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Original Research

Responses of *Gossypium barbadense* L. Cotton Plants to Biofertilizers under Different Levels of Nitrogen Fertilization

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Abstract

Conventional agricultural practices, which rely heavily on polluting agrochemicals, are pushing us towards an unsustainable future. Biofertilizers are multifaceted and span the environmental, agricultural, and economic dimensions. Therefore, this study was conducted to investigate the impact of plant growth-promoting bacteria *Azotobacter chrocooccum* and *Pseudomonas* sp. and their interaction on the growth and productivity of cotton (*Gossypium barbadense* L. vr. Giza CV 97) under different levels of nitrogen fertilization (50, 75, and 100% nitrogen recommended dose). Nitrogen deficiency resulted in lower cotton growth, chlorophyll, and stem anatomical parameters as well as yield or yield components compared to optimal nitrogen fertilization. Application of *A. chrocooccum* and *Pseudomonas* sp. and their interaction mitigated harmful nitrogen deficiency stress. Cotton fiber quality measurements (fiber length, micronaire, and fiber strength) were insignificantly affected by bacterial biofertilizers and their interactions under all levels of nitrogen fertilization. *A. chrocooccum* and *Pseudomonas* sp. interaction was the best treatment at all levels of nitrogen fertilization, producing thicker, wider vascular conductive,

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cortical tissue, and xylem vessel diameters. This treatment also improved cotton growth and gave the highest seed yield, lint percentage, and boll weight.

Keywords: Biofertilizers, Nitrogen deficiency, Azotobacter chrocooccum, Pseudomonas sp., Chlorophyll, Anatomical parameters

Introduction

The cotton plant *Gossypium barbadense* L. belongs to the family Malvaceae and is cultivated for fiber and oil seed production. The main product of cotton is fibers, which are inherently soft and comfortable against the skin due to their natural structure. Cottonseed oil is rich in protein and fat (17-27%, respectively). This oil is already a well-established vegetable oil and has some industrial applications. It is the second source of plant proteins after soybean and the fifth oil-producing plant after soybean, palm oil, canola, and sunflower [1]. Moreover, cottonseed serves as a protein supplement in the animal feed industry. Nitrogen is an essential macronutrient needed for the synthesis of proteins, enzymes, nucleic acids, and chlorophyll molecules. Without enough nitrogen, plants cannot produce enough protein, resulting in stunted growth and yellowing leaves. Plant growth promoting bacteria (PGPR) are found in the soil around the root surface. These biofertilizers promote plant growth directly or indirectly by producing or releasing several chemicals near the root surfaces [2]. Singh and Purohit [3] stated that biofertilizers containing beneficial bacteria or fungi play an essential role in enhancing soil properties, nutrient availability, and crop production. Although they cannot completely replace chemical fertilizers, their combined use can improve soil quality, increase yield, and reduce demand for chemical fertilizers by up to 35%. The genus *Azotobacter* performs many metabolic functions, including nitrogen fixation, the production of certain amino acids (thiamin, riboflavin), and plant hormones (IAA, gibberellin, and cytokines) [4-5].

Nowadays, employing organic fertilizer enriched with native microorganisms has emerged as a suitable technology. The inoculation of microbes serves the purpose of utilizing their decomposition abilities [6] and their role as biofertilizers [7]. Utilizing indigenous microbes yields several benefits, including maintaining ecological balance, producing environmentally friendly products, and enhancing soil and plant nutrition. These indigenous microbes comprise bacteria capable of nitrogen fixation and phosphorus solubilization, thereby contributing to the availability of essential macronutrients.

The use of biofertilizers has gained attention due to the high cost of synthetic chemical fertilizers and their negative impact on the environment. Therefore, the current study aimed to use two biofertilizers (*Azotobacter chrocooccum* and *Pseudomonas* sp.) and examine their interaction under three various levels of nitrogen fertilization to increase the growth and yield of cotton plants (*G. barbadense* L. var. Giza CV 97).

Experimental

Treatments and Crop Management

Field experiments were conducted on clay textured soil located at the Sakha Agricultural Research Station farm, Kafr El-Sheikh Governorate, Egypt, and the laboratory of the Agricultural Botany Department, Faculty of Agriculture, Kafrelsheikh University, Egypt. The present investigation was designed to study the response of cotton plants *G. barbadense* L. var. Giza CV 97 to two bacterial biofertilizers (*Azotobacter chrocooccum* and *Pseudomonas* sp.) under various levels of nitrogen fertilization during the growth seasons (2021 and 2022).

The experiments were conducted according to split plot design (SP) and by testing the effects of two factors. The first factor included three levels of mineral nitrogen treatments, and the second factor involved the use of two bacterial strains (biofertilizers). The experiment consisted of twelve treatments with three replicates, and means were compared utilizing the least significant differences (L.S.D.) at a probability level (0.05). The experimental layout consisted of five rows that were each 4 meters long and 0.7 meters wide. The plot area was 14 m^2. In the first and second seasons, the seeds were sown on May 1 and 3, respectively. In order to fertilize the soil, nitrogen fertilizer in the form of urea (46.5%) was applied in two equal doses to each plot at a rate of 60 kg $N.fed^{-1}$ (100% FRD), 45 $N.fed^{-1}$ (75% FRD), and 30 $N.fed^{-1}$ (50% FRD). The first dose was added after thinning (before the first irrigation), and the second dose was applied before the second irrigation. During soil preparation, phosphorus fertilizer in the form of calcium superphosphate (15.5% P_2O_5) was administered at a rate of 30 kg P_2O_5 fed^{-1}. Potassium in the form of potassium sulfate is administered at a rate of 24 kg K_2O fed^{-1}.

Source of Microorganisms

Two bacterial strains (*Azotobacter chrocooccum* and *Pseudomonas* sp.) as plant growth promoting bacteria were kindly obtained from Prof. Dr. Elsayed B. Belal, Professor of Agricultural Microbiology, Agric. Botany Department, Faculty of Agriculture, Kafrelsheikh University [8].

