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Effect of Combined Application of Organic and Chemical Nitrogen Fertilizers on Physiological Indices and Grain Yield of Maize (*Zea mays* L)

Banafsheh Hatampoor [1] Seyed Keyvan Marashi[2*]

1. MSc. Student, Department of Agronomy, Ahvaz Branch, Islamic Azad University, Ahvaz, Iran.
2. Associate Professor, Department of Agronomy, Ahvaz Branch, Islamic Azad University, Ahvaz, Iran.

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ABSTRACT

BACKGROUND: The integrated management of plant nutrition is always considered as a solution in sustainable development. In this process, the need for chemical fertilizers is reduced and helps to make the environment healthier.

OBJECTIVES: Current study was done to assess effect of different level of fertilizer and biofertlizer on growth curves and crop production.

METHODS: This experiment was conducted via randomized complete block design (RCBD) with three replications at Shahid Salemi field in south west of Iran-Ahvaz. The studied treatments included urea application only, Nitroxin application only, Nitroxin + 25% nitrogen from urea source, Nitroxin + 50% nitrogen from urea source, Nitroxin + 75% nitrogen from urea source, Azotobacter application only, Azotobacter + 25% nitrogen from urea source, Azotobacter + 50% nitrogen from urea source, Azotobacter + 75% nitrogen from urea source.

RESULT: The results indicated that the combined application of organic and chemical nitrogen fertilizers had a significant effect on growth indices. The highest values of dry matter accumulation, leaf area index, relative growth rate, crop growth rate, and net assimilation rate were obtained from the Nitroxin + 75% nitrogen from urea source, Azotobacter + 75% nitrogen from urea source, and Nitrogen supply entirely from urea source treatments. The lowest values of growth indices were observed under 100% Nitroxin and 100% Azotobacter nitrogen source treatments. The combined fertilizer treatment significantly influenced grain yield. The highest grain yield was obtained from Nitroxin + 75% nitrogen from urea source and Azotobacter + 75% nitrogen from urea source treatments with averages of 3670 and 3500 $Kg.ha^{-1}$, respectively. The lowest grain yield with average of 2610 $Kg.ha^{-1}$ was obtained from the treatment using only nitrogen from the Azotobacter source.

CONCLUSION: Overall, the results of the experiment showed that the simultaneous use of organic and chemical nitrogen fertilizers can have a positive effect on physiological indices and grain yield while reducing the use of chemical fertilizers. This can significantly contribute to environmental sustainability and serves as an important strategy towards sustainable agriculture.

KEYWORDS: *Azotobacter, Biofertilizer, Nitroxin, Nutrition, Sustainable agriculture*.

***Corresponding Author:** Seyed Keyvan Marashi ✉ Marashi_47@yahoo.com

1. BACKGROUND

Maize, recognized for its favorable attributes and adaptability to diverse climatic conditions, has gained the global prominence, securing the third position in cultivated acreage after wheat and rice (Noormohamadi *et al*., 2001). In conventional agriculture, nitrogen stands as a vital and impactful nutrient influencing the growth and development of agricultural plants. Particularly in nutrient-deprived soils, nitrogen often plays a limiting role, necessitating supplementation with organic materials. Insufficient amount of N available to plants can hinder the growth and development. Nitrogen can also improve root growth, increase the volume, area, diameter, total and main root length, dry mass and subsequently increase nutrient uptake and enhance nutrient balance and dry mass production. Nitrogen-based fertilizers, frequently mobile in soil, can lead to undesirable consequences such as groundwater and soil contamination (Sit and Krapp, 1999; Diaz *et al*., 2006). Biological fertilizers, composed of various free-living microorganisms, possess the capability to convert essential nutrients from an inaccessible to an accessible form through biological processes. This transformation contributes to enhanced root system development and improved seed germination. The application of biofertilizers can be a probable approach to improve soil microbial status that stimulates the natural soil microbiota therefore influencing nutrient accessibility and decomposition of organic matter (Chaudhary *et al*., 2021). Among these biological fertilizers, nitrogen-fixing bacteria, such as Azotobacter, not only fix nitrogen from the air but also produce antifungal compounds combatting various plant diseases. This dual functionality enhances seed germination and plant structure, ultimately promoting root growth. Additionally, these bacteria balance nutrient uptake, secrete amino acids, and produce antibiotics, fortifying plant roots and aerial components against soil-borne pathogens and, consequently increasing of crop yield (Blak, 2011). The response of cereals to Azotobacter inoculation varies based on bacterial strains, soil conditions, and regional climates. Positive outcomes include yield increases ranging from 7 to 12 percent, with exceptional cases reaching up to 39 percent (Khavari, 2010). The biological fertilizer Nitroxin encompasses some of the most effective nitrogen-fixing bacteria, primarily from the Azotobacter and Azospirillum genera. The bacteria present in Nitroxin biological fertilizer not only fix atmospheric nitrogen and balance essential nutrient uptake but also stimulate the growth and development of plant roots and aerial parts by synthesizing and secreting growth-promoting substances, such as various regulatory hormones (Asadi kopal and Eesazadeh Lzrjan, 2009). Using Nitroxin fertilizer not only helps avoid the consumption of chemical nitrogen fertilizers but also, due to its diverse effects, can lead to increased crop production (Tilake *et al*., 2005). Shaharoona *et al*. (2012) demonstrated under field conditions that the combined application of chemical nitrogen fertilizer significantly amplified the impact of biological fertilizers, resulting in a notable 58% increase in dry matter production compared to the control. N fertilization significantly increased grain yield by enhancing the grain weight, ears ha^{-1}, and grains per ear (Srivastava *et al*., 2018; Wei *et al*., 2019). Lack *et al*. (2008) reported that providing essential nutrients, especially nitrogen, during the critical grain formation period, through an increase in plant growth rate, play a crucial role in determining the number of grains. This situation establishes a robust correlation between the number of grains in the ear and the leaf area index. Recognizing the imperative need for plant nutrition management to enhance and sustain agricultural systems, and acknowledging the potential environmental hazards associated with the direct use of chemical fertilizers.

