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Yield attributes of rice (*Oryza sativa*) as affected by integrated use of zinc oxide and zinc solubilizers

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ABSTRACT

Rice (*Oryza sativa* L.) is the staple food for more than three billion people around the world. Zinc (Zn) deficiency is mostly found in lowland rice due to submergence condition which affect the yield adversely. In view of limited information, a pot experiment was conducted at Department of Soil Science and Agricultural Chemistry, Institute of Agricultural Sciences, BHU, Varanasi, UP during *kharif*, 2018-19 to evaluate the response of rice to different levels of zinc oxide (ZnO) in combination with zinc solubilizing microbes. Zn deficient soil was collected from Agricultural Research Farm, IAS, BHU which were treated with three different doses of ZnO (0, 2.5 and 5 kg ha^{-1}) along with three types of microbial inoculation, *viz.* no inoculation (M_0), *Enterobacter clocae* strain ZnPSBJ-6 (M_1) and zinc solubilizing fungi (M_2). It was found that ZnPSBJ-6 has increased the grain yield by 57.2% and 101.4% while treated with Z_1 (2.5 kg ha^{-1}) and Z_2 (5 kg ha^{-1}) respectively compared to $Z_{0.}$ Whereas, in case of zinc solubilizing fungi, grain yield has increased by 96.4% and 127.1% in case of Z_1 and Z_2 respectively. On the other hand application of Z_1 and Z_2 have significantly increased the average straw yield by 28.9% and 36.5% respectively over the control.

Key words: Biofortification, Grain yield, Rice, Straw yield, Zinc solubilizers

Importance of zinc (Zn) has been realized during the past four decades when widespread deficiencies were observed in soils under intensive agriculture (Katyal 2018). Introduction of high yielding varieties, extension of irrigated areas and use of high analysis micronutrients free NPK fertilizers particularly under intensive agriculture have increasingly catalysed the depletion of finite resources of soil micronutrients, leading to the occurrence of micronutrient deficiencies in post green revolution era. Recently, Shukla *et al.* (2018) reported that 36.5% of soil samples across the country are deficient in Zn. In India, rice (*Oryza sativa* L.) is cultivated in about 162.9 million ha of land with annual production of 494.8 million tonnes (USDA 2019). Several researchers reported that in low land rice field after nitrogen, Zn is the second most deficient nutrient due to formation of insoluble sphalerite (ZnS). Apart from this, high soil pH and high carbonate content as well as low redox potential under submergence reduces Zn availability to rice (Alloway 2009). Excessive concentration of Fe(II) & Mn(II) resulting from the submergence under rice field hinders the uptake of Zn by rice crop. In addition, cereal crops are prone to Zn deficiency as they contain phytase, which reduce the bioavailability of Zn. Zinc deficiency in rice plant was first reported from calcareous soils of northern India (Yoshida and Tanaka 1969). Indeed, Zn plays a vital role in plant as it is an important component of enzymes like carbonic anhydrase, alcoholic dehydrogenase and superoxide dismutase that stimulate the rate of many important metabolic reaction. Therefore, it is recommended to use Zn-efficient rice genotypes in conjunction with judicious Zn fertilizer to enhance Zn content in rice grain. However, on an average, Zn use efficiency is reported to be less than 2% in rice crop. Therefore, major portion applied Zn is converted to carbonates or oxides or phosphates and become unavailable to plant (Zhang *et al.* 2013). In order to combat Zn deficiency in rice, use of Zn solubilizer is presumed to have a great economic importance as it not only enhance the use efficiency of added fertilizer but also solubilize the native form of Zn present in soil. It has been reported that Zn solubilizers are also capable of solubilizing silicate and phosphate present in soil (Dahaji *et al.* 2012, Desai *et al.* 2012). Keeping the above fact in mind, the present study was undertaken to assess the effect of Zn solubilizing microbes along with varying levels of

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zinc oxide (ZnO) on growth parameters and yield of rice.

MATERIALS AND METHODS

Collection of soil sample: One bulk surface (0-15 cm) soil was collected from the Agricultural Research Farm, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi. The soils of Varanasi are basically formed on the alluvium parent material, i.e. weathered rock fragments deposited in the banks of river Ganges. Varanasi is categorized into a semi-arid to sub humid climate with Moisture Deficit Index (MDI) in between 20-40. The soil was high in phosphorus and potassium content with low amount of organic matter. Physico-chemical properties of the initial soil were determined using standard procedures (Jackson 1973).