Cultivation of Plant Growth Promoting Bacteria

Azotobacter chrocooccum and *Pseudomonas* sp. were grown in nutrient liquid medium**.** Then, 200 ml nutrient liquid medium was inoculated with 2 ml of bacterial growth of *A. chrocooccum* or *Pseudomonas*

sp. (nutrient broth medium, 10^8 cfu/ml) and incubated for 3 days at 30°C and 150 rpm. Cultures were incubated for 5 days at 30°C and 150 rpm. Thereafter**,** two bacterial strains were administered to cotton as follows:

Seeds Treatments

Cotton seeds were witted with 10% sugar syrup and thoroughly mixed with bacterial suspension (10^8 cfu / ml) for 30 min at the time of sowing, enough to obtain 10^8 cfu g^{-1} of seeds, and then dried. Seeds were then sown in every plot. Conversely, cotton seeds were completely mixed with a quantity of nutritional broth medium (free of bacterial growth) and submerged in 10% sugar syrup.

Vegetative Growth Traits

Plant growth parameters, i.e., plant height and plant dry weight, were estimated and calculated at the harvesting date. Plant height (cm) values were measured from the soil surface to the stem apical terminal bud. For plant dry weight (g), plant fresh samples were dried at 70°C in an electric oven until a constant weight was achieved. Leaf area/plant (cm^2) was measured after 100 days from sowing using the leaf area meter Model L1 – 3100.

Chlorophyll Pigment Measurement

Chlorophyll pigments (chl. a, b, or total) concentrations (µg/cm^2) were measured as a fresh area from the fourth leaf from the stem top after 100 days from seed sowing in both successive seasons. The chlorophyll pigments were extracted using 5 ml of N-N dimethyl formamide. The selected samples were exposed to darkness for 24 hours in the refrigerator. The absorbance was measured using a spectrophotometer at OD664 and 647nm. The photosynthetic pigment concentration was calculated according to Moran [9].

Anatomical Studies

Selected samples were taken from the second stem internode at the apex after 70 days of sowing. Stem pieces were washed with tap water and cut into appropriate specimens (5 mm in length). The samples were killed, fixed for 48 h in FAA solution (10 ml formalin, 5 ml glacial acetic acid, and 85 ml ethyl alcohol (70%) then washed twice in 70% ethyl alcohol. Dehydration of samples was done by passing the samples in a series of ethyl alcohols, followed by embedding them in paraffin wax at a 60°C melting point. Sectioning at a thickness of 12µm was done with a rotary microtome (model Leica RM 2125), followed by staining with safranin or light green. Samples were cleared in xylene and mounted in Canada balsam, prepared for microscopic examination [10]. The chosen sections were examined microscopically to identify histological characteristics. Vascular conductive tissues (xylem and phloem), vascular cambium, cortex tissue thickness, and xylem vessel diameter were measured using the ImageJ software program (Fiji, http://fiji.sc/Fiji) [11].

Yield and its Components

At first pick, ten guarded plants were randomly chosen from every plot and labeled to calculate the following characteristics: the number of fruiting branches, the number of open bolls per plant, the boll weight (g), and the seed index (100-seed weight). The lint percentage (weight of lint per plant divided by the weight of seed cotton per plant multiplied by 100) and the seed cotton yield/fed (Kentar, i.e., 157.5 kg) were measured. The Earliness Index was determined based on the formula presented by Singh and Purohit [12].

Cotton Fiber Quality

Cotton samples were taken to measure the fiber properties using specific instruments. The digital fibrograph instrument 630 was used to determine fiber length (mm). The Pressley instrument was used to measure fiber strength (g/tex). The micronaire instrument 675 was used to obtain micronaire (Mic) readings. These measurements were conducted according to the standards at the cotton research institute laboratories at the Sakha Agricultural Research Station farm, Kafr El-Sheikh Governorate, Egypt [13]. The collected data from each season was subjected to analysis of variance.

Experimental Design and Statistical Analysis

The experimental design was a split plot design with three replications in every treatment. Results were pooled, and means were taken. An analysis of variance was performed utilizing the statistical package (CO-STATE). Duncan's multiple range tests for comparison of data. The means were deemed significantly different at ≤0.05 [14].

Results and Discussion

Data presented in (Tables (1, 2, 3, 4, and 5) and Figs (1, 2, and 3) show that application of cotton (*G. barbadense* L. var. Giza CV 97) seed treatment by *Azotobacter chrocooccum* and *Pseudomonas* sp. (108 CFU/ml) and their interaction under three different nitrogen fertilizer levels (100, 75, and 50% NRD) on cotton plant parameters (plant height, dry height, and leaf area), chlorophyll pigments, anatomical studies, and yield components during the 2021 and 2022 seasons as follows:

Vegetative Growth Traits

The data presented in Table 1. indicated that the vegetative growth parameters, including plant height, dry weight, and leaf area per plant, were increased under the recommended nitrogen fertilizer dose (100% NRD). Nitrogen is a key macronutrient for cotton plants and other crops, playing a vital role in various physiological processes essential for growth and development. Nitrogen (N) plays a crucial role in cotton growth and development because cotton demands relatively large amounts of N, but its sensitive characteristics necessitate careful management. Efficient N management is critical for maximizing cotton yield while minimizing environmental impact [15]. When cotton experiences a nitrogen deficit (75 and 50% NRD), it triggers a cascade of negative impacts on various growth traits, including plant height, dry weight, and leaf area per plant. Nitrogen is crucial for cell division and elongation, promoting stem growth [16]. Deficient nitrogen limits these processes, leading to shorter internodes and, ultimately, stunted plants with reduced overall height. Nitrogen is a building block for proteins and nucleic acids, vital for biomass production. Insufficient nitrogen hinders photosynthesis and protein synthesis, resulting in a reduction in dry matter accumulation and lower total plant biomass [17].

Table 1. Effect of application of cotton (*G. barbadense* L. var. Giza CV 97) seed treatment by *Azotobacter chrocooccum*, *Pseudomonas* sp. (10^8 CFU/ml) and their interaction under three different nitrogen fertilizer levels (100, 75, and 50% NRD) on plant of cotton parameters (plant height, dry height, and leaf area) during 2021 and 2022 seasons.

