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Research Article
Improvement of Growth, Physiology and Antioxidant System of *Vicia faba* by Algal Treatments

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Abstract

Background and Objective: chemical fertilizers cause damage to the environment and soil. Algae are used as an alternative to the chemical fertilizer for plant growth because they contain a high percentage of nutrients. In this study, algae used to improve the growth of *Vicia faba*. **Materials and Methods:** The algal treatments *(Oscillatoria* sp., *Chlrococcum* sp. and a mixture of two algae *Oscillatoria* sp. and *Chlrococcum* sp.) have been used in faba bean germination. Algae treatments enhanced the growth of faba bean through the improvement of the growth parameters as dry, fresh weight, carbohydrates, amino acids and protein contents. Carotenoid content was amplified by the algal applications *(Chlorococcum*, *Oscillatoria* and mixture of algae) respectively. **Results:** mixture of algae treatment enhanced antioxidant enzyme activities as catalase, ascorbate peroxidase enzyme of faba bean. Algal treatments improved the membrane stability and reduced of MDA. **Conclusion:** The algal mixture treatment is the hopeful treatment that was caused the most improvement in faba growth. The individual application of *Oscillatoria* sp. comes after the algal mixture for the improvement of faba bean.

Key words: Ascorbate peroxidase, bio-fertilizer, catalase, *Chlorococcum*, *Oscillatoria*, *Vicia faba*

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Competing Interest: The author has declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The chemical fertilizers were required for the development of the soil structure, good growth and yield of crop plants but these chemical fertilizers polluted the environment and cause damaging the agricultural soil[1]. For that, there are many studies used to replace chemical fertilizers with bio-fertilizers to prevent its bad impact. Many microorganisms were used as bio-fertilizers for the growth and production of plant. Bio-fertilizers created by microorganisms are ecofriendly and suitable for protection of agricultural soil[2]. Among these organisms are *Azotobacter*, *Azospirillium*, *Azolla*, cyanophyta, P-solubilizing microorganisms, mycorrhizae and *Sinorhizobium*[3].

Algae contained high ratio of important nutrients (macro and micro nutrients) that can be used as a bio-fertilizer for agriculture[4,5]. Cyanophyta can be used as a good bio-fertilizer because they have ability to improve the plant growth and crop yield[6]. Cyanophyta have also the ability to fix the atmospheric nitrogen by nitrogenase enzyme[7] and it can produce very important compounds including phytohormones that adjust the plant growth. Also, cyanophyta produce some enzymes and antioxidant compounds which detoxify oxidative agents and free radicals which have been formed under stresses. Algae can perform very important processes beside to the nitrogen fixation, such as phosphate solubilizing and producing plant hormones[8]. Also algal applications stimulate root growth and caused a good yield[9,10], the effect of cyanobacteria on crop yield occur as a result of release of various active hormones and some substances as gibberellin, cytokinins, auxin[11,12]. Also algae produced some of amino acids, vitamins and polypeptides that improve the plant growth, in addition to some substances that have antimicrobial properties and polymers[13,14].

The effect of cyanophyta as bio-fertilizer has been studied on many plants as in rice and few wheat, maize and cotton. In another study, algal enhanced of the yield of the rice[15]. Another study reported that, algal treatment amplified the nitrogen content and nutrients (sugar, amino acids and protein) in wheat[16]. In another study found that the inoculation of the soil with (*Tolypothrix tenuis* and *Microchaete tenera*) improved some soil enzyme activities and the growth of maize[17]. Where another study proved that, the cyanophyta species (*Nostoc*) treatment increased the maize yield[18]. According to Nayak *et al.*[19] the mixture of bio-fertilizer (blue green algae and Azolla) and urea (as chemical fertilizer) caused highly increasing in chlorophyll content and rice yield in comparing with the control plant. Moreover, Pereira *et al.*[20] reported that soil application with a mixture of cyanobacteria (*Anabaena iyengarii* var. *tenuis, Nostoc commune, Nostoc* sp. and *Nostoc linckia*) decreased the usage of chemical nitrogen fertilizer about half (50%) and produced the same quality and yield of the rice crop.

