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Effect of Biofertilizers as a Partial Substitute for Mineral Fertilizers on Growth, Anatomical Structure, Mineral Elements and Yield of Wheat under Newly Reclaimed Soil Conditions

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A B S T R A C T

Two field experiments were conducted at the Demo Farm, Faculty of Agriculture, Fayoum University during 2013/2014 and 2014/2015 seasons, to study the effect of biofertilizers application (Butassine N as foliar and Biogen as inoculation) and mineral fertilizers (NPK) at 50, 75 and 100% of the recommended dose on growth, yield, anatomical structure and physiological attributes of wheat plants. The results showed that application of biofertilizers in combination with mineral fertilizers (NPK) significantly increased plant height, number of tillers plant^{-1}, number of spikes plant^{-1}, number of spikeletes spike^{-1}, spike length, fresh weight plant^{-1} and dry weight plant^{-1}. This resulted in an increase in grain yield per fed which reached 18.02 and 14.95% during two seasons, respectively by the combined treatment of Butassine N+75% NPK. All treatments greatly increased stem section diameter, leaf pigments, total carbohydrates, protein%, nutrient elements, relative water content (RWC%) and membrane stability index (MSI%) in leaves. However, both total soluble sugar (TSS) and proline declined. From these results it could be recommend using Butassine N in combination with 75% NPK to minimize chemical fertilizers dose under newly reclaimed soil conditions.

Introduction

Wheat (*Triticum aestivum* L.) is one of the most important and strategic crops in the world. It's grains is a staple human food where bread wheat is the main food of people in many countries approximately 70% calories and 80% protein of human diet is supplied from its consumption (Taregh *et al.*, 2011). Also, straw can be used as a fodder for live-stock. The demand for food crops continues to increased due to population growth which has led to increased consumption. This reflects the size of the problem and the efforts needed to increase wheat production. Thus, increased production per unit area appears to be one of the important factors for bridging the gap between wheat production and consumption.

Biofertilizers are known as microbial inoculants and consist of artificially

multiplied cultures of certain soil organisms that can improve soil fertility and crop productivity. Biofertilizers add nutrients through the natural processes of nitrogen fixation, solubilizing phosphorus and incentive plant growth through the synthesis of growth-promoting substances. There use might also reduce the use of chemical fertilizers. Excessive chemical fertilizers may results in low nitrogen use efficiency (NUE) potentially exerts more pressure on the environment (Mahdi and Ismail, 2015). The microorganisms in biofertilizers restore the soil's natural nutrient cycle and build soil organic matter. Through the use of biofertilizers, healthy plants can be grown, while enhancing the sustainability and the health of the soil. Therefore, they are extremely advantageous in enriching soil fertility and fulfilling plant nutrient requirements by supplying the organic nutrients through microorganism and their byproducts (Bohme and Bohme, 2006; Jalili *et al.*, 2009; Abedi *et al.*, 2010; Medani and Taha, 2015). Hence, biofertilizers involved microorganisms could readily and safely convert complex organic material into simple compounds, they maintain the natural habitat of the soil. Increase crop yield by 20-30%, replaces chemical nitrogen and phosphorus by 25%, and stimulates plant growth. They can also provide protection against drought and some soil-borne diseases (Haas and Defago, 2005). Biofertilizers are cost-effective relative to chemical fertilizers. They have lower manufacturing costs, especially regarding nitrogen and phosphorus use.

The objective of the present study was to study the effect of application of biofertilizers with mineral fertilizers on growth, yield and its components, anatomical structure, and chemical analysis of wheat plants grown under new reclaimed soil conditions.

Materials and Methods

A field experiment was carried out in a loamy sand soil (calcareous) at the Experimental Farm, Faculty of Agriculture, Fayoum (at Demo location) during the 2013/2014 and 2014/2015 seasons. Grains Miser 2 variety was obtained from the Agricultural Research Center, Ministry of Agricultural, Egypt. Soil properties of the experimental site are shown in (Table 1).

Mineral fertilizers used in this study were ammonium nitrate (33.5% N), calcium superphosphate (15.5% P_2O_5) and potassium sulphate (48% K_2O). The used biofertilizers were obtained from the Agric. Res. Center, Ministry of Agricultural, Egypt. The activity of biofertilizers were examined in the Microbiology Dep., Fac. Agric., Fayoum Univ. before use. The experimental area was divided into plots 10.5 m^2 (3 x 3.5 m). Wheat grains were drilled handy in rows 20 cm a part at the rate of 60 kg fed^{-1} on 17th and 19th November in the both seasons, respectively. The normal cultural practices for growing wheat were followed.

