Green Microalgae Water Extract and Micronutrients Foliar Application as Promoters to Nutrient Balance and Growth of Wheat Plants

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Abstract: Pot experiment was conducted in the experimental station of the Suez Canal University, Ismailia, Egypt with wheat plants to study the effect of foliar fertilization with algal extract or micronutrients fertilizer or their combinations on the dry matter accumulation in the plant shoots. Nutrient concentrations, uptake and balance were also under investigation. Results showed that the best concentrations of macronutrients were achieved by the algal extract treatments or the higher dose of the micronutrient fertilizer. However, the best uptake, nutrient balance and dry matter accumulation was recorded with combined algal extract and micronutrient fertilizer treatment. [Journal of American Science 2010; 6(9):631-636]. (ISSN: 1545-1003).

Key words: Wheat, Micronutrients, Algae Extract, Dry Weight, Nutrient Balance

1. Introduction

Intensive crop cultivation mostly leads to nutrient depletion of the soil reservoir all over the world. Unfavorable soil conditions such as high pH, salinity and $CaCO_3$ content (Shaaban *et al*., 2004, 2007, 2008) and the antagonism of soil macro and micronutrients (Aulakh and Malhi, 2005), can also affect the availability of some nutrients for plant roots. Micronutrients are essential for growth and development of all higher plants (Marschner, 1995). They serve in the redox systems and as co-enzymes for numerous fundamental processes in the plant cell activities (Hall and Williams, 2003; Salama and Shaaban, 2000). Thus, micronutrients deficiency causes sever problems in plant cell metabolism which finally lead to growth retardation and less yields (El-Fouly *et al*., 2008 and Shaaban *et al*., 2007). Micronutrients as soil application may not meet the crop requirements for growth and nutrient balance within the plant tissues. Thus the alternative effective approach is to apply these micronutrients as foliar spray (Arif *et al*., 2006).

Foliar application on wheat with different micro-nutrients could be equal or more effective than soil applications and used effectively to overcome the problem of micronutrients deficiency (Modaihsh, 1997 and Torun *et al*., 2001). Kassab *et al*. (2004) concluded that the foliar application with a 2% solution from each of Fe, Mn, Zn and Mg significantly increased wheat yield components including grain and straw yields as well as carbohydrates yield.fed^{-1}.

Fresh water green microalgae extracts appeared to be promising natural fertilizers. They contain high macro and micronutrients concentrations in addition to the natural enzymes and hormones (Shaaban, 2001a). Kannan and Tamil, 1990 observed that soil application of seaweed liquid fertilizer of *Enteromorpha clathrata* and *Hypnea musciformis* increased the growth characteristics of green gram, black gram and rice. Furthermore, Whapham *et al*. (1993) observed that the application of seaweed liquid fertilizer of *Ascophyllum nodosum* increased the chlorophyll levels of cucumber cotyledons and tomato plants. Moreover, vegetative growth of olive transplants was markedly enhanced as their root zoon was surrounded by pre-digested *Scenedesmus* bulk at the concentration equal to the recommended nitrogen dose. Nutrient concentrations and balance were also improved by the partial substitution of nitrogen by algal bulk (Abdel-Maguid *et al*., 2004).

The present work aimed at studying the effect of foliar spraying with the green microalgae *Scenedesmus* sp. water extract or/and micronutrients commercial compound and their combinations on the nutrient concentration nutrient balance and dry weight accumulation in wheat plants.

2. Materials and Methods

Pot Experiment was conducted during the growing season 2006/2007 in the experimental station of the Faculty of Agriculture, Suez Canal University, Ismailia, Egypt with wheat (*Triticum aestivum* L.) var. Gemmiza 9. The experiment was designed as randomized complete block (RCB) with three replicates.

2.1. Agricultural practices:

Wheat grains (Gemmiza 9) were purchased for the grain's collection centre of the Agricultural Research Centre, Giza, Egypt and sown in the first half of November. The pots contained 7.0 kg soil. Before sowing, each pot received 1.4 g super mono-phosphate (15.5% P_2O_5). Nitrogen and potassium were added as 3 equal splits in the rate of 2.0 g.pot^{-1}

(ammonium sulphate 20.6% N) and 1.5 $g.pot^{-1}$ potassium sulphate (48.5% K_2O). At the seedling stage, the plants were thinned to leave 20 plants per pot. Irrigation was adopted to maintain the water level at 60% of the field capacity.

