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ROLE OF BIO-AZOTOBACTER AND NITROGEN FERTILIZERS ON GROWTH AND YIELD TRAITS OF SORGHUM (*SORGHUM BICOLOR* L.)

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SUMMARY

A field experiment on sorghum (*Sorghum bicolor* L.) with diverse levels of fertilization, carried out in the spring of 2022 in a randomized complete block design (RCBD) with factorial arrangement, had three replications and two factors at the field of Ibn-Al-Bitar Vocational Preparatory School, District Al-Hussainiya, Kerbala, Iraq. The first factor comprised three sorghum cultivars (V1 = Al-Khair, V2 = Rabeh, and V3 = Bohuth-70), while the second was combinations of nitrogen and bio-fertilizers (F0 = recommended dose of N 320 kg ha^{-1}, F1 = 3/4 of the recommended amount of N with Azotobacter, F2 = 1/2 of the recommended dosage of N + Aotobacter, F3 = 1/4 of the recommended dose of N + Azotobacter, and F4 = Azotobacter + No nitrogen fertilizer). The results showed that the sorghum cultivar Bohuth-70 was significantly superior for enhanced plant height (204.63 cm), number of leaves $plant^{-1}$ (9.807), 1000-grain weight (35.27 g), and the grain yield (6.192 $Mg.ha^{-1}$). However, cultivar Al-Khair produced the highest mean leaf area (4,929 cm^2) and grains per head (4,428 $grains.head^{-1}$). Cultivar Rabeh excelled in performance, with the highest mean value for stem diameter (24.67 mm) and head length (31.29 cm). The F0 treatment recommended dose of N 320 kg ha^{-1} was considerably superior in the number of leaves (9.867 leaves $plant^{-1}$), stem diameter (24.8 mm), and grain yield (6.145 $Mg.ha^{-1}$). The F1 treatment of 3/4 of the recommended dose of N with Aotobacter excelled in plant height (166.57 cm), leaf area (4,936 cm^2), and number of grains (3,916 grain $head^{-1}$). However, the F2 treatment 1/2 of the recommended dose of N + Aotobacter was notably superior in head length (30.18 cm) and 1000-grain weight (29.93 grains). As for the interaction, the sorghum cultivar Bohuth-70 with F0 treatment excelled in the number of leaves $plant^{-1}$ (10.33 leaves) and the grain yield (7.157 $Mg.ha^{-1}$), while the cultivar Al-Khair with F1 treatment excelled in stem diameter (26.70 mm) and leaf area (5,164 cm^2).

Keywords: Sorghum (*Sorghum bicolor* L.), cultivars, nitrogen fertilizer, biofertilizer, growth and yield, Azotobacter

Key findings: Nitrogen and biofertilizer (Azotobacter) significantly affected the growth and yield traits. The studied cultivars also showed varied responses to various fertilization levels. It might be possible to reduce chemical fertilizers and replace them with biofertilizers.

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INTRODUCTION

Sorghum (*Sorghum bicolor* L.) belongs to the family Poaceae and is one of the most important and premeditated cereal crops in many countries (Ibrahim *et al*., 2019). The sorghum ranked fifth after wheat, rice, corn, and barley in terms of cultivated area and production (Saleh *et al.*, 2017). For sorghum, the United States is the largest producer, with an annual production of 18.03%, followed by Nigeria (11.14%), Mexico (8.88%), India (7.81%), Sudan (7.68%), and Ethiopia (7.49%) (FAO, 2020). Excessive use of nitrogen fertilizers leads to environmental pollution and damage to the health of living organisms, as well as, raising the costs of production, which made researchers seek an alternative to use the safe, healthy, environmentally friendly, and less costly methods (Osip *et al.*, 2000; Reddy *et al*., 2014). Biological inoculations (beneficial microbes) serve for fertilization purposes and obtaining a crop free of any chemical pollutants, enhancing the ability of these organisms for plant growth and development, and preventing them from pathogenic microorganisms' propagation, as well as, contributing to different stress resistance (Mahanty *et al.,* 2016; Tomer *et al*., 2016, Al-Myali, 2021).

