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Study the Efficiency of Some Biological Nitrogen Fixers and Mineral Nitrogen Levels on Growth and Productivity of Wheat under Siwa Conditions

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ABSTRACT

This study was carried out in north western desert of Egypt in sandy loam soil of Tegzerty region, at Siwa Oasis region to study efficiency of some biological nitrogen fixers and mineral Nitrogen levels on growth and productivity of wheat under Siwa conditions in two seasons of 2018/2019 and 2019/2020. This soil was salinity, had an EC of $4.63dSm^{-1}$ in the paste extraction and irrigated with water of $1.65dSm^{-1}$. Tegzerty region at Siwa Oasis, located at 29º 11' 32" N and 25º 32' 09" E. Wheat (*Triticum aesitivum* var. Sakha93) was the test plant. The investigated crop was sown in the in plots (3×4m) in rows. The mineral fertilizers was applied as a general treatment using three rates of 50, 75 and 100 kg N/fed. as NH_4NO_3 while one rate for $30kgP_2O_5$ super phosphate ($15.5\%P_2O_5$) and 40 kg K_2O as potassium sulfate. The bio-fertilizers were *Cyanobacteria, Rhizobium and Azospirillium* with double and third mixed between theirs. Obtained results showed a positive effect for biofertilization treatments and N mineral fertilizers dose on total microbial counts and microbial activities in wheat rhizosphere. On another side, Yield components of wheat were increased with increasing rates of mineral nitrogen levels with applied bio-fertilizers , the superior treatment for higher effect on yield components of wheat was mixed biofertilization treatments at 100kgN/fed .While , regarding the effect of biofertilization treatment on yield parameters of wheat as following the *Azospirillium* recording most powerful followed by Rhizobium then Cyanobacteria while mineral N increase yield parameters with increase rates of N levels . The integration between bio and N fertilizers were achieved highest yield components of wheat plant. The superior treatment was Mixed biofertilization treatments with (100kg N/fed) which achieved the highest values of yield components in wheat during two seasons. Biofertilization treatment and Mineral nitrogen fertilizer positively affected nutrients contents during two seasons, but heavy metal content were negatively affected. The mixed biofertilization treatment and higher N mineral rates was higher effect on heavy metal than other single treatments effect. The integration between bio and N mineral fertilizers were achieved highest nutrients content of wheat plant.

Keywords: Rhizobacteris, Mineral fertilization, Melilotus elegons, phosphate dissolving, nitrogen fixers

1. Introduction

Siwa Oasis is a natural depression located in northern part of the Western Desert of Egypt (about 50 km east of the Libyan border and 300 km south of the Mediterranean Sea) in the Sahara desert. Its average depth is around 18 meters below sea level and covers an area of about 250,000 feddans of which 15,000 feddans are currently cultivated based on groundwater available from both of dug wells and natural flowing springs giving a total discharge of about 130 million m^3 /year (Samy, 2010; Brebbia *et al.,* 2012; El Hossary *et al.,* 2016 and Hodge *et al.,* 2013).. Climate in Siwa Oasis is arid to semiarid with a negligible rainfall, the monthly mean maximum temperature range from 20°C in January to 38°C in July, with a yearly average of approximately 30°C. The monthly mean minimum temperature ranges from 4°C in January to 21°C in July. Absolute maximum temperatures

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can reach 50°C while the absolute minimum temperature measured was 4.5°C. Mean monthly relative humidity ranges from 30 to 58% (FAO, 2016)

Wheat (*Triticum aestivum* L.) is the most important cereal crop in Egypt and increasing wheat production is an essential national target to fill the gap between production and consumption. Production could be increased through cultivation of high yielding cultivars and appropriate agronomic practices (Tawfik *et al.,* 2006).

Use of soil microorganisms which can either fix atmospheric nitrogen, solubilize phosphate, synthesis of growth promoting substances or by enhancing the decomposition of plant residues to release vital nutrients and increase humic content of soils, will be environmentally begin approach for nutrient management and ecosystem function(Wu *et al.,* 2005). Application of biofertilizer is considered today to limit the use of mineral fertilizers and supports an effective tool for desert development under less polluted environments, decreasing agricultural costs, maximizing crop yield due to providing them with an available nutritive elements and growth promoting substances (Arafa *et al.,* 2009).

Biofertilizers can play a significant role in fixing atmospheric N and production of plant growth promoting substances. Also, biofertilization has great importance in alleviating environmental pollution and deterioration of nature (Rana *et al.,* 2012). *Azotobacter* sp. and *Azospirillum* sp. are used as biofertilizers in the cultivation of many agricultural crops. The estimated contribution of these free-living N fixing prokaryotes to the N input of soil ranges from 0–60 kg/ha per year (Vessey, 2003). Existence of microbial communities like *Azotobacter* sp. and *Azospirillum* sp. in the rhizosphere promotes the growth of the plant through the cycling and availability of nutrients, increasing the health of roots during the growth stage by competing with root pathogens and increasing the absorption of nutrients and water (Daneshmand *et al.,* 2012). Kandil *et al.,* (2011) studied the effects of inoculation with *Azotobacter* sp. and *Azospirillum* sp. on wheatand observed that inoculated wheat plants gave higher plant height, spike per unit of area, grains per spike, grain weight, biological yield, grain yield and straw yield compared to non-inoculated cultivars. Zorita and Canigia (2009) found that seed inoculation can increase the number of harvested grains by 6.1% and grain yield by 260 kg ha–1 (8.0%) in wheat. Similar results were reported by Sary *et al.,* (2009) and Daneshmand *et al.,* (2012). Some of investigations have suggested that integrated nutrient management strategies involving inoculation of seeds with *Azotobacter* sp. and *Azospirillum* sp. in combination with chemical fertilizers result in improving both growth and yield of crops (Saini *et al.,* 2004; Piccinin *et al.,* 2013).