2.2. Treatments

Foliar spraying treatments were performed two times (at 35 and 66 days after sowing) as present in Table 1.

Table 1. Algae extract & chelated micronutrients treatments and concentrations used

T	Fraction
Con.	Tap water
(T1)	$1g.l^{-1}$ Algal extract
(T2)	$2g.l^{-1}$ Algal extract
(T3)	$1g.l^{-1}$ Modified algal extract
(T4)	$2g.l^{-1}$ Modified algal extract
(T5)	$1g.\ l^{-1}$ Chelated micronutrients
(T6)	$2g.\ l^{-1}$ Chelated micronutrients
(T7)	$1g.l^{-1}$ Modified algal extract + $1g.\ l^{-1}$ chelated micronutrients
(T8)	$2g.\ l^{-1}$ Modified algal extract + $2g.l^{-1}$chelated micronutrients

2.3. Algal extract preparation

The green alga *Scenedesmus sp*. (El-Sayed, 2004) was produced at the Algae Production Station of the National Research Centre (NRC, Dokki, Cairo) within three open ponds ($15m^3$ per each). Continuous centrifugation was employed for harvesting algal bulk contains 75-80 % moisture. The concentrated algal slurry obtained by laboratory cooling centrifugation (contains about 10 % water) was washed with distilled water, re-concentrated and then freezed for 48 hours. The slurry then re-melted at the room temperature and centrifuged again at 5000 rpm to obtain the clear cell sap. The modified algal extract contains 0.3% boron as boric acid (17% B). Major components of the used alga are shown in Table 2.

Table 2: Major constituents of the used alga *Scenedesmus* sp. (n=3)

Constituent (D.w)			%
Crude protein %			50.56
Ether extract %			7.39
Crude fiber %			9.83
Ash %			9.18
Moisture %			4.51
N	P	K	
%			
8.09	2.69	0.65	
Fe	Zn	Mn	Cu
Ppm			
2057	772	747	93

The readymade used chelated micronutrients fertilizer namely Wuxal contained 3% Fe + 3% Mn+ 3% Zn + 0.5% Cu in the EDTA form plus 14% N as urea. The net applied volume was 1-2.0 $g.l^{-1}$.

2.4. Sampling and Soil analysis

Soil physical and chemical characteristics are shown in Table 3. Representative soil samples were taken after soil preparation and before fertilization. The samples were air-dried and passed through 2 mm sieve pores. Soil fractions were determined using the hydrometer method (Bauyoucos, 1954). E.C. and pH were determined in soil/water extract (1:2.5) according to Jackson (1973). $CaCO_3$ content was determined using the calcimeter method according to Black (1965).

Table 3: Physical proprieties and chemical analysis of the experimental soil

Physical characteristics			
pH	$CaCO_3$ (%)		O.M (%)
7.6***	1.2*		0.35*
Sand	Silt	Clay	Texture
(%)			Sand
87.0	2.1	10.9	
Nutrient concentrations			
Exchangeable Macronutrients ($mg.100g^{-1}$soil)			
P	K	Mg	
2.0**	7.7*	16.0*	
Available Micronutrients ($mg.kg^{-1}$ soil)			
Fe	Mn	Zn	Cu
1.5*	4.0*	0.13*	0.7 *

*low; **Adequate; ***High (Ankerman and Large 1974)

Organic matter was determined using the potassium dichromate method according to Walkely and Black (1934). Soil phosphorus was extracted using sodium bicarbonate (Olsen *et al*., 1954). Potassium was extracted using ammonium acetate (Chapman and Pratt, 1978). Iron, manganese; zinc and cupper were extracted using DTPA-solution (Lindsay and Norvell, 1978).

2.5. Plant sampling

Wheat shoots were taken 16 days after the second and last spray to determine the dry weight per plant (g/plant). Part of the sample was washed with tap water, 0.01 N HCl and bi-distilled water and oven dried at 70º C for 24 hours and grinded. The grinded material was dry-ashed in a muffle furnace at 550ºC for 6 hours using 3.0 N HNO_3. The residue then, suspended in 0.3 N HCl.