Sorghum grains contain carbohydrates ranging from 70% to 80%, protein (11%–13%), fats (2%–5%), fiber (1%–3%), and ash (1%–2%). Sorghum is of high nutritional value, with its protein distinguished by being free of gluten. Therefore, it is a suitable food for people with diabetes and digestive disorders (Prasad and Staggenborg, 2010; Rifka *et al*., 2020). Despite the importance of sorghum and its various advantages, its cultivation is limited in Iraq and grown only for fodder purposes (Al-Kubaisi, 2001). The available data on maize production in Iraq lacks clarity concerning the extent of cultivated areas and corresponding yield.

One of the most necessary elements serving the crop is nitrogen fertilizer involved in most of the physiological processes of crop plants. Nitrogen is the first chief determinant of crop production and the prime component of amino acids and carbohydrates, as well as, playing a vital role in the synthesis of carbon and enzymes, metabolism, and nucleic acids, helping form vegetative and flowering buds (Yasari and Wardhan, 2006). An increasing amount of nitrogen beyond the recommended level the plant requires leads to adverse effects, such as, plant lodging with an increase in vegetative growth compared with the reproductive system (Al-Karkhi, 2013). Biofertilizers' use increases the productivity of various crops by about 25% and reduces chemical fertilizers by 25%–50% of nitrogen and 25% of phosphorus (Ghany *et al*., 2013). Therefore, the current study aims to use the reduced dose of nitrogen fertilizer to replace N with inoculation of seeds with nitrogen-fixing bacteria and know its impact on the performance of sorghum cultivars.

MATERIALS AND METHODS

A field experiment on sorghum (*Sorghum bicolor* L.) with diverse levels of fertilization ran in the spring of 2022 in RCBD with factorial arrangements, three replications, and two factors at the field of Ibn-Al-Bitar Vocational Preparatory School, District Al-Hussainiya, Kerbala, Iraq (Latitude: 32° 36' 57.71" N Longitude: 44° 01' 29.57" E). The first factor was three sorghum cultivars (V1 = Al-Khair, V2 = Rabeh, and V3 = Bohuth-70), while the second factor comprised combinations of nitrogen and biofertilizers (F0 = recommended dose of N 320 kg ha^{-1}, F1 = 3/4 of the recommended amount of N with Aotobacter, F2 = 1/2 of the recommended dosage of N + Aotobacter, F3 = 1/4 of the recommended dose of N + Azotobacter, and F4 = Azotobacter + no nitrogen fertilizer).

Azotobacter preparation was at Al-Ameen Center for Research and Biotechnology. The sorghum seeds, contaminated with azotobacter inoculum, had a density of 1.63×10^{7} CFU.Ml^{-1} adds an adhesive substance. Adding biofertilizer followed, with the ingredients mixed well to achieve the maximum possible homogeneity, and the seeds were left to dry, not exposed to direct sunlight for a full hour, and then sown and irrigated directly (Gomare *et al.*, 2013). Sorghum seeds, sown in March 2022, had a distance of 75 cm between rows and 25 cm between plants. The area of each experimental unit was 3 m × 3 m. Phosphate (P) fertilizer addition was 87.2 kg ha^{-1}, and potassium (K) fertilizer was 66.0 kg ha^{-1}. Applying nitrogen was in the form of urea fertilizer according to the treatments in two batches (after germination of 10 days, with 7–9 leaves). The control of corn stem borer (*Sesmia gilica* L.) used diazinon 10% at 6 kg ha^{-1} after 20 and 40 days of germination at the elongation stage in two feeding batches in the growing apices of the stem (Al-Barzanji, 2006). The research followed the recommended production technology for crop maintenance.

Data recorded and statistical analysis

The data recording was on the following traits: Plant height (cm) = calculated when flowering completes for five plants randomly taken from the midlines of each experimental unit by a regular ruler and graded from the soil surface to the highest apex (Elsahookie, 1990); The number of leaves = measured from the same plants used to calculate plant height, randomly taken from the two middle lines for each experimental unit, and then averaged (Elsahookie, 1990); The leaf area (cm^{2}) = measured from the same randomly selected plants, the length of the fourth leaf and its maximum width according to the following mathematical equation (Al-Sahoki and Saddam, 2014):

$$\textbf{\textit{Leaf area}} = \text{length of the fourth leaf} \times \text{maximum width of the leaf} \times 6.18$$

