
<td></td>
<td>Leaf area</td>
<td>18.84 ± 0.29</td>
<td>26.85 ± 0.17</td>
<td>35.45 ± 0.18</td>
<td>34.45 ± 0.21</td>
<td>32.53 ± 0.32</td>
<td>27.21 ± 0.51</td>
</tr>
<tr>
<td></td>
<td>Shoot length</td>
<td>16.02 ± 0.25</td>
<td>23.13 ± 0.28</td>
<td>26.05 ± 0.10</td>
<td>25.25 ± 0.47</td>
<td>22.86 ± 0.34</td>
<td>18.86 ± 0.28</td>
</tr>
<tr>
<td>30&lt;sup&gt;th&lt;/sup&gt; Day</td>
<td>Root length</td>
<td>13.15 ± 0.04</td>
<td>14.06 ± 0.16</td>
<td>16.86 ± 0.25</td>
<td>15.85 ± 0.34</td>
<td>14.85 ± 0.15</td>
<td>13.85 ± 0.23</td>
</tr>
<tr>
<td></td>
<td>Leaf area</td>
<td>25.84 ± 0.19</td>
<td>27.18 ± 0.17</td>
<td>39.45 ± 0.18</td>
<td>35.45 ± 0.21</td>
<td>31.21 ± 0.32</td>
<td>29.21 ± 0.51</td>
</tr>
<tr>
<td></td>
<td>Shoot length</td>
<td>25.72 ± 0.07</td>
<td>28.20 ± 0.19</td>
<td>35.05 ± 0.10</td>
<td>32.35 ± 0.17</td>
<td>29.86 ± 0.24</td>
<td>25.86 ± 0.08</td>
</tr>
<tr>
<td>45&lt;sup&gt;th&lt;/sup&gt; Day</td>
<td>Root length</td>
<td>15.05 ± 0.24</td>
<td>16.06 ± 0.36</td>
<td>20.26 ± 0.15</td>
<td>19.15 ± 0.24</td>
<td>17.05 ± 0.35</td>
<td>15.85 ± 0.45</td>
</tr>
<tr>
<td></td>
<td>Leaf area</td>
<td>34.14 ± 0.19</td>
<td>36.85 ± 0.17</td>
<td>40.45 ± 0.18</td>
<td>38.45 ± 0.21</td>
<td>35.53 ± 0.32</td>
<td>32.21 ± 0.51</td>
</tr>
<tr>
<td></td>
<td>Shoot length</td>
<td>31.12 ± 0.27</td>
<td>33.20 ± 0.19</td>
<td>38.05 ± 0.10</td>
<td>36.35 ± 0.17</td>
<td>34.10 ± 0.24</td>
<td>31.86 ± 0.08</td>
</tr>
<tr>
<td>60&lt;sup&gt;th&lt;/sup&gt; Day</td>
<td>Root length</td>
<td>18.05 ± 0.24</td>
<td>20.06 ± 0.36</td>
<td>25.26 ± 0.15</td>
<td>22.15 ± 0.24</td>
<td>20.85 ± 0.35</td>
<td>18.85 ± 0.45</td>
</tr>
<tr>
<td></td>
<td>Leaf area</td>
<td>40.14 ± 0.19</td>
<td>44.85 ± 0.17</td>
<td>48.45 ± 0.18</td>
<td>44.45 ± 0.21</td>
<td>42.23 ± 0.32</td>
<td>40.21 ± 0.51</td>
</tr>
</tbody>
</table>