2.6. Measurements and determinations

Nitrogen was determined based on microkjeldahl technique using Buchii digestion and distillation apparatus. Phosphorus was Spectro-photometrical determined using the molybdate-vanadate method (Jackson, 1973). Potassium was measured using Dr. Lang -M8D Flame-photometer. Magnesium, Fe, Mn, Zn and Cu were determined using the Atomic Absorption Spectrophotometer (Perkin-Elmer 100 B). Protein content of the alga was determined as N X 6.25, while fat content was determined according to the method of AOAC (1965). Total carbohydrate content was determined according to Dubois *et al*. (1956).

2.7. Statistical analysis

Collected data were subjected to the statistical analysis of Snedecor and Cochran (1990).

3. Results and Discussion

3.1. Macronutrient status

Concentrations of macronutrients in wheat shoots as affected by different treatments of algal extract and chelated micronutrients are shown in Table 4. It is obvious that nitrogen concentration was significantly increased as a response to algal extract at both treatments (T3,T4 & T5) as compared mainly with control treatment or with chelated micronutrients alone (T7&T8). On the other hand, increasing of modified algal extract (T6) exhibited a decrease on nitrogen concentration. This may attributed to the high protein content of the algal extract which split into natural plant amino acids involved directly in the metabolism (Shaaban, 2001a). Also, boron seems to enhance the bioaccumulation of nitrogen in the presence of algal extract (T5). Of this, boron has synergetic effect on nitrogen and other nutrient uptake and utilization by sugar beet plants (Hellal *et al*., 2009).

Table 4: Macronutrients concentration in wheat shoots as affected by different foliar treatments of algal extract and micronutrients (n=3)

T	N %	P%	K%	Mg %
Con.	0.73 ab	0.18 a	0.92 ab	0.20 a
T1	1.10 bc	0.22 b	1.63 cd	0.30 c
T2	1.08 c	0.19 ab	1.87 d	0.22 ab
T3	1.2 c	0.19 ab	1,38 bc	0.27 bc
T4	0.69 ab	0.19 ab	1.43 bc	0.19 a
T5	0.64 a	0.19 ab	1.55 c	0.27 bc
T6	0.78 ab	0.27 c	1.15 b	0.30 c
T7	0.69 ab	0.18 a	0.85 a	0.26 b
T8	0.83 b	0.18 a	1.17 b	0.20 a
$LSD_{0.05}$	0.15	0.04	0.27	0.04

Phosphorus concentrations in wheat shoots only significantly increased with the treatment 2.0 $g.l^{-1}$ micronutrient fertilizer (T8), which may a result of micronutrients effect on enhancing roots growth and then absorb more nutrients from the root medium (El-Fouly *et al*., 1997). It was also followed by the algal extract application (T3). The most increment in K concentration in shoot tissues was obtained by the extra algal extract treatment (T4). This may due to the reasonable percentage of K in the algal extract where it reached more than 1.5% on dry weight basis in different green algae species (El-Fouly *et al*., 1992) as well as the used alga that contain more than 0.5% on dry weight basis.

Magnesium concentrations were superior with the 1.0 $g.l^{-1}$ algal extract (T3) as well as 2.0 $g.l^{-1}$ micronutrients fertilizer (T8). Magnesium found also in a high percentage in the green micro-algae (more than 1.0% on dry weight basis, however, micronutrients fertilizer may considered the cause that led to more Mg absorbance.

As shown in Table 5, the highest N, P, K and Mg-uptakes were recorded with the modified algal extract (T4), modified algal extract + 1.0 $g.l^{-1}$ micronutrients fertilizer (T7), 2.0 $g.l^{-1}$ micronutrients fertilizer (T6) and modified algal extract + 2$g.l^{-1}$chelated micronutrient (T8). Tow nutrient sources might be considered in the case of algal extract treatments. One source is the algal extract itself and the second is its positive effect on the nutrients uptake by the plant roots (Shaaban, 2001a). However, micronutrients fertilizer has only the effect of encouraging roots to absorb more nutrients from the soil medium (Shaaban and Mobarak, 2000). Further studies claimed that algal extract promoting growth as it contains a series of plant growth promoters. Of these, Rama Rao (1991) reported that aqueous extract of *Sargassum wightii* when applied as a foliar spray on *Zizyphus mauritiana* showed an increase in yield and quality of fruits.

