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Original Research

Improving Maize Physiological Attributes by Regulating Urease Activity and Zinc Availability in Rhizosphere Through Bioactivated Zinc-Coated Urea (Engro Zabardast Urea)

Muhammad Hasnain[1], Qudsia Nazir[2]*, Abubakar Dar[3], Azhar Hussain[3], Sajid Mahmood[4], Ifra Saleem[1], Hafiz Tanvir Ahmad[3, 5], Syed Shahid Hussain Shah[1], Muhammad Asif Ali[1], Muhammad Naveed[6], Rashid Iqbal[7], Abd El-Zaher M.A. Mustafa[8], Mohamed Soliman Elshikh[8], Allah Ditta[9, 10]*****

[1]Engro fertilizers Limited, 7th & 8th floor, the Harbor Front Building, HC-3 Marine Drive Block-4, Clifton Karachi-75600, Pakistan
[2]Institute of Soil Chemistry and Environmental Sciences, Ayub Agriculture Research Institute, Faisalabad, 38000, Pakistan
[3]Department of Soil Science, The Islamia University of Bahawalpur-63100, Pakistan
[4]Soil and Environmental Sciences Division, Nuclear Institute of Agriculture and Biology (NIAB), Faisalabad, Pakistan
[5]Provincial Reference Fertilizer Testing Laboratory, Raiwind, Lahore 55150, Pakistan
[6]Institute of Soil & Environmental Sciences, University of Agriculture Faisalabad-38040, Pakistan
[7]Department of Agronomy, Faculty of Agriculture and Environment, The Islamia University of Bahawalpur, Bahawalpur 63100, Pakistan
[8]Department of Botany and Microbiology, College of Science, King Saud University, P.O. 2455, Riyadh 11451, Saudi Arabia
[9]Department of Environmental Sciences, Shaheed Benazir Bhutto University Sheringal, Dir (U), Khyber Pakhtunkhwa 18000, Pakistan
[10]School of Biological Sciences, The University of Western Australia, 35 Stirling Highway, Perth, WA 6009, Australia

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Abstract

The major cause of reduced Zn availability to plants in Pakistan is unfavorable soil factors (alkaline pH and calcareousness). Different strategies can be adopted, to enhance the Zn bioavailability in the rhizosphere and cereals. Among all strategies, a novel technique is the coating of Zn on macronutrient fertilizers like urea. By adopting this technique, dual benefits can be achieved for example Zn and N become available to plants and on the other hand, the loss of urea can be minimized. Firstly, Engro Zabardast Urea was taken from Engro Fertilizers Pvt. Ltd and evaluated for coating effect on

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*e-mail: sbqnazir@gmail.com
**e-mail: rashid.iqbal@iub.edu.pk
***e-mail: allah.ditta@sbbu.edu.pk

urease activity and Zinc release pattern with time (up to 70 days). Then 100%, 90%, 85%, and 80% recommended EZU (Engro Zabardast Urea) were tested to find out the increase in urea efficiency by coating. From the results of the release experiment, it was concluded that EZU @ 90% of the recommended dose showed maximum results of Zn release respective to time and vice versa for urease activity. Secondly, a pot experiment was conducted to evaluate the above-mentioned prepared products on agronomic parameters and Zn and nitrogen concentration in 45 days of maize seedlings. Zn concentration in maize seedlings increased with the application of 90 and 85% of the recommended EZU, respectively. Similar results were found under field conditions with the application of 90% of recommended EZU growth, physiological parameters, and grains and stover yield were improved and Zn contents in grains were enhanced. From the results of all experiments, it can be concluded that EZU is a fantastic product not only to increase Zn bioavailability/fortification but also to reduce nitrogen losses. This product has the potential to reduce the use of plain urea by up to 10%. This ecofriendly approach cannot only reduce extra labor costs but also the ignorance of Zn application by the farming community can also be addressed.