Fertilization level (A)	Treatments (B)	Plant height (cm)		Dry weight (g)		Leaf area/plant (cm^2)	
		2021	2022	2021	2022	2021	2022
100% NRD	Without inoculation	135.64 f	138.45 d	125.23d	139.92cd	2134.12 i	2208.94f
	Pseudomonas sp.	144.58 b	147.22 b	135.99c	145.08c	2768.60 d	2812.00 c
	A. chrocooccum	143.82 c	144.00 c	136.46c	140.37cd	2808.72 c	3206.44 a
	Pseudomonas sp.+ *A. chrocooccum*	150.16 a	150.35 a	159.02a	167.54a	3124.56 a	3184.60 a
	Mean	143.55 a	145.00 a	139.17 a	148.22 a	2709.00 a	2853.00 a
75% NRD	Without inoculation	126.73 j	128.53 j	114.97e	122.89e	1935.25 j	2163.91f
	Pseudomonas sp.	131.88 g	132.76 f	135.24c	141.02cd	2483.67 f	2655.77d
	A. chrocooccum	129.66 h	130.00 h	134.72c	139.61cd	2660.85 e	2906.28 b
	Pseudomonas sp.+ *A. chrocooccum*	138.75 d	137.72 e	157.43ab	159.98b	2911.36 b	2817.67c
	Mean	131.75 b	132.25 b	135.59 b	140.87 b	2497.78 b	2635.81 b
50% NRD	Without inoculation	120.10 l	117.95 l	104.19f	109.54f	1575.93 k	1899.01 g
	Pseudomonas sp.	127.64 i	129.00 i	134.09c	139.46cd	2112.08 i	2555.64 e
	A. chrocooccum	124.44 k	126.06 k	134.48c	136.07d	2218.71h	2772.36 c
	Pseudomonas sp.+ *A. chrocooccum*	136.36 e	131.66 g	153.73b	158.72b	2320.97g	2608.15de
	Mean	127.13 c	126.16 c	131.62 c	135.94 b	2056.92 c	2458.79 c
Average (B)	NRD without inoculation with bacterial strains	127.49 d	128.31 d	114.80 c	124.11 d	1881.76 d	2090.62 d
	NRD + *Pseudomonas* sp.	134.70 b	136.32 b	135.11 b	141.85 b	2454.78 c	2674.47 c
	NRD + *A. chrocooccum*	132.64 c	133.35 c	135.22 b	138.68 c	2562.76 b	2961.69 a
	Ferti. + *Pseudomonas* sp.+ *A. chrocooccum*	141.75 a	139.91 a	156.72 a	162.07 a	2785.63 a	2870.14 b
LSD (0.05%)	(A)	0.01	0.81	2.01	5.37	16.75	41.79
	(B)	0.02	0.99	2.55	2.95	16.44	34.45
	(A*B)	0.03	0.15	4.42	5.12	28.47	59.68

Where, NRD means nitrogen recommended dose. Values within the same column with the same letter are not significantly different at 5% probability level by Duncan's Multiple Range Test (DMRT).

Application of *A. chrocooccum* and *Pseudomonas* sp. strains mitigated the adverse effects of nitrogen deficit levels. Plant height values were higher with *Pseudomonas* sp. application compared to *A. chrocooccum* application during both seasons. On the other hand, *A. chrocooccum* application achieved a higher plant leaf area than *Pseudomonas* sp. under the two different nitrogen deficit levels during the 2021 and 2022 seasons. The interaction between *A. chrocooccum* and *Pseudomonas* sp. yielded the best parameters of plant height, plant dry weight, and plant leaf area under the two different nitrogen deficit levels. Biofertilizers primarily indirectly promote plant growth, although some direct mechanisms also exist. They achieve this through various means, including nutrient availability and plant growth promotion [2-3].

Chlorophyll Pigments

The data presented in Fig. 1. showed that chlorophyll pigment content values (Chl. a, b, and total) were significantly increased under the nitrogen recommended dose compared to the other nitrogen fertilizer levels (75 and 50% NRD). Chlorophyll contents decreased with

