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## Efficiency of Azotobacter, on Growth of Wheat Plants and Its some Anatomical Characteristics

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### ABSTRACT

*Azotobacter chroococcum* and *Azotobacter vinelandii* were isolated and purified from soil samples collected in El-Gharbia, (Qutor), Governorate. These Azotobacter isolates produced the hormones cytokinin, gibberellins and auxin. The current study was carried out to evaluate the impact of a potent Azotobacter strains on growth and productivity of wheat plants. Thus, after inoculation with *Az. chroococcum*, *Az. vinelandii*, and their mixture, the plant height, number of spikes/m<sup>2</sup>, 1000-grain weight, grains yield, straw yield, total chlorophyll (mg g<sup>-1</sup>), leaf nitrogen content, leaf area(cm<sup>2</sup>). Between the various treatments, there were noticeable differences in the total chlorophyll(mg g<sup>-1</sup>),leaf nitrogen content and leaf area(cm<sup>2</sup>). The different treatments (Azotobacter and concentrations of N) showed a substantial difference. The total chlorophyll (mg g<sup>-1</sup>), leaf nitrogen content and leaf area(cm<sup>2</sup>) 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 effects of nitrogen levels and Azotobacter types on wheat protein content .The effects of the two Azotobacter strains (*Az. vinelandii* and *Az. chroococcum*) and the mixture on the protein content were 10.67%, 10.77%, and 10.85%, respectively, according to the data. The blend of Azotobacter and 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.

**Keywords:** Azotobacter, Anatomy, Nitrogen fixers, Wheat crop.

### INTRODUCTION

A microbial-mediated procedure known as "Biological Nitrogen Fixation" (BNF) converts atmospheric nitrogen (N<sub>2</sub>) through the action of the enzyme "Nitrogenase" into ammonium that is easily absorbed by roots. N<sub>2</sub>-fixing bacteria are free-living, extremely diversified, and common throughout the world in agriculture. In agricultural and natural habitats lacking symbiotic N fixation, they provide as a significant natural supply of nitrogen (N). The significance of Azotobacter species as both significant free-living N<sub>2</sub>-fixing bacterium and prospective bacterial biofertilizer with demonstrated efficacy for plant nutrition and biological soil fertility was underlined in this research. We also discussed the characteristics of Azotobacter that help plants grow, such as its ability to use nutrients efficiently, its capacity to synthesize phytohormones, etc. We also provided information on the agronomic characteristics of Azotobacter, which are probably a useful part of an integrated plant nutrition strategy that benefits sustainable agricultural production. In this study, we emphasized the non-symbiotic N<sub>2</sub>-fixing diversity and global distribution of soil-dwelling Azotobacter bacteria. In habitats lacking SNF, this bacterial community can be the main natural supply of nitrogen. reported a rise in the volume and surface area of new leaves. Higher, xylem vessel diameter, phloem tissue width, and cold stress epidermal cell density Hajhashemi *et al.*, (2018). As a result of plant stress, the diameter, wall thickness, hollow pith cavity diameter, the overall number

of vascular bundles, the number of large and small vascular bundles, bundle length and width, phloem tissue thickness, and metaxylem vessel diameter in wheat plants were all reduced. Nassar *et al.*, (2020). The current study intends to assess the ability of two Azotobacter strains (*Azotobacter chroococcum* and *Azotobacter vinelandii*) to form associations with wheat plants, as well as their impact on wheat plant growth and anatomical structure.

### MATERIALS AND METHODS

#### Soil samples collection:

For Azotobacter isolation, soil samples were taken from the rhizosphere region of wheat plants at various sites in El-Gharbia, (Qutor), Governorate.

#### Wheat grains:

Wheat grains (*Triticum aestivum* L.) cv. Giza 171 were kindly obtained from the Crops Res. Inst., Agricultural Research Center (ARC), Giza, Egypt.

#### Isolation and purification of Azotobacter:

Isolation of Azotobacter was carried out according to the method given by Bilal *et al.* (1990).

#### Identification of the isolated Azotobacter:

After purification of Azotobacter by using nutrient agar medium (Oxoid, 1965), the identification isolates of Azotobacter as follows: 250ml Erlenmeyer flasks each containing 100ml of Ashby liquid medium and plates of agarized Ashby medium (Brown *et al.*, 1962 and Mazinani *et al.*, 2013) were inoculated with a loop full of one day old culture of each Azotobacter isolates. For one day, inoculated

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plates and flasks were incubated at 28-30°C. The identification of Azotobacter was done as follows: morphology, reaction to Gram stain, slime production and pigmentation. All tests were carried out on 24 hrs, old cultures using the nitrogen free medium of Stanier *et al.*, (1963).

**Media used:**

Ashby medium (Brown *et al.*, 1962 and Mazinani *et al.*, 2013). Used for isolated of Azotobacter as free fixed nitrogen. & Nutrient agar medium (Oxoid, 1965). Used for testing the purity of Azotobacter isolates from bacteria.

**Determination of phytohormones:**

The regional hub for mycology and biotechnology, Al-Azhar University, used gas liquid chromatography to separate and determine phytohormones (auxin, geberillin, and cytokinin). WinChrome Chromatography Ver. 1.3, equipped with a GBC UV/VIS Detector and Hypercarb (C18, Sum 100x4.6 cm), carried out an HPLC analysis on GBC-Germey at a flow rate of 7 ml/min with 85% Acent: 15% Water. approach as described in Van Staden *et al.*, 1973

**Total nitrogen:**

Total nitrogen in the culture of Azotobacter determined using Jackson, (1973) micro-kjeldahl technique.