Keywords: EZU, maize seedlings, urease activity, physiological parameters

Introduction

Balanced nutrition is an important factor in enhancing the overall yield of different crops [1]. The farming community of Pakistan prefers to use macronutrients i.e. (N) nitrogen, (P) phosphorous, and (K) potassium fertilizers only [2]. However, after N and P, Zn deficiency is widespread, common, and well reported due to unfavorable Pakistani soil conditions i.e., high $CaCO_3$, less organic matter in the calcareous soils, having high pH [3-6]. Zn malnutrition is becoming a severe issue in the developing world and the victims are mostly infants, underaged children, and pregnant women [7]. Moreover, the nitrogen fertilizer losses from the cultivated lands are becoming a major issue due to high temperatures and arid to semi-arid climatic conditions of the country. Nitrogen has a synergistic relationship with Zn and is an essential nutrient for plants and humans [8, 9]. On average 70 $\mu g\ g^{-1}$ Zn is present in the earth's crust (0.02%) by weight [10, 11] but anthropogenic activities are among the major factors affecting Zn availability and release in the soil [12] because Zn is very less mobile within the soil that's why its deficiency in plants is well reported [13]. Optimal Zn in soil is 0.6-1.0 mg kg-1 (DTPA-extractable), and 10-20 mg kg-1 of dry weight in plants [14, 15]. The limits may differ with soil type and crop rotation [16].

Nearly 70% of soils in Pakistan are deficient in Zn [17, 18]. Soils having characteristics such as saline-sodic, high pH, calcareousness, and high cropping intensity are mostly deficient in Zn contents [19, 20]. Its prevalence in the soil is mostly in the form of Zn minerals which are ZnS, ($ZnSO_4$) zinkosite, ($ZnCO_3$) smithsonite, (ZnO) zincite, [$Zn_3(PO_4)_2.4H_2O$] hopeite, ($ZnFe_2O_4$) franklinite, the availability of Zn from these sources depends upon some natural phenomena, for example, weathering of parent rocks, surface dusting and volcanoes [21-23]. Traditionally Zn is applied to soil through broadcasting with almost 50% use efficiency and the remaining gets fixed either with minerals or $CaCO_3$ [24]. Due to its non-availability in Pakistani soils having high pH and calcareousness, the use of coated fertilizers/slow-release fertilizers is becoming popular day by day all over the world. It is among the major strategies to improve environmental quality and sustain agricultural productivity [6, 25-27]. The application of coated fertilizers for better growth and yield is becoming an emerging trend/novel technology in today's agriculture in the future [28]. Various strategies are adopted to increase Zn availability in soil for plants. The second thing is the loss of applied urea in the form of ammonia to the atmosphere is a prominent issue. With the practice of coating, this loss can be minimized up to 15% [29].

Different Zn fertilizers are available to fulfill the requirement of plant Zn but the emerging fertilizer technologies use Zn and urea side by side through Zn coating on urea at varying concentrations [6, 30]. Many benefits can be achieved by coating Zn on urea such as i) the application of Zn and nitrogen to plants side by side because Zn application to crops is neglected among farmers' community, ii) urea loss in the form of ammonia can be minimized up to 10%, iii) slow and continuous supply of nitrogen to plants with very less loss, and iv) Ecofriendly approach with less labor cost [31, 32]. Based on the above discussion, the present study was conducted with the following objectives: i) to test the efficacy of commercialized bioactivated zinc coated urea (EZU) of Engro fertilizers Pvt. Ltd. in terms of soil urease activity and Zn release pattern with specific time intervals, ii) to test the effect of EZU on the growth and Zn contents of maize seedlings in pot studies and iii) to check the improvement in physiological attributes of maize and Zn fortification in grains through application of EZU and common Zn and N sources. It can be hypothesized that the application of EZU may improve the nutritional status of maize and reduce the fixation of Zn and ammonia volatilization in the field.

Experimental

The hypothesis was tested by planning three distinct studies. An incubation study to check the urease activity and release pattern of Zn from EZU at specific time intervals. Pot and field studies at the wirehouse, and field conditions at the Engro center, University of Agriculture, Faisalabad-Pakistan to compare the effect of EZU with $ZnSO_4$ and urea separately for improving the yield, physiology, and Zn fortification in maize grains (hybrid 4040).

