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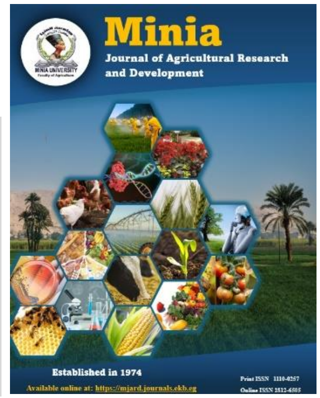

Effects of non-symbiotic nitrogen-fixing bacteria under different levels of nitrogen on the growth rate of wheat

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ABSTRACT

This study was carried out to investigate the effect of inoculation with Cyanobacteria, Azotobacter, and Azospirillum individually or in combination with different mineral-N levels on the growth of wheat plants. A pot experiment was conducted during two successive seasons (2021 and 2022) in the Department of Microbiology, Faculty of Agriculture, Minia University. Results revealed that inoculation with any of the used microorganisms generally encouraged the growth of wheat plants. Also, the treatment with 75 % N + any of the used microorganisms gained the highest plant hight, fresh and dry weight of the plant, highest total N, P, and K contents of the plants. The obtained results indicated that no significant differences in the studied measurements were observed between the treatment with 75 % N + any of the used microorganisms and the application of 100% mineral N. Such results may suggest that inoculation of wheat plants with cyanobacteria or diazotrophic bacteria may preserve nearly 25% of the mineral nitrogen required for wheat production. The most successful inoculum for wheat development was determined to be the mixture of the studied microorganisms, Moreover, all of the microorganisms that were used in this study showed promising biofertilizers for raising crop yields.

Keywords: Cyanobacteria, Azotobacter, and Azospirillum, biofertilizers, diazotrophic bacteria.

INTRODUCTION

Worldwide, wheat (*Triticum aestivum* L.) is a significant staple crop. Due to its involvement in international trade and the regular food shortages that occur, its significance has increased even further. Among cereal crops, wheat is the most important, making up 30% of all cereal food consumed globally and a key source of nutrition for more than one-third of the world's population. It also contributes around 20% of all food calories consumed by humans, either directly or indirectly. In many nations, increasing wheat output to satisfy rising population demands remains a challenge **(Sary *et al*., 2009; Abedi *et al*., 2010** and **Campuzano *et al*., 2012).** Future studies based on long-term inoculations are encouraged to verify the beneficial effects of

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these bacterial strains on nitrogen fixing, soil N mobilization and mineralization and agronomic practices in changing climate on wheat productivity under field conditions. These extensive studies could be beneficial to formulating new inoculants and improving cropping systems in more profitable and eco-friendly environments with cost-effective availability to the farmer community to develop more organic and greener agriculture **López (2023**). The soil is home to a vast array of microorganism species. According to **Gałązka *et al*.** (**2016**), fertile soil can include up to one billion bacteria per one gram of fresh soil material. The composition of microorganisms has a significant impact on both the availability of nutrients in the soil environment and the rate at which they cycle. For the formation of soil humus, the supply of nutrients to plants, and the eradication of disease, microorganisms in the soil are essential. In this context, it is becoming acknowledged that the use of plant growth-promoting bacteria is one of the alternative methods that could reduce N fertilization by up to 25% of the total N applied while also promoting plant growth, N use efficiency, and biological nitrogen fixation [**Galindo (2020) and Jala (2020)**]. *Azospirillum brasilense* inoculation (strains). According to **Jacob** and **Kumar** (**2020**), modern vegetable and crop cultivation greatly depends on fertilizers for a higher yield; however, the widespread use of inorganic chemicals and fertilizers derived from chemicals currently poses a number of risks to human health as well as the environment. As a result, researchers in applied science are focusing increasingly on using microorganisms like algae, fungi, and bacteria. This can lead to a more straightforward, quick, and environmentally sustainable method of growing crops and vegetables. Among these, the fascinating class of microorganisms known as cyanobacteria, or blue green algae (BGA), has a variety of uses in cutting-edge biotechnology for the methods used in crop growth today. By enhancing nutrient availability, maintaining organic carbon, and exhibiting coexistence with higher plants, they achieve this.Nitrogen is one of the crucial limiting factors in wheat production and is considered a challenge to food security. Therefore, sustainable agricultural technologies such as seed inoculation with plant growth-promoting bacteria (PGPBs) can be adopted to promote biological nitrogen fixation (BNF) for higher crop productivity. In this context, the objective of the current study was to evaluate the effects wheat seed inoculations with Cyanobacteria, Azotobacter, Azospirillum and combination of them under differnt levels of nitrogen on agronomic and yield attributes, grain yield, grain N accumulation.Research result showed that all *Azotobacter. chroococcum*strains had positive effect on the yield and N concentrations of wheat. Also, indigenous strain *A. chroococcum*RK49 had the highest effects on yield and increased the production of grain yield by 84% in field experiment and by in 95% pot experiment compared to control treatment without *A. chroococcum*inoculation **Kızılkaya (2008).** The different treatments (Azotobacter and concentrations of N) showed a substantial difference. The total chlorophyll (mg g-1),leaf nitrogen content and leaf area(cm2) were shown to considerably increase with an increase in N fertilization, reaching its maximum at an Azotobacter mixture of 75 kg/Fed., protein content% .The blend of Azotobacte rand 75 kg N/fed had the highest protein content among the treatments on wheat crop, and several anatomical characteristics, including bundle size, leaf thickness, and size of xylem and phloem, increased as a result of the treatments **(El-zawawy *et al*. 2023).**

This study aimed to investigate the effect of inoculation with Cyanobacteria, Azotobacter, Azospirillum each individually or their combination with different mineral-N levels on growth of wheat plants.