**Total chlorophyll content:**

Total chlorophyll content were determined using the formulae of Lichtentaler and Wellburn (1985).

**Leaf area (cm<sup>2</sup>):**

Leaf area (cm<sup>2</sup>) was measured according to Kvet and Marshall (1971).

**Field experiment:**

A field experiment was carried out at Faculty of Agriculture, Al Azhar University, during the winter season of (2022,2023) to study the efficiency of Azotobacter, on wheat growth and yield. The experiment was arranged as a plots measuring 3 m x 3 m (9 m<sup>2</sup>) with three replicates. Azotobacter isolates were present in each main plot. Each subplot contained different urea concentrations [0, 25, 50, and 75 Kg N<sub>2</sub>/fed. Clay loam soil was used, some physicochemical properties according to Page *et al.* (1982) are shown in Table (1).

**Table 1. Physical and chemical properties of the experimental soil**

Property	Clay loamy soil
Sand	40.0 %
Silt	30.0 %
Clay	30.0 %
Chemical analysis meq-L	
Ca <sup>++</sup>	6.5
Mg <sup>++</sup>	4.0
Na <sup>+</sup>	9.0
K <sup>+</sup>	2.1
CO <sub>3</sub> <sup>-</sup>	0.0
HCO <sub>3</sub> <sup>-</sup>	4.5
Cl <sup>-</sup>	9.0
SO <sub>4</sub> <sup>-</sup>	8.1
E.C.	2.4 ds/m
pH	7.7
Total nitrogen	0.1

Of the two split doses of nitrogen provided, the first 2/3 N dose was added before wheat was sown 30 days after the first (1/ 3 N) was sown, the second was added. The treatment were performed as follows: Control (without inoculation), inoculation with *Az. chroococcum*, inoculation with *Az. vinelandii*, and inoculation with their mixture as well four levels from nitrogen four doses. When the wheat seedlings emerged, one seedling was selected to complete a section of a

wheat plant. Wheat grains mixed with an Azotobacter strain were then sown into each plot. Different morphological characteristics of plant growth were assessed: plant height (cm), spike count, dry weight of spike (g), dry weight of grain (g), and weight of 1000- grain. A random sample was taken from threshed grains to measure the 1000-grains weight (g), one thousand air dry wheat grains were weighed.

**Anatomy of plant:**

From each treatment, three wheat leaves were taken. On each leaf, five portions were created. (Yeung *et al.*, 2015) For mol acetic alcohol fixative (90 mL ethanol 50%, 5 mL glacial acetic acid, and 5 mL formalin) was used to fix leaf samples for at least 48 hours. The leaf blade was used as the source for samples that were half a centimetre long. Samples were dried out in a succession of solutions that had ethanol concentrations that ranged from 50% to 100%. In order to embed the samples in paraffin wax, xylol was used as a solvent. A rotary microtome was used to cut sections to a thickness of 15 m, and egg albumin was used as an adhesive to place the sections on slides. Slides were run through a succession of ethanol solutions ranging from 100% to 50% after wax was dissolved in xylol. Safranin T and light green SF were used as double stains, and canada balsam was used to preserve the sections permanently (Ruzin, 1999). A Scmos Digital Camera and a Zeiss microscope were used for all photomicrographs.

**Statistical analysis:**

A totally randomized design was employed, with three copies. Data gathered using the procedures outlined by Steel and Torrie (1980) were subjected to analysis of variance (ANOVA). The mean differences were compared using the least significant difference (LSD) at 5%.

**RESULTS AND DISCUSSION**

**Results**

From selective medium, two isolates were found to be bacterial free Azotobacter after being isolated and purified from soil samples at El-Gharbia (Qutor) Governorate. Two Azotobacter isolates were recognised based on their tests. All tests were conducted on 24-hour to old cultures of Azotobacter using nitrogen-free medium. The morphological characters of the two Azotobacter isolates were studied based on the phenotypic properties, appearance and color of cultures in addition to the microscopic examination and some biochemical tests . The characteristics of the isolated Azotobacter genera are presented in Table (2).

**Table 2. Cultural, morphological and biochemical characterizations of the Azotobacter isolates**

Characters	Azotobacter isolates No.	
	(1)	(2)
Habitat	Soil, water	Soil, water
Gram stain	Negative gram	Negative gram
Cell size (µ)	3.4x1.5	2.0-3.0x3.0-3.6
Cyst development	Present	Present
Shape	Rod	Ovoid to Rod in pairs
Motility	Motile (unseen in old cultures)	Motile, especially in young culture
Pigment properties	Yellowish green, fluorescent (diffusible in water)	Dark brown to black (water insoluble)
	Pigment formation In young cultures	With ageing
Utilizes sodium benzoate	Yes (grows in concentration of 1%)	In some cases only
Utilizes starch	No	Yes
Utilizes mannitol	Yes	Yes
Benzoate	Yes	Yes

Those isolates that exhibited the most favorable properties were found to belong to *Azotobacter chroococcum* and *Azotobacter vinelandii*.