After the completion of flowering, the stem diameter (mm) calculation in five randomly selected plants used a Vernier micrometer (Elsahookie, 1990). The head length (cm) measurement for five randomly selected plants began from the middle lines of each experimental unit at the harvesting stage, using a regular scaled ruler, and then averaged (Al-Alahani, 2017). For the number of grains per head, five heads as chosen representative of the experimental unit had the number of grains per head calculated after forcibly separating each top and then averaged (House, 1985). For 1000-grain weight (g), manually and randomly counting the 1000 grains from plants had the number of grains per head counted and weighed with an electric scale (House, 1985). The grain yield (Mg ha^{-1}) estimation occurred during harvest time. In each experimental unit, the harvest estimate for yield of five plants comprised extraction and multiplication by the plant density, with the data converted to Mg ha^{-1}. The experiment proceeded as per the nested design, using Genestate V.12 program with an RCBD and three replications. The least significant difference ($LSD_{0.05}$) test helped to compare and separate the treatment means (Oehlent, 2010).

RESULTS AND DISCUSSION

Plant height

Significant differences resulted in the sorghum cultivars, combinations of nitrogen and biofertilizers, and their interaction with genotypes for plant height (Table 1). The F1 treatment revealed the highest mean for plant height (166.57 cm). Nonsignificant differences showed for the treatments F0, F2, and F3 for plant height. The F4 treatment emerged with the lowest mean for said trait (159.77 cm). The reason may be due to the role of nitrogen in encouraging vegetative growth and an increase in the process of division and stem elongation, thus, leading to an increase in

Table 1. Effect of bio- and nitrogen fertilizers on plant height in sorghum.

Cultivars Treatments	V1	V2	V3	Means (cm)
F0	143.65	147.33	205.50	165.50
F1	143.47	146.00	210.23	166.57
F2	141.57	140.60	207.13	163.10
F3	139.90	147.30	204.00	163.73
F4	140.50	142.40	196.30	159.77
Means (cm)	141.83	144.74	204.63	
$LSD_{0.05}$	Treatment	Cultivar	Interaction	
	4.075	3.157	7.058	

Table 2. Effect of bio- and nitrogen fertilizers on leaves per plant in sorghum.

Cultivars Treatments	V	V2	V3	Means (leaves $plant^{-1}$)
F0	9.060	10.20	10.33	9.867
F1	8.667	9.800	10.00	9.489
F2	8.650	9.600	10.20	9.483
F3	8.800	9.867	9.200	9.289
F4	8.800	9.133	9.300	9.078
Means (leaves $plant^{-1}$)	8.797	9.720	9.807	
$LSD_{0.05}$	Treatment	Cultivar	Interaction	
	0.3333	0.2582	0.5773	

plant height (Al-Nuaimi, 2011). The sorghum cultivar Bohuth-70 achieved the highest mean for plant height (204.63 cm), whereas cultivar Al-Khair plants showed the minimum plant height (141.83 cm). It may refer to the genetic difference between genotypes, and results are consistent with Attia (2015) and Sayad *et al.* (2014). On interactions, the V3 interacting with F1 had a superiority in plant height (210.23 cm); however, it did not differ significantly from the other three interactions, i.e., V3 × F0, V3 × F2, and V3 × F3. Although, the interaction of V1 × F3 provided the lowest mean value for plant height (139.90 cm).

Leaves per plant

For leaves per plant, the sorghum cultivars, the combination of nitrogen and biofertilizers, and the interaction between them expressed notable differences (Table 2). Treatment F0 excelled by giving the highest number of leaves per plant (9.867), whereas treatment F4 showed the lowest number of leaves (9.078). It is a fact that nitrogen increases vegetative growth by increasing the division and expansion of cells and, thus, encouraging an increase in the number of branches, positively reflecting an escalation in the number of leaves. These results agree with Ates and Tenikecier's (2019) and Kaplan's *et al.* (2019) findings. The sorghum cultivar Bohuth-70 outperformed the rest by producing the highest number of leaves (9.807); however, it was at par with cultivar Rabeh. Cultivar Al-Khair provided the lowest mean for said trait (8.797). It may be due to the genetic variation between sorghum cultivars in the number of leaves characteristic, with such result in line with the study outcomes by Salaheldeen *et al.* (2017). On the interaction between cultivars and combinations of nitrogen and biofertilizers, the interactivity V3 × F0 excelled in the number of leaves (10.33). Yet, it does not differ significantly with other interactions, i.e., V2 × F0, V3 × F1, V3 × F2, V2 × F1, and V2 × F3. The interchange of V1 × F2 exhibited the lowest value for leaves per plant (8.650) in sorghum plants.

