Research Article

Effectiveness of Zinc Coated, Blended and Bio-Activated Zinc Coated Urea to Quality, Biochemical Parameters and Yield in Rice Crop (*Oryza sativa* L.)

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Abstract | Zinc (Zn) is an important microelement not only for animals, plants but for humans as well. Its importance cannot ignore for the plants to improve overall quality and yield. The overall physiology, quality and biochemical parameters also enhanced with optimum application of Zn. By keeping in mind, the facts, it was hypothesized that the use of ZnO (a cheap source of Zn) impregnated urea for rice may enhance grains (paddy) yield. Three types of urea were prepared including Zn coated, bio-activated Zn coated and Zn blended urea at the 1.5% rate of formulate. The bio-activated Zn coated urea was prepared by inoculating the powdered organic material with Zinc solubilizing bacterium and then this material was mixed with ZnO. This bio-active Zn was coated on urea at 1.5% rates to formulate. Moreover, Zn blended urea was prepared by mixing powder ZnO with urea. The comparative efficacies of different types of Zn impregnated urea were compared with $ZnSO_4$ to grains yield, physiology and biochemistry of rice under field conditions. The results showed that 1.5% bio-activated Zn (ZnO) coated urea performed better in promoting yield and biochemical parameters. About 15 to 20% increase was observed in yield physical, biochemical and quality parameters. This suggests that the application of bio-activated Zn coated urea @ 1.5% is greatly active in enhancing quality and yield of rice crop.

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Introduction

Millions of people in the world feeding on cereals like wheat and rice (FAO, 2012). After wheat, rice is utmost imperative essential crop of Pakistan. It contributes approximately 0.6 percent in GDP. During 2018-2019 rice was sown on 2810 thousand hectares with production of 7202 thousand tons (Pakistan Economic Survey, 2018-2019). World's population increasing day by day, therefore, food need is also increasing, while the natural resources are same (United Nations, 2012). Malnutrition is a very popular issue of poor communities and due to reduced bioavailability, micronutrients deficiency is also becoming serious issue (Huang et al., 2002). In the soils of Zinc (Zn) deficient areas, Zn deficiency in humans consider fifth largest reason of deaths (WHO, 2002). Almost 8 to 11 mg per day Zn is required for adults daily while pregnant and lactating mothers require more consumption that is about 12 mg/day (Palanog et al., 2019). Insufficiency of zinc leads to problems of respiration, diarrhea and malaria in many developing countries. Normal reproductive system, immune system, cell growth effects due to Zn deficiency in humans (WHO, 2002). Almost 37% of Pakistani population is suffering in Zn malnutrition (UNDP, 2003). Zinc application to soil enhances growth and yield of plants and recovers overall vigor and plant pigments e.g., sugars and oil contents, over all Zn also improves physiological, bio-chemical and quality of cereals (Khalifa et al., 2011). The possible solution to overcome this problem of low Zn contents in crops is the use of fertilizers but due to economic issues of farmers and fixation of zinc fertilizers in calcareous soils, its usage is not adopted by farmers. By keeping in mind, the importance of Zn for plants and humans, strategies must be employed to increase Zn bioavailability.

As in Pakistan, soils are deficient in Zn because soils having more $CaCO_3$ contents and less organic matter, high soil pH (Hafeez et al., 2013) and high soil phosphorous contents (Singh et al., 1986). Due to Zn deficient soils the crop grown on such soils are also Zn deficient. The people of such poor countries suffer from severe Zn deficiency. To address this issue, so many techniques are in use like clinical treatment of nutrients, biofortication and bioavailability of nutrients etc. (Mayer, 2008). Biofortification of zinc is in practice by so many methods, i.e., genetic breeding and agronomic methods. At the same time, balanced fertilizers management is too a common means used by farmers in the country. Zinc is being used in the form of $ZnSO_4$. Another alternate source of zinc is ZnO but its high price is not permitting farmers to apply it in the field. This insoluble Zn can be solubilizing by ZSB (Zinc solubilizing bacteria). An experiment was conducted in field on rice crop to check the influence of ZnO coated, blended and bio-activated Zn coated urea on physiological, quality, biochemical and rice growth.

Materials and Methods

Rice crop was transplanted under field conditions on research area of University of Agriculture, Faisalabad and comparative effectiveness of Zn blended, Zn coated and bio-activated Zn coated urea was evaluated for physiological, quality and bio-chemical parameters under field conditions for rice crop (Cultivar: Shaheen).