Table 5: Macronutrients uptake by wheat shoots as affected by different foliar treatments of algae and micronutrients

T	Macronutrients ($mg.plant^{-1}$)			
	N	P	K	Mg
Con.	24.6	6.0	31.0	6.7
T1	44.0	8.8	65.2	12.0
T2	37.3	6.5	64.7	8.0
T3	66.1	10.4	76.0	14.0
T4	35.8	9.4	74.3	9.8
T5	23.9	7.1	58.0	10.0
T6	49.2	17.0	73.3	18.9
T7	53.8	14.0	60.1	20.2
T8	50.6	11.0	71.3	12.2

3.2. Micronutrients status

Micronutrients concentrations in wheat shoots were differed significantly with different treatments with certain respect to the examined element (Table 6). In general, the highest Fe, Mn, Zn and Cu concentrations were obtained by the 2g.l^{-1} micronutrient fertilizer treatment (T6). This is a logic result, where the fertilizer contains relatively higher concentrations of these nutrients than other treatments which mostly taken up by the plant shoots and raise their concentrations in the shoot tissues (Kassab *et al*. 2004). This was also true for the uptake of these nutrients (Table 7), where the highest uptake was recorded for the treatment 2.0 g.l^{-1} micronutrient followed by 1.0 g.l^{-1} modified algae + 1.0 gl^{-1} micronutrients fertilizer. So, it is evidence that 1.0 g l^{-1} micronutrient fertilizer can be only substituted by 1.0 g.l^{-1} of algal extract to meet approximate real supplementation of micronutrients for the growth of wheat plants.

Table 6: Micronutrients concentration in wheat shoots as affected by different foliar treatments of algae and micronutrients (n=3)

T	Fe	Mn	Zn	Cu
	mg.kg^{-1}			
Con.	50 a	24 a	4 a	4 b
T1	58 ab	35 b	6 b	5 c
T2	78 bc	23 a	6 b	4 b
T3	97 d	28 ab	7 bc	4 b
T4	65 b	27 ab	6 b	4 b
T5	93 cd	31 ab	7 bc	5 c
T6	109 b	24 a	11 b	4 b
T7	58 ab	31 ab	7 bc	3 a
T8	80 c	27 ab	8 c	3 a
$LSD_{0.05}$	14.0	10.0	2.0	1.0

Table 7: Micronutrients uptake by wheat shoots as affected by different foliar treatments of algal extract and micronutrients fertilizer

T	Micronutrients (µg.plant^{-1})			
	Fe	Mn	Zn	Cu
Con.	16.8	8.0	1.3	1.3
T1	23.2	14.0	2.4	2.0
T2	27.0	7.9	2.4	1.4
T3	53.4	15.4	3.4	2.2
T4	33.8	14.0	3.6	2.1
T5	34.8	11.6	4.1	1.8
T6	68.8	15.1	6.8	2.5
T7	45.2	24.2	5.5	2.3
T8	46.8	16.4	4.8	1.8

3.3. Nutrient balance

Some of the nutrient ratios in the shoot tissues were shown in Fig. 1. The optimum N/P and P/Mn ratios were obtained by the treatment 1.0 g. l^{-1} modified algae + 1.0 g. l^{-1} micronutrient fertilizer (T7). Nirtogen, potassium and iron distribution in the shoot increased significantly. Boron has synergetic effect on nitrogen and other nutrient uptake and utilization. The synergetic effect between B and N may increase N-content in root and shoot tissues (Hellal *et al*., 2009). This can be explained by the integration in nutrient uptake and the balance created by plant regulation mechanisms as presence to sufficient concentrations of the two elements (Shaaban, 2001b). In addition, functions of boron related to cell wall synthesis, lignification, and cell wall structure by cross-linking of cell wall polysaccharides as well as the structural integrity of bio-membrane increases the transport of phosphorus as a result of plasmalemma ATPase induction. (Camacho-Cristobal *et al*., 2008).

