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Dual Inoculation with *Azotobacter chroococcum* MF135558 and *Klebsiella oxytoca* MF135559 Enhance the Growth and Yield of Wheat Plant and Reduce N-Fertilizers Usage

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ABSTRACT

A field experiment was carried out in the farm of faculty of Agriculture, Mansoura University, Mansoura, Egypt during the winter season of 2016 to study the effect of bio-fertilization under different level of nitrogen (75% and 50% of the full dose) on the growth and yield of wheat plant. Obtained results showed that the microbial inoculation leads to a significant increase in growth parameters (plant heights, number of tillers, leaves and dry weights). Also, microbial inoculation leads to a significant increase in NPK-contents as responded to all treatments under investigation in all stages of plant growth. The obtained results show a significant increase in yield parameters and its components (spike length, spike weight , number of grains / spike, weight of 1000 grains, grain and straw yield and NPK contents in grains and straw) as affected by microbial inoculation. The treatment T 9 (*Az. chroococcum* + *K. oxytoca* + 75 % dose of N) gave the highest value of grains and straw yield (19.61 and 3.13 ton/fed., respectively) with an increase by 21.87% and 19.01%, respectively. Also, the bio-fertilization has a pronounced increase in microbial count in comparison with the mineral fertilization. The present study recommend by the possibility of using the dual bacterial inoculum which containing *Azotobacter chroococcum* MF135558 and *Klebsiella oxytoca* MF135559 in the presence of 75% dose of N for enhancing growth and yield of wheat plants and reduce chemical fertilizers usage.

Keywords; Wheat (*Triticum aestivum*), *Azotobacter chroococcum* MF135558**,** *Klebsiella oxytoca* MF135559*, Rhizobium pusense* MF135560 and Cyanobacteria

INTRODUCTION

Wheat (*Triticum aestivum* L.), a member of the Poaceae family, is an important cereal crop in Egypt. Increasing wheat production is an essential national target to fill the gap between production and consumption (Tawfik *et al*., 2006 and James 2009). There is a need to use chemical fertilizers to cope with continuous growth of the human population and increase food production. Chemical fertilizers provide essential nutrients for plants mainly nitrogen, phosphorus and potassium for increasing the yield, but they cause several health hazards. The continuous use of chemical fertilizer in agricultural fields causes the soil pollution and enduces environmental pollution, furthermore, it is damaging to human health (Trujillo-tapia and Ramírez-fuentes, 2016). Recently, the use of bio- fertilizers have considered a promising alternative for the agricultural production and one of the best modern tools for providing nutrients to plants (Bhattacharjee and Dey, 2014). Bio-fertilizers include a wide range of soil microbes, including those, which fix nitrogen, solubilize phosphate, release potassium, produce phytohormones and promote plant growth. (Miransari, 2011). These microbes can provide essential nutrients for plants mainly nitrogen, phosphorus and potassium and can improve the growth and yield of plants and reduce the use of chemical fertilizers (Bashan and de- Bashan, 2005). Thus, focus on studying the effect of microbial inoculation with some PGPR strains individually and in mixture form on growth and yield of wheat plant was the purpose of this work.

MATERIALS AND METHODS

Bacterial strains

Azotobacter chroococcum MF135558 (N2-fixing bacteria)*, Klebsiella oxytoca* MF135559 (P-solubilizing bacteria) and *Rhizobium pusense* MF135560 (K-releasing bacteria) were isolated and identified by Hauka *et al*., (2017).

Cyanobacterial strains

Seven local cyanobacterial strains and another one isolate were supplied from Agric. Res. Microbiol. Dept., Soils, Water & Environ. Res. Inst., A.R.C., Giza, Egypt. The cyanobacterial strains Viz. *Anabaena fertilissma* (I), *Anabaena fertilissma* (II), *Anabaena vriabilis* (I), *Anabaena vriabilis* (II) *Anabaena oryzae*, *Anabaena circinalis*, *Nostoc muscorum* and *Anabaena* sp.

Detection of IAA

Each strain was grown in its specific medium supplemented with 0.1% tryptophan according to Ahmad *et al*. (2005). Production of IAA in the supernatant was assayed using the PC method, as described by Pilet and Chollet (1970). This method was shown to be most sensitive and most specific (Glickmann and Dessaux, 1995).

Methods of inoculation

The individual bacterial strain was grown to maximum density at appropriate period of time up to 4.3×107 cfu/ml for *Azotobacter chroococcum* MF135558 in Modified Ashby's medium (Abd El-Malek and Ishac, 1968), 9.3×108 cfu/ml for *Klebsiella oxytoca* MF135559 in Pikovskaya's medium (Pikovskaya, 1948), 3.3×108 cfu/ml for *Rhizobium pusense* MF135560 in modified Alexandroov's medium (Zahra, 1969). Equal volumes were mixed to make the mixtures of inocula. Seeds were soaked in microbial inoculants for 30 min. Arabic gum (16%) was used as an adhesive agent. An extra of 10 ml culture was added to each plant.

Soil samples

Soil samples were collected at the periods of 30, 60, 90 and 120 days after sowing (DAS) from rhizosphere of wheat plants for microbial count, the initial physical, chemical and biological properties of the experimental soil was showed in Table 1.

Table 1. Initial physical, chemical and biological properties of the experimental soil

Properties	
Texture analysis	
Sand (%)	7.75
Silt (%)	53.70
Clay (%)	38.55
Soil texture	Silty clay loam
Organic matter (%)	1.14
pH soil-water suspension ratio (1:2.5)	8.15
EC(dsm^{-1}) soil-water extract ratio (1:5)	1.26
Soluble cations (meq/L)	
Ca^{++}	7.45
Mg^{++}	2.15
Na^{+}	3.22
K^{+}	0.20
Soluble anions (meq/L)	
CO_3^{--}	0.00
HCO_3^{-}	3.20
Cl^{-}	4.10
$SO4^{--}$	5.72
Available N (ppm)	18.25
Available P (ppm)	7.58
Available K (ppm)	156.0
Bacterial count	
Total bacterial count	2.01×10^6
Azotobacter spp.	1.34×10^4
Phosphate solubilizing bacteria	7.67×10^4
Potassium releasing bacteria	22.7×10^4

Experimental design

A field experiment was carried out in Fac. of Agri., Mansoura Univ., Egypt during the winter season of 2016 to study the effect of bio-fertilization on the growth and yield of wheat plant. Wheat (*Triticum aestivum*, L.) cv. Gemmiza 11 grains were kindly obtained from Agronomy Dept., Fac. of Agri., Mansoura Univ. Grains were sowed at 15th November 2016. The experiment was arranged as a completely randomized design in three replicates. Nitrogen fertilizer in the form of urea (46 %N) at the ratio of 37.5 and 56.25 Kg N/fed which represent 50 and 75 % of the final recommended doses, were applied in two equal doses, before the first and second irrigations. Calcium super phosphate (12.5 % P_2O_5) at the ratio of 150 kg/fed was applied during soil preparation. Potassium fertilizer in the form of potassium sulphate (48 % K_2O) at the ratio of 50 kg/fed was applied with the first dose of nitrogen fertilizer. The treatments were as follows

