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Contribution of zinc oxide and zinc solubilizers on growth parameters of rice (*Oryza sativa*) at different growth stages

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Abstract

Zinc (Zn) is one of the most crucial micronutrients for optimum plant growth. Low land rice (*Oryza sativa* L.) is apparently more susceptible towards Zn deficiency possibly due to the prevailing submergence condition. Zn solubilizing microbes are capable of transforming soil occluded zinc *viz.* zinc oxide (ZnO), zinc carbonate (ZnCO₃), zinc phosphate [Zn₃(PO₄)₂] into plant available forms. In view of limited information, a pot experiment was conducted at Department of Soil Science and Agricultural Chemistry, Institute of Agricultural Sciences (IAS), BHU, Varanasi, UP during *khari*f, 2018-19, to evaluate the growth parameters *viz.* plant height and chlorophyll content of rice at different growth stages (30 DAT & 60 DAT) to different levels of ZnO in combination with Zn solubilizing microbes. Zinc 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 (M₀), *Enterobacter cloacae* strain ZnPSBJ-6 (M₁) and zinc solubilizing fungi (M₂). Plant height was remained statistically at par for M₀, M₁ and M₂. Whereas, highest chlorophyll content was recorded for M₂ in combination with Z₂ which were 35.7% and 39.8% higher over the control (M₀Z₀), respectively.

Keywords: Zn solubilizing microbes, submergence condition, Zn deficient soil

1. Introduction

Zinc (Zn) is essential for the healthy growth and development of living organism (Hafeez *et al.*, 2013) [6]. It plays a significant role as antioxidant and is specifically involved in the metabolism of carbohydrates and auxin in plants (Alloway, 2008; Alloway, 2004) [2, 1]. The normal growth of floral tissues, flowering, fertilization, and fruiting are all regulated by Zn-finger transcription factors (Epstein and Bloom, 2005) [4]. Zinc deficiency in plants results in slowed shoot growth, chlorosis, smaller leaves, heightened sensitivity to heat, light, and fungal infections, as well as effects on grain yield, pollen formation, root growth, water uptake, and transport (Alloway, 2004) [1]. (Tavallali *et al.*, 2010) [13].

Plants can absorb zinc as a divalent cation, but the amount of soluble zinc in soil solution is very small. The residual zinc is present as minerals and insoluble complexes (Alloway, 2008) [2]. One of the most common micronutrient deficiencies, zinc deficiency, arises because zinc is not present in soil. There are many techniques that have been used for a long time to address zinc deficiency. Zinc fertilisers have been used in the form of zinc sulphate (White and Broadly, 2005) [14] or Zn-EDTA (Karak *et al.*, 2005) [9], but their use places pressure on the economy and environment, and these fertilisers transform into insoluble complex forms within 7 days of application (Rattan and Shukla, 1991) [15]. Other techniques include genetic engineering, transgenic strategies, and conventional breeding (Tan *et al.*, 2015) [12]. These methods, however, are pretty expensive, time-consuming, and slower.

Rhizobacteria that can solubilize zinc are a superior alternative to all of these methods. Soil-borne bacteria called plant growth-promoting rhizobacteria (PGPR) colonise the rhizosphere, reproduce, and engage in bacterial competition with one another to support plant growth. By solubilizing and aiding in nutrient uptake, or by releasing phytohormones or biocontrol agents to protect plants from various pathogens, PGPR promote plant growth (Glick, 2012) [5]. Many PGPR have been discovered to be efficient zinc solubilizers. By colonising the rhizosphere and converting complex zinc compounds into simpler ones, these bacteria aid in the improvement of plant growth and development. Acidification is one of the many mechanisms used by microorganisms that solubilize zinc.

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The pH of the nearby soil is decreased and the zinc cations are sequestered by these microbes' production of organic acids in the soil. Additionally, the anions have the ability to chelate zinc and increase its solubility (Jones and Darrah, 1994) [8]. Other potential mechanisms for the solubilization of zinc include the production of siderophores, proton, oxido-reductive systems on cell membranes, and chelated ligands (Saravanan *et al.*, 2011) [11]. Keeping the above fact in mind, the present study was undertaken to assess the effect of Zn solubilizing microbes along with varying levels of zinc oxide (ZnO) on growth parameters of rice.

2. Materials and Methods

2.1 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 (Table 1).

2.2 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 (Z₀), 1.85 (Z₁) and 3.7 (Z₂) 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: T₁-No ZnO (Z₀) with no inoculation (M₀), T₂-inoculation with *Enterobacter cloacae* strain ZnPSBJ-6 (M₁) without ZnO dose (Z₀), T₃ (inoculation with zinc solubilizing fungi (M₂) with no ZnO (Z₀), T₄ (no inoculation with half dose of ZnO, *i.e.* 1.85 mg kg⁻¹(Z₁), T₅- inoculation with *Enterobacter cloacae* strain ZnPSBJ-6 (M₁) with half dose of ZnO, *i.e.* 1.85 mg kg⁻¹ (Z₁), T₆- inoculation with zinc solubilizing fungi (M₂) with half dose of ZnO, *i.e.* 1.85 mg kg⁻¹ (Z₁), T₇-no inoculation (M₀) with full dose of ZnO, *i.e.* 3.7 mg kg⁻¹ (Z₂), T₈- inoculation with *Enterobacter cloacae* strain ZnPSBJ-6 (M₁) with full dose of ZnO, *i.e.* 3.7 mg kg⁻¹ (Z₂), T₉- inoculation with zinc solubilizing fungi (M₂) with full dose of ZnO *i.e.* 3.7 mg kg⁻¹ (Z₂). Seedling root dip was done with desired microbial inoculation with the aid of some adhering substances like Jaggery 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: P₂O₅: K₂O @ 40.1: 40.2: 26.8 mg kg⁻¹ 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). Growth parameters, *i.e.* plant height and chlorophyll content were also recorded at 30 DAT and 60 DAT using a meter scale and SPAD meter, respectively.