Values are mean ± SD; Sample (n) =6

Table 3. Effect of different concentration of *Hypnea musciformis* seaweed liquid fertilizer (SLF) on fresh and dry weight of shoot and root, number of branches and root nodules in *Vigna mungo* 60<sup>th</sup> day.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>1%</th>
<th>2%</th>
<th>4%</th>
<th>6%</th>
<th>8%</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of Branches</td>
<td>7-10 ± 2</td>
<td>10-15 ± 3</td>
<td>16-20 ± 2</td>
<td>14-18 ± 3</td>
<td>11-14 ± 4</td>
<td>10-13 ± 3</td>
</tr>
<tr>
<td>No. of Nodules</td>
<td>32-45 ± 5</td>
<td>45-50 ± 4</td>
<td>60-70 ± 3</td>
<td>58-65 ± 5</td>
<td>52-55 ± 4</td>
<td>45-48 ± 3</td>
</tr>
<tr>
<td>Fresh weight of Shoot (mg/g Fw)</td>
<td>90.195 ± 7.651</td>
<td>110.130 ± 6.710</td>
<td>125.172 ± 8.152</td>
<td>120.134 ± 5.213</td>
<td>112.313 ± 4.322</td>
<td>103.101 ± 3.120</td>
</tr>
<tr>
<td>Fresh weight of Root (mg/g Fw)</td>
<td>11.050 ± 1.413</td>
<td>12.050 ± 1.413</td>
<td>17.011 ± 1.213</td>
<td>15.013 ± 1.521</td>
<td>12.117 ± 1.331</td>
<td>11.100 ± 1.110</td>
</tr>
<tr>
<td>Dry weight of Shoot (mg/g Fw)</td>
<td>0.040±0.003</td>
<td>0.049±0.004</td>
<td>0.058±0.003</td>
<td>0.052±0.002</td>
<td>0.043±0.004</td>
<td>0.040±0.003</td>
</tr>
<tr>
<td>Dry weight of Root (mg/g Fw)</td>
<td>0.038±0.003</td>
<td>0.046±0.003</td>
<td>0.051±0.002</td>
<td>0.040±0.003</td>
<td>0.038±0.002</td>
<td>0.034±0.002</td>
</tr>
</tbody>
</table>

Values are mean ± SD; Sample (n) =6

more layers. These cells are compactly arranged without intracellular spaces. Its thickness is 8-10μm. Palisade parenchyma cells contain more chloroplasts than the spongy parenchyma cells. Spongy parenchyma lies below the palisade parenchyma. Spongy cells are irregularly shaped. These cells are very loosely arranged with numerous airspaces. As compared to palisade cells, the spongy cells contain lesser number of chloroplasts. Spongy cells facilitate the exchange of gases with the help of air spaces. Its thickness is 5-10μm.

Vascular tissues are present in the veins of leaf. Vascular bundles are conjoint, collateral and closed. Xylem cell individual length and width is 8-10μm, its thickening size is 3-5μm. Xylem is present towards the upper
epidermis, while the phloem towards the lower epidermis.

Vascular bundles are surrounded by a compact layer of arencymatous cells called bundle sheath or border parenchyma. Xylem consists of metaxylem vessels and protoxylem vessels. Protoxylem vessels are present towards the upper epidermis. Phloem consists of sieve tubes, companion cells and phloem parenchyma. Phloem fibres are absent. Phloem cell individual length and width is 10-14μm, its thickening size is 5-7μm. Xylem consists of vessels and xylem parenchyma. Tracheids and xylem fibres are absent, when compared to control (fig. 2a, and d).

SLF 2% treated plant stem, epidermal cell is a single layer of parenchymatous rectangular cells. The cells are compactly arranged without intercellular spaces. Outer walls of the epidermal cells have a single layer called cuticle. Cross section, measuring thickness of cortex is 10-15μm. Endodermis is made up of single layer of barrel shaped parenchymatous cells. Casparian strips are absent in the endodermal cells which are located opposite to the protoxylem elements.

All the tissues present inside endodermis comprise the stele. Pericycle is generally a single layer of parenchymatous cells found inner to the endodermis. Lateral roots originate from the pericycle. Vascular tissues are in radial arrangement. The tissue by which xylem and phloem are separated is called conjunctive tissue. Thickness of xylem is 8-12μm. Xylem is in exarch condition. Each phloem patch consists of sieve tubes, companion cells and phloem parenchyma. Metaxylem vessels are generally polygonal in shape, when compared to control (fig. 2c and e).

SLF of treated plant root nodules, mainly three types of tissues constitute the earliest detectable nodule, i.e., cortical, meristematic and the central bacterial cells with feebly developed vascular elements. The nodule meristem develops as crescent shaped as a result only spherical nodules are formed which surround the central area “Bacteroid zone” but separated by a few layers of parenchymatus cells (fig. 2h). Meristem cells present a more or less brick-like cross section, measuring 8.5-10.0μm x 5.5-9.0μm when compared to control (fig. 2g).