However, there are some algal species of the genera (*Anabaena*, *Microcystis*, *Nostoc* and *Oscillatoria*) cause the inhibition of plant growth through their toxic compounds such as microcystins as secondary metabolites[21,22]. And these toxins were accumulated in plant tissues and then carried through the food to reach to human and animal[23].

The main objective of this study, to investigated the influence of *Oscillatoria* sp. and *Chlorococcum* sp. and mixture of these 2 species (*Oscillatoria* sp. and *Chlorococcum* sp.) on the growth, physiology and antioxidant system of crop (*Vicia faba*)".

MATERIALS AND METHODS

The experiment was carried out in the winter of 2018 at the physiological laboratory of Botany and Microbiology department of faculty of Science-Assiut University. Algal species (*Oscillatoria* sp. and *Chlrococcum* sp.) isolated from Elabrhmia canal, Assiut, Egypt and algal was enriched using (BG11) medium and incubated at 30°C under fluorescent light having light intensity of 45 µmol photon m^{-2} sec^{-1}. Algal species were centrifuged and then pellet was washed with sterilized distilled water to remove traces of growth medium. About 20 mL of each algal culture (0.5 OD) were used as treatments for plant germination.

Vicia faba (faba beans) seeds obtained from faculty of Agriculture, Assiut University. Sterilized seeds surface by ethyl alcohol 95% and then washed with distilled water. Petri dishes containing sterile seeds and 20 mL of sterile distilled water as the control (untreated plant). And the treatments were 20 mL of algal culture of *Oscillatoria* sp. and *Chlrococcum* sp. culture individually and mixture of (10 mL of *Oscillatoria* sp. and 10 mL of *Chlrococcum* sp.) for faba beans germination. The experiment was took place under room temperature for 20 days and each treatment was replicated 3 times. Then faba beans were harvested and measured the following parameters:

- Root and shoot lengths of faba beans seedlings were measured as cm
- Dry and fresh weights of (roots and shoot) were measured weight as g/plant
- Photosynthetic pigments Chlorophyll a, b and carotenoids of leave have been estimated as µg g^{-1} plant[24]

- Soluble proteins were determined using alkaline reagent solution as mg g^{-1} FW[25]
- Total free amino acids were determined as mg g^{-1} FW[26]
- Soluble carbohydrates were estimated by the anthrone-sulphuric acid as mg g^{-1} FW[27,28]
- Lipid peroxidation has been estimated by measuring malondialdehyde (MDA) as nmol g^{-1} FW[29]
- Hydrogen peroxide (H_2O_2) content was determined[30]
- Superoxide anion (O^{-}_2) production rate has been determined[31] as µg g^{-1} FW

Enzymatic antioxidants: Plant tissues were ground in liquid N_2 and then homogenized in 5 mL of 100 mM potassium phosphate buffer (pH 7.8) which containing (0.1 mM ethylenediaminetetraacetic acid (EDTA) and 0.1 g polyvinylpyrrolidone). Then mixture was centrifuged for 10 min at 4°C at 18000 rpm. Then the supernatant has been used for enzymes activities determination:

- Catalase (CAT) was assayed as U mg^{-1} protein g^{-1} FW[32] min^{-1}
- Guaiacol peroxidase (POD) was measured as U mg^{-1} protein g^{-1} FW [33] min^{-1}
- Ascorbate peroxidase (APX) activity was estimated as µmol mg^{-1} protein g^{-1} FW[34] min^{-1}

RESULTS

The influence of constant optical density of three treatments (*Chlorococum* sp., *Oscillatoria* sp. and mixture of 2 algae (*Chlorococum* sp. and *Oscillatoria* sp.)) was studied on the growth, physiology and antioxidant system of the dicot plant (*Vicia faba*).

Shoot and root length: This study showed that the shoot length of *Vicia faba* was improved by algal treatments and the highest length was showed at cyanophyta species (*Oscillatoria* sp.) treatment (about 23.5 cm). Shoot length was increased by mixture treatment (*Chlorococum* sp. and *Oscillatoria* sp.) to about 75% more than the shoot length that recorded in the control and other treatments as showing (Fig. 1). Root length was showed different response to algal treatment than in the shoot. It was recorded the highest root length (about 8.1 cm) at chlorophyta species (*Chlorococum* sp.) as showing (Fig. 1). No change was detected in the root length of faba beans at other algal treatments comparing to the control.