Table 1: Initial characteristics of experimental soil (Ash *et al.*, 2020) [13].

Parameter	Value
pH1:2	7.8
EC1:2 (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

3. Result and Discussion

3.1 Plant height: A critical study of the data presented in table 2. revealed that height of rice plants upon inoculation with M₁ showed non-significant difference among the treatment at 30 DAT as compared to M₀ (83.8 cm). However, it was the highest plant height recorded at 30 DAT *i.e.* 86.9 cm which is statistically at par with the plants inoculated with M₂ (85.7 cm). Almost similar trend was noticed with the plant height recorded at 60 DAT.

It is also found that rice plants treated with different concentration of ZnO resulted in non-significant difference in plant height. However the highest plant height was recorded in the plants treated with Z₂ (86.9 cm) which is statistically at par with the plants treated with Z₁ (86.4 cm). Almost similar trend was noticed with the plant height recorded at 60 DAT. Iqbal *et al.* (2010) [7] found maximum increase in all plant growth parameters in *Vigna radiata* when seedlings were treated with ZSB isolate 102. Maximum increase in seedling length (35.10 cm) was recorded in case of ZSB isolate 36 with 1 mM zinc phosphate showed the as compared to un inoculated control. It is probably due the catalytic activity of Zn in biosynthesis of tryptophan, precursor of auxin which enhances the plant height.

Table 2: Effect of zinc solubilizing microbes and different levels of ZnO on plant height of rice crop

Treatments	Plant Height (cm)	
	30 DAT	60 DAT
M ₀	83.8	96.7
M ₁	87	101.9
M ₂	85.7	97.3
SEm±	1.85	1.68
CD (0.05)	5.51	5.00
Z ₀	83.2	97.5
Z ₁	86.4	98.0
Z ₂	86.9	100.4
SEm±	1.85	1.68
CD (0.05)	5.51	5.00
Interaction	NS	NS
CV	6.50	5.12

3.2 Chlorophyll content: Data pertaining to chlorophyll content (leaf SPAD value) are presented in table 3. Significant increase in chlorophyll content (leaf SPAD value, 47.1) at 30 DAT was recorded with the application of M₁ over M₀ (leaf SPAD value, 42.5). Results also revealed that increase in chlorophyll content at 30 DAT was recorded as leaf SPAD

value 47.7 when it was inoculated with of M_2 . Almost similar trend was noticed with the chlorophyll content recorded at 60 DAT (table 4). It is also found that rice plants treated with different level of ZnO resulted in significant difference in chlorophyll content. At 30 DAT highest chlorophyll content was recorded in the plants treated with Z_2 (leaf SPAD value 49.9) which is significantly superior to plants treated with Z_0 (leaf SPAD value 43.8). Results also revealed that increase in chlorophyll content (leaf SPAD value, 43.5) at 30 DAT was recorded when it was treated with of Z_1 . Almost similar trend was noticed with the chlorophyll content recorded at 60 DAT. Interaction between different level of ZnO and ZSM has increased significant chlorophyll content (leaf SPAD value, 59.9) at 30 DAT was recorded with the application of M_2Z_2 over M_0Z_0 (leaf SPAD value, 41.5). Almost similar trend was observed with the chlorophyll content recorded at 60 DAT. Samreen *et al* (2017) [10] reported that different levels of Zn had positively influenced the chlorophyll content in Mungbeans plant (*Vigna radiata*). It was probably due to the Zn plays a role in chlorophyll synthesis.

Table 3: Effect of zinc solubilizing microbes and different levels of ZnO on chlorophyll content (SPAD value) of rice crop at 30 DAT

Z*M	M ₀	M ₁	M ₂	Mean
Z ₀	41.5	43.5	46.5	43.8
Z ₁	46.0	47.7	36.7	43.5
Z ₂	39.9	50.1	59.9	49.9
Mean	42.5	47.1	47.7	
SEm±	M=1.43 Z=1.43 M*Z =2.47			
CD	M=4.24 Z=4.24 M*Z=7.34			

Table 4: Effect of zinc solubilizing microbes and different levels of ZnO on chlorophyll content (SPAD value) of rice crop at 60 DAT

Z*M	M ₀	M ₁	M ₂	Mean
Z ₀	40.4	42.8	36.1	39.8
Z ₁	41.8	47.7	41.7	43.7
Z ₂	36.2	50.7	56.5	47.8
Mean	39.5	47.1	44.8	
SEm±	M=1.05 Z=1.05 M*Z =1.81			
CD	M=3011 Z=3.11 M*Z =5.39			

4. Conclusion

Intervention of ZnO of Zn solubilizing microbes has a significant positive effect on chlorophyll content as Zn plays a very important role in chlorophyll synthesis but the treatments ae found have non-significant effect on plant height.

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