Physico-chemical characteristics of soil

Soil samples were taken randomly from various sites of field and samples were spreaded and dried in the air. All samples were passed through sieve having mesh size of 2 mm after grinding. Method suggested by Moodie et al. (1959) was used to determine soil textural class that was sandy clay loam. All laboratory analysis was accomplished applying methods of Handbook No. 60 of USDA (1969) or otherwise referred. Total nitrogen and available phosphorus in soil was determined according to methods of Jackson (1962) and Watanabe and Olsen (1965), respectively. Likewise, concentration of zinc in plant samples was determined by using method of Soltanpour and Workman (1979).

Preparation of 1.5% Zn coated, Bio-activated Zn coated and Zn blended urea

Pre-isolated Zn solubilizing bacterial strain *Bacillus* sp. AZ6 (accession number KT221633) (Hussain et al., 2015) was taken University of Agriculture, Faisalabad, Pakistan. Zinc solubilizing bacteria were isolated from rhizosphere of maize plants using dilution plate method and agar medium (Hussain et al., 2015)). Inoculum of the strain AZ6 was made by rising it in 1000 mL flask having Bunt and Rivera basal medium (Bunt and Rovira, 1955). Glass flasks used for inoculation purpose were incubated at temperature of 28±10°C for total time of 72 hours on an incubator of orbital shaking type. Before use, an optical density of 0.5 at 535 nm was adjusted. Mixture of bacteria so prepared was utilized for the formulation of zinc coated urea. The powder organic material (plant residues) was first dried in an oven at 800°C. It was inoculated with bacterial strain AZ6 and incubated for 72 h at 30 ± 20 °C in an incubator. Then this bio-augmented organic material was thoroughly mixed with 300-400 mesh size ZnO in the ratio of 40:60 (powder ZnO: bio-augmented organic material). This mixture was again incubated for 3 days at 30 ± 20°C to attain extreme chelation of zinc through organic complexes. The bio-active Zn was coated on urea at 1.5% rates to formulate bio-activated Zn coated urea. Before impregnation/coating on urea granules, the bio-active Zn complex was once again passed through 300-400 mesh size sieves. All the precautions were used and there was no change in the composition of urea. In Zn coated urea strain AZ6 was not added while in Zn blended urea 1.5% Zn (ZnO) was only mix with urea.

Experimental description

The field experiment was conducted with six treatments including T0= control (no Zn), T1= $ZnSO_4$ (Recommended), T2= ZSB (Zinc solubilizing bacteria), T3= 1.5% Zn coated urea, T4= 1.5% bio-activated Zn coated urea, T5= 1.5% Zn blended urea, each treatment was repeated thrice. NPK recommended (180, 115 and 90 kg ha^{-1}) was applied from urea, di-ammonium phosphate (DAP) and sulfate of potash (SOP). Zinc @ 5 kg ha^{-1} was applied. At maturity, rice plants were harvested. Data regarding different growth parameters were noted.

Physiological parameters

Photosynthetic rate (A), transpiration rate (E), stomatal conductance parameters were measured. In the same way efficiency of water use by rice plants was also noted using CIRAS-3 System. Efficiency of water use was calculated as under:

Water use efficiency (A/E) = Photosynthetic rate (A) / Transpiration rate (E)

Method of Arnon (1949) was used for the determination of chlorophyll a and b and carotenoids. Electrolyte leakage was measured by Lutts et al. (1995) and Carbonic Anhydrase Activity Enzyme was detected by method of Dwivedi and Randhawa (1974).

Quality parameters

Oil contents were determined by Soxhelt apparatus. Oil contents were as:

Oil %age = Weight of ether extract / Weight of flour sample x 100

Ash/minerals, dry matter and moisture contents of rice grains were determined by given expressions:

Ash (%) = weight of reside / weight of sample x 100
Dry matter (%) = weight of oven dry sample / weight of sample before drying x 100
Moisture (%) = weight of sample – oven dry weight of sample / weight of sample x 100

Nitrogen content and crude protein from rice grains was determined using methods of Wolf (1982) and Shih et al. (1999), respectively.

Statistical analysis

Software Statistix 8.1 was used for the analysis of variance of all collected data (Steel et al., 1997).

