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Full Length Article

Effect of *Chlorella vulgaris* as Bio-fertilizer on Growth Parameters and Metabolic Aspects of Lettuce Plant

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ABSTRACT

Seeds of *Lactuca sativa* were germinated in culture medium containing microalga (*Chlorella vulgaris*) and grown for 3, 6, 9, 12 and 15 days in order to study its effect on growth parameters and some physiological response of seed germination and growth. In general, microalgal treatment significantly increased the growth with a significant decrease in soluble carbohydrate, soluble protein and total free amino acids compared with those of the control (sterilized culture medium) of seed germination. Addition of microalga to the culture medium or soil significantly increased fresh and dry weight of seedlings as well as pigments content. The best treatments were 2 and 3 g dry alga kg^{-1} soil.

Key Words: *Lactuca sativa*; *Chlorella vulgaris*; Carbohydrates; Amino acids; Proteins

INTRODUCTION

In Egypt, soil fertility is diminishing gradually due to soil erosions, loss of nutrient, accumulation of salts and other toxic elements, water logging and un-balanced nutrient compensation. Organic wastes and bio-fertilizers are the alternate sources to meet the nutrient requirement of crops and to bridge the future gaps. Farming regions that emphasizing heavy chemical application led to adverse environmental, agricultural and health consequences. Many efforts are being exercised to combat the adverse consequences of chemical farming.

The bio-fertilizer, organic manuring and bio-control of agricultural have emerged as a promising component of integrating nutrient supply system in agriculture. Microbiological fertilizers are important to environment friendly stainable agricultural practices (Bloemberg *et al.*, 2000). Bio-fertilizers include mainly the nitrogen fixing, phosphate solubilizing and plant growth- promoting microorganisms (Goel *et al.*, 1999). Among bio-fertilizers benefiting the crop production are Azotobacter, Azospirillium, blue green algae, Azolla, P-solubilizing micro organisms, mycorrhizae and Sinorhizobium (Hegde *et al.*, 1999). Green manures were also found to stimulate root growth and produce good yields (Boussiba, 1987; Mandimba *et al*., 1998). Dry green algae contain high percentage of macronutrients, considerable amount of micronutrients and amino acids (El Fouly *et al.*, 1992; Mahmoud, 2001). They can be conveniently produced on sewage and brackish water and partially substituted the chemical fertilizers to avoid environmental pollution.

Objectives of this work were to study the effect of fresh and dry green microalgae (*Chlorella vulgaris*) as soil additive on growth and some physiological responses of lettuce seedlings and to determine the importance of bio-fertilizer application in order to improve the yield quality and productivity and avoid environmental pollution.

MATERIALS AND METHODS

Plant material. The experimental plant used in this study was (*lactuca sativa* cv.) lettuce. The seeds were obtained from the Agricultural Research Center (ARC), Cairo, Egypt.
Algal culture. Microalga (*Chlorella vulgaris* Beijerinck) was isolated from River Nile Sohag, Egypt and enriched using algae nutritive medium specific for chlorophyta (Stein, 1966). The final pH of this medium was 6.8, after being autoclaved. The nutritive media were inoculated, with algal suspension from agar slants. The algal cells were grown at a temperature of 25 ± 1^0C and surface light of about 2500 lux (measured by Gossen Luxmeter). Filtered dry air was let to bubble in the culture vessels to provide carbon dioxide and to prevent settling of cells.
Effect of culture media after growth of green microalga on seed germination of lettuce. In this experiments, culture media of green microalga after growth of alga for 3, 6, 9, 12 and 15 days were harvested for assay and analytical procedures.
Determination of cell number and pigments content of alga. Cell number was determined by taking 0.1 mm deep having Improved Naubauer Hemocytometer ruling (A.O. Spencer "Bright Line"). Data were given as cell per mL. The pigments were determined spectrocoloimetertcally using the method recommended by Metzner *et al.* (1965).