**Ability of Azotobacter nitrogen fixation:**

The results in Table (3) are shown that both strains of *Az. chroococcum* and *Az. vinelandii* varied in their ability to fix atmospheric nitrogen. Amount of fixed nitrogen were increased with an increasing culture age in both strains of Azotobacter.

**Table 3. Amounts of fixed-nitrogen by Azotobacter strains (mg N/100 ml-culture)**

Azotobacter strains Culture age (days)	<i>Az. vinelandii</i>	<i>Az. chroococcum</i>
Two	6.3	7.2
Four	13.9	15.6
Eight	19.8	21.5

**Production of phytohormoes by Azotobacter strains**

Data in Table (4) demonstrated that both *Az. chroococcum* and *Az. vinelandii* generated auxin (Indole 3 acetic acid, IAA) at 7.61 and 6.99 ug/100 ml, gibberellins (Gibberellic acid, GA<sub>3</sub>) at 9.12 and 6.12 ug/100 ml, and cytokinin (Zeatin) at 5.65 and 4.11 ug/100 ml, respectively.

**Table 4. Composition and analysis of phytohormones of *Az. chroococcum* and *Az. vinelandii* (µg/100 ml).**

Phytohormones	<i>Az. vinelandii</i>	<i>Az. chroococcum</i>
Indole 3 Acetic Acid (IAA)	6.99	7.61
Gibberellic Acid (GA <sub>3</sub> )	6.12	9.12
Cytokinin (Zeatin)	4.11	5.65

**Growth parameters of Wheat:**

**Total chlorophyll (mg g<sup>-1</sup>), leaf nitrogen content and leaf area (cm<sup>2</sup>)**

Between the various treatments, there were noticeable differences in the total chlorophyll (mg g<sup>-1</sup>), leaf nitrogen content and leaf area (cm<sup>2</sup>). In Tables (5, 6 and 7) the different treatments (Azotobacter and concentrations of N) showed a substantial difference.

**Table 5. Effects of Azotobacter strains and nitrogen levels on total chlorophyll (mg g<sup>-1</sup>) in wheat plant.**

Nitrogen level /Fed.	Without inoculation (control)	<i>Az. vinelandii</i>	<i>Az. chroococcum</i>	Mixture of Azotobacter
0	4.01	4.38	4.75	5.49
25	4.28	4.88	5.33	5.95
50	4.71	5.58	5.65	6.38
75	5.33	5.98	6.12	6.84

LSD = 0.05 Nitrogen 0.80

**Table 6. Effects of Azotobacter strains and nitrogen levels on leaf nitrogen content in wheat plant.**

Nitrogen level /Fed.	Without inoculation (control)	<i>Az. vinelandii</i>	<i>Az. chroococcum</i>	Mixture of Azotobacter
0	1.85	2.21	2.48	2.60
25	2.19	2.55	2.62	2.81
50	2.51	2.65	2.70	3.12
75	2.77	2.81	2.82	3.23

LSD = 0.05 Nitrogen 0.30

**Table 7. Effects of Azotobacter strains and nitrogen levels on leaf area (cm<sup>2</sup>) in wheat plant.**

Nitrogen level /Fed.	Without inoculation (control)	<i>Az. vinelandii</i>	<i>Az. chroococcum</i>	Mixture of Azotobacter
0	16.22	22.94	23.44	23.76
25	22.17	23.73	24.59	32.60
50	23.63	24.56	32.10	33.88
75	24.02	32.52	33.11	34.18

LSD = 0.05 Nitrogen 0.97

The total chlorophyll (mg g<sup>-1</sup>), leaf nitrogen content and leaf area (cm<sup>2</sup>) were shown to considerably increase with an increase in N fertilization, reaching its maximum at an Azotobacter mixture with 75 kg/Fed. of N.

**Protein content (%) of wheat plants**

The effects of nitrogen levels and Azotobacter strains on protein content of wheat plants are shown by the results in Table (8). The effects of the two Azotobacter strains (*Az. vinelandii* and *Az. chroococcum*) and the mixture on the protein content were 10.67%, 10.77%, and 10.85%, respectively. The blend of Azotobacter and 75 kg N/fed had the highest protein content among the treatments.

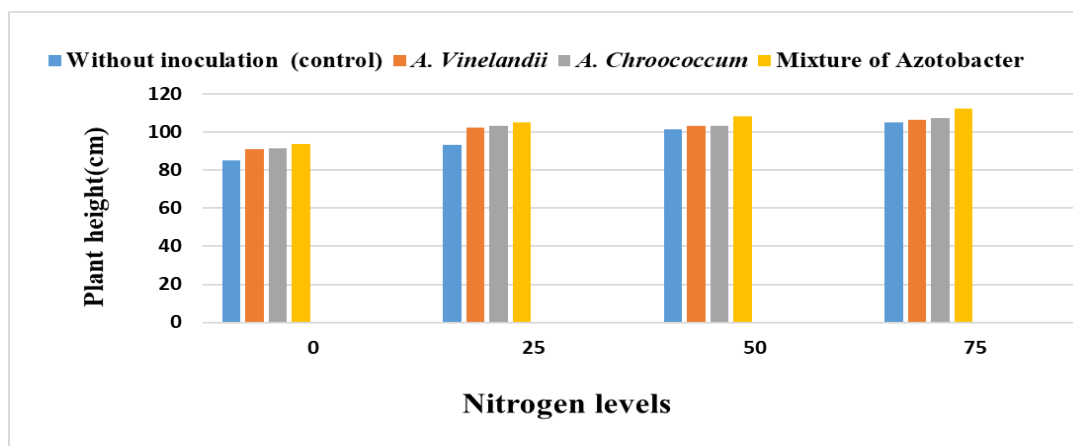
**Table 8. Effects of Azotobacter strains and nitrogen levels on protein content (%) in wheat plants.**

