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MAIZE SEED INOCULATION WITH BIOAGENT AND ITS EFFECTS ON THE GROWTH AND YIELD TRAITS

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SUMMARY

The presented study sought to use the plant growth-promoting microorganism (PGPM) as a biofertilizer in maize (*Zea mays* L.) seeds and determine its effects on the growth and productivity of maize, with two levels of mineral fertilizer (25% and 50% of chemical fertilizer) under Iraqi conditions. Laboratory studies confirmed no antagonism between *Azotobacter chroococcum* and other microorganisms used in this study. Field experiments carried out during crop season 2021 were in two different regions, Mosul (36°20′6″N, 43°7′8″E) and Kirkuk (35°28′5.02″N, 44°23′31.99″E) in the north of Iraq. The result showed biofertilizer superiority when combined with 25% and 50% doses of the recommended mineral fertilizer for maize growth and yield traits in the experiments in both locations. In Kirkuk city, the biofertilizer combined with 25% chemical fertilizer recorded superiority without significant difference from the biofertilizer combined with 50% mineral fertilizer. However, in Mosul city, the biofertilizer combined with 50% chemical fertilizer expressed a more superior and significant difference than other treatments for growth and yield traits in maize. The difference between the two regions might be due to chemical fertilizer residues in the soil.

Keywords: Maize (*Zea mays* L.), biofertilizer, plant growth-promoting microorganisms (PGPM), *Azotobacter chroococcum*, bio-agents, mineral fertilizer, growth and yield traits

Key finding: Biofertilizer treatment with 25% and 50% doses of the recommended mineral fertilizer lead to significantly enhance the growth and yield parameters of maize. However, the use of mineral fertilizer depends upon the available fertilizer residues in the soil.

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INTRODUCTION

Maize (*Zea mays* L.) production has doubled worldwide for the past three decades. Maize is one of the most valuable cereal crops globally, following wheat and rice. Generally, maize is high yielding, easy to process, readily digested, and cheaper than other cereals. It is also a versatile crop growing across agroecological zones. Maize is a multipurpose crop, with every part having an economic value, such as, grains, leaves, stalks, and cobs that can convert into various foods and feeds (Beyranvand *et al*., 2013). In Iraq, the maize yield is approximately 4.6 t h^{-1} (CSO, 2022).

The significant increase in yield might be due to the breeding of high-yielding cultivars, mineral fertilizers, and crop management. Maize has very high nutrients' requirement, and using chemical fertilizers has caused severe environmental issues, such as, air pollution, water eutrophication, and soil acidification (Mateo-Sagasta *et al.,* 2017; Zeffa *et al.,* 2019; Ali and Alshugeairy, 2023). The negative environmental impact of mineral fertilizers demands an alternative source of nutrients, i.e., beneficial soil microorganisms, also known as plant growth-promoting microorganisms (PGPM) (Majeed *et al.,* 2020).

The PGPM is a term describing soil bacteria and fungi that colonize the rhizosphere of plants, growing around and on the plant tissues and stimulating plant growth by several mechanisms (Perez-Montano *et al.,* 2014), either by facilitating nutrients uptake and increasing nutrient availability in the rhizosphere (Majeed, 2020). Using PGPMs is one of the potential ways to alleviate the harmful effects of the continued use of chemicals in agriculture, like mineral fertilizers, herbicides, and pesticides (Santos *et al.,* 2020). PGPMs also indirectly affect crop plants by inhibiting pathogens and suppressing diseases (Yasmin *et al.,* 2016).

PGPMs can provide crop plants with nutrients by fixing nitrogen, solubilizing phosphate-producing phytohormones and siderophore compounds, i.e., *Rhizobium sp., Azotobacter sp., Bacillus sp., Pseudomonas sp.,* and *Cyanobacteria*. Plant growth-promoting microorganisms as a biofertilizer increase the crop growth and yield when applied complementary and as an alternative to chemical fertilizers. The interaction between the plant and PGPMs is critical in enhancing the growth and health of crop plants (Majeed, 2020).

The PGPMs can also suppress the diseases of crop plants by several mechanisms via producing antibiotics, hydrolytic enzymes like pancreatic lipase and lysosomal lipase, and competing with other organisms inducing systemic resistance (Mustafa *et al*., 2019). Therefore, the presented study addresses the influence of adding PGPMs on the growth and productivity of the maize crop grown in Iraq.

MATERIAL AND METHODS

Microorganisms used in this study came from the Plant Protection Directorate, Ministry of Agriculture, Abu-Greab, Baghdad, Iraq (Table 1).

Laboratory experiment

The interaction test proceeded to determine the type of interaction between the *Azotobacter chroococcum* and other microorganisms used in this study and detect if any antagonism between the *Azotobacter* and other microorganisms occur*.* The said experiment ran in the Biofertilizer Laboratory,

Table 1. Microorganisms used in this study.