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The data were given as µg/mL algal suspension.

Treatment of lettuce seeds. Seeds were surface sterilized with 5% sodium hypochlorite for 8 min, then rinsed with distilled water several times before germination. The seeds were classified into two groups. The first group of 50 seeds were floated in each petridish containing 10 mL of sterilized culture medium of *Chlorella vulgaris*. The second group of 50 seeds were floated in each petridish containing 10 mL of supernatant after centrifuged culture medium and after grown the alga for 3, 6, 9, 12 and 15 days. Petridishes were maintained in green house under a 16 h photoperiod at 20-25^{0}C for one week. At the end of the experiment total fresh weight, length of shoots and roots per plant were determined. The seedlings were washed twice with distilled water, oven-dried at 75^{0}C for 72 h to obtain the dry weight and then ground to a fine powder. Three replicates were used.

***Chlorella vulgaris* as bio-fertilizers for lettuce seedlings growth.** The algal culture were grown in two big container (5 L) for 7 days. After this period the algal suspension of the first container was centrifuged and the clear growth medium was decanted. The algal cells were precipitated. 1/2, 1, 2 and 3 g of fresh algal cells were added to the pots containing 1 kg soil before sowing. The algal suspension in the second container was centrifuged and the algal cells were dried over night in an oven at 105^{0}C. 1/2, 1, 2 and 3 g dry algal cells were added to another groups of pots containing 1 kg soil before sowing and then 20 seeds were planted in each pot and placed in greenhouse under a 16 h photoperiod at 20-25^{0}C. After 30 days of sowing, samples of each treatment were taken for studying the metabolic analysis.

Metabolic samples analysis. The photosynthetic pigments (chlorophyll a, b & carotenoids) were determined using method of Metzner *et al.* (1965). The dry matter yield was used (in replicate) for chemical analysis for the determination of water-soluble sugars, a known weight of the powdered tissue was hydrolyzed in distilled water for 2 h in a boiling water bath. After cooling, the hydrolysis was filtered and filtrate was made up to a known volume, after which the water-soluble sugars were determined by the anthrone sulphuric acid method (Fales, 1951). Free amino acids were extracted from the plant tissues and determined according to the method of Moore and Stein (1948).

To estimate the soluble proteins, powdered tissue samples were boiled in distilled water for 2 h. After cooling the water extract was centrifuged and supernatant was decanted and made up to known volume with distilled water. The water insoluble protein residue was treated with NaOH. The plant protein content was determined according to Lowry *et al.* (1951), using bovine serum albumin as standard.

Statistic analysis. The measurements of growth parameters and metabolic aspects were subjected to one-way analysis of variance (ANOVA) to test difference among means corresponding to alga levels via STATISTICA computer software distributed by Stat Soft. Inc.

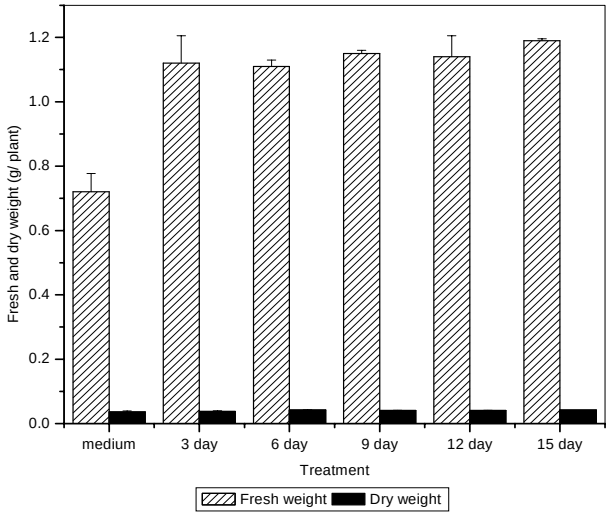
RESULTS AND DISCUSSION

Growth parameters (cell number & pigment contents) of alga. The growth (cell number & pigments) of alga after 15 days, was followed. A gradual increase in cell number was recorded after 3, 6, 9, 12 and 15 days (Table Ia). The pigment (chlorophyll a, chlorophyll b & carotenoids) and consequently the total pigments were generally increased after 3, 6, 9, 12 and 15 days (Table Ib). This increase was highly significant ($p<0.0001$). Also, the pigment fraction (chl. a/chl. b) was significantly increased ($p<0.05$).

