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EFFECT OF BIO FERTILIZERS AND INORGANIC FERTILIZERS ON GROWTH, PRODUCTIVITY AND QUALITY OF BREAD WHEAT CULTIVARS

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ABSTRACT. Integrated nutrient management strategies involving chemical and biological fertilizer is a real challenge to stop using the high rates of agrochemicals and to enhance sustainability of crop production. In order to study the effects of biofertilizers (Cerialin and Nitrobein) and chemical nitrogen levels (0, 85,170 and 250 kg N ha^{-1}) on yield and yield attributes of two wheat cultivars (Sakha 94 and Gemmeiza 10), an agricultural experiment in the form of strip-split factorial design with three replications was conducted in Kafr El-Sheikh region, Egypt, in 2014/2015 and 2015/2016 growing seasons. The objective of this study was evaluation of the effects of these fertilizers separately and in integrated forms, and setting out the best fertilizer mixture. The results showed that treatment with biofertilizers and chemical nitrogen increased the growth, yield attributes, biological and grain yield. Both grain and biological yield produced a better result during the combination of nitrogen fertilizer and biofertilizers than using either method alone. Using biofertilizers increased biological yield through increase in number of grains spike^{-1}, number of spikes m^{-2} and 1000 grain weight, which cause to increase in grain yield with significant changes in harvest index, as well as protein content. We may conclude that using biofertilizers (Cerialin or Nitrobein) and chemical nitrogen fertilizer (170 or 250 kg N ha^{-1}) together had the maximum impact on yield. Then, we can decrease use of chemical fertilizers through using biofertilizers.

Keywords: chlorophyll content; protein content; leaf area inde; wheat; grain yield

INTRODUCTION

Wheat is the most important crop in the world in terms of area and production and it is a stable food for more than one third of the world population. Wheat contributes more calories and protein in the world diet than any other food crop. In Egypt, wheat is the main winter cereal crop. It is used as a stable food grain for urban and rural societies and as a

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major source of straw for animal feeding. The wheat area over the last 20 years (1998-2016) has been expanded in the old cultivated land from (1.521 to 3.100 million feddan (1 feddan = 2.38 ha) and about 0.6 million feddan in new lands out valley. The wheat cultivated area in Egypt researched about 3 million fed. (1.2 ha) in 2015-2016 season.

The expected production is about 8.0 million tons. The average yield in the old land reached 18.5 ardab/fed. (6.60 t ha^{-1}). In 2015-2016, the area grown to wheat in the northern region of Delta (Kafr El-Sheikh, Dakhleia, Behera and Domiate governorates) is about 800.000 fed. (340.000 ha), expected to produce 1.5 million tons (FAO, 2016). However, total wheat consumption has increased drastically due to overall population growth of about 2.5% per year. Therefore, Egypt imports about 45% of wheat requirements. This reflects the size of the problem and the efforts needed to increase wheat production. Thus, increasing production per unit area of wheat, appears to be one of the important factors for narrowing the wheat production gab, due to breeding for high yielding varieties and by the application of improved agro-technique (Badr *et al*., 2009).

Nitrogen fertilization has contributed greatly in raising grain yield per ha in every important producing area. Nitrogen fertilization is the plant nutrient most universally needed to meet the fertilizer requirement for the high crop yields obtained in Egypt. However, the use of nitrogen fertilizer is a very important tissue due to increasing production costs, as well as environmental pollution that cause many hazards to human health (Scheiner *et al*., 2002). This led to draw considerable interest into biofertilization with N_2-fixation bacteria, especially in new lands, out Nile Valley. Fortunately, biological nitrogen fertilizers may act as a safe and cheap practice that contributes productivity directly by supplement fixed nitrogen and growth hormones to wheat plants, and indirectly by enhancement soil fertility. Most of the developed countries have adapted environmentally based policies to sustain the earth ecosystem and reduce the consumption of non renewable sources of energy of manufacturing fertilizers (Bahrani *et al*., 2010). This led to considerable interest in biological N_2-fixation and other biofertilizer technology. Using such technology, considerable portions of chemical nitrogen requirement could be saved (Chen, 2006). Moreover, Yasari and Patwardhan (2007) reported that the inoculation with *B. polymyxa* and *A. brasilense* can save 41.6 and 37.5% of nitrogen fertilizer, respectively. In addition, many researchers proved that excess nitrogen was compensated in grain produced by inoculation with bacteria (Badawy *et al*., 1998). However, rate of profit from using biofertilizers depends on soil properties, characters of the N_2-fixing bacteria, method of inoculation, genotype of the plant cultivar, culture practices and climatic conditions (Kotb, 1998).