Microorganisms	Source
Azotobacter chroococcum (Azt.)	Biofertilizer Laboratory, Plant Protection Directorate
Azospirillum brasilense (*Azs*.)	-do-
Pseudomonas fluorescens (*P.f.*)	-do-
Bacillus megaterium (*B.m*.)	-do-
Trichoderma harzianum (*Th*.)	-do-

Plant Protection Directorate, Abu-Greab, Baghdad, Iraq, by following Hewedy *et al.* (2010). The succeeding interactions underwent assessment:

A. chroococcum against *Bacillus megaterium*
A. chroococcum against *P. fluorescens*
A. chroococcum against *Azospirillum brasilense*
A. chroococcum against *Trichoderma harzianum*

The test used two types of medium: nutrient agar for bacteria, suitable for all kinds of bacteria (Majeed *et al.,* 2020; Sparks *et al*., 2020). Another medium was potato dextrose agar (PDA) appropriate for fungi. The test had three replications for each treatment and control of 54 plants (12 plants for treatments on each medium and 15 plants for control, also on each medium). The inoculation plants received incubation for three days at 28 °C±2 °C, with the data tacked by comparing the treatments with the control, according to Kucuk and Kivance (2003).

Field experiments

The maize field experiments transpired in the crop season 2021 at two different sites, i.e., Mosul, Al Namrood City, and Kirkuk, Al-Hawija City, Iraq. Experiments in both locations used the maize cultivar Drakhma Mayes, laid out in a randomized complete block design. The drying of coated seeds by air ensued in the shade for half an hour, then sown in the experimental subplots (5 m^2 × 5 m^2) with three replications. All the subplots for various treatments had 10 rows, with a 0.5 m row-to-row spacing.

Biofertilizer preparation had the bacteria grown and activated in nutrient growth medium (1000 ml) and incubated in a cool-shacked incubator at 28 °C±2 °C for two days to attain a cell density of 10^8 cfu/ml. Bacterial concentration determination used a viable count method (Majeed, 2020). Loading the bacteria on a specific sterilized carrier (charcoal, peat 3:1, and Arabic gum 10%) followed the process by Majeed *et al.* (2017). The inoculation carrier with bacteria gained incubation for three days at 28 °C±2 °C with daily shacked to ensure the optimum growth of bacterial cells. *Trichoderma harzianum* was in powder form, concentrated at 10^8 spore/ml. The various treatments of biofertilizer in combination with mineral fertilizer are in Table 2. Moistened maize seeds with warm water continued mixing extensively with biofertilizer and *Trichoderma* for perfect coating (Majeed, 2020).

In the experiments at both sites, plant sample collection occurred at harvest time (20 October 2021) from the five central rows of each subplot (Sandini *et al*., 2019). Ten plants collected from each subplot served as specimens to determine the plant dry weight (removing the cobs with the plants dried at 75 °C until reaching a constant weight), cob weight (g), grains per cob, grain weight per cob, 1000-grain weight, and grain yield ha^{-1} (taking grain yield in each treatment, then converting to yield per hectare).

Table 2. Treatments of biofertilizer in combination with mineral fertilizers used in the study.

No.	Treatments details
T1	AZS + AZT + B.m. + T.h. + 25% chemical fertilizer
T2	AZS + AZT + B.m. + T.h. + 50 % chemical fertilizer
T3	AZS + AZT + B.m. + P.f. + 25% chemical fertilizer
T4	AZS + AZT + B.m. + P.f. + 50 % chemical fertilizer
T5	AZS + AZT + B.m. + T.h. + P.f. + 25% chemical fertilizer
T6	AZS + AZT + B.m. + T.h. + P.f. + 50% chemical fertilizer
T7	100% chemical fertilizer (50 kg Dab + 60 kg urea)

Azospirillum brasilense (*Azs*), *Azotobacter chroococcum* (Azt), *Bacillus megaterium* (*B.m.*), *Trichoderma harzianum* (*Th.*), *Pseudomonas fluorescens* (*P.f.*).

Statistical analysis

Analysis of variance used the Genstat computer software package. The comparison and separation of means had an LSD at 5% of probability.

RESULTS

In maize experiments at both locations, superiority was notable in the biofertilizer treatments compared with mineral fertilizers for growth and yield traits (Tables 3 and 4).

Table 3. Effect of PGPMs combined with chemical fertilizers on maize crop in Kirkuk City, Iraq.