T1	Full dose of N	
T2	75 % dose of N	
T3	50 % dose of N	
T4	*Azotobacter chroococcum* MF135558	75 % of N dose
T5	Cyanobacterial Mixture (*N. muscorum, A. fertilissma* (II), *A. vriabilis* (II) and *A. oryzae*)	
T6	*Klebsiella oxytoca* MF135559	
T7	*Rhizobium pusense* MF135560	
T8	*Az. chroococcum* MF135558+ Cyanobacterial Mixture	
T9	*Az. chroococcum* MF135558+ *K. oxytoca* MF135559	
T10	*Az. chroococcum* MF135558+ *R. pusense* MF135560	
T11	Cyanobacterial Mixture + *K. oxytoca* MF135559	
T12	Cyanobacterial Mixture + *R. pusense* MF135560	
T13	*K. oxytoca* MF135559 + *R. pusense* MF135560	
T14	*Az. chroococcum* MF135558 + Cyanobacterial Mixture + *K. oxytoca* MF135559 + *R. pusense* MF135560	
T15	*Azotobacter chroococcum* MF135558	50 % of N dose
T16	Cyanobacterial Mixture (*N. muscorum, A. fertilissma* (II), *A. vriabilis* (II) and *A. oryzae*)	
T17	*Klebsiella oxytoca* MF135559	
T18	*Rhizobium pusense* MF135560	
T19	*Az. chroococcum* MF135558+ Cyanobacterial Mixture	
T20	*Az. chroococcum* MF135558+ *K. oxytoca* MF135559	
T21	*Az. chroococcum* MF135558+ *R. pusense* MF135560	
T22	Cyanobacterial Mixture + *K. oxytoca* MF135559	
T23	Cyanobacterial Mixture + *R. pusense* MF135560	
T24	*K. oxytoca* MF135559 + *R. pusense* MF135560	
T25	*Az. chroococcum* MF135558 + Cyanobacterial Mixture + *K. oxytoca* MF135559 + *R. pusense* MF135560	

Wheat plant parameters

Samples were collected at 60, 90, 120 days after sowing (DAS) and at the harvest stages.

A) Growth parameters

Plant height (cm), number of leaves/plant, number of tillers/plant, fresh weight (g/plant), dry weight (g/plant) and NPK content in plant were measured.

B) Yield parameters

Spike length, spike weight, number of grains/spike, weight of 1000 grains, straw yield (t/fed), grain yield (ardab/fed) and NPK content in grains and straw were measured.

Phytochemical determinations

The finely powdered dry plant material were first digested with sulphoric-perchloric acids mixture according to the method cited in (piper, 1950).

A) Nitrogen

Total nitrogen content was determined calorimetrically at 420 nm by Nessler reagent (Lindner, 1944).

B) Phosphorus

Phosphorus content was determined calorimetrically at 660 nm by the method of Boltz and Mellon (1948) modified by Hemalatha *et al*. (2013).

C) Potassium

Potassium content was determined by atomic absorption spectroscopy (Jackson, 1973).

Microbial count determination

A) Total bacteria

Total bacterial count was done on nutrient agar medium (Skerman, 1967) using pour-plate method. Colony counts were obtained after three days of incubation at 30°C.

B) *Azotobacter* spp.

Azotobacter was counted on Ashby's medium (Abd-El-Malek and Ishac, 1968), using Most Probable Number (MPN) technique. Tubes were incubated at 30°C for 15-21 days. At the end of incubation period the presence of characteristic surface *Azotobacter* pellicle was checked.

C) Phosphate-solubilizing bacteria

Phosphate-solubilizing bacteria was counted on Pikovskaya's medium (Pikovskaya, 1948) by plate method. Clear zones around the colonies were recorded after 7 days incubation at 30°C.

D) Potassium- releasing bacteria

Potassium-releasing bacteria was counted on modified Alexandroov medium (Zahra, 1969) by plate method. Plates were incubated at 30°C for 5 days.

Statistical analysis

The obtained experimental data were statistically analyzed using COSTAT (2005) software of analysis of variance (Gomez and Gomez, 1984). The means were compared using Duncan multiple range test at p = 0.05 as outlined by Snedecor and Cochran (1980).

RESULTS AND DISCUSSION

Cyanobacteria

Results in Table 2 showed the most active cyanobacterial strains were *N. muscorum*, *A. fertilisima* II, *A. variabilis* II and *A. oryzae*. Their corresponding nitrogenase values were 37.86, 34.11, 34.11 and 23.16 µmole C_2H_2 /100 ml culture / day against 11,89, 7.06, 7.06 and 1.11 µmole C_2H_2 /100 ml culture / day for *Anabaena* sp., *A. fertilisima* , *A. variabilis* I and *A. circinalis*.

Table 2. Nitrogenase activity of N_2 fixing local cyanobacterial strains.

Cyanobacteria spp.	Nitrogenase activity µmole C_2H_2 /100 ml / day
Nostoc muscorum	37.86
Anabaena fertilissma (II)	34.11
Anabaena vriabilis (II)	34.11
Anabaena oryzae	23.16
Anabaena sp.	11.89
Anabaena vriabilis (I)	7.060
Anabaena fertilissma (I)	7.060
Anabaena circinalis	1.110

The highest nitrogenase activity (N_2- fixing capacity) was due to *N. muscorum* (37.86 µmole C_2H_2 /100 ml culture / day), while the lowest value of 1.11 µmole C_2H_2 /100 ml culture / day was due to *A. circinalis*.

Indole acetic acid (IAA) production

Table 3 showed the production of indole acetic acid (IAA) by *Azotobacter chroococcum* MF135558, *Klebsiella oxytoca MF135559* and *Rhizobium pusense MF135560* up to 8 days of incubations. As shown from the results, all bacterial strains could produce IAA but not in equal efficacy. For *Azotobacter chroococcum* MF135558, IAA was not detected in the first and the second days of incubations, however IAA was detected from the 3rd day and gradually increased up to the 7^{th} day (20.15 µg/ml) then the production decreased. For *Klebsiella oxytoca MF135559*, IAA was detected from the first day and gradually increased up to the 6^{th} day (30.24 µg/ml) then the production decreased. For *Rhizobium pusense* MF135560, the maximum IAA production was observed in the 1^{st} day (58.05 µg/ml) then the production gradually decreased. Results are in agreement with those obtained by Ahmad *et al*. (2005) ; Jha and Kumar (2007) ; Celloto *et al*. (2012) ; Ali *et al*. (2015) ; Bergottini *et al*. (2015) and Mondal *et al*. (2017).

Table 3. IAA production by bacterial strains

Incubation time (days)	IAA (µg/ml) *A. chroococcum* MF135558	*K. oxytoca* MF135559	*R. pusense* MF135560
1	ND	6.78	58.05
2	ND	11.33	33.78
3	2.33	15.01	30.05
4	4.19	16.92	27.51
5	8.87	19.24	20.51
6	18.05	30.24	17.24
7	20.15	22.05	13.96
8	15.01	5.33	9.33

ND: Not detectable

Growth parameters of wheat

The effect of chemical fertilization and microbial inoculation of wheat plants on plant heights (cm), number of tillers and leaves / plant and dry weights (g) / plant is presented in Table 4. Data revealed that the plant's heights of wheat plants increased by increasing N-fertilizer up to the full dose of N. In general, bacterial inoculation led to significant increase in plant heights during all stages of plant growth (60, 90 and 120 DAS). The treatment T6 (*K. oxytoca* + 75 % dose of N) was the best treatment in increasing the plant height (108.46 cm), followed by T14 (mixture + 75 % dose of N) which gave 107.26 cm at 120 DAS. Also, treatments number T8, T11, T13, T10, T25, T17 and T9 gave values 106.80, 106.63, 105.36, 105.20, 104.90 and 103.26 which were better than the value 102.86 cm of T1 (control, full dose of N). At the same trend, there was no significance between treatments number T9, T12, T5, T24, T22, T23, T4, T19, T20 and T16 which gave values 103.26, 102.60, 102.03, 102.00, 101.66, 101.16, 100.66, 100.10, 99.20 and 99.03 cm, respectively and T1 (control, full dose of N). The treatments number T7, T15 and T18 gave shorter plants 97.80, 97.50 and 95.50 cm, respectively in comparison to T1 (control, full dose of N).