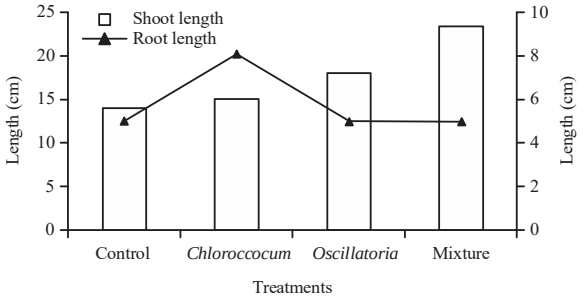

Fig. 1: Shoot and root length of *Vicia faba* under the effect of control, *Chlorococcum*, *Oscillatoria* and mixture of algal species (*Chlorococcum* and *Oscillatoria*) treatments

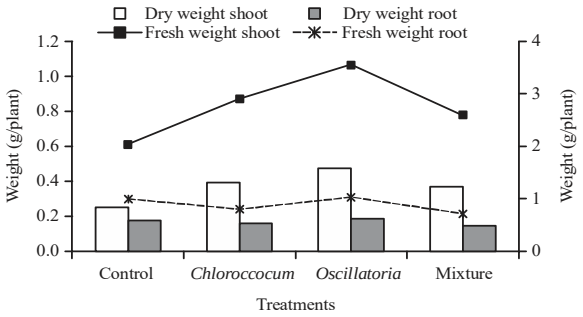

Fig. 2: Fresh and dry weight of *Vicia faba* under the effect of control, *Chlorococcum*, *Oscillatoria* and mixture of algal species (*Chlorococcum* and *Oscillatoria*) treatments

Fresh and dry weight: *Chlorococcum* and *Oscillatoria* improved fresh and dry weight of shoot of faba beans and the highest was noted at *Oscillatoria* treatments. The application of mixture of 2 algal species restricted the shoot development (dry weight and fresh weight) comparing to the control (germinated in water) as (Fig. 2). In chlorophyta species (*Chloroccocum*) and mixture of algal species (*Chlorococcum* and *Oscillatoria*) treatments had almost the same influence on (fresh and dry) weight of shoot of faba beans. While slightly drop was appeared in (fresh and dry) weight of root at all the algal treatments comparing to the control plant (Fig. 2).

Photosynthetic pigments: Slightly develop was carried in the chlorophyll a content at *Oscillatoria* and approximately no alteration in its content by chlorophyta species (*Chlorococum*) treatment. The mixture of 2 algal species was improved the chlorophyll a content (about 2.5 µg g^{-1} plants) comparing to with the control and other treatments (Fig. 3). No changes were detected in chlorophyll b content when adding of any one of the algal species individually comparing with the

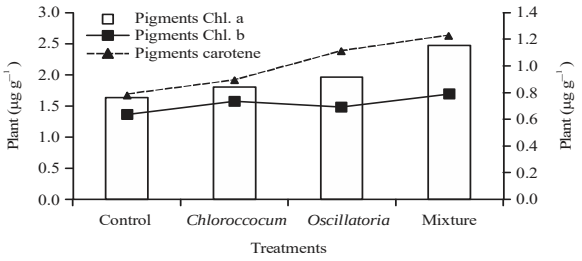

Fig. 3: Chlorophyll a, b and carotenoid content of *Vicia faba* under the effect of control, *Chlorococcum*, *Oscillatoria* and mixture of algal species (*Chlorococcum* and *Oscillatoria*) treatments