Treatments	Plant dry weight (g)	1000- grain weight (g)	Grain weight cob^{-1} (g)	Grains cob^{-1}	Cob weight (g)	Grain yield (t ha^{-1})
T1	440.3	314	163.78	531.6	206.67	16.38
T2	435.4	306	162	529.4	200.3	16.2
T3	457	309	165.11	510.8	190.9	16.5
T4	478.9	300	165.4	571.2	195.3	16.5
T5	506.4	319	178.3	558.8	214.6	17.8
T6	461	301	163.4	549.3	207	16.04
T7	409.4	266	155.53	510	180.2	15.50
$LSD_{0.05}$	4.8	4.07	4.8	3.67	5.38	0.48

Table 4. Effect of PGPMs combined with chemical fertilizers on the maize crop in Mosul City, Iraq.

Treatments	Plant dry weight (g)	1000- grain weight (g)	Grain weight cob^{-1} (g)	Grains cob^{-1}	Cob weight (g)	Grain yield (t ha^{-1})
T1	366.4	297	100.75	367.7	133.25	7.56
T2	393.8	316	154.06	487.5	186.17	11.15
T3	380.6	305	108.25	407.8	121.3	7.78
T4	400	314	166.3	495	200	11.98
T5	450	317	145	491	210	11.02
T6	467	320	170	500	216	12.4
T7	368	274	68.28	223.9	101.2	5.12
$LSD_{0.05}$	23.42	7.17	3.62	4.55	4.9	0.26

Maize experiment at Kirkuk City

The superiority of treatment T5 prevailed compared with other treatments for the growth and yield-related traits except for grains per cob, for which T4 led with a recorded maximum number of grains per cob (571.2 grains/cob) and a significant difference with other treatments (Table 3).

Treatment T5 (506.4 g/plant) for maize plant dry weight emerged with the best results, followed by T4 (478.9 g/plant) with significant differences from other treatments. The lowest plant dry weight showed in T7 (409.4 g/plant). For 1000-grain weight, the treatment T5 also provided highest importance and significant increase (319 g), followed by T1 (311 g) and T3 (309 g), with no significant differences among these three treatments.

Treatment T5 dominated by showing the highest grain weight per cob (178.3 g), revealing a significant difference with other treatments of maize (Table 3). However, the rest of the dilutions have no significant differences, while T7 showed the least grain weight per cob (155.53 g). For the number of grains per cob, T4 came out with the highest number of grains per cob (571.2 grain/cob), with a significant difference from other treatments. The treatments T3 and T7 provided the lowest and equal number of grains per cob (510.8 and 510, respectively).

For maize cob weight, treatment T5 (214.6 g) also showed superiority with significant differences from other treatments, followed by two others, i.e., T6 and T1 (207 g and 206.67 g, respectively) (Table 3). Treatment T5 (17.8 tons ha^{-1}) still leads for maize grain yield per hectare, recording superior over other doses. Treatment T7 had the lowest grain yield (15.5 tons ha^{-}). All other treatments showed moderate and similar grain yield per hectare.

Maize experiment at Mosul City

In the maize experiment at Mosul city, Iraq, treatment T6 rose with the best results for all the studied traits, except 1000-grain weight (320 g), showing a nonsignificant difference with other treatments, i.e., T2, T4, and T5 (316 g, 314 g, 317 g, respectively) (Table 4). The control treatment T7 appeared with the lowest values for all the traits, except plant dry weight (368 g), with nonsignificant differences from T1 and T3 (366.4 and 380.6 g, respectively) (Table 4). For plant dry weight, the treatments T6 and T7 (467 g and 450 g, respectively) exhibited significant increases in the said trait compared with other dilutions.

Treatment T6 displayed the highest grain weight per cob (170 g), with a notable difference from other treatments, whereas treatment T7 came out with the lowest value for the said trait (68.28 g). For grains per cob, treatment T6 also gave the best performance and maximum grains per cob (500 grain/cob), followed by T4 and T5 (495 and 491 grains/cob, respectively) (Table 4). Moreover, treatment T6 indicated a significant increase in the grain yield (12.4 t ha^{-1}), followed by T4 (11.98 t ha^{-1}), over all other treatments, revealing considerable differences from the rest. The control (T7) provided the lowest grain yield (5.12 t ha^{-1}) (Table 4).

The results authenticated that adding biofertilizers, combined with 25% and 50% doses of mineral fertilizer, improved maize plants' growth and yield-related traits at both locations (Tables 3 and 4). In Mosul City, the biofertilizer combined with 50% chemical fertilizer was the best combination over treatments with biofertilizers and 25% chemical fertilizer. However, at Kirkuk City, the biofertilizer with 25% mineral fertilizer was the best combination, recording the best performance for most growth and yield-related traits. This contradiction might be due to varied levels of fertilizer residues from the previous season crops found in the soil of both locations, resulting in differences when adding chemical fertilizer combined with biofertilizer based on the soil properties (Agbodjato *et al.,* 2021).

