Improvement of Growth Parameters of *Zea mays* and Properties of Soil Inoculated with Two *Chlorella* Species

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Abstract: The effects of two green microalgal strains, *Chlorella oocystoides* and *Chlorella minutissima*, on some growth parameters of *Zea mays* and on some soil physico-chemical properties were studied. The growth mass, chlorophyll content, phosphorous content, and potassium content of maize plants were significantly increased. The increase of other parameters such as plant length, number of leaves, and total nitrogen was not statistically significant. Concerning the studied soil physicochemical properties, the soil total nitrogen significantly increased by inoculation with algal strains. Also the soil organic matter significantly increased with all the used concentrations of the two algae. The pH significantly decreased with 1%, 2.5%, and 5% of *C. oocystoides*, while there was no significant change with the second alga.

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1. Introduction

Successive culturing of soil leads to decreasing of soil fertility due to many factors including, soil erosion, loss of nutrients, accumulation of salts and other toxic elements, water logging, and un-balanced nutrient compensation (Faheed & Abd-el Fattah, 2008). Decreasing soil fertility leads to decreasing of its productivity. The rapid increasing of world population and the continuous increasing of the world food demand make it necessary to find safe solutions for increasing soil fertility and productivity. The conventional practice to solve this problem is to use chemical fertilizers, however it is not safe. Chemical fertilizers are chemical substances composed of known quantities of nitrogen, phosphorus, and potassium. Using of these fertilizers spoil soil and causes air and ground water pollution (Kennedy & Tchan, 1992; Mytton, 1993; and Youssef & Eisa 2014). Finding safe and environment friendly fertilizers is one of the urgent issues among researchers worldwide.

Biofertilizer can be any substance that contains living microorganisms, which can colonize rhizosphere or the interior of the plants and promote growth of plants by improving their nutrient status (Vessey, 2003). A variety of organisms such as plant growth promoting rhizobacteria (Bloemberg & Lugtenberg, 2001) which include symbiotic nitrogen fixing bacteria (Noel *et al*., 1996), free nitrogen fixing bacteria (Kandil *et al*., 2011), and phosphorus solubilizing bacteria (Kucey *et al*., 1989), in addition to cyanobacteria (Song *et al*., 2005), green Algae (Faheed & Abd-el Fattah, 2008), and seaweeds (Bokil *et al*., 1972), have high potential to be used as biofertilizers.

Algae are photoautotrophic organisms that can produce unlimited mass utilizing light energy and CO_2. They can fix CO_2 to produce oxygen, organic matter, and extracellular metabolites in their vicinity. Microalgae are employed in agriculture systems as biofertilizers and soil conditioners especially the nitrogen-fixing cyanobacteria (blue-green algae) which have an important role in maintenance and increasing soil fertility of rice fields (Song *et al*., 2005). It is well known that freshwater algae, such as *Chlorella vulgaris*, contain high amounts of macro and micronutrients, as constituents or metabolites, like carbohydrates and proteins (Wake *et al*., 1992). Also some bacteria present in the rhizosphere can be entrapped along with *Chlorella* to improve soil fertility (Raposo *et al*., 2011). Recently, a consortium containing *Anabaena variabilis*, *Chlorella vulgaris*, and *Azotobacter* sp. was found to improve germination and growth of rice plants and it is recommended as a biostimulator and a biofertilizer for crops (Zayadan *et al*., 2014).

In this work we studied the effect of two *Chlorella* spp. on some growth parameters of *Zea mays* plant and on some physico-chemical properties of soil.

2. Materials and Methods

2.1. Algal material

Two algal strains named *C. oocystoides* and *C. minutissima* were provided from Algae and Plant Physiology Laboratory, Faculty of Science, Al-Azhar

University, Assiut, Egypt. The medium BG11 (Stanier *et al*., 1971) was used for culturing of algae using 1000 ml conical flasks containing 600 ml of BG11 medium supplied by continuous air bubbling under continuous illumination about 3500 lux for 7 days.

2.2. Preparation of algal material

After harvesting, algal material was separated by centrifugation at 5000 rpm for ten minutes, and then washed two times with distilled water. The washed algal pellet of 0.5 g was resuspended in 1000 ml sterilized distilled water to be used for inoculation of pots.

2.3. Pot experiment

A pot experiment was conducted to evaluate the influence of two algal strains on growth parameters of maize plant and on some soil chemical properties i.e. EC (Electric Conductivity), pH, O.M. (Organic Matter), available-N in summer season of 2014 at the Agricultural Experimental Farm of Soils and Water Sci. Dept., Faculty of Agric., Al-Azhar Univ., Assiut, Egypt. Some physical and chemical properties of the experimental soil sample, collected before planting of maize plants, are presented in Table (1).