However, several investigators have revealed that rate of profit depends on soil properties, characters of the bacteria, methods of inoculation agricultural practices, climatic conditions and genotype of the plant cultivar. Therefore, the present investigation was conducted to evaluate the response of the two wheat cultivars, Gemmeiza 10 and Sakha 94, to N_2-fixing biofertilizers under four nitrogen fertilizer applications at Delta.

MATERIAL AND METHODS

The present study was carried out in Experimental Farm Station of the Faculty of Agriculture, Kafr El-Sheikh University, Egypt. This investigation was performed during the two successive growing seasons 2014/2015 and 2015/2016 to study: 1) the effect of two vulgar wheat cultivars (*Triticum aestivum* vulgar L.) (*Table 1*); 2) the effect of four nitrogen levels; 3) the effect of N_2-fixing biofertilization. The mechanical and chemical analysis of the experimental soil was done before the sowing, as shown in *Table 2*.

Table 1 - Pedigree and characteristics of the two tested cultivars

Cultivar	Year of release	Pedigree	General characteristics
Sakha 94	2005	OPATA/RAYON//KAUZ CMBW 90Y3180-OTOPM-3Y-010M-010M-010Y-10M-015Y-OY-OAP-0S	white grains high tillering resistant to yellow rust resistant to leaf rust
Gemmeiza 10	2005	MAYA 74 "S"/ON//1160-147/3/BB/GLL/4/CHAT "S"/5/CROW "S".CGM 5820-3GM-1GM-2GMOGM	white grains high tillering resistant to yello rust resistant to leaf rust

Table 2 - The mechanical and chemical analysis of the experimental soil during the two seasons of study

Variable	Seasons	
	2014/2015	2015/2016
Mechanical analysis		
Fine sand (%)	12	11
Silt (%)	32	33
Clay (%)	56	57
Soil texture class	clayey	clayey
Chemical analysis		
Soil reaction (pH)	8.02	8.1
Organic matter (%)	1-55	1.65
Available N ppm	16.3	17.6
Available P ppm	13.9	14.8
Available K ppm	25.5	26.7
EC (m-mhos/cm)	2.1	2.3

Experiments and treatments

Effect of nitrogen levels and N_2-fixing biofertilization on growth, yield and yield components of two wheat cultivars were studied as follows: 1) The horizontal plots is nitrogen levels (N): N1 = 0.0 kg N ha^{-1}, N_2 = 85 kg N ha^{-1}, N3 = 170 kg N ha^{-1}, N4 = 250 kg N ha^{-1}; 2) The vertical plots is cultivars (V): V1 = Sakha 94, V2 = Gemmeiza 10; 3); Sub-plots is N_2-fixing biofertilization (B): B1= non inoculated seed (control), B2 = inoculated seed with Cerialin: *Azospirillum brasilense* + *Bacillus polymyxa*, B3 = inoculated seed with Nitrobein: *Azotobacter* spp. + *Azospirillum* spp.

The biofertilizers used in the present study were produced by the General Organization for Agricultural Equalization Fund, Ministry of Agriculture, and consists of mixture of N_2-fixing bacteria, e.g. *Azospirillum, Azotobacter* and *Bacillus* (Mitkees *et al.,* 1996). The biofertilizers were added at rate 1200 g ha^{-1}. Wheat seeds were wetted with some water, added to the mixture of biofertilizers and gum, mixed carefully and spread on plastic sheet far from the direct sun effect for a short time before the sowing and the soil was irrigated immediately after the sowing. The experiments were grown using A strip split plot in four RCBD. The horizontal plots were devoted the four nitrogen levels, as above mentioned. The vertical plots were allocated with two cultivars as above mentioned. The subplots were occupied by treatments of biofertilization, the area of subplot was 10.5 m^2 (3 m width and 3.5 m length), each plot contains 20 rows with 15 cm apart. Plots were separated by 1 m allays. The preceding crops were maize in both seasons. In this experiment, the seeds were inoculated with Nitrobein and Cerialin, immediately before the sowing. The sowing was done by using hand drill machine on 22nd of November in both seasons. The soil was irrigated after the sowing. Four nitrogen fertilizer levels (0, 85, 170, 250 kg N ha^{-1}.) in the form of urea (46.5% N) were applied in two equal doses at life irrigation (21 days after planting) and two dose was applied 21 days after life irrigation. Phosphatic fertilizer was applied at the rate of 37.5 kg P_2O_5 ha^{-1} (250 kg calcium super phosphate), during seedbed preparation. The ordinary agricultural practices were applied during the growing seasons. At harvest, the 16 middle rows were harvested to determine grain and straw yields.