Also, data indicated that the treatment T9 (*Az. chroococcum* + *K. oxytoca* + 75 % dose of N) was the best treatment in increasing tillering 5.33 and 8.00 tillers/ plant at 60 and 90 DAS, respectively followed by T6 (*K. oxytoca* + 75 % dose of N) which gave 5.00 and 7.33 tillers / plant at 60 and 90 DAS, respectively. On the other hand, the treatment T4 (*Az. chroococcum* + 75 % dose of N) was the best treatment in increasing number of leaves per plant (29.00 leaves / plant) followed by T6 (*K. oxytoca* + 75 % dose of N) and which gave 27.33 leaves / plant at 90 DAS. Generally, applying bacterial inoculation with 75% dose of N has enhancing tillering and number of leaves better than inoculation with 50% dose of N. Also, data revealed that the increase in fresh and dry weights of wheat plants was at the same trend, the increase of nitrogen fertilization leads to pronounced and significant increase in fresh and dry weights. Also, the effect of bio-fertilization was pronounced. The treatments T9, T6 and T4 enhanced wheat growth significantly and gave values greater than T1 (control, full dose of N), respectively, at 120 DAS. The highest increase in dry weights (36.58 g/plant) was scored by wheat plants inoculated with T9 (*Az. chroococcum* + *K. oxytoca* + 75 % dose of N) at 120 DAS. The data also showed that at 120 DAS there were no significant effect in dry weights between T1 (control, full dose of N) and T5, T13, T17, T10, T11, T20, T8 and T12 which gave values of 29.28, 28.66, 28.48, 28.36, 28.33, 28.15, 27.96 and 27.71 g/plant, respectively. The beneficial effect of the used rhizobacteria can be attributed to nitrogen fixation, nutrients availability and the ability of the used bacteria to exert high quantities of auxins like indole acetic acid, which have a pronounced effects on plant growth. Results are in agreement with those obtained by Iniguez *et al*. (2004) ; Sachdev *et al*. (2009) ; Abou-Aly and Mady (2009) ; Saber *et al*. (2012) and Desoky and El-sayed (2016).

Table 4. Growth parameters of wheat plants as affected by bio-fertilization treatments

Treatments	Plant height (cm)			Number of tillers /plant		Number of leaves /plant		Dry weight (g/plant)		
	60 DAS	90 DAS	120 DAS	60 DAS	90 DAS	60 DAS	90 DAS	60 DAS	90DAS	120 DAS
T1	57.63^{ab*}	89.66^{c-e}	102.86^{c-h}	4.33^{a-d*}	5.66^{cd}	20.66^{a}	23.33^{d}	4.33^{a}	8.20^{h}	28.31^{de}
T2	41.73^{j-l}	81.43^{gh}	96.00^{kl}	3.66^{c-f}	4.00^{e-g}	13.33^{f-j}	17.66^{h-l}	1.77^{c-g}	5.77^{jk}	21.02^{i}
T3	33.43^{o}	68.93^{m}	89.86^{l}	2.33^{g}	3.33^{g}	8.66^{l}	14.66^{m}	0.84^{i}	4.18^{l}	17.18^{k}
T4	45.03^{f-i}	88.90^{de}	100.66^{f-j}	4.33^{a-d}	6.00^{bc}	15.00^{d-g}	29.00^{a}	2.33^{c}	12.65^{c}	31.10^{c}
T5	48.76^{c-e}	86.03^{ef}	102.03^{e-h}	4.00^{b-e}	6.00^{bc}	19.33^{ab}	23.66^{de}	2.27^{cd}	9.07^{g}	29.28^{d}
T6	57.73^{ab}	96.83^{a}	108.46^{a}	5.00^{ab}	7.33^{ab}	19.33^{ab}	27.33^{b}	4.21^{a}	14.51^{a}	33.99^{b}
T7	45.76^{e-g}	84.30^{fg}	97.80^{i-k}	3.00^{e-g}	3.66^{fg}	14.33^{e-h}	17.33^{h-k}	1.89^{c-f}	7.56^{i}	23.89^{fg}
T8	54.60^{b}	95.33^{ab}	106.80^{a-c}	4.33^{a-d}	5.66^{cd}	17.66^{b-d}	21.33^{ef}	3.79^{a}	9.79^{ef}	27.96^{de}
T9	47.43^{d-f}	95.13^{ab}	103.26^{c-g}	5.33^{a}	8.00^{a}	17.66^{b-d}	26.66^{bc}	2.19^{cd}	13.30^{b}	36.58^{a}
T10	51.20^{c}	91.80^{b-d}	105.20^{a-e}	4.33^{a-d}	5.33^{c-e}	15.00^{d-g}	25.33^{c}	1.96^{c-e}	9.25^{fg}	28.36^{de}
T11	56.03^{ab}	93.00^{a-d}	106.63^{a-d}	4.66^{a-c}	5.00^{c-f}	16.33^{c-e}	23.66^{d}	2.31^{cd}	9.14^{fg}	28.33^{de}
T12	55.26^{b}	93.63^{a-c}	102.60^{d-h}	4.00^{b-e}	4.00^{e-g}	18.00^{a-c}	23.33^{d}	3.81^{a}	9.95^{e}	27.71^{e}
T13	58.60^{a}	87.33^{ef}	105.36^{a-e}	4.33^{a-d}	6.00^{bc}	17.33^{b-d}	21.33^{ef}	4.17^{a}	11.70^{d}	28.66^{de}
T14	51.53^{c}	89.36^{c-e}	107.26^{ab}	4.66^{a-c}	4.66^{c-e}	19.66^{ab}	22.33^{de}	3.20^{b}	9.80^{ef}	24.41^{fg}
T15	40.43^{kl}	77.03^{i-k}	97.50^{jk}	2.66^{fg}	3.66^{fg}	10.33^{kl}	18.00^{g-i}	1.29^{f-i}	5.70^{jk}	20.65^{i}
T16	43.63^{g-k}	80.33^{g-i}	99.03^{h-k}	3.33^{d-g}	4.00^{e-g}	12.33^{g-k}	18.00^{g-i}	1.36^{e-i}	5.79^{jk}	22.48^{h}
T17	49.56^{cd}	78.66^{h-j}	103.70^{b-f}	3.66^{c-f}	4.33^{d-g}	16.00^{c-f}	18.66^{gh}	1.68^{d-g}	5.37^{jk}	28.48^{de}
T18	39.26^{lm}	71.70^{lm}	95.50^{kl}	3.00^{e-g}	3.33^{g}	13.66^{e-i}	16.66^{i-l}	0.93^{hi}	4.24^{l}	18.91^{j}
T19	45.30^{f-h}	74.63^{j-l}	100.10^{f-j}	2.66^{fg}	4.33^{d-g}	12.33^{g-k}	16.00^{k-m}	1.55^{e-h}	6.07^{j}	24.47^{fg}
T20	41.90^{i-l}	72.93^{k-m}	99.20^{g-k}	3.33^{d-g}	4.66^{c-g}	12.00^{h-k}	18.00^{g-j}	1.20^{g-i}	5.86^{j}	28.15^{de}
T21	35.06^{no}	74.66^{j-l}	103.46^{b-f}	2.66^{fg}	4.33^{d-g}	12.33^{g-k}	21.33^{ef}	0.95^{hi}	5.64^{jk}	25.34^{f}
T22	43.83^{g-j}	74.50^{j-l}	101.66^{e-i}	3.66^{c-f}	5.00^{c-f}	11.00^{i-l}	19.66^{fg}	1.16^{g-i}	5.45^{jk}	23.58^{gh}
T23	42.30^{h-l}	76.50^{i-k}	101.16^{e-j}	2.66^{fg}	4.00^{e-g}	12.33^{g-k}	15.33^{lm}	1.29^{f-i}	5.48^{jk}	22.37^{h}
T24	37.16^{mn}	73.50^{kl}	102.00^{e-h}	2.66^{fg}	4.00^{e-g}	10.33^{j-l}	16.33^{j-m}	1.53^{e-h}	5.91^{j}	24.50^{fg}
T25	36.30^{m-o}	73.00^{k-m}	104.90^{a-e}	3.00^{e-g}	3.66^{fg}	9.00^{l}	18.33^{g-i}	0.97^{hi}	5.11^{k}	20.43^{i}

