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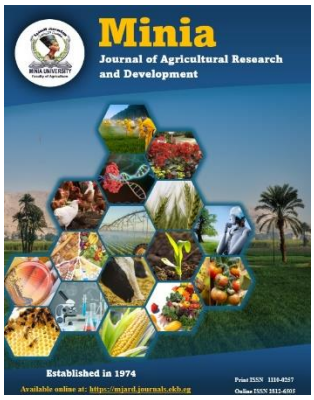


# Response of wheat cultivar (Beni suef 5) to nitrogen fixing and phosphorus solubilizing inocula

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## ABSTRACT

Inoculation of the wheat (Beni suef 5) with *Azotobacter chroococcum* (AZ1 or AZ2) as nitrogen fixers, or *Bacillus megaterium* (BM1 and BM2) as phosphate dissolvers significantly increased the grain yield, plant height, spikelets/spike, kernels/spike and grain weight of 10 spike as compared to un-inoculated plants. The inoculation with *A. chroococcum* gave a significant increase in plants nitrogen content (N%) as compared to the un-inoculated plants. No significant increase in nitrogen % was observed in plants treated with *B. megaterium*. Plant phosphorus content in plants treated with *B. megaterium* showed significant increase as compared to control plants (un-inoculated) or those inoculated with *A. chroococcum*. No significant difference was recorded in potassium content (K%) of plants treated with *A. chroococcum* or *B. megaterium* as compared to un-inoculated plants. *A. chroococcum* and *B. megaterium* inocula were subjected to determination of their abilities in producing indole acetic acid (IAA), gibberellins ($GA_3$), and Siderophores. *A. chroococcum* isolates exhibited high efficiency in producing indole acetic acid (IAA), gibberellins ($GA_3$) and Siderophores as compared to *B. megaterium* (BM1 and BM2). Nitrogenase activity in wheat root soil treated with *A. chroococcum* AZ1 or AZ2 was significantly higher than in the control plants. The highest enzyme activity in the root zone of wheat inoculated with *A. chroococcum* or *B. megaterium* was recorded at 45 days after planting.

**Keywords:** *Azotobacter, Bacillus megaterium*, Wheat

## INTRODUCTION

Due to its significance in the Egyptian diet, wheat is a strategic cereal crop in Egypt. Since domestic consumption is not met by local wheat production, increasing local wheat production is a national objective to close the gap between production and consumption and enhance food security **(.Yigezu *et al*., 2021).**

This goal can be achieved by increasing productivity through the use of a number of techniques, including the use of advanced

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agricultural techniques, cultivation of high-yielding varieties and the use of biofertilizers.

Because it is a key component of chlorophyll, the substance that allows plants to use solar energy to convert carbon dioxide and water into sugars (a process known as photosynthesis), nitrogen is extremely important to wheat. Additionally, nitrogen plays a significant role in amino acids, which are the building blocks of proteins **(Zayed *et al*., 2023).**

Phosphorus is additionally a basic supplement for wheat growth because it could be a key component in numerous physiological and biochemical forms. Phosphorus could be a component of each living cell, and cannot be replaced by any other component. It happens in numerous complicated compounds such as hereditary particles (DNA and RNA) conjointly the phospholipids, which form all cell membranes **(Majid *et al*., 2025).**

In Egypt, several problems have been recorded regarding the problem of soil fertility including poor soil fertility and poor content of organic matter. The poverty of Egyptian soils in organic matter is often accompanied by low level of available of nitrogen. On the other hand, the majority of the Egyptian soils are alkaline, (pH 7 to 9), therefore, phosphorus can be converted to precipitated form unavailable for plant absorption **(Elbasiouny *et al.* 2020).**

Inoculation of wheat seeds with N-fixing and P-dissolving bacteria, can, promote plant growth and minimize the used chemical fertilizers. The aim of the current study was to apply this co-inoculation process in order to provide wheat plants cultivated in the soil of Beni Suef Governorate- Egypt with available nitrogen and available phosphate

## MATERIALS AND METHODS

### 1- The used Seeds (grains) and bacterial inocula:

Seeds (grains) of wheat were Beni Suef 5 (durum wheat). These seeds were developed and kindly supplied by the Agricultural Research Center (ARC).