Treatments

Mineral fertilizers were used at rates of 50, 75 and 100% NPK of the recommended dose (224, 200 and 50 kg fed^{-1} of NPK fertilizers, respectively) according to Ministry of Agriculture, Egypt. Nitrogen fertilizer was added in 2 equal doses, directly before the first and the second irrigation while, the P fertilizer was added during soil preparation and K fertilizer was given before the second irrigation. Grains were immersed in Arabic Gum solution (16%) as a sticking agent, before, grains were mixed with the tested powder of mixed biofertilizer. Biogen (containing nitrogen fixing bacterium (i.e. *Azotobacter chroccocum Dutch; Beijerinck*) was added at rate of 500 g fed^{-1}.

Inoculated grains were allowed to dry before sowing (Allen, 1971). Plants were also sprayed with the Butassine N (potassium dissolving bacterium namely, *Bacillus circulans* Jordan) at rate of 300 l fed^{-1}., in two equal doses at 20 and 30 days after sowing. The experiments were arranged in a complete randomized blocks design, with five treatments and three replicate plots for each.

Plant growth and yield analyses

When plants were 130 days-old, wheat plants (n=15) were carefully removed from each experimental plot and dipped in a bucket of water. Plants were shaken gently to remove all adhering soil particles and plant height was measured using a meter scale. Number of tillers plant−1 were counted. The plants were weighted to record their fresh weights and then placed in an oven at 80 °C for 48 h. The dried plants were weighed to record their dry weights. At harvest, all yield characters, i.e. number of spikes $plant^{-1}$, number of spikeletes $spike^{-1}$, spike length, 1000-grain weight and grain yield per fed were measured.

Chemical Analysis

Determination of leaf photosynthetic pigment concentrations

Total chlorophyll and carotenoids concentrations (mg g^{-1} FW) were estimated according to procedure of Cherry (1973). Leaf discs (0.2 g from each replicate of each treatment (n=10) were homogenized in 50 ml 80% (v/v) acetone and centrifuged at 10,000 × *g* for 10 min. The absorbance of each acetone extract was measured at 665, 649, and 440 nm using a UV-160A UV-visible spectrophotometer (Shimadzu, Kyoto, Japan).

Determination of proline, total soluble sugar concentrations and protein%

Proline contents (mg/100g) were measured in dry leaf tissue using the rapid colorimetric method outlined by Bates *et al.* (1973). It was extracted from 0.5 g DW of leaf tissue by grinding in 10 ml of 3% (v/v) sulfosalicylic acid. The mixture was centrifuged at 10,000 × *g* for 10 min, then placed in a test tube, with 2 ml of the supernatant followed by 2 ml of freshly prepared acid–ninhydrin solution. The tubes were incubated in a water bath at 90 °C for 30 min and the reaction was terminated in an ice-bath. Each reaction mixture was then extracted with 5 ml of toluene and vortex-mixed for 15 s. The tubes were allowed to stand for at least 20 min in the dark at room temperature to separate the toluene and aqueous phases. The toluene phase was then collected carefully into a test tube and its absorbance was read at 520 nm. Proline concentrations were determined from a standard curve prepared using analytical grade proline.

Total soluble sugars (TSS) were determined as suggested by Irigoyen *et al.* (1992). A sample of 0.2 g dried leaf was homogenized in 5 ml of 96% (v/v) ethanol then washed with 5ml 70% (v/v) ethanol. The extract was centrifuged at 3500 × *g* for 10 min and the supernatant was stored at 4 °C prior to measurement. TSS concentrations were determined by reacting 0.1 ml of the ethanolic extract with 3ml of freshly-prepared anthrone reagent [150 mg anthrone plus 100 ml of 72% (v/v) sulphuric acid] and placing it in a boiling water bath for 10 min. After cooling, the absorbance of the mixtures was recorded at 625 nm using a Bausch and Lomb-2000 Spectronic Spectrophotometer.

Determination of total nitrogen in grains was carried out with Micro-Kjeldahel method (A.O.A.C., 1990). Protein was determined according to the formula: Protein = Total N × 6.25.