Physicochemical Characteristics of Soil

Soil samples were mix thoroughly after drying, grinding and passing through 2 mm mesh size sieve, determined for physicochemical characteristics: texture, clay loam; organic matter, 0.77% [33]; similarly, pH, 8.22 EC, 1.27 dSm^{-1} [34]; available phosphorus, 9.17 mg kg^{-1} [35]; extractable potassium 95 mg kg^{-1} [34]; and plant available Zn, 0.97 mg kg^{-1} [36].

Characteristics of EZU Engro Fertilizers Pvt. Ltd.

The Zabardast Urea is a synergetic hybrid of urea, Bioactive Zinc (BAZ)© and Bioactive Coating (BAC)©; a consortium of Zn and other nutrients solubilizing and mobilizing bacteria. BAZ© is organically encapsulated Zn that is less prone to fixation, sandwiching, and trapping in soil structure. BAZ© is gradually released in the rhizosphere as per plant demand that supports an uninterrupted continuous supply of Zn during the crop cycle. In addition, BAC© enhances root growth, mobilizes other nutrients present in the rhizosphere, and triggers induced systemic resistance of plants to healthily pass through stress conditions. The coating cover of BAZ© and BAC© encapsulates urea prills induces a slow N release mechanism, contributes to reducing N losses, and enhances N use efficiency. Collectively, Zabardast urea is a revolutionary fertilizer suitable for all types of soils, climates, and crops [37].

Experimental Description

Experiments were conducted with six treatments along with one control (no Zn) and three replications in the experiments treatment plan as described in Table 1. ZSB was taken from the Department of Soil Science, The Islamia University, Bahawalpur. To conduct the first and second experiments Completely Randomized Design (CRD) was used with three repetitions. Randomized complete block design (RCBD) with three blocks was adopted in the third experiment. Fertilizers were applied as 275, 125, and 75 kg ha^{-1} NPK recommended doses along with zinc dose as 15 kg ha^{-1}. For the irrigation, tap water was used in the first and second experiments while tube well water was used in the third experiment. In the first experiment, Zn release with time and urease activity was evaluated while in the second experiment maize seedlings of 1.5 months were collected for analysis. In the third experiment, the crop was harvested at maturity, and plant height, biomass, cob length, cob diameter, and yield (stover yield, grain yield, grains Zn concentration, and physiological parameters (with CIRUS) were taken.

Measurement of Zn in Grain

Wet digestion of grain samples was done by following the method described by Jones and Case, [38] and Zn contents in grain were determined from the digested sample on Atomic Absorption Spectrophotometer (PerkinElmer, Analyst 100, Waltham, USA).

Statistical Analysis

The data regarding different parameters was collected and subjected to analysis of variance (ANOVA), through Statistix v. 8.1 (Analytical Software, USA) software. The least significant difference (LSD) test at a 5% probability was used to evaluate/compare the treatment means [39]. The significance of the treatment was presented through alphabetical lettering. The treatment means having the same letters was considered statistically non-significant at $p \leq 0.05$.

Table 1. Treatment description of the experiment.

Treatments	Description
T_0	Control (recommended N, P, and K with no Zn).
T_1	ZSB (Zinc solubilizing Bacteria) along with (RD) recommended dose of N, P, and K.
T_2	$ZnSO_4$ + urea and recommended P and K.
T_3	100% of EZU with recommended P and K
T_4	90% of EZU with recommended P and K
T_5	85% of EZU with recommended P and K
T_6	80% of EZU with recommended P and K.

