

Research Article

Effectiveness of Bio-Activated Zinc Coated Urea (Engro Zabardast Urea) for Growth, Yield, and Biochemical Parameters of Rice Crop (*Oryza sativa* L.)

Muhammad Hasnain¹, Qudsia Nazir², Ifra Saleem^{2*}, Syed Shahid Hussain Shah¹, Muhammad Asif Ali¹ and Noor ul Ain³

¹Engro Fertilizer Ltd., Pakistan; ²Institute of Soil Chemistry and Environmental Sciences, AARI, Pakistan; ³Soil Fertility Research Institute, Pakistan.

Abstract | Zinc (Zn) bio-fortification is an effective, low-cost in grains of staple food like rice and wheat and possible choice to reduce/decrease the Zn deficiency among humans of poor world. Around the globe main/basic reasons of Zn deficiency are intensive agriculture and ignorance to micronutrient fertilization. Soils with less organic matter (OM), high in pH and calcareousness contribute in Zn deficiency. Moreover, efficiency of zinc (Zn) in soils is usually very little due to fixation of Zn in the soil matrix. So, a study was executed to find out the combined effects of urea coated with bio-augmented Zn 1% (Engro zabardast urea (EZU)-a fertilizer source of N and Zn) on rice growth, yield, and Zn bio-fortification. The applied treatment (EZU) was compared with control (recommended N, P and K) and ZnSO₄ + urea and P, K. Results clearly indicated that the application of EZU showed improved Zn concentration significantly in rice grains, which were maximum (66.8%) and minimum (55.3%) increase was in rice observed as compared to where no (Zn) was applied (in control) at Faisalabad and Chiniot (FSD and CHT), respectively. EZU also showed improved results of Zn contents in grains as compare to the treatment where ZnSO₄ and urea was applied separately. On the other hand, with application of EZU, agronomic parameters including straw and grains yield, and panicle length were also improved significantly (2.7, 18.7 and 16.4%, respectively), as compared to urea and ZnSO₄ application separately. Therefore, it was concluded that the application of EZU played role as a slow-release fertilizer that improved Zn bio fortification in rice grains and Zn needs of humans will be fulfilled by consumption of zinc (Zn) enrich grains of rice.

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***Correspondence** | Ifra Saleem, Institute of Soil Chemistry and Environmental Sciences, Pakistan; **Email:** ifrasaleem17@gmail.com

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Keywords | EZU, Growth, Zn contents, Rice grains, Yield, Zn uptake



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Introduction

In the 820 million people of the world, malnourishment had been observed (<https://www.wfp.org/>)

and food security is a major challenge of 21th century (Nazir *et al.*, 2021) because of world's population is increasing very sharply. Micronutrients deficiency is well documented not only in plants,

animals and humans as well. Among all micronutrients Zinc (Zn), Iron (Fe) and iodine are widespread deficient nutrients. Zn deficiency among humans had been observed in 34% population of developing world that is a main reason of less growth and diseases like pneumonia, diarrhea, less neuropsychological activity, and abnormal fetal development (Hambidge, 2000). Its requirement for adults is nearly 8 to 11 mg per day, on the other hand, Palanog *et al.* (2019) showed almost 12–13 mg day⁻¹ Zn requirements for lactating and pregnant mothers. Its deficiency can be reduced by taking supplements in the form of tablets and capsules or using fortified/diversified foods; this technique is ineffective due to higher prices and the poor of developing countries cannot afford with a serious risk of food security and health abnormalities (Nazir *et al.*, 2016). In the poor world, most of the people depend on cereals (wheat, rice and maize) to fulfill their daily caloric requirements. Husain *et al.* (2012) and his coworkers stated that, in poor countries, cereals are commonly grown in those soils which has less inherent Zn in soils. The Zn enhancement in the edible portion of cereals (rice, wheat and maize) is an economical and feasible approach to enhance Zn contents of low-income population (Mayer *et al.*, 2008); this purpose of enhancing Zn in cereal grains can be achieved through bio-fortification process (Prasad, 2010; Nazir *et al.*, 2021). Genetic engineering and conventional breeding methods can be used for biofortification of Zn but these approaches are less effective, time consuming and costly (Pfeifer *et al.*, 2007). Grains Zn concentration can be improved/enhanced by Zn application in soil (Husain *et al.*, 2012; Alloway, 2009). Thus, to encourage the growers for application of Zn in poor or developing countries, the improvement in efficiency of soil zinc can be seen with Zn application in soils. In other words, we can say that they are reluctant to use Zn due to many marketing, availability and cost issues (Nazir *et al.*, 2021). On the other hand, the calcareous soils have less efficiency of Zn (Bun-Quin *et al.*, 2017), The Zn make complexes in the calcareous soils and became unavailable to the plants (Alloway, 2009), Zn fertilizers application have tendency to be converted into insoluble forms of Zn in alkaline calcareous soils, as it has low organic matter and high pH of soil. Furthermore, (Hussain *et al.*, 2015) has been declared that bioaugmentation of ZSB (Zn solubilizing bacteria) enhance the bioavailability of (Zn) for plants, (Mumtaz *et al.*, 2019), in the calcareous soils. It has been identified that there are many microbial strains