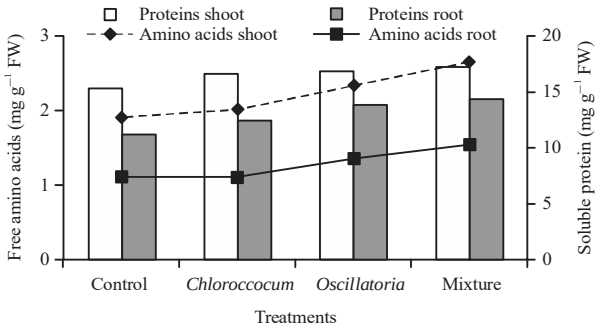

Fig. 4: Protein and amino acids of (shoot and root) of *Vicia faba* under the effect of control, *Chlorococcum*, *Oscillatoria* and mixture of algal species (*Chlorococcum* and *Oscillatoria*) treatments

control (untreated plant) as in (Fig. 3). Slightly improvement of chlorophyll b was observed at the mixture algal treatment (*Chlorococcum* and *Oscillatoria*). Carotenoid content of faba beans was amplified by the algal application in this order (control > *Chlorococcum* > *Oscillatoria*> mixture) as showing in the Fig. 3.

Soluble protein and total free amino acids: Algal treatments have effects on both protein and total amino acid content. Protein content in shoot was enlarged to almost similar level at all algal treatments whatever algal species used to about 17.2 mg g^{-1} FW. Algae treatments increased the protein content in root and the highest content of protein was showed at algal treatment mixture followed by *Oscillatoria* treatment in related to the control plant (Fig. 4). The same response of amino acid content was recorded in both shoot and root as showing in Fig. 4. Amino acid content of shoot was improved by algal mixture (2.65 mg g^{-1} FW), followed by *Oscillatoria* sp., (2.33 mg g^{-1} FW) in related to the control (Fig. 4).

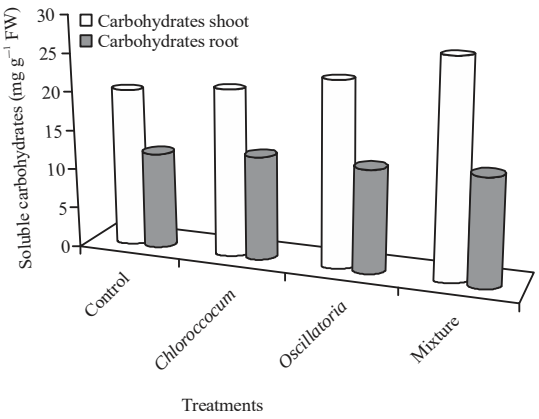

Fig. 5: Carbohydrates of (shoot and root) of *Viciafaba* under the effect of control, *Chlorococcum*, *Oscillatoria* and mixture of algal species (*Chlorococcum* and *Oscillatoria*) treatments

Soluble carbohydrate: Different behavior was appeared in carbohydrate contents in the shoot and root. Shoot carbohydrate contents were affected by the algal application, while no changes in root carbohydrate contents were noted comparing to the control (Fig. 5). *Oscillatoria* sp. treatments amplified of carbohydrate content in the shoot of faba beans (23.54 mg g^{-1} FW) more than an increase by *Chlorococcum* sp. (21.7 mg g^{-1} FW) as shown in (Fig. 5). A mixture of (*Chlorococcum* and *Oscillatoria*) treatment was recorded the highest content of carbohydrate (27.3 mg g^{-1} FW) at a shoot comparing to the control and each of algal species individually.

Catalase enzyme activity: The algal treatments have an effect on antioxidant enzymes in faba bean. Catalase activity of the shoot was affected by the algal treatments more than in the root. Both the algal species (*Chlorococcum* and *Oscillatoria)* almost the same increase in catalase enzyme activity in shoot when they applied individually. When mixing the 2 algae (*Chlorococcum* and *Oscillatoria)*, enhancement of the catalase activity to the highest level (11.8 U mg^{-1} protein g^{-1} FW min^{-1}) comparing to the control and other treatments (Fig. 6). Algal mixture (*Chlorococcum* and *Oscillatoria)* caused slightly development in catalase activity of root comparing to the control. No change in catalase activity of the root under application of each algal species individually.

Lipid peroxidation (MDA): The MDA content was decreased by algal treatments at the shoot and root. The lowest level of MDA was noted at the mixture of (*Chlorococcum* and *Oscillatoria*) treatment comparing to the control (Fig. 7).

