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RESEARCH ARTICLE

Enhanced Zinc Nutrient and Enzyme Activity of Rice Crop by Zinc Solubilizing Bacteria with Zn sources in Zn Deficient Rice Soil

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ABSTRACT

A pot experiment was conducted during 2017 at Department of Soil Science and Agricultural Chemistry, Tamil Nadu Agricultural University, Coimbatore to study the effect of Zinc Solubilizing Bacteria (ZSB) and different Zn sources on zinc nutrient and enzyme activity of rice crop in a Completely Randomized Block design. The treatments consisted of four zinc sources (No zinc, $ZnSO_4$, ZnO and Zn-EDTA) and with and without ZSB inoculation. Beneficial effects of ZSB and Zn sources on Zn content and uptake in above ground and root at critical growth stages and enzyme activity at panicle initiation stage were observed, with significant increases in all the determined parameters, in comparison with the control. Among the Zn sources, $ZnSO_4$ @ 25 kg ha^{-1} significantly increased the Zn content in rice grain (32.6 and 42.2 mg kg^{-1}), straw (25.9 and 30.2 mg kg^{-1}), above ground (29.0 and 36.3; 33.5 and 38.4 mg kg^{-1} at active tillering and panicle initiation stage respectively) for without and with ZSB respectively. The root Zn content decreased with rice growth stages and ZSB with $ZnSO_4$ recorded the highest Zn content of 44.0, 39.5 and 34.8 mg kg^{-1} at active tillering and panicle initiation and harvest stage respectively compared with other Zn sources. The application of $ZnSO_4$ with ZSB markedly increased the above ground shoot Zn uptake (0.41 and 1.73 at active tillering and panicle initiation stage respectively), root Zn uptake (0.48, 0.54 and 0.74 g plant^{-1} at active tillering, panicle initiation and harvest stage respectively), grain and straw Zn uptake (1.96 and 1.32 g plant^{-1}) compared to ZnO and Zn-EDTA. The activity of Zn requiring enzymes viz., superoxide dismutase (SOD) and carbonic anhydrase (CA) also significantly increased by the application of $ZnSO_4$ with ZSB (232 and 12.15 EU mg^{-1} FW) followed by ZnO with ZSB (124 and 10.94 EU mg^{-1} FW) and Zn-EDTA with ZSB (120 and 10.56 EU mg^{-1} FW).

Keywords: *Rice, Zn sources, ZSB, Zn content, enzyme activity, SOD, CA.*

INTRODUCTION

Micronutrients are important for the optimum growth and productivity of crop plants. Though these elements are required in lesser amounts, they are very important for plant development and profitable crop production because they work ‘behind the scene’ as activators of many plant functions (Sunitha kumari *et al.,* 2014). After nitrogen (N), phosphorus (P) and potassium (K), widespread zinc (Zn) deficiency has been found responsible for yield reduction in many crops particularly rice. Zinc is an important component of various enzymes that are responsible for driving many metabolic reactions in plants. In addition, zinc is a co-factor of over 300 enzymes and proteins involved in cell division, nucleic acid metabolism and protein biosynthesis (Marschner, 1986). Indian soils are generally low in zinc (Zn) and 49 per cent soil samples of the country have been found to be Zn deficient (Shukla and Behera, 2011). Zn deficiency is one of the most prevalent nutritional constraints in crop plants, especially cereals. Cereal based diet system especially prone to Zn deficiency, which affects about two billion people (Sperotto *et al.,* 2012). People in the developing countries are at great risk as they are not able to meet the required zinc due to deficiency in plants. In Asia and Africa, it is estimated 500-600 million people are at risk for low zinc intake, while, in developed countries, nearly seventy per cent of the micronutrients are supplied by meat and dairy foods. Cereals are the most important staple crops being the major source of vitamins, minerals and rare amino acids. But cereals in general, contain a low level of micronutrients, most of which is lost during processing for food or feed (Cheng and