Regard to effect of mineral nitrogen fertilization on yield components of wheat, Campillo *et al.,* (2010) reported that the optimal physical N rate (OPR) in both seasons ranged from 290 to 339 kg ha^{-1}, whereas optimal economic N rate (OER) ranged from 248 to 274 kg ha-1, with yields between 10.2 and 10.1 t ha^{-1}. Nitrogen use efficiency associated to OER was high in both seasons (36.9 and 41.2 kg grain kg^{-1} N) and fluctuated in similar ranges. Nitrogen rate increased hectoliter weight and grain protein, but decreased NUE. Gomaa *et al*. (2015) stated that the effective treatments for spike length (cm), number of grains/spike, number of spikes/m^2, number of spikelets/spike, 1000-grain weight, straw, grain and biological yield (tons/fed) were obtained from applying 20 m^3/fed, organic with adding of 70 kg N/fed. In both seasons the highest values of nitrogen, phosphorus, Potassium percentages and crude protein content were recorded by using 20m^3/fed organic with applying 70 kg N/fed. Attia and Abd El Salam (2016) reported that the highest grain yield was obtained by using rate 100% NPK mineral fertilizer, 20 m3/fed organic manure with adding bio-fertilizer. (bio-fertilized were Microbein as Pseudomonas sp., *Azotobacter sp., Azospiirillum sp., and B.megatherium*) in the first and second season, respectively. El Metwally *et al.* (2018) reported that the mineral NPK fertilizers with bio fertilizer (Cerealin) had significant effect on yield and its components Mineral NPK fertilizers with bio fertilizer (Cerealin) had significant effect on yield and its components. The highest grain yield (4.31 and 4.07 ton/fed) in both seasons, respectively was recorded by sowing 65 kg seed fed with adding 65 kg N + 37.5 kg P_2O_5 + 36 kg K_2O + Cerealin inoculation compared with other tested treatments. El-Samie *et al.* (2018) decided that the recommended fertilizing wheat plants was poultry manure at the rate of 10m3/fad and foliar spraying with mixture micronutrients (Fe, Mn and Zn at 100g/200L water/fed) as well as addition 80kg N/fed, to improve the productivity of wheat yield under newly reclaimed soil conditions in Fayoum Governorate.

The effect of integration mineral fertilizers especially N with bio fertilizers on yield components of wheat plants were showed by Fawy *et al.* (2015) they reported that the integration treatment (Bio and mineral fertilizers) for yield, nutrients and biochemical components contents of wheat was (P4+Zn1 plus Mycorrhizae + Azotobacter) which achieved 5.45 and 2.21ton/fed for straw and grains respectively in sandy soil, while being 9.5 and 4.16 ton/fed in clay soil of New Valley, Egypt. Attia *et al.* (2015) reported that the integration between bio and mineral fertilizers was P_2+ (AZ) + (SD) + (PDB) + Zn_1 under conditions of the irrigation of every 10 days which gave 2.34, 11.1, 0.99 and 1.82 for weight straw, seeds, oil and fiber (Mg/ha^{-1}) of flax plant respectively in the first season, while in the second season it achieved 2.48, 11.4, 1.09 and 1.89 (Mg/ha^{-1}). EL-Sharabasy *et al.* (2018) reported that, the application of bio fertilizer at rate 1:1/4:1:1/4 (v/v) induced significant increases in the Leaf nutrient elements content (N, P, K, Fe, Mn, Zn, and Cu over control treatment. So, it can be recommended to use plant growth promoting rhizobacteria (PGPR) as a source of nitrogen (*Azotobacter chrococcum and Azospirillum brasilense*), phosphorus (*Bacillus megatherium*) and potassium (*Bacillus circulans*) at rate 1:1/4:1:1/4 (v/v) to improve the vegetative growth, increase chemical compositions in leaves and improved nutrients uptake of date palm plants grown under saline stress conditions.

The purpose of this research was to study the effect of bio-fertilizers, and mineral fertilizers application especially N on behavior of heavy metal and production of wheat plant under Siwa soil conditions.

2. Materials and Methods

A field experiment was conducted at two successive years (2019 and 2020) completely randomized field experiments with three replications for each treatment was carried out in Tegzerty region at Siwa Oasis, located at 29º 11' 32" N and 25º 32' 09" E. Wheat (*Triticum aesitivum* var. Sakha93) was the test plant. The investigated crop was sown in the in plots (3×4m) in rows. The mineral fertilizers was applied as a general treatment using three rates of 50, 75 and 100 kg N/fed. as NH_4NO_3 while one rate for 30kg P_2O_5/fed super phosphate (15.5%P_2O_5) and 40 kg K_2O/fed as potassium sulfate during soil preparation. The bio-fertilizers were Cyanobacteria, Rhizobium and Azospirillium with double and third mixed between theirs. Some physico- chemical properties and available nutrients of the studied soils were measured according to Page *et al.* (1982) obtained results reported in Table (1).

Table 1: Physico- chemical properties and available nutrients of the experimental soil*.