The used bacteria were *Azotobacter chroococcum* (AZ1 and AZ2) as nitrogen fixing bacteria and *Bacillus megaterium* (BM1 and BM2) as phosphate dissolving bacteria. These bacteria were kindly provided by the Central Lab. Of Organic Agriculture in Egypt. Liquid culture of each strain of these bacteria ($10^8$ cfu/ml) were prepared separately to be used as inoculum. Wheat seeds (grains) were treated as below:

a- Inoculation with *Azotobacter chroococcum* (AZ1)

b- - Inoculation with *Azotobacter chroococcum* (AZ2)

c- Inoculation with mixture of *Azotobacter chroococcum* (AZ1 + AZ2)

d- Inoculation with *Bacillus megaterium* (BM1)

e- Inoculation with *Bacillus megaterium* (BM2)

f- Inoculation with mixture of *Bacillus megaterium* (BM1+ BM2)

g- Uninoculated plants (control)

Three replicates for each inoculation treatment were involved. The used bacteria were kindly provided by the Central Lab. Of Organic Agriculture in Egypt.

### 2- Data collected:

- Number of spikelets per spikes, which was determined by counting the number of spikelets per spikes
- Number of kernels/spike i.e. average number of kernels per spike (Main spike) counted manually.
- Grain Weight for 10 Spike i.e. the weight of 100-spike from the bulk of the guarded plants in grams.
- Grain yield/plant i.e. average grain weight of individual guarded plants, in grams.

- Plant height [ PH ] in cm :- The distance from the base of the culm to the tip of the-spike of the main culm excluding awns.
- Spike length in cm. [SL]:- It was measured from the base of the main spike to its tip excluding awns
- Biological yield (biomass) per plant [BY/P] in gm:- The total dry matter yield produced by the crop during the growing season of individual plant (excluding roots) .
- Number of towers per spike: it was determined by counting the number of towers per spik

**3- Mean performance and analysis of variance:**

Analysis of variances for randomized complete block design (RCBD) according to the method outlined by **(Snedecor and Cochran، 1967)** was used. using computer soft ware (GENSTAT) program. The L.S.D at 5% was utilized for comparing the means performance of the different treatments according to **Steel and Torrie (1980).**

**4- Determination of soil enzyme activities**

Nitrogenase activity ($N_2$ – ase) activity was estimated in plant rhizosphere soil after 45 and 60 days after planting. The measurements based on the reduction of acetylene to ethylene as quantities by gas chromatography. Acetylene reduction was carried out using modified version of the procedure **(Silvester and Harris, 1989).** Injected acetylene was produced directly from calcium carbide chips submerged in water into each jar to give a final concentration as 10% (v/v). Jars were incubated at ambient temperature and analyzed for ethylene ($C_2H_4$) after 1 hr. Ethylene was analyzed by standard flame ionization gas chromatography (Shimadzu GC8A) standardized with pure ethylene and results expressed as N mole $C_2HV$/g dry soil. The alkaline phosphatase activity was measured in plant rhizosphere 45 and 60 days after planting according to the method described by **Tabatabai (1982).**

**5- Microbial hormone production**

Microbial strains were checked for their ability to produce gibberellins GA3 and Indole acetic acid as described by **Grolamys and Servando (1997)** using Isco model 2360 gradient liquid chromatograph equipped with a V-UTS-250 UV.

Also microbial strains were checked for the ability to produce Siderophores (qualitatively) using Chrome Azurol (CAS) agar as modified by **Alexander and Zuberer (1991).**

## RESULTS AND DISCUSSION

**Inoculation of wheat with nitrogen fixing and phosphate dissolving bacteria**

Data in **Table (1 )** indicated that inoculation of the wheat cultivar (Beni suef 5) with the two strains of *Azotobacter chroococcum* (AZ1 or AZ2) each individually or both together significantly increased the values of biological yield, grain yield, plant height, spikelets/spike, kernels/spike and grain weight of 10 spike as compared to control plants (un-inoculated). **Aasfar *et al.* (2021)** reported that *Azotobacter* spp. have the ability to fix nitrogen and release phytohormones of indolic and gibberellinic nature, that promote plant growth, photosynthesis and absorption of nutrients. Furthermore, **Chaudhary *et al*. (2013)** reported that enhancement of wheat yield parameters indicate that inoculation with *Azotobacter* strains resulted in improving growth and yield of wheat under the study.