OBJECTIVES

Current study was done to assess effect of different level of fertilizer and biofertlizer on growth curves and crop production.

3. MATERIALS AND METHODS

3.1. Field and Treatments Information

This experiment was conducted in the crop year (2015-2016) at Shahid Salemi Farm in Ahvaz (Fig. 1), utilizing a randomized complete block design with three replications. The farm's geographical coordinates were 31 degrees and 20 minutes north latitude, 48 degrees and 40 minutes east longitude, and an elevation of 23 meters above sea level. Before the experiment, soil samples were collected at a depth of 0-30 centimeters for physical and chemical analysis (Table 1).

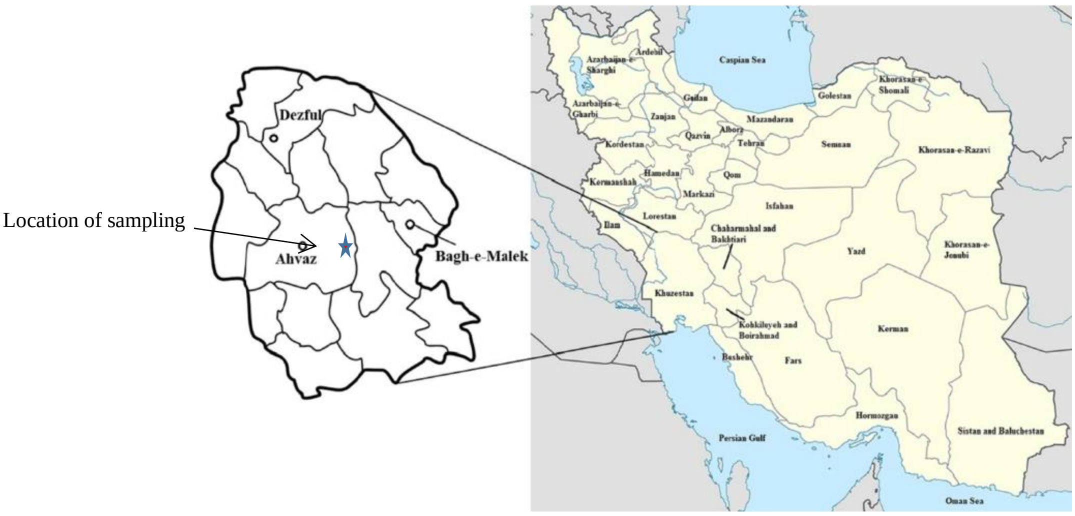

Fig. 1. Location of sampling

This experiment was conducted in the form of a randomized complete block design with three replications. The nitrogen treatments included:

1. Urea application only (T1)
2. Nitroxin application only (T2)
3. Nitroxin + 25% nitrogen from the urea source (T3)
4. Nitroxin + 50% nitrogen from the urea source (T4)