Results and Discussion

The applications of 1.5% bio-activated Zn coated urea significantly (*P<0.05*) increased the chlorophyll contents (both a and b) of rice as compared to 1.5% Zn coated urea and 4.7% increase was observed in case of chlorophyll a and 7.3% in chlorophyll b (Table 1) In case of carotenoids contents 1.5% bio-activated Zn coated urea showed maximum results and it was almost 57% increase as compared to recommended Zn ($ZnSO_4$), while 46 and 31% increase was observed in stomatal conductance as compared to control and recommended Zn ($ZnSO_4$). It was also found that plants receiving 1.5% bio-activated Zn coated urea showed maximum photosynthetic and transpiration rate, the percent increase was 20 and 11.5, respectively obtained with the application of 1.5% bio-activated Zn coated urea as compared to recommended Zn ($ZnSO_4$). Water use efficiency which is the ratio of Photosynthetic and transpiration rate showed statistically significant effect by the application of Zn in the form of Zn coated, blended and bio-activated Zn coated urea All treatments showed statistically significant results as compared to control (*P<0.05*), while 1.5% bio-activated Zn coated urea showed maximum results in case of water use efficiency. Almost similar results were obtained in 1.5% Zn blended and 1.5% Zn coated urea and there was 27.7% increase as compared to control. An increase of 18.7% was noted with the application of ZSB. About 16% increase was observed with 1.5% bio-activated Zn coated urea as compared to Zn ($ZnSO_4$).

In all physiological parameters of rice crop 1 to 15 percent increase was observed with the application of 1.5% bio-activated Zn (ZnO) coated urea as compared to recommended Zn ($ZnSO_4$).

Different methods of Zn application showed statistically significant results (*P<0.05*) as compared to control where no Zn was applied (Table 2). Ash contents (6.7%) were recorded with the application of 1.5% bio-activated Zn coated urea and it was 55%

Table 1: *Comparative effectiveness of zinc blended, zinc coated and bio-activated zinc coated urea with respect to physiological parameters of rice.*

Treatment	Choloro-phyll a (mg g^{-1})	Cholo-rophyll b (mg g^{-1})	Caroti-noides (mg g^{-1})	Stomatal conductance (μmol-^{2}s^{-1})	Sub stoma-tal $CO_{2\,(\mu mol\ mol^{-1})}$	Photosyn-thetic rate (A) (μmol m^{-1} S^{-1})	Transpira-tion rate (E) (mmol H_2O m^{-2} S^{-1})	Water use efficiency (A/E) (μmol m^{-2} S^{-1}/ mmol H_2O m^{-2} S^{-1})
Control	20.5±0.45 e	19±0.5 e	0.5±0.03 b	60.6±0.2 f	370.4±29.8 a	5.4±0.2 d	1.6±0.2 b	2.6±0.2 c
Recommended Zn	40.5±0.2 c	23.4±0.6 c	0.8±0.05 b	88.2±0.2 d	240±11.5 c	9.0±1.2 bc	2.5±0.4 a	3.6±0.2 b
ZSB	29±0.5 d	21.4±0.3 d	0.7±0.2 b	76.6±0.6 e	336.6±3.5 b	7.7±0.2 c	2.3±0.2 a	3.2±0.1 b
1.5% Zn coated urea	42.5±0.5 b	24.4±0.2 b	0.9±0.06 b	101.5±0.3 b	199.3±0.6 d	10.2±0.2ab	2.5±0.3 a	3.6±0.06 b
1.5% bioactivated Zn coated urea	44.5±2.5 a	26.2±0.2 a	1.9±0.2 a	112.6±1.7 a	197±1.2 d	11.2±0.2 a	2.6±0.1 a	4.3±0.2 a
1.5% Zn blended urea	41.5±0.8 bc	24.2±0.1 b	0.9±0.18 b	94.5±0.5 c	241.3±0.7 c	9.4±0.3 b	2.5±0.1 a	3.6±0.3 b
LSD	1.6350	0.3338	0.4097	0.5088	9.9923	1.5031	0.5181	0.4248

Means sharing the same letters within the column do not differ significantly ($P<0.05$). Values shows mean±SE.

