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Green Microalgae Water Extract as Foliar Feeding to Wheat Plants

Mahmoud M. Shaaban
Botany Department, National Research Centre, Dokki, Cairo, Egypt

Abstract: The effect of green alga *Chlorella vulgaris* cell extract in water as foliar feeding on nutrient status, growth, and yield of wheat plants (*Triticum aestivum* L. var. Giza 69) was studied and compared to a micronutrient foliar fertilizer. Algae cell extract was superior in increasing both concentrations and uptake of all the studied nutrients in plant shoots. As a reflection to the nutrient status improvement, fresh and dry biomass accumulation in the shoots increased. 50 % v/v algae cell extract (T_2) was the best significant in fresh weight increase (led to 60.7 % increase over control). 100 % algae cell extract (T_4) was the best significant, where it led to 95 %, 60 % and 160 % increase over control in dry weight, spikes weight and 100 grains weight respectively. However, T_2 resulted in significant increases in yield (more than 140 % over control) and grain weight (more than 40 % over control). Micronutrient foliar fertilizer increased the spikes weight and 100 grains weight by only 90 % and 22 % over control, respectively.

Key words: Wheat, green algae extract, foliar feeding, nutrient status, yield

Introduction

Fertilizer use is one of the basic practices in agriculture. Due to soil physical and chemical problems, foliar fertilization becomes more and more desirable treatment for crops. Foliar fertilizing may also has interventionist effect and it is then, the only way to supply the lacking elements to the plant.

Foliar application of inorganic micronutrients is well-studied (El-Fouly, 1983, 1984; Mengel and Kirkby, 1987; Marchner, 1995). Combinations of macro and micronutrients in foliar fertilization were also tested (Fageria *et al.*, 1997). Recently, foliar application of biochemical organic substances, which supply both macro and micronutrients, is of increased demand because they have the advantage that they are safe to the environment.

Fresh water green microalgae contain high percentage of macro and micronutrients bounded in their major biochemical constituents such as carbohydrates and proteins (El-Fouly *et al.*, 1992).

The present work aims at evaluating the effect of the water extract of the single cell green alga *Chlorella vulgaris* as foliar fertilizer on the nutrient uptake, nutrient balance and consequently, growth, yield and some yield components of wheat plants.

Materials and Methods

A pot experiment was carried out with wheat (*Triticum aestivum* L. cv. Giza 69) in the greenhouse of the Programme "Micronutrients and Other Plant Nutrition Problems in Egypt", National Research Centre, Dokki, Cairo, Egypt. Seeds were sown in November 1998 in Mitscherlich pots containing 7.0 Kg soil. Soil physical and chemical characteristics and nutrient status evaluation according to Ankerman and Large (1974) are shown in Table 1.

Agricultural practices: Before sowing, each pot received 1.0g super-phosphate (15.5 % P_2O_5) and one third of 1.0 g potassium sulfate (48 % K_2O) + 2.0 g ammonium sulfate (20.6 % N). Two other splits of equal quantities of N and K were applied 10 days after sowing and before tillering. At seedling stage, the plants of each pot were thinned to 20 plants. Irrigation was applied to maintain the water level at 60 % of the field capacity. No pesticides were used during the course of study.

Treatments: A concentrated slurry of the microalga *Chlorella vulgaris* (contains about 10 % water) was washed with distilled water, reconcentrated by centrifugation and freezed and then remelted at the room temperature. The melted slurry was then centrifuged at 5000 rpm to obtain a clear cell sap. Major components and nutrient content of the algae extract is show in Table 2.

Table 1: Mean values of physical and chemical soil characteristics

Physical characteristics		Nutrient concentrations	
		Exchangeable Macronutrients (mg/100g soil)	
pH	7.9	P	3.32*
E.C. (dS/m)	1.2	K	37.6*
$CaCO_3$ (%)	1.2	Mg	22.6**
O.M. (%)	3.5	*Available Micronutrients*	
Sand (%)	22.8	(mg/Kg soil)	
Silt (%)	38.0	Fe	15*
Clay (%)	39.2	Mn	5.9**
Texture	Loam	Zn	14.7*
		Cu	10.2*

* Adequate ** Low

Table 2: Major chemical composition and elemental contents of *Chlorella* cell extract and its protein amino acid composition