MATERIALS AND METHODS

1- Soil used:

In this study, two different kinds of soil were employed. Clay soil was collected from the Faculty of Agriculture's Farm in Minia. Loamy Sand freshly reclaimed soil that was taken from the Agricultural Research Center, Farm of Shosha Minia Governorate. After being air dried and sieved through a 2 mm sieve, soil samples were kept until further examination. The primary physical and chemical characteristics of the used soils were analysed at Soil, Plant, and Water Analysis Service Laboratory,Minia University's Faculty of Agriculture **Table 1** lists some of the primary attributes of utilized soil.

Table (1) Physical and chemical analyses of the tested soils

Analysis		Clay Sample	Sand Sample
PH (Ratio 1;2.5)	H_2O	8.20	8.50
	KCL	7.80	8.15
EC (Ratio 1;2.5) ms/cm		.62	.16
Total Carbonate $CaCo_3$ %		2.30	8.10
Total Organic Carbon %		1.36	.18
Organic Matter %		2.34	.31
Available Phosphorus ppm		46.00	20.00
Particle Size Analysis	Sand %	32.50	83.94
	Slit %	20.00	2.34
	Clay %	47.50	13.72
	Texture	Clay	Loamy sand

2- Wheat grains:

Wheat grains (Sides 13) were kindly supplied by Department of Crops, Faculty of Agriculture, Minia University.

3- Selection of most efficient nitrogen-fixing bacterial isolates;

From the rhizosphere of wheat plants cultivated in various localities in Minia Governorate, twenty representative isolates of each Azotobacter and Azospirillum were randomly isolated. The nitrogen fixing capability of these isolates was achieved using ambient assay of N-ase activity according to **(Postage**,**1972**). The most efficient five isolates of either Azotobacter or Azospirillum were selected to be used as inocula.

Cyanobacteria

Anabaena oryzae was kindly provided by Department of Microbiology, Faculty of Agriculture, El-Mansoura University.

Chemical Fertilizers:

Three types of mineral fertilizers were used. As a source of nitrogen, urea fertilizer (46%N) was used at rates of 130kg/feddan with clay soil and 217 kg/feddan with loamy sand soil. The mentioned amounts of urea were divided into two equal doses, which was added at 0 and 30 days after sowing. Superphosphate (15% P_2O_5) and potassium sulphate (48% K_2O) were applied as sources of phosphate, and potassium, respectively. Superphosphate at a rate of 100 Kg/feddan and potassium sulphate at a rate of 50 Kg/feddan were added once before sowing, for the two types of used soil. All of the other agricultural practices were performed as usual.

Preparation of nitrogen fixing inocula :

The most efficient nitrogen-fixing isolates (Azotobacter or Azospirillum) were propagated individually in 250 ml conical flasks containing 50 ml/flask of the nutrient broth medium. Each flask was inoculated with a loop-full of 48 hours old culture and incubated for 48 hrs. at 30°C. After propagation every prepared liquid culture contained 10^7 to 10^8 cfu/ml as determined by the plate count method. The prepared liquid cultures were used for grain inoculation. In the case of inoculation with the mixed bacterial cultures (Azotobacter and Azospirillum), equal amounts of each bacterial suspension were thoroughly mixed just before inoculation.

Preparation of cyanobacterial inoculum:

Anabaena oryzae was cultured in Allen's BG110 medium for a month at 30 ± 2°C under continuous illumination to obtain enriched cultures. The cyanobacterial isolate was counted and adjusted to be 3.5×10^5 cell/g using a MPN as described by **Shalaby and Mehessen (2014)**. wheat grains were thoroughly mixed with the prepared inoculum after being moistened with 10% sugar solution. Grain- inoculates were exposed to air drying in shade for 30 min before planting **(El-Khateeb, 2019)**.

Design of the experiments:

Pot experiments were set up during 2021\2022 growing seasons to study the effects of inoculation with cyanobacteria, Azotobacter, Azospirllum, and their combination on growth and nutrition of wheat plants. Plastic post of 35cm depth and 30cm diameter were used for cultivation of the above plants using clay loam soil and loamy sand soil. Each pot was filled with 8-kg air-dried soil. Wheat grains were inoculated before cultivated with used microorganisms at different levels on nitrogen fertilizer (75%N, 50%N, 25%N and without N) as well as (control) uninoculated with 100%N and without N. The pots were divided into four groups and each group was divided into 4 subgroups. The first group was inoculated with cyanobacteria and amended with four levels of Nitrogen, while the seconded group, was inoculated with Azotobacter and amended with four levels of nitrogen. Moreover, the third group was inoculated with Azspirillum and amended with four levels of nitrogen. While, the fourth group was inoculated with mixed inocula and amended with four levels of nitrogen. Cultivation process was performed by sowing four grain of wheat cultivar, Sides 13.. Plant samples were taken after 30-, 45-, 60-, and 75-days from sowing for determination of total counts of bacteria Azotobacter and Azospirillum using **(Ashby, 1947 and Mazinani *et al*., 2012)** for total Azotobacter and Semi-solid Malate Medium **(Cassán *et al*., 2015)** was used for counting Azospirillum.

Plant Measurements:

Wheat and plants grown in pots containing clay loam soil or loamy sand soil were carefully uprooted after 60 days of growth. Then, the plants were thoroughly hand-washed to remove soil particles from the roots, then, heights of plants, plant fresh weight and dry weight were recoded.