Pot experiment: A pot experiment was conducted at Department of Soil Science and Agricultural Chemistry, Institute of Agricultural Sciences, BHU, Varanasi, UP during *kharif*, 2018-19 with a rice cultivar characterized as dwarf non-aromatic high yielding variety (HYV), *viz.* Swarna-sub-1. Seedlings of four-leaf stage were obtained from the Agricultural Research Farm, IAS, BHU. For this purpose, earthen pots (Height- 30 cm, Diameter- 22.5 cm) were filled with 10 kg of processed soil. Zinc oxide (ZnO) was added at the rates of 0 (Z_0), 1.85 (Z_1) and 3.7 (Z_2) mg kg^{-1} of soil, which are equivalent to field application of 0, 4.16 and 8.33 kg ha^{-1}. Treatment combination were designed as follows: T_1-No ZnO (Z_0) with no inoculation (M_0), T_2-inoculation with *Enterobacter clocae* strain ZnPSBJ-6 (M_1) without ZnO dose (Z_0), T_3 (inoculation with zinc solubilizing fungi (M_2) with no ZnO (Z_0), T_4 (no inoculation with half dose of ZnO, i.e. 1.85 mg kg^{-1}(Z_1), T_5- inoculation with *Enterobacter clocae* strain ZnPSBJ-6 (M_1) with half dose of ZnO, i.e. 1.85 mg kg^{-1} (Z_1), T_6- inoculation with zinc solubilizing fungi (M_2) with half dose of ZnO, i.e. 1.85 mg kg^{-1} (Z_1), T_7-no inoculation (M_0) with full dose of ZnO, i.e. 3.7 mg kg^{-1} (Z_2), T_8- inoculation with *Enterobacter clocae* strain ZnPSBJ-6 (M_1) with full dose of ZnO, i.e. 3.7 mg kg^{-1} (Z_2), T_9- inoculation with zinc solubilizing fungi (M_2) with full dose of ZnO i.e. 3.7 mg kg^{-1} (Z_2). Seedling root dip was done with desired microbial inoculation with the aid of some adhering substances like jaggary and gum acacia. Transplanting was done in such a way that each pot consists of four hills and each hill contents two to three seedlings. All nine treatment combinations were replicated thrice and the experiment was laid out in factorial completely randomized design. A uniform basal dose of N: P_2O_5: K_2O @ 40.1: 40.2: 26.8 mg/kg were added in solution form in each pot through urea, potassium dihydrogen phosphate and muriate of potash, respectively. After establishment of plant, they were thinned to a uniform population of four plants per pot. Water level was maintained at 3 cm above the soil surface until harvest. Two top dressings of N, each at the rate of 20.1 mg/kg, were done at 30 and 60 days after transplanting (DAT). Yield attributes, i.e. number of tillers per hill and number of panicles per plant were also recorded at 30 DAT. The harvesting of paddy was done at 120 DAT by cutting the stem close to ground and storing in paper bags separately from each pot. Harvested plants were washed and then dried in hot air oven at 60±2°C. After attaining constant weight, grain and straw yields were separately measured using electronic balance and expressed in g/pot.

DTPA extractable zinc: Available Zn in soil samples were determined by the method of Lindsay and Norvell (1978). In this method, 10 g of soil was extracted with 20 ml of DTPA extracting solution containing (0.005 M DTPA +0.01 M $CaCl_2$+0.1 M TEA, pH 7.3) and was shaken for 2 hours in an environmental shaker at 25℃. The suspension was then filtered with Whatman no 42 filter paper and Zn present in the extract was determined by atomic absorption spectrophotometer (UNICAM-969).

RESULTS AND DISCUSSION

Zn content in soil: DTPA extractable soil Zn as affected by different treatments has been graphically depicted in Fig 1. It was predominantly found that at 60 DAT, DTPA extractable Zn has increased significantly by 177.8% and 125.3% in treatment inoculated with M_2 (1.75 ppm) as compared to M_1 (1.42 ppm) when treated with similar doses of Zn, i.e. 3.7 mg kg^{-1} over the absolute control (0.63 ppm) (fig.1). So it may be said that interaction effect between different level of ZnO and ZSM has increased DTPA extractable soil Zn content (ppm) significantly irrespective of different growing period of rice. This result invariably shows the worth of using Zn solubilizing microbes along with a insoluble source of Zn. The decrease in DTPA extractable soil Zn content (ppm) at post harvest may be due to the plant uptake. Wang *et al.* (2005) reported that there was a significant increase in the DTPA extractable Zn content when the rice seedlings were inoculated with endophytic microbes, viz. SaMR12 and SaCS 20 isolated from *Sedum alfredii* which is a Zn hyper accumulating plant.