Major components of the cell extract and alga protein amino acid composition		Elements content of the cell extract Major components %	
Protein	35.0	Macro-elements %	
Fats	7.0	N	5.6
Carbohydrates	9.0	P	0.46
Amino acid composition		K	1.6
(g/100g protein)*		Ca	0.25
Arginine	6.9	Mg	1.02
Histidine	2.0	Na	0.23
Isoleucine	3.2	**Micro-elements (ppm)**	
Lucien	9.5	Fe	245
Lysine	6.4	Mn	131.2
Methionine	1.3	Zn	111.5
Phenylalanine	5.5	Cu	28
Threonine	5.3		
Tryptophan	1.5		
Valine	7.0		

* Source: El-Fouly *et al.* (1992)

The treatments were carried out in three replicates. The plants were treated with the following treatments as foliar feeding 25 days after sowing:

Table 3: Macro and micronutrient concentrations in wheat plant shoots as affected by foliar treatments with micronutrients (MN) and different concentrations of algae cell extract

Treatments	Macronutrients (%)					Micronutrients (ppm)			
	N	P	K	Mg	Ca	Fe	Mn	Zn	Cu
Control	2.13	0.25	2.13	0.28	0.73	30.5	61.0	51.0	6.5
MN	2.48	0.29	2.10	0.32	0.80	45.0	68.0	79.0	10.0
T_1	2.22	0.72	1.96	0.24	0.83	71.3	65.0	57.0	11.2
T_2	2.43	0.80	2.25	0.28	0.86	83.3	61.0	60.5	11.2
T_3	2.27	0.77	2.19	0.27	0.82	86.6	66.0	63.0	11.8
T_4	2.38	0.77	1.98	0.28	0.80	84.3	81.0	56.2	14.8
Mean ± SD	2.13±0.13	0.6±0.25	2.10±0.11	0.27±0.02	0.80±0.08	66.8±23.5	67.0±7.4	61.1±9.6	10.9±2.6
r	0.29	0.73	-0.07	-0.1	0.34	0.81	0.71	-0.13	0.87

SD = standard deviation r = correlation coefficient

Control: distilled water

MN: Micronutrient liquid fertilizer contains 5.2 % manganese, 0.65 % zinc, 0.65 % copper and 0.02 % molybdenum (v/v) in a concentration of 2ml/l in the spray solution.

T_1: 25 % (v/v) algae cell extract in distilled water

T_2: 50 % (v/v) algae cell extract in distilled water

T_3: 75 % (v/v) algae cell extract in distilled water

T_4: 100 % (v/v) algae cell extract

Sampling and sample analysis

Soil samples: A representative soil sample was taken after soil preparation but before fertilization. Soil samples were air-dried and passed through a 2.0 mm sieve pores. Mechanical analysis of soil samples was carried out using hydrometeric method (Bauyoucos, 1954); pH and E.C (electric conductivity) were determined in soil/water extract (1:2.5) (Jackson, 1973); Calcium carbonate ($CaCO_3$) content of the soil was determined using Calcimeter (Black, 1965); Organic matter (OM) was determined using potassium dichromate method (Walkely and Black, 1934)

Soil phosphorus was extracted using sodium bicarbonate (Olsen *et al.*, 1954). Potassium (K) and magnesium (Mg) were extracted using ammonium acetate (Chapman and Pratt, 1978), while Fe, Mn, Zn and Cu were extracted using DTPA solution (Lindsay and Norvell, 1978).

Plant sampling: Leaf samples were taken 15 days after treatments (40 days after sowing). The samples were washed with tap water, 0.01 N HCl and bidistilled water, sequentially, oven dried at 70°C for 24 hours and ground. Plant material was dry-ashed in a muffle furnace at 550 °C for 6 hours using 3.0 N HNO_3. The residue was, then suspended in 0.3 N HCl.

Determinations and measurements: Protein content of the algae extract was calculated as total nitrogen x 6.25. Algae extract fat content was determined in its ether extract using A.O.A.C. (1965) method. Total carbohydrate content was determined according to Dubois *et al.* (1956).

Wheat leaf and algae extract nitrogen content was determined using Bauschi digestion and distillation apparatus. Phosphorus was photometrically determined using the Molybdate-Vanadate method and measured using the UVNIS Spectrophotometer (Perkin-Elmer Lambda2). Potassium and Ca were measured in the extract using (Jenway PFP7) Flame photometer. Magnesium, Fe, Mn, Zn and Cu were measured using the Atomic Absorption Spectrophotometer Perkin-Elmer (HGA 700).

Dry weight determination: The samples were weighed for the fresh weight and oven dried at 70 °C for 24 hours, then weighed again and the dry weight was calculated.

Yield determinations: The plants were harvested at maturity stage. The complete mature spikes per pot (20 spikes) as well as 100 grains were weighed.