5. Nitroxin + 75% nitrogen from the urea source (T5)
6. Azotobacter application only (T6)
7. Azotobacter + 25% nitrogen from the urea source (T7)
8. Azotobacter + 50% nitrogen from the urea source (T8)
9. Azotobacter + 75% nitrogen from the urea source (T9)

3.2. Farm Management

The specified nitrogen fertilizer from the urea source was considered at a rate of 200 kilograms of pure nitrogen per hectare. The experiment had three replications, and each replication included nine experimental plots. Each experimental plot consisted of six rows of crops, each 5 meters in length, with 75-centimeter spacing between rows and 15-centimeter spacing between seeds in the rows. A 1.5-meter distance was maintained between each experimental replication, and no line was drawn between each plot. Land preparation involved plowing with a moldboard plow and disc harrowing, followed by leveling with a harrow. The base fertilizer used in the field included 200 $Kg.ha^{-1}$ of triple superphosphate and 150 $Kg.ha^{-1}$ of potassium sulfate. Biological fertilizer was applied through seed inoculation, while chemical nitrogen fertilizer was applied in two stages (50% at planting and 50% at the six-leaf stage). For seed inoculation with Azotobacter, seeds were first moistened and then spread on a plastic surface. According to the manufacturer's recommendation, 100 grams of Azotobacter per hectare was applied, mixed with the amount of seeds needed for the hectare. For seed inoculation with Nitroxin, the liquid fertilizer was sprayed completely over the seeds according to the manufacturer's recommendation, with a rate of 1 liter per 35 kilograms of seeds. The sowing operation took place in late December by hand. The first irrigation was applied after seed sowing, and subsequent irrigations were performed at the required plant intervals. Manual weeding was conducted for weed control. For grain yield measurement, after removing 0.5 meters from both ends of the central lines in each plot, all ears present in the 3-meter-long central rows were manually harvested.

Table 1. Physiochemical characteristics of field soil

Soil depth (cm)	Clay (%)	Silt (%)	Sand (%)	O.C (%)	N (%)	P (ppm)	K (ppm)	pH	EC ($ds.m^{-1}$)
0-30	41.5	37.5	21	0.6	0.085	9.1	143	7.7	4.62

3.3. Measured Traits

After hand threshing of plants, grain yield was determined. To calculate the relative growth rate (RGR), the following equation was used, where W_1 and W_2 represent the dry weight of each sample at times t_1 (ZG60) and t_2 (ZG71), respectively (Koochaki and Sarmadnia, 1990).

$$RGR= (LnW_2-LnW_1)/ (t_2-t_1)$$

Crop growth rates (CGR) and net assimilation rate (NAR) were calculated in grams per square meter per day.

$$CGR= \frac{W2-W1}{t2-t1} \times \frac{1}{GR}$$

$NAR = \frac{CGR}{LAI}$

(W_2-W_1): Dry matter weight produced in two consecutive harvests.

(t_2-t_1): Time interval between two consecutive harvests.

(GA): Ground area occupied by the plant at the time of sampling.

(CGR: Crop growth rate.

(LAI): Leaf area index.

3.4. Statistical Analysis

Analysis of variance and mean comparisons were done via SAS (Ver.8) software and Duncan multiple range test at 5% probability level.

4. RESULT AND DISCUSSION

4.1. *Leaf Area Index (LAI)*

In various nitrogen treatments, the Leaf Area Index (LAI) gradually decreased as the plant growth stage progressed, such that at the male flowering stage, the LAI in all experimental treatments was higher than at the grain filling stage. The difference in LAI among the treatments was significant (Table 2). At the male flowering stage, the highest LAI, with an average of 4.66, was related to the treatment with Nitroxin+ 75% nitrogen from urea source (T5). The lowest LAI at this stage was observed in the treatment with 100% nitrogen from Azotobacter source (T6), with an average of 3.65. A significant difference in LAI was observed at the grain filling stage among different treatments, similar to the male flowering stage, with the highest LAI in the Nitroxin+ 75% nitrogen from urea source treatment (T5), showing no significant difference with the treatment of Azotobacter + 75% nitrogen from urea source (T9) (Table 3). The reduction in LAI at the grain filling stage compared to the male flowering stage may be due to leaf senescence, which progresses as the plant advances through its growth stages, leading to aging in photosynthetic tissues and leaves. Researchers have shown that the corn LAI increases up to 70 days after planting and then decreases due to leaf loss and aging. Moreover, the LAI of corn decreases under unfavorable conditions in the final growth stages due to adverse environmental emergence (Cakir, 2004). The reduction in LAI with the advancement of growth stage in this experiment was consistent with the findings of Cakir (2004). Therefore, it can be concluded that by appropriately integrating chemical and biological fertilizers, chemical fertilizer consumption can be reduced in fields, and growth traits such as LAI can be enhanced. Researchers like Burris (2000) and Wani (1988) have reported in their experiments that reducing the amount of fertilizer usage while increasing traits such as LAI, tiller number, and biological yield are among the optimal effects of using biological fertilizers in combination with chemical fertilizers. These effects might be due to increased nitrogen fertilizer absorption, as well as the production of tryptophan (a precursor to the hormone auxin) by the microorganisms in biological fertilizers.