Results

Wirehouse and field area of Engro center, University of Agriculture, Faisalabad was used for Pot and field experiments to find out the efficiency of EZU on growth, yield, and physiology of maize crop and results are given as follows:

Axenic Conditions Study

Zn Release Pattern and Urease Activity

The results regarding Zn release depicted an increasing trend in the release pattern of Zn by application of EZU than control (no Zn) as shown in Fig. 1a). A constant and gradual increase was observed in the pots, which received 90% EZU. On the first day, Zn concentration was 0.76 mg kg^{-1}, which raised to 1.92 mg kg^{-1} Zn at the end of incubation, which was 68% more Zn contents than first-day readings. While an increase of 66% was obtained with the application of 85% EZU. In the treatments where $ZnSO_4$ and plain urea were applied, 1.1 mg kg^{-1} Zn contents were determined after incubation. Furthermore, 85% EZU produced similar results as $ZnSO_4$ was applied with plain urea after 60 days, no further increase was observed with time. All the results that were obtained showed a statistically significant interactive effect of EZU. On the other hand, the application of ZSB alone has caused a slow increase (12%) in Zn contents.

As compared to all other treatments, 90% EZU has caused a continuous increase in urease activity throughout the incubation period. At the start of the experiment, the urease activity of 10-12 mg NH_4-N kg^{-1} h^{-1} was observed under 90% EZU application and a gradual decline of 5.5% in urease activity was observed. Under control (without Zn) 70 mg NH_4-N kg^{-1} h^{-1} activity at the end of the incubation. While 66 mg NH_4-N kg^{-1} h^{-1} activity was observed under 90% EZU application. Furthermore, almost similar and constant results were detected from 50-70 days. The experimental units receiving 85 and 80 EZU showed 66 and 67 mg NH_4-N kg^{-1} h^{-1} activities, respectively. On the 70th day, the soil analysis showed that with the application of ZnSO4 and plain urea separately 70 mg NH_4-N kg^{-1} h^{-1} activity was recorded (Fig. 1b).