Characters studied

A. Growth analysis

A1. Leaf area index (LAI). LAI express the ratio of leaf area tom, according to Daughtry and Hollinger (1984)

A2. Flag leaf area. Ten flag leaves were used to determine the average of flag leaf area at 125 days after sowing for each sub-plot, determined by using leaf area meter.

A3. Chlorophyll content. Hand-held chlorophyll meter (SPAD-502; Minolta Sensing Co., Ltd, Japan) was used to record SPAD readings from the topmost fully expanded leaves on each main stem at heading stage. SPAD values were measured at three different points along the flag leaf blade, and then the readings were averaged to have a single value for a plant (Markwell *et al.*, 1995).

A4. Days to heading, determined as number of days from sowing up to 50% of head emerged from sheat.

A5. Days to maturity, determined as number of days after sowing up to 50% physiological maturity**.** These character was estimated on the basis of 10 spikes randomly collected from each plot.

B. Yield and yield components

B1. Plant height (cm), measured at harvest from soil surface to the tip of the spike of the main stem.

B2. Spike length (cm), measured at harvest from the main stems, which were used for estimation of plant height.

B3. Number of grains/spike. These character was estimated on the basis of 10 spikes randomly collected from each plot.

B4. Number of spikelets/spike, determined as number of fertile and sterile spikelets of ten spikes from each plot at harvest.

B5. Number of spikes/m^{-2}. According to the number of spikes per 1 m^2, the number of spikes/m^2 was calculated.

B6. 1000 grain weight (g), determined from the three random samples each contained 1000 grains, taken from each treatment.

B7. Grain yield (t ha^{-1}), determined from the harvested area of each plot in terms of kg/plot and converted to t ha^{-1}.

B8. Straw yield (t ha^{-1}), determined as the difference between biological and grain yield of subplot in terms of kg / plot and converted to t ha^{-1}.

B9. Biological yield = grain yield + straw yield.

B10. Harvest index = grain yield/biological yield × 100, where biological yield = grain yield + straw yield.

C. Grain quality

*Protein percentage***.** The micro-Kjeldahl method was used to determine the total nitrogen in the grains and multiplied by 5.75 to obtain the percentage of grain protein according to A.O.A.C. (1990).

Statistical analysis

All data collected were subjected to statistical analysis of variance, as described by Snedecor (1956). The mean values were compared according to Duncan's Multiple Range Test (Duncan, 1955). All statistical analysis were performed using analysis of variance technique by means of "MSTAT-C" computer software package.

RESULTS AND DISCUSSION

A. Growth analysis (*Table 3*)

A1. Leaf area index (LAI)

The differences among the two cultivars in leaf area index was significant at the first and the second growth stages in the second season only and the third growth stages in the two seasons. The higher values of leaf area index were recorded by Sakha 94 in the two seasons Gharib *et al.* (2016). Leaf area index was significantly increased with increasing nitrogen levels at the three sampling periods in both seasons. In general, the highest leaf area index was obtained by highest nitrogen level (250 t ha^{-1}) at all growth stages. Biofertilization showed highly significant increase on leaf area index at three samples in both seasons with Nitrobein. These results are in conformity with the findings of Rana *et al.* (2012).

A2. Flag leaf area (cm^2)

Highly significant effects were detected among the two tested cultivars. Sakha 94 surpassed Gemmeiza 10 in flag leaf area. Nitrogen application levels had significant effect on flag leaf area, up to 250 kg N ha^{-1} in the two seasons Hafez *et al.* (2015). Biofertilization had significant effect on flag leaf area, where inoculation with Nitrobein in the first season significantly increased flag leaf area and inoculation with cerialin or nitrobein in the second season significantly increased flag leaf area.

The interaction between nitrogen fertilization levels and biofertilizers were significant in the first season, where 250 kg N ha^{-1} + inoculation with Cerialin or Nitrobein gave significantly increased flag leaf area. The results were corroborated with findings of Kizilkaya (2008).