The experiment was carried out in a randomized complete block design with three replicates. Different concentrations of each algae were used; 1- Without algae (Control 0%), 2- 0.1%, 3- 1%, 4- 2.5%, and 5- 5%. Black plastic pots (15 cm in diameter and 20 cm height) were filled with 5 Kg of soil sample. Five grains of maize were planted in each pot which was later thinned to 2 stands per pot and the algal materials were applied into pots with irrigation water at three equal doses each 10 days.

2.3. Determination of growth parameters of maize plant

2.3.1. Chlorophyll

Chlorophyll *a*, *b* and total chlorophyll were determined in leaf samples after 35 days of planting in mg/gm fresh weight according to the method described by Wettestein (1957).

2.3.2. Length, fresh, and dry weights

The plants were harvested at 45-days age by cutting the plants just 2 cm above the soil surface. The length of the plants, their fresh and dry weights, and their leaf number were determined. The harvested plants were dried at 70°C for 72 h till constant dry weight and the dry weight per pot was recorded.

2.4. Plant chemical analysis

The dried plants were grinded using stainless steel mill and kept for chemical analysis. Plant chemical analysis was determined according to Page *et al.* (1982).

2.5. Soil chemical analysis

Soil analysis was determined according to method described by (Jackson, 1973).

2.6. Statistical analysis

The obtained data were statistically analyzed using the analysis of variance method according to Snedecor & Cochran (1990). Duncan's Multiple Range Test (DMRT) at the 5% level of probability was used to compare means of treatments.

3. Results

3.1. Effect of algal inoculation on plant growth parameters

In this experiment we measured some growth parameters to study the effect of two *Chlorella* species, named *C. oocystoides* and *C. minutissima*, on maize plant. The measured growth parameters were plant length, number of leaves, fresh and dry weights, root length, Chlorophyll *a*, chlorophyll *b*, total chlorophyll, phosphorus, potassium, and total nitrogen. The statistically analyzed data are presented in Tables (2, 3, 4, and 5). Some of these parameters significantly increased with algal treatments, while some others were not affected. The fresh weight increased significantly when treated with 1% and 2.5% (v/w algae to soil) of *C. oocystoides* with maximum increase of 44.3% at the concentration of 2.5% of this alga (Table 2).

Also the fresh weight significantly increased at 1%, 2.5% and 5% (v/w algae to soil) of *C. minutissima* with maximum increase of 50% at the concentration of 2.5% of alga (Table 2). The dry weight significantly increased with algal treatments at ratios of 1%, 2.5% and 5% of both *C. oocystoides* and *C. minutissima.* The maximum increases were 65% at 2.5% of *C. oocystoides* and 71.3% at 2.5% of *C. minutissima* (Table 3).

Chlorophyll *a* content of maize plant also significantly increased with treatment of *C. oocystoides* at ratios of 1%, 2.5%, and 5% while the increase was significantly only at ratio of 2.5% of *C. minutissima*. Total chlorophyll increased significantly with treatment of *C. oocystoides* at ratios of 1%, 2.5% and 5% while the increase was significant only at ratio of 2.5% of *C. minutissima*.

The plant phosphorus content increased significantly with the treatment of *C. oocystoides* at ratios of 1%, 2.5% and 5%, while the significant increase was recorded only at the concentration of 2.5% of the second algae. The potassium content of maize plant increased significantly with the treatment of both of the two algae at ratios of 1%, 2.5% and 5% (Table 5). The increase of the other parameters such as plant length, number of leaves, chlorophyll *b* and total nitrogen was not statistically significant.

Table 1. Some physical and chemical properties of the soil before culturing

Parameter		Value	Parameter	Value
Particle size dist.	Sand (%)	77.2	Cations (cmol /kg soil)	
	Silt (%)	13.4	Ca^{++}	37.53
	Clay (%)	9.4	Mg^{++}	16.58
Texture grade		Loamy sand	Na^{+}	54.04
Bulk density (g/cm^3)		1.62	K^{+}	8.59
Saturation percent (%)		21.50	Anions (cmol /kg soil)	
Field Capacity (%)		10.75	CO_3^{--}	---
pH soil past		7.07	HCO_3^{-}	9.64
E.Ce (dSm^{-1}) soil past		1.422	Cl^{-}	96.5
C.E.C ($cmol_c\ kg^{-1}$)		2.01	SO_4^{--}	11.65
N-Available (ppm)		21	Total-N (%)	0.12
O.M (%)		0.21	Total $CaCO_3$ **(%)**	8.47