Moreover, application of *Bacillus megaterium* BM1 and BM2 each separately or their mixture as phosphate dissolving biofertilizer to the wheat cultivar (Beni suef 5) resulted in significant increase in values of biological yield, grain yield, plant height, spikelets/spike, kernels/spike and grain

weight for 10 spike as compared to control plants (uninoculated). The use of phosphate dissolving bacteria play an important role in dissolving the insoluble phosphorus. These results are in agreement with those obtained by **Alam *et al.* (2022)**, who reported that inoculation with P-dissolving bacteria improved nutrients availability and consequently enhanced growth and yield of plants.

**Table (1): Effect of inoculation of wheat cultivar (Beni suef 5) with Azotobacter *chroococcum*(AZ 1 and/or AZ 2) and *Bacillus megaterium* (BM1 and/or BM2) on growth and yield of wheat plants.**

| Parameters / Treatments | Biological Yield (Kg/plot) | Grain Yield (Kg/plot) | Plant Height (Cm) | Spike Length (Cm) | Spikelets/ Spike | Kernels/ Spike | Grain Weight for 10 Spike (Kg) |
|---|---|---|---|---|---|---|---|
| **Control** | 12.96 | 6.01 | 102.2 | 7.7 | 19.00 | 609 | 0.033 |
| **AZ 1** | 14.30 | 8.01 | 109.8 | 8.60 | 20.96 | 782 | 0.036 |
| **AZ 2** | 15.66 | 8.79 | 110.6 | 8.96 | 21.70 | 798 | 0.035 |
| **BM 1** | 14.96 | 7.90 | 112.3 | 8.99 | 20.60 | 787 | 0.038 |
| **BM 2** | 16.56 | 7.68 | 111.3 | 8.97 | 20.97 | 799 | 0.036 |
| **Mix1(AZ1 + AZ2)** | 16.96 | 9.16 | 114.6 | 9.96 | 22.59 | 796 | 0.040 |
| **Mix2(BM1+ M2)** | 16.93 | 8.98 | 115.0 | 9.10 | 22.46 | 779 | 0.036 |
| **L.S.D ( 5% Level )** | 1.9764 | 0.8511 | 6.189 | 0.4749 | 1.252 | 161.9 | 0.0084 |

Towers/spike and spike length (cm) were estimated at post-harvestin Durum Wheat (Beni suef 5) subjected to inoculation with the above mentioned inocula. The obtained results cleared in **(Table 2)** indicate that this wheat cultivar exhibited significant increase in values of the towers/spike and spike length over the control. These results clearly confirm the importance of application of nitrogen fixing and phosphate dissolving bacterial inocula to improve and increase the yield.

**Table (2): Towers/spike and spike length at post-harvest in Durum Wheat (Beni suef 5) cultivar subjected to bacterial inocula.**

| Treatment | Towers/Spike | Spike Length (Cm) |
|---|---|---|
| **Control** | 19.93 | 7.73 |
| **AZ 1** | 21.53 | 8.99 |
| **AZ 2** | 21.23 | 8.96 |
| **BM 1** | 20.66 | 8.86 |
| **BM 2** | 20.97 | 8.73 |
| **Mix1(AZ1 + AZ2)** | 22.53 | 8.96 |
| **Mix2(BM1 + BM2)** | 22.46 | 8.99 |
| **L.S.D (5% Level)** | 1.10 | 1.20 |

**Nitrogen , phosphorous and potassium presentin wheat and plant inoculated with *Azotobacter chroococcum* and *B. megaterium***

Results in **Table (3)** indicated that inoculation of the wheat plants (Beni Suef 5) with *Azotobacter* resulted in significant increase in plants nitrogen content (N%) as compared to the uninoculated plants. These results may indicate the high efficiency of the applied *Azotobacter* in fixing nitrogen and supplying the growing plants with high amount of nitrogen. **Pandey and Kumar (1989**) reported that *Azotobacter* is a beneficial bacterium that can be used as bio-fertilizer because of its ability to rapidly grow and fix high amounts of nitrogen.