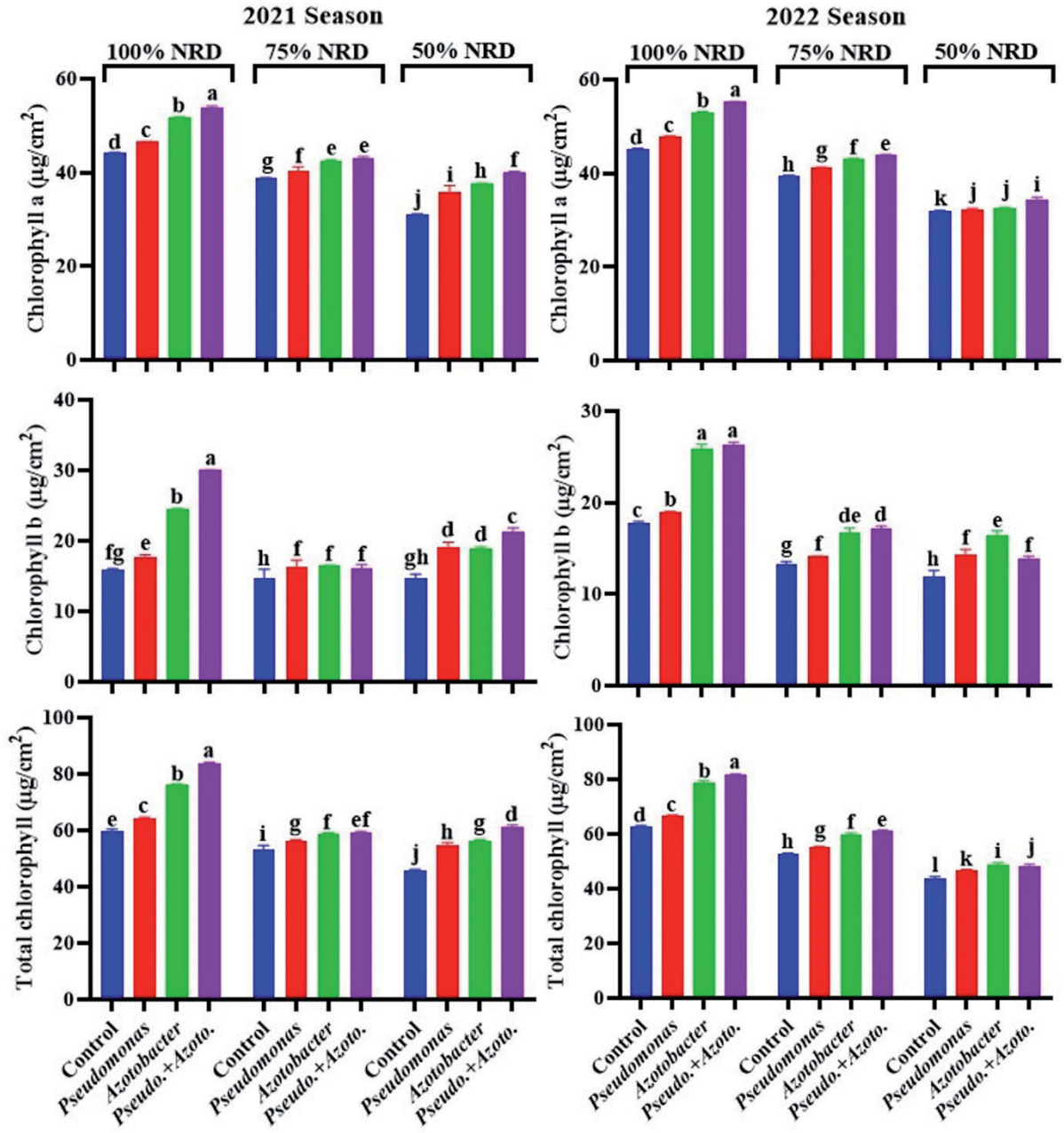

Fig. 1. Effect of application of *Azotobacter chrocooccum*, *Pseudomonas* sp. and their interaction on chlorophyll (chl. a, b and total) content of cotton (*G. barbadense* L. var. Giza CV 97) under three different nitrogen fertilizer levels (100, 75 and 50% NRD) during 2021 and 2022 seasons, where, NRD means nitrogen recommended dose, Pseudo.*:* means *Pseudomonas* sp. and Azoto: means *A. chrocooccum.*

increasing nitrogen fertilizer deficit levels compared to the recommended dose (100% NRD) during the 2021 and 2022 seasons. The application of *A. chrocooccum* and *Pseudomonas* sp. Strains, as well as their interactions, increased chlorophyll content under the recommended nitrogen fertilizer dose compared to the control (100% NRD). *A. chrocooccum* and *Pseudomonas* sp. strains and their interactions mitigated the harmful effects on chlorophyll contents under the two different nitrogen deficits (75 and 50% NRD). The highest chlorophyll content values were observed with the interaction between *A. chrocooccum* and *Pseudomonas* sp. under all nitrogen fertilizer levels. Nitrogen is essential for chlorophyll synthesis, giving leaves their green color and enabling photosynthesis. Nitrogen deficiency leads to chlorophyll breakdown and/or a reduction in chlorophyll biosynthesis, causing leaf chlorosis (yellowing) and reduced leaf area. This directly impacts