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Hardy, 2003). Zinc deficiencies also result in the inability of the rice plant to support root respiration during flooded conditions (Slaton *et al.,* 2005). To ameliorate Zn deficiency, Zn fertilizers are being used but its availability to the plant is less due to very high fixation in soils. The fertilizer-use-efficiency of inorganic Zn sources is very low and on the other hand, synthetic chelated-Zn compounds are a source of readily available Zn (Mortvedt *et al.*, 1972), but their application is limited because they are expensive. It is documented that soil microflora had good potential to improve the availability of nutrients in the soil through various mechanisms. Several bacterial species belonging to *Pseudomonas*, *Bacillus*, *Gluconacetobacter, Burkholderia* and *Acinetobacter* have been already reported to solubilize soil zinc (Fasim *et al.*, 2002; Saravanan *et al.*, 2007). Zinc solubilization can be achieved by a range of mechanisms through excretion of metabolites such as organic acids, proton extrusion, or production of chelating agents (Vaid *et al.*, 2013). In this context, the use of beneficial rhizosphere microorganisms as bio-inoculants in combination with zinc sources to increase the availability of zinc to crop nutrition has become imperative and with this background, the present study was carried out to find out the effect of Zn sources and ZSB on root, straw and grain Zn content, uptake and enzyme activity of rice crop in a Zn deficient soil.

MATERIAL AND METHODS

A pot experiment was conducted at Department of Soil Science and Agricultural Chemistry, Tamil Nadu Agricultural University, Coimbatore during 2017 to study the effect of ZSB on zinc content and uptake by root, shoot, grain and enzyme activity of rice crop. A bulk soil sample collected from the field at Anaimalai block, Coimbatore was processed and eight kilogram of soil was weighed and placed into the pots. The experimental soil was low in available N (226 kg ha^{-1}), high in P (27.4 kg ha^{-1}) and K (620 kg ha^{-1}). The soil was deficient in DTPA-Zn (0.82 mg kg^{-1}) and sufficient in other micronutrients such as 1.68 mg kg^{-1} for DTPA Cu, 15.52 mg kg^{-1} for DTPA Fe and 6.66 mg kg^{-1} for DTPA Mn respectively.

Treatment Details

The experiment was carried out in a completely randomized design with three replications. Treatments consisted of four zinc sources *viz.,* control, $ZnSO_4$ @ 25 kg ha^{-1}, ZnO @ equivalent to Zn in $ZnSO_4$ @ 25 kg $ha^{-1}$and Zn-EDTA @ 5 kg ha^{-1} and with Zinc Solubilizing Bacteria 20 ml (10^8 cfu) per pot having 8 kg soil and without ZSB. The bacterial culture was isolated from maize rhizosphere soils of maize crop and the isolate identified as *Bacillus Cereus* (IMTECH, Chandigarh, Ref. No. RC 2096-ZSB SM-1). The pots were replicated thrice and maintained at the submerged condition. Five seeds of rice (CO 51) were sown per pot and 10 days after germination, three robust seedlings per pot were maintained. Recommended levels of NPK (150: 50: 50 kg ha^{-1}) was given in solution form uniform to all pots. As a basal dose 50 per cent of N and K, full dose of P were applied 10 days after sowing, while 50 per cent of the remaining N and K was given at active tillering and panicle initiation stages. Zn sources *viz.,* $ZnSO_4$, ZnO, Zn-EDTA were applied as per treatments after the application of basal dose of NPK. ZSB inoculum was applied in liquid form as per treatments after application of basal dose of NPK.

Bacterial Strain and Culture Condition

Bacillus cereus strain ZSB SM-1, isolated and characterized from the rhizosphere of maize, capable of solubilizing insoluble compounds *viz.,* ZnO (20.62 µg/ml of soluble Zn), $ZnCO_3$ (17.03 µg/ml) and $Zn_3(PO_4)_2$ (8.89 µg/ml) was used for this study. The strain was routinely grown in Bunt and Rovira medium containing 0.1% ZnO with and without agar (1.5%) at 30°C in an incubator (Lab Companion, USA).

Analysis of Soil and Plant Samples

The soil sample used for conducting the study was air dried, broken with a wooden mallet and sieved through 2mm sieve. The characteristics of the initial soil sample was analysed for various properties using standard procedure (Table 1) and the initial physico chemical properties of the experimental soils are given in Table 2. The plants were uprooted at critical stages viz., active tillering, panicle initiation and harvest stages from all the treatments. The plant sample were washed with water to remove the adhering soil particles then with dilute 1:1 HCl and water to remove the stain, air dried and oven dried 65^0 C for 72 hours. The dry biomass was recorded and the samples were separated into shoot and root, ground in a wiley mill and analyzed Zn content in plant samples using triacid extract (Table 1) with the help of Atomic absorption spectrophotometer (GBS Scientific, Australia). Grain samples were collected separately and analysed for Zn content. The fresh plant samples collected at panicle initiation stage were analysed for enzyme activity of Superoxide dismutase and Carbonic anhydrase (Table 1). The data obtained from the present investigation was subjected to the analysis of variance to find out the significance as suggested by Panse and Sukhatme (1985).