which are documented in literature and have the ability to solubilize less/ insoluble forms of Zn in soil. It can perform as a potential tool for better Zn use efficiency (ZUE).

According to researchers (Mumtaz *et al.*, 2017; Nazir *et al.*, 2016), *Bacillus* spp. had been documented as the most leading Zn solubilizing bacteria (ZSB) in bacterial classes. It may convert fixed Zn in to an available or soluble fraction. For the improvement in availability of soil (Zn) and biofortification, it also has appeared as eco-friendly method and an innovative sustainable method. The present study was conducted to estimate the effectiveness of EZU (a coated urea with Zn [(1%) in the form of ZnO]) bioaugmented with (ZSB). It is hypothesized that in rice crop, this fertilizer will not only increase the contents of (Zn) in grains but also increase ZUE. The source of zinc as zinc oxide (ZnO) is cheaper in comparison to zinc sulphate (ZnSO₄ 33 or 21% Zn) fertilizer. It also reduces the costs of Zn fertilizers. Nazir *et al.* (2021) has been observed that this permeated fertilizer would save the labor costs for the application of biofertilizers, urea and zinc individually. Hussain *et al.* (2020) reported the coating of urea with bioactivated ZnO to support cereal yield, physiology and growth of crop. In Pakistan, after wheat the rice crop is the (2nd) most important cereal crop and was preferred for Zn biofortification evaluation. Rice was sown on an area of (2810 thousand ha) with production of (7202 thousand tons) reported by Pakistan Economic Survey (2018–2019). Therefore, rice grains with Zn biofortification will increase the ZUE and can also help in decreasing in deficiency of (Zn) in humans.

Materials and Methods

Physico-chemical characteristics of soil

To evaluate the effect of EZU with ZnSO₄ and urea separately for improving the content of Zn in grains, yield and growth of rice (Cultivar: Shaheen), two field trials were executed; one at the research area of Soil Chemistry Section, Ayub Agriculture research Institute (AARI), FSD-Pakistan; and other on a farmer field at CHT district. The pre-sowing soil samples were taken from both locations of FSD and CHT. The soil samples were grinded, sieved (2mm mesh size) and mixed to get the uniform samples. The samples were analyzed for physico-chemical attributes including organic matter, (Moodie *et al.*, 1959); available phosphorus, (Watanabe and Olsen, 1965);

extractable potassium (U.S. Salinity Laboratory Staff, 1954); EC, (U.S. Salinity Laboratory Staff, 1954), texture of soil (Moodie *et al.*, 1959), pH (U.S. Salinity Laboratory Staff, 1954). The crop was harvested at maturity, yield parameters and straw and grain yield was recorded. The grain samples were collected from both the locations and plant available Zn (Soltanpour and Workman, 1979) was measured. The EZU was collected from Engro fertilizers Pvt. Ltd. Faisalabad to be used at both the locations.

The OM (0.76 and 0.85%), available P (8.79 and 9.11 mg kg⁻¹), extractable K (84 and 91 mg kg⁻¹), EC (1.36 and 1.24 dSm⁻¹) and pH (8.21 and 8.14) of the soil samples collected from both locations (CHT and FSD, respectively) were determined. The soils were found free from salinity, low in OM. The P and K content were found in the moderate range. The available Zn content was found 0.95 and 0.71 mg kg⁻¹ at CHT and FSD, respectively (Table 1).