Depth cm	pH	E.C dS/m	OM	$CaCO_3$	Sand %	Silt	Clay	C.E.C Cmol/kg	Texture
0-30	8.36	1.51	2.97	27.4	68.09	16.02	15.89	12.13	S.L
30-60	8.44	1.65	2.15	30.6	60.48	21.16	18.36	15.10	S.L

Soluble cations and anions in soil (me/L)							
Depth	**Na^+**	**K^+**	**Ca^{++}**	**Mg^{++}**	**HCO_3^-1**	**Cl^-**	**SO_4^-**
0-30	3.87	0.58	4.90	5.75	0.80	9.67	4.63
30-60	4.56	0.60	5.39	5.95	0.85	10.44	5.21
Available nutrients in soil (µg/g)							
Depth	**N**	**P**	**K**	**Fe**	**Mn**	**Zn**	**Cu**
0-30	43.4	10.4	81	4.47	3.03	0.89	0.37
30-60	41.1	8.81	87.5	5.54	3.47	1.05	0.41

Wheat plants was the investigated crop, sown in the in plots (3×4m) in rows. The mineral fertilization was applied as a general treatment using three rates of 50, 75 and 100 kg N/fed as NH_4NO_3 while 30 kgP_2O_5/fed. as calcium super phosphate(15.5%P_2O_5) mixed with the soil during soil preparation. The K applied at 40 kgK_2O/fed as potassium sulfate. The P and K fertilizers were added at one rate of 30 kgP2O5/fed and 40 kg K_2O as potassium sulfate. The N and K divided into three equal doses applied at seedling, flowering and full grains stages. The dose of 10m3 organic manure was added by mixing with 0-20 surface layer before sowing. Physical and chemical analysis soil are presented in (Table1).

Bio-fertilizers treatments were: 1) control, 2) Cyanobacteria, 3) Rhizobium, 4) Azospirillium, 5) Azospirillium. + Rhizobium, 6) Azospirillium + Cyanobacteria, 7) Rhizobium + Cyanobacteria, 8) Rhizobium + Cyanobacteria and 8) mixed treatment its.
Rh: *Rhizobium legumenozarum,*
Cyanobacteria: *Anabena orayza*
Azo: *Azospirillum liboferum,*

Fresh liquid culture of *Azospirillium and Rhizobium* were used for soil applications single, dual or in combination at the rate of 10^8colony forming unit (cfu/ml).

Rhizosphere soil samples were collected at heading and harvesting stages. The samples were analysed for total counts of microorganisms according to Nautiyal (1999). For counting rhizobium according to Woomer *et al.* (1990). For counting and growing Azospirillum according to Usha and Kanimozhi (2011) Grain and straw samples were taken at harvesting from each treatment, dried at 70°, and ground using stainless steel equipments for the determination of N,P, K, Pb, Ni, Cd and Co Plant nutrients were determined as follows: Total nitrogen using the micro kjeldahl method (A.O.A.C, 1980). Phosphorus, potassium, using dry ashing technique according to Cottenie *et al.* (1982).

Growth parameters: at heading and harvesting stages plants were taken from each plot for estimating plant height, fresh and dry weights.

Statistical analysis: All the obtained data from each season were exposed to the proper statistical analysis of variance according to Gomez and Gomez (1984). LSD at 0.05 level of significance was used for the comparison between means.

3. Results and Discussion

3.1. Isolation of *Azospirrilum*

Azospirillum isolates were isolated from different localities from Siwa soil rhizosphere of different growing plants, bacteria was grown on its specific media for growth.

Obtained isolates were examined for their Total counts in rhizosphere, specific activity of Azospirillum isolates in nitrogen fixation by measuring total nitrogen, nitrogenase activity and their ability for pghytohormone production by measuring Indole acetic acid (IAA). The most active isolate was selected for application in field experiment. Isolate no. 2 was selected as most active isolate and will be used in field experiment

Table 2: Azospirillum counts, total nitrogen, N2-ase activity and indole acetic acid (IAA) of Azospirillum isolated.

No.	Azospirillum counts ($\times 10^3$ cells dry soil)	Total nitrogen (ppm)	N2-ase activity (ml C_2H_4/L/day)	IAA (µg/ml)
1	65	63	11.43	8.5
2	78	102	19.6	8.6
3	74	91	18.82	8.4
4	70	77	14.68	12.0
5	67	70	13.3	13.1
6	60	52	9.41	7.3
7	71	84	15.2	3.0
8	52	43	7.41	0.9
9	61	56	10.3	0.8
10	57	49	8.22	4.0

3.2. Effect of studied factors on yield and yield attributes of wheat under Siwa conditions

3.2.1. Microbial determinations

3.2.1.1. Total microbial counts

Represented data in Table 3 indicated that, microbial counts increased with increasing mineral nitrogen application in the two studied seasons while the second season was little about the first season. *Azospirillium* was superior compared to other single treatments, mixed treatment recorded highest values compared to all dual inoculations (Table 2). The superior treatments on total account of bio-fertilizers was Mixed with 100kgN/fed for total microbial counts. This results agreed with finding of Abdel Gawad and Zeinab (2009).

Table 3: Effect of inoculation with Cyanobacteria, Rhizobia and *Azospirillum* on total microbial counts of Wheat plants under Siwa condition.