RESULTS AND DISCUSSION

In this study, zinc nutrient was significantly affected by bacterial inoculation with Zn treatments over Zn fertilizers alone.

Zn content and Uptake

Effect of ZSB and different Zn sources on Zn content and uptake of above ground plant

The application of Zn sources and ZSB influenced the Zn content by rice crop at different growth stages (Fig 1). The application of $ZnSO_4$ + ZSB registered the highest Zn concentration (36.3 and 38.4 mg kg^{-1}) followed by ZnO with ZSB (30.0 and 35.0 mg kg^{-1}) and Zn-EDTA with ZSB (29.5 and 34.2 mg kg^{-1}) at active tillering and panicle initiation stage of rice crop respectively. The straw zinc content varied from 20.2 to 30.2 mg kg^{-1} (Fig 1). Among the zinc sources, $ZnSO_4$ followed by Zn EDTA recorded the higher values of 25.9 and 24.4 mg kg^{-1} respectively. Whereas with ZSB, $ZnSO_4$ followed by ZnO (30.2 and 28.7 mg kg^{-1} respectively) recorded higher straw Zn content.

Table 1. Methods of Analysis of Soil and Plant Samples

Parameter	Method	Reference
I. Physical properties		
Particle size analysis	International pipette method	Piper (1966)
II. Physio-Chemical properties		
Soil reaction (pH)	Potentiometry (1:2.5 soil: water suspension)	Jackson (1973)
Electrical conductivity	Conductometry (1:2.5 soil: water suspension)	Jackson (1973)
III. Chemical Properties		
Available Nitrogen (N)	Alkaline $KMnO_4$	Subbiah and Asija (1956)
Available Phosphorus (P)	$NaHCO_3$ extraction	Olsen *et al.* (1954)
Available Potassium (K)	Neutral normal NH_4OAc	Hanway and Heidal (1952)
DTPA micronutrients	DTPA extraction – AAS	Lindsay and Norvell (1978)
Total Zinc	Diacid (HNO_3 and $HClO_4$)	Benitez and Dubois, (1999)
IV. Plant Analysis		
Zinc content	Tri acid digestion and Atomic Absorption Spectrophotometer	Lindsay and Norvell (1978)
V. Enzyme Analysis		
Super Oxide Dismutase	Calorimeter	Dhindsa *et al.,* 1981
Carbonic Anhydrace	Spectrophotometer	Hatch and Burnell,1990

In general, there was an increasing trend of Zn uptake at all the stages of rice towards maturity (Table 3). Zn removal by rice shoots was appreciably influenced by the application of Zn sources and ZSB.

Table 2. Characteristics of the initial soil sample of the pot experiment

Properties	Values
Physical Properties	
Soil texture	Sandy Clay Loam
Sand (%)	57.30
Silt (%)	6.40
Clay (%)	32.70
Physico-Chemical Properties	
pH	6.85
EC	0.10
CEC	16.40
Chemical Properties	
Available N (kg ha^{-1})	226.32
Available P (kg ha^{-1})	27.40
Available K (kg ha^{-1})	620.25
DTPA Zn (mg kg^{-1})	0.82
DTPA Cu (mg kg^{-1})	1.68
DTPA Fe (mg kg^{-1})	15.52
DTPA Mn (mg kg^{-1})	6.66
Total Zn (mg kg^{-1})	60.78

Addition of $ZnSO_4$ with ZSB recorded the highest shoot uptake at two growth stages (0.41 and 1.73 g $plant^{-1}$ at active tillering and panicle initiation respectively) followed by the application of ZnO > Zn-EDTA. The Zn sources with ZSB showed an increase in Zn content from 0.12 to 0.41 and 0.80 to 1.73 g $plant^{-1}$ at active tillering and panicle initiation stages respectively.