Quality parameters

Table 2: *Comparative effectiveness of zinc blended, zinc coated and bio-activated zinc coated urea with respect to quality parameters of rice.*

Treatment	Ash contents (%)	Moisture contents (%)	Dry matter contents (%)	Oil contents (%)	Protein contents (%)	Nitrogen concentration (%)
No Zn	3±0.1 d	9.2±0.04 a	90.6±0.2 c	0.04±0.002 c	11.9±0.6 b	1.9±0.3 c
Recommended Zn	6±0.05 a	6.4±0.09 d	93.4±1.9 a	0.07±0.002 ab	15±1.2 a	2.4±0.2 ab
ZSB	4±0.02 c	8.3±0.2 b	91.7±0.6 bc	0.05±0.002 bc	13±0.6 b	2.1±0.06 bc
1.5% Zn coated urea	6.3±0.02 a	7.3±0.2 c	92.7±0.7 ab	0.08±0.005 a	15±0.3 a	2.5±0.3 a
1.5% bio-activated Zn coated urea	6.7±0.1 a	6±0.09 d	94±1.7 a	0.08±0.005 a	15.7±0.2 a	2.5±0.5 a
1.5% Zn blended urea	5±0.2 b	8.5±0.3 b	91.5±0.8 bc	0.06±0.006 abc	15±0.6 a	2.4±0.2 ab
LSD	0.8277	0.5448	1.5423	0.0279	1.9102	0.3186

Means sharing the same letters within the column do not differ significantly ($P<0.05$). Values shows mean±SE.

control where no Zn was used and minimum in the treatment where 1.5% bio-activated Zn coated urea was applied and percent decrease was observed as 34.7%. Dry matter and oil contents were showed statistically significant effect as compared to control, 3.6 and 50% increase was observed respectively. While the treatments where ZSB, 1.5% Zn coated urea, 1.5% bio-activated Zn coated urea and 1.5% Zn blended urea was applied showed 2.1, 2.4, 2.5 and 2.4% nitrogen contents respectively. Crude protein was also determined; with the application of 1.5% bio-activated Zn coated urea 4.4% increase as compared to recommended Zn ($ZnSO_4$) was obtained. In all above-mentioned quality parameters showed almost 4 to 15 percent increase by the use of 1.5% bio-activated Zn (ZnO) coated urea as compared to Zn ($ZnSO_4$).

Biochemical and yield parameters

Regarding the effect of Zn application in the form of Zn coated, Zn blended, bio-activated Zn coated urea and ZSB (*Bacillus* sp.), a statistically significant (*$P<0.05$*) results in the electrolyte leakage of rice was recorded (Figure 1). The maximum electrolyte leakage was observed in the control where no Zn was applied, and minimum value of electrolyte leakage was noted in 1.5% bio-activated Zn coated urea. In 1.5% bio-activated Zn coated urea 45% decrease as compare to control was observed. And the results presented in Figure 2 showed that the application of Zn significantly increased the carbonic anhydrase activity of rice crop as compared to control (no Zn). Maximum activity in case of carbonic anhydrase was obtained in the treatment where 1.5% bio-activated Zn coated urea was applied (405 umol CO_2/kg/s) and it was 40 and 6.1% increase as compared to control and recommended Zn ($ZnSO_4$), respectively. Figure 3 clearly showed the statistically significant results (*$P<0.05$*) of biomass production (tons ha^{-1}) as compared to control (no Zn). the treatment with 1.5% bio-activated Zn coated urea showed 31% increase as compared to control (no Zn). The treatments with 1.5% Zn coated and 1.5% Zn blended urea was applied showed 14.25 and 14 tons ha^{-1} biomass. With the application of only ZSB (*Bacillus* sp.) the biomass production was 10.25 tons ha^{-1}. Grain's yield (tons ha^{-1}) of rice is very important parameter in this aspect, the application of 1.5% bio-activated Zn coated urea showed maximum results, in this the percent increase was 23.5 as compared to control (no Zn) (Figure 4). On the other hand, the treatment with recommended Zn ($ZnSO_4$) and 1.5% Zn coated urea showed almost similar results i.e., 4.5 tons ha^{-1} grains yield. After that the treatment where 1.5% Zn blended urea was applied showed 4.3 tons ha^{-1} grains yield of rice. Only Zn solubilizing bacteria showed 3.6 while control showed 3.5 tons ha^{-1} grains yield of rice. Almost 1.5 % increase was observed with 1.5% bio-activated Zn (ZnO) coated urea as compared to recommended Zn ($ZnSO_4$).