Mineral Nitrogen	Bio Fertilizers	Total microbial counts (10^5 cfu / g dray soil) First season			Second season		
	Days	45	60	120	45	60	120
50 Units	Control	56	76	63	51	73	60
	Cyanobacteria	67	93	71	62	90	68
	Rhizobium	72	96	69	67	93	76
	Azospirillium	75	100	83	70	97	80
	Azo. +Rh.	80	102	87	75	99	84
	Azo. + Cyano.	86	109	95	81	106	91
	Rh. + Cyano.	82	104	90	77	101	87
	Mix	89	118	98	84	115	96
75 Units	Control	84	116	96	80	113	91
	Cyanobacteria	97	123	102	93	120	97
	Rhizobium	104	129	110	100	126	105
	Azospirillium	108	134	115	104	131	110
	Azo. +Rh.	113	139	119	109	136	114
	Azo. + Cyano.	120	148	127	116	145	123
	Rh. + Cyano.	115	141	119	112	138	115
	Mix	123	152	133	120	147	129
100 Units	Control	93	125	106	88	120	100
	Cyanobacteria	97	133	112	92	128	106
	Rhizobium	114	139	123	109	134	118
	Azospirillium	118	143	128	113	138	123
	Azo. +Rh.	124	150	135	119	145	129
	Azo. + Cyano.	132	161	143	127	156	138
	Rh. + Cyano.	127	153	138	122	148	133
	Mix	138	167	149	133	162	145
LSD $_{0.05}$ Mineral Nitrogen			0.1993			0.1984	
LSD $_{0.05}$ Bio-Fertilizers			0.3239			0.2952	
LSD $_{0.05}$ Interaction (Min. x Bio.)			0.5611			0.5638	

Cyano: Anabena Orayza (Cyanobacteria), **Rh:** Rhizobium leguminosarum**, Azo.:** Azospirillum lipoferum,

Regarding the effect of mineral fertilization levels and biofertilization treatments on Azospirillum counts, obtained data in Table 4 clearly showed that, the Counts of Azospirillum increased in first season and decreased the second season, Azospirillum treatment either in single, dual and mixed was superior to other treatment. Also, Azospirillum counts increased with increasing mineral fertilization dose

3.3. Growth and yield parameters

Regarding to the effect of bio-fertilization and N mineral fertilizer doses on growth and yield parameters of wheat as shown in table 5. Growth and yield parameters of wheat were increased with increased mineral nitrogen rates. For biofertilization treatments growth and yield components increased with *Azospirillum* treatment than other single treatments (Cyanobacteria, Rhizobia), mixed treatment was superior to other treatment at 100kgN/fed. (Table 6). The same trend was recorded with shoot and root dry weights g/plant as shown in Tables 5 and 6. These results compatible with Campillo *et al.,* (2010) and Gomaa *et al.,* (2015).

Table 4: Effect of inoculation with Cyanobacteria, Rhizobia and Azospirillum on counts of Rhizobium of Wheat plants under Siwa condition

Mineral nitrogen	Bio Fertilizers	Counts of Rhizobium (10^4 cfu / g dray soil)					
		First season			Second season		
	Days	45	60	120	45	60	120
50 Units	Control	36	54	42	32	49	39
	Cyanobacteria	47	71	50	43	67	48
	Rhizobium	52	74	58	47	71	55
	Azospirillium	55	77	62	52	75	59
	Azo. +Rh.	60	79	65	58	78	62
	Azo. + Cyano.	66	86	73	63	83	69
	Rh. + Cyano.	62	81	67	60	79	64
	Mix	69	94	76	67	91	70
75 Units	Control	63	94	76	58	90	71
	Cyanobacteria	76	101	82	72	98	79
	Rhizobium	83	107	90	80	103	88
	Azospirillium	86	112	95	84	110	93
	Azo. +Rh.	92	117	99	90	115	97
	Azo. + Cyano.	98	126	106	97	122	103
	Rh. + Cyano.	93	119	99	92	118	99
	Mix	102	130	111	100	127	108
100 Units	Control	71	104	85	67	101	80
	Cyanobacteria	85	112	91	80	110	89
	Rhizobium	92	118	101	90	115	99
	Azospirillium	94	122	105	93	120	104
	Azo. +Rh.	100	128	112	98	125	110
	Azo. + Cyano.	107	137	118	104	133	118
	Rh. + Cyano.	102	130	108	102	128	109
	Mix	112	141	122	110	135	122
LSD $_{0.05}$ Mineral Nitrogen		0.2174			0.2214		
LSD $_{0.05}$ Bio-Fertilizers		0.3550			0.3474		
LSD $_{0.05}$ Interaction (Min. x Bio.)		0.6149			0.2554		

Cyano: Anabena Orayza (Cyanobacteria), **Rh:** Rhizobium leguminosarum**, Azo.:** Azospirillum lipoferum,

Table 5: Effect of inoculation with Cyanobacteria, Rhizobia and Azospirillum on plant height of wheat plants under Siwa condition.

Mineral nitrogen	Bio Fertilizers	Plant height (cm)					
		First season			Second season		
	Days	45	60	120	45	60	120
50 Units	Control	46.5	55.5	67.2	41.6	51.4	64.3
	Cyanobacteria	66.6	76.2	84.8	61.5	72.3	81.8
	Rhizobium	70.4	78.9	89.5	65.4	75.1	86.5
	Azospirillium	73.1	81.8	91.8	68.8	77.9	88.2
	Azo. +Rh.	78.5	87.3	93.9	73.6	83.4	90.8
	Azo. + Cyano.	84.9	89.9	98.1	79.8	85.9	95.1
	Rh. + Cyano.	80.5	88.2	95.8	75.5	84.2	92.8
	Mix	89.4	93.6	99.7	84.3	89.6	96.7
75 Units	Control	49.8	58.2	73.2	46.7	54.3	70.2
	Cyanobacteria	70.1	80.4	90.8	67.2	76.5	87.5
	Rhizobium	73	83.2	95.1	70.8	79.9	92.1
	Azospirillium	78.5	86.9	79.9	75.6	82.8	95.1
	Azo. +Rh.	84.8	92.4	99.9	81.8	88.5	97.2
	Azo. + Cyano.	91.5	98.2	105.1	88.4	94.2	102.3
	Rh. + Cyano.	89.1	95.3	102.3	85.7	91.4	99.1
	Mix	95.8	102.4	109.1	92.5	98.4	106.3
100 Units	Control	54.9	64.8	89	50.8	59.8	86.5
	Cyanobacteria	75.3	86.2	97.4	71.2	82	92.4
	Rhizobium	78.4	89.9	99.6	75.1	85.1	96.3
	Azospirillium	83.5	94.2	102.2	79.8	89.4	99.7
	Azo. +Rh.	89.9	99.8	106.3	85.7	94.8	101.3
	Azo. + Cyano.	97.1	108	113.2	93.4	103.7	110.1
	Rh. + Cyano.	94.2	104	110.1	90.2	99.8	107
	Mix	101	110	118	96.1	105	114
LSD $_{0.05}$ Mineral Nitrogen			0.0922			0.0880	
LSD $_{0.05}$ Bio-Fertilizers			0.1290			0.1588	
LSD $_{0.05}$ Interaction (Min. x Bio.)			0.2769			0.2607	