***Means followed by different letter(s) in the column are significantly different**

NPK content in wheat plants

The effect of chemical fertilization and microbial inoculation application of wheat plants on NPK content after 60, 90 and 120 DAS is presented in Table 5. Data shows that the N-contents were significantly responded to all treatments under investigation. The highest values in N-content (15.37, 37.02 and 58.43 mg/g dry weight after 60, 90 and 120 DAS, respectively), was scored by wheat plants inoculated with T9 (*Az. chroococcum* + *K. oxytoca* + 75 % dose of N). Also, treatments number T4, T12, T5, T6, T23, T14, T16, T13 and T11 gave values of 49.64, 47.60, 41.62, 38.62, 38.17, 37.94, 36.59, 34.07 and 33.39 mg/g dry weight after 120 DAS, respectively, which were better than the value of T1 (control, full dose of N). There was no significance between treatments number T25, T8, T7 and T10 which gave values of 33.01, 30.12, 29.70 and 28.72 mg/g dry weight after 120 DAS, respectively, and T1 (control, full dose of N). However, the highest value in P-content (5.716 mg/g dry weight after 120 DAS) was scored by wheat plants inoculated with T13 (Mixture + 75 % dose of N). Also, treatments T12, T9 and T10 gave values (5.070, 4.526 and 4.320 mg/g dry weight after 120 DAS, respectively), which were better than the value of T1 (control). There was no significance between treatments T14 and T6 which gave values of 4.210 and 3.993 mg/g dry weight after 120 DAS, respectively, and T1 (control). On the other hand, the highest value in K-content (30.38 mg/g dry weight after 120 DAS) was scored by wheat plants inoculated with T5 (Cyanobacteria + 75 % dose of N). Also, treatments T9, T12, T10, T6, T7, T4, T11, T18, T13, T15, T8 and T24 gave values (29.53, 29.39, 28.47, 28.35, 27.69, 27.51, 27.29, 27.23, 26.28, 26.58 and 26.42

mg/g dry weight after 120 DAS, respectively), which were better than the value of T1 (control). There was no significance between the treatment (T19) which gave value (26.09 mg/g dry weight after 120 DAS) and T1 (control). In fact, the increase of nutrients content in plant can be explained by an enhancement of the biological N_2-fixation and/or production of organic acids to solubilize P and K and/or production of certain growth promoting substances, which positively affect root development and consequently their function in the uptake of both water and nutrients. Results are in agreement with those of Iniguez *et al*. (2004) ; Ghazal *et al*. (2011) ; Salim *et al*. (2013) and Desoky and El-sayed (2016).

Table 5. NPK content (mg/g dry weight) of wheat plants after 60, 90 and 120 days after sowing (DAS) as affected by bio-fertilization treatments

Treatments	N -content in plant (mg/g)			P -content in plant (mg/g)			K -content in plant (mg/g)		
	60DAS	90DAS	120 DAS	60DAS	90DAS	120 DAS	60DAS	90DAS	120 DAS
T1	11.82^{de*}	18.04^{hi}	30.72^{fg}	0.893^{e*}	1.783^{de}	3.983^{ef}	12.83^{cd*}	16.18^{h}	25.89^{i}
T2	8.16^{f-h}	16.03^{jk}	28.36^{gh}	0.696^{h-j}	1.566^{f}	3.313^{g}	9.40^{k}	15.62^{j}	20.85^{n}
T3	5.36^{j}	8.63^{n}	15.61^{l}	0.543^{k-m}	1.373^{h-l}	2.420^{jk}	7.29^{o}	12.65^{p}	18.67^{p}
T4	7.66^{f-i}	30.04^{b}	49.64^{b}	1.000^{d}	1.116^{m}	3.333^{g}	13.02^{c}	14.87^{l}	27.51^{de}
T5	8.29^{fg}	29.78^{bc}	41.62^{c}	1.106^{c}	1.293^{j-l}	3.850^{f}	12.52^{e}	14.93^{l}	30.38^{a}
T6	13.48^{a-d}	21.70^{de}	38.62^{d}	0.806^{e-g}	1.723^{e}	3.993^{ef}	12.24^{f}	20.24^{d}	28.35^{c}
T7	7.50^{f-i}	20.70^{d-f}	29.70^{g}	0.530^{lm}	1.783^{de}	3.323^{g}	9.38^{k}	16.13^{h}	27.69^{d}
T8	15.10^{ab}	17.95^{h-j}	30.12^{g}	1.470^{a}	1.536^{fg}	3.370^{g}	11.01^{h}	15.91^{i}	26.46^{gh}
T9	15.37^{a}	37.02^{a}	58.43^{a}	0.880^{ef}	1.483^{f-i}	4.526^{c}	14.74^{a}	16.29^{h}	29.53^{b}
T10	13.66^{a-d}	21.87^{de}	28.72^{g}	1.266^{b}	1.910^{b-d}	4.320^{cd}	10.45^{i}	21.51^{b}	28.47^{c}
T11	14.75^{a-c}	22.49^{d}	33.39^{e}	1.003^{d}	1.806^{c-e}	3.773^{f}	14.54^{a}	20.44^{d}	27.29^{de}
T12	14.69^{a-c}	27.98^{c}	47.60^{b}	0.793^{f-h}	1.980^{b}	5.070^{b}	12.58^{e}	18.75^{e}	29.39^{b}
T13	12.12^{de}	21.15^{d-f}	34.07^{e}	0.766^{gh}	1.940^{bc}	5.716^{a}	14.54^{b}	21.10^{c}	26.82^{fg}
T14	13.25^{b-d}	19.15^{f-h}	37.94^{d}	1.473^{a}	2.483^{a}	4.210^{de}	12.63^{de}	23.17^{a}	24.19^{k}
T15	5.22^{j}	11.19^{m}	24.45^{ij}	0.623^{j-l}	0.960^{n}	2.946^{h}	11.99^{g}	14.48^{m}	26.58^{g}
T16	6.96^{f-j}	18.56^{gh}	36.59^{d}	0.460^{m}	1.270^{kl}	2.863^{hi}	8.33^{n}	14.35^{m}	25.28^{j}
T17	6.29^{g-j}	11.60^{m}	20.92^{k}	0.486^{m}	1.426^{f-j}	2.886^{hi}	9.55^{k}	15.17^{k}	23.67^{l}
T18	5.42^{j}	12.97^{k}	22.15^{jk}	0.316^{n}	1.240^{lm}	2.870^{hi}	8.63^{m}	15.83^{i}	27.23^{ef}
T19	5.51^{j}	12.97^{lm}	21.67^{k}	0.466^{m}	1.503^{f-h}	2.443^{jk}	8.22^{n}	14.12^{n}	26.09^{hi}
T20	6.25^{h-j}	12.49^{m}	20.10^{k}	0.496^{m}	1.343^{i-l}	3.250^{g}	8.56^{m}	13.67^{o}	20.27^{o}
T21	6.17^{ij}	14.55^{kl}	19.49^{K}	0.490^{m}	1.386^{h-k}	2.633^{ij}	10.09^{j}	18.21^{f}	24.95^{j}
T22	11.31^{e}	16.15^{i-k}	26.03^{hi}	0.726^{g-i}	1.556^{f}	2.953^{h}	9.06^{l}	18.25^{f}	24.24^{k}
T23	13.09^{c-e}	14.48^{kl}	38.17^{d}	0.633^{i-k}	1.393^{g-k}	2.420^{jk}	10.60^{i}	17.81^{g}	18.78^{p}
T24	8.61^{f}	20.32^{e-g}	21.49^{k}	0.603^{j-l}	1.456^{f-i}	3.476g	8.68^{m}	13.84^{o}	26.42^{gh}
T25	8.78^{f}	15.75^{k}	33.01^{ef}	0.760^{gh}	1.236^{lm}	2.320^{k}	10.40^{i}	14.30^{mn}	22.84^{m}

