RESEARCH ARTICLE

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THE PROMOTIVE EFFECT ANABAENA FLOS AQUAE AS BIO-FERTILIZER ON THE GROWTH OF GLYCINE MAX SEEDLINGS

ABSTRACT:
In the present study, the interactive effect of culture filtrates of the cyanobacterium Anabaena flos aquae and the fungus Penicillium citrinum were studied on the growth criteria of soybean Glycine max cultivar "Crawford". The cyanobacterial filtrates of Anabaena flos aquae provoked growth parameters in soybean. Moreover, significant promoted growth of soybean seedlings was recorded when the cyanobacterial filtrate of Anabaena flos aquae combined together with the fungal culture filtrate of Penicillium citrinum, an increase in plant length by 37.32%, shoot length by 51.09%, plant fresh weight by 54.37% and shoot fresh weight by 47.49% as compared to the controls.

INTRODUCTION:
Biofertilizers are biologically active products or microbial inoculants of bacteria, algae and fungi (separately or in combination), which may help biological nitrogen fixation for the benefit of plants. The need for the use of bio-fertilizers has arisen, primarily for two reasons – to increase productivity and to reduce the damage to the soil and environment by the chemical fertilizers. Therefore, the use of biofertilizers is both economical and environment friendly. Watanabe (1986) confirmed that the application of blue green algae as bio-fertilizer increased the yield of rice.

The plant growth promoting fungi (PGPF) are associated with plant roots, and they secrete a number of secondary metabolites including gibberellins in the rhizosphere (Hamayun et al., 2009). Gibberellin secretion by PGPF was reported by several researchers (Kawaide, 2006; Vandenbussche, 2007), who showed the importance of Gibberellin producing fungi in plant growth and development.

GA₅₅ are diterpenoid plant hormones, first detected in the 1920s from culture filtrate of Gibberella fujikuroi, a known pathogen of rice plants (Ogas, 2000). GA₅₅ appear to be involved in every aspect of plant growth and development, but their most typical property involves the enhancement of stem growth (Nishijima et al., 1995). GA₅₅ may modify the sex expression of flowers, induce the parthenocarpic development of the fruit, and delay senescence. They obviate the need for exposure to red light in the germination of seeds and spores, and the need for vernalization in the growth of bulbs and tubers. They are associated with the breaking of winter dormancy and stimulate the formation of hydrolytic enzymes in germinating cereal grain (Martin, 1983).

KEY WORDS:
Biofertilizers, Anabaena flos aquae, Penicillium citrinum, Glycine max.

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This work aims to study the effect of the cyanobacterium *Anabaena flos aquae* alone and in combination with the fungus *Penicillium citrinum* on the growth criteria of soybean.

**MATERIAL AND METHODS:**

**Biofertilizer culture:**

Axenic algal culture of *Anabaena flos aquae* was grown in Watanabe (1951) under continuous fluorescent white light. The light intensity was kept at 2000 Lux and temperature 28 ± 2°C up to their appropriate logarithmic phase, the cyanobacterial biomass was separated from the culture medium by centrifugation under sterile conditions. The supernatant containing extracellular products. The supernatant was separated from the culture filtrate by centrifugation at 5,000xg at 4°C for 15 min and sterilized by 0.22 mm pore size Millipore membrane. Pure fungus culture of *Penicillium citrinum* kindly provided by Plant Pathology Institute, Agricultural Research Center, Giza, Egypt. The pure fungus culture was grown on potato dextrose agar (PDA) medium. For Gibberellin (GA) production, the fungus was incubated at 30°C for 7 days in Czapek broth medium, containing 1% glucose and peptone (Hasan, 2002).

**Test plant:**

Healthy seeds of (*Glycine max*) cultivar "Crawford," kindly provided by Field Crops Research Institute, Agricultural Research Center, Giza, Egypt. The seeds of soybean were surface sterilized using 0.1% HgCl₂ solution for 5 minutes followed by several washing with distilled water for about one hour.

**Biofertilization treatments:**

Selected seeds were distributed on water agar Petri dishes 0.5% agar and (10 ml) of the following different treatments were added as recommended by Nishijima *et al.* (1995):

1- Filtrate of *Anabaena flos-aquae*.

2- Filtrate of *Anabaena flos aquae* and *Penicillium citrinum* cultures.

3- In control Petridishes the cyanobacterial and fungal filtrate (10 ml) were substituted with water for comparison. Each treatment was repeated in triplicates and arranged in complete randomized design.

Well developed 5 days old seedlings of the plant were transferred to (500 ml) capacity bottles containing 1% autoclaved water-agar media and exposed to the same previous treatments. The growth criteria (i.e. plant length, shoot length, plant fresh weight and shoot fresh weight) were recorded after 10 days of the treatment and compared with the control. All data were statistically analyzed by one-way ANOVA (Armitage, 1971).