Nitrogen content of the shoots and roots was determined by micro-kjeldahlprocedure (**Eastin**, **1978**), phosphorus contents had been calorimetrically determined as described by **Olsen *et al*. (1954**) and total potassium in plant material were determined in the acid solution of the digested samples using flam photometer (model ILAE 201 Fisher Scientific company) as described by **Jackson (1973)**.

Data collected were statistically analysed by the analysis of variance using the general linear model (GLM) procedure of statistical analysis system **(SAS,2011)**.

RESULTS AND DISCUSSION

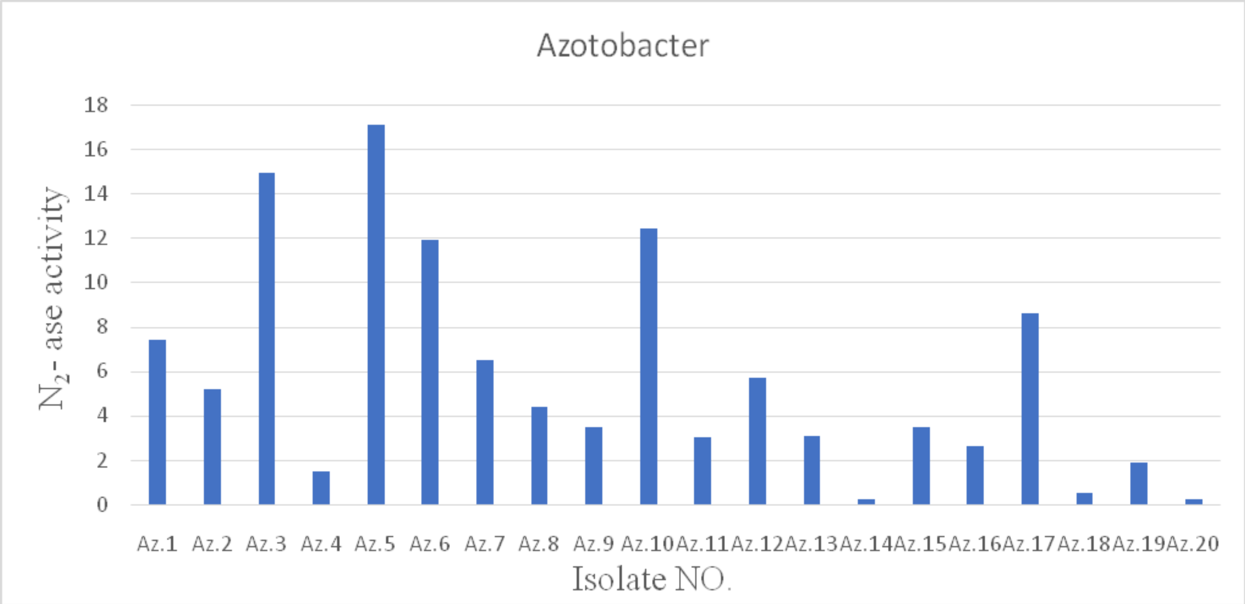
1-Selection of most efficient nitrogen – fixing bacterial isolates.

Result presented in **Table(2) and Fig. 1** indicate that, the production of C_2H_4 by the twenty *Azotobacter* isolates ranged from 0.13 to 17.1 nano moles C_2H_4 $ml^{-1}hr^{-1}$, where the most efficient five isolates of *Azotobacter* wer AZ5, AZ3, AZ10, AZ6 and AZ17. Result presented in **Table (3) and Fig. 2** indicate that, the production of C_2H_4 by the twenty isolates of ***Azospirillum*** produced 0 to 5.02 nano moles $C_2H_4ml^{-1}$ hr^{-1}. where the most efficient five isolates of Azospirillum were AS14, AS20, AS1, AS15 and AS18. Therefore, these isolates were subjected to further studies.

Table (2) Nitrogenase activity (nanomole C_2H_4 ml^{-1} hr^{-1}) of either Azotobacter isolates.

Azotobacter isolates

Isolate NO.	N_2- ase activity	Isolate No.	N_2-ase activity
Az.1	7.4	11	3.0
Az.2	5.2	12	5.7
Az.3	14.9***	13	3.1
Az.4	1.5	14	0.24
Az.5	17.1***	15	3.5
Az.6	11.9***	16	2.6
Az.7	6.5	17	8.6***
Az.8	4.4	18	0.50
Az.9	3.5	19	1.9
Az.10	12.4***	20	0.25
CO.	0.13	CO.	0.13