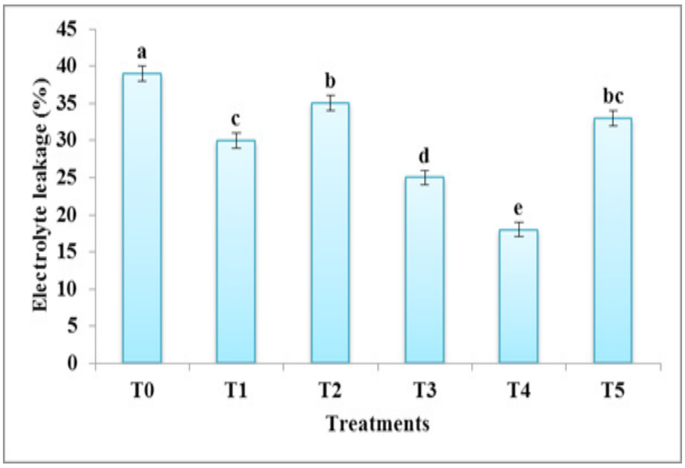

Figure 1: *Comparative effectiveness of different Zn treatments on electrolyte leakage of rice. Bar showing the same letters do not differ significantly (P<0.05) (LSD= 3.7579).*

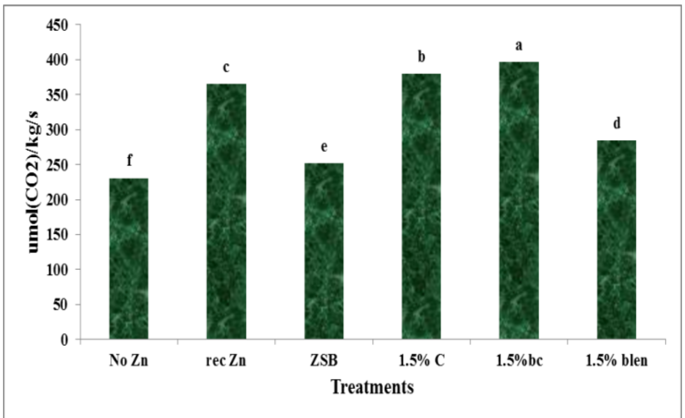

Figure 2: *Comparative effectiveness of different Zn treatments on Carbonic Anhydrase activity of rice Bar showing the same letters do not differ significantly (P<0.05) (LSD=22.771)*

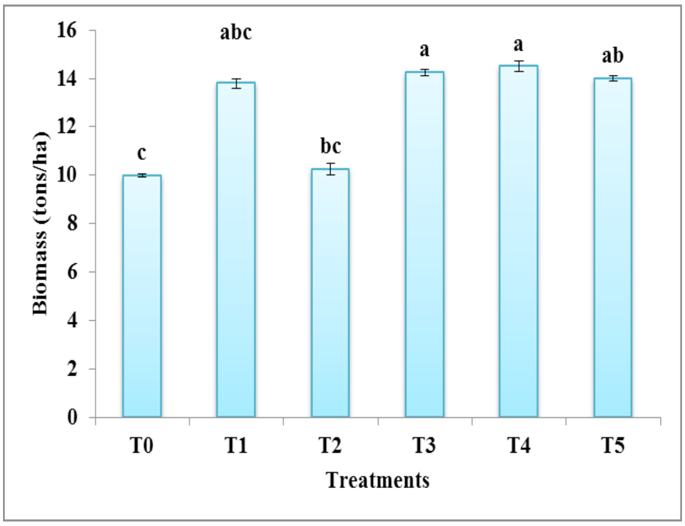

Figure 3: *Comparative effectiveness of different Zn treatments on biomass yield of rice Bar showing the same letters do not differ significantly (P<0.05) (LSD=0.4243).*