Data analysis: Data were statistically analyzed using Costate Statistical Package (Anonymous, 1989).

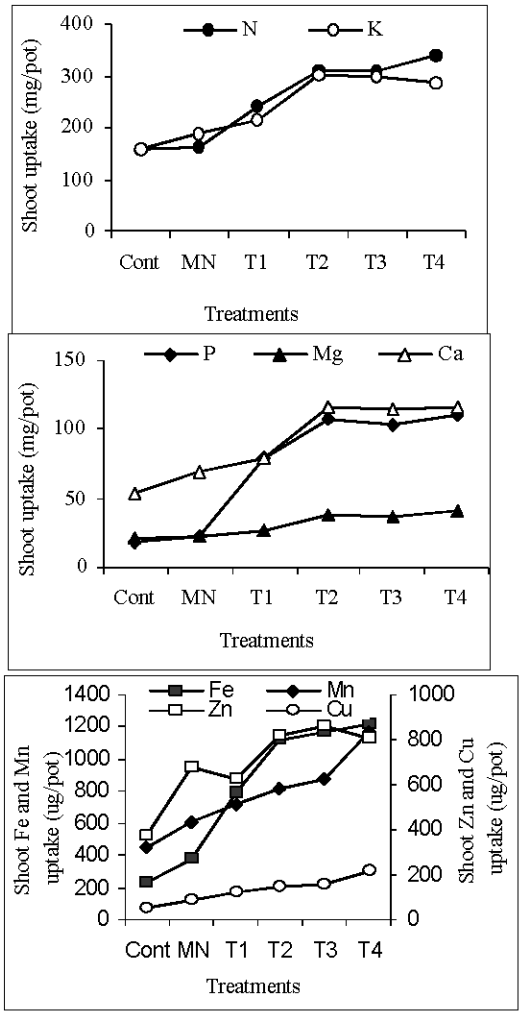

Fig. 1: Macro-and micronutrients uptake by wheat shoots as affected by foliar fertilization with micronutrients and different concentrations of algae cell extract

Results and Discussion

Nutrient status

Nutrient concentrations: Use of algae cell extract increased the concentrations of some nutrients in wheat shoots (Table 3). Highly positive correlations were found between the increased concentrations of the extract and phosphorus, iron, manganese and copper concentrations in shoots. This may attribute to the presence of reasonable percentage of these elements in the alga extract, especially phosphorus, which is not present in many of the known foliar fertilizers.

Nutrient uptake: Foliar application of algae cell extract was found to increase the uptake of most nutrients by shoots of wheat plants (Fig.1). Nitrogen, P, K, Ca, Fe, Mn, Zn and Cu uptakes were increased and the treatment T_2 seemed to be adequate for the desired nutrient uptakes. Nutrient uptake increases may be due to nutrients present in the cell extract, which mostly are in an organic form and can be directly involved in the metabolism. On the other hand, the amino acids derived from proteolysis can work as chelating agents, facilitating the penetration of elements through leaves