Scanning electron microscopy studies with energy dispersive spectroscopic analysis

The EDS analysis of different chemical elements present in the cell wall of leaf 2% SLF treated and control of V. mungo. Totally eleven elements namely N, P, K, Ca, S, Na, Si, Mg, Mn, Zn and Fe were observed. Among the elements, maximum value of Ca was detected followed by P and N both in control and 2% SLF treated plants. But the higher value of N, lower value of P and almost similar value of Ca were recorded in 2% SLF than control. The order of chemical elements from epidermal portion of the leaf of SLF treated V. mungo and control was as follows; Ca>P>N>Na>K>Si>Mg>Mn>S>Fe>Zn, Ca>N>P>Na>Si>Mg>Mn>K>Zn>S>Fe respectively (fig. 4). The H. musciformis (SLF) 2% treated plant leaf, the cuticle is thickest layer. Stoma was used for transpiration and gas exchange. Cross section, measuring cuticle thickness is 10-15μm. The epidermis is usually made up of a single layer of cells that are closely packed. It is 8-13μm thickness. The cuticle on the upper epidermis is thicker than that of lower epidermis. Stomata are more in number on the lower epidermis than the upper epidermis (fig. 3a).

The SLF treated plant stem is very thickening and separated individual cell. Epidermal cell is a single layer of parenchymatous rectangular cells. The cells are compactly arranged without intracellular spaces. The outer walls of the epidermal cells have a layer called cuticle. Cross section, measuring epidermal cell is thickness is 15-20μm. Cortex lies below the epidermis. Its measuring thickness is 12-16μm. Cambium consists of brick shaped and thin walled meristematic cells. It is two to three layers in thickness. The large central portion of the stem is called pith. It is composed of parenchyma cells with intercellular spaces (fig. 3b).

The outermost layer of the root is known as rhizodermis. It is made up of a single layer of parenchyma cells which are arranged compactly without intercellular spaces. The cells are oval or rounded in shape. Cross section, measuring thickness of cortex is 10-15μm. Endodermis is made up of single layer of barrel shaped parenchymatous cells. The tissue by which xylem and phloem are separated is called conjunctive tissue. Thickness of xylem is 8-12μm (fig. 3c).
Three types of tissues constitute the earliest detectable nodule, i.e., cortical, meristematic and the central bacterial cells with feebly developed vascular elements. The nodule meristem develops as crescent shaped as a result only spherical nodules are formed which surround the central area “Bacteroid zone” but separated by a few layers of parenchymatous cells. Meristem cells present a more or less brick-like cross section, measuring 8.5-10.0μm x 5.5-9.0μm (fig. 3d).

Table 4. Effect of seaweed liquid fertilizer (SLF) on biochemical constituents of *Vigna mungo* (*Hypnea musciformis*) 30th day.

<table>
<thead>
<tr>
<th>Biochemical Constituents</th>
<th>Control</th>
<th>1%</th>
<th>2%</th>
<th>4%</th>
<th>6%</th>
<th>8%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorophyll ‘a’ (µg/g/ Fw)</td>
<td>290 ± 5.270</td>
<td>300 ± 6.177</td>
<td>350 ± 7.132</td>
<td>330 ± 4.181</td>
<td>310 ± 6.541</td>
<td>282 ± 3.768</td>
</tr>
<tr>
<td>Chlorophyll ‘b’ (µg/g/ Fw)</td>
<td>188 ± 5.787</td>
<td>210 ± 7.813</td>
<td>246 ± 5.236</td>
<td>230 ± 4.897</td>
<td>208 ± 8.231</td>
<td>178 ± 4.670</td>
</tr>
<tr>
<td>Xanthophyll + Carotenes (µg/g/ Fw)</td>
<td>151 ± 4.176</td>
<td>158 ± 4.785</td>
<td>176 ± 9.167</td>
<td>165 ± 6.096</td>
<td>155 ± 7.496</td>
<td>147 ± 7.561</td>
</tr>
<tr>
<td>Total Carbohydrates (Mg/g/Fw)</td>
<td>1.8 ± 0.351</td>
<td>1.9 ± 0.235</td>
<td>2.8 ± 0.257</td>
<td>2.5 ± 0.318</td>
<td>2.1 ± 0.278</td>
<td>1.6 ± 0.535</td>
</tr>
<tr>
<td>Total Protein (mg/g/Dw)</td>
<td>1.1 ± 0.125</td>
<td>1.2 ± 0.115</td>
<td>1.5 ± 0.185</td>
<td>1.4 ± 0.212</td>
<td>1.2 ± 0.282</td>
<td>0.9 ± 0.370</td>
</tr>
<tr>
<td>Lipid (mg/g/Dw)</td>
<td>0.28 ± 0.028</td>
<td>0.35 ± 0.018</td>
<td>0.45 ± 0.183</td>
<td>0.41 ± 0.052</td>
<td>0.38 ± 0.071</td>
<td>0.25 ± 0.065</td>
</tr>
</tbody>
</table>