*Each value in this table is the mean of 3 replicates

Table 2. Impact of algal inoculation on length, leaves number, and fresh weight/plant of maize plant

Treatments	Length (cm)		No. leaves /plant		Fresh weight (gm)	
	C. oocystoides	*C. minutissima*	*C. oocystoides*	*C. minutissima*	*C. oocystoides*	*C. minutissima*
Without algae	36.5 b	37.7 ab	6.0 a	5.8 a	9.10 c	9.22 c
0.1% algae	37.5 ab	38.0 ab	6.1 a	6.0 a	9.70 c	11.38 b
1% algae	39.8 ab	43.7 ab	6.5 a	6.2 a	12.73 ab	13.33 a
2.5% algae	41.0 ab	45.0 b	6.7 a	6.5 a	13.13 a	13.85 a
5% algae	40.0 ab	40.7 ab	6.5 a	6.0 a	9.10 c	13.62 a

Mean values in the same column followed by the same letters are not significantly different according to Duncan`s multiple range test ($P < 0.05$)

Table 3. Impact of algal inoculation on dry weight/plant, length of root, and chlorophyll *a* of maize plants

Treatments	Dry weight (gm)		Length of root (cm)		Chlorophyll a (mg/g)	
	C. oocystoides	*C. minutissima*	*C. oocystoides*	*C. minutissima*	*C. oocystoides*	*C. minutissima*
Without algae	0.98 d	1.11 bcd	11.33 c	11.67 bc	3.323 c	4.493 bc
0.1% algae	1.03 cd	1.36 ab	11.83 bc	12.50 abc	3.711 c	5.762 ab
1% algae	1.33 abc	1.45 a	12.50 abc	13.00 abc	5.798 ab	6.178 ab
2.5% algae	1.50 a	1.55 a	13.83 ab	14.17 a	6.084 ab	6.792 a
5% algae	1.49 a	1.50 a	12.67 abc	13.17 abc	5.896 ab	6.396 ab

Mean values in the same column followed by the same letters are not significantly different according to Duncan`s multiple range test ($P < 0.05$)

Table 4. Impact of algal inoculation on chlorophyll *b*, Total chlorophyll, and nitrogen contents of maize plants

Treatments	Chlorophyll *b* (mg/g)		Total chlorophyll (mg/g)		N (%)	
	C. oocystoides	*C. minutissima*	*C. oocystoides*	*C. minutissima*	*C. oocystoides*	*C. minutissima*
Without algae	2.704 a	2.434 a	6.026 c	6.925 bc	2.85 ab	3.02 a
0.1% algae	2.946 a	2.908 a	7.322 abc	8.668 ab	3.16 a	3.55 a
1% algae	3.418 a	2.989 a	8.880 ab	9.164 ab	3.13 a	3.20 a
2.5% algae	3.429 a	3.264 a	9.178 ab	9.720 a	3.30 a	3.57 a
5% algae	3.284 a	2.997 a	9.037 ab	9.389 a	2.82 ab	3.18 a

Mean values in the same column followed by the same letters are not significantly different according to Duncan`s multiple range test ($P < 0.05$)

Table 5. Impact of algal inoculation on P and K concentrations of maize plants

Treatments	P (ppm)		K (ppm)	
	C. oocystoides	*C. minutissima*	*C. oocystoides*	*C. minutissima*
Without algae	0.109 c	0.124 bc	35.49 d	37.14 d
0.1% algae	0.124 bc	0.136 ab	41.41 cd	41.50 cd
1% algae	0.137 ab	0.142 ab	47.50 bc	48.07 bc
2.5% algae	0.139 ab	0.145 a	54.14 ab	61.66 a
5% algae	0.128 ab	0.139 ab	47.97 bc	50.27 bc

Mean values in the same column followed by the same letters are not significantly different according to Duncan`s multiple range test ($P < 0.05$)

3.2. Effect of algal inoculation on some soil properties

The statistically analyzed data concerning the effect of algal inoculation on soil properties are presented in Tables (6 & 7). Some soil properties such as total nitrogen content, electric conductivity, organic matter, and pH were studied. Inoculation of the soil with *C. oocystoides* at ratios of 1%, 2.5% and 5% significantly increased the soil nitrogen content. The increasing of nitrogen was also significant with inoculation with *C. minutissima* at all studied ratios (Table 6). The electric conductivity increased significantly with inoculation with 1%, 2.5% and 5% of the algae *C. oocystoides*, while decreased with all treatments of *C. minutissima* and the decrease was significant with 5%.

The soil organic matter increased significantly with all treatments of *C. oocystoides*, while the increment was significant with inoculation of *C. minutissima* at ratios of 1%, 2.5%, and 5%. The pH decreased significantly with 1%, 2.5%, and 5% of *C. oocystoides*, while there was no significant change with the second algae.