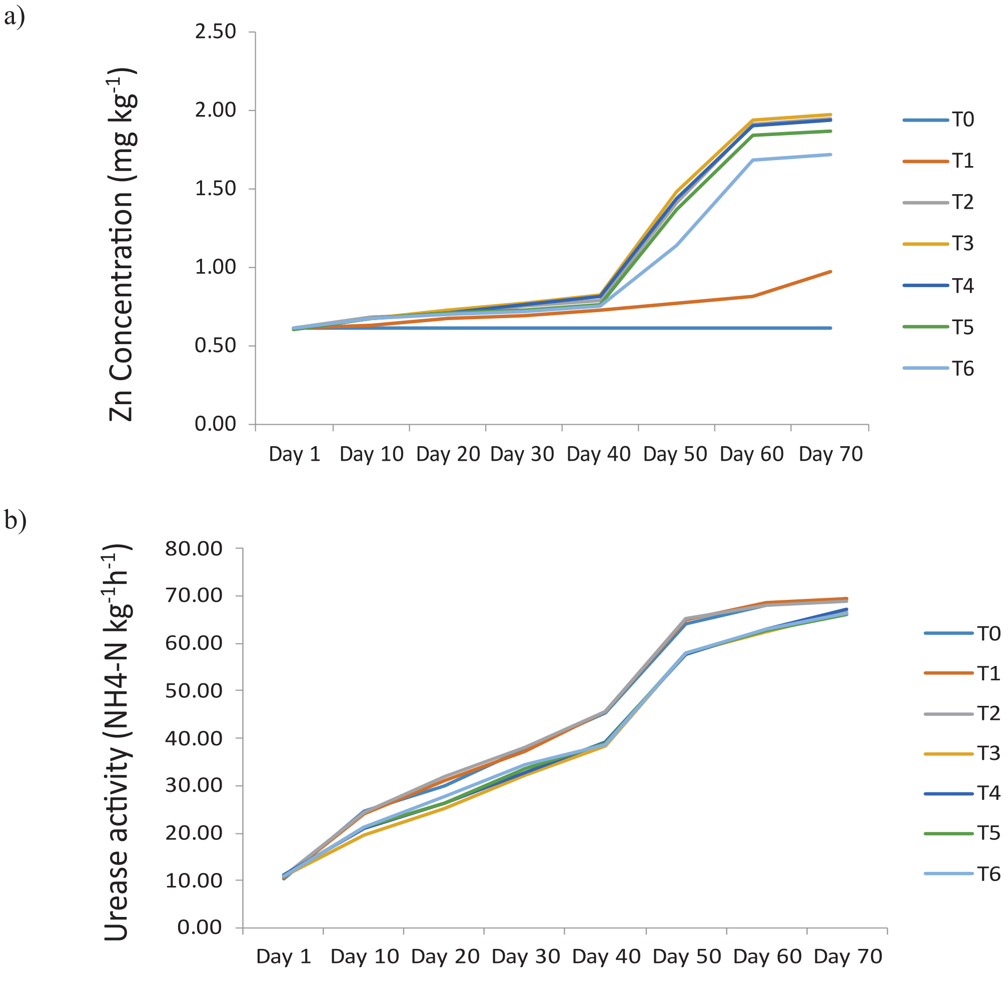

Fig. 1. Temporal release of Zn a) and urease activity b) from ZnSO4 and EZU under controlled conditions.

Pot Experiment

Plant Height and Biomass Production of Maize Seedlings

Different levels of EZU (100, 90, 85, and 80% of EZU) had a significant effect on plant height and biomass (g) of maize seedlings. With the application of different levels of EZU, an increasing trend was obtained in the biomass (g) of maize seedlings. Only the individual application of ZSB showed a minor increase in biomass production. Statistical analysis depicted significance at different levels of EZU; however, the maximum biomass (10.8 g) was recorded under 90% EZU treatment while the minimum height (7.6 g) was recorded without Zn application. With the application of 90% EZU, a 42% increase was observed as compared to control, similar results were found in plant height (Table 2).

Zn and N Concentration in Maize Seedlings

The impact of different levels of EZU (100, 90, 85, and 80% of EZU) showed statistically significant results on Zn and N concentration of maize seedlings. From the results presented, it was clear that 60.7 (µg g^{-1}) Zn and 1.56% N were found in maize seedlings with the application of 90% EZU. In the concentration of Zn and N a maximum of 46% and 12% increase was obtained respectively. On the other hand, 43 and 7% increase was obtained in maize seedlings respectively with the application of 85% of EZU (Table 2).

Field Experiment

Effect of EZU on Cob Length and Cob Diameter of Maize

Regarding the effect of different levels of EZU, 90% of EZU depicted a significant (p<0.05) increase in the cob length and cob diameter. There was observed a 6 and 7.6% increase in cob length and cob diameter, respectively, for T0 (no Zn). On the contrary, the treatment where $ZnSO_4$ was applied with plain urea instead of EZU, showed 4 and 6.3% less cob length and cob diameter as compared to 90% EZU. Significant (statistically) results were found in the treatments where 85 and 80% EZU was applied than control and $ZnSO_4$ and urea application separately (Table 3).

Effect of EZU on the Physiology of Maize

Physiological parameters; net photosynthesis, transpiration rate, substomatal CO_2, stomatal conductance, and chlorophyll contents were determined because Zn has a major role in plant physiology. From the results, it was observed that a significant effect at different levels of EZU was noted. Maximum net photosynthesis, substomatal CO_2 concentration, stomatal conductance, and chlorophyll contents were found in the treatment where 90% EZU was applied, it was 15, 21.5, 31, and 45% increase respectively. An increase of 8.5, 20.6, 14.5, 17, and 39% was noted in net photosynthesis, transpiration rate, substomatal CO_2 concentration, stomatal conductance, and chlorophyll contents, respectively with the application of 85% of EZU (Table 3).

Effect of EZU on Stover Yield, Grain Yield, and Grain Zn and N Contents in Maize

Results of yield attributes depicted statistically significant results at different levels of EZU noted on stover yield, grain yield, and Zn and N contents in grains (Fig. 2). Maximum results were found in the treatment where 90% EZU was applied. Stover and grains yield (8.83 and 6.04 t ha^{-1}) was obtained with the application of 90% EZU. The application of 85% EZU showed 7.9 and 6 t ha^{-1}, stover, and grains yield respectively (Fig. 2-A). On the other hand, a similar trend in grain Zn contents was observed (Fig. 2-B). Zn contents 53.4, 51.2, and 44 µg g^{-1} were observed with the application of 90,

Table 2. Efficacy of EZU against $ZnSO_4$ in improving Plant height, maize biomass, Zinc, and nitrogen concentration in maize seedlings in a pot experiment.