Nitrogen level /Fed.	Without inoculation (control)	<i>Az. vinelandii</i>	<i>Az. chroococcum</i>	Mixture of Azotobacter
0	7.95	9.44	9.85	9.99
25	8.99	9.72	10.02	10.03
50	9.76	9.99	10.10	10.66
75	10.00	10.67	10.77	10.85

LSD = 0.05 Nitrogen 0.15

**Plant height (cm)**

The effects of inoculation at various nitrogen levels on wheat plant height (cm) are shown by the data in Fig. 1.



**Fig. 1. Effect of Azotobacter strains and nitrogen levels on plant height(cm) in wheat plant**

The results showed that when wheat was inoculated with both *Azotobacter* strains (*Az. chroococcum* and *Az. vinelandii*) as well as their mixture, wheat plants grew to a height of 85.22 to 112.02 cm in response to the effects of 0 and 75 kg of nitrogen /fed., respectively.

However, in both treatments, of 75 kg N/fed with a mixture of *Azotobacter* inoculation were gave the wheat plant height a highly significant difference from the control.

**Number of spikes/m<sup>2</sup>**

Figure 2 depicts the impact of *Azotobacter* isolates and nitrogen levels on the number of spikes/m<sup>2</sup> in wheat, which revealed extremely significant differences. Results. As a result, the Mixture of *Azotobacter* combination with 75 kg N/fed. produced the highest value (409.22), while the nitrogen control without *Azotobacter* inoculation gave the lowest value (215.88). The best *Azotobacter* combinations for this trait contain 75 kg of nitrogen per meal.

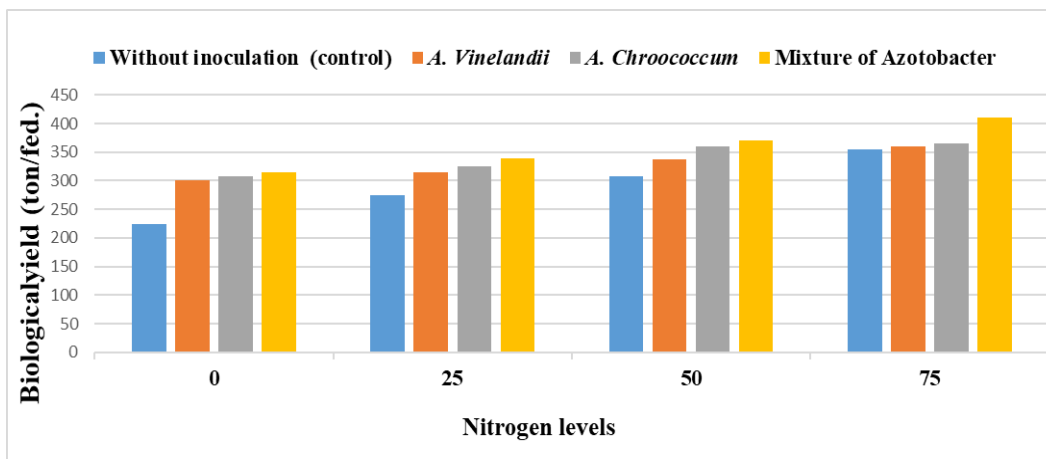


Fig. 2. Effect of *Azotobacter* strains and nitrogen levels on number of spikes/m<sup>2</sup> in wheat plant.

**Weight of 1000- grain (g)**

Utilizing an *Azotobacter* mixture boosted grain weight by one thousand. The most weight per thousand

grain was obtained utilizing an *Azotobacter* combination (Fig. 3). However, when *Azotobacter* was omitted, the lowest 1000-grain weight was generated.

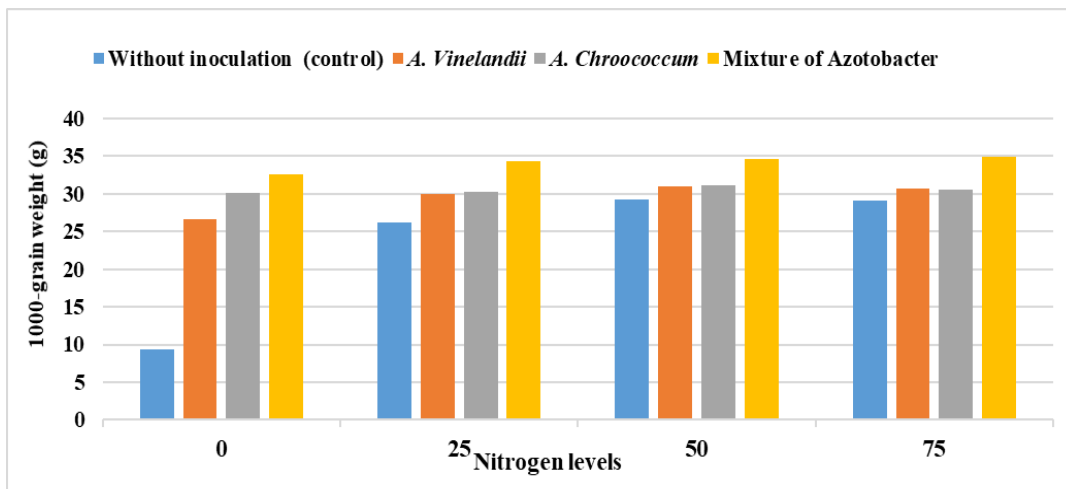


Fig. 3. Effect of *Azotobacter* strains and nitrogen levels on 1000-grain weight (g) in wheat plant.

