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Full Length Article

Influence of Zinc Impregnated Urea on Growth, Yield and Grain Zinc in Rice (*Oryza sativa*)

Qudsia Nazir[1,*], Muhammad Arshad[1], Tariq Aziz[1] and Muhammad Shahid[2]
[1]*Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad, 38040, Pakistan*
[2]*Department of Biochemistry, University of Agriculture Faisalabad-38040, Pakistan*
[*]For correspondence: sbqnazir@gmail.com

Abstract

Zinc (Zn) is critically required by plants, animals and human beings. About one third of total world's poor population is at the high risk of Zn deficiency because they rely on cereals for their daily caloric intake. By keeping in mind this scenario it is hypothesized that the use of ZnO (a cheap source of Zn) impregnated urea for rice may enhance Zn contents in grains. Three types of urea were prepared including bio-activated Zn coated, Zn coated and Zn blended urea. 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 three rates to formulate 0.5, 1 and 1.5% bio-activated Zn coated urea. Moreover, Zn blended urea was prepared by mixing powder ZnO with urea. The comparative efficacy of different types of Zn impregnated urea were compared with $ZnSO_4$ to enhance growth, yield and grains Zn concentration of rice grown in pots. The results showed that 1.5% bio-activated Zn (ZnO) coated urea performed better in promoting growth, yield and grain Zn content than other treatments. About 15 to 20% increase was observed in yield and grain Zn concentration. This suggests that the application of 1.5% bio-activated Zn coated urea is highly effective in enhancing growth, yield and quality of rice.

Keywords: Zn impregnated urea; Zn contents in grains; Phytate; Zn; Rice

Introduction

Population of the world is increasing day by day, therefore, food demand is also increasing, while the natural resources are limited (United Nations, 2012). On the other hand, malnutrition is a very popular issue of poor community due to reduced bioavailability, micronutrients deficiency is also severe threat among them (Huang *et al.*, 2002). In these areas Zn deficiency is the fifth largest cause of deaths and disorders (WHO, 2002). Its deficiency is responsible for about 16% of respiratory disorders, 10% of diarrhea and 18% malaria with 800,000 deaths annually in the developing countries. Its deficiency also affects the immune system, normal reproductive system and normal cell growth and causes skin disorders and cancer (WHO, 2002). Almost 37% of Pakistani population is suffering in Zn malnutrition (UNDP, 2003; Jamil *et al.*, 2015). Zinc application to soil not only increases the growth and yield of plants but also improves overall vigor and plant pigments e.g., sugars and oil contents (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 applied Zn fertilizers in soils, the use of Zn in soils is ignored by farming community. By keeping in view the importance of Zn for humans, strategies must be employed to increase Zn bioavailability.

Millions of people in the world feeding on cereals like wheat and rice (FAO, 2012). After wheat, rice is second most important cereal and staple crop grown in Pakistan. It contributes about 0.7 percent in GDP. During 2013–2014 rice was grown on an area of 2789 thousand hectares with 6798 thousand tones production (Pakistan Economic Survey, 2013–2014). As in Pakistan soils are deficient in Zn because soils having more $CaCO_3$ contents and less organic matter, high soil pH (Kiekens, 1995) 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 in severe Zn deficiency.

For this purpose, many strategies have been employed including supplementation (nutrients as clinical treatment), fortification (add particular nutrient in food items), food modification/diversification (cooking and processing of food on nutritional point of view) and bio-fortification which is a process of enhancing the bioavailable nutrient contents in the edible portion of crops (Mayer, 2008). Zinc bio-fortification can be done by various ways such as genotype selection and improvement. This can be achieved

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by using genetic engineering and conventional breeding methods. However, this is a time consuming method. On the other hand, fertilizer management is also an effective approach, the most commonly used source of Zn in Pakistan is $ZnSO_4$, but due to its high cost and not availability in time, farmers are reluctant to use it. ZnO is an effective source due to containing 80% Zn contents but in insoluble form. This insoluble Zn can be solubilizing by ZSB (Zinc solubilizing bacteria). By keeping in mind the above mentioned facts a pot experiment was conducted on rice to check the effect of ZnO coated, blended and bio-activated Zn coated urea on growth, yield and Zn bio-fortification in rice.