Seed germination and seedling growth of lettuce plants. The results of growth parameters obtained for the germination of lettuce seeds subjected to culture media after growth of microalga for 3, 6, 9, 12 and 15 days treatments

Fig. 1. Effect of culture medium containing *Chlorella vulgaris* grown for 3, 6, 9, 12 and 15 days on growth parameters (a: fresh and dry weight, b: shoot and root length) of seed germination of lettuce plants

(a)



(b)

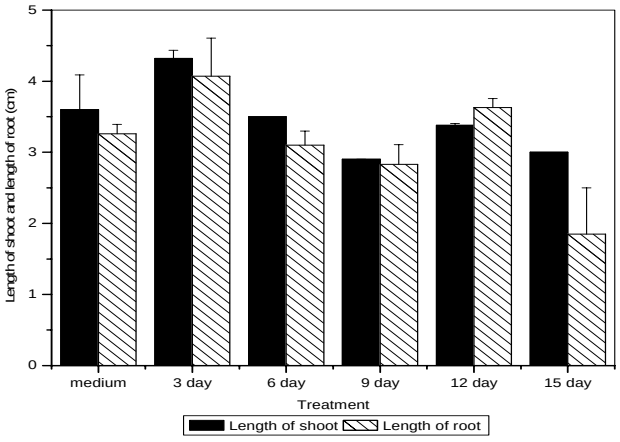


Table I. Growth parameters (a) cell number 10^4 mm^3/mL algal suspension and (b) pigments content µg/mL) of *Chlorella vulgaris* for 3, 6, 9, 12 and 15 days under control laboratory conditions (Mean ± SD, n=3)

(a)

Treatment (day)	Cell number
0 time	4
3	6
6	15
9	20
12	43
15	66

(b)

Treatment (day)	Chl.a	Chl. b	Carot	Total	Chl.a/Chl.b
0 Time	2.472±0.084	1.159± 0.137	0.995±0.001	4.627±0.227	2.149±0.192
3	6.023±0.068	2.667±0.438	2.244±0.109	10.934 ±0.417	2.301±0.532
6	8.664±0.116	3.685±0.265	3.633±0.173	15.982 ±0.341	2.363±0.207
9	12.239±0.032	5.711±0.208	5.072±0.105	23.023±0.226	2.146±0.193
12	14.088±0.171	6.815±0.114	6.290±0.214	27.193±0.437	2.067±0.019
15	22.633±0.167	10.326±0.529	10.544±0.113	43.503± 0.431	3.661±1.291

Table II. Effect of culture medium containing *Chlorella vulgaris* grown for 3, 6, 9, 12 and 15 days on pigments content (mg/g f. wt.) of seed germination of lettuce plants (Mean ± SD, n=3)

Treatment	Day	Chl a	Chl b	Carot	Total	Chl. a/Chl. b
Sterilized culture medium	0 time	0.202±0.002	0.089±0.001	0.073±0.007	0.372±0.017	2.389±0.172
Culture medium after growth alga	3	0.202±0.002	0.089±0.001	0.071± 0.001	0.362±0.002	2.280±0.007
	6	0.230±0.004	0.094±0.004	0.088±0.008	0.412±0.012	2.440±0.040
	9	0.268±0.008	0.121±0.001	0.092±0.004	0.481±0.001	2.209±0.009
	12	0.371±0.001	0.089±0.009	0.109±0.002	0.568±0.009	4.216±0.008
	15	0.270±0.005	0.125±0.000	0.094±0.003	0.489±0.003	2.168±0.002