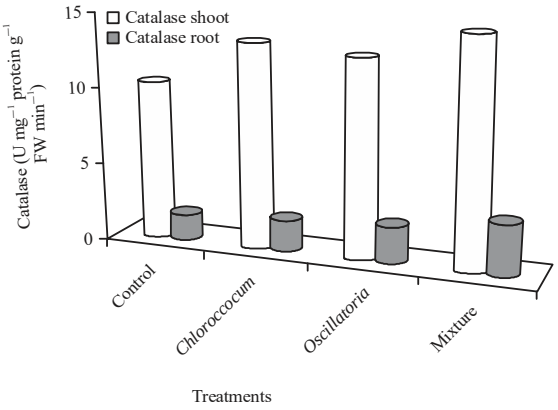

Fig. 6: Catalase enzyme activity of (shoot and root) of *Vicia faba* under the effect of control, *Chlorococcum*, *Oscillatoria* and mixture of algal species (*Chlorococcum* and *Oscillatoria*) treatments

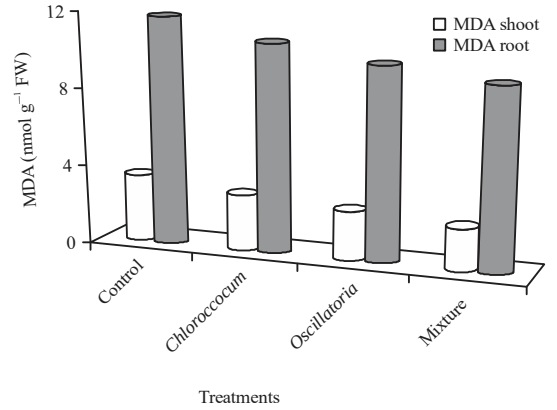

Fig. 7: MDA of (shoot and root) of *Vicia faba* under the effect of control, *Chlorococcum*, *Oscillatoria* and mixture of algal species (*Chlorococcum* and *Oscillatoria*) treatments

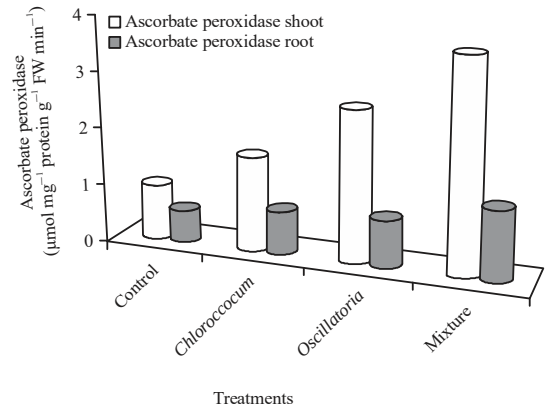

Fig. 8: Ascorbate peroxidase enzyme activity of (shoot and root) of *Vicia faba* under the effect of control, *Chlorococcum*, *Oscillatoria* and mixture of algal species (*Chlorococcum* and *Oscillatoria*) treatments

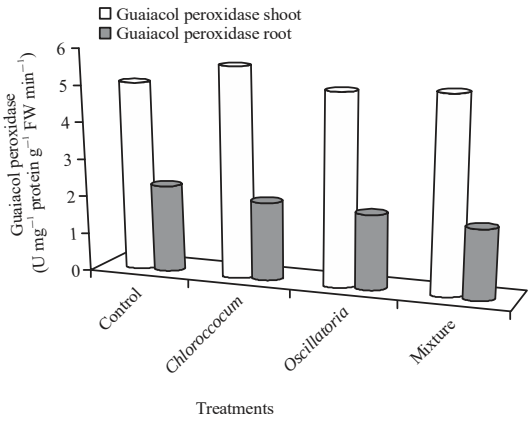

Fig. 9: Guaiacol peroxidase enzyme activity of (shoot and root) of *Vicia faba* under the effect of control, *Chlorococcum*, *Oscillatoria* and mixture of algal species (*Chlorococcum* and *Oscillatoria*) treatments

Ascorbate peroxidase (ASP) activity: Algal mixture had a similar effect on ascorbate peroxidase (ASP) activity in both stem and root of faba bean. That ASP enzyme activity showed the highest activity at mixture treatment in both shoot and roots comparing to the control. Slightly improvement was observed in ASP activity at root under *Oscillatoria* and *Chlorococcum* treatments each one individually. The ASP activity was improved by algal application in shoot in this order (control > *Chlorococcum* > *Oscillatoria*> mixture of algal species) as showing in Fig. 8.