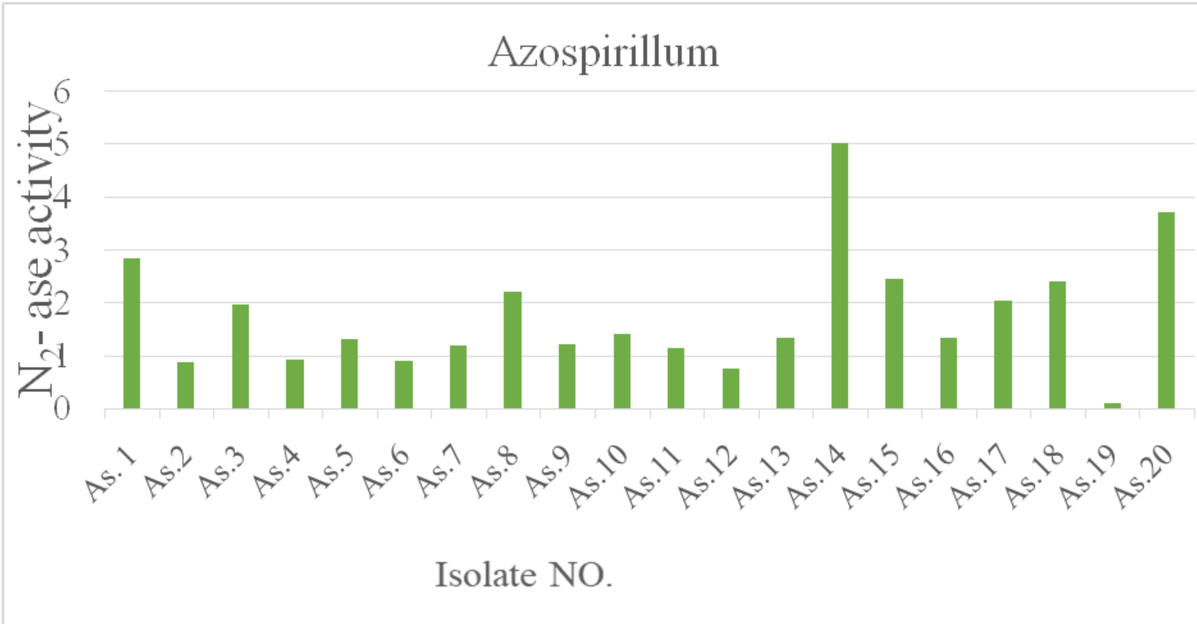


"***Most efficient isolates"

Fig 1. Nitrogenase activity (nanomole C2H4 ml-1 hr-1) of Azotobacter isolates.

Table (3) Nitrogenase activity (nanomole C_2H_4 ml^{-1} hr^{-1}) of Azospirillum isolates.

Azospirillum isolates			
Isolate NO.	**N_2- ase activity**	**Isolate No.**	**N_2-ase activity**
As. 1	2.84***	As.11	1.13
As.2	0.87	As.12	0.74
As.3	1.96	As.13	1.33
As.4	0.92	As.14	5.02***
As.5	1.3	As.15	2.44***
As.6	0.9	As.16	1.33
As.7	1.18	As.17	2.03
As.8	2.2	As.18	2.4***
As.9	1.2	As.19	0.0
As.10	1.4	As.20	3.71***
CO.	0.7	CO.	0.7



"***Most efficient isolates"

Fig 2. Nitrogenase activity (nanomole C2H4 ml-1 hr-1) of Azospirillum isolates.

2- Densities of different microorganisms in rhizosphere soil of inoculated plants:

The total numbers of cyanobacteria, Azotobacter and Azospirillum in rhizosphere soil of wheat plants, grown in clay loam and loamy sand soils were determined to ensure the establishment of the applied inocula.

a- Total count of Azotobacter:

Data in **Tables (4 and 5)** showed that, inoculation with Azotobacter increased the count of Azotobacter in rhizosphere soil at high level of nitrogen 75% which reached 11× 10^5cfu /g (cells g^{-1}) dry clay loam and loamy sand soils of wheat after 45 and 60 days of sowing thereafter decreased to reach up to 49 ×10^4 and 46 ×10^4 cfu/g^{-1} dry clay loam and loamy sand soils after 75 days, respectively. In case of Azospirillum inoculation the numbers are less than those of the Azotobacter which reached 4.9× 10^5 and 4.6× 10^5 cfu/g^{-1} dry clay loam and loamy sand soils, respectively at nitrogen 75% and decreased to reach up to 3.5× and 2.7x 10^5 , respectively after 75 days. In the case of Cyanobacteria and mixed inoculation the number of azotobacter reached 11× 10^5 cfu/g^{-1} at nitrogen 75% in both soils after 45 days of sowing. On the other hand, the highest number of Azotobacter was obtained in the case of inoculated compared to uninoculated plants. The rhizosphere of plants is clearly a fertile habitat, as evidenced by the fact that Azotobacter spp. have been found in rhizosphere soil (**Brenner *et al*., 2005; Sivasakthi *etal*., 2017**).

Numerous environmental parameters, such as soil qualities (pH, organic matter content, moisture content, fertility, C/N ratio), or climatic circumstances, have an impact on the occurrence and population size of this particular species of bacteria **(Tejera *et al*., 2005)**. The quantity of Azotobacter-genus nitrogen-fixing bacteria under perennial cereal crops was measured by **Siebielec *et al*. (2015)** in various soil types. Eutrophic brown soil, brown rendzina, black earth, and brown alluvial soils had the highest numbers of them. The type of crop grown has an impact on the abundance of these bacteria, which are more prevalent in rhizosphere soil **(Kaviyarasan *et al*., 2020)**. The results obtained were in agreement with **Abd el-Halim 2009; Ki-Yoon kim *et al*., 2010** and **Mahmoud *et al*., 2011.**

b- Total count of Azospirillum:

Tables (4 and 5) show that in case of Azospirillum inoculation the number of Azsospirillum increased gradually up to 45 days at 75% nitrogen then decreased sharply up to 75 days in both two soil. Whereas, in clay loam soil inoculated with Azospirillum or mixed inoculation the numbers were higher which reached to 11×10^5 cfu/g^{-1} dry clay loam soil after 60 days then decreased to reach 46×10^4 cfu/g^{-1} dry clay loam soil. Therefore, it was clear that single or mixed inoculation caused light increase in number of both Azotobacter and Azospirillum compared to control without inoculation. The results obtained were in agreement with those of **Ali, Nadia *et al*. (2002); Abd el-Halim (2009); Ki-Yoon Kim *et al*. (2010)** and **Mahmoud *et al*. (2011).**

Table (4) Count of Azotobacter and Azospirillum in rhizosphere of wheat plant under different levels of nitrogen fertilizer in clay loam soil (10^4 cfu/g dry soil).