A3. Chlorophyll content (SPAD)

The influence of cultivars on chlorophyll content was highly significant in both seasons. The superiority of Gemmeiza 10 cultivar in chlorophyll content was noted in both seasons. Increasing nitrogen levels caused significant increases in chlorophyll content of wheat in both seasons.

The highest chlorophyll content was recorded at 250 kg N ha^{-1} in both seasons. Biofertilization showed significant effects on chlorophyll content in both seasons Hafez and Abou El-Hassan (2015). The interaction between cultivars and nitrogen levels showed significant effect on chlorophyll content in the first season. Gemmeiza 10 wheat cultivar gave the highest chlorophyll content at 250 kg N ha^{-1}.

The interaction between nitrogen levels and biofertilization showed positively significant effect on chlorophyll content at 250 kg N ha^{-1} with Nitrobein in the first season. The results were similar with the findings of Kızılkaya (2008).

A4. Days to 50% heading

The two cultivars differed significantly for the two seasons, in number of days to heading.

The earliest cultivar was Sakha 94 (95.54 days in the first season and 95.47 days in the second season), while Gemmeiza 10 was the latest one (99.52 days in the first season and 98.75 days in the second season). Increasing nitrogen up to 105 kg N/fed delayed significantly heading with 6-7 days. Inoculation with biofertilizers caused significant decrease in days to heading, but no more than 1-2 day.

The interaction effect between wheat cultivars and nitrogen fertilizer were significantly detected in both seasons Hafez (2016). Gemmeiza 10 headed the latest, while Sakha 94 was the earliest in both seasons, in spite of their delay by increasing nitrogen up to 250 kg N ha^{-1}.

The interaction effect between nitrogen fertilizer and biofertilizers had highly significant effect in the first season only, where 250 kg N ha^{-1} + Nitrobein or Cerialin caused decrease in days to heading, as compared with control.

The interaction effect between wheat cultivars and biofertilizers had highly significant effect in both seasons, where Gemmeiza 10 was the latest cultivar in days to heading in both seasons and Sakha 94 was the earliest cultivar with Nitrobein (Kizilkaya, 2008).

Table 3 - Growth attributes of two wheat cultivars as affected by different nitrogen levels and biofertilizers in 2014/2015 and 2015/2016 seasons

Character	Cultivar (C)		N levels (kg N ha^{-1}) (N)				Biofertilizers (B)			Interaction			
	Sakha 94	Gemmeiza 10	0.0	85	170	250	Control	Cerialin	Nitrobein	CN	CB	NB	CNB
					2014/2015								
LAI (80 DAS)	3.37a	3.29b	2.96d	3.75c	3.86b	3.96a	3.13c	3.29b	3.59a	NS	NS	**	NS
Flag leaf area (cm^2)	44.67a	38.45b	32.56d	35.87c	38.98b	42.54a	35.65c	38.87b	39.87a	NS	NS	NS	NS
Chlorophyll content (SPAD)	44.33a	38.23b	36.22d	39.54c	44.23b	47.23a	34.76c	42.76b	44.87a	**	NS	NS	NS
Days to 50% heading	90.65	91.74	96.11d	92.65c	92.12 b	92.41a	91.14 b	92.25 b	94.36 a	NS	NS	NS	NS
Days to 50% maturity	147.54	148.25	150.36 d	148.65c	148.85b	148.95a	147.57c	148.51b	150.54a	NS	NS	NS	NS
					2015/2016								
LAI (80 DAS)	3.20a	3.10b	2.80d	3.09c	3.25b	3.49a	3.01c	3.19b	3.25a	NS	NS	**	NS
Flag leaf area (cm^2)	46.34a	39.87b	34.99d	37.24c	40.24b	43.87a	36.87c	39.66b	40.12a	NS	NS	NS	NS
Chlorophyll content (SPAD)	45.12a	40.87b	37.77d	40.87c	46.35b	48.87a	37.76c	44,87b	45.43a	**	NS	NS	NS
Days to 50% heading	92.88	93.88	94.44c	94.57c	94.92b	94.88a	93.02	94.58	96.27	NS	NS	NS	NS
Days to 50% maturity	150.19	151.02	150.58c	151.33c	151.95b	152.91a	150.25	151.44	153.11	NS	NS	NS	NS

Means designated by the same letter are not significantly different at 5% level, according to Duncan's Multiple Range Test.
*,** and N.S. indicate $P< 0.0$, $P< 0.01$ and not significant, respectively.

