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Effect of Zn fertilizers and Zn solubilizing bacteria on yield, nutrient uptake and economics of rice (*Oryza sativa* L.)

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ABSTRACT

*A field experiment was performed to study the effect of Zn sources combined with Zn solubilising microorganisms-ZSM (*Acinetobacter calcoaceticus + Pantoea agglomerans*) on yield, nutrient uptake, phytic acid and economics of rice (Oryza sativa L.). The experiment was conducted for two consecutive years (2018 and 2019) at Banaras Hindu University, Varanasi. The experiment was designed in randomized block design with twelve treatments replicated thrice. The data indicated that 25 kg $ZnSO_4.7H_2O$ ha^{-1} + ZSM significantly increased tillers over other treatments, but 9.8 kg ZnO ha^{-1} + ZSM improved panicle length, filled spikelet $panicle^{-1}$ and test weight. The phytic acid content in rice was found superior in control treatment (6.5 g kg^{-1}), whereas significantly reduced in 25 kg $ZnSO_4.7H_2O$ ha^{-1}+ ZSM treatment. Moreover, the maximum grain yield (5.01 t ha^{-1}), straw yield (7.85 t ha^{-1}), net return (Rs 58.94 $\times 10^3$ ha^{-1}) and benefit: cost ratio (1.88) was recorded with 9.8 kg ZnO ha^{-1} + ZSM. The higher uptake of nitrogen and potassium was recorded in 25 kg $ZnSO_4.7H_2O$ ha^{-1}+ ZSM treated plot. However, phosphorous uptake was maximum in control. The maximum total Zn uptake (1364.8 g ha^{-1}) was documented with 9.8 kg ZnO ha^{-1} + ZSM + cow dung.*

Keywords: Zn fertilizer, rice, zinc solubilizing bacteria, nutrient uptake, phytic acid, yield

INTRODUCTION

Rice (*Oryza sativa* L.) is a dietary and protein currency for both Indian and global populations, as it is a staple food for more than half of the world's population and provides 20 and 31% of total calories required by the world and Indian populations, respectively (Cakmak and Kutman, 2018). Zinc is the fifth most important yield-limiting nutrient in India's upland crops after N, P, K, and S, and it is second only to N in lowland crops like rice. In India, about 40% of soil samples tested for available Zn was found to be deficient (Das *et al.* 2019). Hence, growing cereal crops on Zn deficient soils further decreases grain Zn concentration. Zinc is important in plants as a structural component and a regulatory co-factor for a variety of enzymes and proteins involved in various biochemical pathways. These pathways include photosynthesis, sugar to starch conversion, carbohydrate, protein and auxin metabolism, pollen formation, maintenance of the integrity of biological membranes, and resistance to infection by certain pathogens. There are multiple reasons for the increasing incidences of Zn deficiency in India, including large Zn removal due to high crop yields and intensive cropping systems, lesser application of organic manures, use of high analysis fertilizers, increased use of phosphatic fertilizers resulting in P-induced Zn deficiency, and the use of poor quality irrigation water with high calcium carbonate content. Zink bioavailability in the soil is also altered by a number of edaphic factors like coarse texture (sandy/loamy sand), high pH and low organic carbon or calcareous soil. The management of Zn deficiency depends on crop, soil types, and severity of deficiency, source, method, time, rates and frequency of application. Out of the several Zn sources evaluated, $ZnSO_4 \cdot 7H_2O$ proved to be better or equal with other sources in correcting the Zn deficiency, however in some studies seedling root dipping with ZnO proved to be efficient. Unfortunately, exogenous application of chemical fertilizers alone will not help in the long run to combat soil Zn deficiency because 96.0–99.0% of the applied Zn is converted to unavailable Zn pools by precipitation to carbonates, oxides, phosphates, and other compounds (Zhang *et al.* 2017). As a result, a viable alternative would be to take advantage of certain soil microorganisms' to solubilize these fixed forms of Zn into labile Zn forms for increased availability and subsequent uptake by plants (Prajapati *et al.* 2021). Some

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genera of bacteria such as *Pseudomonas* sp., *Gluconacetobacter* sp., *Thiobacillus* sp., *Bacillus* sp., *Acinetobacter* sp., etc. are reported as potential Zn solubilizers. Hence, the present study was designed to study the effect of Zn fertilizers and zinc solubilizing bacteria on yield, nutrient uptake and economics of rice.