Determination of membrane stability index and relative water content

Membrane stability indices (MSI) were estimated, in 10 samples for each treatment, using duplicate 0.2 g samples of fully-expanded leaf tissue (Rady, 2011). One sample of each duplicate was placed in a test tube containing 10 ml of double-distilled water and heated at 40 °C in a water bath for 30 min. The electrical conductivity (C_1) of the solution was recorded using a conductivity bridge. The second sample was boiled at 100 °C for 10 min, and the conductivity was also measured (C_2). The formula: MSI (%) = $[1- (C_1 \div C_2)] \times 100$ was applied for calculating the MSI. Excluding the midrib, fresh 2 cm-diameter fully-expanded leaf discs (n=10) were used to determine the relative water contents (RWC). The discs were weighed (fresh mass; FM) and immediately floated on double-distilled water in Petri dishes for 24 h, in the dark, to saturate them with water. Any adhering water was blotted dry and the turgid mass (TM) was measured. The dry mass (DM) was recorded after dehydrating the discs at 70 °C for 48 h. The RWC was then calculated using the formula of Hayat *et al.* (2007) as follows: RWC (%) = $[(FM - DM) \div (TM - DM)] \times 100$.

Determination of elements (Macro and micronutrients)

Percentage of nitrogen, phosphorus, potassium, iron, manganese and zinc in wheat plants were determined using a Perkin-Elmer Model 3300 Atomic Absorption Spectrophotometer (Chapman and Pratt, 1961) after digesting the plant material in a mixture of concentrated sulfuric acid and perchloric acids. K^+ ion contents (mg g^{-1} DW) were assessed using a Perkin-Elmer Model 52-A Flame Photometer (Glenbrook *et al.*, 1982).

Anatomical Study

During the first season 90 days after sowing, samples were taken for anatomical study. Specimens (0.5 cm in length) from the middle part of the flag leaf were killed and fixed in an FAA solution (10 ml formalin+5 ml glacial acetic acid+50 ml ethyl-alcohol 95%+35 ml distilled water) for 72 hours. Thereafter, samples were washed in 50% ethyl alcohol, dehydrated, cleared in n-butyl alcohol series and embedded in paraffin wax of 56-58 °C m.p. Cross sections of 20µ thick were cut, using a rotary microtom, adhered to slides by Haupt's adhesive. Slides were then stained with the Crystal violet erythrosine combination, cleared in carbol xylene and mounted in Canada balsam. Slides were microscopically analyzed and sections were microphotographed. An average of five readings was calculated by using a micrometer eyepiece (Nassar and El-Sahhar, 1998).

Statistical Analysis

All data were subjected to analysis of variance (ANOVA) for a randomized complete blocks design, after testing for homogeneity of error variances according to the procedure outlined by Gomez and Gomez (1984). Data from two seasons was analyzed separately and significant differences between treatments were compared at $P \leq 0.05$.

Results and Discussion

Growth attributes

Mineral fertilizers at 75% NPK combination with biofertilizer Butassine N increased plant height, weight plant^{-1}, dry weight

plant^{-1} and number tillers plant^{-1} which scored greater values in comparison to the other treatments (Table 2). These increases were 2.56 and 2.56%, 37.07 and 40.57%, 24.08 and 29.26% and 39.21 and 44.53% for plant height, fresh weight plant^{-1}, dry weight plant^{-1} and number of tillers plant^{-1} at the first and second seasons, respectively compared with control (100% NPK and non-inoculated). It is clear from these results that application of Butassine N+75 NPK increased all growth characters of wheat plant. This increase in plant height may be attributed to the enhancement of cell elongation or cell division. might also be, due to N stimulating amino acids which are the building blocks of proteins. Amino acids are used in forming protoplasm, the site for cell division and thus for plant growth and development.

In addition, K is vital for plant growth because K is known to be an enzyme activator that promotes metabolism, which lead to increase in dry matter in leaves of wheat plant. These results are in agreement with those of Harridy and Amara (1998) and Shaalan *et al.* (2001). Soil microbes are also able to enhance the availability of different nutrients including N, P and micronutrients. These results are in agreement with those obtained by Singh *et al*., (1997); Mohiuddin *et al.,* (2000); Das *et al*., (2001) and Zahran *et al*., (2002) on wheat plants.