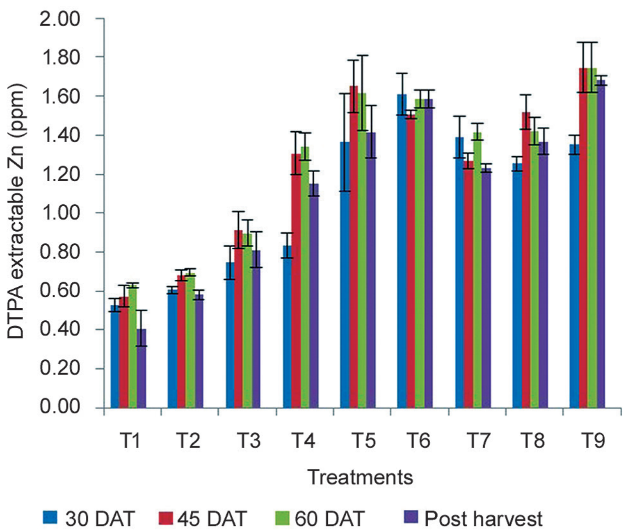

Fig 1 Effect of zinc solubilizers and ZnO on DTPA extractable Zn at different growth period of rice (Values are presented in the bar as mean ± SD). (For treatment details refer the section Materials and Methods).

Table 1 Initial characteristics of experimental soil

Parameter	Value
$pH_{1:2}$	7.8
$EC_{1:2}$ (dS m^{-1})	0.48
Clay %	15.7
Silt %	23.1
Sand %	61.2
Texture	Sandy loam
Organic carbon (g kg^{-1})	16
Cation exchange capacity [cmol(p^+) kg^{-1}]	20.3
DTPA extractable Zn (ppm)	0.54

Tillers per hill: It has been found that mean value regarding number of tillers per hill was increased from 24 (in M_0) to 25.2 and 26.3 under M_1 and M_2, respectively (Table 2). Although number of tillers per hill has been significantly increased in case of M_2 unlike M_1 which is found to be statistically at par with M_0. Increased level of zinc (1.85 mg/kg) resulted in significant enhancement of this particular growth parameter but beyond this level such effect was absent. Interaction effect of these two factors (zinc solubilizers and ZnO) was found to be significant. Zinc act as an essential component of many enzymes and controls several biochemical processes in the plants such as cytochrome and nucleotide synthesis, enzyme activation, chlorophyll production, maintenance of membrane activity, increase rate of seed and stalk maturation (IRRI 2000). Increase in number of tillers per hill in this experiment may be attributed to the positive effect of Zn on photosynthetic rate, chlorophyll synthesis, protein synthesis. A Similar kind of result was reported earlier by Kadam *et al.* (2018).

Panicles per plant: Among the treatments, average panicles per plant has been increased significantly due to application of M_1 (16.8) and M_2 (18.8) as compared to M_0 (15) (Table 3). On the other hand, application of 1.85 mg/kg Zn has resulted in 42.5% increment in this particular yield attribute over control irrespective of any type of microbial inoculation. Adequate supply of Zn might have increased the availability and uptake of other essential nutrients required in the growth of crop. This result lies in parallel to the findings of Maqsood *et al.* (1999) who reported that adequate supply of Zn results in greater number of panicles per plant.

Table 2 Effect of zinc solubilizing microbes and different levels of ZnO on tillers per hill of rice crop

Zinc fertilizer	Tillers per hill			
	Microbial inoculation			
	M_0	M_1	M_2	Mean
Z_0	20.3	24.6	25.0	23.3
Z_1	25.0	27.3	26.0	26.1
Z_2	26.6	23.6	28.0	26.1
Mean	24.0	25.2	26.3	
CD (0.05)	M=1.58 Z=1.58 M*Z=2.74			

Grain yield: On an average, grain yield of rice was increased with increasing level of applied ZnO (Table 4). Mean grain yield was increased from 13.2 (Z_0) to 21.7 and 28.7 g/pot under Z_1 and Z_2, respectively. Among the microbial treatments, on an average, highest grain yield was recorded with M_2 (24.5 g/pot) followed by M_1 (21.1 g/pot) and M_0 (18.1 g/pot). Maximum yield (2.69 times) was obtained in paddy which was inoculated with zinc solubilizing fungi in combination with Z_2.

Effect of zinc application on grain yield of rice was significantly modified by zinc solubilizers. For example, application of both zinc solubilizing bacteria, i.e. *Enterobacter clocae* strain ZnPSBJ-6 as well as zinc solubilizing fungi resulted in concomitant increase in grain yield with the increasing levels of zinc, i.e. in Z_1 and Z_2. Zinc solubilizing bacteria has increased the grain yield by 57.2% and 101.4% while treated with Z_1 and Z_2 respectively over the control. Result was found to be more conspicuous in case of zinc solubilizing fungi which showed 96.4% and 127.1% increase in grain yield in case of Z_1 and Z_2 respectively over the control. So interactive effect of zinc fertilization and zinc solubilizers was found to be significant in increasing the grain yield of rice.