A5. Days to maturity

The two cultivars differed significantly for the two seasons in number of days to maturity. Sakha 94 was the earliest in days to maturity (146.95 days) in 2014/2015 season and 147.56 days in 2015/2016 season, while Gemmeiza 10 was the latest (148.54 days) in 2014/2015 season and 148.91 days in 2015/2016 season. Increasing nitrogen fertilizer levels caused significant delay in days to maturity up to 250 kg N ha^{-1} in both seasons (Hafez and Seleiman, 2017).

Biofertilization caused significant decrease in days to maturity in both seasons, where the corresponding data were 147.65 days for inoculated with Cerialin and 147.25 days with Nitrobein in the first season, 147.62 days for inoculated by Cerialin and 148.25 days by Nitrobein in the second season versus 148.34 days in the first season and 148.84 days in the second season for non-inoculated. The interaction between wheat cultivars and nitrogen fertilizer were significantly detected in the first season. Results were changed with respect to cultivar differences and nitrogen levels. Gemmeiza 10 was the latest, while Sakha 94 was the earliest, in spite of their delay by increasing nitrogen up to 250 kg N ha^{-1}.

The interaction between nitrogen fertilizer levels and biofertilizer in both seasons indicates that biofertilization may promote earliness at 250 kg N ha^{-1} + Nitrobein in the first season and Cerialin in the second season (Abou El Hassan *et al.*, 2014). The interaction between wheat cultivars and biofertilization were significant in the first season. Gemmeiza 10 was the latest cultivar in days to maturity in first season and Sakha 94 was the earliest cultivar with Nitrobein. The interaction of cultivars × nitrogen levels × biofertilizers showed significant effect on days to maturity in both seasons. Gemmeiza 10 was the latest at 250 kg N ha^{-1} + Nitrobein. Similar results were reported by Rana *et al.* (2012).

B. Yield and yield components (*Table 4*)

B1. Plant height (cm)

Highly significant effects were detected among the two cultivars for plant height, where, Sakha 94 was taller than Gemmeiza 10 in both seasons, Sakha 94 was 107.99 and 107.61 cm in both seasons, respectively, while Gemmeiza 10 was 96.01 and 93.85 cm in both seasons, respectively. Also, nitrogen application showed significantly positive effect on plant height. The tallest plants were recorded at 250 kg N ha^{-1} (111.69 and 111.45 cm, respectively) in both seasons. Inoculation with biofertilizers (Cerialin or Nitrobein) significantly increased plant height in both seasons. The interaction between cultivars and nitrogen levels showed significant effect on plant height in the first season only, where Sakha 94 had the tallest plants at 105 kg N/fed in the first season. The interaction between nitrogen levels and biofertilization had significant in the first season only, where the highest plants were produced with 250 kg N ha^{-1} + biofertiizers (Cerialin or Nitrobein). Results were corroborated with the findings of Kandil *et al.* (2011).

B2. Spike length (cm)

The longest spike was detected for the long spike Sakha 94.

The longest spike was recorded by 250 kg N ha^{-1} in both seasons. Inoculation with biofertilizers had significant effect in the second season only, where Cerialin gave the highest spike length in the second season Kandil *et al.* (2011).

B3. Number of grains/spike

Sakha 94 wheat cultivar gave the highest number of grains/spike in both seasons. The highest number of grains/spike was obtained by 250 kg N ha^{-1} in both seasons. Inoculation with biofertilizers had highly significant effect in the first seasons, where Nitrobein gave the highest number of grains/spike. It might be due to the fact that increasing dose of nitrogen might have resulted in higher rate of photosynthesis, which increased the carbohydrate content in grains. Results were corroborated with the findings of Kandil *et al.* (2011) and Hafez *et al.* (2014).

B4. Number of spikelets/spike

Sakha 94 wheat cultivar gave the highest number of spikelets/spike in both seasons. Increasing nitrogen levels led to significant increase in number of spikelets /spike in both seasons. The highest number of spikelets/spike was obtained by 250 kg N ha^{-1} in both seasons. Biofertilization did significantly on affect number of spikelets/spike in both seasons. It increased number of spikelets/spike with Nitrobein in the first season and Cerialin in the second season **(**Hafez and Geries, 2018). The interaction between wheat cultivars and nitrogen levels showed significant effects on number of spikelets/spike at the first season only. Results were changed with respect to cultivar differences and nitrogen levels. Sakha 94 producted the greatest number of spikelets/spike (18.56 spikelet/spike) at 250 kg N ha^{-1}. The interaction between nitrogen fertilizer levels and biofertilizer were significant in the first season only on number of spikelets/spike.