**Grains weight (ton/fed.)**

The data in Fig. 4. It was evident that *Azotobacter* in combination with nitrogen fertilization had a considerable impact on grain weight, with the combination producing the greatest grain weight value.

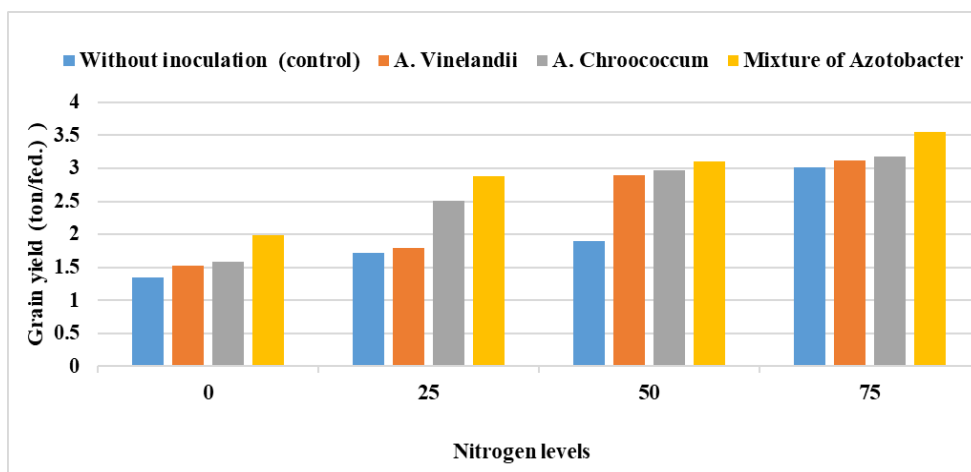
Found that the weight of the grains as influenced by *Azotobacter* varied greatly. demonstrated that the range of grains yield in wheat ranged from 1.35 to 3.55 (ton/fed.) under the influence of two different *Azotobacter* as well as the nitrogen levels after inoculation with different two locally adapted species of *Az. Vinelandii*, *Az. chroococcum*, and their mixture of *Azotobacter*. Mixture of *Azotobacter* with 75 kg of nitrogen / fed. was the most effective treatment combination.

**Straw yield (ton/fed.)**

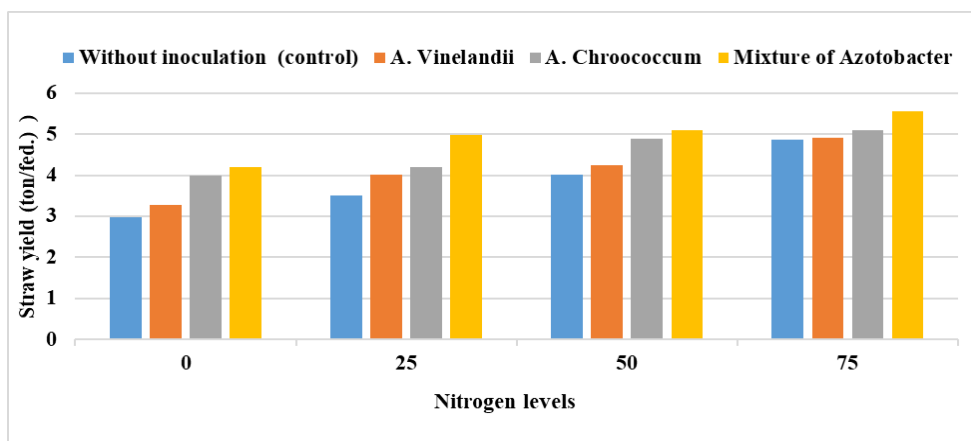
According to data in Fig. 5, the combination of *Azotobacter* and 75 kg N/fed produced the maximum straw yield (5.57 tons/fed). While, the cheapest straw (2.99 ton/ fed.) was made from nitrogen-free, non-inoculated soil.