Treatment	Plant height (cm)	Biomass (g pot^{-1})	Zn concentration (µg g^{-1})	N concentration (%)
T_0	59.1 d	7.6 d	32.5 f	1.37 d
T_1	62.8 c	9.1 c	54.5 e	1.52 a
T_2	67.9 ab	10 b	58.4 bc	1.56 a
T_3	68.4 ab	9.9 b	59.1 b	1.54 a
T_4	69.4 a	10.8 a	60.7 a	1.56 a
T_5	67.9 ab	9.2 c	58 c	1.48 b
T_6	67.9 b	9.0 c	56.8 d	1.41 c
LSD ($p \leq 0.05$)	1.9	0.48	0.96	0.035

In a column, means sharing the same letters are statistically non-significantly different at $p \leq 0.05$.

Table 3. Efficacy of EZU against $ZnSO_4$ in improving agronomic and physiological attributes of maize under field experiment.

Treatment	Cob length (cm)	Cob diameter (cm)	Pn (mmol m^{-2} s^{-1})	E (mmol m^{-2} s^{-1})	Ci (mmol m^{-2} s^{-1})	Gs (mmol m^{-2} s^{-1})	Chlorophyll contents (SPAD value)
T_0	12.64 c	11.56 d	12.65 e	4.34 d	200.3 d	0.24 c	19.4 d
T_1	12.67 c	11.73 c	12.94 c	4.56 d	215.3 c	0.26 c	22.0 d
T_2	12.89 b	11.89 c	14.96 a	5.68 ab	249.7 a	0.34 ab	35.4 a
T_3	13.6 a	12.54 ab	14.97 a	5.78 a	255.4 a	0.38 a	36.1 a
T_4	13.5 a	12.7 ab	14.95 a	5.68 ab	255.0 a	0.35 a	35.4 a
T_5	13.68 a	12.49 b	13.830 b	5.47 b	234.4 b	0.29bc	32.2 b
T_6	12.8 bc	11.85 c	12.76 d	5.07 c	219.7 c	0.26 c	28.6 c
LSD ($p \leq 0.05$)	0.17	0.16	0.08	0.23	11.00	0.0569	3.06

In a column, means sharing the same letters are statistically non-significantly different at $p \leq 0.05$. Pn: Net Photosynthesis, E: Rate of transpiration, Ci: Substomatal CO_2 concentration, and gs: Stomatal conductance

85 and 80% EZU. The treatment where ZnSO4 was applied with plain urea showed 51 µg g^{-1} Zn which is comparable to 85% EZU (Fig. 2-C).

Nitrogen contents in grains (%) were increased with the application of EZU (Fig. 2-D), and with the application of 90% EZU 30.4% N contents in grains were increased than control where recommended plain urea was applied with no Zn. Similarly, a 24.8% increase with 90% EZU was obtained as compared to the application of ZSB only. The application of recommended plain urea with $ZnSO_4$ showed comparable results with the treatment where 80% EZU was applied.