***Means followed by different letter(s) in the column are significantly different**

Yield parameters

Data in Table 6 show the effect of different N-levels and / or microbial inoculation on some wheat yield parameters i.e., spike length (cm), spike weight (g), number of grains/spike and weight of 1000 grains (g). Obtained results showed that these parameters significantly responded to the treatments. Data indicated that the treatment T8 (*Az. chroococcum* + cyanobacteria + 75 % dose of N) and T 9 (*Az. chroococcum* + *K. oxytoca* + 75 % dose of N) gave the highest values of spike length (13.10 and 13.00 cm, respectively). However, the treatments T14 (Mixture + 75 % dose of N) and T6 (*K. oxytoca* + 75 % dose of N) gave the highest values of spike weight (8.28 and 8.05 g) followed by treatments T11, T9 and T8 which gave values (7.87, 7.53 and 7.47 g). On the other hand, the treatments T9 (*Az. chroococcum* + *K. oxytoca* + 75 % dose of N), T5 (Cyanobacteria + 75 % dose of N), T12 (Cyanobacteria + *R. pusense* + 75 % dose of N) and T13 (*K. oxytoca* + *R. pusense* + 75 % dose of N) gave the highest numbers of grains / spike (57.6, 55.2, 53.4 and 51.2), respectively. There was no significant difference between the other treatments and T1 (control, full dose of N). However T19 (*Az. chroococcum* + Cyanobacteria + 50 % dose of N) and T 24 (*K. oxytoca* + *R. pusense* + 50 % dose of N) gave the lowest values in compared to control. Also, data indicated that the treatment T 9 (*Az. chroococcum* + *K. oxytoca* + 75 % dose of N) gave the highest values of weight of 1000 grains (53.12 g) followed by the treatments T6 and T5 which gave values (50.44 and 50.07 g, respectively). The pronounced increase in wheat yield parameters is due to N_2-fixation performance as well as P and K mobilization, phytohormone production and saving the bio-protection against phytopathogens, also the role of nitrogen nutrient in the anabolic plant processes, which has very important function as main constituent of protein, amino acids and many essential compounds in plant system. Similar observations were reported by (Hussain and Hasnain (2011) ; Saber *et al*. (2012) ; Salim *et al*. (2013) and Desoky and El-sayed (2016) who noted that using bio-fertilizers improves wheat yield parameters i.e., spike length (cm), spike weight (g), number of grains/spike and weight of 1000 grains.

Grain and straw yield

Data in Table 6 indicated that the treatment T 9 (*Az. chroococcum* + *K. oxytoca* + 75 % dose of N) gave the highest value of grain yield (19.61 ardab/fed.) with an increase by 21.87% over the control treatment (T1), followed by treatments T10, T6, T12 and T8 which gave values (18.56, 17.44, 17.22 and 17.06 ardab/fed., respectively) with an increase by 15.35%, 8.39%, 7.02% and 6.02%, respectively over the treatment T1 (control). On the other hand, the treatments T9 (*Az. chroococcum* + *K. oxytoca* + 75 % dose of N) and T13 (Mixture + 75 % dose of N) gave the highest value of straw yield (3.13 and 2.95 ton/fed., respectively) with an increase by 19.01% and 12.16%, respectively over the control treatment T1 (control). The beneficial increase in wheat yield is due to the nutrient availability in the rhizosphere as affected by microbial inoculation and to the secretion of plant growth hormones, which might be supporting such increase. Also, the increase of the yield by increasing the N supply might due to the increase in the dry weight of vegetative organs, which could be considered as a criterion for the photosynthetic efficiency of the plant. Similar observations were reported by (Hussain and Hasnain (2011) ; Saber *et al*. (2012) and Desoky and El-sayed (2016) who noted that using bio-fertilizers improves grain and straw yield.

Table 6. Yield parameters of wheat plants as affected by bio-fertilization treatments