MATERIALS AND METHODS

A field experiment was conducted for two consecutive years (2018 and 2019) at the research farm BHU (Uttar Pradesh) located at 25°18' North latitudes, 83°03' East longitude. The experimental soil was sandy loam in texture. It had 176.4 kg ha^{-1} available nitrogen, 33 kg ha^{-1} available phosphorus, 143.6 kg ha^{-1}available K and 2.5 g kg^{-1} organic carbon. The soil was alkaline in nature (pH 7.3) and had 0.78 mg kg^{-1} DTPA extractable Zn. The experiment was laid out in randomized block design with twelve treatments and three replications. Treatments included T_1: control (Zn_0), T_2:12.5 kg $ZnSO_4.7H_2O$ ha^{-1}, T_3: 25.0 kg $ZnSO_4.7H_2O$ ha^{-1}, T_4: microbial consortia (ZSM) (*Acinetobacter calcoaceticus* × *Pantoea agglomerans*), T_5:12.5 kg $ZnSO_4.7H_2O$ ha^{-1}+ ZSM, T_6: 25.0 kg $ZnSO_4.7H_2O$ ha^{-1} + ZSM, T_7:4.9 kg ZnO ha^{-1} + ZSM, T_8: 9.8 kg ZnO ha^{-1} + ZSM, T_9:14.8 kg ZnO ha^{-1} + ZSM, T_{10}: 4.9 kg ZnO ha^{-1} + ZSM + 200 kg cow dung ha^{-1}, T_{11}: 9.8 kg ZnO ha^{-1} + ZSM + 200 kg cow dung ha^{-1}, T_{12}: 14.8 kg ZnO ha^{-1} + ZSM + 200 kg cow dung ha^{-1}. The recommended dose of fertilizer (RDF) 120 kg N+ 40 kg P_2O_5 + 60 kg K_2O was applied uniformly in all the treatment through urea, di-ammonium phosphate, and muriate of potash, respectively. Half dose of N and full doses of P and K were applied as basal and remaining half of N was applied in two equal splits at tillering and panicle initiation stage. All the sources of Zn were applied as soil application before the transplanting. For the application of ZSM, strains were inoculated in Bunt and Rovira broth and incubated at rotary shaker (28±℃) for two days, followed by dilution of culture suspension with distilled water to make up the bacterial population to (~ × $10^8/10^9$ colony-forming unit ml^{-1}). Thereafter, sticking solution (water + 10% (w/v) jaggery + 10% (w/v) gum acacia) was prepared and mixed well with the microbial culture. During transplanting, seedlings were dipped in microbial consortia for half an hour before transplanting. Twenty-five days old seedlings of rice variety HUR-105 (Malviya Sugandha-105) were transplanted per hill at 20 cm x 15 cm in the second fortnight of July in both the years. At harvest, the number of effective tillers m^{-2}, panicle length (cm), filled spikelet $panicle^{-1}$, unfilled spikelet $panicle^{-1}$ and 1000 grain weight were recorded. The grain and stover yields were also recorded. Phytate content in rice seed was determined by modified colorimetric method (OD-500) using wade reagent describe by Gao *et al.* (2007). The plant samples (grain and straw) were digested with a di-acid mixture (HNO_3: $HClO_4$:: 9:4, v/v). Phosphorous in digest was determined by Vanado–molybdo phosphoric yellow color, K by flame photometer and Zn by atomic adsorption spectrophotometer. Nitrogen content was determined by micro-kjeldahl method. Nutrient uptake was calculated as a function of their concentration and yield. The net returns were calculated by subtracting the total cost of cultivation from the gross returns. The data was then statistically analyzed for various parameters using the analysis of variance technique at a 5% level of significance.

RESULTS AND DISCUSSION

Yield attributes

Yield attributes of rice like tillers m^{-2}, panicle length, filled spikelet $panicle^{-1}$, fertility percentage and test weight varied significantly due to zinc fertilizers and microbial inoculation (Table 1). The maximum tiller m^{-2} (441.3) was recorded with application of 25 kg Zn sulphate ha^{-1} combined with Zn solubilizing bacteria. However, panicle length (27.7cm), filled spikelet $panicle^{-1}$ (136), fertility percentage (84.2 %) and test weight (25.1 g) were found maximum with 9.8 kg ZnO ha^{-1} +ZSM, which were statistically comparable with 25.0 kg zinc sulphate ha^{-1} + ZSM, and 12 kg ZnO ha^{-1} +ZSM. The increased number of tillers due to Zn application may be explained by the fact that more photosynthates were produced, and available for tiller initiation, and their growth under these treatments. Alone application of 25.0 kg $ZnSO_4.7H_2O$ ha^{-1} produced significantly higher yield attributes like tiller m^{-2} (441.3), panicle length (26.9 cm), filled spikelet $panicle^{-1}$ (129.5), unfilled spikelet $panicle^{-1}$ (24), fertility percentage (84.4 %), 1000 grain weight (25.0 g), than 12.5 kg $ZnSO_4.7H_2O$