Leaf area

Remarkable differences appeared among the sorghum cultivars, nitrogen and biofertilizer combinations, and their interactions for leaf area (Table 3). The F1 treatment recorded the highest mean value for leaf area (4,936 cm^2), which was at par with the F0 treatment, but the F4 treatment displayed the lowest leaf area (4487 cm^2). The reason for this may be due to the role of nitrogen in increasing the division of cells and their rapid growth and, thus, the expansion and elongation of cells increased the number of leaves (Table 2), which led to an increase in the leaf area. Said outcome aligns with Ziki's *et al.* (2019) and Abera's (2020) findings. The cultivar Al-Khair gave the highest value for leaf area (4,929 cm^2); however, it did not differ significantly from the cultivar Rabeh. The cultivar Bohuth 70 showed the minimum leaf area (4,428 cm^2). It may be because of the variation in the genetic composition of the cultivars and the extent to which each of the cultivars included in the study responds or adapts to the environmental conditions, with this outcome consistent with the results of Al-Jubouri and Safaa (2013). In the interaction between cultivars and fertilizer combinations, the exchange of V1 and F1 expressed the highest leaf area (5,164 cm^2); however, it did not differ substantially from other interactions, i.e., V2 × F0, V1 × F0, V1 × F2, V1 × F3, V2 × F1, and V3 × F1. The interaction of V3 and F4 provided the slightest leaf area (4,194 cm^2).

Table 3. Effect of bio- and nitrogen fertilizers on leaf area in sorghum.

Cultivars Treatments	V1	V2	V3	Means (cm^2)
F0	5111	4975	4564	4883
F1	5164	4894	4752	4936
F2	4953	4665	4276	4631
F3	4696	4562	4356	4538
F4	4720	4548	4194	4487
Means (cm^2)	4929	4729	4428	
$LSD_{0.05}$	Treatment 269.1	Cultivar 208.5	Interaction 466.1	

Table 4. Effect of bio- and nitrogen fertilizers on stem diameter in sorghum.

Cultivars Treatments	V1	V2	V3	Means (mm)
F0	25.40	25.73	23.33	24.8
F1	26.70	23.93	20.80	23.81
F2	24.53	24.67	21.13	23.44
F3	23.07	23.07	19.73	21.96
F4	23.10	22.93	20.60	22.21
Means (mm)	24.56	24.67	21.12	
$LSD_{0.05}$	Treatment 1.827	Cultivar 14.415	Interaction 3.164	

Stem diameter

Considerable variations occurred from sorghum cultivars, nitrogen and biological fertilization, and their interactions for stem diameter (Table 4). The biofertilizer treatment F0 excelled by showing the maximum stem diameter (24.8 mm), but did not differ significantly from the other formulas, F1 and F2. The biofertilizer treatment F3 recorded the lowest mean value for the said trait (21.96 mm). It may be due to the increase in the leaf area (Table 3) and the chlorophyll content, which led to photosynthesis buildup, increasing dry matter, and expanding the stem diameter. The study results are in line with the findings of Al- Al-Doughi (2001). Sorghum cultivars Rabeh and Al-Khair achieved equal highest value for the stem diameter (24.67 mm). Cultivar Bohuth-70 recorded the minimum mean value for stem diameter (21.12 mm). The reason may refer to the discrepancy between cultivars in the stem diameter with the shading of the lower leaves, which leads to elongation of the internodes due to the evidence of leaf area, consistent with the findings of Ahmed and Abbood (2016). The interaction between the cultivars and fertilizer combinations resulted in V1 × F1 with a massive stem diameter (26.70 mm); however, the said interaction had no significant difference from other exchanges, i.e., treatments V1 × F0, V1 × F2, V2 × F0, V2 × F1, and V2 × F2. The interchange V3 × F3 indicated the least stem diameter (19.73 mm).