Values are mean ± SD; Sample (n) = 6

**Discussion**

It has been suggested that the growth promoting activity of seaweed extracts was due to macro and micro elements as well as growth promoting substances like cytokinin. Germination of pepper seeds was examined after priming seeds in a 10% Maxicrop solution at dilutions up to 1:1,000. Fertilizer
plus a simple autoclaved extract of the brown seaweed, Rosenviga intricata, stimulated lateral root development, increased pigment content and the number of leaves and vegetables over the fertilizer controls when tested with the crop plant Abelmoschus esculentus16. A proprietary marine extract has been shown to improve the leaf content of macronutrients, promote growth, and impart resistance to drought stress in grapes32. Increases in phenolic components, flavonoids and antioxidant levels were reported in leaves from spinach plants watered with soluble A. nodosum extract33.

SLF treatment increased the number of branches and concentration of photosynthetic pigments34. Crop cultivation using organic fertilizers has contributed for deposition of residues, improving physical and chemical properties of soil that is important for biological development55. This enhanced growth is thought to be due to various organic compounds present in the seaweed extract. More specifically it is understandable to be due to the presence of phytohormones, mainly cytokinins in the seaweed extracts36. Our findings coincide with those of earlier studies in Cajanus cajan37, Oryza sativa38, Abelmoschus esculentus39, Cyamopsis tetragonoloba40, Lycopersicon esculentum31, 42 Vigna radiata43 and Triticum aestivum44. This is in accordance with the earlier reports that the application of Enteromorpha intestinalis extract increased the chlorophyll contents of Seasamum indicum35. Biochemical analysis of the experimental plants showed that the foliar treated plants showed more photosynthetic pigments compared to root treated plants, whereas the accumulation of total protein, total carbohydrate and total lipid content was found maximum in root treated plants than the foliar treated plants46. Syliva et al., reported that higher dilutions of LSFs are more effective than lower dilutions47. Ramamoorthy and Sujatha reported linear growth of both root and shoot in black gram seeds, similar typical growth promotion was observed in this study at lower concentration of the Sargassum wightii extract48.

The ultrastructure of these cells in its general features is similar to those described49, 50. In general, the fine structure of nodule meristem is in agreement with that reported by Dart and Mercer51. Seaweed fertilizers are better than other fertilizers and are very economical. This simple practice of application oriented ecofriendly seaweed liquid fertilizers to pulse crops may be useful for the growers for attaining better germination, growth and yield52.

Diverse physiological and biochemical processes are involved for both plants and animals. The observed effects from foliar or soil applications may be direct or indirect as can occur due to changes in the rhizosphere community for plants, cultivated with seaweed products53.

Among the organic fertilizers, the seaweed fertilizer is significantly better than that of untreated one. This can be attributed to easy decomposability of its carbonaceous matter and also due to the presence of micronutrients. This is conformity with the observation of Broadbent and Norman that the case of decomposition of the soil organic matter is apparently a function of the available energy material added to the soil54. This observation is further supported by the fact that the performance of Hypnea musciformis seaweed liquid fertilizer, which contains higher proportion of alginic acid and analogues compounds, is relatively superior with respect to both the yield and quality attributes. Hypnea musciformis is eco-friendly, easily manageable input farming and a self-regenerating system which provide nutrients and maintains health status of soil. Energy dispersive spectroscopic analysis (EDS) study elucidates the various constituents within a cell which differed in their chemical composition, certain elements being specific to individual cell component.

Conclusion
The cost effective cultivation of leguminous crops depends on effective symbiotic nitrogen fixation in their nodules that relies on the rhizobial population of the soil. Present investigation showed that the application of seaweed liquid fertilizer as seed treatment enhances the nodule number and their quality in both the legume crops tested and its application in consortium with Rhizobium further enhanced their vegetative growth and yield.

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