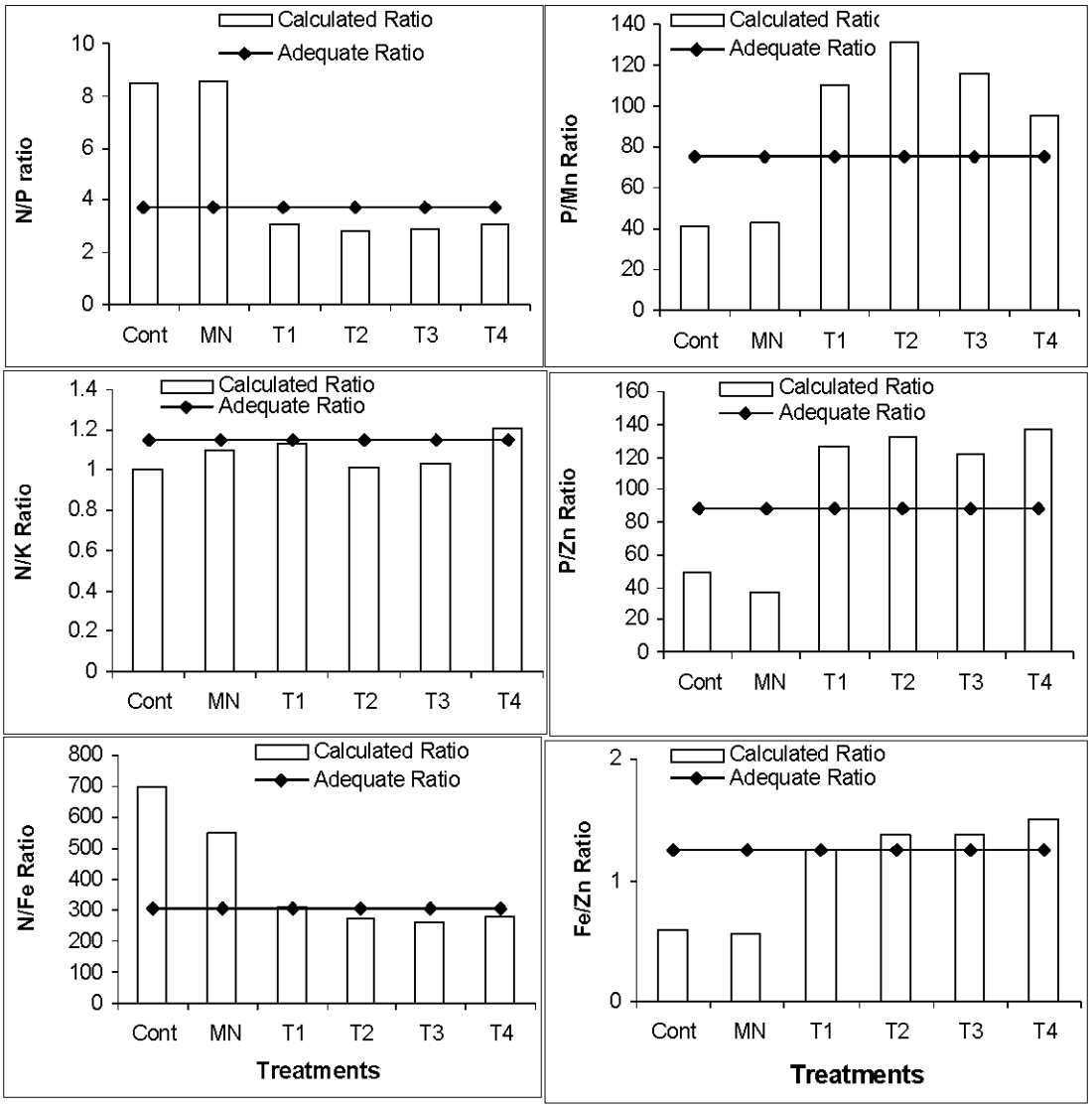

Fig. 2: Calculated present nutrient ratios in the shoots of wheat plants as affected with MN and different algae extract concentrations compared to the adequate ratios calculated after Reuter, 1986 and Fageria *et al.*, 1997)

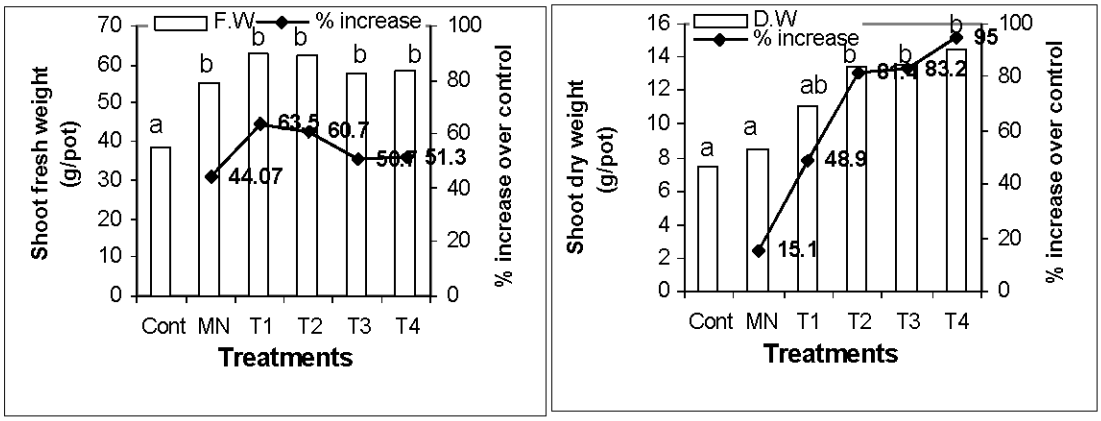

Fig.3: Fresh and dry biomass of wheat shoots (g/pot) as affected by micronutrients and different concentrations of algae extract (bars *with same letter are not significantly different, P = 0.05*)