Head length

For head length, the sorghum cultivars and combinations of nitrogen and biofertilizers and their interactions showed significant differences (Table 5). The treatment F2 gave the highest mean value for head length (30.18 cm); however, no substantial difference from other fertilization treatments, i.e., F0, F1, and F3, showed. The treatment F4 recorded the lowest mean value for head length (27.27 cm). It is attributable to the fact that supplying the crop with sufficient quantities of nitrogen in the different growth stages as a result of the presence of organisms responsible for fixing atmospheric nitrogen reflected positively on the crop's growth due to the plant's necessity for it; thus, increasing the inflorescence length, confirming the work of Escasinas *et al.* (1981). Sorghum cultivar Rabeh provided the highest mean value for head length (31.29 cm), whereas the cultivar Bohuth-70 recorded with the lowest (27.67 cm). It may be due to the difference in genetic factors between cultivars, as Hassan *et al.* (2005) and Al-Khazali *et al*. (2013) found, who confirmed the existence of genetic differences from one cultivar to another in the trait of head length. Regarding interactions, head length superiority came from the interaction V2 × F2 (33.33 cm); however, no considerable differences appeared with other interactions, i.e., V2 × F0, V2 × F3, and V1 × F2. The interchange V3 × F0 confirmed with the smallest value for head length (25.40 cm).

Table 5. Effect of bio- and nitrogen fertilizers on head length in sorghum.

Cultivars Treatments	V1	V2	V3	Means (cm)
F0	30.40	32.73	25.40	29.51
F1	29.85	30.13	29.10	29.69
F2	31.01	33.33	26.20	30.18
F3	30.13	32.58	27.53	30.08
F4	28.13	27.67	26.00	27.27
Means (cm)	29.91	31.29	27.67	
$LSD_{0.05}$	Treatment	Cultivar	Interaction	
	1.448	1.121	2.508	

Grains per head

The results revealed the nitrogen and biofertilizer combinations showed notable differences for grains per head (Table 6). The F1 treatment stood out with the highest amount of grains per head (3,916). Yet, such treatment did not differ significantly from the treatment F0, while the lowest value for grains per head (3,582) emerged in the treatment F4. It may be due to an increase in the plant's leaf area (Table 3), increasing the efficiency of the carbon metabolism process, and amplifying the amount of nutrients produced during the flowering period (Abdullah *et al*., 2011; Al-Kubaisi, 2001). Cultivar Al-Khair performed best with the highest mean value (4,428 grains head^{-1}), whereas the lowest mean value (3,276 grains head^{-1}) was with sorghum cultivar Bohuth-70. The superiority of the Al-Khair

Table 6. Effect of bio- and nitrogen fertilizers on the number of grains per head in sorghum.

Cultivars Treatments	V1	V2	V3	Means (grains head^{-1})
F0	4630	4149	3743	3841
F1	4442	4010	3295	3916
F2	4401	3075	3270	3582
F3	4420	3411	3225	3685
F4	4250	3720	2848	3606
Means (grains head^{-1})	4428	3473	3276	
$LSD_{0.05}$	Treatment	Cultivar	Interaction	
	229.7	177.9	397.9	

Table 7. Effect of bio- and nitrogen fertilizers on 1000-grain weight in sorghum.

Cultivars Treatments	V1	V2	V3	Means (g)
F0	26.97	25.44	35.90	29.43
F1	25.90	21.93	36.17	28.00
F2	26.47	26.94	36.38	29.93
F3	25.06	24.61	34.27	27.98
F4	25.65	21.09	33.63	26.79
Means (g)	26.01	24.00	35.27	
$LSD_{0.05}$	Treatment	Cultivar	Interaction	
	1.629	1.262	2.821	

cultivar in leaf area (Table 3) explains this, and as a result, increasing the vegetative total (the size of the source), which leads to the processing of the downstream with the products of photosynthesis, as confirmed by Al-Moaini (2017). In interactions between cultivars and combinations of nitrogen and biofertilizers, V1 × F0 gave the highest mean value (4,630 grain head^{-1}), which also did not differ significantly from other interactions, i.e., V1 × F1, V1 × F2, V1 × F3, and V1 × V4. The lowest number of grains per head occurred in the interchange of V3 × F4 (2,848 grains head^{-1}).