Characteristics of EZU declared by engro fertilizers Pvt. Ltd.

The Zabardast Urea is 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 rhizosphere as per plant demand that support uninterrupted continuous supply of Zn during crop cycle. In addition, BAC[®] enhances root growth, mobilizes other nutrients present in rhizosphere and triggers induced systemic resistance of plants to healthily pass-through stress conditions. Coating cover of BAZ[®] and BAC[®] encapsulates urea prills induce slow N release mechanism, contribute in reducing N losses and enhance N use efficiency. Collectively, Zabardast urea is revolutionary fertilizer suitable to all types of soils, climates, and crops (Hussain *et al.*, 2020).

Experimental description

Experiments were conducted with three treatments and three replications on both locations including T₀ = control (recommended N, P and K with no Zn), T₁ = ZnSO₄ with Urea and recommended P and K, T₂ = EZU with recommended P and K. The plot size was 35 m² for each treatment, to conduct the experiments. The recommended dose of Zn (8 kg ha⁻¹) was applied to soil with recommended doses of other fertilizers

such as nitrogen (140 kg ha⁻¹), phosphorous (80 kg ha⁻¹) and potassium (65 kg ha⁻¹). The di-ammonium phosphate (DAP), sulphate of potash (SOP) and urea was also applied. For these experiments, Randomized Complete Block Design (RCBD) was used. For irrigation, tubewell (1.34 dSm⁻¹) and canal water (0.97 dSm⁻¹) was used and rice crop was harvested at maturity. At the end, data regarding grains, Zn uptake, Zn concentration, grain yield, yield and growth parameters (1000 grain weight, grains per panicle, panicle length and straw yield) harvesting index (HI) and chlorophyll contents were taken.

Measurement of grains Zn

Samples of grains were digested for assessing Zn concentration in grains through a procedure depicted by (Jones and Case, 1990) and determined Zn contents with Atomic Absorbance Spectrophotometer (PerkinElmer, Analyst 100, Waltham, USA).

Statistical analysis

Data related to various parameters were subjected to analysis of variance (ANOVA). To estimate the means of treatment, LSD test (Least significant difference) was used at a (5%) probability (Steel *et al.*, 1997). After this, the significance of treatment means was showed by alphabetical lettering. The means of treatments having same letters were considered non-significant statistically at level of significance ($p \leq 0.05$). Analysis of variance was made by using software (Statistix 8.1) (Analytical Software, USA).

Results and Discussion

Effect of Zn application as ZnSO₄ and Engro Zabardast Urea (EZU) on the yield traits and harvest index

At Faisalabad paddy trial, results showed that the Engro Zabardast Urea (EZU) and Zn application in the form of zinc sulfate (ZnSO₄) significantly increased the length of panicle over control treatment (RD NPK). At two different locations CHT and FSD, results of yield traits are illustrated in the (Table 2). The EZU showed maximum panicle length increase of 2.7% over the control treatment. Engro Zabardast Urea (EZU) gave maximum number of grains per panicle as compared to the other treatments but statistically at par with treatment receiving the (Zn as ZnSO₄) at both locations. The similar results trend was found in the CHT trial where EZU depicted a 13.4% increase in panicle length compared to control. The 1000 grain weight which is an important

Table 1: The basic soil analysis of two soil sites before sowing of rice.

Locations	pHs	ECe (dSm-1)	O.M (%)	Available P (mg kg ⁻¹)	Extractable K (mg kg ⁻¹)	Extractable Zn (mg kg ⁻¹)	Texture
FSD	8.14	1.24	0.85	9.11	91	0.71	Clay loam
CHT	8.21	1.36	0.76	8.79	84	0.95	Loam

Table 2: Effect of ZnSO₄ and EZU on the yield contributing traits and harvest index.

