Treatment of broad bean seeds with algal suspensions to study their effects on certain growth and yield parameters

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1. Introduction

Broad bean (Viciafaba L.) is a crop grown primarily for its edible seeds (beans) and is a major legume seed consumed by humans worldwide. The dried seeds are cooked, canned or frozen (Mohiuddin Das and Ghosh, 2000). Microalgae have multi-functional properties in agriculture, nutrient uptake, improving crops, physiological and tolerance to a biotic stress (Ronga et al., 2000).

Algae are considered a rich source for various products that positively affect both growth and yield of most of plant crops (De Morais et al., 2015). Because of their high content of protein, algae have been extensively used as nitrogen rich biofertilizer and also as nutritious animal feed (Gaese, 2012). It has been reported that the gelatinous sheath of some algal species was able to chelate Fe, Cu, Mo, Zn, Co, Mn, and other elements essential for their growth (Lange, 1976). The sheath was also thought to influence the availability of elements to other organisms (Belnap and Harper, 1995).

Exopolysaccharides secreted by many microalgae species provide organic carbon for the growth and development of beneficial microbes, leading to the formation of useful biofilms in the rhizo sphere (Xiao and Zheng, 2016).

In general, soil fertility could be diminished due to soil erosions, loss of nutrients, accumulation of salts and other toxic elements. Organic and bio-fertilizers are the alternative sources to meet the nutrient requirements of different crops. Nowadays, algal have been emerged as a valuable component for integrating nutrient supply system in agriculture.

Several studies have already showed a host of beneficial effects of algal application on plants, such as enhanced seed germination, improved crop and yield, resistance to biotic and abiotic stresses (Mohsen et al., 2016; Kawalekar, 2013; Sengar et al., 2010).

Therefore, this work was designed to study the effect of some algal suspensions as priming solution for seeds before cultivation on growth of some metabolic activities and yield parameters of broad bean plant.

2. Materials and Methods

Algal biomass and growth medium

Nostoc muscorum was grown on BG11 medium (Rippka et al., 1979) and Chlorella vulgaris was grown on Khul medium (Kühl, 1962) for 12 days. The culture was incubated under continuous fluorescent light of intensity 55 µ mole photon m-2 s-1 and temperature of 25°C ± 2°C with constant aeration. The algae were isolated and purified according to Stein, 1973. All cultures were harvested at the beginning of stationary phase; cells were rinsed three times and washed with sterilized distilled water to remove traces of the growth medium (Rogers and Burns, 1994). Experimental design

Seeds of broad bean were selected then sterilized in 1% sodium hypochlorite solution for 15 minutes. The seeds were washed thoroughly with distilled water,
and then soaked overnight (12 hours) the following solutions:  
a) Distilled water (control).  
b) Suspensions of algal samples as soaking solutions each at 1% fresh weight biomass of Chlorella vulgaris and Nostoc muscorum. Afterwards, the seeds were sown in pots (35 cm diameter and 30 cm depth) containing equal amounts of loamy soil. Seeds were sown at 2 cm depth as 10 seeds per pot. The pots were kept in the green house under normal conditions of light, temperature and irrigated with tap water. Chemical fertilizers applied were ammonium nitrate and super phosphate (as the recommended dose of the Ministry of Agriculture for each plant) added as referred to the surface area of one feddan. Plant samples were taken after 15 days old (germination stage) and yield (fruits stage).

1-Growth parameters  
Root and shoot length, fresh and dry weights were recorded at the seedling stage.

2-Some metabolic activities  
Total pigments were estimated quantitatively Metzner et al. (1965). Carbohydrates in the plant root and shoot were estimated quantitatively Nelson (1944) and Naguib (1963). Borate buffer extract was used to estimate the direct reducing (DRV) and total reducing values (TRV) of carbohydrates. Total protein was estimated Bradford (1976). The activity of some antioxidant enzymes (peroxidase, catalase) were measured Kato and Shimizu (1987). Lipid peroxidation was estimated the concentration of malondialdehyde (Uchiyama and Mihara, 1978).

3-Yield parameters  
Some productivity criteria including the number of pods/plant, number of seeds/pod, number of seeds/plant, weight of 100 seeds (g) were estimated at fruiting stage.

Statistical Analysis  
Statistical analysis was performed with one-way ANOVA, using SAS program version 6.12 at p ≤ 0.05 level of significance (Snedecor, 1970).