Inoculation	Fertilization	Azotobacter				Azospirillum			
		Days after sowing							
		30	45	60	75	30	45	60	75
Cyanobacteria	75% N	24.0	110.0	46.0	29.0	20.0	110.0	42.0	20.0
	50% N	21.0	110.0	29.0	27.0	21.0	110.0	29.0	19.0
	25% N	15.0	46.0	21.0	21.0	15.0	46.0	19.2	17.0
	0% N	9.3	29.0	15.0	9.3	9.3	23 .0	15.0	9.3
Azotobacter	75% N	49.0	110.0	110.0	49.0	29.0	110.0	49.0	35.0
	50% N	46.0	110.0	110.0	46.0	21.0	110.0	46.0	29.0
	25% N	35.0	110.0	110.0	35.0	21.0	110.0	29.0	27.0
	0% N	35.0	110.0	110.0	29.0	15.0	110.0	24.0	21.0
Azospirllum	75% N	29.0	110.0	110.0	35.0	49.0	110.0	110.0	46.0
	50% N	21.0	110.0	46.0	29.0	46.0	110.0	110.0	46.0
	25% N	21.0	110.0	29.0	27.0	35.0	110.0	110.0	35.0
	0% N	15.0	110.0	24.0	21.0	29.0	110.0	110.0	29.0
(Cy- Az-As)	75% N	49.0	110.0	110.0	49.0	46.0	110.0	110.0	46.0
	50% N	49.0	110.0	110.0	49.0	35.0	110.0	110.0	35.0
	25% N	46.0	110.0	110.0	46.0	29.0	110.0	110.0	29.0
	0% N	29.0	110.0	110.0	35.0	21.0	110.0	110.0	21 .0
Uninoculated	100%N	21.0	46.0	29.0	21.0	21.0	٤٦.٠	٢9.0	21.0
	0%N	9.3	9.3	15.0	3.4	9.3	٩.٣	١٥.٠	3.4

Table (5) Count of Azotobacter and Azospirillum in rhizosphere of wheat plant under different levels of nitrogen fertilizer in loamy sand soil (10^4 cfu/g dry soil).

Inoculation	Fertilization	Azotobacter				Azospirillum			
		Days after sowing							
		30	45	60	75	30	45	60	75
Cyanobacteria	**75% N**	24.0	110.0	46.0	29.0	21.0	110.0	46.0	27.0
	50% N	21.0	110.0	29.0	27.0	21.0	110.0	29.0	21.0
	25% N	15.0	46.0	21.0	21.0	15.0	29.0	21.0	15.0
	0% N	9.3	29.0	15.0	9.3	9.3	21.0	15.0	9.3
Azotobacter	**75% N**	46.0	110.0	110.0	46.0	29.0	110.0	35.0	29.0
	50% N	46.0	110.0	49.0	35.0	21.0	110.0	29.0	21.0
	25% N	35.0	110.0	46.0	29.0	21.0	110.0	21.0	19.0
	0% N	29.0	110.0	35.0	21.0	15.0	110.0	15.0	15.0
Azospirllum	**75% N**	29.0	110.0	110.0	35.0	49.0	110.0	110.0	46.0
	50% N	21.0	110.0	46.0	29.0	46.0	110.0	49.0	35.0
	25% N	21.0	110.0	29.0	27.0	35.0	110.0	46.0	29.0
	0% N	15.0	46.0	24.0	21.0	21.0	110.0	35.0	21.0
(Cy- Az-As)	**75% N**	46.0	110.0	49.0	46.0	35.0	110.0	46.0	35.0
	50% N	46.0	110.0	49.0	35.0	35.0	110.0	46.0	35.0
	25% N	35.0	110.0	46.0	29.0	29.0	110.0	35.0	29.0
	0% N	21.0	110.0	35.0	21.0	4.60	110.0	29.0	21.0
	100%N	15.0	35.0	21.0	16.0	9.3	21.٠	16.0	15.0
Uninoculated	**0%N**	9.3	9.3	15.0	3.4	9.3	15.0	9.3	3.4

3- Effect of bacterial seed inoculation with different levels of nitrogen on growth of wheat after 60 days from sowing during 2021 and 2022 growing season.

The data **(Table 6)** show that the vegetative growth of wheat received chemical N-fertilizer sources were generally better than plants of the control treatments. Moreover, the growth of wheat plants inoculated with either non symbiotic nitrogen fixation and/or their mixture was generally higher than plants of the control treatment. The highest values of vegetative growth parameters were obtained in inoculated plants with 75% nitrogen fertilizer compared to those of any other level of fertilizer. This increase in vegetative growth was recorded in plant height, fresh and dry weight/plant. Data in **Table (6)** indicated that with mixed inoculation the plant height was higher than single inoculum under any level of nitrogen fertilizer.