Locations	Treatments	Panicle length (cm)	No. of grains panicle ⁻¹	1000 grain weight (g)	Harvest index (%)
FSD	RD NPK	25.9 ± 0.24 ^b	68 ± 1.45 ^b	25.5 ± 0.40 ^c	0.58 ^b
	RD NPK + ZnSO ₄	25.7 ± 0.40 ^a	72 ± 1.16 ^{ab}	26.0 ± 0.32 ^b	0.59 ^a
	EZU + PK	26.6 ± 0.35 ^a	75 ± 1.86 ^a	27.5 ± 0.17 ^a	0.59 ^a
CHT	RD NPK	23.1 ± 0.39 ^b	70 ± 0.88 ^b	24.7 ± 0.76 ^b	0.56 ^b
	RD NPK + ZnSO ₄	25.9 ± 0.45 ^a	76 ± 0.58 ^a	25.3 ± 0.44 ^{ab}	0.57 ^b
	EZU + PK	26.2 ± 0.32 ^a	76 ± 1.20 ^a	27.6 ± 0.68 ^a	0.62 ^a

indicator of grain yield showed positive response towards the application of EZU at both the locations. The increase in 1000 grain weight at CHT and FSD is 11.7 and 7.8%, respectively. The maximum harvest index was observed in the CHT trial compared to the FSD trial in the EZU treatment. At CHT location, the EZU showed significantly higher HI compared to the other two treatments. At FSD location, the EZU and treatment with ZnSO₄ showed significantly similar results of HI.

Effect of Zn application as ZnSO₄ and Engro Zabardast Urea (EZU) on the grain and straw

The maximum grain yield (positive response) was observed in the EZU treatment over control at the FSD location followed by EZU treatment at the CHT location. The grain and straw yield of paddy trials grown at two different locations are illustrated in the (Figure 1). The grain yield in ZnSO₄ treatment at CHT location showed significantly similar results compared to the EZU. The EZU showed 7.6 and 15.7% increase in grain yield over ZnSO₄ and control treatment, respectively. The highest straw yield was found in EZU treatment at the FSD location and gave an increase of 8.8% over the ZnSO₄ treatment. The control treatments at FSD and CHT showed minimum straw yield of 8.59 and 7.83t ha⁻¹, respectively, compared to the other treatments. The ZnSO₄ and EZU treatments showed statistically significant results at the CHT location.

Effect of Zn application as ZnSO₄ and Engro Zabardast Urea (EZU) on the chlorophyll content

The results of the chlorophyll content found at both

locations are presented in the Figure 2. The maximum chlorophyll was found in the EZU treatment at the CHT location. The chlorophyll content observed in the treatment where ZnSO₄ was applied showed statistically similar results when compared with the EZU treatment (43.3 SPAD value). The treatments where Zn was applied showed significantly higher SPAD value compared to the control (RD NPK) at the CHT location. The chlorophyll content was also found maximum in the EZU treatment and was statistically similar to the ZnSO₄ treatment. The EZU showed significantly higher chlorophyll compared to control at FSD location. This increase of EZU was 9.3 and 15.8 % more compared to control at FSD and CHT locations, respectively.

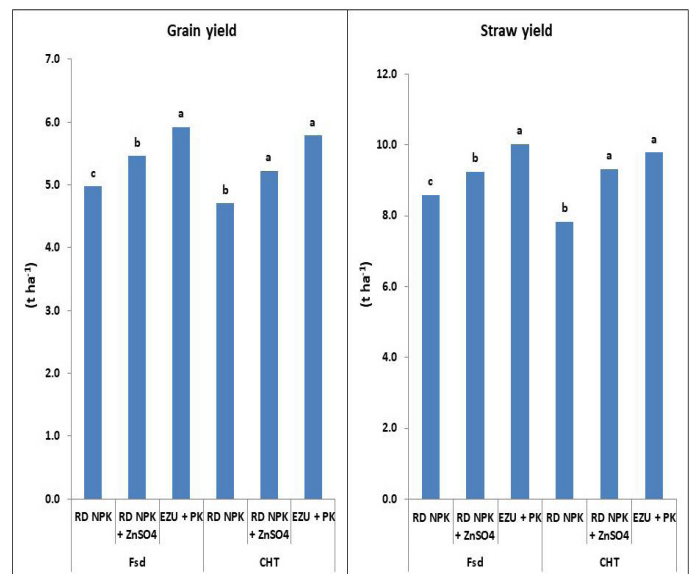


Figure 1: Effect of ZnSO₄ and EZU on the grain and straw yield of rice at both the locations (FSD & CHT).