Table 2. The results of analysis of variance for leaf area index (LAI)and total dry matter (TDW), relative growth rate (RGR), Crop growth rate (CGR), net assimilation rate (NAR) and grain yield

S.O.V	df	At the first of anthesis stage (ZG60)				
		LAI	TDM	RGR	CGR	NAR
Replication	2	2.77 ns	27.7 ns	0.002 ns	34.94 ns	4.1 ns
Treatments	8	25.7**	341.5 **	0.5 **	411.7 **	23.2 *
Error	16	3.72	42.2	0.003	25.8	5.5
C.V (%)	-	8.19	9.43	7.52	8.23	11.44

ns, * and **: no significant, significant at 5% and 1% of probability level, respectively.

Continue table 2.

S.O.V	df	At the first Grain filling stage (ZG71)					Grain yield
		LAI	TDM	RGR	CGR	NAR	
Replication	2	2.41 ns	27.2 ns	0.002 ns	36.17 ns	3.8 ns	537711ns
Treatments	8	21.4 **	388.4 **	0.32 **	295.36**	62.2 **	4998329*
Error	16	3.8	32.2	0.002	25.1	5.2	372448
CV (%)	-	10.34	12.07	7.52	6.72	12.31	13.41

ns, * and **: no significant, significant at 5% and 1% of probability level, respectively.

4.2. *Total Dry Weight (TDW)*

The effect of different nitrogen treatments at two stages, namely the appearance of male tassel and grain filling, on the accumulation of total dry matter was significant (Table 2). The highest amounts of dry matter at growth stages belonged to the urea-only treatment (T1), Nitroxin + 75% nitrogen from the urea source (T5), and Azotobacter + 75% nitrogen from the urea source (T9). The lowest amount was observed in the Azotobacter-only treatment (T6) and Nitroxin-only treatment (T2) (Table 3). Zahir *et al*., (2004) stated that the application of biofertilizers in corn leads to an increase in dry matter yield in plants. This is because these fertilizers, in addition to their ability to fix nitrogen, contribute to the production of various growth-promoting substances such as indole acetic acid, gibberellins, and vitamins. These substances enhance nutrient absorption and, consequently, improve plant growth. Mirza *et al*. (2000) reported that the application of non-nitrogen-fixing biofertilizers induces the production of auxins, which stimulate root development, increase nutrient and nitrogen uptake, and enhance plant dry matter production. Ashkavand *et al*. (2013) reported a 32% increase, and Zahir *et al*. (2004) reported an 18% increase in corn dry matter production due to seed inoculation with biofertilizers combined with nitrogen application, which is consistent with the results of this study.

Table 3. Mean comparison different level of fertilizer combination on studied traits

Treatments	At the first of anthesis stage (ZG60)				
	LAI	TDM ($g.m^{-2}$)	RGR ($g.g^{-1}.day^{-1}$)	CGR ($g.m^{-2}.day^{-1}$)	NAR ($g.m^{-2}.day^{-1}$)
T1	4.35ab*	735b	0.038a	32b	7ab
T2	3.7e	543e	0.028d	24.7e	6.67b
T3	4.0cd	645cd	0.032c	27.5d	6.87ab
T4	4.33b	682c	0.036ab	29.2c	6.74ab
T5	4.66a	770a	0.04a	35a	7.4a
T6	3.65e	522e	0.027d	24e	6.57b
T7	4.1cd	627d	0.031c	28d	6.82ab
T8	4.27bc	665cd	0.034ab	29.5c	6.9ab
T9	4.54a	740b	0.037a	33.5ab	7.33a

*Mean which have at least once common letter are not significant different at the 5% level using (DMRT).
T1: Urea application only, T2: Nitroxin application only, T3: Nitroxin + 25% nitrogen from the urea source, T4: Nitroxin + 50% nitrogen from the urea source, T5: Nitroxin + 75% nitrogen from the urea source, T6: Azotobacter application only, T7: Azotobacter + 25% nitrogen from the urea source, T8: Azotobacter + 50% nitrogen from the urea source, T9: Azotobacter + 75% nitrogen from the urea source.