**Biological yield (ton/fed.)**

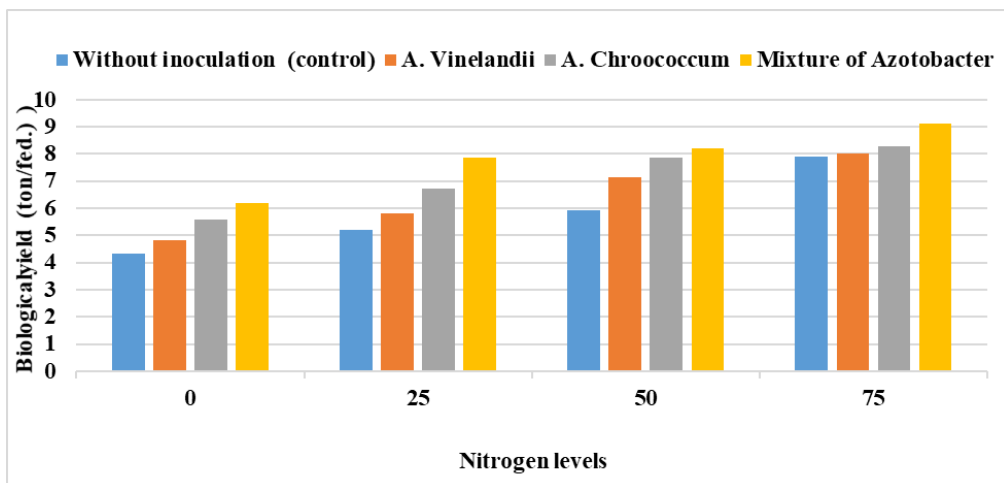
The biological yield (ton/fed.) of wheat is affected by *Azotobacter* strains and nitrogen levels, as shown in results in Fig. 6. The effects of the two different inoculated *Azotobacter* as well as the nitrogen levels led to a wheat biological yield ranged from 4.34 to 9.12 (ton/fed.) in both tests using various two locally sources *Azotobacter* strains (*Az. chroococcum* and *Az. vinelandii*). The *Azotobacter* mixture and 75 kg N/fed treatments worked best as a pair.



**Fig. 4.** Effect of Azotobacter strains and nitrogen levels on grain yield (ton/fed.) in wheat plant.



**Fig. 5.** Effect of Azotobacter strains and nitrogen levels on Straw yield (ton/fed.) in wheat plant.



**Fig. 6.** Effect of Azotobacter strains and nitrogen levels on biological yield (ton/fed.) in wheat plant.

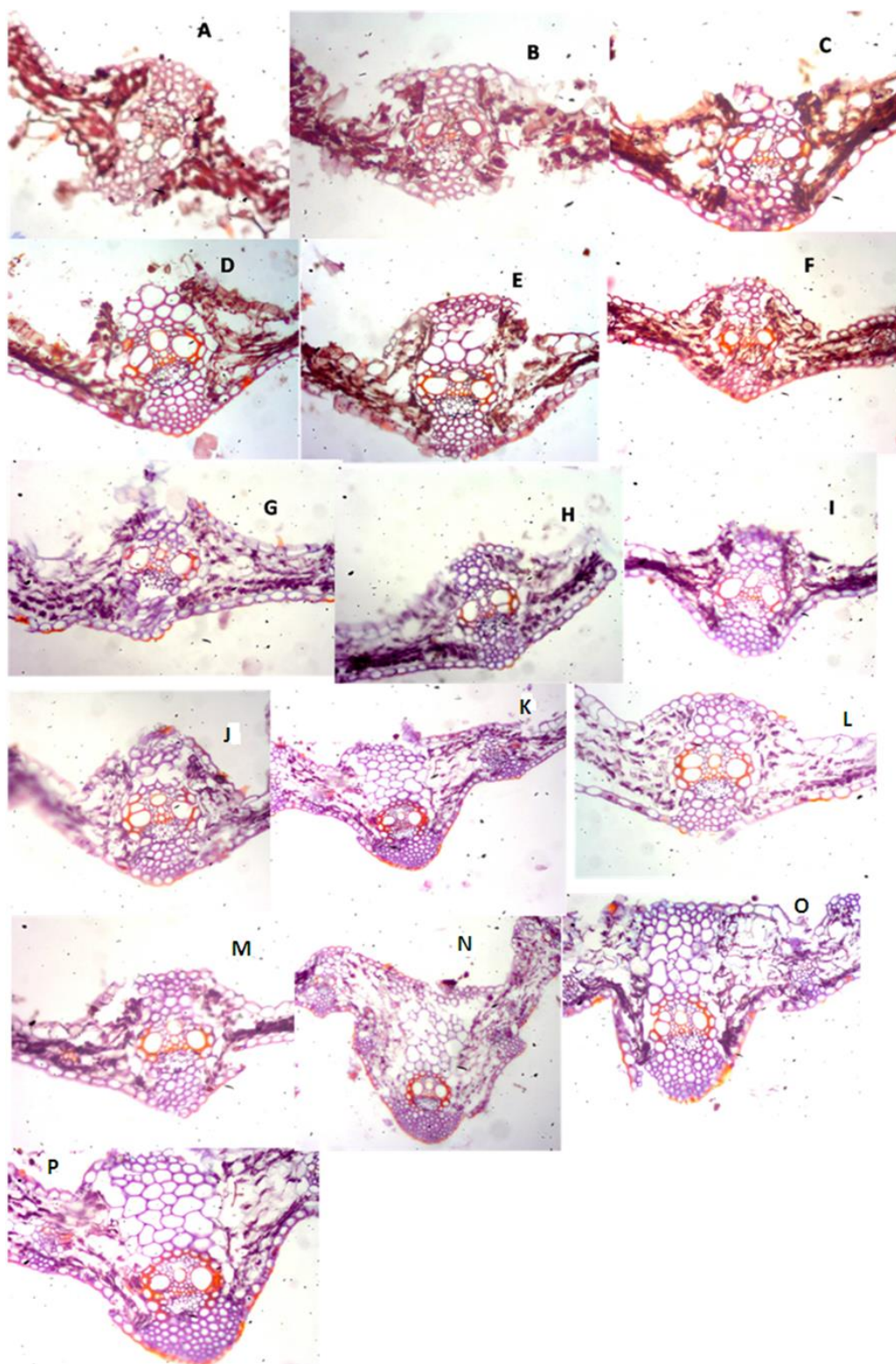
**Anatomical differences of treatment wheat leave.**