Table III. Mean values of pigment contents (mg/g fresh weight) in seedling of lettuce plants as affected by different levels of fresh and dry alga as soil additives (Mean ± SD, n=3)

Treatment		Chl.a	Chl.b	Carot	Total	Chl.a/Chl.b
Control		0.673±0.091	0.236±0.068	0.197±0.112	1.105± 0.047	2.992±0.472
Fresh alga	1/2 g/kg soil	0.597±0.121	0.258±0.056	0.211±0.020	1.066±0.197	2.320±0.039
	1	0.823±0.036	0.345±0.024	0.271±0.011	1.438±0.061	2.390±0.058
	2	0.662±0.035	0.264±0.006	0.130±0.013	1.056±0.043	2.516±0.182
	3	0.551±0.056	0.303±0.107	0.098±0.011	0.951±0.062	1.818±0.084
Dry alga	1/2 g/kg soil	0.602± 0.064	0.306±0.067	0.096±0.002	1.003±0.129	2.017±0.229
	1	0.570±0.054	0.234±0.051	0.114±0.004	0.918±0.008	2.610±0.794
	2	0.574±0.029	0.262±0.051	0.117±0.001	0.952± 0.021	2.336±0.581
	3	0.577±0.003	0.289±0.050	0.120±0.001	0.986±0.049	2.062±0.368

are given in Table II, Fig. 1a and b. Fresh weight and chlorophyll contents were more increased ($p<0.0001$) after 3, 6, 9, 12 and 15 days, compared with those of control, while dry weight, shoot and root length indicated no pronounced increase except at 3 day.

In case of seedling growth of lettuce plants, fertilization treatment by fresh and dry green microalga increased the values of growth parameters and pigments content (Table III & Fig. 3) as compared with those of un-fertilized plants. Fresh and dry weights (Fig. 3) were found to be high significantly increased ($p<0.0001$) with the addition of increased fresh and dry alga levels. Most effective treatments were 2, 3 g dry alga kg^{-1} soil than fresh alga. Increase in fresh and dry weight resulted from the improved nutrient status of plant due to the presence of alga in the soil. Similar trends were found by Al-Gosaibi (1994) and Shaaban and Mobarak (2000). The stimulatory effects of alga as bio-fertilizer on some growth parameters of lettuce are in accordance with the results obtained by Rani and Sathiamoorthy (1997). Mahmoud and Amara (2000) who found that all treatments significantly increased plant growth parameters compared with un-treated plant. In addition, similar trend was observed by Mekki *et al.* (1999) and Galal *et al.* (2000). Such increases might reduce chemical fertilizers and consequently reduce pollution and health hazard as reported for pearl millet Verma (1996). Furthermore, enhancement in the growth parameter attributes leads to improved crop productivity (Ghosh & Mohiuddin, 2000). These results are in agreement with those obtained by Mehta *et al.* (1995), Snehal *et al*. (1998), Mahmoud and Amara (2000) and Das *et al*. (2001).

Carbohydrates content in seed germination and seedling growth of lettuce plants. The seed germination of lettuce plants germinate under culture media after growth of green

Fig. 2. Effect of culture medium containing *Chlorella vulgaris* grown for 3, 6, 9, 12 and 15 days on soluble carbohydrate, soluble protein and total free amino acids (mg/g dry weight) of seed germination of lettuce plants