Table 6: Effect of inoculation with Cyanobacteria, Rhizobia and Azospirillum on Shoot dry weight of wheat plants under Siwa condition.

Mineral nitrogen	Bio Fertilizers	Shoot dry weight (g / plant)					
		First season			Second season		
	Days	45	60	120	45	60	120
50 Units	Control	16.3	21.6	33.52	14.8	20.2	32.2
	Cyanobacteria	20.4	28.4	41.6	18.9	26.3	40.3
	Rhizobium	23.5	33.5	47.2	22	32.1	45.4
	Azospirillium	26	36.2	51.5	24.6	35.3	50.1
	Azo. +Rh.	31	39.6	55.2	29.7	38.5	54.2
	Azo. + Cyano.	37.2	44.9	65.8	35.8	43.2	63.9
	Rh. + Cyano.	33.2	41.3	59.9	31.3	40.1	57.8
	Mix	39.1	46.8	69.2	37.6	45.4	67.3
75 Units	Control	19.5	33.4	47.2	18.4	31.8	45.6
	Cyanobacteria	26.8	39.9	53.8	25.4	38.4	52.1
	Rhizobium	30.2	45.2	59.6	28.8	43.7	58.2
	Azospirillium	37.6	48.3	64.2	36.1	47.3	62.5
	Azo. +Rh.	41.8	50.5	68.8	40.3	49.9	66.8
	Azo. + Cyano.	50.3	63.8	79.9	48.7	62.1	78.2
	Rh. + Cyano.	45.1	56.7	73.4	43.6	54.3	72
	Mix	54.8	66.1	83.6	51.9	64.6	81.3
100 Units	Control	23.2	40.7	57.2	21.6	39.3	56
	Cyanobacteria	30.5	45.2	63.8	29.1	44.1	63.1
	Rhizobium	33.8	48.8	68.2	32.4	48	67.2
	Azospirillium	37.9	55.5	74.8	36.5	54.8	73
	Azo. +Rh.	44.3	62.8	79.4	43.1	60.5	76.1
	Azo. + Cyano.	63.1	71.4	87.1	61.3	70.1	85.6
	Rh. + Cyano.	55	65.7	82.3	54.8	64.7	81.8
	Mix	66	74.2	89.2	64.7	73.6	88.2
LSD $_{0.05}$ Mineral Nitrogen			0.9296			0.9144	
LSD $_{0.05}$ Bio-Fertilizers			1.4425			1.5295	
LSD $_{0.05}$ Interaction (Min. x Bio.)			2.6292			2.5864	

Table 7: Effect of inoculation with Cyanobacteria, Rhizobia and Azospirillum on Root dry weight of wheat plants under Siwa condition

Mineral nitrogen	Bio Fertilizers	Root dry weight (g / plant)					
		First season			Second season		
	Days	**45**	**60**	**120**	**45**	**60**	**120**
50 Units	**Control**	8.2	10.1	13.8	6.9	8.5	9.9
	Cyanobacteria	9.8	11.4	15.4	8.5	9.9	11.8
	Rhizobium	11.2	12.8	15.9	9.7	11.6	12.9
	Azospirillium	11.9	13.6	17.3	10.8	13.1	13.8
	Azo. +Rh.	12.4	14.7	19.1	11.9	14.1	14.4
	Azo. + Cyano.	14.3	15.4	22.2	13.8	15.2	18.3
	Rh. + Cyano.	12.9	14.9	20.1	12.8	14.5	16.4
	Mix	15.5	16.9	24.3	15.2	16.3	19.9
75 Units	**Control**	17.2	22.8	27.8	15.3	21.2	24.2
	Cyanobacteria	20.8	25.1	30.9	18.1	24.6	26.8
	Rhizobium	24.1	26.9	33.5	20.4	28.7	28.4
	Azospirillium	25.7	32.2	36.7	22.8	31.8	31.2
	Azo. +Rh.	29.1	34.7	38.9	25.1	34.7	34.4
	Azo. + Cyano.	33.6	38.8	40.5	32.6	37.9	38.8
	Rh. + Cyano.	30.7	36.1	34.6	29.2	35.6	35.7
	Mix	35.4	40.2	43.5	34.8	40.2	43.1
100 Units	**Control**	18.9	23.6	27.8	17.8	20.2	26.2
	Cyanobacteria	23.7	27.9	32.6	20.7	23.8	29.5
	Rhizobium	27.6	28.7	35.2	23.5	25.9	33.4
	Azospirillium	29.5	33.8	37.4	26.2	28.7	35.9
	Azo. +Rh.	31.2	36.1	39.7	29,2	31.2	38.2
	Azo. + Cyano.	35.6	40.6	44.2	34.8	35.4	42.6
	Rh. + Cyano.	32.8	36.9	40.9	31.5	33.1	40.1
	Mix	37.9	42.5	46.8	37.1	39.9	45
LSD $_{0.05}$ Mineral Nitrogen			0.6313			0.6428	
LSD $_{0.05}$ Bio-Fertilizers			0.3063			0.3077	
LSD $_{0.05}$ Interaction (Min. x Bio.)			0.5328			0.5306	