3. Results  
Data present in Table 1 show the effect of the different algal treatments (Chlorella vulgaris and Nostoc muscorum) on some growth parameters. All algal treatments induced an increase in root and shoot length, fresh weight and dry weight of root and shoot. The stimulatory effect induced by algal presoaking on the roots and shoots length of broad bean seedlings show that Nostoc muscorum maintained higher effect compared to Chlorella vulgaris.

Results show that treatment with C. vulgaris increased the fresh weight by 33.3% for root and 82.7% for shoot, dry weight by 50% for root and 90% for shoot and water content by 29.2% for root and 81.7% for shoot in compared to control, on the other hand treatment with Nostoc muscorum showed the highest increase in fresh weight by 2 fold for root and 1.5 fold for shoot, dry weight by 66.7% for root and 1.6 fold for shoot and water content by 1.9 fold for root and 1.5 fold for shoot in compared to control (Table 1).

Table 1: Effect of seeds priming in algal suspensions on various growth parameters of 15-day old broad bean seedlings

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Root length (cm)</th>
<th>Shoot length (cm)</th>
<th>Fresh weight (g)</th>
<th>Dry weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Root</td>
<td>Shoot</td>
<td>Root</td>
<td>Shoot</td>
</tr>
<tr>
<td>Control (H₂O)</td>
<td>0.3 ±0.17</td>
<td>0.2±0.17</td>
<td>0.005 ±0.03</td>
<td>0.05 ±0.81</td>
</tr>
<tr>
<td>Chlorella vulgaris</td>
<td>0.1±0.63</td>
<td>0.1±0.27</td>
<td>0.008±0.04</td>
<td>0.01±0.48</td>
</tr>
<tr>
<td>Nostoc muscorum</td>
<td>12.50±0.83</td>
<td>0.2±0.09</td>
<td>0.002±0.01</td>
<td>0.01±0.08</td>
</tr>
</tbody>
</table>

F(value) = 238.44*** 93.41*** 255.51*** 7883*** 929.22*** 99999.9*** 99999.99*** 99999.9***

Each value represents the mean of three readings ± the standard error.

Results presented in Table 2 show increase in the contents of chlorophyll a, b and carotenoids in leaves of broad bean seedling produced from algal-treated seeds over control. The highest stimulatory effect was observed in plants presoaked in Nostoc muscorum.

Table 2: Effect of seed priming in algal suspensions on the photosynthetic pigments of broad bean leaves of 15-day old seedlings

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Chl. a (mg/g D.wt)</th>
<th>Chl. b (mg/g D.wt)</th>
<th>Carotenoids (mg/g D.wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (H₂O)</td>
<td>0.04±0.001</td>
<td>0.04±0.0087</td>
<td>0.05±0.027</td>
</tr>
<tr>
<td>Chlorella vulgaris</td>
<td>0.05±0.131</td>
<td>0.08±0.121</td>
<td>0.05±0.40</td>
</tr>
<tr>
<td>Nostoc muscorum</td>
<td>0.05±0.137</td>
<td>0.05±0.128</td>
<td>0.005±0.061</td>
</tr>
</tbody>
</table>

F(value) = 99999.9*** 99999.9*** 77469.59***

Each value represents the mean of three readings ± the standard error.

Results presented in Table 2 show increase in the contents of chlorophyll a, b and carotenoids in leaves of broad bean seedling produced from algal-treated seeds over control. The highest stimulatory effect was observed in plants presoaked in Nostoc muscorum.
Nostoc muscorum treatment (1.7 fold) and (78%) in case of Chlorella vulgaris.

Table 3: Effect of seed priming in algal suspensions on carbohydrates and protein content of broad bean leaves of 15-day old seedlings

<table>
<thead>
<tr>
<th>Treatment</th>
<th>DRV (mg/g D.wt)</th>
<th>TRV (mg/g D.wt)</th>
<th>Protein (mg/g D.wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Root</td>
<td>Shoot</td>
<td>Root</td>
</tr>
<tr>
<td>(Control H&lt;sub&gt;2&lt;/sub&gt;O)</td>
<td>±0.005± 21.43</td>
<td>±0.05± 32.73</td>
<td>0.000± 26.77±</td>
</tr>
<tr>
<td>Chlorella vulgaris</td>
<td>±0.005± 22.67</td>
<td>±0.01± 35.63</td>
<td>0.001± 29.37±</td>
</tr>
<tr>
<td>Nostoc muscorum</td>
<td>24.7±0.002± 36.9±0.01±</td>
<td>±0.02± 30.7</td>
<td>0.02± 42.4±</td>
</tr>
<tr>
<td>F(value)</td>
<td>260.24±**** 139.38±***</td>
<td>400.91±* 196.5±**</td>
<td>2077.28±***</td>
</tr>
</tbody>
</table>

Each value represents the mean of three readings ± the standard error.