**RESULTS AND DISCUSSION:**

Cyanophyceae are a diverse group of prokaryotes, it distributed worldwide and improve the growth and development of the plants. Hedge *et al.* (1999) documented that application of algal biofertilizers is useful for the reclamation of marginal soils such as saline-alkali and calcareous soils.

The algal and fungal culture filtrates used in this study, either separately or in combination have stimulatory effect on plant and shoot lengths and also increased plant and shoot weights (Table 1).

**Table 1. Effect of filtrate of *Anabaena flos aquae* and *Penicillium citrinum* on growth characters of soybean seedlings.**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Plant length (cm)</th>
<th>Shoot length (cm)</th>
<th>Plant fresh Wt. (g)</th>
<th>Shoot fresh Wt. (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SE)</td>
<td>Mean (SE)</td>
<td>Mean (SE)</td>
<td>Mean (SE)</td>
</tr>
<tr>
<td>Control</td>
<td>25.00 ± 0.57</td>
<td>15.66 ± 0.57</td>
<td>1.90 ± 0.10</td>
<td>1.33 ± 0.0577</td>
</tr>
<tr>
<td>Filtrate of <em>Anabaena flos aquae</em></td>
<td>28.00 ± 0.58</td>
<td>19.00 ± 1.00</td>
<td>2.26 ± 0.057</td>
<td>1.50 ± 1.33</td>
</tr>
<tr>
<td>Filtrate of <em>Anabaena flos aquae</em> and <em>Penicillium citrinum</em></td>
<td>34.33 ± 0.88</td>
<td>23.66 ± 1.53</td>
<td>2.93 ± 0.058</td>
<td>1.96 ± 1.500</td>
</tr>
</tbody>
</table>

According to Duncan (1955) test, means with the same letters are not significantly different.

Adam (1999) reported that when seeds of wheat, sorghum, maize and lentil exposed to either live *Nostoc muscorum* algal extract or killed algal extract, the germination was significantly promoted, and growth parameters and content of nitrogenous compounds increased compared with control.

These increases could be attributed to the nitrogenase, nitrate reductase activities and growth promoting regulators. Mohamed (2001) showed that soaking rice seeds in cyanobacterial filtrates of *Anabaena oryzae*, *Nostoc calciola*, *Microchaete tenera* and *Cylindrospermum muscicola* each alone had increased germination percentage, as well as shoots and roots lengths than those treated with water only. This has been attributed to the growth promoting substances secreted by the cyanobacteria strains in their filtrates. Sudha Rani *et al.* (2008) realized the
importance of algae as biofertilizers in supplementing the use of chemical fertilizers by providing optimal conditions for soil biological activity.

Gibberellins secretion by the plant growth promoting fungi was reported by several researchers (MacMillan, 2002; Kawaide, 2006; Vandenbussche et al., 2007; Khan et al., 2008). They showed the importance of plant growth promoting fungi in plant growth and development, especially under nutrient deficient conditions.

Gibberellins producing Phoma herbarum and Chrysosporium pseudomerdarium have been reported from soybean (Hasan, 2002). Gibberellin secretion by the plant growth promoting fungi was reported by Vandenbussche et al. (2007) and Hamayun et al. (2009), they showed the importance of gibberellin-producing fungi in plant growth and development, especially under nutrient deficient conditions.

The marked increase in growth parameters of the seedlings can be easily attributed to the activity of plant growth promoting secondary metabolites from fungal culture filtrate (Choi et al., 2005; Khan et al., 2008; Hamayun et al., 2009), in addition to the growth promoting substances secreted by cyanobacterial strain (Mohamed, 2001).

The growth bioregulators present in the algal filtrate increased phytohormone levels of the plant over control, according to this the percentage of increase was 12% in plant length and 19.26% in plant fresh weight, but in case of combination of filtrate of the alga and the fungus cultures percentage was 37.32% in plant length and 54.37% in plant fresh weight over control, in this connection there is a general agreement on the induction of the endogenous hormone levels by exogenous application of different growth regulators (El-Shahaby, 1992).

Significant promoted growth of soybean seedlings when the cyanobacterial filtrate of Anabaena flos aquae combined with the fungal culture filtrate of Penicillium citrinum was observed (Figs 1 & 2). The increase in shoot length of seedling was 26.67% and 19.26% in the plant fresh weight compared to control.
The use of biofertilizers is both economical and environment friendly and can be also used as biofertilizer for improvement of growth of many important crops.

REFERENCES:

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التأثير المحفز للـ Anabaena flos aquae

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تمت دراسة تأثير راشج طحلب Anabaena flos aquae وقططاء Penicillium citrinum على نمو Glycine max. وجدت ازدهار نبات الفول الصويا بنسبة 54.37% وزيادة وزن الساق بنسبة 47.49% بالمقارنة بالكонтور.

المحكمون:
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