On the other hand, no significant increase in nitrogen content was recorded in plants inoculated with *B. megaterium*. *i.e.* the used isolate of *B. megaterium* was un-efficient in fixing the atmospheric nitrogen. Whereas, plant phosphorus content in *B. megaterium* inoculated plants showed significant increase as compared to control plants (un-inoculated) or those inoculated with *Azotobacter chroococcum* **(Table 3)**. This may indicate that *B. megaterium* has the ability to produce organic acids and dissolve un- soluble phosphors. **Sang-Mo, *et al.* (2014)** stated that inoculation with *B. megaterium* could improve plant growth because its ability to produce organic acids and hence supplying the growing plants with soluble phosphors.

Moreover, no significant difference was recorded in potassium content (K%) of plants treated with either *Azotobacter chroococcum* or *B. megaterium* as compared to control plants (un-inoculated).

**Table (3) :Nitrogen , phosphorous and potassium (%) in the wheat plant inoculated with *Azotobacter chroococcum* and *B. megaterium*.**

| Treatments | N% | P% | K% |
|---|---|---|---|
| **Control** | 1.40 | 0.60 | 1.64 |
| **AZ1** | 1.64 | 0.72 | 1.69 |
| **AZ2** | 1.67 | 0.69 | 1.73 |
| **BM1** | 1.39 | 0.81 | 1.79 |
| **BM2** | 1.35 | 0.85 | 1.81 |
| **AZ1+ AZ2** | 1.72 | 0.72 | 1.80 |
| **BM1 + BM2** | 1.43 | 0.86 | 1.85 |
| **L.S.D (5% Level)** | 0.21 | 0.13 | 0.30 |

**Gibberellins ($GA_3$)Indole acetic acid (IAA)and Siderophores released by *Azotobacter chroococcum*and *B. megaterium* (in vitro study)**

*Azotobacter chroococcum* and *B. megaterium* isolates which used in this study as inocula were subjected to determination of their abilities in producing indole acetic acid (IAA), gibberellins ($GA_3$) and Siderophores.

Results presented in **Table (4)** indicated that *Azotobacter chroococcum* isolates (AZ1 and AZ2) exhibited high efficiency in producing indole acetic acid (IAA), gibberellins ($GA_3$) and Siderophores as compared to *B. megaterium* (BM1 and BM2). Moreover, amounts of indole acetic

acid (IAA), gibberellins ($GA_3$) and Siderophores releases by *Azotobacter chroococcum* AZ2 were much higher than those produced by *Azotobacter chroococcum* AZ1. Furthermore, the amounts of indole acetic acid (IAA), gibberellins ($GA_3$) and Siderophores released by *B. megaterium* (BM1 and BM2) were much lower than those produced by *Azotobacter chroococcum* (AZ1 and AZ2). The obtained results are in agreement with those obtained by **Brakel and Hilger (1965)** who stated that in *in-vitro* studies *Azotobacter* released indol-3-acetic acid (IAA) when tryptophan was added to the medium.

Siderophores are metabolites secreted by different microorganisms, they chelate ferric iron ($Fe^{III}$) with high affinity. Soil microorganisms produce siderophores that transform precipitated iron into a dissolved form that can be used by plants and microorganisms **(Siwen Zhang *et al*., 2023).** Moreover, **Siwen Zhang *et al*. (2023)** compared plant growth in plants inoculated with siderophore-producing bacteria (SPB) and un-inoculated ones, and they found that plant growth significantly increased (up to 30%) in plants inoculated with siderophore-producing bacteria as compared to control plants (un-inoculated).