3-1. Plant height

Data in **Table (6)** showed that, inoculation with cyanobacteria, Azotobacter, and Azospirillum significantly increased wheat plant height in both clay loam and loamy sand soils under different levels of chemical nitrogen fertilizer after 60 days from sowing.The plant height increased with increasing nitrogen fertilizer. The highest plant height was recorded with 75 % nitrogen which reached 68.5 and 55.66 cm. with Cyanobacteria, 66 and 48.50 cm. with Azotobacter, 66 and 50 cm. with Azospirillum and 69.50 and 56.83 cm/plant with mixed inocula in clay loam and loamy sand soils, respectively. The plant height gradually decreased with decreasing nitrogen fertilizer in both clay loam and loamy sand soils. Similar results were obtained by **Rajaa *et al.* (2021)** who studied the combined effect of *Chryseobacter iumbalustinum*, *Pseudomonas simiae* and *Pseudomonas fluorescens* were used as PGPR strains and *Calothrix sp.* and *Anabaena cylindrica* were used as cyanobacterial strains on *Triticum aestivum* L. The study results indicate that the five isolates gave the high performance in terms of growth parameters. Combination of cyanobacteria and PGBR strains increased plant hight by 36% and increased dry wight of shoot from 76% to 80%. Azotobacter affected growth and yield of crop through, biosynthesis of biologically active substances, stimulation of rhizosphere microorganisms and producing phytopathogenic inhibitors **Lenart (2012).** Also *Azotobacter* has beneficial effects on crop growth and yield through, biosynthesis of biologically active substances, stimulation of rhizospheric microbes, producing phyopathogenic inhibitors **(Lenart, 2012).** Seed inoculation with selected strains of plant growth-promoting rhizobacteria (PGPR) is reported to increase plant growth and grain yield in maize **(Martins *et al.* 2018),** rice **(Dartora *et al*. 2013), and** wheat **(Neiverth *et al*. 2014),** among other cereal crops. Both root and shoot length in inoculated plants were signifantly higher than control. Root long was associated with the IAA productively in early stages. IAA increased in inoculated plants than control and so increased root and shoot length due to bacterial phyto-hormones. Due to the interaction between inoculation and N-fertilizer levels, the splendid results of wheat growth parameters, yield and its components were for wheat plants inoculated with the bacterial mixture inoculum which showed significantly a positive response **(Desoky and Hala, 2016).**

3-2: Frish and dry weight

Data in **Table (6)** indicated that, inoculation with Cyanobacteria, Azotobacter, Azospirillum and mixed inocula significantly increased fresh and dry weight of wheat plant in both clay loam and loamy sand soils under different levels of chemical nitrogen fertilizer after 60 days from sowing. Fresh and dry weights of plants were increased with increasing nitrogen fertilizer. The highest fresh and dry weights /plant inoculated with any microbe or mixed were recorded with 75 % nitrogen which reached 36.05 - 32.62 gm. and 12.72 - 12.01gm. in the case of Cyanobacteria, 34.17 - 30.39 gm. and 11.00 - 10.41 gm. with Azotobacter, 34.53 - 30.59 gm. and 11.43 - 10.51 gm. with Azospirillum and 38.39 - 35.09 gm. and 15.22 - 13.66 gm. with mixed inocula in clay loam and loamy sand soils respectively. The fresh and dry weights / plant gradually decreased with decreasing nitrogen fertilizer to be lowest weight in control without nitrogen fertilizer in both soils.

Many secondary metabolites produced by cyanobacteria have beneficial properties for plants, such as enhancing resistance to plant diseases or increasing plant growth. Numerous secondary metabolites can also lessen the impacts of biotic and abiotic stress. Moreover, the cyanobacteria's biofilms have the potential to improve soil properties including greater water retention **(Kollmen and Strieth, 2022).** When comparing the inoculated plant seedlings' dry weight to the control, the inoculated seedlings' roots increased by more than three times, and their shoots were stimulated by more than 36%. The co-inoculation of *Azospirillum brasilense* and *Anabaena cylindrica* increased initial corn growth without altering the photosynthetic pigment levels **(Osvaldo *et al*., 2022).**

Table (6) Effect of inoculation with cyanobacterium, Azotobacter,Azospirillum and mixture of them on plant height, fresh and dry weight of wheat plant after 60 days from sawing during 2021 and 2022 growing season.

Inoculation	Fertilization	Plant height (cm)		Frish weight (gm/plant)		Dry weight (gm)/plant	
		Clay soil	Loamy sand	Clay soil	Loamy sand	Clay soil	Loamy sand
Cyanobacteria	75%N	68.50ab	55.66 a	36.05b	32.62b	12.72b	12.01 b
	50%N	65.66cde	45.33 cde	33.35de	28.75cd	10.80cd	9.52 ef
	25%N	61.83g	36.33 fgh	30.70gh	24.37e	9.61ef	7.41g
	0%N	58.50h	32.16ij	28.80ij	17.89f	8.65fgh	5.78i
Azotobacter	75%N	66 cd	48.50 bc	34.17cd	30.39 bc	11 cd	10.41cde
	50%N	63.66efg	45 cd	31.52fg	27.05d	9.3٣efg	7.82 g
	25%N	59.33h	36.3 ٣fgh	29.57hi	24.47 e	8.50 gh	5.64 i
	0%N	57.50hi	34.16ghi	27.81`jk	20.27f	6.89i	4.52j
Azospirillum	75%N	66 cd	50 b	34.53 cd	30.59 bc	11.43c	10.51 cd
	50%N	63.16fg	44 de	31.84 fg	26.74d	9.63 ef	7.70 g
	25%N	59.33h	37.66fg	28.96 ij	24.57e	7.90h	6.19 hi
	0%N	56.16i	33.6hi	26.63k	20.09f	6.35i	3.99jk
(Cy- Az-As)	75%N	69.50a	56.83a	38.39a	35.09 a	15.22 a	13.66 a
	50%N	67bc	47.50bcd	35.33bc	32.11 b	12.87 b	11.19 bc
	25%N	62.83fg	38.66f	32.42 ef	28.77 cd	10.75cd	9.74 def
	0%N	58.50h	33.50hi	29.01ij	22.29ef	9.69ef	7.01gh
Uninoculated	100%N	64.16def	42.66 e	32 fg	28.76 cd	10.11 de	9.18 f
	0%N	43.66j	29j	21.50l	13.65h	5.10j	3.37k
Stander Error		0.71	1.22	0.45	0.75	0.34	0.31

6- Effect of bacterial seed inoculation and different levels of nitrogen on percentage of Nitrogen, Phosphorus and Potassium of wheat plant after 60 days from sowing during 2021 and 2022 growing season.