1000-grain weight

Sorghum cultivars, combinations of nitrogen and biofertilizer, and their interactions remarkably showed differences for 1000-grain weight (Table 7). The treatment F2 excelled by giving the highest 1000-grain weight (29.93 g), which did not differ significantly from the F0 treatment. The treatment F4 came out with the lowest mean value for the trait (26.79 g). An explanation for this is the ability of nitrogen to increase the outputs of carbon metabolism, reflecting an increase in the fullness of the grain, thus, amassing its weight and keeping the leaves green. It is consistent with the results that Shalaji (2000) obtained. Sorghum cultivar Bohuth-70 achieved the highest mean value for the 1000-grain weight (35.27 g), and cultivar Rabeh produced the lowest 1000-grain weight (24.00 g). It may be due to the adoption of the principle of compensation in yield and its components, as cultivar Bohuth 70 recorded the lowest number of grains per head (Table 6), which led to reducing competition between grains for manufactured materials at the source (leaf) causing an increase in grain weight. The findings agree with Abdel-Hamid (2016) and Abbood *et al*. (2017), who confirmed the existence of differences between the sorghum cultivar and the weight of 1000 grains. On interactions, V3 × F2 showed the highest mean 1000-grain weight (36.38 g), which had no substantial difference from other interactions of the same sorghum cultivar with the rest of the fertilizer combinations. However, the interchange of V2 × F4 resulted in the lowest mean value for the 1000-grain weight (21.09 g).

Table 8. Effect of bio- and nitrogen fertilizers on grain yield in sorghum.

Cultivars Treatments	V1	V2	V3	Means (Mg ha^{-1})
F0	6.677	4.600	7.157	6.145
F1	6.135	4.691	6.396	5.741
F2	6.217	4.420	6.405	5.680
F3	5.889	4.479	5.905	5.424
F4	5.815	4.374	5.100	5.096
Means (Mg ha^{-1})	6.147	4.513	6.192	
$LSD_{0.05}$	Treatment	Cultivar	Interaction	
	0.5119	0.3965	0.8866	

Grain yield

Table 8 shows significant differences between the sorghum cultivars, the combinations of nitrogen and biofertilizer, and their interactions for grain yield. The treatment F0 excelled by producing the highest mean grain yield (6.145 $Mg.ha^{-1}$); however, it has no considerable distinction from other applications, F1 and F2. The treatment F4 provided the lowest mean grain yield (5.096 $Mg.ha^{-1}$), which may be because the increase in nitrogen levels also increases the length of the head, boosting the number of grains in the head. It is also due to the stimulation of nitrogen for the growth and development of flowering facilities. In addition to its positive role in increasing the weight of 1000 grains, combining all these increase the total yield of grains, consistent with the study results of Ramshe *et al.* (1985). Cultivar Bohuth-70 stood out by giving the highest grain yield (6.192 $Mg.ha^{-1}$), which has a nonsignificant difference from cultivar Al-Khair. Cultivar Rabeh recorded the lowest mean value for such a trait (4.513 $Mg.ha^{-1}$). The reason for the superiority of the Bohuth 70 cultivar in the grain yield trait may be due to its excelling in the plant height and number of leaves features, which led to an increase in the weight of 1000 grains, thus reflecting an upsurge in the grain yield and its components. This cultivar could have balanced the duration of vegetative growth and reproductive progress, in addition to the efficiency of this cultivar in transferring photosynthetic products from the source to the downstream. Yassin and Nazem (2017) and Jassim (2018) observed similar results, indicating remarkable differences between sorghum cultivars in grain yield. The interaction V3 × F0 revealed superior by showing the highest grain yield (7.157 $Mg.ha^{-1}$) but also did not differ meaningfully from three other interactions, i.e., V3 × F1, V3 × F2, and V1 × F0. However, the interrelation V2 × F4 gave a minimum grain yield (4.374 $Mg.ha^{-1}$).

CONCLUSIONS

Applying 50% of the recommended amount of nitrogen fertilizer in conjunction with biofertilizer (Azotobacter) resulted in the superior performance of plant characteristics, including plant height, stem diameter, head length, 1000-grain weight, and grain yield. However, adding 100% nitrogen fertilizer revealed a nonsignificant difference in yield results. Hence, adopting a combination of chemical and biological fertilizers as a recommendation for enhancing grain yield production requires immediate action.

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