**Table (4): Amount of indole acetic acid (IAA) and gibberellins ($GA_3$), Siderophores released by *Azotobacter chroococcum* (AZ1 and AZ2) and *B. megaterium* (BM1 and BM2) in *in-vitro* study.**

| Bacterial isolates | $GA_3$ (µg/100 ml) | IAA (µg/100 ml) | Siderophores (mg/L) |
|---|---|---|---|
| **AZ1** | 40.3 | 30.4 | 1.10 |
| **AZ2** | 42.4 | 31.2 | 1.23 |
| **BM1** | 28.7 | 21.9 | 0.91 |
| **BM2** | 27.9 | 22.4 | 0.83 |

**Effect of inoculation of wheat plants with *Azotobacter chroococcum* and *B. megaterium* on activities of nitrogenase, and phosphatase in rhizosphere soils.**

Data presented in **Tables (5)** indicated that at any sampling time (45 and 60 days after planting), nitrogenase activity in wheat root zone soil treated with *Azotobacter chroococcum* AZ1 or AZ2 was higher significantly than in the un-inoculated plants (control). Moreover, in root zone soil of plants inoculated with *Azotobacter chroococcum* AZ2, nitrogenise activity was higher than in case of inoculation with *Azotobacter* AZ1. In rhizosphere soil of plants inoculated with the mixture of the two isolates of *Azotobacter chroococcum* (AZ1 +AZ2) nitrogenise activity was significantly higher than in case of inoculation with each *Azotobacter chroococcum* isolate individually.

Furthermore, in plants inoculated with *B. megaterium* (BM1 or BM2), nitrogenise activity in the rhizosphere soil was lower than that recorded in case of inoculation with *Azotobacter chroococcum*. In the root zone soil of plants inoculated with mixture of the two *B. megaterium* isolates (BM1 +BM2), nitrogenise activity was significantly higher than in case of inoculation with each isolate separately.

The highest nitrogenase activity in the rhizosphere soil of wheat inoculated with either *Azotobacter chroococcum* or *B. megaterium* was recorded at 45 days after planting. The partial pressure of oxygen

affects nitrogen fixation and nitrogenase activity of *Azotobacter chroococcum*. Bacteria grown in low $pO_2$ (0·09 atm) exhibited the highest enzyme activities at a $pO_a$ of 0·05 atm **(Drozd and Postgate, 1970).**

**Table (5): Effect of microorganisms treatments on nitrogenase activity (µl $C_2H_4$ $g^{-1}$/ dry rhizosphere soil ) of wheat after 45 and 60 days from planting**

| Treatments | Sampling time (days after planting) | |
|---|---|---|
| | **45** | **60** |
| **Control** | 10.10 | 8.30 |
| **AZ 1** | 40.44 | 38.20 |
| **AZ 2** | 50.30 | 40.73 |
| **BM 1** | 23.71 | 20.25 |
| **BM 2** | 22.50 | 21.40 |
| **MIX 1 (AZ1+AZ 2)** | 62.42 | 50.90 |
| **MIX 2 (BM1+BM2)** | 31.21 | 30.90 |
| **L.S.D (5% Level)** | 4.1 | 3.2 |

**Phosphatase activity**

**Keller *et al.* (2013)** stated that certain species of N-fixing bacteria accelerate soil P cycling due to increase activity of phosphatase enzyme**.**

As shown in **Table (6),** the obtained results indicated that at any sampling time (45 or 60 days after planting), activity of phosphatase in wheat rhizosphere soil treated with *Azotobacter chroococcum* (AZ1 or AZ2) or those inoculated with *B. megaterium* (BM1 or BM2) was significantly higher than in un-inoculated plants (control). Moreover, activity of phosphatase in wheat rhizosphere soil treated with *Azotobacter chroococcum* AZ2 was higher than in case of inoculation with *Azotobacter chroococcum* AZ1. Furthermore, at any sampling time phosphatase activity in rhizosphere soil of plants inoculated with *B.megaterium* BM2 was much higher than in case of inoculation with *B.megaterium* BM1.

The obtained results also indicated that phosphatase activity in wheat rhizosphere soil treated with *B. megaterium* (BM1 or BM2) was significantly higher as compared with in the rhizosphere soil of plants inoculated with *Azotobacter chroococcum* (AZ1 or AZ2).