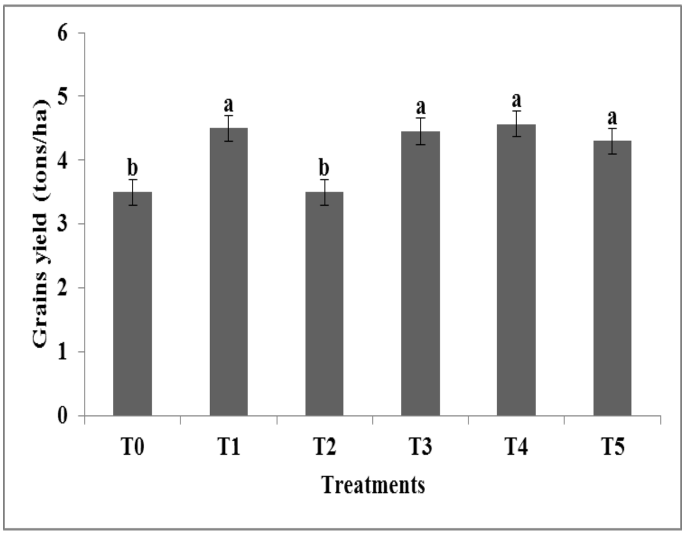

Figure 4: *Comparative effectiveness of different Zn treatments on grains yield of rice Bar showing the same letters do not differ significantly (P<0.05) (LSD= 3.8659.*

Zinc is an essential nutrient required not only for plants but also for humans and microorganisms as well. Humans require Zn throughout their lives to complete the growth, development and physiological functions (Hambidge and Krebs, 2007). Zinc deficiency ranked as the fourth main micronutrient deficiency in humans, it affects approximately 66% of the world's population (Zhang et al., 2011). In Pakistan the current practice to overcome the Zn deficiency is the use of $ZnSO_4$ in soils but its use is problematic for farmers community due to costly and the poor quality available in the market (Shivay et al., 2008). Zinc sulfate contains 33% Zn contents while ZnO has 80% Zn but in insoluble form. The effect of ZnO coating on urea is well documented on growth and yield attributes as compared to the use of Zn instead of coating in rice wheat cropping system. The coated fertilizers such as Zn coated urea have direct contact with plant roots and enhance nutrient availability by reducing its adsorption on clay complexes (Shivay et al., 2008). The application of PGPR (Zn solubilizes) to improve growth and crop yield is an emerging trend in contemporary agriculture in the near future. In the same way bio-activation of Zn insoluble source i.e., ZnO and then coating of this bio-activated Zn (ZnO) on urea also preferred for enhancing Zn bio-availability in soil and for achieving the purpose of bio-fortification of Zn.