Materials and Methods

Physico-chemical Characteristics of Soil

A pot experiment was conducted in the wire house of the Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad, Pakistan to evaluate the comparative effectiveness of three types of urea viz., Zn blended, Zn coated and bio-activated Zn coated urea for improving the growth, yield and Zn content in edible portion of rice (Cultivar: Shaheen). Soil was air dried, ground and after passing through 2 mm sieve it was mixed thoroughly and the pots were filled with 12 kg soil. Soil used in pots was analyzed for physic-chemical characteristics: texture, sandy clay loam (sand, 51.2%; silt, 29.6%; clay, 19.2%) (Moodie *et al*., 1959); pH, 7.9 (U.S. Salinity Laboratory Staff, 1954); EC, 1.41 dS m^{-1} (U.S. Salinity Laboratory Staff, 1954); organic matter, 0.68% (Moodie *et al*., 1959); total nitrogen, 0.06% (Jackson, 1962); available phosphorus, 8.79 mg kg^{-1} (Watanabe and Olsen, 1965); extractable potassium 84 mg kg^{-1} (U.S. Salinity Laboratory Staff, 1954); and plant available Zn, 0.65 mg kg^{-1} (Soltanpour and Workman, 1979).

Preparation of Bio-activated Zn Coated, Zn Coated and Zn Blended Urea

Pre-isolated Zn solubilizing bacterial strain *Bacillus* sp. AZ6 (accession number KT221633) (Hussain *et al.,* 2015) was taken from Environmental Sciences Laboratory, Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad, Pakistan. Hussain *et al.* (2015) were isolated zinc solubilizing bacteria from the rhizosphere of the maize by using dilution plate technique on nutrient agar medium. The inoculum of the strain AZ6 was prepared by growing it in 1000 mL conical flask containing Bunt and Rivera basal medium (Bunt and Rovira, 1955). The inoculated flasks were incubated at 28 ± 1^0C for 72 h in the orbital shaking incubator at 100 rpm. Before use, an optical density of 0.5 at 535 nm was adjusted [10^8–10^9 colony-forming units (cfu) mL]. This suspension of the bacterial strain was used for urea coating for formulation of bio-activated Zn coated urea. The powder organic material (plant residues) was first dried in an oven at 80^0C. It was inoculated with bacterial strain AZ6 and incubated for 72 h at 30 ± 2^0C 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 ± 2^0C to achieve maximum chelation of Zn with organic complexes. The bio-active Zn was coated on urea at three rates to formulate 0.5, 1 and 1.5% bio-activated Zn coated urea. Before impregnation/coating on urea granules, the bio-active Zn complex was once again passed through a 300–400 mesh size sieves. All the precautions were used and there was no change in the composition of urea.

Experimental Description

The pot experiment was conducted with twelve treatments including T0= control (no Zn), T1= $ZnSO_4$, T2= ZSB, T3= 0.5% Zn coated urea, T4= 1% Zn coated urea, T5= 1.5% Zn coated urea, T6= 0.5% bio-activated Zn coated urea, T7= 1% bio-activated Zn coated urea, T8= 1.5% bio-activated Zn coated urea, T9= 0.5% Zn blended urea, T10= 1% Zn blended urea and T11= 1.5% Zn blended urea. Three seedlings were transplanted per pot and treatments were arranged according to completely randomized design (CRD), each treatment was repeated thrice. Recommended dose of NPK (180, 115 and 90 kg ha^{-1}) was applied by using urea, di-ammonium phosphate (DAP) and sulfate of potash (SOP). Zinc was applied at the rate of 5 kg ha^{-1}. Tap water was used for irrigation.

The crop was harvested at maturity and data regarding growth (plant height, root length, fresh shoot biomass, fresh root biomass, dry root biomass, dry shoot biomass), yield (100 grains weight and total grain yield per pot) and plant Zn and grains Zn and phytate contents were taken.