Chlorophyll content and number of tillers of wheat plants were affected by biofertilization treatments. These data also clarify the role of biofertilization type in increasing chlorophyll content, whereas the effectiveness order was as follows: Mixed> dual treatment > Azospirillum and the least one cyanobacteria at second season of plant growth.

This trend might be attributed to the enhancement of both microorganisms and plant roots in stimulating and producing humic materials which contribute in binding soil separates.

Also obtained results showed that total chlorophyll and number of tillers of wheat plants were increased with N mineral fertilizer and mixed bio-fertilizer under Siwa condition as shown in Table 8. The present results are in compatible with El Ghany *et al.* (2010), which reported that inoculation nitrogen fixers stimulated growth parameters of wheat plant under siwa conditions.

Table 8: Effect of inoculation with Cyanobacteria, Rhizobia and Azospirillum on total chlorophyll and number of tillers of Wheat plants under Siwa condition after 60 days.

Mineral nitrogen	Bio Fertilizers	Total chlorophyll		Number of tillers per plant	
	Seasons	**1st**	**2nd**	**1st**	**2nd**
50 Units	**Control**	46	46.02	2.9	3.8
	Cyanobacteria	46.14	46.17	3.1	4
	Rhizobium	46.16	46.2	3.3	4.2
	Azospirillium	46.45	46.5	3.4	4.4
	Azo. +Rh.	46.66	46.7	3.9	5
	Azo. + Cyano.	47.32	47.35	4.6	5.6
	Rh. + Cyano.	46.9	46.92	4.4	5.4
	Mix	47.52	47.55	4.8	5.8
75 Units	**Control**	46.45	46.5	3.3	4.2
	Cyanobacteria	47.14	47.22	3.6	4.5
	Rhizobium	47.26	47.29	3.7	4.8
	Azospirillium	47.46	47.5	4.9	5
	Azo. +Rh.	47.71	47.75	4.2	5.2
	Azo. + Cyano.	48.52	48.57	4.7	5.8
	Rh. + Cyano.	47.73	47.75	4.6	5.6
	Mix	48.97	49.22	5	6
100 Units	**Control**	47.65	47.72	4.3	4.5
	Cyanobacteria	48.58	48.8	4.6	4.8
	Rhizobium	48.86	48.95	4.9	5.1
	Azospirillium	49.28	49.42	5.3	5.4
	Azo. +Rh.	49.7	49.8	5.4	5.4
	Azo. + Cyano.	50.61	50.7	5.8	6.2
	Rh. + Cyano.	50.52	50.55	5.6	5.8
	Mix	51.8	51.92	6.2	6.4
LSD 0.05 Mineral Nitrogen		0.1273		0.1891	
LSD 0.05 Bio-Fertilizers		0.1519		0.1953	
LSD 0.05 Interaction (Min. x Bio.)		0.2094		0.2600	

The earing features of wheat plants under Siwa condition also take the same trends of others yield component of wheat as presented Table 9. This results in compatible with those reported by Abou Tahoun *et al.,* (2020).

The grain yield and straw yield of wheat plants under Siwa condition was increased with *Azospirillium* inoculation and high level applied N mineral fertilizers, grain yield and straw yield increasing with increase N mineral fertilizers dose, while in biofertilization treatment were increased with mixed bio-fertilizers under high level applied of mineral N (100Nkg/fed) as recorded in Table10. Maaboud and Abd EL Gawad (2014) reported that yield parameters and nutrient contents of wheat increased with increasing mineral fertilization dose

The Nitrogen ,Phosphorus and potassium contents of wheat grains under Siwa condition were increased with N mineral applied and applied bio-fertilizers where that the superior treatment was mixed under 100kgn/fed as shown in Table 11 .

The present results are in harmony with those of Mohamed *et al.* (2019) they reported that Nutrient uptake of wheat grain increased also by both fertilizer and dual application with either sludge or compost with biofertilizers.

Table 9: Effect of inoculation with Cyanobacteria, Rhizobia and Azospirillum on earing features of wheat plants under Siwa condition