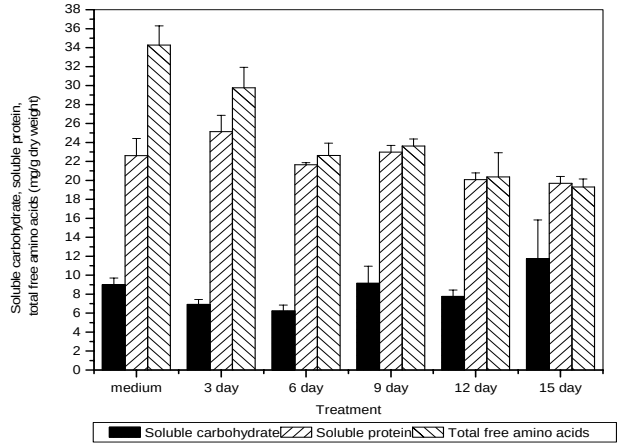


Fig. 3. Mean values of fresh and dry weight (g/plant) in seedling of lettuce plants as affected by different levels of fresh and dry alga as soil additives

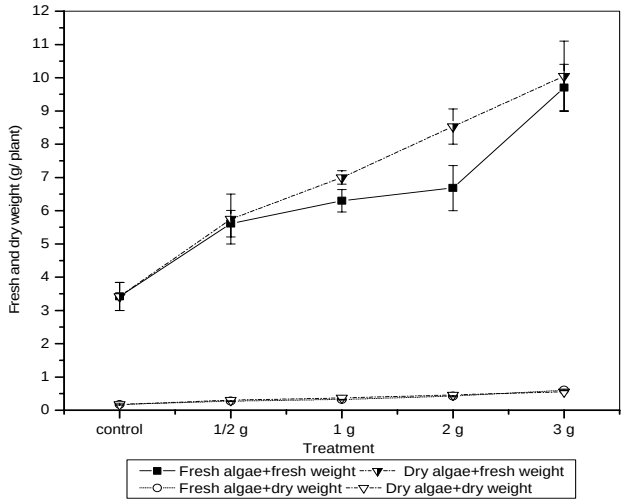


Fig. 4. Mean values of soluble carbohydrate (mg/g dry weight) in seedling of lettuce plants as affected by different levels of fresh and dry alga as soil additives

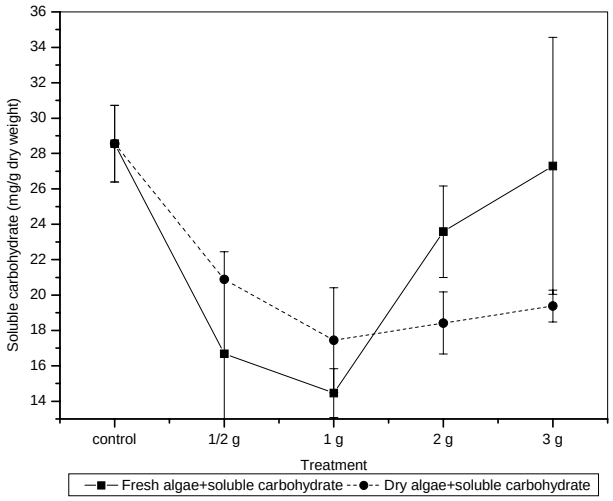
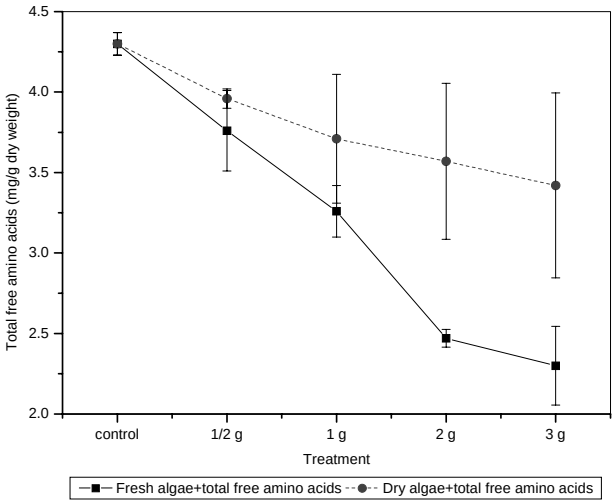


Fig. 5. Mean values of total free amino acids (mg/g dry weight) in seedling of lettuce plants as affected by different levels of fresh and dry alga as soil additives