Anatomical structure stem

The highest increase (14.79%) in diameter of stem was obtained by 75% of NPK application and Butassine N application (Table 3; Fig. 1). This result was due to the increase of thickness of seclerenchymatous tissue (50%) by increasing cell number (16.67%) and diameter (5.38%), length and width of vascular bundles (50 and 55.56%, respectively), average diameter of meta-xylem vessel (10.72 %) as compared to the control (100 % NPK)**.**

Yield and its components

Means in (Table 4) indicate that superiority in number of spikes plant^{-1}, number of spikeletes spike^{-1}, spike length, 1000-grain weight and grain yield/ha^{-1} were achieved when sprayed with Butassine N+75% NPK. These increases were 41.75 and 40.14% in number of spikes plant^{-1}, 4.47 and 4.65% in number of spikeletes spike^{-1}, 8.44 and 3.84% in spike length, 10.30 and 10.35% in1000-grain weight and 18.02 and 14.95% in grain yield per fed compared to the control treatment (100% NPK and non-inoculated) in the both seasons, respectively.

Such increase in yield and its components due to application of Butassine N combined with full NPK or Biogen combined with full NPK might be due to the role of biofertilizer Biogen (*Azotobacter*) in enhancing soil biological activity, which improved nutrient mobilization from organic and chemical sources. Also, biofertilizer plays a significant role in regulating the dynamics of organic matter decomposition and the availability of plant nutrients and in increasing nitrogen fixation. In this order, Sharief *et al.,* (1998); El-Garhi *et al*., (2007) and Bahrani *et al.,* (2010) reported that positive effect on yield and yield components of wheat when inoculated with biofertilizer. In the same trend, Khavazi *et al.,* (2005) found that yield improvements of more than 20% have been observed for wheat as a result of application of *Azotobacter* and *Azospirillum* inoculums.

Chlorophyll content a, b and carotenoids

The application of mineral fertilizers with biofertilizers on wheat led to increased chlorophyll content especially by 75%

NPK+biofertilizer (Butassine N) followed by 75% NPK+Biogen as compared to the control (100% NPK). These increases were 2.83 and 1.11% in chlorophyll a, 32 and 5.79% in chlorophyll b and 5.85 and 7.17% in carotenoids, at the first and second seasons, respectively as compared to the control. Chlorophylls were less with 50% NPK combination with Biogen or Butassine N than 100% NPK. The results in (Table 5) show that application of biofertilizers increased chlorophyll a, b and this increase was due to increasing of photosynthesis process. Rajendran *et al.,* (2008) reported that using of biofertilizers such as rhizobium and bacillus could increase leaf chlorophyll content.

Total soluble sugar (TSS (mg/g)), proline (mg/g) and protein%

TSS% in wheat treated with of 75% NPK combination with Biogen or Butassine N was less than the control (Table 5). However, both the treatments 50% NPK combination with Biogen or Butassine N had greater TSS% than the control. At the first season these increases were 28.06 and 7.09%, respectively as compared to the control. The second season had the same trend. The proline content in plants treated with 75% NPK combination with Biogen or Butassine N was less than the control. However, the application of 50% NPK combination with Biogen or Butassine N increased content of proline by 25.0 and 12.5%, respectively compared to the untreated plants. In the first season, the protein percentage was greater with 75% NPK+Biogen or Butassine N, compared to 100% NPK. The wheat plants treated with 50% NPK+ Biogen or Butassine N had less protein than 100% NPK.

These results are accordance with those obtained by Khan *et al.*, (2007). Rana *et al.* (2012) reported enhancement of 18.6% in protein content with biofertilizer inoculation in wheat. It has been found that in case of an adequate supply of N in the soil, leaf senescence is slower and the plant is able to supply its seeds with N and photoassimilate for a longer period, which results in higher protein content and grain yield (Azeez, 2009 and Abedi *et al*., 2010).

Table.1 Some physical and chemical properties of soil samples collected from the experimental locations during 2013/2014 and 2014/2015 seasons. *

Characters	2013/2014	2014/2015
Physical analysis		
Clay (%)	11.40	10.00
Course sand (%)	49.30	49.50
Fine sand (%)	25.80	25.60
Silt (%)	13.50	14.90
Soil texture	Sand Loamy	Sand Loamy
Chemical analysis		
PH 1: 2.5	7.10	7.50
EC 1: 2.5 ds/m	6.35	6.85
Calcium carbonate (%)	3.90	4.09
Organic matter (%)	0.72	0.76
N (ppm)	15.84	16.75
P (ppm)	7.66	8.10
K (ppm)	1.10	1.15

*All analyses were done in Center Lab of Soil, Water and Plant Analysis (Iso-17025) Faculty of Agriculture, Fayoum University, Egypt.