However, the highest number of spikelets/spike (17.91 spikelet/spike) was producted at the rate of 250 kg N ha^{-1} + Nitrobein. The interaction between wheat cultivars and biofertilization were significant in the second season. Sakha 94 gave the greatest number of spikelets/spike with Cerialin (19.66 spikelet/spike) or Nitrobein (19.56 spikelet/spike). It might be due to the fact that increasing dose of nitrogen might have resulted in higher rate of photosynthesis, which increased the carbohydrate content in grains. Results were corroborated with the findings of Kandil *et al.* (2011).

B5. Number of spikes m^{-2}

Number of spikes/m^2 differed significantly among cultivars in both seasons. Gemmeiza 10 produced the highest number of spikes/m^2 (405.00S/m^2) in the first season and 359.31S/m^2 in the second season and significantly affected by increasing nitrogen levels up to 250 kg N ha^{-1} in both seasons. Inoculation with biofertilizers had highly significant effect in both seasons, where Nitrobein gave the highest number of spikes/m^2 Hafez and Gharib (2016).

The interaction between cultivars and nitrogen levels led to significant effect on number of spikes/ m^2 for both seasons. Gemmeiza 10 produced the highest number of spikes/ m^2 at 250 kg N ha^{-1} in first season and at 170 or 250 kg N ha^{-1} in the second season. It might be due to the fact that increasing dose of nitrogen might have resulted in higher rate of photosynthesis, which increased the carbohydrate content in grains. Results were corroborated with the findings of Kandil *et al.* (2011).

B6. 1000 grain weight (g)

Sakha 94 accounted for the heaviest kernels (52.54 g) in the first season, 53.30 g in the second season. 1000 grain weight decreased with increasing nitrogen levels up to 250 kg N ha^{-1}. Inoculation with biofertilizers had not significant in both seasons. It might be due to the fact that increasing dose of nitrogen might have resulted in higher rate of photosynthesis, which increased the carbohydrate content in grains.

Results were corroborated with the findings of Ghaderi-Daneshmand *et al.* (2012).

B7. Grain yield (t ha^{-1}) (*Table 5*)

Insignificant differences were obtained between Sakha 94 and Gemmeiza 10 for this criterion. Increasing N level, up to 250 kg N ha^{-1}, caused significant and consistent linear increase in grain yield of wheat for both seasons. Inoculation with biofertilizers had highly significant effect in both seasons, where Nitrobein gave the highest grain yield/ha.

The interaction between cultivars and nitrogen levels showed significant effect on grain yield/fed in the first season only. Gemmeiza 10 wheat cultivar gave the highest grain yield. The interaction between nitrogen levels and biofertilization were significantly effective on grain yield/fed in the first season only. The highest grain yield/fed was produced by using the rate of 250 kg N ha^{-1} + Nitrobein, due to the role of biofertilizer (*Azotobacter* and *Azospirillum*) in enhancing soil biological activity, which improved nutrient mobilization from organic and chemical sources.

Also, the biofertilizer plays a significant role in regulating the dynamics of organic mater decomposition and the availability of plant nutrients and in increasing nitrogen fixer. In this case, Zeidan *et al.* (2005) and El-Garhi *et al.* (2007) found positive effect on yield and yield attributes of wheat, when inoculated with biofertilizer. 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.

Table 4 - Yield attributes of two wheat cultivars as affected by different nitrogen levels and biofertilizers in 2014/2015 and 2015/2016 seasons