Positive effect of Zn on grain yield may be due to higher carbonic anhydrase activity, which is localized in cytoplasm and chloroplast facilitating the transfer of CO_2 or HCO_3^- during CO_2 assimilation phase of photosynthesis through increasing the rate of equilibrium between CO_2 and

Table 3 Effect of zinc solubilizing microbes and different levels of ZnO on panicles per plant of rice crop

Zinc fertilizer	Panicles per plant			
	Microbial inoculation			
	M_0	M_1	M_2	Mean
Z_0	12.0	14.0	14.0	13.3
Z_1	16.0	19.0	22.0	19.0
Z_2	17.0	17.6	20.6	18.4
Mean	15.0	16.8	18.8	
CD (0.05)	M=1.10 Z=1.10 M*Z=1.90			

CD (0.05): Critical difference at 5% level of significance

Table 4 Effect of zinc solubilizers and different levels of ZnO on grain yield (g/pot) of rice crop

Zinc fertilizer	Grain yield (g/pot)			
	Microbial inoculation			
	M_0	M_1	M_2	Mean
Z_0	11.8	13.8	14.0	13.2
Z_1	15.8	21.7	27.5	21.7
Z_2	26.6	27.8	31.8	28.7
Mean	18.1	21.1	24.5	
CD (P=0.05)	M=1.90 Z=1.90 M*Z=3.20			

CD (0.05): Critical difference at 5% level of significance

Table 5 Effect of zinc solubilizers and different levels of ZnO on straw yield (g/pot) of rice crop

Zinc fertilizer	Straw yield (g/pot)			
	Microbial inoculation			
	M_0	M_1	M_2	Mean
Z_0	23.8	25.3	26.4	25.2
Z_1	29.6	34.9	33.0	32.5
Z_2	34.3	34.7	34.2	34.4
Mean	29.3	31.6	31.2	
CD (P=0.05)	M=1.90 Z=1.90 M*Z=N/A			

CD (0.05): Critical difference at 5% level of significance

HCO_3^- in solution. On the other hand SOD helps to scavenge free radicles and protects the photosynthetic apparatus from oxidative damage (Potarzycki and Grzebisz 2009). Stomatal conductance is also increased due to higher bioavailability of Zn. Zn also plays a major role in anaerobic root respiration of rice as it is a constituent of alcoholic dehydrogenase enzyme which catalyzes the conversion of acetaldehyde to ethanol. Enhancement in two major yield attributes, *viz.* tillers per hill and panicles per plant resulted in higher grain yield of rice. This result is also in line with findings of Phattarakul *et al.* (2012) and Rehman *et al.* (2012).

Straw yield: Data represented in Table 5 shows the effect of zinc solubilizers and zinc fertilizer on straw yield of rice. Mean value of straw yield has increased by 7.2% over control due to application of zinc solubilizing bacteria, whereas this increment is only 6.4% in case of zinc solubilizing fungi. On the other hand application of 1.85 mg $kg^{-1}$and 3.7 mg kg^{-1} of ZnO has significantly increased the average straw yield by 28.9% and 36.5% respectively. However the combined effect of zinc solubilizing microbes and zinc fertilization was found to be non-significant.

Enhanced straw yield may be attributed to the fact that Zn helps in re-translocation of photosynthates in phloem which facilitates vegetative growth and concomitant increase in dry matter content.

Results, therefore, revealed that practically realizable doses of ZnO in combination with zinc solubilizers had significant positive effect on soil Zn content, tillers per hill, panicles per plant, grain as well as straw yield of paddy. DTPA extractable Zn content in soil also enhanced significantly while inoculated with zinc solubilizing fungi along with higher level of Zn (Z_2). ZnPSBJ-6 has increased the grain yield by 57.2% and 101.4% while treated with Z_1 and Z_2 respectively over to Z_0. Whereas, in case of zinc solubilizing fungi, grain yield has increased by 96.4% and 127.1% in case of Z_1 and Z_2, respectively over the control. Putting it into perspective, it seems that the higher (5 kg ha^{-1}) dose of Zn had more pronounced effect on grain yield as compared to the straw yield. It can be concluded that there is a considerable positive influence of zinc solubilizers in combination with ZnO in increasing biomass yield of rice crop, which should be exploited by the farmers particularly in the alluvial Inceptisol of UP.

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