Effect of Zn application as ZnSO₄ and Engro Zabardast Urea (EZU) on Zn uptake by grain and the Zn concentration of grain

The results of the paddy trials conducted at two locations showed positive response to Zn application either as ZnSO₄ or EZU. Zinc application increased the concentration of Zn significantly than that treatment where no Zn was applied (in control). The EZU showed an increase of 18% in terms of Zn concentration in grain compared to the ZnSO₄ application at the FSD location. The results of Zn concentration at CHT location were of similar trends found at FSD. The results of grain Zn uptake and concentration of Zn in the grains is illustrated in the (Figure 3).

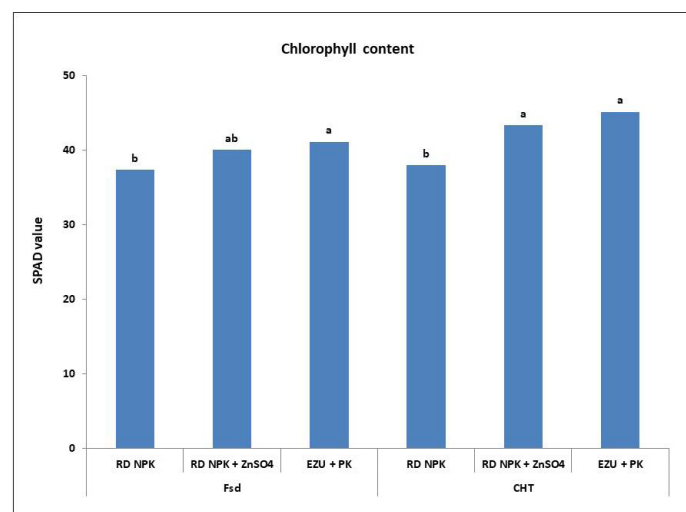


Figure 2: Effect of ZnSO₄ and EZU on the chlorophyll content of rice at both the locations (FSD & CHT).

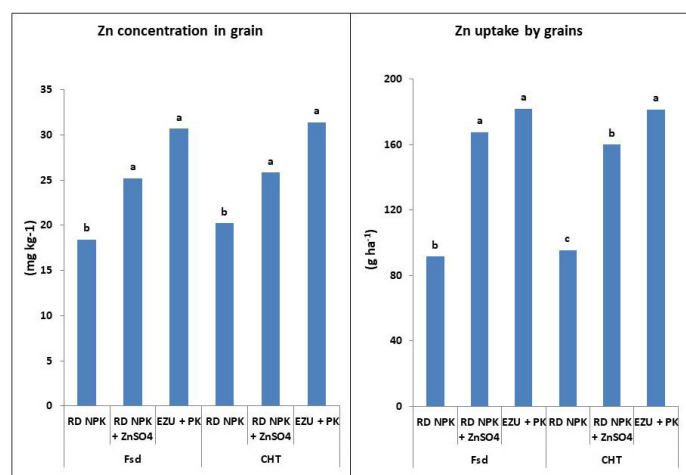


Figure 3: Effect of ZnSO₄ and EZU on the Zn concentration and Zn uptake by grains at both the locations (FSD & CHT).

At the FSD, the EZU and ZnSO₄ application gave statistically similar results (181.8 and 167.5 g ha⁻¹). The application of Zn indicated positive response towards uptake of zinc by the grain and showed

increased Zn uptake in both treatments of EZU and ZnSO₄ over the control. The control treatment gave minimum Zn uptake of 91.5 g ha⁻¹. The results of the trial conducted at the CHT location showed that the maximum uptake was found in the EZU treatment (182.5 g ha⁻¹) followed by the ZnSO₄ application (160 g ha⁻¹), both the treatments were found statistically different. The minimum uptake was calculated in the control treatment at the CHT location as there was no exogenous application of Zn.