Results presented in Table 4 show that the activities of peroxidase, catalase (µM /g F.wt) and the values of lipid peroxidation measured as Malondialdehyde (µmol MDA g<sup>-1</sup> F. wt) in leaves of broad bean seedlings were also reduced in response to both algal treatments. The reduction in lipid peroxidation was more intense in case of Nostoc muscorum than Chlorella vulgaris treatment.

Table 4: Effect of seed priming in algal suspensions on antioxidant enzymes and lipid peroxidation content of Broad bean leaves of 15-day old seedlings

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Peroxidase (µmol MDA g&lt;sup&gt;-1&lt;/sup&gt; F. wt)</th>
<th>Catalase (µmol MDA g&lt;sup&gt;-1&lt;/sup&gt; F. wt)</th>
<th>Lipid peroxidation (mg/g D.wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (H&lt;sub&gt;2&lt;/sub&gt;O)</td>
<td>0.05±7.71</td>
<td>0.002±0.15</td>
<td>0.003±0.6</td>
</tr>
<tr>
<td>Chlorella vulgaris</td>
<td>0.06±2.1</td>
<td>0.005±0.12</td>
<td>0.005±0.5</td>
</tr>
<tr>
<td>Nostoc muscorum</td>
<td>0.05±1.84</td>
<td>0.005±0.08</td>
<td>0.005±0.5</td>
</tr>
<tr>
<td>F(value)</td>
<td>26343.98±**** 731.10±***</td>
<td>733.70±****</td>
<td></td>
</tr>
</tbody>
</table>

Each value represents the mean of three readings ± the standard error.

Table 5: Effect of seed priming in algal suspensions on various yield parameters of broad bean plant

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N&lt;sub&gt;o&lt;/sub&gt; of pods / plant</th>
<th>N&lt;sub&gt;o&lt;/sub&gt; of seeds / pods</th>
<th>No of seeds / plant</th>
<th>W eight of 100 seeds (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (H&lt;sub&gt;2&lt;/sub&gt;O)</td>
<td>0 ±3</td>
<td>0.05±3.0</td>
<td>0.1±18.0</td>
<td>0±53.7±***</td>
</tr>
<tr>
<td>Chlorella vulgaris</td>
<td>0±6.0</td>
<td>0.05±3.0</td>
<td>0.5±18.0</td>
<td>0±57.0±***</td>
</tr>
<tr>
<td>Nostoc muscorum</td>
<td>0±6.33±</td>
<td>0.08±3.3</td>
<td>0.005±19.0</td>
<td>0±57.3±***</td>
</tr>
<tr>
<td>F(value)</td>
<td>4±25**</td>
<td>1±0.57±</td>
<td>3.66±*</td>
<td>35±3.30±***</td>
</tr>
</tbody>
</table>

Each value represents the mean of three readings ± the standard error.

Table 6: Effect of seed priming in algal suspensions on carbohydrates and protein content of harvested seeds

<table>
<thead>
<tr>
<th>Treatment</th>
<th>D RV (m g/g D.wt)</th>
<th>Starch (m g/g D.wt)</th>
<th>Sucrose (mg/g D.wt)</th>
<th>Total protein (m g/g D.wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (H&lt;sub&gt;2&lt;/sub&gt;O)</td>
<td>39±0.2±0.8</td>
<td>10±0.02±1</td>
<td>45±2.0±5</td>
<td>83±1.1±1bac</td>
</tr>
<tr>
<td>Chlorella vulgaris</td>
<td>40±0.07±0.8</td>
<td>10±2.33±1</td>
<td>45±73±0.1</td>
<td>10±3.1±1.1</td>
</tr>
<tr>
<td>Nostoc muscorum</td>
<td>41±33±0.8</td>
<td>10±4.77±7</td>
<td>46±47±1.1</td>
<td>11±0.1±0.8</td>
</tr>
<tr>
<td>F(value)</td>
<td>97±25***</td>
<td>39.35±**</td>
<td>18±60.33*</td>
<td>16±291.99***</td>
</tr>
</tbody>
</table>

Each value represents the mean of three readings ± the standard error.
**Discussion**

In the present work soaking of broad bean seeds in different algal suspensions (Chlorella vulgaris and Nostoc muscorum) caused a marked increase in the measured growth parameters of the produced root and shoot (length, fresh and dry weight) in compared to control (Table 1).