Treatments	Spike length (cm)	Spike weight (g)	No. of grains / spike	Weight Of 1000 grains	Grain yield	Straw yield
T1	10.80^{c-e*}	5.75^{e-i}	45.6^{d-g}	48.25^{bc*}	16.09^{ef}	2.63^{c-h}
T2	10.22^{ef}	5.15^{h-j}	39.2^{f-h}	42.92^{g-j}	14.88^{g-i}	2.39^{h-j}
T3	9.54^{f}	4.47^{j}	37.6^{h}	40.15^{j}	13.77^{j}	1.94^{l}
T4	12.32^{ab}	5.81^{e-i}	47.6^{c-e}	44.34^{d-i}	16.08^{ef}	2.64^{c-h}
T5	12.58^{ab}	6.25^{d-h}	55.2^{ab}	50.07^{ab}	16.56^{de}	2.80^{b-e}
T6	12.24^{ab}	8.05^{a}	42.4^{e-h}	50.44^{ab}	17.44^{c}	2.84^{a-c}
T7	10.76^{c-e}	6.39^{d-g}	45.6^{d-g}	49.26^{b}	16.16^{ef}	2.66^{c-h}
T8	13.10^{a}	7.47^{a-c}	46.4^{c-f}	47.42^{b-e}	17.06^{cd}	2.88^{a-c}
T9	13.00^{a}	7.53^{a-c}	57.6^{a}	53.12^{a}	19.61^{a}	3.13^{a}
T10	12.02^{a-c}	6.57^{c-e}	47.2^{c-e}	47.52^{b-d}	18.56^{b}	2.56^{d-i}
T11	12.60^{ab}	7.87^{ab}	48.0^{c-e}	46.93^{b-f}	16.82^{c-e}	2.77^{b-f}
T12	12.24^{ab}	6.39^{d-g}	53.4^{a-c}	48.21^{bc}	17.22^{cd}	2.74^{b-g}
T13	12.44^{ab}	6.97^{b-d}	51.2^{a-d}	43.74^{e-j}	16.73^{c-e}	2.95^{ab}
T14	12.00^{a-c}	8.28^{a}	44.0^{d-h}	47.29^{b-e}	16.42^{de}	2.86^{bc}
T15	12.22^{ab}	5.34^{f-j}	44.0^{d-h}	41.51^{h-j}	14.50^{i}	2.07^{kl}
T16	11.42^{b-e}	4.95^{ij}	43.2^{e-h}	40.41^{j}	14.63^{hi}	2.09^{kl}
T17	11.66^{b-d}	6.45^{d-f}	41.6^{e-h}	43.53^{f-j}	15.38^{f-h}	2.53^{e-i}
T18	10.54^{d-f}	6.34^{d-g}	47.2^{c-e}	44.24^{d-i}	14.69^{g-i}	2.31^{i-k}
T19	11.26^{b-e}	5.61^{e-i}	36.8^{h}	44.85^{c-h}	15.13^{g-i}	2.72^{b-g}
T20	12.24^{ab}	5.40^{f-j}	46.4^{c-f}	40.89^{ij}	15.47^{fg}	2.47^{g-j}
T21	11.48^{b-e}	5.28^{g-j}	41.6^{e-h}	41.57^{h-j}	14.65^{g-i}	2.50^{f-j}
T22	11.58^{b-d}	5.95^{d-i}	42.4^{e-h}	45.41^{c-g}	14.90^{g-i}	2.25^{jk}
T23	11.56^{b-d}	6.38^{d-g}	48.8^{b-e}	42.36^{g-j}	14.71^{g-i}	2.30^{i-k}
T24	12.06^{a-c}	6.11^{d-h}	38.4^{gh}	41.89^{g-j}	14.96^{g-i}	2.61^{c-h}
T25	11.78^{a-d}	5.73^{e-i}	43.2^{e-h}	44.61^{c-i}	14.84^{g-i}	2.53^{e-i}

***Means followed by different letter(s) in the column are significantly different**

NPK content in grains and straw

Data in Table 7 shows the effect of chemical fertilization and microbial inoculation of wheat plants on grain's NPK-content. N, P and K-contents were significantly responded to all treatments under investigation. The highest value in N-content (21.32 mg/g dry weight) was scored by wheat plants inoculated with T9 (*Az. chroococcum* + *K. oxytoca* + 75 % dose of N) with an increase 7.35 % over the control treatment. Also, treatments T12, T4, T10, T11, and T14 gave high values of 20.50, 20.39, 20.15, 20.14 and 20.04 mg/g dry weight, respectively, there was no significance between these treatments and the value of T1 (control, full dose of N). However, the highest values in P-content 3.71, 3.59 and 3.57 mg/g dry weight was scored by wheat plants inoculated with T5, T9 and T10, There was no significance between these treatments and the value of T1 (control, full dose of N). On the other hand, the highest values in K-content 4.78, 4.62, 4.56, 4.28, 3.78, 3.75 and 3.66 mg/g dry weight was scored by wheat plants inoculated with T6, T5, T13, T24, T9, T20 and T7, with an increase 38.95%, 34.30%, 32.55%, 24.41%, 9.88% and 6.39%, respectively) which were better than the value of T1 (control, full dose of N). The pronounce increase in grain's NPK content of wheat plant is due to the nutrient availability in the rhizosphere as affected by microbial inoculation. Results are in agreement to those obtained by Iniguez *et al*. (2004) ; Abou-Aly and Mady (2009) ; Ghazal *et al*. (2011) ; Salim *et al*. (2013) and Desoky and El-sayed (2016).

Table 7. N, P and K –content (mg/g dry weight) in grains and straw of wheat plant as affected by bio-fertilization treatments

Treatments	Grains			Straw		
	N	P	K	N	P	K
T1	19.86^{b-e*}	3.579^{ab}	3.44^{f-h}	7.16^{de*}	1.144^{d-g}	7.73^{d}
T2	18.44^{e-j}	3.492^{b}	3.14^{k-m}	6.15^{f-i}	0.918^{m}	6.51^{h-g}
T3	15.21^{k}	2.773^{i}	2.59^{p}	5.68^{h-j}	0.674^{p}	5.36^{l}
T4	20.39^{a-c}	3.481^{b}	3.29^{h-k}	7.52^{cd}	0.977^{lm}	6.81^{gh}
T5	19.63^{b-f}	3.710^{a}	4.62^{b}	8.75^{ab}	1.023^{j-l}	6.56^{hi}
T6	19.34^{b-g}	3.045^{e-g}	4.78^{a}	6.83^{d-f}	1.041^{i-k}	9.02^{b}
T7	19.46^{b-g}	3.189^{c-e}	3.66^{de}	6.15^{f-i}	1.103^{e-i}	9.65^{a}
T8	18.94^{c-h}	3.214^{cd}	3.41^{f-i}	7.59^{cd}	1.149^{d-f}	6.45^{ij}
T9	21.32^{a}	3.597^{ab}	3.78^{d}	8.99^{a}	1.241^{b}	9.18^{b}
T10	20.15^{a-d}	3.579^{ab}	3.33^{g-j}	6.98^{d-f}	1.105^{e-h}	7.80^{d}
T11	20.14^{a-d}	3.191^{c-e}	3.49^{fg}	8.10^{bc}	1.190^{b-d}	8.27^{c}
T12	20.50^{ab}	3.040e^{-g}	3.40^{f-i}	6.41^{e-i}	1.087^{f-i}	9.93^{a}
T13	19.04^{b-g}	3.332^{c}	4.56^{b}	7.58^{cd}	1.300^{a}	6.99^{fg}
T14	20.04^{a-d}	3.081^{d-g}	3.51^{ef}	8.15^{a-c}	1.210^{bc}	9.67^{a}
T15	18.01^{g-j}	2.753^{i}	2.85^{o}	6.36^{e-i}	0.733^{o}	6.02^{k}
T16	18.22^{f-j}	2.817^{hi}	2.93^{no}	5.85^{g-j}	0.918^{m}	5.68^{l}
T17	17.49^{h-j}	2.819^{hi}	3.05^{mn}	6.10^{f-i}	0.846^{n}	7.37^{e}
T18	18.84^{d-i}	2.991^{fg}	3.22^{j-l}	5.10^{j}	0.805^{n}	7.02^{fg}
T19	17.39^{ij}	2.935^{gh}	3.25^{i-l}	6.17^{f-i}	0.920^{m}	5.53^{l}
T20	18.48^{e-j}	3.114^{d-f}	3.75^{d}	6.49^{e-h}	1.154^{c-e}	7.38^{e}
T21	19.63^{b-f}	2.365^{k}	3.23^{j-l}	5.48^{ij}	1.080^{h-j}	7.29^{ef}
T22	18.15^{f-j}	2.794^{hi}	3.12^{lm}	6.78^{d-g}	1.085^{g-j}	5.40^{l}
T23	18.84^{d-i}	2.493^{jk}	3.14^{k-m}	5.75^{h-j}	1.008^{kl}	5.41^{l}
T24	17.20^{j}	3.104^{d-f}	4.28^{c}	5.73^{h-j}	1.085^{g-j}	6.26^{i-k}
T25	17.48^{h-j}	2.527^{j}	2.81^{o}	5.47^{ij}	1.108^{e-h}	6.17^{jk}