6-1: Percentage of Nitrogen:

The results of inoculating wheat with Cyanobacteria, Azotobacter, Asospirillum and their mixture under various nitrogen fertilizers are displayed in **Table (7).** The effect inoculation with any inoculum on percentage of nitrogen was significant compared with uninoculated plants (control) in the clay loam and loamy sand soils. The highest percentages of nitrogen were obtained when nitrogen fertilizer was administered at a rate of 75%; these values were 1.99% and 1.51%, with Cyanobacteria, 2.48% and 1.65% with Azotobacter, 2.89 and 1.66% with Azospiriullum and 2.89 and 1.66 % with mixed inocula, in clay loam and loamy sand soils, respectively. Higher nitrogen percentage of wheat plant were obtained at 75% nitrogen fertilizer with inoculation than 100% nitrogen fertilizer without inoculation reached 1.86% and 1.43% in the clay and loamy sand soils respectively. These results may be due to the role of inoculation of wheat plant with *Anabaena cylindrica* revealed inoculation increased surface soil soluble organic carbon

(SolC), microbial biomass carbon (C), total nitrogen (N), mineral N, available phosphorus (P), and microbial activity. At day 42 after sowing, wheat plant N was higher in inoculated than non-inoculated plants**.** In addition, microalgae produce multiple bioactive compounds such as exopolysaccharides, phytohormones, vitamins, and amino acids, which are the focus of continuous investigations as plant growth promoters and biocontrol agents **(Ramakrishnan *et al.,* 2023 and Ferreira *et al.***, **2023).**

The treatment of 75 % N + Cyanobacteria gained the highest wheat grain and straw yields, highest total N, P and K contents for both grains and straw **(Ghazal *et al.*, 2018)**.The blend of Azotobacter and 75 kg N/fed had the highest protein content among the treatments on wheat crop (**El-Zawawi *et al*., 2023).** The inoculation with *Azospirillum brasilense* increase wheat grain yield, and N uptake, and overall farm profitability. The increased total N accumulation observed occurred mainly as a result of the enhanced N-NH4+ accumulation in wheat root and shoot **(Galindo *et al*., 2022).** These results are in accordance with those obtained by **Basham *et al*. (2014); Otang (2018); Soliman (2018) and Bredaa *et al.* (2019).**The observed results in **Table (7)** indicated that, mixed inoculation caused higher increase in nitrogen percentage of wheat plant than those of any single inoculation at all different levels of nitrogen fertilizer. The combined treatment of bio-fertilizer and mineral nitrogen recorded increasing plant height, plant dry weight, leaf area, chlorophyll pigments concentration, spike length, dry weight, grains number spike-1,1000-grain weight and N-content in grains, grain yield and plant growth **(El-Khateeb and Metwaly, 2019 and Al-Farouk *et al*., 2023).** These results are agreement with those obtained by **Namvar and Khanvan (2013); Bageshwar *et al*. (2017); Bredaa *et al.* (2019)** and **López (2023).**

6-2: Percentage of Phosphorus:

Data presented in **Table (7)** show the effect of wheat inoculated with Cayanobacteria on phosphorus percentage in the two soils. It could be seen that the highest percentage of phosphorus in wheat plants were obtained with application of 75% nitrogen fertilizer (0.73) and 0.66% in clay and loamy sand respectively). The lowest percentages of phosphorus obtained when chemical fertilizer was applied at rate of 0% were 0.33 and 0.58% in clay and loamy sand respectively. The effect of inoculation with Cyanobacteria on percentage of phosphoruswas significant compared with uninoculated plants (control) in the clay and loamy sand soils. This effect may be due to the role of inoculation, which increased available phosphorus (P), and microbial activity.Similar results were obtained by **Alvarez *et al*., (2024), Ramakrishnan *et al*. 2023** and **Ferreira *et al*. 2023.**

It is obvious from the results given in Table (7) that the highest percentage of phosphorus was obtained with inoculated wheat with Cyanobacteria, Azotobacter, Azospirillum and combination of them in the two used soils compared with uninoculated plants, Biofertilizers are rising alternatives to supply major limiting nutrients in agriculture such as nitrogen (N) and phosphorus (P) and to replace or reduce the dependence on chemical fertilizers (FAO 2022 and Macik *et al*., 2020). These results are agreement with obtained by Ghazal *el al*. (2018), yandigeri *et al*. (2011), Aasfar *et al*. (2021), Alvarez *et al*. (2021), Jain *et al*. (2021) and Rajaa (2021). Data in Table (7) indicated that there were no significant differences in phosphorus percentage as affected by the interaction between inoculation and nitrogen levels in the two used soils.