Among the zinc sources, $ZnSO_4$ followed by Zn EDTA recorded the higher values of straw Zn uptake of 1.51 and 1.38 g $plant^{-1}$ respectively. Whereas with ZSB, $ZnSO_4$ followed by ZnO and Zn-EDTA (1.96, 1.50 and1.50 g $plant^{-1}$ respectively) recorded higher straw Zn uptake. Increase in Zn acquisition through the inoculation of plant growth promoting rhizobacteria have been reported Rana *et al.* 2012. The increased Zn content and uptake might be accelerated due to the improvement of enzyme activity, auxin metabolism and carbohydrate metabolism in plants (Mandal and Das, 2013).

Effect of ZSB and different Zn sources on root Zn content and uptake

In general, the zinc concentration in root tended to decrease gradually from active tillering to harvest stage (Fig 2). The application of Zn sources and ZSB differed significantly among themselves in influencing root Zn content. The application of $ZnSO_4$

Table 3. Zn fertilizers and ZSB on above ground Zn uptake (g plant^{-1}) at critical growth stages of rice crop

	Active Tillering		Panicle Initiation		Harvest			
					Straw Zn uptake		Grain Zn uptake	
Zn sources	Without ZSB	With ZSB	Without ZSB	With ZSB	Without ZSB	With ZSB	Without ZSB	With ZSB
No Zn	0.07	0.12	0.55	0.8	0.98	1.15	0.49	0.60
ZnO	0.16	0.28	0.79	1.32	1.26	1.50	0.62	1.16
$ZnSO_4$	0.23	0.41	1.08	1.73	1.51	1.96	0.95	1.32
Zn-EDTA	0.18	0.23	0.86	1.19	1.38	1.50	0.71	1.03
	SEd	CD (p=0.05)	SEd	CD (p=0.05)	SEd	CD (p=0.05)	SEd	CD (p=0.05)
Zn	0.014	0.028**	0.044	0.088**	0.050	0.100**	0.051	0.104**
ZSB	0.009	0.018**	0.028	0.036**	0.032	0.064**	0.032	0.065**
ZnxZSB	0.020	0.040**	0.062	0.126**	0.071	0.142**	0.071	0.155**

with ZSB recorded the highest mean root Zn content of 44.0, 39.5 and 34.8 mg kg^{-1} followed by ZnO + ZSB (38.0, 33.0 and 26.7 mg kg^{-1}) and Zn-EDTA+ ZSB (38.5, 29.6 and 24.7 mg kg^{-1}) at active tillering, panicle initiation and harvest stage respectively. The microbial activity might have resulted in quantitative and qualitative alterations of root exudates composition due to the degradation of exudates and the release of microbial metabolites might have increased the root growth, root volume and alternately increased the root Zn content. A similar finding of an increase in Zn content by the inoculation of ZSB in shoot and root in soybean and wheat crops was reported by Madhaiyan *et al.* (2010).

Table 4. Zn fertilizers and ZSB on root Zn uptake (g plant^{-1}) at critical growth stages of rice crop

	Active Tillering		Panicle Initiation		Harvest	
Zn sources	Without ZSB	With ZSB	Without ZSB	With ZSB	Without ZSB	With ZSB
No Zn	0.05	0.21	0.09	0.15	0.09	0.13
ZnO	0.15	0.35	0.19	0.40	0.31	0.50
$ZnSO_4$	0.21	0.48	0.29	0.54	0.43	0.74
Zn-EDTA	0.15	0.29	0.23	0.27	0.36	0.39
	SEd	CD (p=0.05)	SEd	CD (p=0.05)	SEd	CD (p=0.05)
Zn	0.010	0.020**	0.014	0.028**	0.016	0.033**
ZSB	0.006	0.013**	0.009	0.017**	0.010	0.021**
ZnxZSB	0.014	0.028**	0.019	0.039**	0.023	0.046**

The data revealed that the application of Zn sources and ZSB significantly influenced the root Zn uptake of rice at active tillering, panicle initiation and harvest stages (Table 4). The application of Zn sources, with ZSB influenced the root Zn uptake markedly and the values were ranged from 0.21 to 0.48 g plant^{-1}, 0.15 to 0.54 g plant^{-1} and 0.13 to 0.74 g plant^{-1} at active tillering, panicle initiation and harvest stages respectively. The interaction between Zn sources and ZSB was significant at all the growth stages.