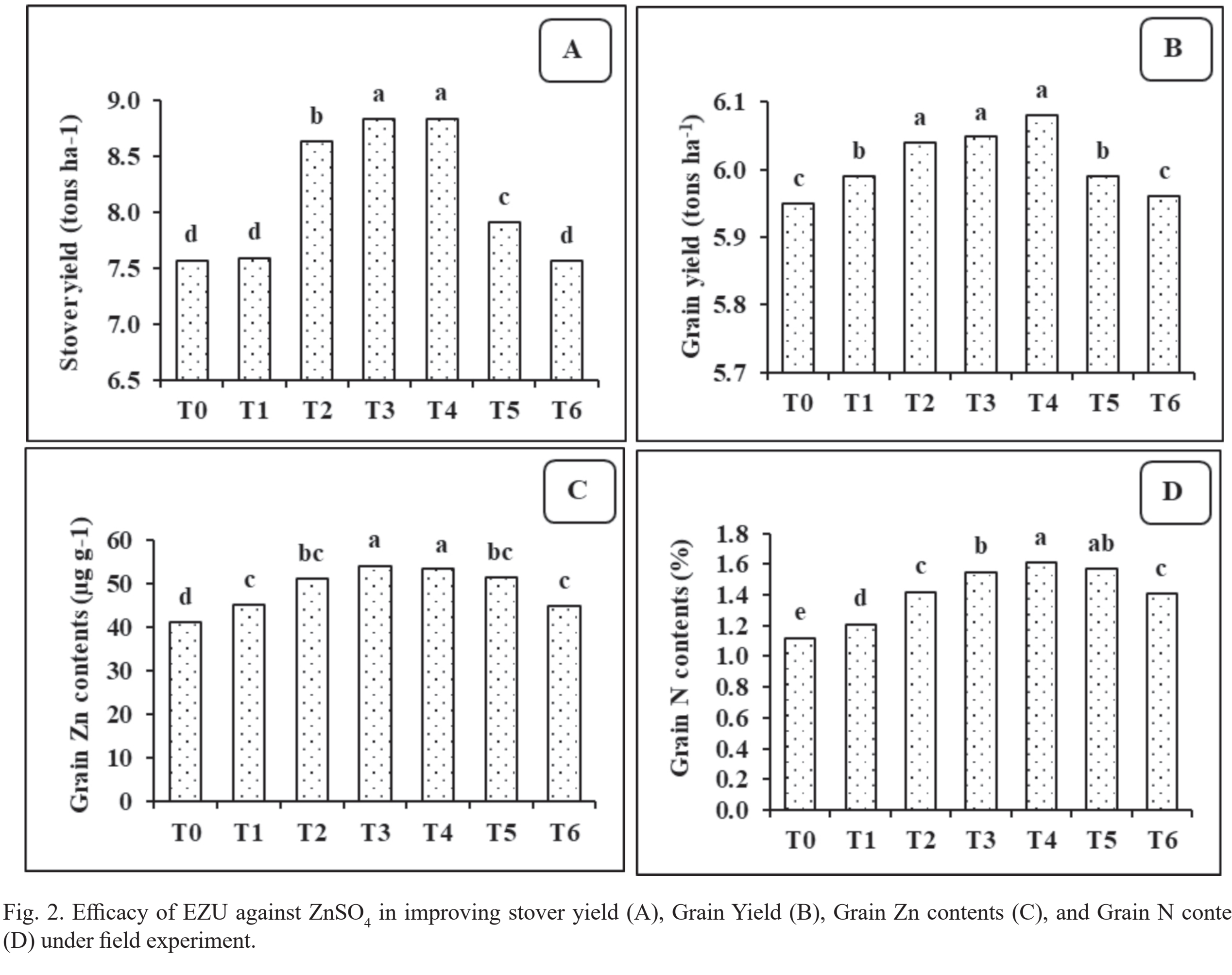

Fig. 2. Efficacy of EZU against $ZnSO_4$ in improving stover yield (A), Grain Yield (B), Grain Zn contents (C), and Grain N contents (D) under field experiment.

Discussion

Zinc sulfate is the most common Zn source, but its use is limited due to its high price and not availability in time. On the contrary, ZnO is a cheaper source of Zn and contains 80% of Zn but an insoluble source. This insoluble Zn contents in ZnO can be made soluble through Zinc solubilizing bacteria (ZSB) [40]. The solubilization of ZnO with ZSB could be termed as bioactivation of Zn which bacteria do through the production of various organic acids and metabolites [41]. Further, Zn is a vital metallic ion that performs as a cofactor in almost all six classes of enzymes, considered the second most significant metal after iron present in living organisms [42]. However, its deficiency in various crop plants is well reported due to fewer uptakes in Pakistani soils with alkaline pH, calcareousness, and low organic matter. Most of the Pakistani soils are Zn deficient and to overcome Zn deficiency farmers are using commercial fertilizer of Zn in the form of $ZnSO_4$ [6, 43, 44]. To avoid Zn deficiency one effective method is the coating of Zn on urea, in this way, dual benefits can be achieved, the first benefit is the simultaneous application of Zn and nitrogen side by side, and the second benefit is the reduction of urea loss and improvement in nitrogen uptake [45].