In the anatomical study, all treatments were different their effect on the epidermis. They reached their highest value in the where they were 15.9 μm in treatment mixture of Azotobacter with 75N for the upper epidermis and 14.9 μm for the lower epidermis. While, its lowest value was 11.2 μm and 12.4 μm in the without inoculation (control). In addition, the lowest value for measuring the mean vein area was 304.2 μm in treatment 25 N and its maximum value was present in treatment mixture of Azotobacter with 75N which is 561.7 μm, as it was acquired Also, treatment Azotobacter with 75N had the highest value for both the length and width of the

vascular, and it was 151.7 μm and 173.4 μm respectively, while the lowest value for the length of the vascular was present in treatment 25 N, where it was 94.2 μm, and for the lowest width of the vascular it was present in treatment without inoculation (control), where it was diameter 97.2 μm. It also found different effects of the treatments on the Phloem area, so treatment *Az. chroococcum* with 75N had the highest value for Phloem which is 48.1 μm and the lowest value is 30.8 μm in without inoculation (control) while, the treatment led to an increase in the Xylem area, reaching a maximum in treatment mixture of Azotobacter with 75N, which is 87.9 μm, and the lowest value in treatment without inoculation

(control) where it was 44.3  $\mu\text{m}$ . Also, the effect extended to the diameters of the vessels, the highest thickness of the vessel reached mixture of *Azotobacter* with 75N, where it was 46.3  $\mu\text{m}$ . The lowest thickness was in 25 N, and it was 31.2  $\mu\text{m}$ . (Fig. 7 and Table 9). The effect of the treatments was clear on both the mesophyll and leaf thickness, as the thickness of the

mesophyll ranged from 55.7  $\mu\text{m}$ ) in treatment control to 83.4 $\mu$  in treatment mixture of *Azotobacter* with 75N. As for the thickness of the leaf, it was the lowest value in treatment control which was 90.2 $\mu$  and the highest in mixture of *Azotobacter* with 75N it was 117.1.  $\mu$  (Fig. 7 and Table 9).



**Fig. 7.** Vertical sections of treated wheat leaves(x150): (A) Without inoculation (control); (B) 25 N; (C) 50N; (D) 75N ; (E) *Az. vinelandii* ; (F) *Az. vinelandii*+ 25N ; (G) *Az. vinelandii* +50N; (H) *Az. vinelandii*+ 75N; ( I) *Az.chroococcum*; (J) *Az. chroococcum*+25N; (K) *Az. chroococcum*+50N (L); *Az. chroococcum*+75N; (M) Mixture of *Azotobacter*; (N) Mixture of *Azotobacter* +25N; (O) Mixture of *Azotobacter* +50N; (P) Mixture of *Azotobacter* +75N.

**Table 9. Effects of Azotobacter strains and nitrogen levels on anatomy in wheat leaves.**

Treatments	Parameters (µm)									
	1	2	3	4	5	6	7	8	9	10
	Upper epidermis thickness	Lower epidermis thickness	Mean vein diameter	Vascular bundle length	Vascular bundle width	Phloem thickness	Xylem thickness	Xylem vessels diameter	Mesophyll thickness	Blade thickness
Without inoculation (control)	11.2	12.4	307.6	96.8	97.2	30.8	44.3	32.9	55.9	90.2
25 N	11.7	12.63	304.2	94.2	116.1	31.6	48.1	31.2	66.8	91.1
50N	12.7	12.83	391.9	98.5	135.7	32.7	62.97	32.3	66.2	99.4
75N	11.7	12.9	380.1	110.2	118.2	33.8	64.25	33.6	64.2	92.7
<i>Az. vinelandii</i>	11.7	12.61	347.7	96.3	120	33.6	65.53	32.2	57.7	96.5
<i>Az. vinelandii</i> + 25N	11.6	12.9	381.1	98.3	99.2	41.8	62.6	33.7	66.3	92.2
<i>Az. vinelandii</i> + 50N	12.7	14.9	387.8	125.3	129.8	38.1	78.9	30.8	72.1	100
<i>Az. vinelandii</i> + 75N	14.3	14.5	492.3	141.2	155.7	43.5	86.6	42.5	80.1	101.1
<i>Az. chroococcum</i>	12.2	12.6	387.4	124	138.3	35.7	55.3	34.9	55.6	93.2
<i>Az. chroococcum</i> +25N	13.9	13.8	453.2	151.7	145.7	41.1	87.7	36.6	80.9	101.1
<i>Az. chroococcum</i> +50N	12.6	13.7	491.3	141.2	152.3	42.2	83.6	34.7	79.1	101.1
<i>Az. chroococcum</i> +75N	14.1	14.5	553.2	143.2	159.7	48.1	87.7	46.2	80.9	101.1
Mixture of Azotobacter	12.2	12.9	388.4	121.9	148.9	43.6	61.9	35.1	62.8	90.6
Mixture of Azotobacter +25N	12.4	12.7	461.7	143.2	144.5	41.7	87.9	36.8	80.1	101.1
Mixture of Azotobacter +50N	14.8	14.1	425.8	125.7	150.8	42.6	83.4	40.8	75.6	100
Mixture of Azotobacter +75N	15.9	14.9	561.7	151.7	173.4	44.6	87.9	46.3	83.4	117.1