Measurement of Zn in Root Shoot and Grains of Rice

Air dried ground material (1 g) was placed in the digestion flask. 10 mL of nitric acid and perchloric acid (2:1 ratio) was added into it and let it stand overnight. Next day samples were heated on the hot plate carefully until the production of red NO_2 fumes has ceased. The flasks were cooled down and then added a small amount (2–4 mL) of 70% $HClO_4$. The samples were heated again and allowed evaporating to a small volume. When the vapors were condensed, contents of the flask were transferred to 50 mL volumetric flask and volume was made with distilled water. Each batch of digestion samples contained two reagent blanks (no plant material). Samples were then filtered and used for determination of Zn by atomic absorption spectrometry.

Phytic Acid Contents in Grains of Rice

The grains phytic acid content was determined by the modified colorimetric procedure (Wade reagent) as described by Gao *et al*. (2007). The half gram sample of rice flour was thoroughly mixed with 2.4% HCl and shaken for 16 h and centrifuged at 9600 rpm for 10 min. The crude extracts were then transferred to another centrifuge tube containing 1 g NaCl. The contents were shaken for 20 min and 1 mL of clear supernatant was diluted to 25 mL with distilled water. Three milliliters of the diluted sample was combined with 1 mL of Wade reagent (0.03% $FeCl_3.6H_2O$ + 0.3% sulfosalyclic acid) and contents were read by spectrophotometer. The concentration of phytate and Zn in rice grains was used to calculate Phytate: Zn ratio.

Statistical Analysis

The data recorded were subjected to analysis of variance using computer software Statistix v. 8.1 (Analytical Software, USA). The treatment means were compared by least significant difference test (Steel *et al*., 1997).

Results

Growth Parameters

Table 1 shows the effect of different levels of Zn coated, Zn blended and bio-activated Zn coated urea on the growth parameters (plant height, no. of tillers per plant, panicle length, total shoot biomass, and dry biomass per pot) of rice grown in pots. It was found that all growth parameters were significantly ($P<0.05$) increased under different levels of Zn coating, blending and bio-activation of Zn. But the interactive effect of ZSB and ZnO in the form of 1.5% bio-activated Zn coated urea showed best results in all above mentioned parameters the maximum value (138, 11, 26, 77 and 23.5, respectively) was recorded. As a comparison, in plant height, no. of tillers per plant, panicle length, total shoot biomass and root dry biomass 26, 18, 19.2, 10.7 and 14.8% increase was observed with the application of 1.5% bio-activated Zn coated urea as compared to recommended Zn (ZnSO4). After that the treatment where 1.5% Zn coated urea was applied showed 97 cm plant height, 10 no. of tillers per plant, 23.33 cm panicle length, 73.7 g shoot biomass and 21.7 g root dry biomass, respectively it was 10.6, 50, 37, 28 and 31% increase as compared to control (no Zn). While in the pots where 1.5% Zn blended urea was used showed 28.5, 54.5, 31, 27 and 23% increase with respect to control. The treatment where only ZSB was applied showed 8% increase in case of plant height, 22% in panicle length, 28% in no. of tillers per plant, 6.5% in shoot biomass and 15.8 in root dry biomass, respectively as compared to control (no Zn). From the results mentioned regarding all growth parameters, it was noticed that in all treatments three levels of bio-activated, coating and blended urea i.e., 1.5% showed maximum results. 1.5% bio-activated Zn (ZnO) coated urea performed better even from recommended Zn ($ZnSO_4$).

Yield Parameters

The effect of Zn application in the form of bio-activated Zn coated, Zn coated and Zn blended urea on the 100 grains weight of rice was observed (Fig. 1). The treatment in which 1.5% bio-activated Zn coated urea was applied showed 38.2% increase as compared to control where no Zn was applied, and the results were statistically non-significant as compared to recommended Zn ($ZnSO_4$). In the control, 100 grains weight was (1.19 g) and after that the treatment in which 1.5% Zn coated urea and 1.5% Zn blended urea was applied showed 3.05 g and 2.79 g, respectively was observed in case of 100 grains weight. The percent increase with respect to control was statistically 8.6%.