A field experiment was conducted to find out the comparative effectiveness of zinc blended, zinc coated and bio-activated zinc coated urea to physiological, quality, biochemical parameters and yield in Rice (*Oryza sativa* L.). For the purpose of bio-activation, pre-isolated and identified bacterial strain (*Bacillus* Sp. AZ6) was used. It is well reported in many previous studies that the application of ZSB in cereals affects the overall growth, yield and grains Zn concentration, because of having the ability to produce the organic acids and many other mechanisms to solubilize the insoluble sources of Zn such as ZnO and $ZnCO_3$ (Saravanan et al., 2003). Prasad and his coworkers reported in 2013 (Prasad et al., 2013) that the major benefit of Zn coated urea is saving in the amount of Zn to be applied, only 2.83 kg Zn ha^{-1} was applied with Zn coated urea as against 6 kg Zn ha^{-1} in the case of soil + foliar application of ZnSO4. The Zn coated urea is therefore a favorable fertilizer in developing countries with small holding farmers (Shivay et al., 2015). In rice crop all physiological parameters were improved with application of bio-activated Zn coated urea. The physiological parameters such as in enzymes especially carbonic anhydrase (CA) activity in which Zn act as cofactor improves significantly with the application of Zn in Zn deficient soils especially in rice cultivated areas (Reed and Graham, 1980). Carbonic anhydrase activity decreases in many plants as a concern of Zn deficit conditions (Gibson and Leece, 1981); CA activity is directly related with the Zn concentration in plants. In severe Zn deficient conditions, no activity of CA was observed (Guliev et al., 1992). For more activity of CA in the mesophyll cells, Zn application is necessary. The activity of CA is an indicator for the levels of physiologically active Zn (Gibson and Leece, 1981). The gaseous exchange characteristics such as photosynthetic rate, respiration rate, stomatal and sunstomatal conductance were improved by bio-activated Zn coated urea. This increase in physiological parameters due to the increase in CA activity. In fact, CA fixes the CO_2 for photosynthesis. The increased rate of photosynthesis automatically increased the all other physiological parameters and biomass of crops, the above-mentioned parameters improved only due to increase in Zn concentration (Escudero-Almanza et al., 2012). The used ZSB having the ability to produce Auxin and as auxin is the reason to increase the root growth of plants, ultimately increase the nutrients uptake (Kamilova et al., 2006). Membrane permeability and electrolyte leakage effects badly due to Zn deficiencies. It is the direct indication of membrane permeability. With the application of Zn the decrease value of electrolyte leakage was observed but maximum reduction in electrolyte leakage was observed in the plot which receives 1.5% bio-activated Zn coated urea (Welch, 1995). the quality parameters like proteins, nitrogen concentration in grains, oil, ash and dry matter contents in rice and wheat were evaluated and it was observed that these parameters also improved significantly with the application of Zn and maximum increase was observed with 1.5% bio-activated Zn coated urea (Table 2), these results showed similar findings by Seadh et al. (2009); and Soleymani et al. (2009). Increase in quality parameters is just due to the contribution of Zn in photosynthesis, chlorophyll, starch metabolism of starch enzyme carbonic anhydrase activity and formation, carbohydrate formation, the requirement of Zn depends upon the above-mentioned processes in plants. It also starts glutamic dehydrogenase activity, RNA and DNA synthesis which are main protein components of gluten accumulated in the later stages of grain filling (Singh et al., 2012; Soleymani et al., 2009). As N and Zn have synergistic effect so, with the proper application of Zn improved N concentration in grains were obtained (Rehman et al., 2002). The dry matter (%) increases and moisture (%) decreases with Zn fertilization and these results are in agreement with the Sowokinos and Preston, (1988). The oil contents in the cereals also increases with the application of proper Zn (Ikenie et al., 2004). The yield parameters such as grain yield and biomass production increased significantly with the application of Zn and improved results were obtained with 1.5% bio-activated Zn coated urea (Sadras, 2007). Grain's production is an important parameter contributing towards yield, with the application of Zn a significant effect on grain yield in rice was observed. But according to some scientists in the recent released varieties due to having more yield potential and dilution effect the overall minerals contents becomes low (Zhao et al., 2009), due to dilution effect the starchy grain endosperm enhances in size and becomes rich in minerals as compared to other parts. Many direct and indirect mechanisms are involved to improve the micronutrients availability, root growth improvement is one of the main mechanisms (Khalid et al., 2004). Due to increase in root growth the nutrients availability also increases and over all plant vigor and root-shoot growth enhances. As in grains the Zn concentration increases, this might be due to the pH reduction in rhizosphere, reduction in rhizospeheric pH increases the micronutrients availability to plants (Yu et al., 2011). This reduction in pH is due to the organic acids produced by the ZSB used for the bio-activation process. Zinc application is necessary for the proper yield of cereals.

Conclusions and Recommendations

Zinc application in the form of 1.5% bio-activated Zn (ZnO) coated urea has a significant effect on physiological, quality, biochemical and yield parameters of rice crop. The grains yield (tons/ha) also enhance in this way. The use of Zn and urea separately increases the labor cost but with the use of this strategy of coating the extra labor cost and farmers ignorance to Zn use can be minimized. ZnO contains more Zn contents as compare to other Zn sources. Bio-activation of the insoluble Zn contents makes it soluble and easily available for plants. The bio-activation by ZSB and then coating on the urea is an environmentally friendly approach and the purpose of improving quality of grains also achieved successfully in rice. This approach of Zn supply to plants is novel due to eco-friendly, less costly and less time consuming as compared to the others. The farmers of poor community can get maximum benefit by bio-activated Zn (ZnO) coated urea from their limited resources. It can be concluded that for the cereals grown on the Zn deficient sites 1.5% bio-activated Zn (ZnO) coated urea is effective not only for the increase the yield of cereals grains but also improves grains quality.

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Novelty Statement

Zinc coated urea is effective for cereals under Zn deficient soils

Author's Contribution

Qudsia Nazir: Conception and design of work and conduction of experiment
Muhammad Aftab: Interpretation of data and excel work for graphs making
Ghulam Sarwar: Overall supervision and guidance about manuscript write up
Aneela Riaz: Interpretation of data and excel work for graphs making
Sarfraz Hussain: Final editing and proof reading
Ifra Saleem and Amina Kalsom: Helped in lab. work
Noor-us-Sabah: Elaborated results and discussion
Mukkram Ali Tahir: Participated in materials and methodology portion
Ateeq-ur-Rehman: Participated in introduction portion
Muhammad Arif: Statistical analysis of data

Conflict of interest
The authors have declared no conflict of interest.

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