Mineral nitrogen	Bio Fertilizers	Earing Features					
		First season			Second season		
	Earing features	1	2	3	1	2	3
50 Units	Control	10.4	1.5	41	10.8	1.6	43
	Cyanobacteria	11.7	1.9	47	12.4	1.9	50
	Rhizobium	12.9	2.1	53	13.5	2.2	55
	Azospirillium	13.3	2.4	56	13.9	2.5	57
	Azo. +Rh.	14.3	2.6	60	14.8	2.8	63
	Azo. + Cyano.	15.1	3.2	69	15.9	3.4	73
	Rh. + Cyano.	14.6	3	64	15.1	3.2	66
	Mix	15.5	3.3	74	16.2	3.6	77
75 Units	Control	13.6	1.8	46	13.9	1.9	49
	Cyanobacteria	13.8	2	49	14.1	2.2	53
	Rhizobium	14.2	2.2	53	14.9	2.4	57
	Azospirillium	14.6	2.4	58	15.3	2.6	63
	Azo. +Rh.	15.4	2.5	63	15.9	2.8	66
	Azo. + Cyano.	16.8	2.7	78	17.5	3.2	81
	Rh. + Cyano.	16.2	2.6	70	16.3	3.02	69
	Mix	17.1	2.8	82	17.9	3.3	85
100 Units	Control	14.2	2.1	49	14.5	2.1	50
	Cyanobacteria	14.9	2.4	53	15.3	2.4	55
	Rhizobium	15.8	2.6	57	16.2	2.9	59
	Azospirillium	17.1	2.7	60	17.7	2.8	63
	Azo. +Rh.	18.3	2.9	66	18.9	2.9	69
	Azo. + Cyano.	19.8	4.95	79	20.1	4.9	83
	Rh. + Cyano.	18.7	4.6	73	19.2	4.2	73
	Mix	20.2	5.3	86	20.3	5.4	87
LSD $_{0.05}$ Mineral Nitrogen		0.2833	0.033	1.1222	0.300	0.0433	1.328
LSD $_{0.05}$ Bio-Fertilizers		0.1067	0.497	1.0706	0.1051	0.0432	1.1151
LSD $_{0.05}$ Interaction (Min. x Bio.)		0.2176	0.0500	2.3104	0.2174	0.1067	2.2838

Table 10: Effect of inoculation with Cyanobacteria, Rhizobia and Azospirillum on grain yield and straw yield of wheat plants under Siwa condition after 120 days.

Mineral nitrogen	Bio Fertilizers	Grain yield (Ardab / fed)		Straw yield (Ton / fed)	
	Seasons	1st	2nd	1st	2nd
50 Units	Control	10.6	10.3	3.8	3.65
	Cyanobacteria	10.8	10.6	4.4	4.33
	Rhizobium	11.2	10.8	4.7	4.61
	Azospirillium	11.4	11.1	5.01	4.95
	Azo. +Rh.	11.75	11.5	5.45	5.22
	Azo. + Cyano.	13.25	12.4	6.4	6.15
	Rh. + Cyano.	12.2	11.8	5.85	5.67
	Mix	13.6	12.9	7.1	6.98
75 Units	Control	11.2	10.8	4.3	4.21
	Cyanobacteria	12.35	10.9	4.95	4.78
	Rhizobium	12.7	11.58	5.35	5.1
	Azospirillium	12.85	11.99	5.5	5.22
	Azo. +Rh.	12.99	12.5	5.78	5.64
	Azo. + Cyano.	13.7	13.9	7.11	6.89
	Rh. + Cyano.	12.8	12.8	6.55	6.25
	Mix	13.9	14.3	7.83	7.63
100 Units	Control	11.55	11.3	4.65	4.48
	Cyanobacteria	11.9	11.6	5.15	5.01
	Rhizobium	12.3	12.1	5.8	5.67
	Azospirillium	12.8	12.2	5.95	5.84
	Azo. +Rh.	13.4	13.1	6.7	6.6
	Azo. + Cyano.	16.8	16.1	8.15	7.95
	Rh. + Cyano.	15.2	14.8	7.11	6.89
	Mix	17	16.65	9.85	9.58
LSD 0.05 Mineral Nitrogen		0.1018	0.0494	0.0928	0.0428
LSD 0.05 Bio-Fertilizers		0.0921	0.0391	0.1407	0.0352
LSD 0.05 Interaction (Min. x Bio.)		0.1891	0.0877	0.1664	0.1007

Table 11: Effect of inoculation with Cyanobacteria, Rhizobia and Azospirillum on Nitrogen and Phosphorus contents of Wheat grains under Siwa condition after 120 days.

Mineral nitrogen	Bio Fertilizers	N , P and K % in Grains					
		N%	P%	K%	N%	P%	K%
	Seasons		1st			2nd	
50 Units	Control	1.62	0.26	1.5	1.6	0.25	1.52
	Cyanobacteria	1.72	0.31	1.58	1.71	0.3	1.6
	Rhizobium	1.75	0.32	1.6	1.73	0.31	1.62
	Azospirillium	1.77	0.32	1.72	1.75	0.32	1.75
	Azo. +Rh.	1.79	0.34	1.75	1.77	0.33	1.78
	Azo. + Cyano.	1.86	0.37	1.8	1.85	0.36	1.82
	Rh. + Cyano.	1.82	0.35	1.77	1.79	0.34	1.8
	Mix	1.93	0.38	1.81	1.9	0.37	1.83
75 Units	Control	1.74	0.28	1.62	1.72	0.27	1.64
	Cyanobacteria	1.85	0.32	1.77	1.83	0.31	1.79
	Rhizobium	1.87	0.33	1.8	1.85	0.32	1.82
	Azospirillium	1.88	0.34	1.82	1.86	0.33	1.84
	Azo. +Rh.	1.88	0.34	1.83	1.87	0.34	1.85
	Azo. + Cyano.	1.92	0.36	1.88	1.9	0.35	1.91
	Rh. + Cyano.	1.9	0.35	1.85	1.88	0.34	1.87
	Mix	1.94	0.36	1.89	1.93	0.35	1.93
100 Units	Control	1.82	0.29	1.65	1.8	0.28	1.67
	Cyanobacteria	1.94	0.33	1.86	1.92	0.31	1.88
	Rhizobium	2	0.34	1.89	1.99	0.33	1.9
	Azospirillium	2.1	0.34	1.91	2.08	0.34	1.93
	Azo. +Rh.	2.16	0.35	1.93	2.14	0.34	1.95
	Azo. + Cyano.	2.19	0.36	1.96	2.16	0.35	1.98
	Rh. + Cyano.	2.17	0.35	1.94	2.15	0.34	1.94
	Mix	2.23	0.36	1.97	2.2	0.36	1.99
LSD $_{0.05}$ Mineral Nitrogen		0.1654	0.0185	0.0355	0.4782	0.0166	0.032
LSD $_{0.05}$ Bio-Fertilizers		0.3369	0.0159	0.01351	0.3407	0.0172	0.01926
LSD $_{0.05}$ Interaction (Min. x Bio.)		0.4782	0.0339	0.0724	0.4831	0.0329	0.0681