Fixation and adsorption processes are the causative factors of Zinc deficiency under calcareous and high pH soils (Duffner *et al.*, 2012). The Zn application is a precondition observed by Prasad *et al.* (2013), to cereal crops for improved nutritional status of the grains of cereal crops. The application of urea is a dire need to fulfill the nitrogen (N) requirement of the crops as soils of Pakistan are deficient in nitrogen. The problem with the application of the urea is its environmental losses and low recovery efficiency (Li *et al.*, 2017). Only 30-40% of the applied N in the form of urea becomes available to the plants. Yang *et al.* (2013) has been stated that to overcome the low recovery efficiency of urea fertilizer and to reduce the gaseous emissions, (Ke *et al.*, 2017), and the use of slow-release coated fertilizer is a practical approach. The Engro Zabardast urea (EZU) is a Zn coated urea, commercially available in the market with a theme to slower down the release of N due impregnated Zn coating.

Yang *et al.* (2011) and his co-workers showed similar results that the Zn application enhanced the zinc uptake by plants and availability of Zn which proved in betterment in plants metabolic activities which also increased length of panicle. The present experiment depicted that the EZU application resulted in the significant increased the number of grains per panicle. The results of the yield traits showed that the EZU showed higher panicle length compared to control and ZnSO₄ application treatment. Results matched with many studies of (Li *et al.*, 2018; Geng *et al.*, 2015), that the EZU a controlled release fertilizer showed the improved 1000 grain weight and grains per panicle. By application of EZU, straw and grain yields were found maximum. So, the current trail results were in resemblance with results of Hassan *et al.* (2019) research and he gave similar results that application of Zn improved the 1000 grain weight which is a direct indicator of the yield. Guo *et al.* (2016) reported that

use of same dose of coated urea resulted in the higher grain yield which was in accordance with the present study results. The studies of (Kiran *et al.*, 2010; Yang *et al.*, 2012) showed that the application of coated urea improved the yield of the rice grain. Nadim *et al.* (2013) showed similar results that the application of Zn significantly enhanced the production of dry matter. Chen *et al.* (2020) stated that controlled urea improved the yield contributing parameters that collectively play role to increase the yield.

The results showed increased Zn content in the grain where EZU was applied. It ultimately resulted in the bio fortification of the grains as proved by many scientists in various studies (De Steur *et al.*, 2014). Jiang *et al.* (2008) stated that the application of Zn increased the movement of Zn to leaves during the vegetative growth which later on allocated to the grains which are metabolically active sinks of the plant. Ishimaru *et al.* (2011) and his colleagues clearly indicated that Zn fertilizers application is a main source for availability of Zn for the plants and increasing Zn content in grains. The EZU improved the Zn uptake by rice grains compared to the control (RD NPK). The EZU is a slow-release fertilizer that slowed down the N release improved the Zn availability due to impregnated Zn in it. The synchronized availability of the N and Zn improved the chlorophyll content (Samreen *et al.*, 2017). The improved chlorophyll contents higher the photosynthetic rate resulted in the improved yield. The permeated Zn in EZU, (which is a derivative of the yield and Zn content), showed significant increase in Zn uptake in plants and results matched with study of Johnson-Beebout *et al.* (2016).

Conclusions and Recommendations

It was concluded from the experiments executed at two different locations (CHT and FSD) that the zinc application has positive impact on the grain yield and also increased the concentration of Zn in the grain significantly. The EZU (the coated urea with 1% Zn) indicated increased grain yield significantly and bio fortified the rice grains may also be helpful to get better the nutritional status of human masses.

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Chemistry and Environmental Sciences, (Soil Chemistry Section), Ayub Agriculture Research Institute (AARI), Faisalabad, Pakistan.

Novelty Statement

This research deals with the bio-fortification of Zn, with the use bio-activated Zn coating on urea. The coating contributes to the Zn availability along with the slow release of the Nitrogen for a longer period to the rice crop.

Author's Contribution

Muhammad Hasnain: Conceived the idea, Practical execution of the trial in the research field, Data collection.

Qudsia Nazir: Wrote abstract, Introduction and Methodology. Chemical analysis of the samples.

Ifra Saleem: Wrote Results and discussion, statistical analysis of the data performed, Overall Management of the article.

Syed Shahid Hussain Shah: Technical Input at every step and proof read of the article.

Muhammad Asif Ali: Make all the inputs available at appropriate time. Technical Input at every step.

Noor ul Ain: Zn analysis and References portion.

Conflict of interest

The authors have declared no conflict of interest.

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