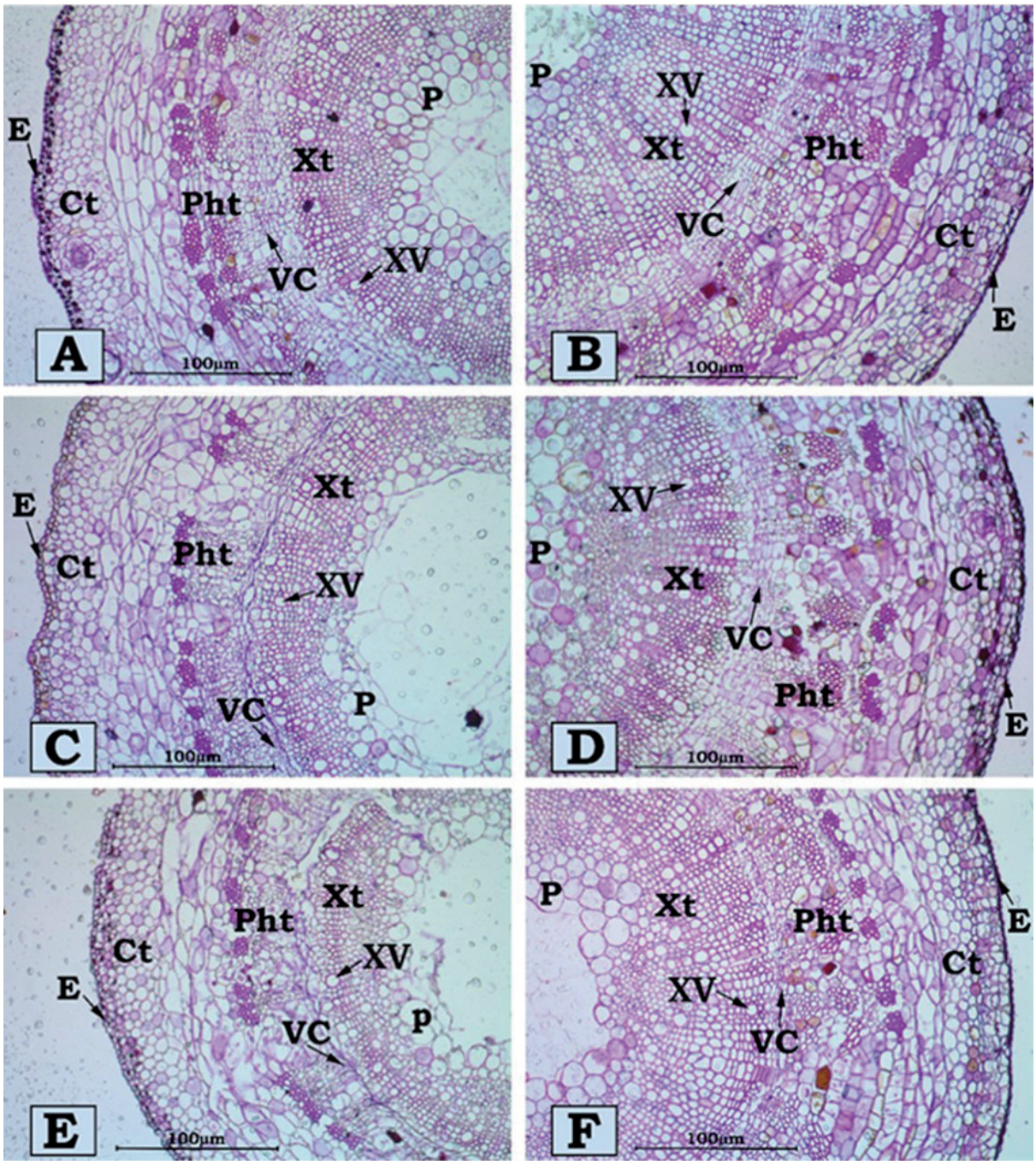

Fig. 2. Effect of application of *A. chrocooccum*, *Pseudomonas* sp. and their interaction on transverse sections of cotton under three different nitrogen fertilizer levels as affected by different levels of nitrogen fertilization, where (A: treated only with 100% NRD, C: 75% NRD and E: 50% NRD) and (B: means the interaction between *A. chrocooccum*, *Pseudomonas* sp. under different nitrogen levels (B:100% NRD, D: 75% NRD and F: 50% NRD)), epidermis tissue (E), cortex tissue (Ct), phloem tissue (Pht), vascular cambium tissue (VC), xylem tissue (Xt), pith tissue (P) and xylem vessel (XV).

light interception and photosynthetic capacity [18]. A reduction in chlorophyll content can significantly decrease the rate of photosynthesis in plants, leading to stunted growth and smaller leaves [19].

Anatomical Studies

The data demonstrated in Fig. 2 and Table 2 showed that nitrogen deficit levels (75 and 50% NRD) decreased the thickness of the xylem, phloem, vascular cambium, and cortex tissues, as well as xylem vessel diameters of cotton stems compared to the nitrogen recommended dose (100% NRD) treatment. The application of the interaction between *Azotobacter chrocooccum* and *Pseudomonas* sp. increased stem anatomical parameters under all nitrogen level treatments. The highest values of these anatomical parameters were noted by the application of the *A. chrocooccum* and *Pseudomonas* sp. interaction under the nitrogen recommended dose (100% NRD). On the other hand, stem cortex tissue thickness values increased with a reduction in nitrogen levels. Moreover, the interaction between *A. chrocooccum* and *Pseudomonas* sp. reduced stem cortex tissue thickness under all nitrogen fertilization treatments. Nitrogen deficit levels (75 and 50% NRD) resulted in narrow xylem vessels compared to 100% NRD. The use of the *A. chrocooccum* and *Pseudomonas* sp. interaction produced wider xylem vessels under all nitrogen levels than the untreated plant with plant growth promoting bacteria (*A. chrocooccum* or *Pseudomonas* sp.). Reductions in internal anatomical stem parameters (vascular conductive tissues (xylem and phloem), cortex tissues, and xylem vessel diameter) were reduced under nitrogen deficiency, and this may be due to insufficient

Table 2. Effect of application of *A. chrocooccum*, *Pseudomonas* sp. and their interaction on cotton stem (*G. barbadense* L. var. Giza CV 97) anatomical parameters under three different nitrogen fertilizer levels (100, 75 and 50% NRD) during 2021 and 2022 seasons.

Treatments	Thickness (µm)				Xylem vessels diameter
	Xylem tissue	Phloem tissue	Vascular cambium	Cortex	
100%NRD	46.45d	44.59b	25.41a	75.43ab	8.68ab
100%NRD + *Pseudomonas* sp. + *A. chrocooccum.*	90.22a	68.76a	26.37a	64.65c	10.19a
75%NRD	31.08e	36.12c	13.08b	82.67a	5.73c
75%NRD *Pseudomonas* sp. + *A. chrocooccum.*	74.74b	64.31a	22.44a	69.61bc	8.54ab
50%NRD	23.92e	29.82d	6.60c	80.09a	3.62d
50%NRD+ *Pseudomonas* sp. + *A. chrocooccum.*	57.43c	45.24b	12.56b	68.48bc	7.97b
LSD (0.05%)	8.56	4.93	3.29	6.62	1.43

Where, NRD means nitrogen recommended dose. Values within the same column with the same letter are not significantly different at 5% probability level by DMRT.

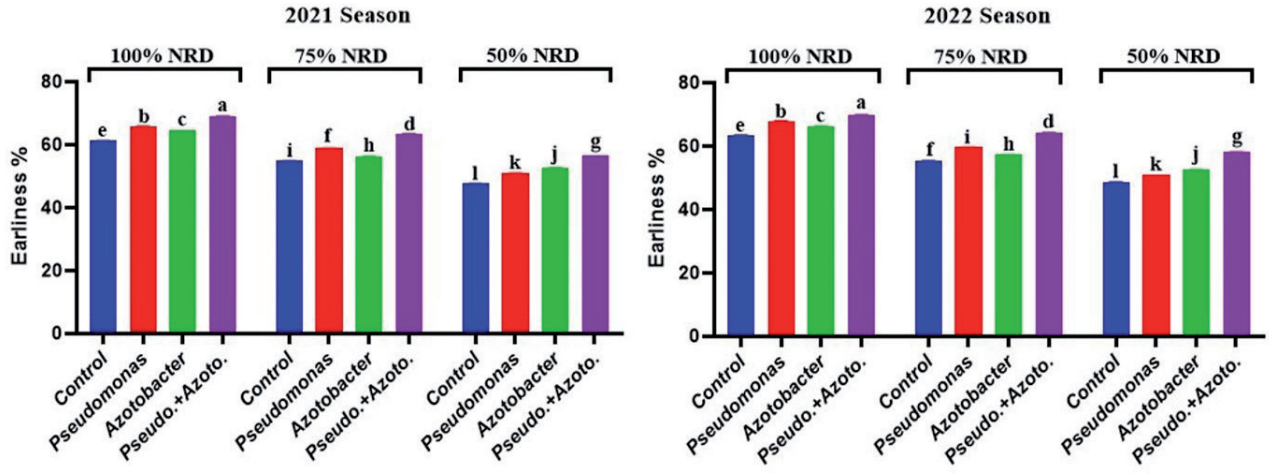

Fig. 3. Effect of application of *A. chrocooccum*, *Pseudomonas* sp. and their interaction on Earliness% of cotton (*G. barbadense* L. var. Giza CV 97) under three different nitrogen fertilizer levels (100, 75 and 50%) during 2021 and 2022 seasons. where, NRD means nitrogen recommended dose, Pseudo.: means *Pseudomonas* sp. and Azoto: means *A. chrocooccum.*

nitrogen nucleic acids and protein synthesis, resulting in decreasing cell division and elongation as well as cell differentiation. Anatomical differences in cotton stems are associated with external plant morphology. A reduction in cotton stem tissue thickness due to a reduction in thickness of the cortex and vascular conductive (phloem and xylem) tissue resulted from an inhibition of cambial cell activity and/or reduced DNA content, resulting in reduced cell division and expansion [20-21]. Vessels with a larger diameter offer less resistance to water flow, leading to faster and more efficient transport [22].