Character	Cultivar (C)		N levels (kg N ha^{-1}) (N)				Biofertilizers (B)			Interaction			
	Sakha 94	Gemmeiza 10	0.0	85	170	250	Control	Cerialin	Nitrobein	CN	CB	NB	CNB
2014/2015													
Plant height (cm)	117.11 a	94.44 b	97.77 d	101.45c	102.88b	104.77 a	101.65c	102.32b	104.41a	ns	**	NS	NS
Spike length (cm)	9.10 a	8.18 b	8.57 c	8.62 c	9.06 b	9.39a	8.65 c	9.45 b	9.85a	NS	NS	NS	NS
Spikelet number/spike	18.95 a	17.75b	16.96c	16.63c	17.41b	18.52a	16.45c	17.32 b	18.21 a	*	**	*	NS
Spike number m^{-2}	337.65a	297.45b	308.12d	312.74c	316.21b	319.13a	312.58 c	314.34 b	316.30 a	**	NS	NS	NS
Grain number/spike	57.83a	49.58b	50.07 d	50.83 c	51.3 b	54.75 a	50.83 c	53.27 b	55.77 a	*	**	NS	**
1000 grain weight (g)	47.55a	45.80b	45.17d	45.91c	47.55b	46.66d	45.91c	47.66b	49.55a	NS	NS	NS	NS
2015/2016													
Plant height (cm)	119.75a	96.13b	99.27 d	104.11 c	105.47 b	106.58 a	103.44c	104.91 b	106.80a	NS	**	NS	NS
Spike length (cm)	10.16a	9.82b	9.50 d	10.08 c	10.20 b	10.30 a	9.98 c	10.16 b	10.34 a	NS	NS	NS	NS
Spikelet number/spike	20.30a	19.08b	16.52 d	17.25 c	18.98 b	20.27 a	18.16 c	19.19 b	20.55a	*	**	*	ns
Spike number m^{-2}	341.02a	301.91b	312.16 d	316.69 c	320.47 b	329.94 a	316.50c	318.30 b	320.30a	**	NS	NS	NS
Grain number/spike	59.83a	51.58b	51.47 d	53.83 c	55.30 b	56.75 a	52.83 c	55.27b	57.77a	*	**	NS	**
1000 grain weight (g)	48.55a	46.80b	51.77 a	49.91 b	47.55 c	45.66 d	47.91c	49.66b	51.55a	NS	NS	NS	NS

Means designated by the same letter are not significantly different at 5% level, according to Duncan's Multiple Range Test.
*,** and N.S. indicate $P< 0.0$, $P< 0.01$ and not significant, respectively.

Table 5 - Yield and quality of two wheat cultivars as affected by different nitrogen levels and biofertilizers in 2014/2015 and 2015/2016 seasons

Character	Cultivar (C)		N levels (kg N ha^{-1}) (N)				Biofertilizers (B)			Interaction			
	Sakha 94	Gemmeiza 10	0.0	85	170	250	Control	Cerialin	Nitrobein	CN	CB	NB	CNB
2014/2015													
Grain yield (t ha^{-1})	6.67 a	6.14b	5.80d	6.30c	6.56b	6.95a	6.10c	6.45b	6.79a	NS	NS	NS	NS
Straw yield (t ha^{-1})	13.52a	12.33b	12.01d	12.93c	12.66b	13.38a	12.38c	12.90b	13.36a	NS	NS	NS	NS
Biological yield (t ha^{-1})	20.21a	18.48b	17.31d	18.33c	18.71b	20.36a	18.50c	19.36b	20.14a	NS	NS	NS	NS
Harvest index (%)	34.28 a	33.25 b	30.66 d	31.70c	33.97b	34.15a	32.95 b	33.33 b	34.69 a	NS	NS	NS	NS
Protein content (%)	11.24a	10.54b	9.24d	9.98c	10.65b	11.26a	9.78c	10.78b	11.02a	NS	NS	NS	NS
2015/2016													
Grain yield (t ha^{-1})	7.21a	6.69b	6.05d	6.55c	6.90b	7.50a	6.64c	7.00b	7.33a	NS	NS	NS	NS
Straw yield (t ha^{-1})	14.48a	13.31b	13.06d	13.88c	14.21b	14.33a	13.33c	13.86b	14.31a	NS	NS	**	NS
Biological yield (t ha^{-1})	21.71a	19.98b	18.81d	19.43c	20.21b	21.86a	20.00c	20.86b	21.64a	NS	NS	NS	NS
Harvest index (%)	34.52 a	33.49 b	31.27 d	32.05c	33.16b	34.31a	33.21 b	33.56 b	34.88 a	NS	NS	NS	NS
Protein content (%)	11.54a	10.87b	9.45d	10.23c	10.78b	11.46a	9.98c	10.88b	11.26a	NS	NS	NS	NS

Means designated by the same letter are not significantly different at 5% level, according to Duncan's Multiple Range Test.
*,** and N.S. indicate $P< 0.0$, $P< 0.01$ and not significant, respectively.