Guaiacol peroxidase enzyme: Guaiacol peroxidase enzyme was slightly affected by addition of algae treatments in the shoot and root. A little improvement of guaiacol peroxidase enzyme activity was caused by *Chlorococcum* treatment in comparing with the control (untreated with algae) in shoot (Fig. 9). Slightly decline of guaiacol peroxidase activity was noted under algae treatments in faba bean root in comparing with the control (Fig. 9).

Total antioxidants: Total antioxidants were slightly affected by algal treatment in shoot. A little increase was present in total antioxidant at algae treatments in the shoot. All algal treatments didn't cause any different in total antioxidant content. The total antioxidant of the root was affected by the algal treatment in this order (control > *Chlorococcum*> *Oscillatoria* >algal mixture) as showing Fig. 10.

DISCUSSIONS

Faba beans is one of the most important crops in Egypt so, the improvement of the crop yield of bean is one of the

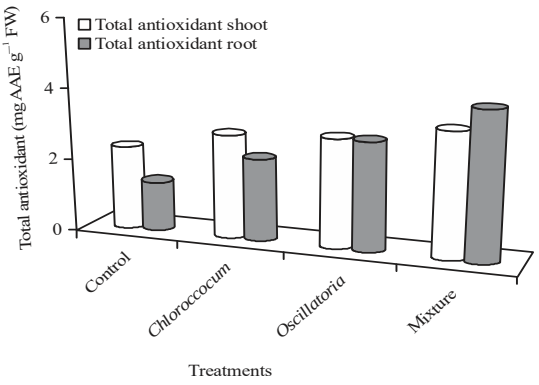

Fig. 10: Total antioxidant of (shoot and root) of *Vicia faba* under the effect of control, *Chlorococcum*, *Oscillatoria* and mixture of algal species (*Chlorococcum* and *Oscillatoria*) treatments

main targets in the researches. In this study, cyanophyta and chlorophyta used as bio-fertilizer which are widely distributed in all the world and they can improve the growth of the some plants, because of their performance as bio-fertilizer to soil in many ecosystems.

According to this study, the shoot length was increased by the mixture of (*Chlorococum* sp. and *Oscillatoria* sp.) in comparing with the control in faba bean. The results are compatible with Shariatmadari *et al.*[35], which recorded the enhancement in the shoot length by the algal treatment plant. According to Shariatmadari *et al.*[35] and Svircev *et al.*[36] the plant growth was enriched by inoculation with cyanobacterium, even without organic nitrogen fertilizer application. Improvement effects of cyanophyta treatment documented, for many crops such as wheat, bean, rice, soybean, tomato, radish, oat, sugarcane, cotton, chili, maize, lettuce and muskmelon[18,22,26,35].

Algal treatments improved fresh and dry weight of shoot of faba beans. *Oscillatoria* treatment has been caused the highest fresh and dry weight. Results of this study are compatible with Wang *et al.*[16] and Adam[37], which found that presoaking of seeds of the different plants in cyanophyta enhanced the plant growth (root depth, shoot length, dry weights). Algal application improved the nutrients in plant growth culture which enhanced the fresh weight and dry weight of faba beans[38-41]. The shoot growth of faba beans improved more than that formed in the root growth by algal treatments. That may be due to growth regulator hormones produced by algal, so plant didn't require more increased in the root system growth[42,43].

Minor improvement was observed in the chlorophyll a and b by the algal treatment of faba beans. The result of this study was well-matched with Naresh *et al.*[44] and Osman *et al.*[45] that found, the treatment with *Oscillatoria angustissima* didn't increase of chlorophyll a, b and carotenoid content, also *Nostoc entophytum* did not cause an increase in the content of chlorophyll a and carotenoids, but the content of chlorophyll b was improved.