The integration of $ZnSO_4$ + ZSB recorded the highest root Zn uptake of 0.48, 0.54 and 0.74 g plant^{-1} followed by ZnO (0.35, 0.40 and 0.50 g plant^{-1}) at active tillering, panicle initiation and harvest stages respectively. Increased in root weight may be due to increased availability of Zn from rhizosphere by mechanisms such as pH decrease, release of organic acids due to decomposition of manures, chelating and other compounds resulting in root growth that promoted the increase in nutrient uptake, per unit root volume (Hamad Raza Ahmad *et al.,* 2012).

Effect of ZSB and different Zn sources on grain Zn content and uptake

The zinc content of grain was found to be increased by the application of Zn sources and ZSB (Fig 3). The highest grain Zn was registered in the $ZnSO_4$ + ZSB (42.2 mg kg-^{1}), followed by ZnO + ZSB (38.5 mg kg-^{1}) and Zn-EDTA + ZSB (34.6 mg kg-^{1}). The Zn sources recorded a lower Zn content ranged from 22.0 to 32.6 mg kg^{-1} without ZSB, whereas with ZSB,

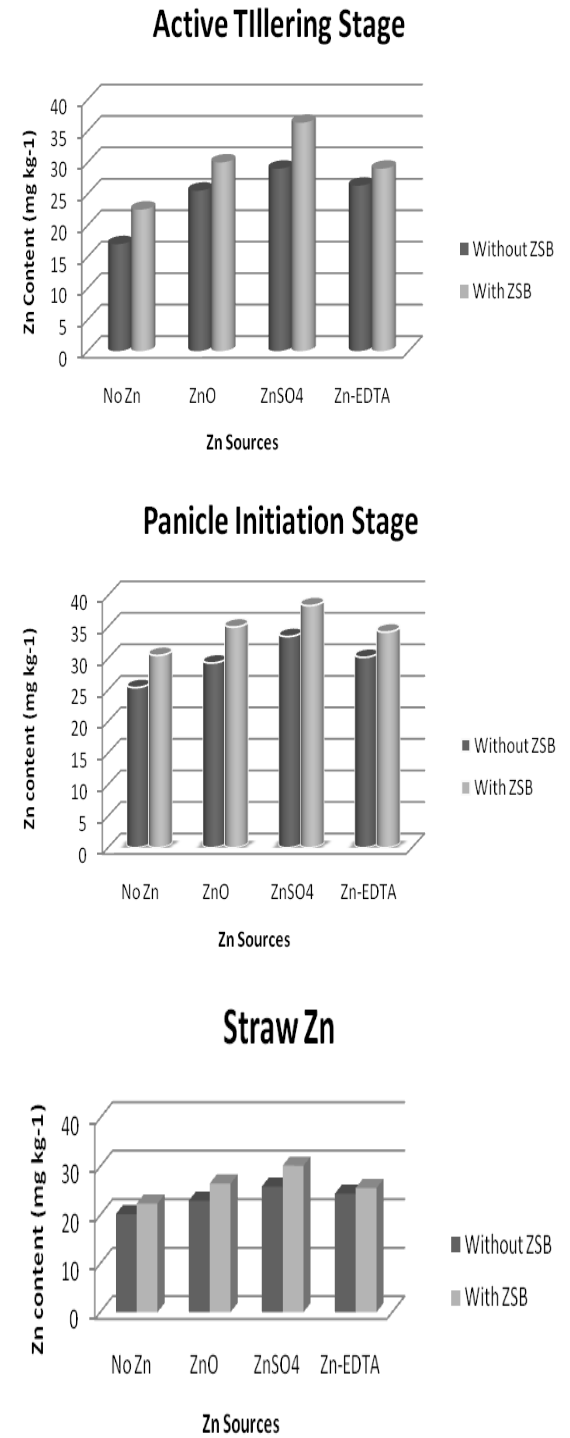


Figure 1. Effect of ZSB and different Zn sources on above ground Zn content (mg kg-1)

a higher Zn content from 23.6 to 42.2 mg kg^{-1} was recorded. Addition of $ZnSO_4$ alone recorded a higher grain Zn content followed by Zn-EDTA of 32.6 and 26.7 mg kg^{-1} respectively. Whereas with ZSB, $ZnSO_4$ followed by ZnO (42.2 and 38.5 mg kg^{-1} respectively) recorded the highest grain Zn content.