B8. Straw yield (t ha^{-1})

Straw yield was significantly different among cultivars. The superiority of Sakha 94 cutivar in straw yield/fed in both seasons. Increasing nitrogen levels caused significant increases in straw yield of wheat. The highest straw yield/ha was recorded at 105 kg N ha^{-1} in both seasons.

Inoculation with biofertilizers had highly significant effect in both seasons, where Nitrobein gave the highest straw yield/ha. It might be due to the fact that nitrogenous fertilizers enhanced the vegetative growth of crop, which resulted in the higher production of stover yield. Results were corroborated with the findings of Ghaderi-Daneshmand *et al.* (2012). It might be due to the fact that increasing dose of nitrogen and inoculation might have resulted in higher rate of photosynthesis, which resulted in an increase in the carbohydrate content in straw. Similar results were reported by Abedi *et al.* (2010) and Hafez and Kobata (2012).

B9. Biological yield (t ha^{-1})

The superiority of Sakha 94 cultivar in biological yield/fed was noted in both seasons. The highest biological yield/ha was recorded at 250 kg N ha^{-1} in both seasons. Inoculation with biofertilizers had highly significant effect in both seasons, where Nitrobein gave the highest biological yield/ha. The interaction between nitrogen levels and biofertilization showed positively significant effect on biological yield/ha up to 250 kg N ha^{-1} + Nitrobein in the first season and Cerialin in the second season. The rhizosphere competence of native bacteria for C sources was major determinant for the success of inoculants (Kizilkaya, 2008). As free living, non-photosynthetic bacteria depend on soil organic matter as a food source, enhanced bacterial populations in the mixtures possibly increased competition for energy sources in the soil. Plant growth promoting activity was partially independent of bacterial population size on roots (Kizilkaya, 2008). The nutrient competition between plant and high bacteria population probably limited plant growth (Kandil *et al.*, 2011).

Mixed microbial cultures allow their components to interact with each other synergistically, thus, stimulating each other through physical or biochemical activities (Ghaderi-Daneshmand *et al.*, 2012).

The interaction of N_2-fixing bacteria with other bacteria could also inhibit their diazotrophic activity (Kandil *et al.,* 2011). Soil microbial cultures with similar or different functions might express beneficial actions in a soil or rhizosphere (Abedi *et al.*, 2010).

B10. Harvest index (%)

The superiority of Gemmeiza 10 cutivar in harvest index was noted in both seasons.

The highest harvest index was recorded at 170 or 250 kg N/ha in both seasons. Inoculation with biofertilizers had highly significant effect in both seasons, where Cerialin gave the highest harvest index. The interaction between cultivars and nitrogen levels showed significant effect on harvest index in the first season only. Gemmeiza 10 wheat cultivar gave the highest harvest index at 170 or 250 kg N ha^{-1} (Abedi *et al*., 2010 and Ghaderi-Daneshmand *et al*., 2012).

C. Grain guality

Protein content (%)

Sakha 94 was higher than Gemmeiza 10 in both seasons. The two cultivars were in a limited range (11.24-10.54 %) in the first season and 11.54-10.87% in the second season. The results showed that protein content in grains increased significantly and gradually by increasing nitrogen level up to 250 kg N ha^{-1}. Significant effects for biofertilization on protein content in grains were obtained for both seasons. Protein content increased by using Cerialin, compared to Nitrobein.

The interaction between wheat cultivars and nitrogen levels showed significant effects on protein content at both seasons. Sakha 94 recorded the highest protein content with 250 kg N ha^{-1}. Significant differences for the interaction between nitrogen fertilizer levels and biofertilizer in both seasons on protein content. However, the highest value was recorded at 250 kg N ha^{-1} + Nitrobein. Sakha 94 gave the greatest protein content with Nitrobein. It might be due to the fact that dual inoculation (nitrogen fixer solubilizers) improved the nutritional value of the crop, which resulted in higher protein content in the grain of wheat. Similar results were also reported by Daneshmand *et al*. (2012). Also, due to the fact that the higher nitrogen doses increased nitrogen content in straw, which is resulted in higher protein content. Similar results were also reported by Abedi *et al*. (2010) and Abou-Khadrah *et al*. (2014).

CONCLUSIONS

Biofertilizer and nitrogen fertilizer have increased grain yield through increase in number of grains $spike^{-1}$, number of spikes m^{-2} and 1000 grain weight, which cause to an increase in biologic yield and harvest index. We may conclude that using biofertilizer and nitrogen fertilizer together could reduce N application without any significant reduction of grain yield.

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