ha^{-1}. The increase in yield attributes with 25.0 kg $ZnSO_4.7H_2O$ ha^{-1} might be due to the greater amount of Zn uptake compared to 12.5 kg $ZnSO_4.7H_2O$ ha^{-1}application. Das *et al.* (2019) found similar result with basal application of 25 kg $ZnSO_4.7H_2O$ ha^{-1}. The perusal of data related to phytic acid (PA) revealed that control treatment had maximum content (6.5 g kg^{-1}), whereas the lowest PA was observed with 25.0 kg Zn sulphate ha^{-1} + ZSM. Except for the number of unfilled grains, all of the treatments that received cow dung significantly reduced yield attributes. The detrimental effect of cow dung on crop growth was due to early decomposition of organic matter formed numbers of complexes and chelates of varying solubility which reduced soil solution concentration of Zn.

Table 1: Effect of Zn fertilizer and Zn solubilizing bacteria on yield attributes and quality of rice (mean of 2 years)

Treatment	No. of tillers m^{-2}	Panicle length (cm)	Filled spikelet $panicle^{-1}$	Unfilled spikelet $panicle^{-1}$	Fertility %	1000 grain weight (g)	Phytic acid (g kg^{-1})
T_1	354.9	23.8	98.5	31.5	75.8	22.6	6.5
T_2	399.5	24.3	113.0	72	79.0	23.7	6.0
T_3	416.0	26.1	124.5	25.5	83.0	24.1	5.6
T_4	333.7	23.4	95.5	24	79.9	22.0	5.9
T_5	413.9	25.7	124.5	27	82.2	24.1	5.9
T_6	441.3	26.9	129.5	24	84.4	25.0	5.3
T_7	422.5	25.9	122.5	31.5	79.6	24.6	5.7
T_8	433.6	27.7	136.0	25.5	84.2	25.1	6.4
T_9	428.5	27.2	133.5	24	85.8	25.2	6.2
T_{10}	427.0	26.0	120.0	28.5	82.2	24.5	5.6
T_{11}	429.0	27.4	125.0	22.5	84.7	24.6	5.8
T_{12}	413.8	25.7	121.0	26.5	86.4	25.1	5.4
SEm ±	5.11	0.31	1.71	0.21	2.5	0.34	0.07
CD(P=0.05)	15.1	0.91	5.1	1.01	2.8	0.8	0.22

T_1: control (Zn_0), T_2:12.5 kg $ZnSO_4.7H_2O$ ha^{-1}, T_3: 25.0 kg $ZnSO_4.7H_2O$ ha^{-1}, T_4: microbial consortia (ZSM) (Acinetobacter calcoaceticus × Pantoea agglomerans), T_5:12.5 kg $ZnSO_4.7H_2O$ ha^{-1}+ ZSM, T_6: 25.0 kg $ZnSO_4.7H_2O$ ha^{-1} + ZSM, T_7:4.9 kg ZnO ha^{-1} + ZSM, T_8: 9.8 kg ZnO ha^{-1} + ZSM, T_9:14.8 kg ZnO ha^{-1} + ZSM, T_{10}: 4.9 kg ZnO ha^{-1} + ZSM + 200 kg cow dung ha^{-1}, T_{11}: 9.8 kg ZnO ha^{-1} + ZSM + 200 kg cow dung ha^{-1}, T_{12}: 14.8 kg ZnO ha^{-1} + ZSM + 200 kg cow dung ha^{-1}