The grain Zn uptake values ranged from 0.49 to 0.95 and 0.60 to 1.32 g plant^{-1} respectively for with and without ZSB respectively (Table 3). The $ZnSO_4$ in combination with ZSB recorded the highest grain

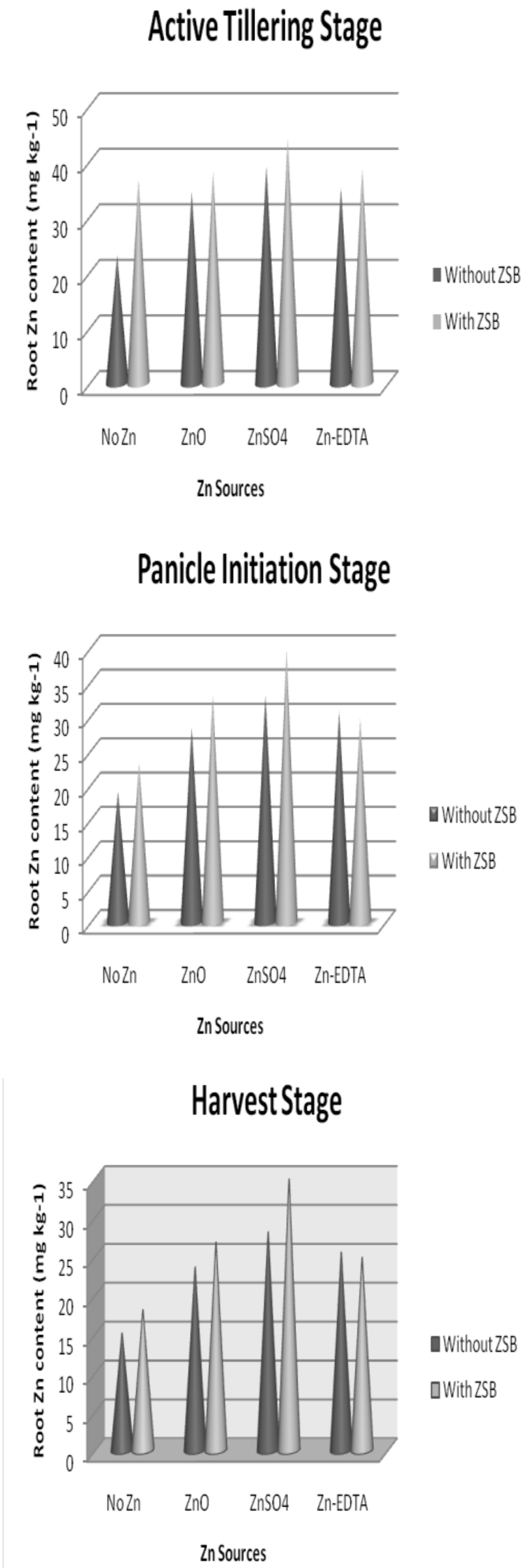


Figure 2. Effect of ZSB and different Zn sources on root Zn content (mg kg-1)

Zn uptake of 1.32 g plant^{-1} followed by ZnO and Zn EDTA in similar combination. The lower Zn uptake

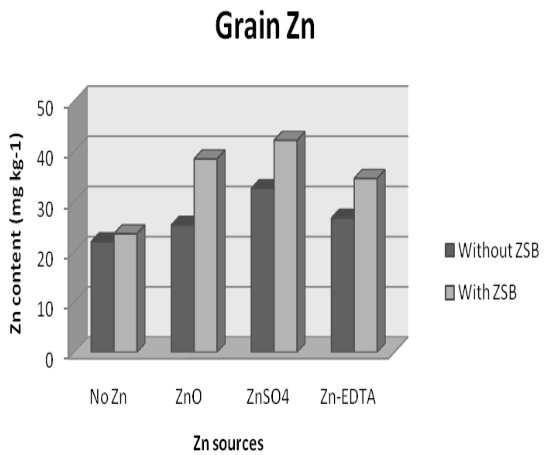
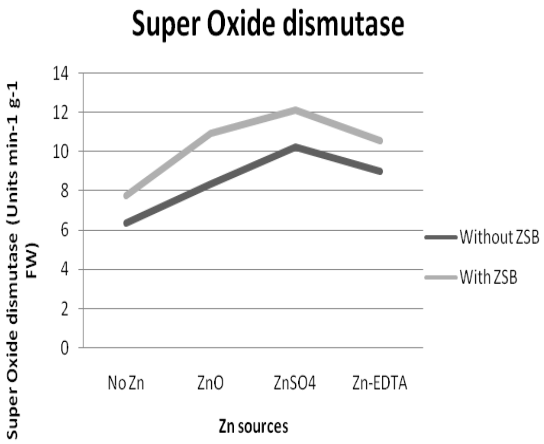