***Means followed by different letter(s) in the column are significantly different**

Also, data in Table 7 shows the effect of chemical fertilization and bacterial inoculation application of wheat plants on straw NPK content. N, P and K-contents were significantly responded to all treatments under investigation. The highest value in N-content (8.99 mg/g dry weight) was scored by wheat plants inoculated with T9 (*Az. chroococcum* + *K. oxytoca* + 75 % dose of N) with an increase 25.55 % over the control treatment. Also, treatments number T5, T14 and T11 gave values of 8.75, 8.15 and 8.10 mg/g dry weight with an increase 3.22%, 2.66% and 1.46%, respectively, which were better than the value of T1 (control, full dose of N). However, the highest values in P-content 1.30, 1.24 and 1.21 mg/g dry weight was scored by wheat plants inoculated with T13, T9 and T14 with an increase 14.03%, 8.77% and 7.00%, respectively. There was no significance between treatments (T11, T20 and T8) and the value of T1 (control, full dose of N). On the other hand, the highest values in K-content (9.93, 9.67, 9.65, 9.18, 9.02, 8.27 mg/g dry weight) was scored by wheat plants inoculated with (T12, T14, T7, T9, T6 and T11), with an increase 28.46%, 25.09%, 24.83%, 18.75%, 16.68% and 6.98%, respectively. There was no significance between treatment number T10 which gave value 7.80 mg/g dry weight and the value of T1 (control, full dose of N). The pronounce increase in straw's NPK content of wheat plant is also due to the nutrients availability, which lead to the increase in plant height, tillers, leaves and weights. Results are in agreement with those obtained by Iniguez *et al*. (2004) ; Ghazal *et al*. (2011) ; Salim *et al*. (2013) and Desoky and El-sayed (2016).

Total bacterial count

The effect of chemical fertilization and microbial inoculation on total bacterial count in the rhizosphere of wheat plant is presented in Table 8.

Table 8. Total bacterial count (10^6 cfu/g dry soil) in rhizosphere of wheat plants at the periods of 30, 60, 90 and 120 days after sowing (DAS) as affected by bio-fertilization treatments

Treatments	Days after sowing (DAS)			
	30	60	90	120
T1	18.46	61.03	132.91	122.17
T2	14.25	52.07	112.73	110.39
T3	12.97	40.54	102.39	96.24
T4	42.47	101.15	154.08	148.89
T5	25.54	109.68	196.42	131.99
T6	32.37	155.78	200.85	130.03
T7	32.97	89.20	174.27	124.16
T8	19.75	93.04	222.02	166.56
T9	18.41	168.58	259.43	191.31
T10	15.44	97.73	229.90	180.71
T11	22.72	90.48	163.44	138.67
T12	16.33	89.62	257.96	181.88
T13	13.81	88.77	236.79	175.60
T14	19.60	78.95	218.08	186.60
T15	19.75	118.22	201.61	158.31
T16	20.64	64.44	184.11	124.14
T17	17.52	92.61	177.22	128.85
T18	20.79	70.84	187.56	156.35
T19	15.74	72.12	164.91	141.03
T20	14.10	76.82	201.34	183.85
T21	21.38	85.78	168.85	164.99
T22	21.09	73.83	186.08	126.89
T23	19.15	66.15	147.68	130.03
T24	14.85	94.32	165.90	138.67
T25	17.52	103.28	190.51	118.64

Initial count 2.01 × 10^6 cfu / g dry soil.

It is obvious from the results that the total bacterial count in rhizosphere of inoculated treatments were higher than those in rhizosphere of uninoculated ones. So, it can say that the bio-fertilization has a pronounced increase in total bacterial count in comparison with the mineral fertilization. Also, it was observed that the highest values of bacterial numbers were obtained at 90 DAS under all applications, the counts were gradually increased until 90 DAS, however it decreased at 120 DAS, this is due to the decrease in roots exudates of old plants. In all stages of growth, by increasing N-fertilizer, the bacterial count increased. Data show that the highest total bacterial count in wheat rhizosphere being 259.43 x 10^6 cfu / g dry soil was recorded in rhizosphere of wheat plants inoculated with T9 (*Az. chroococcum* + *K. oxytoca* + 75 % dose of N), followed by T12, T13, T10, T8, T14, T15 and T20 which gave also higher values, respectively at 90 DAS. Results are in agreement with those obtained by Hauka *et al*. (2010) who noted that the total bacterial count increased by increasing N-fertilizer in all stages of growth. Also, the role of biofertilization in stimulating the growth and reproduction of microorganisms was clear.

***Azotobacter* spp.**

The effect of chemical fertilization and microbial inoculation application on *Azotobacter* spp. count in rhizosphere of wheat plants is presented in Table 9.

Table 9. Counts of *Azotobacter* spp. (10^4/g dry soil) in rhizosphere of wheat plants at the periods of 30, 60, 90 and 120 days after sowing (DAS) as affected by bio-fertilization treatments

Treatments	Days after sowing (DAS)			
	30	60	90	120
T1	1.03	3.54	3.58	1.64
T2	2.52	4.87	6.27	3.88
T3	1.63	4.13	5.88	2.00
T4	2.67	4.22	32.49	4.71
T5	1.41	2.95	14.08	1.64
T6	5.68	7.97	11.19	8.02
T7	3.83	4.09	8.02	2.82
T8	1.79	2.95	13.66	2.00
T9	2.06	6.01	14.03	9.31
T10	2.65	4.86	11.96	5.19
T11	1.79	2.52	15.36	7.30
T12	3.32	4.72	8.02	3.06
T13	2.17	3.10	5.53	5.19
T14	2.06	2.17	23.76	4.83
T15	1.40	1.63	25.51	1.29
T16	2.07	2.68	4.72	1.64
T17	2.68	2.95	8.02	2.00
T18	1.77	4.09	5.19	1.41
T19	2.07	2.95	4.99	1.41
T20	1.78	3.32	5.19	2.00
T21	2.06	3.07	3.11	1.41
T22	3.65	2.17	3.10	2.00
T23	1.79	2.51	5.19	2.00
T24	2.68	2.95	4.15	1.64
T25	2.07	3.58	10.33	1.29

Initial count 1.34 × 10^4 / g dry soil.

Results show that the *Azotobacter* spp. count increased with the increase of N-fertilizer, but at the full dose of *Azotobacter* spp. count slightly decreased. Also, it was observed that the highest values of *Azotobacter* spp. count were obtained at 90 DAS under all applications, then they decreased at 120 DAS. Data show that the highest *Azotobacter* spp. count being 32.49 x 10^4 / gram dry soil was recorded in rhizosphere of wheat plants inoculated with T4 (*Az. chroococcum* + 75 % dose of N), followed by T15 (*Az. chroococcum* + 50 % dose of N). Also, T14, T11, T5, T9, T8, T10, T6, T25, T7, T12 and T17 which gave also higher values and enhance *Azotobacter* spp. count, respectively at 90 DAS. Similar observations were obtained by Selim (2010) who reported that at the high level of N-fertilizer 60 unit N/fed, *Azotobacter* count decreased. Also, results are in agreement with those obtained by Zayed (1999) and Nain *et al*. (2000). But, data are in contrast with Hauka *et al*. (2010) who mentioned that the negative effect of addition 120 kg N/fed. on *Azotobacter* counts is not appeared, it may be related to the decrease in N-content of the used sandy soil.