Carotenoid content of faba beans recorded the highest level at mixture treatments (*Chlorococcum* and *Oscillatoria*) comparing to the control. This study was well-matched with Naresh *et al.*[44] and Osman *et al.*[45] that, the application of a mixture of 2 cyanobacterial species was amplified the contents of carotenoids.

Carbon metabolites (protein, amino acid and carbohydrates) have been influenced by algal treatments in faba beans. Both protein and total amino acids content have been stimulated by the application of different algal treatments in faba beans. The increased that was noted may be due to the production of growth algal bioregulators and that may be involved in enhancing saccharides and nitrogen metabolism[46].

Carbohydrate contents greatly have been stimulated by application of mixture of (*Chlorococcum* and *Oscillatoria*) followed by *Oscillatoria* and *Chlorococcum*. The amplification of carbohydrates by the mixture treatment (*Chlorococcum* and *Oscillatoria*) were caused due to rising in photosynthetic electron transport rather than enhancement of the photosynthetic pigments in faba beans and that may be because of the slightly development of (chlorophyll a, b and carotenoids) under effect of the algal treatments comparing to the control. Results of this study well-matched with Naresh *et al.*[44] and Bograh *et al.*[47] that found, an increasing in the carbohydrate content of pea has been noted under algal treatments, that may be as a result of the increase in the photosynthetic pigments and also, due to the increasing in the photosynthetic electron transport rate. Carbohydrate content in this study was increased under algal application may be because of the increasing of CO_2 fixation[48].

The antioxidant system has been affected by different algal treatments and they developed the performance of faba bean. Algal treatments enhanced the activity of catalase enzymes of faba beans. This result like-minded with Essa *et al.*[49], which found that, amplifies of catalase enzyme activity through cyanobacteria treatment in compared with untreated plant. The enhancement of catalase activity that recorded at algal mixture (*Chlorococcum* and *Oscillatoria*) in faba bean may be as a consequence of the phytohormones production by algae and that has been developed several enzymes activities. That approved with Osman *et al.*[45] and Kumar *et al.*[50] that found, enhancement in enzymes activities in pea plant under algal treatments.

The MDA content of faba beans was decreased by all algal treatments. That's because, algal treatment developed the growth and oxidative system, that appeared by decline the level of cell damage as a result of growth regulators and phytohormones produce by algae.

The algal mixture improved the activity of the peroxidase ascorbate (ASP) and better than that of algal treatments individually. That means highly positive outcome was carried out by the application of the mixture of algal species on faba beans rather than applied on individually. But guaiacol peroxidase activity has been slightly affected by algal treatments. This result attributed to, the algal release of various biologically active substances such as auxin, cytokinins and gibberellin[10,16]. Also algae produced amino acids, vitamins, polypeptides, antimicrobial substances and polymers[7,13,14]. The important of algal application on plant growth coming from the capacity of initiation of growth promoting substances phytohormones such as auxins, vitamin B12, amino acids, some sugars and folic, pantothenic and nicotinic acids according to Misra and Kaushik[51,52]. The enhancement in the growth, oxidative system and enzymes activities of faba beans under treated with *Oscillatoria* and *Chlorococcum* algal species are compatible with Misra and Kaushik[51,52].

From the previous results and discussions it can be concluded that, the mixture of *Oscillatoria* and *Chlorococcum* can be used as bio-fertilizer for faba bean. So, some algal species can be recommended as bio-fertilizer for growth of some plants.

CONCLUSION

The application of a mixture of algal species (*Oscillatoria* and *Chlorococcum*) improved some growth parameters (fresh, dry weight, protein, amino acid and carbohydrates) of the plant. The algal mixture also improved the oxidative system and this was demonstrated by improving catalase enzyme, ascorbate peroxidase enzyme activities and total antioxidant of faba bean and that is better than each algal treatment individually. Faba bean growth has been improved by application of *Oscillatoria* more than *Chlorococcum* application.

SIGNIFICANCE STATEMENT

This study discovered the use of some alga species as bio-fertilizers as an alternative of chemical fertilizers, because alga species improved the growth of some plants. Such improvements may be reducing the use of chemical fertilizers which lead to the reduction of pollution and health hazard effects on environment.

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