Comparative effectiveness of various Zn sources such as ZSB, $ZnSO_4$ and Zn (ZnO) coated, blended and bio-activated coated urea on total paddy yield per pot is evident from Fig. 2. It was observed that the treatment in which 1.5% bio-activated Zn coated urea was applied showed maximum response and there was 59% increase as compared to control (no Zn). In the control total paddy yield was (4.6 g) and after that the treatment in which 1.5% Zn coated urea and 1.5% Zn blended urea was applied showed 10.33 g and 10.25 g yield respectively. The effect of ZSB with respect to control was statistically non-significant ($P<0.05$), in case of this parameters. Statistically significant effect of the 1.5% bio-activated Zn coated urea was noted as compared to all other treatments. Almost 12% increase was noted with the use of 1.5% bio-activated Zn coated urea as compared to separate use of $ZnSO_4$ and urea.

Root, Shoot and Grain Zn and Phytate Contents of Rice

Table 2 shows the data related to Zn concentration in root, shoot and grains of rice affected by different Zn treatments. It was found that 1.5% Zn coated urea and 1.5% blended urea significantly improved zinc acquisition ranged 32 to 40% in root and shoot of rice crop as compared to control (No Zn). While in grains the Zn contents were increase 42 to 46% with these two treatments. The application of zinc solubilizing bacterial strain AZ6 showed minimum increase i.e. 12.5, 29.5 and 20% increase was found in root, shoot and grains Zn concentration, respectively as compared to control. On the other hand, the pots which received 1.5% bio-activated Zn (ZnO) coated urea showed maximum increase in Zn acquisition in root, shoot and grains i.e. 9.3, 13 and 18% increase, respectively as compared to recommended Zn ($ZnSO_4$).

Data regarding phytate concentration in grains showed the reducing trend with the application of Zn. The maximum reduction in phytate contents were observed with the application of 1.5% bio-activated Zn (ZnO) coated urea i.e. 326.7 $\mu g\ g^{-1}$. While in the control (no Zn) 1100 $\mu g\ g^{-1}$ phytate contents were observed. Almost 40% decrease was observed with 1.5% bio-activated Zn (ZnO) coated urea as compared to recommended Zn ($ZnSO_4$).

Table 1: Effect of different treatments of zinc impregnated urea on the growth parameters of rice

Treatments	Plant height (cm)	No. of tillers per plant	Panicle length (cm)	Shoot bio-mass (g/pot)	Dry root biomass (g/pot)
Control (No Zn)	86.7 i	5 f	14.7 h	53.0 f	14.9 g
$ZnSO_4$	102.0 ef	9 bc	21.0 ef	68.7 c	20.0 c
ZSB	95.0 h	7 e	18.0 g	56.7 e	17.7 e
0.5% Zn coated urea	101.0fg	8 d	19.7 f	57.0 e	15.9 f
1% Zn coated urea	114.0 d	10 b	21.4 de	62.0 d	17.2 e
1.5% Zn coated urea	97.0 gh	10 b	23.4 bc	73.7 b	21.7 b
0.5% bio-activated Zn coated urea	103.0 e	8 d	20.0 ef	58.0 e	17.3 e
1% bio-activated Zn coated urea	122.0 b	10 b	24.4 b	66.0 c	19.3 d
1.5% bio-activated Zn coated urea	138.0 a	11 a	26.0 a	77.0 a	23.5 a
0.5% Zn blended urea	104.0 e	7 e	19.7 f	59.0 e	15.8 f
1% Zn blended urea	118.0 c	9 cd	22.0 cd	66.7 c	18.8 d
1.5% Zn blended urea	121.4 b	11 a	21.4 de	73.0 b	19.4 cd
LSD	*3.1275*	*0.8882*	*1.5888*	*2.9188*	*0.6864*

Data are shown as mean of three replicates; Values showing different alphabets within the column are statistically ($p \leq 0.05$) different from others ZSB: Zn solubilizing bacteria

Table 2: Effect of different treatments of zinc impregnated urea the root, shoot and grain Zn and phytate concentration of rice