Table 6. Impact of algal inoculation on available nitrogen and organic matter content of soil

Treatments	N (ppm)		O.M. (%)	
	C. oocystoides	*C. minutissima*	*C. oocystoides*	*C. minutissima*
Without algae	110.33 ef	90.73 f	0.190 cd	0.155 d
0.1% algae	134.73 de	126.53 e	0.312 ab	0.209 cd
1% algae	159.13 cd	165.13 cd	0.330 ab	0.262 bc
2.5% algae	209.20 ab	176.60 c	0.345 ab	0.321 ab
5% algae	227.93 a	187.40 bc	0.379 a	0.392 a

Mean values in the same column followed by the same letters are not significantly different according to Duncan`s multiple range test ($P < 0.05$)

Table 7. Impact of algal inoculation on Electric Conductivity (EC) and pH of soil

Treatments	EC (1:2.5)		pH susp. (1:2.5)	
	C. oocystoides	*C. minutissima*	*C. oocystoides*	*C. minutissima*
Without algae	51.50 c	42.20 de	8.61 a	8.57 a
0.1% algae	53.47 c	41.93 de	8.49 ab	8.56 a
1% algae	83.07 a	34.93 ef	8.26 b	8.54 ab
2.5% algae	64.10 b	33.40 ef	8.25 b	8.51 ab
5% algae	45.50 cd	23.10 f	8.24 b	8.43 ab

Mean values in the same column followed by the same letters are not significantly different according to Duncan`s multiple range test ($P < 0.05$)

4. Discussion

Generally, increased populations of soil microflora such as algae and bacteria will help improve soil fertility and plant growth (Mallavarapu, 2001**).** The plant growth parameters are strongly related to soil fertility and any improvement in soil fertility should be reflected into the plant growth. In our experiment we noticed significant increasing in the soil organic matter and nitrogen content as a result of inoculation with *Chlorella* spp. Soil organic matter increased due to algal growth which adds new organic matter to the soil as a result of photosynthesis. The increase in soil nitrogen may be due to increasing of rhizosphere bacteria, which may contain free-living nitrogen fixers, as a result of the addition of *Chlorella* spp. Lopez *et al*. (2013) noticed that *Chlorella sorokiniana* favored bacterial richness, which may have occurred by growth of the bacterial population. Some authors suggested that the increase of soil nitrogen by *Chlorella* may be due to absorbing of ammonia and nitrogen oxides from air (Fogg, 1956).

The increase of electric conductivity may be due to increase of soluble ions released by microbial increased exploration of the soil for available nutrients and delivers more mineral nutrients. In our experiment soil pH decreased as a result of algal treatment and these results agree with that obtained by (Eletr, *et al*., 2013). Some algae have the ability to excrete a number of extracellular compounds, such as polysaccharides, peptides, lipids, organic acids that can decrease the soil pH (El-Ayouty, *et al*., 2004). Our study showed that, addition of *Chlorella* spp. enhanced many of the studied plant growth parameters. Fresh weight, dry weight, and chlorophyll content increased significantly after algal addition. Similar results were obtained by (Shaaban, 2001) when he used *Chlorella vulgaris* as soil additive for maize plant. Increasing of fresh and dry biomass of corn seedling treated with *Chlorella* sp. is also reported by (Grzesik & Romanowska-Duda, 2014). Application of *Chlorella* sp. can be beneficial to growth, development, and metabolic activity of corn seedling; it increase and accelerate germination, chlorophyll content in leaves, and activity of net photosynthesis (Grzesik & Romanowska-Duda, 2014). Similar results were obtained on different plants other than *Zea*, for example (Faheed & Abd-El Fattah, 2008) observed the increase of germination, fresh and dry weights, and pigment content of *Lactuca sativa* seedlings treated with *Chlorella vulgaris*.

Increasing of the plant growth parameters by addition of *Chlorella* may be due to improving the nutrient status of plant which in turn enhances all physiological reactions (Shaaban, 2001). Also it is a powerful phytoprotector against phytoparasites such as nematodes (Bileva, 2013), fungi (Ibraheem & Mohamed, 2002), and Bacteria (Ingham, *et al*. 1985).

5. Conclusion

Each of the used algal strains improved the plant growth parameters and soil physico-chemical properties. *C. oocystoides* is more effective than *C. minutissima* as it showed higher improvement in plant growth parameters and soil properties even at lower concentrations. Intensive studies are needed to understand the effects of algal inoculation on plant growth parameters and soil properties and to discover a super algal species or algal consortium that can be used as biofertilizer to improve soil fertility and productivity.

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