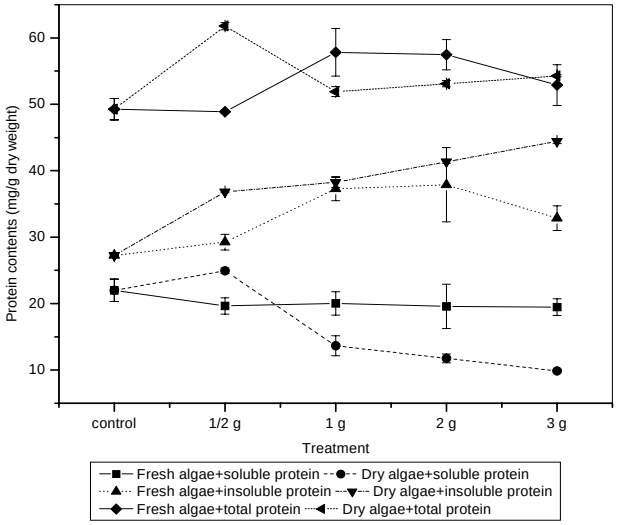


microalga for 3, 6, 9, 12 and 15 days, significantly ($p<0.05$) decreased the soluble carbohydrate compared with those of control (sterilized culture medium) seeds germination (Fig. 2). The results of soluble carbohydrate in lettuce seedling grown under different fertilizers (1/2, 1, 2 & 3 g kg^{-1} soil) levels are given in Fig. 4. The soluble carbohydrates were significantly ($p<0.01$) lower in fertilized compared with unfertilized plants. This reduction was more pronounced in dry alga ($p<0.001$) than in fresh alga ($p<0.01$) especially at levels 2 and 3 g kg^{-1} soil. These results are in accordance with those obtained by Goel *et al*. (1999) who reported that the inoculation with certain plant growth-promoting rhizobacteria may enhance crop production either by making the other nutrients available or protecting plant from pathogenic micro organism (allelopathic effects). Zodape (2001) also concluded that, the increase yield productivity with bio-fertilizer application is due to microelement and plant growth regulator contained in the fertilizer.

Amino acids and proteins content in seed germination and seedling growth of lettuce. Little investigations were carried out to study in case of seeds germination. The addition of microalga after growth of on the total free amino acids, protein contents revealed that total free amino acids and soluble proteins (Fig. 2) were significantly ($p<0.001$) increased on germinated seeds in control compared with those of seeds treated with *Chlorella vulgaris* for 3, 6, 9, 12 and 15 days.

Total free amino acids of fertilized lettuce plants with dry alga were significantly ($p<0.0001$) lower than those of the un-fertilized control plants (Fig. 5). A significant

Fig. 6. Mean values of proteins content (mg/g dry weight) in seedling of lettuce plants as affected by different levels of fresh and dry alga as soil additives



difference between dry ($p<0.0001$) and fresh alga ($p<0.01$) treatments and control was detected in case of soluble, insoluble and total proteins content. In fertilized plants (Fig. 6), the contents of insoluble protein as well as total protein exhibited generally a significant increase than those of the control plants, while the contents of soluble protein showed a significant decrease than those of control plants. These results are in accordance with those obtained by Tiwana *et al*. (1992), Sharma and Namdeo (1999). Similar trend was found by Adam (1999) who showed that the improvement of the growth and nitrogen contents in response of application of cyanobacteria as bio-fertilizers on seed and related processes of wheat, sorghum, maize and lentil could be attributed to the nitrogen as well as nitrate reductase activities.

CONCLUSION

It can be concluded that dry microalgae as soil additives improve plant nutrient, which in turn enhances all the physiological reactions that lead to a good growth. A quantity of 2 and 3 g dry algae per kg soil can improve soil fertility, plant nutrient required for obtaining good yields and leads to less environmental pollution.

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