In the present study, Zn-coated urea (EZU) was obtained from Engro Fertilizers Pvt. Ltd. and tested on maize crops, first, the Zn release and urease activity were evaluated. We found that their release was slow and steady which indicated that with coating, urea becomes available for a longer period to the plants, and its loss can be minimized up to 15-20% as described by Ali et al. [46]. The effect of EZU on growth and yield attributes of maize crops is more as compared to only the use of Zn as $ZnSO_4$ and plain urea. Application of nitrogen (90%) with EZU showed maximum results in agronomic parameters because of its availability for longer periods, higher uptake in plants, and reduced adsorption on clay and precipitation with other salts as mentioned by Mirbolook et al. [47]. It is evident from the literature that with the application of ZSB, the insoluble Zn compounds can be solubilized in calcareous, high pH soils as these bacteria are blessed with certain mechanisms of metals solubilization mainly organic acids production, siderophore production, and EPS production [48]. Bioactivated zinc-coated urea (EZU) can solubilize Zn by producing organic acids, especially in cereals, and increasing overall growth, yields, and grain Zn concentration through mineral solubilization as described by Ali et al. [43].

Zinc amount can also be saved by the application of Zn-coated urea, as 2.8 kg Zn ha^{-1} was applied with Zn ZU whereas 6 kg Zn ha^{-1} is recommended in soil and foliar applications as compared by Prasad et al. [49]. Hence, it is an effective, eco-friendly, and economical fertilizer in poor countries with smallholding farmers [47]. Maximum results in stover and grain yields were obtained in 90% EZU and comparable results were found in 85% EZU with $ZnSO_4$ and plain urea. From the studies, it was found that with the help of coating almost 10-15% urea could be saved. The present findings are in line with the findings of Pooniya et al. [50]. Many researchers have done work on coated fertilizers and found similar results because with coating the hydrolysis of urea is delayed. We can say that a slow and constant supply of N can be achieved with the application of coated fertilizers [51].

By coating and bioactivation (EZU) urease activity was regulated and slow nitrogen availability was observed. Zinc uptake was also improved by plants. Zn acts as a cofactor in many enzymes that's why with the application of EZU all physiological parameters, for example, net photosynthesis, transpiration, and chlorophyll contents were improved as described by Mumtaz et al. [52]. Plant physiological parameters were improved at maximum level with the application of 90% EZU as compared to plain urea; the possible reason is that Zn acts as a cofactor in carbonic anhydrase enzyme as described by many scientists [48, 53, 54]. Nazir and her coworkers [6] concluded that the farmers of poor/developing communities can get the maximum benefit from the use of this economical, efficient, and eco-friendly bio-activated Zn-coated urea (EZU) by their limited resources and more nutrition in maize grain can be obtained.

Conclusions

It can be concluded from our salient findings that the application of Bioactivated Zn-coated urea (EZU) has a marked effect not only on plant growth but also on (Zn) concentration in plants as well. Different levels of EZU (90, 85, and 80 %) were used but 90% EZU showed maximum increase in maize seedlings Zn concentrations. N acquisition. Moreover, the uptake of Zn and N improved the physiological attributes (photosynthesis, transpiration rate, stomatal conductance, etc.) that are responsible for higher maize yields. On the other hand, we can say that it is an economical, novel, and easy technique as compared to the use of commercial Zn as $ZnSO_4$ and urea distinctly. This approach is very effective in saving 10 to 15% urea without sacrificing the yield. Ongoing research, farmer education, and support through regulatory incentives and research funding are essential for the successful implementation and sustainability of these strategies, contributing to more resilient and productive agricultural systems.

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Conflict of Interest

The authors declare no conflict of interest.

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