Table.2 The effect of mineral fertilizers alone or in combination with biofertilizers on growth characters of wheat at 2013/2014 and 2014/2015 seasons

Treatments	Characters			
	Plant height (cm)	FW plant^{-1} (g)	DW plant^{-1} (g)	No. tiller plant^{-1}
	2013/2014 Season			
100 % NPK	91.00	6.77	2.45	5.51
75% % NPK+ Bi* inoculated	91.00	7.85	2.57	7.03
75 % NPK + Bu foliar**	93.33	9.28	3.04	7.67
50 % NPK + Bi inoculated	83.67	6.59	2.29	4.05
50 % NPK + Bu foliar	89.67	4.44	1.42	5.00
LSD (5%)	**3.41**	**0.89**	**0.27**	**1.93**
	2014/2015 Season			
100 % NPK	90.57	6.31	2.05	5.21
75% % NPK+ Bi inoculated	90.53	7.43	2.17	6.95
75 % NPK + Bu foliar	92.89	8.87	2.65	7.53
50 % NPK + Bi inoculated	83.43	6.17	1.81	4.03
50 % NPK + Bu foliar	89.13	4.03	0.97	4.95
LSD (5%)	**3.21**	**0.90**	**0.36**	**1.35**

*Bi=Biogen and **Bu= Butassine N

Table.3 The effect of mineral fertilizers alone or in combination with biofertilizers on anatomical structure of wheat stem

Treatments	Characters					
	Seclerenchymatous tissue			Ground tissue		
	Thickness (µ)	No. of cell layers	Av. diameter of cells	Thickness (µ)	No. of cell layers	Av. diameter of cells
100 % NPK	75.0	6	11.9	460	9	85.0
75% % NPK+ Bi* inoculated	100.0	7	15.6	600	9	90.0
75 % NPK + Bu foliar**	112.5	7	12.5	410	7	62.5
50 % NPK + Bi inoculated	100.0	5	13.8	450	9	72.5
50 % NPK + Bu foliar	87.5	7	13.8	330	9	67.5
	Vascular bundles			Av. diameter of mx vessels (µ)	Av. diameter of hollow pith (µ)	Section Diameter (µ)
	Length (µ)	Width (µ)	Number			
100 % NPK	150.0	112.5	33	33.1	2037.5	3212.5
75% % NPK+ Bi inoculated	157.5	126.7	28	28.1	1687.5	3300.0
75 % NPK + Bu foliar	225.0	175.0	33	36.7	1662.5	3687.5
50 % NPK + Bi inoculated	200.0	100.0	28	33.8	1400.0	2600.0
50 % NPK + Bu foliar	162.5	118.8	26	30.3	1500.0	2750.0

*Bi=Biogen and **Bu= Butassine N

Table.4 The effect of mineral fertilizers alone or in combination with biofertilizers on yield and its components of wheat at 2013/2014 and 2014/2015 seasons

Treatments	Characters				
	No. spikes plant^{-1}	No. spikeletes Spike^{-1}	Spike length (cm)	1000-grain weight (g)	Grain yield (t per fed)
	2013/2014 Season				
100 % NPK	5.03	15.00	8.33	39.21	2.22
75% % NPK+ Bi* inoculated	6.91	15.00	9.00	41.89	2.42
75 % NPK + Bu foliar**	7.13	15.67	9.00	43.25	2.62
50 % NPK + Bi inoculated	4.01	14.00	7.33	38.11	2.10
50 % NPK + Bu foliar	4.89	14.67	7.33	36.93	2.02
LSD (5%)	**1.83**	**1.85**	**0.97**	**3.23**	**0.25**
	2014/2015 Season				
100 % NPK	5.01	14.61	8.07	39.03	2.14
75% % NPK+ Bi inoculated	6.25	14.49	8.69	41.71	2.30
75 % NPK + Bu foliar	7.03	15.29	8.38	43.07	2.46
50 % NPK + Bi inoculated	4.03	13.63	7.09	37.95	2.14
50 % NPK + Bu foliar	4.73	14.27	7.11	36.79	2.02
LSD (5%)	**1.71**	**1.84**	**0.59**	**3.21**	**0.21**

*Bi=Biogen and **Bu= Butassine N

Table.5 The effect of mineral fertilizers alone or in combination with biofertilizers on chemical characters of wheat at 2013/2014 and 2014/2015 seasons