Continue **table 3.**

Treatments	At the first of anthesis stage (ZG60)					Grain yield ($kg.ha^{-1}$)
	LAI	TDM ($g.m^{-2}$)	RGR ($g.g^{-1}.day^{-1}$)	CGR ($g.m^{-2}.day^{-1}$)	NAR ($g.m^{-2}.day^{-1}$)	
T1	3.6b	1194a	0.025ab	24.5b	6b	34.50b
T2	2.5d	942c	0.018bc	15.5e	5.5c	2830d
T3	3.2c	1009bc	0.02b	21d	6b	3050cd
T4	3.5b	1067b	0.023ab	23c	6.2b	3411b
T5	3.8a	1203a	0.03a	28a	6.56a	3670a
T6	2.56d	933c	0.016c	16e	5.45c	2600e
T7	3.25c	996bc	0.018bc	20.5d	5.94b	3111ce
T8	3.58b	1045b	0.023ab	23.3c	6b	3323bc
T9	3.7b	1185a	0.024ab	27a	6.75a	3550ab

*Mean which have at least once common letter are not significant different at the 5% level using (DMRT).
T1: Urea application only, T2: Nitroxin application only, T3: Nitroxin + 25% nitrogen from the urea source, T4: Nitroxin + 50% nitrogen from the urea source, T5: Nitroxin + 75% nitrogen from the urea source, T6: Azotobacter application only, T7: Azotobacter + 25% nitrogen from the urea source, T8: Azotobacter + 50% nitrogen from the urea source, T9: Azotobacter + 75% nitrogen from the urea source.

4.3. *Relative Growth Rate (RGR)*

The relative growth rate at different growth stages was significantly affected by combined nitrogen treatments at a 1% probability level (Table 2). The impact of various combined nitrogen treatments on the changes in relative growth rate before the appearance of male inflorescence and grain filling stages is illustrated in table 3. In both growth stages, the highest RGR values were attributed to the treatment of nitrogen supply solely from urea source (T5), nitrogen-fixing bacteria + 75% nitrogen from urea source (T9), and urea application alone (T1), while the lowest values were associated with the treatment of obtaining nitrogen solely from Azotobacter source (T6). The RGR value is a function of the overall plant photosynthetic surface, and therefore, it decreases with the age of the plant and an increase in respiration at the end of the growth stage. Under suitable conditions and in the absence of nutrient deficiencies, due to better access to water and nutrients and increased photosynthesis, the formation of growth and reproductive organs accelerates, resulting in an increase in the relative growth rate of the plant under favorable conditions (Lawrence *et al*., 2008). Seyed Sharifi *et al*. (2012) stated that Nitroxin and Azotobacter-containing bacteria act as plant growth promoters by expanding the root surface and depth and efficiently fixing nitrogen and producing growth-regulating hormones. This is effectively achieved by increasing nitrogen absorption in combined treatments in maize. It seems that the increased nitrogen absorption through the Nitroxin and Azotobacter biological fertilizers under 75% nitrogen utilization from the urea source has been higher compared to other research treatments. In this regard, Hamzei and Sarmadi (2010) stated that biological fertilizers, in combination with nitrogen, provide the conditions for increased crop production in maize by improving nitrogen absorption efficiency, which is consistent with the findings of this study.

4.4. *Crop Growth Rate (CGR)*

The crop growth rate was significantly influenced by the application of different nitrogen treatments at both the male inflorescence emergence and grain filling stages, with statistical significance at the 1% level (Table 2). The highest CGR at both stages was observed in treatments with Nitroxin + 75% urea-derived nitrogen (T5) and Azotobacter + 75% urea-derived nitrogen (T9). Conversely, the application of 100% nitrogen sourced from Nitroxin (T2) and 100% nitrogen sourced from Azotobacter (T6) resulted in a reduced crop growth rate compared to the other combined treatments, achieving the lowest rates at both developmental stages (Table 3). This finding aligns with previous research indicating that the CGR typically correlates with the extent of solar radiation absorption, and it tends to decline towards the end of the growth period due to increased shading by leaves, which reduces light interception and photosynthetic activity, subsequently decreasing CGR (Campillo *et al*., 2010). In this study, the variability in plant growth rates across different nitrogen fertilization treatments can largely be attributed to differences in the Leaf Area Index (LAI), as changes in plant growth rate are contingent upon alterations in both LAI and the net assimilation rate.