Figure 3. Effect of ZSB and different Zn sources on Grain Zn content (mg kg^{-1})

by grain was registered by the control treatment. Zn concentration of rice grain was reported to increase with soil Zn application. The increase in Zn content in grains might be due to the presence of the increased amount of Zn in soil solution by the application of $ZnSO_4$ that facilitated greater absorption and it also



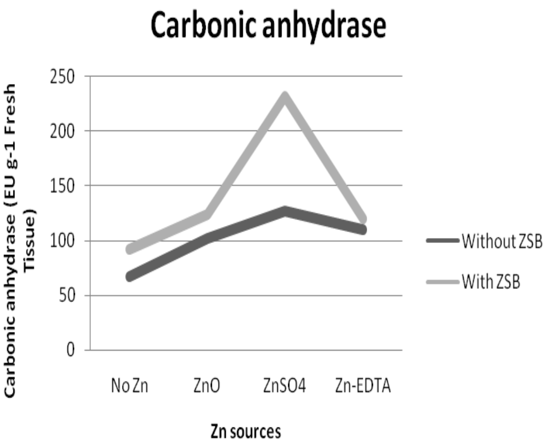


Figure 4. Effect of ZSB and different Zn sources on Carbonic anhydrase (EU g^{-1} Fresh Tissue) and Super Oxide dismutase (Units min^{-1} g^{-1} FW) of Rice crop

enhanced uptake and translocation of sugars and carbohydrate accumulation in grains which was in line with Sudha and Stalin (2015) in rice crop. Jana *et al.* (2010) also observed that soil application of Zn EDTA led to higher content and uptake of Zn by rice grain and straw. The zinc solubilizing bacterial isolates improved Zn acquisition in inoculated plants and also the production of Indole Acetic Acid (Vaid *et al.,* 2013).

Enzyme analysis

Assessing the Zn requiring enzymes is essential to understand the Zn dynamics in soils and plants and hence the enzymes namely, Super Oxide Dismutase (SOD) and Carbonic Anhydrase (CA) which are activated by Zn^{2+} were estimated in this present study.

The application of Zn sources and ZSB increased the Super Oxy dismutase and Carbonic anhydrase activity (Fig 4) at the panicle initiation stage. The highest enzyme activity was recorded with the application of $ZnSO_4$+ ZSB (232 and 12.25 Units min^{-1} g^{-1} FW) for Super Oxide Dismutase (SOD) and Carbonic Anhydrase (CA) respectively followed by ZnO + ZSB (124 and 10.94 Units min^{-1} g^{-1} FW) and Zn-EDTA+ZSB (120 and 10.56 Units min^{-1} g^{-1} FW), whereas the control recorded the lowest SOD and CA activity in the panicle initiation stage. Similar results were reported by Sudha and Stalin (2016) and Vahid Tavallali (2010) in rice crop. The activity of CA and SOD enzymes are closely related Zn content in C3 plants (Uma Sanker Ram *et al.,* 2017).

CONCLUSION

From the study, the results revealed that the application of ZSB @ 20 ml (10^8 cfu) per pot with Zn fertilizers influenced the zinc content, uptake by different parts and enzyme activity of rice crop. The inoculation of ZSB increased the Zn content and uptake and enzyme activity which might have increased the rice crop yield. It will facilitate efficient nutrient's uptake which ultimately produces plants of superior quality making agriculture more productive with zinc enriched grains, the latter may have greater control in eliminating malnutrition. Among the different Zn sources, $ZnSO_4$ followed by ZnO with ZSB enhanced the Zn content and uptake along with improved SOD and CA enzyme activities in a zinc-deficient soil. It may be concluded from the study that ZSB applied in combination with $ZnSO_4$ @ 25 kg ha^{-1} could be a better choice for sustainable crop production.

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