Yield and Yield Components

Earliness %

The data in Fig. 3 showed that earliness percentage significantly reduced under nitrogen fertilization deficiency (75 and 50% NRD) compared to optimal nitrogen fertilization (100% NRD). The use of *Azotobacter chrocooccum* and *Pseudomonas* sp. and their interaction mitigated the reduction in earliness percentage under all nitrogen fertilization levels. Application of the interaction between both plant growth promoting bacteria gave the best earliness% values compared to individual plant growth promoting bacteria

Table 3. Effect of application of *A. chrocooccum*, *Pseudomonas* sp. and their interaction on number of fruiting branches and open bolls per plant and lint% of cotton (*G. barbadense* L. var. Giza CV 97) under three different nitrogen fertilizer levels (100, 75, and 50%) during 2021 and 2022 seasons.

Fertilization level (A)	Treatments (B)	No. of fruiting branches / plant		No. of open bolls/plant		Lint %	
		2021	2022	2021	2022	2021	2022
100%NRD	Without inoculation	13.32 cde	14.04 bcd	16.05 b	16.00 a-d	40.04 abc	39.87 c
	Pseudomonas sp.	14.62 ab	15.11 ab	16.26 b	17.00 a	39.82 cde	39.61 e
	A. chrocooccum	14.00 bcd	14.28 bcd	16.15 b	16.11 abc	39.43 f	39.10 i
	Pseudomonas+ A. chrocooccum	15.37 a	16.00 a	17.00 a	17.05 a	39.51 ef	39.26 h
	Mean	14.32 a	14.85 a	16.36 a	16.54 a	39.46 c	39.70 b
75%NRD	Without inoculation	12.87 ef	13.06 de	14.11 c	14.00 e	40.08 abc	39.98 ab
	Pseudomonas sp.	14.04 bcd	14.73 abc	14.02 c	16.82 a	40.32 a	39.82 c
	A. chrocooccum	13.36 cde	14.00 bcd	14.31 c	14.60 cde	39.44 f	39.22 h
	Pseudomonas+ A. chrocooccum	14.36 bc	15.00 ab	13.90 cd	16.38 ab	39.66 def	39.43 g
	Mean	13.65 b	14.19 ab	14.08 b	15.45 a	39.61 b	39.87 ab
50%NRD	Without inoculation	12.18 f	12.00 e	13.00 e	12.98 e	40.23 ab	40.02 a
	Pseudomonas sp.	13.26 de	13.76 bcd	13.16 e	14.44 cde	40.01 bc	39.96 b
	A. chrocooccum	13.00 def	13.46 cde	14.21 c	14.33 de	39.82 cde	39.52 f
	Pseudomonas+ A. chrocooccum	14.00 bcd	13.98 bcd	13.32 de	14.65 b-e	39.95 bcd	39.68 d
	Mean	13.11 c	13.30 b	13.42 c	14.10 b	39.79 a	40.00 a
Average (B)	NRD without inoculation	12.79 c	13.03 c	14.38 b	14.42 b	39.75 a	40.11 a
	NRD + *Pseudomonas* sp.	13.97 b	14.53 ab	14.48 ab	16.08 a	39.79 b	40.05 a
	NRD + *A. chrocooccum*	13.45 b	13.91 b	14.89 a	15.01 b	39.28 d	39.56 b
	NRD + *Pseudomonas+ A. chrocooccum*	14.57 a	14.99 a	14.79 ab	16.02 a	39.45 c	39.71 b
LSD (0.05%)	(A)	0.33	1.25	0.57	1.18	0.05	0.18
	(B)	0.54	0.85	0.41	1.00	0.03	0.16
	(A*B)	0.94	1.47	0.70	1.73	0.28	0.05

Where, NRD means nitrogen recommended dose. Values within the same column with the same letter are not significantly different at 5% probability level by DMRT.

treatment under nitrogen fertilization deficiency (75 and 50% NRD). Nitrogen deficiency in plants indeed has a profound impact on various processes that directly affect their growth and development, including stunted growth, limiting photosynthesis, and hindering protein synthesis. This results in reduced dry matter accumulation and, ultimately, lower overall plant biomass [2, 3, 17].

Number of Fruiting Branches and Open Bolls/Plant and Lint Percentage

Nitrogen deficiency resulted in a reduction in the number of fruiting branches and open bolls per plant compared to NRD treatment (Table 3). Application of *A. chrocooccum*, *Pseudomonas* sp., and their interaction increased both traits compared to the control under nitrogen recommended dose (100% NRD). Relatively, the use of *A. chrocooccum* and *Pseudomonas* sp., and their interaction increased the number of fruiting branches/plant under two nitrogen fertilizer deficit levels (75 and 50% NRD). Moreover, the application of *A. chrocooccum* and *Pseudomonas* sp. interaction gave the best results in this respect under all nitrogen level treatments. Conversely, the nitrogen deficit insignificantly increased lint% compared to the nitrogen recommended dose. Furthermore, the treatment of *A. chrocooccum*, *Pseudomonas* sp., and their interaction reduced the lint percentage under all nitrogen level treatments. The highest value of lint percentage was

Table 4. Effect of application of *A. chrocooccum*, *Pseudomonas* sp. and their interaction on Seed index, boll weight and seed yield of cotton (*G. barbadense* L. var. Giza CV 97) under three different nitrogen fertilizer levels (100, 75 and 50%) during 2021 and 2022 seasons.