Treatments	Root Zn concentration ($\mu g\ g^{-1}$)	Shoot Zn concentration ($\mu g\ g^{-1}$)	Grain Zn concentration ($\mu g\ g^{-1}$)	Grain Phytate concentration ($\mu g\ g^{-1}$)	Phytate/Zn
No Zn	14.5 f	9.45 f	17.4 k	1100 a	63.15 a
Recommended Zn ($ZnSO_4$)	22.8 b	15.67 b	35.08 c	436.67 f	12.5 g
ZSB	16.58 e	13.42 d	21.83 j	900 d	41.22 c
0.5% Zn coated urea	17.08 e	13.48 d	24.5 h	1095 a	44.69 b
1% Zn coated urea	18.58 d	14.32 c	28.5 f	1009.3 bc	35.41 d
1.5% Zn coated urea	21.62 c	15.8 b	32.5 d	400 fg	12.5 g
0.5% bio-activated Zn coated urea	17.5 e	12.08 e	39.8 b	1066.7 b	26.75 ef
1% bio-activated Zn coated urea	21.16 c	14.4 c	42.5 ab	736.67 e	17.33 f
1.5% bio-activated Zn coated urea	25.16 a	18 a	43 a	326.67 g	7.59 i
0.5% Zn blended urea	16.83 e	12.08 e	22.5 i	960 cd	42.67 bc
1% Zn blended urea	18.81 d	13.4 d	27.58 g	766.67 e	27.79 e
1.5% Zn blended urea	21.5 c	14.7 c	30.5 e	426.67 f	13.98 h
LSD	*0.9657*	*0.6100*	*0.7626*	*76.570*	*2.5029*

Data are shown as mean of three replicates; Values showing different alphabets within the column are statistically ($p \leq 0.05$) different from others; ZSB: Zn solubilizing bacteria

The Phytate/Zn ratio was decrease due to the reduction in phytate contents with the application of Zn. In case of phytate/Zn recommended Zn ($ZnSO_4$) and 1.5% Zn coated urea showed almost similar results and 12.5 value was observed. Maximum reduction in ratio was observed where 1.5% bio-activated Zn (ZnO) coated urea was applied as compared to control and recommended Zn ($ZnSO_4$).

Discussion

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 roots and maximum availability due to less adsorption on clay complexes (Shivay *et al.,* 2008). The use of PGPR (Zn solubilizers) to enhance plant growth and crop yield is predicted to become 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 prefered for enhancing Zn bio-availability in soil and for achieving the purpose of bio-fortification of Zn.

A pot experiment was conducted to find out the best level of bio-activated ZnO coated, ZnO coated and ZnO blended urea. For the purpose of bio-activation, pre-isolated and identified bacterial strain (*Bacillus Sp.* AZ6) was used. It has been 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}

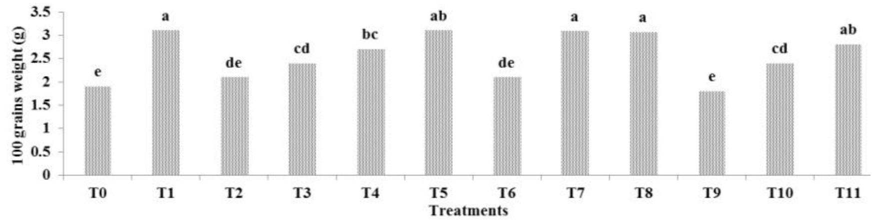

Fig. 1: Effect of different treatments of zinc impregnated urea on 100 grains weight (g) of rice
Bar sharing the same letters do not differ significantly ($p<0.05$) (LSD= 0.3961)
Treatments Description: T0= No Zinc; T1= Recommended $ZnSO_4$; T2= ZSB (Zinc solubilizing bacteria); T3= 0.5% Zn coated urea (ZnO); T4= 1% Zn coated urea (ZnO); T5= 1.5% Zn coated urea (ZnO); T6= 0.5% bio-activated Zn coated urea (ZnO); T7= 1% bio-activated Zn coated urea (ZnO); T8= 1.5% bio-activated Zn coated urea (ZnO); T9= 0.5% Zn blended urea (ZnO); T10= 1% Zn blended urea (ZnO); T11= 1.5% Zn blended urea (ZnO