The heavy metals content of wheat grains and straw revealed the nutrients behavior. So we found increased N mineral applied decreased heavy metal content also mixed bio-fertilizers decreased heavy metal while the second season increased than first season as shown in Table 12. The previous results agree with obtained by Wei *et al.* (2020).

Heavy metal content were measured for soil under study (Siwa) recorded 26.1, 9.52, 2 and 13.45 ppm for Pb, Ni, Cd and Co respectively, Also , heavy metal content for compost and Mineral fertilization were measured recorded 27.1, 3.5,0.75 and 4.5 for compost, Ammonium sulfate heavy metal contents were 39.2, 0.69, 1 and 2.9 respectively. While Superphosphate heavy metal contents were 42.3, 4.73, 4.12 and 1.5 respectively. Potassium sulfated recorded 9.0, N.D, 0.6 and 1.1 respectively for Pb, Ni, Cd and Co.

Biofertilization treatments efficiently reduced heavy metal contents in wheat grains and straw after inoculation.

Table 12: Effect of inoculation with Cyanobacteria, Rhizobia and Azospirillum on some heavy metals content of Wheat grains and straw after 120 days.

Mineral nitrogen	Bio Fertilizers	Grains				Straw			
		First season				Second season			
	Heavy metals	Pb	Ni	Cd	Co	Pb	Ni	Cd	Co
50 Units	Control	9.5	3.75	0.18	10.2	12.35	5.5	0.31	9.2
	Cyanobacteria	8.6	2.6	0.17	9.9	11.1	5.2	0.3	9.1
	Rhizobium	8.1	2.4	0.16	9.1	10.3	4.9	0.3	9
	Azospirillium	7.55	2.1	0.15	8.5	10.1	3.75	0.29	8.8
	Azo. +Rh.	6.1	1.8	0.14	7.8	9.5	3.25	0.28	8.6
	Azo. + Cyano.	5.4	0.9	0.12	6.7	4.7	2.8	0.25	8.3
	Rh. + Cyano.	5.7	1.2	0.14	6.9	8.6	3.1	0.26	8.4
	Mix	5.1	0.6	0.1	6.3	6.78	2.25	0.25	8.2
75 Units	Control	9.61	3.85	0.22	11.3	10.75	6.2	0.32	10.2
	Cyanobacteria	8.72	2.81	0.21	10.2	10.5	5	0.31	10.1
	Rhizobium	8.13	2.52	0.19	10.5	10	4.8	0.3	9.9
	Azospirillium	7.59	2.22	0.17	9.4	9.55	4.5	0.29	9.8
	Azo. +Rh.	6.2	1.9	0.15	8.6	9.25	4.4	0.28	9.7
	Azo. + Cyano.	5.45	0.95	0.13	7.5	8.8	3.8	0.27	9.6
	Rh. + Cyano.	5.65	1.28	0.14	7.9	9	4	0.28	9.7
	Mix	5.25	0.66	0.12	7.1	8.6	3.4	0.26	9.5
100 Units	Control	9.72	3.92	0.34	12.4	9.3	7.6	0.33	11.4
	Cyanobacteria	8.82	2.89	0.32	11.9	9.1	6.45	0.31	11.2
	Rhizobium	8.25	2.61	0.31	11.4	9.2	6.3	0.31	11
	Azospirillium	9.65	2.32	0.3	10.95	9	6.2	0.3	10.8
	Azo. +Rh.	6.31	1.95	0.28	10.4	8.9	6.1	0.29	10.6
	Azo. + Cyano.	5.58	0.98	0.24	8.3	8.8	5.4	0.28	10.4
	Rh. + Cyano.	5.69	1.3	0.26	8.9	8.7	5.8	0.27	10.5
	Mix	5.3	0.69	0.22	7.75	8.5	5.25	0.28	10.1
LSD $_{0.05}$ Mineral Nitrogen		0.0632	0.0521	0.0155	0.0491	0.0752	0.537	0.0533	0.1537
LSD $_{0.05}$ Bio-Fertilizers		0.094	0.0275	0118	0.0618	0.0381	0.034	0.0794	0.182
LSD $_{0.05}$ Interaction (Min. x Bio.)		0.1059	0.194	0.128	0829	0.1081	0.2170	0.136	0.115

4. Conclusions

From obtained results in the current investigation we can concluded that, response of the wheat to different nitrogen fertilization doses was varied. Different biofertilization treatments used for improving the wheat productivity and achieve the optimal level of agricultural sustainability. Also, the application of biofertilization treatments resulted in positive improvements in the yield and quality of wheat. Azospirillum inoculation significantly produce higher yield and better quality in the presence of the optimal fertilizer application 100 units. Significant increment in the grain yield and grain quality were achieved by mixed bio fertilization, which were found to be the greatest combined treatments for maximize the grain yield under the environmental conditions of the experimental location.

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