Fertilization level (A)	Treatments (B)	Seed index (gm)		Boll weight (gm)		Seed cotton yield (kg/f)	
		2021	2022	2021	2022	2021	2022
100%NRD	Without inoculation	9.69 e	8.99 h	2.46 c	2.44 cd	8.96 de	8.53 d
	Pseudomonas sp.	10.53 cd	10.75 c	2.56 b	2.60 ab	9.50 b	8.84 c
	A. chrocooccum	11.46 ab	11.46 b	2.59 ab	2.53 bc	9.33 c	9.29 b
	Pseudomonas+ *A. chrocooccum*	12.00 a	11.66 a	2.62 a	2.64 a	9.87 a	9.98 a
	Mean	10.92 a	10.71 a	2.55 a	2.56 a	9.41 a	9.16 a
75%NRD	Without inoculation	9.32 ef	8.90 i	2.12 h	2.06 f	6.64 i	6.56 j
	Pseudomonas sp.	10.49 d	9.61 g	2.34 e	2.46 cd	8.94 e	7.18 h
	A. chrocooccum	11.20 bc	9.91 e	2.40 d	2.43 d	7.94 f	7.62 f
	Pseudomonas+ *A. chrocooccum*	11.73 ab	9.93 d	2.43 c	2.56 ab	8.99 d	7.85 e
	Mean	10.68 ab	9.50 b	2.37 b	2.32 b	8.12 b	7.30 b
50%NRD	Without inoculation	8.94 f	8.81 j	1.78 i	1.87 g	5.78 j	5.66 k
	Pseudomonas sp.	10.40 d	9.57 g	2.18 g	2.39 d	7.18 h	6.49 j
	A. chrocooccum	10.67 cd	9.98 d	2.21 g	2.18 e	7.17 h	6.81 i
	Pseudomonas+ *A. chrocooccum*	11.46 ab	9.83 f	2.25 f	2.44 cd	7.42 g	7.29 g
	Mean	10.37 b	9.54 c	2.22 c	2.11 c	6.88 c	6.58 c
Average (B)	NRD without inoculation	9.31 d	8.90 c	2.12 d	2.12 d	7.12 d	6.95 d
	NRD + *Pseudomonas sp.*	10.47 c	9.97 b	2.48 b	2.36 c	8.54 b	7.50 c
	NRD + *A. chrocooccum*	11.11 b	10.45 a	2.38 c	2.40 b	8.14 c	7.90 b
	NRD + *Pseudomonas*+ *A. chrocooccum*	11.73 a	10.47 a	2.45 a	2.43 a	8.76 a	8.37 a
LSD (0.05%)	(A)	0.37	0.02	0.06	0.03	0.04	0.07
	(B)	0.40	0.03	0.05	0.02	0.02	0.05
	(A*B)	0.70	0.06	0.04	0.08	0.04	0.09

Where, NRD means nitrogen recommended dose. Values within the same column with the same letter are not significantly different at 5% probability level by DMRT.

achieved with the nitrogen recommended dose during the 2022 season.

Seed Index, Boll Weight, and Seed Yield

The data in Table 4. showed that seed index (gm), boll weight (gm), and seed yield (kg/fed.) was reduced under two nitrogen deficit levels (75 and 50% NRD) compared to the nitrogen recommended dose (100% NRD). Application of *A. chrocooccum* and *Pseudomonas* sp. and their interaction significantly increased seed index or seed yield during both successive seasons. The highest values of seed index, boll weight, or seed yield were achieved with the interaction between *A. chrocooccum* and *Pseudomonas* sp. at all nitrogen levels.

Cotton yield parameters, including open bolls per plant, lint percentage, seed index, boll weight, and seed yield were associated with plant growth improvement and enhancement of chlorophyll content [23]. The optimal nitrogen fertilizer (NRD) directly influences the growth and yield of cotton. When cotton encounters a nitrogen deficit, it experiences stress. This stress triggers the plant's defense mechanisms, leading to increased production of ethylene, a gaseous hormone, which increases boll shedding [24]. The productivity of cotton greatly relies on the importance of nitrogen as a crucial nutrient [25]. Published reports overwhelmingly support the claim that applying the appropriate N doses can significantly benefit cotton production in several ways, including stimulating vegetative growth, leading to increased leaf area, thicker stems, and more bolls per plant [26].

On the way to clean agriculture, biofertilizers are fascinating and sustainable alternatives to traditional

Table 5. Effect of application of *A. chrocooccum*, *Pseudomonas* sp. and their interaction on Fiber length, micronaire and fiber strength of cotton (*G. barbadense* L. var. Giza CV 97) under three different nitrogen fertilizer levels (100, 75 and 50%) during 2021 and 2022 seasons.