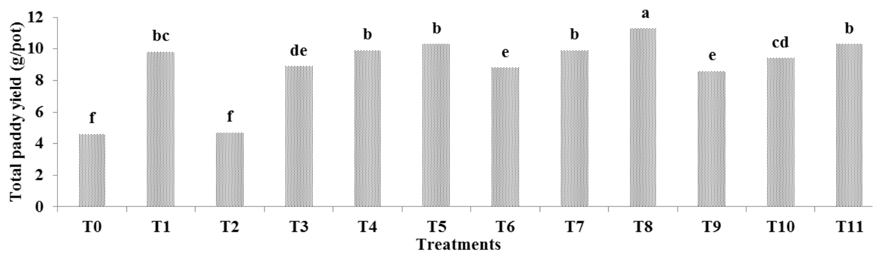

Fig. 2: Effect of different treatments of zinc impregnated urea on total paddy yield per pot
Bar sharing the same letter(s) do not differ significantly ($p<0.05$) LSD for total paddy yield= 0.5168
Treatments Description: T0= No Zinc; T1= Recommended $ZnSO_4$; T2= ZSB (Zinc solubilizing bacteria); T3= 0.5% Zn coated urea (ZnO); T4= 1% Zn coated urea (ZnO); T5= 1.5% Zn coated urea (ZnO); T6= 0.5% bio-activated Zn coated urea (ZnO); T7= 1% bio-activated Zn coated urea (ZnO); T8= 1.5% bio-activated Zn coated urea (ZnO); T9= 0.5% Zn blended urea (ZnO); T10= 1% Zn blended urea (ZnO); T11= 1.5% Zn blended urea (ZnO)

was applied with Zn coated urea as against 6 kg Zn ha^{-1} in the case of soil + foliar application of $ZnSO_4$. The Zn coated urea is therefore a favorable fertilizer in developing countries with small holding farmers (Shivay *et al.*, 2015). In pot experiment (rice) 61% increase in total paddy yield was obtained as compared to control (no Zn) while the increase in grains Zn concentration was 46% in the treatment, where 1.5% bio-activated Zn coated urea was applied these results are in agreement with the results of Yilmaz *et al.* (1997), they reported the clear cut increase in grains Zn concentration by the application of Zn in alkaline calcareous soils. The reason of this response is the application of Zn and ZSB in combined form, the alkaline calcareous soils are deficient in plant available Zn. So, the Zn application response is generally observed in such soils (Alloway, 2008). On the other hand, Zn application in the form of ZnSO4 increased Zn concentration in different parts of plant. While different scientists reported that the calcareousness of soils (like Pakistan) reduces Zn phytoavailability (Alloway, 2009; Hussain *et al.*, 2011). According to some authors the wheat varieties which are cultivated now-a-days are low in grains Zn concentration as compared to those which were cultivated in early nineties. So, the rapid solution of Zn deficiency among humans is application of Zn to soil or sometimes the breeding approaches becomes necessary (Cakmak, 2008; Fan *et al.*, 2008). The yield parameters such as grains yields 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). The number of grains per pot is an important parameter contributes towards yield, with the application of Zn a significant effect on grain yield in rice was observed (Zhao *et al.*, 2009; Imran *et al.*, 2015). Due to increase in root growth with Zn application the nutrients availability also increases and over all plant vigor and root-shoot growth also increases (Khalid *et al.*, 2004). Zinc application is necessary for the proper growth and yield of cereals.

Conclusion

Zinc application in the form of 1.5% bio-activated Zn (ZnO) coated urea has a significant effect on growth, yield and Zn acquisition in root, shoot and grains of rice crop. The Zn contents also enhance and phytate contents reduce 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 environmental friendly approach and the purpose of Zn bio-fortification 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 of Zn contents in cereals grains but also to fulfills the humans requirements.

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