**Table (6):Effect of microorganisms treatments on phosphatase activity (µg p Np $g^{-1}$ $h^{-1}$ dry rhizosphere soil ) of two wheat varieties after 45 and 60 days from planting.**

| Treatments | Sampling time (days after planting) | |
|---|---|---|
| | **45** | **60** |
| **Control** | 11.93 | 11.23 |
| **AZ 1** | 15.62 | 16.42 |
| **AZ 2** | 16.46 | 17.82 |
| **BM 1** | 20.30 | 23.70 |
| **BM 2** | 26.40 | 25.90 |
| **MIX 1 (AZ1+AZ 2)** | 17.30 | 16.32 |
| **MIX 2 (BM1+BM2)** | 28.90 | 27.90 |
| **L.S.D (5% Level)** | 2.20 | 2.30 |

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## الملخص العربي

# استجابة صنف القمح (بني سويف 5) للتلقيح بالبكتيريا المثبتة للنيتروجين والمذيبة للفوسفات

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[2] المعمل المركزي للزراعة العضوية، مركز البحوث الزراعية، الجيزة

أدى تلقيح صنف القمح (بني سويف 5 ) بسلالتين من البكتيريا *Azotobacter chroococcum* (AZ1 و AZ2) بشكل منفصل أو معًا كمثبتات للنيتروجين، وكذلك التلقيح بسلالتين من البكتيريا *Bacillus megaterium*(BM1 وBM2) بشكل منفصل أو معًا كمذيبات للفوسفات إلى زيادة معنوية في قيم المحصول البيولوجي، ومحصول الحبوب، وطول النبات، وعدد السنيبلات/السنبلة، وعدد الحبوب/سنبلة، ووزن الحبوب لكل عشرة سنابل، مقارنةً بنباتات الكنترول (غير الملقحة) .كما أدت عملية التلقيح إلى زيادة معنوية في محتوى النباتات من النيتروجين (N%)مقارنة بالنباتات غير الملقحة. في المقابل، لم تُسجل أي زيادة معنوية في محتوى النيتروجين في النباتات الملقحة بالبكتيريا *B. megaterium*.في حين أظهر محتوى الفوسفور في النباتات الملقحة بـ*B. megaterium*زيادة معنوية مقارنةً بالنباتات غير الملقحة (الكنترول) أو تلك الملقحة بـ *Azotobacter chroococcum*.لم يُسجل أي فرق كبير في محتوى البوتاسيوم (K%) في النباتات الملقحة إما بالبكتيريا *Azotobacter chroococcum*أو البكتيريا*B. megaterium* مقارنة بالنباتات غير الملقحة (مجموعة الكنترول).

خضعت عزلات*Azotobacter chroococcum* و*B. megaterium*المستخدمة في هذه الدراسة كلقاح لتحديد قدرتها على إنتاج الجبرلين (GA3) وحمض الإندول الأسيتيك (IAA) والسيدروفورات . وقد أظهرت عزلات *Azotobacter chroococcum*كفاءة عالية في إنتاج الجبرلين (GA3) وحمض الإندول الأسيتيك (IAA) والسيدروفورات مقارنة بالبكتيريا *B. megaterium* (BM1وBM2). كان نشاط إنزيم النيتروجيناز في تربة منطقة الجذور للقمح الملقح بالبكتيريا *Azotobacter chroococcum*( AZ1أو AZ2) أعلى بكثير من نباتات الكنترول .تم تسجيل أعلى نشاط للنيتروجيناز في تربة منطقة الجذور للقمح الملقح بـ*Azotobacter chroococcum*أو*B. megaterium* بعد 45 يومًا من الزراعة. أظهرت النتائج أيضا أن نشاط إنزيم الفوسفاتيز في نباتات تربة منطقة الجذور الملقحة بالبكتيريا *B. megaterium* (BM1 أو BM2 ) أعلى بشكل ملحوظ مقارنة بتربة منطقة الجذور للنباتات الملقحة بالبكتيريا
*Azotobacter chroococcum* (AZ1 أو AZ2 )