DISCUSSION

Biofertilizer addition helps make available all the important elements for plant growth, by nitrogen fixation, phosphorus solubilization, production of different enzymes, phytohormones, and substances that make the important minerals easily absorbed by crop plants (Majeed, 2020). Microorganisms added as a biofertilizer, such as, *Azospirillum, Pseudomonas,* and *Azotobacter*, produce the growth stimulants which improve the root growth and subsequent increase in the water and nutrient uptake rate, eventually enhancing the grain yield, in addition to their vital role as available minerals for crop plants (Namazari *et al*., 2012).

Biofertilizers like *Bacillus megaterium, Azotobacter chroococcum, Pseudomonas fluorescens,* and *Trichoderma harzianum* also produce vitamins, amino acids, and growth-promoting substances, such as, indol acetic acid (IAA) and gibberellins, resulting in better plant growth by enhancing nutrient uptake and translocation (Umesha *et al*., 2014). Microorganisms can easily make available the nutrients for crop plants in alkaline soil, such as, Iraqi soil, since the chemical fertilizer residues are multiple and exist in a complex form in the soil particles, which are unavailable to the plants. Biofertilizer is crucial in decreasing the PH and disassembling the particles of elements held in the soil, converting them into soluble forms suitable for absorption by crop plants (Abou-El-Seoud and Abdel-Mageed, 2012).

In the presented study, the inoculated plants with microorganisms led to reduce nitrogen fertilizer by 50%–75%, although the yield of maize increased by about 14.8% in

Kirkuk City and 58.0% in Mosul City of Iraq. The increase in the maize yield is due to the production of an enzyme by the microorganisms (1. Aminocyclopropane – 1 – carboxylate) ACC-deaminase, performing better when the level of N decreased in the soil (Shaharoona *et al.,* 2006). Bioagents producing ACC-deaminase can hydrolyze ACC (the precursor of ethylene) in higher plants, resulting in weak ethylene synthesis and improving the growth and yield of inoculated maize plants (Glick *et al.,* 1998).

Using biofertilizers, plants can adapt to diverse environmental conditions, such as, salt and water-deficit stresses, protecting plant roots from many pathogenic fungi, enhancing the plant growth, and eventually improving yield (Sandini *et al.,* 2019). Adding biofertilizer to maize crop reduced mineral fertilizer applications, thus helping decrease the cost of production and alleviate the negative impacts of chemical fertilizers (Hungria *et al.,* 2016). Saravanakumar *et al.* (2017) reported that the use of *Trichoderma harzianum* strain CCTCC-RW0024 as a biofertilizer to the maize crop increased plant growth and significantly reduced the infection of fusarium stalk rot by 86.66%.

The rise in grain quantity can refer to the enhanced translocation of dry matter from the source to the downstream (grain), thereby promoting the successful development of grains (Farhood *et al*., 2022). It can be due to fertilizer application, particularly during the flowering phase, which enhances the plant's capacity to produce highly viable pollen grains, leading to successful fertilization and a subsequent increase in grain count (Mohammed *et al*., 2021).

The existing results were in harmony with the findings of Gholami *et al.* (2009), who observed that the treatment of maize seeds with plant growth-promoting rhizobacteria (PGPR), such as, *Pseudomonas fluorescens* strain R-93*, P. fluorescens* DSM50090*, P. putida* DSM291, *Azospirillum brasilense* DSM 1690, and *Azospirillum lipoferum* DSM 1691*,* significantly increased plant height, seeds per cob, 100-seed weight, and shoot dry weight, including promoting seed germination and seedling vigor and stimulating plant growth and development.

The presented results were also in analogy with the findings of Zeffa *et al.* (2019), who reported that seeds inoculation with *Azospirillum brasilense* Ab-V5 under nitrogen deficiency conditions strengthened the plant growth and improved the biological traits. Sandini *et al.* (2019) used the *Pseudomonas fluorescens* as growth-promoting agent in maize crops, which reduced the need for nitrogen chemical fertilizer and enhanced the grain yield with a lesser cost of production and soil pollution.

CONCLUSIONS

Engaging bioagents as a biofertilizer, in conjunction with mineral fertilizers at varying proportions (25% and 50% of the recommended dosage), has incurred to yield positive effects on maize crops. These effects include increasing plant dry weight, 1000-grain weight, grain weight per cob, grains per cob, and ultimately grain yield. The optimal quantity of chemical fertilizer necessary to achieve ideal outcomes is contingent upon the available soil characteristics and the residual chemical fertilizers. The cities of Kirkuk and Mosul in Iraq demonstrated optimal maize growth and yield when chemical fertilizer supplements with biofertilizer have rates of 25% and 50%.

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