Treatments	Characters							
	Chl a (mg/g)	Chl b (mg/g)	Carotenoids (mg/g)	TSS (mg/g)	Proline (mg/g)	Protein %	RWC %	MSI %
	2013/2014 Season							
100 % NPK	7.78	6.53	2.05	3.10	0.64	9.01	72.22	77.66
75% % NPK+ Bi* inoculated	7.78	6.60	2.07	2.50	0.56	9.25	72.29	77.65
75 % NPK + Bu foliar**	8.00	6.85	2.17	2.63	0.52	10.01	75.82	80.40
50 % NPK + Bi inoculated	5.72	4.65	1.91	3.97	0.80	8.23	71.32	75.77
50 % NPK + Bu foliar	6.67	5.57	1.91	3.32	0.72	7.73	72.05	77.07
LSD (5%)	**2.41**	**2.11**	**0.41**	**0.32**	**0.13**	**0.93**	**1.74**	**3.72**
	2014/2015 Season							
100 % NPK	7.19	6.21	1.95	3.05	0.63	8.91	71.13	76.67
75% % NPK+ Bi inoculated	7.21	6.29	1.93	2.43	0.51	9.13	71.21	76.63
75 % NPK + Bu foliar	7.27	6.57	2.09	2.29	0.47	9.95	74.79	79.35
50 % NPK + Bi inoculated	5.13	4.33	1.85	3.91	0.79	8.17	70.25	74.79
50 % NPK + Bu foliar	6.05	5.21	1.87	3.27	0.65	7.69	71.03	76.01
LSD (5%)	**2.27**	**2.15**	**0.39**	**0.33**	**0.17**	**0.93**	**1.78**	**3.65**

*Bi=Biogen and **Bu= Butassine N

Fig.1 Transverse sections of wheat as affected by mineral fertilizers alone or in combination with biofertilizers

treatments A: control (100% NPK), B: 75% NPK+Bi* inoculated C: 75% NPK+Bu** foliar, D: 50% NPK+Bi inoculated, E: 50% NPK+Bu foliar (gt= ground tissue, s= sclerenchyma and vb= vascular bundle). Scale bar= 491 µ