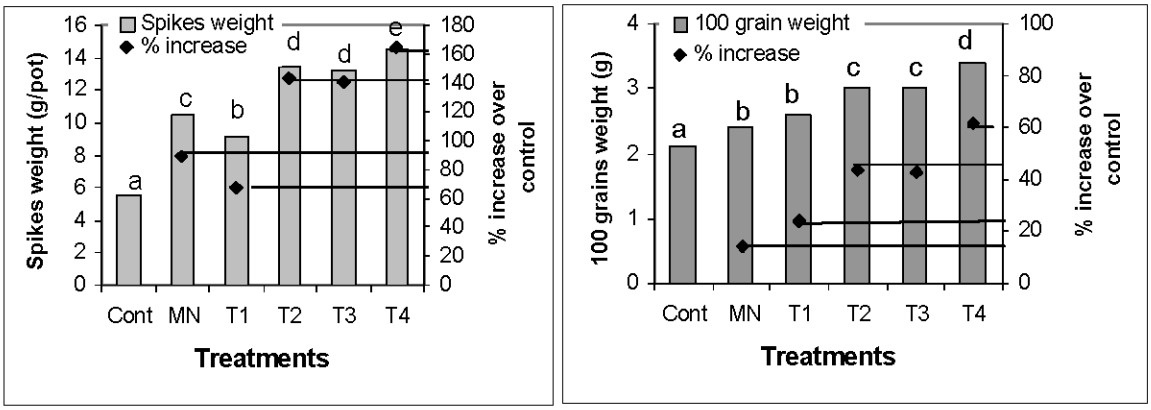

Fig. 4: Spikes weight (g/pot) and 100 grains weight (g) as affected by micronutrients (MN) and different concentrations of the algae extract (bars *with same letters are not significantly different, P = 0.05*)

(El-Fouly *et al.*, 1997). Amino acids can also migrate to roots and play a role as phytosiderophores, facilitating the absorption of micronutrients through the root hairs (Lindsay, 1974; Cakmak *et al.*, 1994; Marschner and Roemheld, 1996; Shaaban and Mobarak, 2000).

Nutrient balance: Fig. 2 shows the nutrient ratio in the shoots of wheat plants. N/P and N/Fe of control plants and those treated with micronutrients foliar spray were far away above the adequate ratios, while they are very near in the plants sprayed with algae cell extract. This definitely attributed to the low concentrations of phosphorus and iron in the shoot tissues of control and MN-sprayed plants. The ratios P/Mn, P/Zn and Fe/Zn were in the opposite situation, where they were faraway down the adequate levels in control and MN-sprayed plant shoots. Deviation of the ratios from the adequate levels causes disturbance in the physiological behaviour of the nutrients in the plant tissues, which in turn, leads to low yields (Fawzi *et al.*, 1996; Shaaban and Abou El-Nour, 1996; El-Fouly and Shaaban, 1999).

Growth and yield

Fresh and Dry biomass formation: Even they are significantly increased over control, fresh weight of the shoots treated with micronutrients or different concentrations of algae cell extract are not significantly different (Fig. 3). However, dry biomass formation of the shoots treated with alga extract was significantly different. The treatment T2 (50 % v/v alga extract in water) which leads to 81.4 % dry weight increase over control seems to be practically suitable, where it is not significantly different from the higher treatments. Dry biomass increase due to alga extract treatments is a reflection of the increase of nutrient uptake and concentrations, which led to a nutrient balance near the recommended adequate values (Fawzi *et al.*, 1996, El-Fouly and Shaaban, 1999).

Yield: Wheat yield is expressed as dry spikes weight per pot (Fig. 4). Significant increase was found in spikes weight and 100 grain weight using micronutrients or different concentrations of the algae extract as foliar spray. Algae extract treatments higher than 25% (i.e.50 % algae extract and up-words) led to higher yield than that obtained using micronutrients. The best significant treatment was T4 (100 % algae water extract), however, satisfactory yield can be also obtained using the treatment T2 (50 % algae extract) Yield and grain weight increases are reflection to the increase in dry biomss accumulation. On the other hand, algae extract as a natural plant cell sap contains certain amounts of

hormones, enzymes and vitamins that may improve nutrient assimilation and solute translocation from leaves to grains which, led to significant increases in yield and grain weight.

Conclusions

From the present work it can be concluded that: Green algae cell extract in water is superior than micronutrients as foliar feed to wheat plants.

A concentration of 50 % (v/v) algae extract as one time foliar spray (25 days after sowing) can lead to more than 140 % yield increase and more than 40 % grain weight increase.

Further studies should be done to estimate costs of the algae cell extract as foliar fertilizer, on the large scale, compared to other foliar fertilizers present in the market.

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