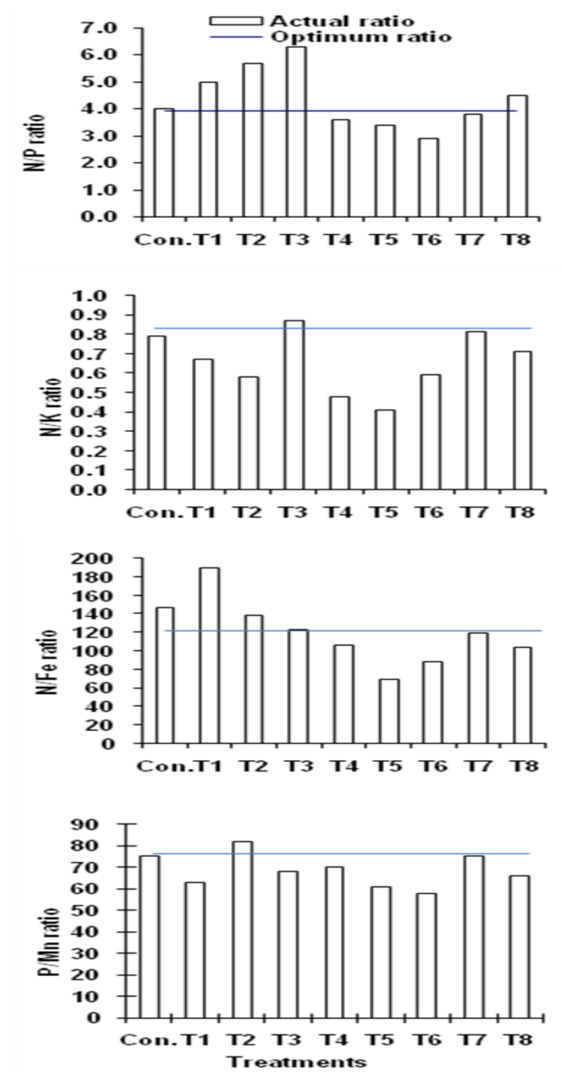

Fig. 1: Actual nutrient ratio within wheat shoots as affected by different treatments of algal extract and micronutrients fertilizer

It is clear that the treatment 1.0 $g.l^{-1}$ modified algal extract + 1.0 $g.l^{-1}$ micronutrient fertilizer (T7) is superior in realizing the best nutrient balance among other treatments. It is known that deviation of the nutrient ratios above or down the optimum ratios causes disturbance in the physiological behavior of the nutrients which, in turn, leads to low dry matter accumulation and low yields (Fawzi *et al*., 1996, Shaaban and Abou El-Nour, *1996*).

3.4. Dry weight accumulation

Dry matter accumulation in the wheat shoots were determined 66 days after sowing (Fig. 2). The highest values were recorded with the treatment 1.0 $g.l^{-1}$ modified algal extract + 1.0 $g.l^{-1}$ micronutrient fertilizer (T7). As previously mentioned, this treatment was the best to realize the nearest nutrient ratios to the optimums.

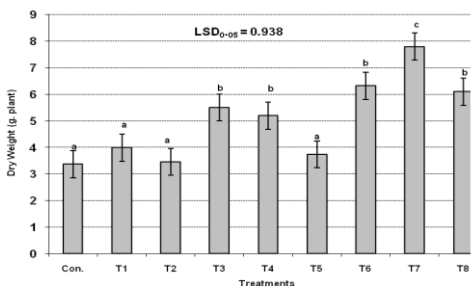

Fig.2: Dry weight accumulation in the wheat shoots as affected by different treatments of algal extract, micronutrient fertilizer and their combinations (n=3)
Columns with the same letters are no significantly different at $p \leq 0.05$.

Similar results were achieved by Shaaban (2001b), Shaaban and Abou El-Nour (1996). Abscisic acid and cytokinins present in *Ascophyllum nodosum* (Kingman and Moore 1982) found to have beneficial effects, such as increased crop yield, increased uptake of nutrients and resistance to frost and stress conditions, when such algae applied to soil. Optimization of the nutrient balance could prevent nutrient accumulation, nutrient fixing and nutrient toxicity, enabling every element to play its role in a harmony with other nutrients, which leads, in turn, to best dry matter accumulation and higher yields.

4. Conclusions

From the present work it could be concluded that a) Micronutrients foliar fertilization is very important for the growth of winter wheat plants, b) Algal extract can be partially substitute micronutrient foliar fertilizers and best to be complementary portion of the spray solution and c) Most important for the growth of wheat plants is the nutrient balance within the plant tissues. The nearest to the optimum the best to realize better dry matter accumulation.

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