*Bi=Biogen and **Bu= Butassine N

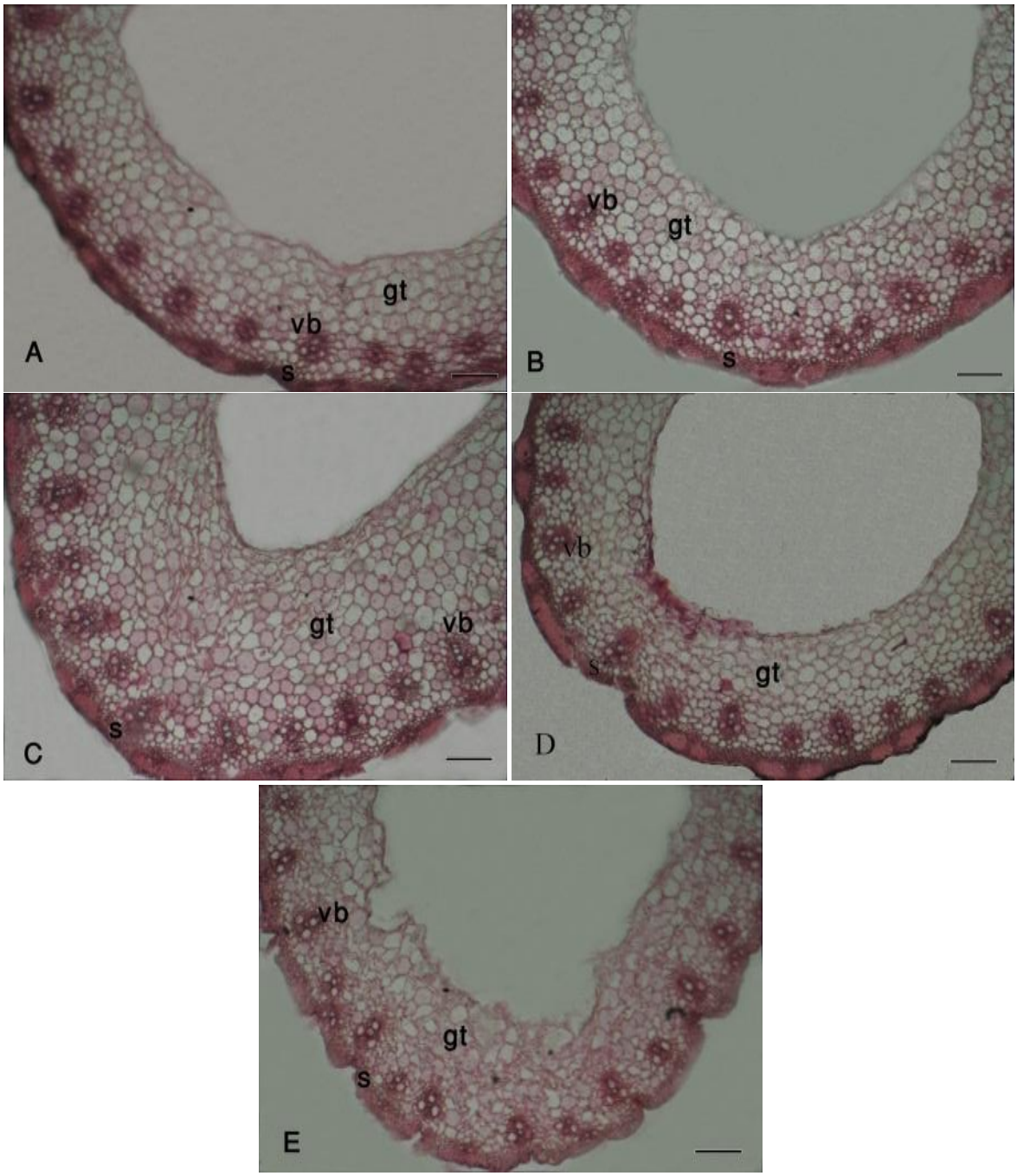

Table.6 The effect of mineral fertilizers alone or in combination with biofertilizers on mineral content of wheat at 2013/2014 and 2014/2015 seasons.

Treatments	Characters					
	N%	P%	K%	Fe%	Zn%	Mn%
	2013/2014 Season					
100 % NPK	4.88	7.74	30.23	1.04	0.75	0.12
75% % NPK+ Bi* inoculated	6.65	8.84	30.37	1.24	0.98	0.14
75 % NPK + Bu foliar**	7.33	9.63	32.06	1.43	1.30	0.17
50 % NPK + Bi inoculated	4.13	7.11	28.40	0.77	0.73	0.08
50 % NPK + Bu foliar	4.36	7.61	29.81	0.97	0.74	0.11
LSD (5%)	**0.67**	**0.79**	**1.65**	**0.17**	**0.15**	**0.03**
	2014/2015 Season					
100 % NPK	4.45	7.31	30.01	0.99	0.63	0.09
75% % NPK+ Bi inoculated	6.17	8.43	30.23	1.17	0.85	0.13
75 % NPK + Bu foliar	6.89	9.27	31.89	1.33	1.19	0.17
50 % NPK + Bi inoculated	3.63	6.79	28.25	0.71	0.61	0.05
50 % NPK + Bu foliar	3.81	7.25	29.67	0.95	0.67	0.11
LSD (5%)	**0.71**	**0.83**	**1.63**	**0.13**	**0.16**	**0.04**

*Bi=Biogen and **Bu= Butassine N

Macro and micro elements

As shown in (Table 6) the application of mineral fertilizers alone or interaction with biofertilizers effected the percentage of elements in dry leaves. The application of 75% NPK+Butassine N increased N, P, K, Fe, Zn and Mn% in dry leaves. These increments were 50.20 and 54.83%, 24.42 and 26.81%, 6.05 and 6.26%, 37.50 and 34.34%, 73.33 and 88.89% and 41.67 and 88.89% in both seasons, respectively as compared to the 100% NPK.

According to obtained results, it could be suggested that using microorganisms is very useful if the environment conditions are optimum. These microorganisms need organic matter in the soil to survive (Javaid and Shah, 2010). The results might be due to role of biofertilizer in improved physiological functions in wheat plants.

In conclusion, the use of biofertilizers became important to minimize the environmental pollution, resulting from using mineral fertilizers and to improve the yield and quality of wheat plants needed. Application of biofertilizers combined with mineral fertilizers significantly increased all characters except total soluble sugar (TSS) and proline compared to the control (100% NPK). We recommended using Butassine N with 75% NPK. We could save 25% of the recommended dose of NPK when wheat plants are cultivated in newly reclaimed lands.

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