6-3: Percentage of Potassium:

The results in **Table** (**7**) showed that inoculating wheat with Cyanobacteria, Azotobacter, Azospirillum and their combination, increased potassium % substantially in comparison to the control. The highest values of potassium percentage were observed in the treatment of inoculation with 75% of nitrogen fertilizer which reached 4.65 and 3.96%, 6.06 and 4.23, 4.59 and 4.23% and 4.78 and 4.78% in clay soil for Cyanobacteria, Azotobacter, Azospirillum and their mixture, respectively. The percentage of potassium in uninoculated wheat plants at 100% N were 4.43 and 3.58% in clay loam and loamy sand soils, respectively. The result also showed that, inoculation with diazotrophic bacteria can increase root hair growth through physiological changes in plants that have increased the production of plant growth hormones such as indole-3-acetic acid, cytokinin's, gibberellins and ethylene, which could influence the ability of plant roots to penetrate into the soil for greater water and nutrient absorption. Inoculation wheat with Growth-Promoting Bacteria *Azospirillum brasilense* resulted in greater accumulation of S and K in the shoot of the wheat cultivar, as well greater accumulation of Cu (**Boleta *et al.*, 2020**). Similar results were obtained by ; **Ghazal *et al.* (2018); Li *et al*. (2016); Zeffa *et al.* (2019) and Eckshtain-Levi *et al.* (2020).**

Tale (7) Effect of inoculation with Cyanobacterium, Azotobacter, Azospirillum and their mixture on percentage of Nitrogen, phosphorus and potassium of wheat plant after 60 days from sawing during 2021 and 2022 growing season.

Inoculation	Fertilization	N%		P%		K%	
		Clay soil	Loamy sand	Clay soil	Loamy sand	Clay soil	Loamy sand
Cyanobacteria	75%N	1.99bcde	1.51 ab	0.77ab	0.66 ab	**4.65 bc**	**3.96 ab**
	50%N	1.90 bcd	1.41 a	0.67abc	0.62 ab	**4.59 b**	**3.54 ab**
	25%N	1.75bcde	1.41ab	0.43d	0.61ab	**4.03 d**	**3.55 ab**
	0%N	1.0f	1.21ab	0.33abc	0.58 ab	**3.31bc**	**3.44ab**
Azotobacter	75%N	2.48a	1.65 a	0.90a	0.62ab	**6.06a**	**4.23a**
	50%N	2.23 abc	1.35ab	0.89ab	0.59 ab	**4.33 bc**	**3.45 ab**
	25%N	2.13ab	1.24ab	0.82 a	0.49 bc	**4.01bc**	**3.27 ab**
	0%N	1.32 ef	1.22ab	0.56bcd	0.44bc	**2.97 d**	**3. 17ab**
Azospirillum	75%N	2.60 dce	1.68 a	0.70abc	0.76 a	**4.59 bc**	**4.23 a**
	50%N	2.43 a	1.40 ab	0.70abc	0.54 abc	**4.55 b**	**3.54 ab**
	25%N	0.94 f	1.38 ab	0.69abc	0.52 abc	**4.33 bc**	**3.38ab**
	0%N	0.90bcd	1.34ab	0.67abc	0.66ab	**3.47cd**	**3.32ab**
(Cy- Az-As)	75%N	2.89 bcd	1.66 ab	0.78bcd	0.67 ab	**4.78 bc**	**4.78 ab**
	50%N	2.05abc	1.60a	0.76 ab	0.66 ab	**4.75 b**	**4.48 ab**
	25%N	1.98abcd	1.60 a	0.66 cd	0.66 ab	**2.78 d**	**3.22 a**
	0%N	1.49be	1.25ab	0.44ab	0.42bc	**4.33bc**	**2.39b**
Uninoculated	100%N	1.86bcd	1.43 b	0.73 ab	0.60 c	**4.43 bc**	**3.58 ab**
	0%N	0.78bcde	0.90 a	0.62abc	0.44 ab	**2.7bc**	**2.20a**
Stander error		0.16	0.25	0.08	0.07	**0.31**	**0.49**

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الملخص العربي

تأثير البكتيريا المثبتة للنيتروجين غير التكافلية تحت مستويات مختلفة من النيتروجين على معدل نمو القمح

رشا حسن أحمد حسن – سامية فرحات محمد احمد – سمير أحمد سيد محمد حداد - عمر عبد اللطيف عمر سعد

قسم الميكروبيولوجيا الزراعية – كلية الزراعة – جامعة المنيا

من أجل تقييم تأثير التلقيح بالسيانوبكتيرياوالأزوتوباكتروالازوسبيريللام وخليط منهم على نمو القمح، أجريت تجربة أصص خلال موسمي ٢٠٢١ و٢٠٢٢ في قسم الميكروبيولوجيا الزراعية جامعة المنيا للتحقيق في تأثير العزلات الميكروبية المثبتة للنيتروجين على نباتات القمح، إما بشكل فردي أو بالاشتراك مع مستويات مختلفة من النيتروجين المعدني. أظهرت النتائج أن التلقيح بالكائنات الحية الدقيقة المستخدمة يشجع بشكل عام نمو نباتات القمح، كما أن التسميد بمعدل ٧٥% مع التلقيح بأي من الميكروبات اعطي أعلى ارتفاع للنبات ووزن طازج وجاف للنبات وأعلى محتوى إجمالي من النيتروجين والفوسفور والبوتاسيوم للنبات. وأظهرت النتائج التي تم الحصول عليها أن التلقيح بأي من الميكروبات المستخدمة مع التسميد بمعدل ٧٥% من النيتروجين كانت أعلي منه في حالة التسميد بمعدل ١٠٠% نيتروجين بدون تلقيح. وتشير التوقعات الأولية إلى أن تلقيح القمح بالبكتيريا الزرقاء أو البكتيريا الدايازوتروفية قد يكون قادراً على تخفيض ما يقرب من ٢٥% من النيتروجين المعدني المطلوب لإنتاج القمح. كما تبين أن التلقيح المختلط كان أكثر نجاحاً في تنمية القمح، وأظهرت جميع البكتيريا التي تمت دراستها نتائج واعدة كسماد حيوي لزيادة إنتاج المحاصيل.