4.5. *Net Assimilation Rate (NAR)*

The impact of combined nitrogen treatments on the net assimilation rate was statistically significant at a 5% level during the male inflorescence appearance stage and reached significance at a 1% level during the grain-filling stage (Table 2). In treatments combining various nitrogen sources, the net assimilation rate exhibited a meaningful increase as nitrogen levels from the urea source rose. Notably, the treatments Nitroxin + 75% nitrogen from the urea source (T5) and Azotobacter + 75% nitrogen from the urea source (T9) demonstrated the highest net assimilation rates during both growth stages. The comparison between these two treatments revealed no significant difference in terms of net assimilation rate. Conversely, treatments involving biological fertilizers and those not combined with urea fertilizer experienced a reduction in the net assimilation rate, with the lowest values attributed to the treatments with 100% nitrogen from the Nitroxin source (T2) and 100% nitrogen from the Azotobacter source (T6) (Table 3). Given that the reproductive growth stage aligns with elevated photosynthetic rates and overall plant growth, the absence of nutrient deficiencies during this stage leads to improved photosynthesis and, consequently, an increase in the net assimilation rate. As noted by Nemat and Seyed Sharifi (2014), the net photosynthesis rate does not remain constant over time and exhibits a declining trend with the plant's age during growth and development. This decline is further accelerated in unfavorable conditions, coupled with water and nutrient deficiencies. Results from Lawrence *et al*. (2008) also support a decrease in the net assimilation rate under conditions of nutrient limitation in maize plants. Evidently, the net assimilation rate is influenced by various factors, making its measurement complex and often challenging to discern. For example, Sturz and Christe (2003) found that an increase in the leaf area index led to a reduction in the net assimilation rate, while Sharma (2012) asserted that changes in the net assimilation rate have a positive correlation with the leaf area index. Overall, in this experiment, the net assimilation rate emerges as a parameter exhibiting more stability compared to plant growth rate and leaf area index.

4.6. *Grain Yield*

The analysis of variance results indicates a significant effect of nitrogen treatments on grain yield (Table 2). In this experiment, the treatment Nitroxin + 75% nitrogen from the urea source (T5) exhibited the highest grain yield, followed by the Azotobacter + 75% nitrogen from the urea source (T9) treatment, which showed the second-highest grain yield. The lowest grain yield was observed in the treatment using Azotobacter alone (T6) (Table 3). Plant inoculation with growth-promoting bacteria not only led to a 30% to 35% reduction in nitrogen fertilizer consumption but also increased plant performance through enhancements in leaf area index, total dry matter, and net assimilation rate. Seyed Sharifi *et al*. (2012) reported that the combination of biological and chemical fertilizers contributes to increased maize productivity, aligning with the findings of this study. Naseri Rad *et al*. (2011) stated that the biological fertilizer Nitroxin, containing nitrogen-fixing bacteria, enhances biological nitrogen fixation ability, root surface area, optimal water and nutrient absorption, and the production of certain vitamins, resulting in improved quantitative and qualitative plant growth and increased yield. Singh *et al*. (2004) highlighted the nitrogen-fixing bacteria's ability to produce antifungal compounds against various plant diseases, promoting seed germination and plant architecture, consequently enhancing basal plant growth. In another study, Oliverira *et al*. (2006) suggested that the lack of a sole positive impact of biological fertilizer application on maize yield could be attributed to the timing and the presence of bacteria in the soil, emphasizing the need for an optimal timeframe for the manifestation of positive effects on plant growth and yield, aligning with the results of this research.

5. CONCLUSION

The results of this experiment indicate that among the studied treatments, the most favorable conditions for physiological indices such as leaf area index, total dry matter, crop growth rate, and net assimilation rate ultimately leading to increased grain yield are associated with Nitroxin + 75% nitrogen from the urea source, Azotobacter + 75% nitrogen from the urea source, and the sole supply of 100% nitrogen through urea. As there were no statistically significant differences in most cases regarding physiological indices and grain yield among these treatments, biofertilizers can be recommended as a viable strategy to reduce nitrogen fertilizer consumption. This substantial contribution aids in environmental sustainability and represents a great step to sustainable agriculture.

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FOOTNOTES

AUTHORS' CONTRIBUTION: All authors are equally involved.

CONFLICT OF INTEREST: Authors declared no conflict of interest.

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