Yield and economics

The plot with the highest grain yield (5.01 t ha^{-1}) was fortified with 9.8 kg ZnO ha^{-1} + ZSM, which was statistically at par with 14.8 kg ZnO ha^{-1} +ZSM (4.95 t ha^{-1}) and 25 kg zinc sulphate ha^{-1} + ZSM (4.89 t ha^{-1}). The positive effect of ZnO over Zn sulphate might be due to comparatively lower chemical transformation of ZnO than letter one under anaerobic conditions. Zhang *et al.* (2017) reported that water-soluble Zn fertilizer tends to be readily precipitate into insoluble compounds than sparingly soluble sources. The increase in biomass may be due to greater enzyme activities, significant drop in rhizosphere pH and Zn redistribution among native zinc pools which helped in increased zinc availability for crop acquisition under Zn deficient soil. The lowest grain (4.06 t ha^{-1}) and straw yield (6.67 t ha^{-1}) was recorded with ZSM treatment (Table 2). The performance of different treatments in terms of grain yield was in the order of; 9.8 kg ZnO ha^{-1} + ZSM > 14.8 kg ZnO ha^{-1} +ZSM > 25 kg Zn sulphate ha^{-1} +ZSM > 9.8 kg ZnO ha^{-1} + ZSM + 200 kg cow dung ha^{-1} > 25 kg Zn sulphate ha^{-1} > 4.9 kg ZnO ha^{-1} +ZSM > 14.8 kg ZnO ha^{-1} + ZSM + 200 kg cow dung ha^{-1} > 12.5 kg Zn sulphate ha^{-1} + ZSM > 4.9 kg ZnO ha^{-1} + ZSM + 200 kg cow dung ha^{-1} > 12.5 kg Zn sulphate ha^{-1} > control (no Zn) > ZSM. As the fertilizer dose increased above 9.8 kg ZnO ha^{-1} reduction was observed in rice growth and yield parameters. This may be attributed to the fact that Zn utilization efficiency decreases as the Zn fertilizer application increases per the Law of Diminishing return. All treatments having cow dung reduced grain and straw yield, with the greatest reduction occurring with 14.8 kg ZnO ha^{-1} + ZSM + 200 kg cow dung ha^{-1}, which could be attributed to higher Zn content inhibiting bacterial efficiency, resulting in a decrease in biomass. The involvement of Zn in numerous metallic enzyme systems, regulatory functions, auxin manufacturing, carbohydrate synthesis, and transport to grain formation sites has been attributed to higher yield due to Zn fertilization (Cakmak and Kutman 2018). This result was in corroboration with the findings of Mondal *et al.* (2020). The maximum net return (Rs 58.94 ×10^3 ha^{-1}) and benefit: cost ratio (1.88) was recorded with the application of 9.8 kg ZnO ha^{-1} when combined with Zn solubilizing bacteria (Table 2). It is self- evident that as grain and stover yields increased, so net returns also rise. When compared to the control, integrated use of inorganic Zn fertilizers and zinc solubilizing bacteria produced more yield after subtracting the extra cost of treatment, resulting in higher net returns. Similar result was observed by Shahane *et al.* (2019)

Table 2: Effect of Zn fertilizer and Zn solubilizing bacteria on yield, total nutrient uptake and economics of rice (mean of 2 years)

Treatment	Grain yield (t ha^{-1})	Straw yield (t ha^{-1})	Nitrogen (kg ha^{-1})	Phosphorous (kg ha^{-1})	Potassium (kg ha^{-1})	Zinc (g ha^{-1})	Net return (Rs ×10^3 ha^{-1})	B:C ratio
T_1	4.17	6.92	108.6	17.4	150.8	645.1	45.12	1.51
T_2	4.32	7.11	114.5	13.2	190.9	815.9	46.59	1.49
T_3	4.62	7.55	127.7	13.6	223.8	1171.5	50.82	1.57
T_4	4.06	6.67	107.7	11.4	141.5	593.1	43.12	1.44
T_5	4.48	7.41	124.4	13.3	232.5	879.2	49.51	1.59
T_6	4.89	7.88	142.8	14.1	252.6	1110.9	55.79	1.72
T_7	4.56	7.30	124.2	16.1	194.1	1101.9	51.56	1.68
T_8	5.01	7.85	141.8	15.4	238.4	1305.5	58.94	1.88
T_9	4.95	7.80	140.9	14.0	235.3	1166.3	57.14	1.78
T_{10}	4.41	7.16	127.3	15.2	205.0	1235.0	48.57	1.58
T_{11}	4.77	7.61	146.1	19.2	184.7	1364.8	54.35	1.72
T_{12}	4.55	6.91	133.2	14.2	188.4	1151.3	49.74	1.54
SEm ±	0.09	1.14	1.72	0.21	3.01	16.17	0.79	0.02
CD(P=0.05)	0.26	3.38	5.07	0.63	9.01	48.01	2.34	0.07