Fertilization level (A)	Treatments(B)	Fiber length (mm)		Micronaire (Mic)		Fiber strength (g/tex)	
		2021	2022	2021	2022	2021	2022
100%NRD	NRD	33.79 abc	33.68 cd	4.47 abc	4.49 abc	10.81 abc	10.79 abc
	NRD+ *Pseudomonas* sp.	33.80 ab	33.77 ab	4.48 abc	4.53 a	10.83 ab	10.80 ab
	Fertilization +*Azoto.*	33.82 a	33.78 a	4.50 ab	4.50 ab	10.84 ab	10.83 a
	NRD + *Pseudomonas* sp. + *A. chrocooccum*	33.84 a	33.80 a	4.52 a	4.53 a	10.86 a	10.82 a
	Mean	33.81 a	33.76 a	4.49 a	4.51 a	10.83 a	10.81 a
75%NRD	NRD	33.70 cde	33.66 cde	4.40 def	4.45 b-e	10.75 cde	10.68 d
	NRD+ *Pseudomonas* sp.	33.75 a-d	33.68 cd	4.43 cde	4.42 c-f	10.78 bcd	10.68 abc
	NRD+ *A. chrocooccum*	33.77 abc	33.78 ab	4.44 bcd	4.44 b-e	10.78 bcd	10.75 bc
	Fertilization + *Pseudomonas* sp. + *A. chrocooccum*	33.79 abc	33.79 a	4.45 bcd	4.46 a-d	10.80 abc	10.74 c
	Mean	33.75 b	33.72 b	4.43 b	4.44 b	10.77 b	10.73 b
50%NRD	NRD	33.65 e	33.60 e	4.37 ef	4.36 f	10.66 f	10.60 e
	NRD + *Pseudomonas* sp.	33.66 e	33.62 de	4.36 f	4.38 ef	10.69 ef	10.67 d
	NRD+ *A. chrocooccum*	33.68 de	33.70 bc	4.40 def	4.39 def	10.70 ef	10.67 d
	NRD+ *Pseudomonas* sp.+ *A. chrocooccum*	33.72 b-e	33.74 abc	4.45 bcd	4.39 def	10.73 de	10.68 d
	Mean	33.76 c	33.66 c	4.39 c	4.38 c	10.69 c	10.65 c
Average (B)	NRD	33.71 b	33.64 b	4.41 b	4.43 a	10.74 b	10.69 b
	NRD+ *Pseudomonas* sp.	33.73 ab	33.69 b	4.42 b	4.44 a	10.76 ab	10.73 a
	NRD+ *A. chrocooccum*	33.75 ab	33.75 a	4.44 ab	4.44 a	10.77 ab	10.75 a
	NRD + *Pseudomonas* sp. + *A. chrocooccum*	33.78 a	33.77 a	4.47 a	4.46 a	10.79 a	10.76 a
LSD (0.05%)	(A)	0.04	0.01	0.03	0.02	0.03	0.03
	(B)	0.05	0.04	0.03	0.03	0.03	0.03
	(A*B)	0.08	0.07	0.06	0.07	0.06	0.05

Where, NRD means nitrogen recommended dose. Values within the same column with the same letter are not significantly different at 5% probability level by DMRT.

chemical fertilizers. They offer a host of benefits for both plant growth and the environment [27]. The use of biofertilizers has gained attention due to the scarcity and high cost of chemical fertilizers, as well as their negative impact on the environment. So, the judicious combination of biofertilizers and mineral nitrogen fertilizers can optimize plant growth and yield. Application of *Azotobacter chrocooccum*, *Pseudomonas* sp., and their interaction mitigated the adverse effects of cotton vegetative and reproductive growth, primarily due to the activity of these PGPR in decomposing organic substances. Also, it has a beneficial role in lowering the pH level in soils by releasing organic acids like propionic, acetic, fumaric, or succinic acids, which lead to the dissolution of nutrients bound to organic matter and render them available for plants [28].

This activity leads to improved soil properties and enhances the release of nutrients in forms that are easily taken up by plant roots by fixing atmospheric nitrogen and solubilizing phosphates. Under the same trend, biofertilizers produce growth-promoting substances like phytohormones and other biochemicals that enhance nutrient mobilization in crops, leading to immediate utilization by the plants and ultimately improving cotton growth and productivity [29-30]. It has been reported that supplementation of plant growth regulators in cotton crops improves the antioxidant defense systems, water use efficiency, nutrient availability, and uptake [31].

Paungfoo-Lonhienne et al. [32] suggested that the idea of combining mineral fertilizers with plant growth-promoting rhizobacteria (PGPR), *i.e., Azotobacter chrocooccum*, and *Pseudomonas* sp., in a hybrid fertilization approach can be effective in achieving comparable yields to those obtained with mineral fertilizers alone. Thus, the application of *A. chrocooccum*, *Pseudomonas* sp., and their interaction alleviated the harmful effects of nitrogen deficits on the growth or yield parameters. The positive impact of inoculation had a marked effect on the growth of the plant, which was reflected in an increase in cottonseed yield. This increment might be attributed to the effect of nitrogen and some growth regulators such as IAA, GA3, and cytokinens which bacteria strains produce [33-34]. Release of phosphates and micronutrients, non-symbiotic nitrogen fixation, and enhancement of disease-resistance mechanisms [35-36].

Utilization of phosphate-dissolving bacteria (*Pseudomonas* sp.) as a biofertilizer led to a decrease in soil pH, resulting in improved solubility of certain nutrients like phosphorus (P), iron (Fe), zinc (Zn), manganese (Mn), or copper (Cu) [37-38]. This, in turn, facilitated greater nutrient absorption by plants. *Azotobacter chrocooccum* and *Pseudomonas* sp. interaction was more effective than plant growth-promoting rhizobacteria (PGPR) individual treatments under all nitrogen fertilization treatments. Application of two plant growth-promoting bacteria (PGPB) and their interaction may induce cambium differentiation to yield xylem and phloem tissues, improve absorption and conduct water to the growing organs, and, in addition, improve translocation of photo-assimilates thus increasing cotton plant growth and productivity [39-40]. Furthermore, plant growth and yield analyses of cottonseed and fibers analyses showed that both strains possess multiple plant growth promoters (PGP) features, which indirectly might explain the observed results.

Cotton Fiber Quality

Data in Table 5. revealed that cotton fiber quality measurements, including fiber length and fiber fineness (micronaire), as well as fiber strength, were significantly affected under the 50% NRD. Application of *A. chrocooccum* and *Pseudomonas* sp. interaction induced higher fiber quality under all different nitrogen levels during both seasons. *A. chrocooccum* and its interaction with *Pseudomonas* sp. significantly increased all fiber traits under the nitrogen recommended dosage during both seasons. Nitrogen is an essential nutrient for plant growth and development, and it plays a crucial role in determining cotton fiber quality. It is a major component of proteins, which are essential for building cell walls and other structural components of the fiber. Nitrogen also plays a role in chlorophyll production, which is necessary for photosynthesis and energy production in the plant [15-19].

Conclusions

Overall, the application of biofertilizers *Azotobacter chrocooccum*, *Pseudomonas* sp., and their interaction in combination with varying levels of mineral nitrogen fertilizer represents a promising approach for sustainable cotton production. The application of these bacterial strains and their interaction mitigated the harm of nitrogen deficiency stress. The results indicated that the application of both bacterial strains' interactions produced the highest growth and yield parameters. The present study indicated that co-inoculation of *A. chrocooccum* and *Pseudomonas* sp. interaction allows minimization of nitrogen fertilization doses up to 75% on cotton growth and productivity.

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Conflict of Interest

Authors state no conflict of interest with respect to research, authorship, and/or publication of this article.

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