**Discussion**

Chemical fertilizer application, which was largely intended to increase yield, interfered with unintended microbial activities and decreased quality. Microbial inoculants, in particular free living nitrogen fixers, have been tested as an alternative to or as a supplement to artificial nitrogen in an effort to lessen these harmful effects (Wang, and Li 2019). Microbial inoculants may lessen the negative impacts of artificial fertilisers while also preserving quality and lowering growing costs. Azotobacter, an aerobic free-living bacterium, has been used to improve crop performance by giving plants the extra N they need for growth and production. Application of Azotobacter would lessen reliance on synthetic nitrogen sources (Li, *et al.*, 2018). The goal of the experiment was to determine how different nitrogen levels and Azotobacter interacted to determine the ideal treatment combination for maximising the growth, yield and quality of while minimising costs (Zaidi,*et al.*, (2017). This is comparable to the reported results by Jnawali *et al.*, (2015). Azotobacter spp. are heterotrophic, free-living, non-symbiotic bacteria that can fix 20 kg of nitrogen on average each year (Stanier *et al.*, 1963). These microorganisms are known as plant growth promoting rhizobacteria (PGPR), which produce growth factors that promote the growth and development of plants and hinder the growth of phytopathogenic microorganisms by secreting inhibitors Tantawy and Atef (2010). Additionally, it aids in the absorption of nutrients and generates various biochemical molecules like protein and amino acids. Azotobacter increases crop growth rate (CGR) and benefits seed germination. For better crop response, it helps to boost nutrient availability and restore soil fertility. Due to its large contribution to the long-term sustainability of the soil, it is a crucial part of an integrated nutrient management system. Future study is required to examine Azotobacter's potential role in soil fertility. A further application of Azotobacter increased the yield by 21.17% above the control (chemical fertilisers) and caused a 3.5% increase in the LAI of the plant crop. 37 the rate of expansion in leaf area controls the plant's capability for photosynthetic activity, which improves the assimilation of food and increases output. The number of

branches, pods per plant, and 1000 grain weight were all reported to rise in plants that had been inoculated with *Azotobacter* spp. 40, 41. When cultivated in culture conditions supplemented with various carbon and nitrogen sources, azotobacter are capable of generating amino acids. These rhizobacteria create substances like amino acids that are involved in numerous mechanisms that explain the encouragement of plant growth. Schulze and Drevon (2005) performed a biochemical examination of chlorophyll, nitrogen, and infected plants in comparison to non-inoculated control plants. In addition, to fixing nitrogen, Azotobacter also makes Gibberalin, Nicotin, Thiomine, Riboflavin, and Indol Acetic Acid. Seed germination is significantly enhanced when Azotobacter is administered to the seeds. Indol-3-Acetic Acid (IAA) production by Azotobacter was demonstrated by Hakeem *et al.*, (2017). In the anatomical work, the parameters caused an increase in all of the plant's parts, including the size of the epidermis and cuticle. These results are comparable to those of Azoz *et al.*, (2016) and El-Afry *et al.*, (2012). This was accompanied by an increase in leaf thickness and mesophyll area (Zhang *et al.*, 2015 and Elsharkawy *et al.*, 2022). The mean vein area has increased as a result of the treatments, and the increase is mainly due to the increase in length and width, which is due to the increase in the phloem and xylem area, as well as in the diameter of the xylem vessels (Selim *et al.*, 2022 and Zhang *et al.*, 2015). Hajhashemiet *et al.*, (2018). The increase in new leaf mass and area was reported. Cold stress epidermal cell density, xylem vessel width, and phloem tissue width are all higher.

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## تقييم نشاط الازوتوبكتري على نمو محصول القمح وبعض صفاته التشريحية

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### الملخص

تم عزل وتنقية بكتريا الازوتوبكتري كرووكوم و الازوتوبكتري فينيلانديا من عينات التربة التي جمعت من قطور بمحافظة الغربية. أنتجت عزلتي الازوتوبكتري هرمونات السيبتوكينين (الزيتين) والأكسين (اندول ٣ اسيتك اسد) و الجبريلين (حمض الجبريليك). اظهرت نتيجة تلقيح نبات القمح بالازوتوبكتري كرووكوم و الازوتوبكتري فينيلانديا، وخليطهما زيادة في كلا من ارتفاع النبات وعدد السنابل / م<sup>٢</sup> ووزن ١٠٠٠ حبة ومحصول الحبوب ومحصول القش ومحتوى الأوراق من الكلوروفيل (مجم/جم)، محتوى الأوراق من النيتروجين، مساحة الورقة (سم<sup>٢</sup>) ومحتوى البروتين٪ على محصول القمح. كما ظهرت العديد من الخصائص التشريحية نتيجة للمعاملات، بما في ذلك حجم الحزمة، وسمك الورقة، وحجم النسيج الخشبي واللحاء.