Phosphate-solubilizing bacteria

The effect of chemical fertilization and microbial inoculation application on phosphate-solubilizing bacterial count in rhizosphere of wheat plants is presented in Table 10.

Table 10. Counts of phosphate-solubilizing bacteria (10^4 cfu/g dry soil) in the rhizosphere of wheat plants at the periods of 30, 60, 90 and 120 days after sowing (DAS) as affected by bio-fertilization treatments

Treatments	Days after sowing (DAS)			
	30	60	90	120
T1	25.74	43.10	106.82	79.74
T2	22.27	39.69	81.22	65.21
T3	18.31	23.90	71.38	47.92
T4	44.55	49.08	112.73	108..81
T5	36.63	102.43	139.81	92.71
T6	34.65	118.22	148.67	87.60
T7	22.27	35.85	98.95	51.85
T8	21.78	29.87	191.50	110.39
T9	25.24	41.39	121.10	108.42
T10	24.75	33.29	130.45	101.74
T11	52.97	45.66	162.95	127.67
T12	19.80	53.35	193.47	141.42
T13	33.17	36.70	199.38	145.35
T14	24.75	26.46	184.11	122.96
T15	35.64	51.21	114.70	109.99
T16	40.59	42.25	122.08	114.71
T17	34.16	29.87	81.72	69.14
T18	18.81	29.02	62.52	45.17
T19	17.82	39.69	104.36	96.64
T20	20.79	46.09	106.33	71.49
T21	29.20	43.96	57.59	41.24
T22	40.59	107.12	138.33	115.49
T23	57.42	67.00	69.41	57.35
T24	30.69	50.36	108.30	60.89
T25	20.79	64.02	88.61	36.53

Initial count 7.67 × 10^4 cfu / g dry soil.

The counts were gradually increased until 90 DAS, however they decreased at 120 DAS. Data show that the highest phosphate-solubilizing bacterial count being 199.38 x 10^4 cfu / g dry soil was recorded in rhizosphere of wheat plants inoculated with T13 (*K. oxytoca* + *R. pusense* + 75 % dose of N), followed by T12, T8 and T14 which gave also higher values, respectively at 90 DAS. The obtained results are in agreement with those obtained by Shahaby *et al*. (2000) and Khafagy (2003).

Potassium-releasing bacteria

The effect of chemical fertilization and microbial inoculation application on potassium-releasing bacteria in rhizosphere of wheat plant is presented in Table 11. At the same trend, the counts were gradually increased until 90 DAS, however they decreased at 120 DAS. Data in Table 11 show that the highest potassium-releasing bacteria being 332.79 x 10^4 cfu / g dry soil was recorded in the rhizosphere of wheat plants inoculated with T5 (Cyanobacteria + 75 % dose of N), followed by T9 (*Az. chroococcum* + *K. oxytoca* + 75 % dose of N) which gave value 300.79 x 10^4 cfu / g dry soil. Also, T6, T11 and T10 gave higher values (296.36, 291.43 and 290.94 x 10^4 cfu /g dry soil), respectively at 90 DAS. The obtained results are in agreement with that results obtained by Khafagy (2003).

Table 11. Counts of potassium-releasing bacteria (10^4 cfu/g dry soil) in rhizosphere of wheat plants at the periods of 30, 60, 90 and 120 days after sowing (DAS) as affected by bio-fertilization treatments

Treatments	Days after sowing (DAS)			
	30	60	90	120
T1	75.25	87.92	186.08	139.46
T2	50.99	75.54	162.95	121.39
T3	29.70	60.60	150.15	102.92
T4	92.08	101.15	264.85	143.39
T5	61.88	100.72	332.79	145.74
T6	85.15	91.76	296.36	134.74
T7	70.79	82.37	277.65	155.17
T8	26.73	93.04	271.25	98.99
T9	67.33	100.29	300.79	132.39
T10	65.34	119.07	290.94	175.99
T11	75.25	81.51	291.43	155.96
T12	77.23	87.49	264.85	121.78
T13	70.23	87.06	268.30	153.99
T14	58.41	116.94	265.34	164.60
T15	24.75	53.35	222.02	103.32
T16	45.54	77.25	225.96	108.42
T17	41.09	74.26	224.48	128.85
T18	68.32	69.14	234.33	133.96
T19	29.70	73.41	206.27	112.35
T20	39.60	52.07	197.90	135.14
T21	42.08	71.27	237.77	157.53
T22	28.21	58.04	224.97	150.06
T23	57.92	71.70	277.65	117.46
T24	35.64	40.97	213.65	138.67
T25	57.92	76.82	199.87	135.53

Initial count 22.7 × 10^4 cfu / g dry soil.

Recommendations

From the current study, it could be recommend the possibility of using the dual bacterial inoculum, which containing *Azotobacter chroococcum* MF135558 and *Klebsiella oxytoca* MF135559 in the presence of 75% dose of N for enhancing growth and yield of wheat plants and minimize the request of chemical fertilizers.

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التلقيح المزدوج ببكتيريا أزوتوباكتر كروكوكم وكليبسيلا أوكسيتوكا يحسن من نمو وإنتاجية نبات القمح ويقلل من استخدام الأسمدة النيتروجينية.

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أجريت تجربة حقلية فى مزرعة كلية الزراعة جامعة المنصورة مصر خلال الموسم الشتوى ٢٠١٦ ، لدراسة تأثير التسميد الحيوى مع التسميد النيتروجينى (٧٥% و ٥٠% من معدل النيتروجين الموصى به) على نمو وإنتاجية نبات القمح. أدى التلقيح الميكروبى إلى زيادة معنوية فى صفات النمو (طول النبات وعدد الأشطاء وعدد الأوراق والوزن الجاف للنبات) ، كما أدى إلى زيادة معنوية فى محتوى النباتات من النيتروجين والفوسفور والبوتاسيوم فى جميع مراحل نمو النبات. أدى أيضاً التلقيح الميكروبى إلى زيادة معنوية فى الصفات المحصولية لنبات القمح (طول ووزن السنبلة وعدد الحبوب فى السنبلة ووزن الألف حبة ومحصول الحبوب والقش و محتوى الحبوب و القش من النيتروجين والفوسفور والبوتاسيوم) ، حيث أعطت المعاملة رقم ٩ (أزوتوباكتر كروكوكم + كليبسيلا أوكسيتوكا + ٧٥% من معدل النيتروجين الموصى به) أعلى قيمة لمحصول الحبوب والقش ١٩.٦١ أردب/فدان و ٣.١٣ طن/فدان بزيادة ٢١.٨٧ % و ١٩.٠١ % عن معاملة الكنترول على الترتيب. أدى التسميد الحيوى إلى زيادة واضحة فى أعداد ميكروبات التربة بالمقارنة بالتسميد المعدنى. لذلك توصى الدراسة من خلال النتائج التى تحصلنا عليها ، بإمكانية استخدام اللقاح الثنائى المكون من بكتيريا أزوتوباكتر كروكوكم وكليبسيلا أوكسيتوكا فى وجود ٧٥% من معدل النيتروجين الموصى به لتحسين نمو وإنتاجية نبات القمح.