Other Experimental studies tested the action of microalgae under open-field and greenhouse conditions, have demonstrated that they stimulate germination, seeding growth, shoot, and root biomass in several crops such as lettuce, red amaranth, tomato and pepper (García-Gonzalez and Sommerfeld, 2016; Barone et al., 2018; El Arrousséït et al., 2018).

Furthermore, Likhitkar and Tarar (1995) found that soaking of cotton seeds in extract of Nostoc muscorum increased germination, total length of seedlings and length of radical after 10 days.

Results of the current study showed that all pigment contents (chl. a, b and carotenoids) of leaves of broad bean were significantly increased in response to treatments with both algal suspensions (Table 2) in compared to control, which agreed with the studies of Abd–Allah et al.(1994); Abou-Khadrah et al. (2000) who reported that the cyanobacterial extract enhanced chlorophyll bio-synthesis. Such results could be attributed to some bioactive substances which have been produced by some algae and cyanobacteria, as gibberellic, auxin [including indole-3-acetic acid, indole-3-propionic acid, indole-3-butyric acid and 1-naphthalene acetic acid], cytokinins (Hashtroudi et al., 2011), vitamins, amino acids and exopolysaccharides (Osman et al., 2005), thus simulating growth and chlorophyll biosynthesis. The obtained data concerning carbohydrates content (Table 3) in broad bean plants showed increase values in response to different algal treatments in compared with control in both stages, indicating a stimulation of the photosynthetic process in response to algal pretreatment of seeds.

These results are in accordance with Haroun and Hussein (2003), who showed that the increase in pigments production in Lupinus leaves pretreated with the algal filtrate of Cylindrospermum led to an increase in the photosynthetic activity and carbohydrates content of plant tissue.

Results (Table 3) also showed that protein of broad bean plants has increased markedly in response to treatment with both algal suspensions in comparison with control; More or less similar results were reported by Gururag and Mallikarjunaiah (1995).

It is worth to attribute the increase in protein content to the increase of nitrogen uptake and consequently amino acid level contents which play a prominent role in the building of protein structure (Haroun and Hussein, 2003). However, the increase in total protein level content may be attributed to increased respiration, resulting in increased keto acids (Krebs cycle) which are the main precursor for amino acid biosynthesis. On the other hand, Cytokinin present in the algae could stimulate also protein biosynthesis in the treated plants (Norrie and Hiltz, 1999; El-Sheekh and El-Saied, 2000). Ghallab and Salem (2001) indicated that blue green treatment of wheat plant increased growth criteria, nutrients, sugars, amino acids and growth regulators as well as crude protein content.

In addition, pre-soaking of broad bean seeds in different algal suspensions caused different changes in antioxidant enzymes and lipid peroxidation in seedlings (Table 4). Broad bean peroxidase activity, catalase and Lipid peroxidation have reduced in broad bean seedlings in response to seeds presoaking in both algal suspensions.

Carocho and Ferreira (2013) stated that antioxidant enzymes can act as scavenging chain initiating radicals like hydroxyl, alkoxyl, or peroxyl, quenching singlet oxygen, decomposing hydro peroxides, and chelating prooxidative metal ions.

It can be concluded that pre-soaking of seeds in both algal suspensions has brought about changes in antioxidant enzymes and lipid peroxidation causing improvement and accelerating the different metabolic process of plants through some activates (Chen et al., 2010; Barron, 2010; Wu et al., 2010).

Yield parameters of broad bean plant, carbohydrates and protein content of the harvested seeds have significantly increased (Table 5 and Table 6) in response to both algal treatments.

This increase could be related to the significant increase in photosynthetic activity of plant in response to algal treatment.

**Conclusion**

Priming of broad bean seeds before cultivation in different algal suspensions (Chlorella vulgaris and Nostoc muscorum) stimulated growth parameters and some metabolite biosynthesis in the seedling causing a marked increase in the yield of broad bean plant. We may recommend the use of this method (seeds priming in some tested algal suspensions) as alternative method than using chemicals for the improvement of crop yield and quality.

**References**