Total nutrient uptake

The total nitrogen uptake varied from 107.7 to 146.1 kg ha^{-1} under various treatments. It is apparent from the data (Table 2) that maximum uptake (146.1 kg ha^{-1}) was found in treatment receiving 9.8 kg ZnO ha^{-1} + ZSM + 200 kg cow dung ha^{-1}, which showed a 34.5 % increase over control (no Zn). During the later stages of decomposition, the positive effect of cow dung on N content was observed as decomposition greatly altered soil pH as a result of the release of basic cations, NH_3 and NH_4^+ production during decomposition, and increased microbial activity. The dilution effect of increasing biomass production was attributed to a decrease in N content in 9.8 kg ZnO ha^{-1} + ZSM and 25 kg Zn sulphate ha^{-1}. Higher uptake of N in Zn sulphate and ZSM inoculated treatment (8.6% and 11.8%) at both levels over alone application of 12.5 and 25 kg zinc sulphate ha^{-1} illustrated the contribution of microbial inoculation. Similar positive effect of zinc fertilizers and microbial inoculation on nitrogen uptake was reported by Shahane *et al.* (2019). The total P uptake in rice crop varied from 13.2 to 17.4 kg ha^{-1} and maximum value (19.2 kg ha^{-1}) was recorded with 9.8 kg ZnO ha^{-1} + ZSM + 200 kg cow dung ha^{-1}, whereas the ZSM treatment showed the lowest uptake (11.4 kg ha^{-1}). With higher levels of Zn, there was a corresponding decrease in P uptake, with the highest reduction (24.1%) with 12.5 kg Zn sulphate ha^{-1}. It could be due to zinc has a strong antagonistic interaction with P and prevent mobilization from the root to the top of the plant. Yadav and Singh (2021) reported similar antagonistic effect of Zn on P. The potassium uptake varied from 141.5 to 252.6 kg ha^{-1} and among the treatments, maximum value was observed with 25 kg zinc sulphate ha^{-1} + ZSM, followed by 9.8 kg ZnO ha^{-1} +ZSM (235.3 kg ha^{-1}), owing to higher nutrient concentrations in rice grain and straw, as well as higher biological yields of rice. The application of both treatments registered 67.5 and 56.0 % increase in total K uptake, respectively over control. The combination of ZSM and Zn sulphate fertilizer at both levels, significantly improved the K uptake by 41.6 and 28.8 kg ha^{-1} over non-inoculated. Suganya *et al.* (2020) also reported similar result. The total Zn uptake varied from 593.1 to 1364.8 g ha^{-1} and maximum uptake was recorded with 9.8 kg ZnO ha^{-1} +ZSM + cow dung (1364.8 g ha^{-1}). The transformation of OM occurs during decomposition as a result of hydrolysis, oxidation, or depolymerization, which may result in increased dissolved OM release and metal availability in solution phase. Zink uptake in rice crop significantly increased up to 9.8 kg ZnO ha^{-1}, above this level, a decrease in Zn uptake was observed. The application of 200 kg cow dung ha^{-1} showed a respective increase of 91.4, 111.6 and 78.5 in total Zn uptake over control. Many other compounds like humic substances, organic acids and amino acids are also produced during decomposition of OM that could increase metal availability by forming complexes with metals. The presence of increased amounts of zinc in soil solution due to zinc application may have facilitated zinc absorption through the phloem, resulting in an increase in zinc uptake in grain and straw at harvest. Similar result was reported by Prajapati *et al.* (2021). In our experiment, both sources were taken with combination of Zn solubilizing microbes which further quicken the solubilization process and greater root interception for translocation of zinc.

From the results, it may be concluded that application of 9.8 kg ZnO ha^{-1} with potential Zn solubilizing bacteria produced maximum yield and higher net return than 25 kg Zn sulphate ha^{-1}, hence can be used in place of Zn sulphate fertilizer. Further application of 14.8 kg ZnO ha^{-1} apparently reduced the yield due to nutrient imbalance in soil. Cow dung applications after transplanting significantly reduce the grain yield due to anaerobic decomposition and reduction was marked maximum with 14.8 kg ZnO ha^{-1}. The best economical return was found with combine application of microbial consortia with 9.8 kg ZnO ha^{-1}.

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