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In vivo Efficacy of Zinc Solubilizing Bacteria on Available Zinc Content, Growth and Yield Attributes of Paddy (*Oryza sativa*)

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Authors' contributions

This work was carried out in collaboration with all authors. Author SGM wrote the protocol and wrote the first draft of the manuscript. Authors Mahadevaswamy and YR designed the study and helped to perform the statistical analysis. Authors MNN and RCG managed the analyses of the study. Author Mahadevaswamy managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The experiment was aimed at assessing the effect of zinc solubilizing isolates on the available zinc content, growth and yield attributes of paddy. The study was conducted using a randomized block design at the experimental plots of Agricultural Research Station, Dhadesugur. The isolates namely MZSB 6 and MZSB 8 were tested for *in vitro* solubilization of the zinc and later brought under field condition. 25-day-old paddy seedlings were dipped in lignite based biofertilizer slurry and transplanted according to treatments. Data on growth and yield parameters of paddy were taken at regular intervals of 30 DAT, 60 DAT and 90 DAT and available plant zinc content were estimated using the Inductively Coupled Plasma Mass Spectrometry. Growth and yield parameters of paddy showed a significant increase in the treatment that received combination of MZSB 6, MZSB 8 and 75% recommended dose of fertilizer (RDF) as compared to control and other treatments. Results

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also showed the highest available zinc of 46.18 mg kg^{-1} of plant estimated using the ICP-MS. Thus, the results revealed that the combination of both isolates with 75% RDF was found to be efficient in enhancing growth and yield of paddy.

Keywords: Zinc; in vitro; lignite; RDF; ICP-MS; etc.

1. INTRODUCTION

Rice (*Oryza sativa* L.) is one of the significant sustenance crops and the biggest yield developed on the planet as far as both area and production are concerned [1,2]. The greater part of the total populace relies upon rice, particularly in developing nations. It gives around 90% of carbohydrates and 8% of protein. All-around world, rice is developed in an area about 161.40 million hectares and production of about 487.50 million tonnes with a productivity of 3.14 tons per hectare. In India, rice is being produced with an area of 43.993 million hectares and positions second underway (109.698 million tons) alongside China. India sends out 9.3 million tons of rice to the nations around the globe. The significant rice-growing states in India are Karnataka, Andhra Pradesh, Bihar, Uttar Pradesh, Madhya Pradesh, West Bengal, and Punjab. In Karnataka, rice is being grown in an area of 1.03 million hectares with the yearly generation of 2.604 million tones and productivity is observed to be 2494 kg ha^{-1}.

The primary situation in India for rice production entails excessive rainfall/drought stipulations, prolonged utilization of typical varieties due to scarcity of elevated seed types or lack of talents in farmers about them, heavy infestation of weeds, pests and diseases, low soil fertility, indiscriminate use of fertilizers and many other which effects in reduced rice production. Amongst these, low soil fertility is an important component which now not only influences the rice production but also reduces the quality of the rice. Chaudhary et al. [3] suggested Zn deficiency as the main component which decides the rice production in a number of constituents of India [4]. In accordance with Singh [5], 48% of soils in India are dealing with Zn deficiency. In rice, Zn deficiency factors a couple of symptoms that most of the time show up 3 weeks after transplanting the seedlings; leaves advance brown blotches and streaks that will fuse to quilt older leaves, vegetation remain stunted and in severe instances could die, even as these which recover exhibit lengthen in maturity and reduction in yield [6].

One of the vital viable approaches to develop crop productiveness and food quality without causing any damage to the ecosystem is the usage of plant growth-promoting rhizobacteria (PGPR). There are a couple of reviews in which PGPR were proven as good replacement to chemical fertilizers for increasing the plant development and yield which can aid in minimizing agrochemicals usage. The PGPR colonize the rhizosphere, root surface, and internal tissues and accordingly render improvements to the nutrient availability and hinder the pathogens close the roots. The mechanisms wherein PGPR enhance plant growth include N-fixation, inorganic P solubilization, siderophore production, phytohormone synthesis and capability in controlling plant pathogens [7]. Distinct plant growth-promoting bacteria including free-living and associative bacteria such as *Azospirillum, Azotobacter, Bacillus,* and *Pseudomonas* which were used in agricultural practices as biofertilizers for their benefits on plant growth [8].

The study was conducted with the aim of selecting the efficient combination of zinc solubilizing bacterial isolates based on their effect on the plant growth and yield parameters. This study was also aimed to develop inoculants into commercialization potential along with proper dosage of fertilizers which is a major challenge preventing the bio-inoculants production technology. Such isolates blend with a satisfactory amount of inorganic composts and expand the bioavailability of zinc to the rice plant, promote the nutrient recycling and increase the growth and yield of the crop simultaneously contributing to the sustainable ecosystem.

2. MATERIALS AND METHODS

The zinc solubilizing bacteria used in this experiment were obtained from the Department of Agricultural Microbiology, UAS Raichur. The isolates were previously studied for its ability to solubilize the inorganic of insoluble zinc under *in vitro* conditions and various plant growth-promoting properties such as the production of indole acetic acid, siderophore and phosphate solubilization.

2.1 Field Preparation and Experimental Design

The main field was well prepared for transplantation and divided into plots as experimental units. Randomized complete design with three replications was used. The 25-day-old rice seedlings were uprooted from the nursery, treated with inoculants accordingly and transplanted into the plots with the spacing of 25 cm × 25 cm.

2.2 Inoculum Preparation

The 24 hrs old cultures were inoculated into a 250 ml conical flask containing 100 ml sterilized nutrient broth; incubated on a shaker for 3 days for development of mother culture. Simultaneously, two liters of nutrient broth was prepared in a round bottom flask separately for each inoculant. 40 ml of mother culture were inoculated into flasks and incubated for development of inoculum to achieve 10^7 CFU ml^{-1} that was confirmed by serial dilution technique and agar plating method.

2.3 Carrier Material

Lignite powder was used as a carrier material. It was sterilized using an autoclave at 121°C, 15 lb pressure for 30 minutes. Later, the broth culture was mixed thoroughly with the sterilized carrier material in the ratio of 1:2.5 shade-dried to bring down the moisture levels to 30%.

2.4 Seedling Root Dip

One kg of bioinoculant was mixed with five litres of water in a bucket and mixed thoroughly. The roots of the seedling bundles were dipped in the bioinoculant suspension for about 30 minutes and taken out. The seedlings root coated with biofertilizer were transplanted immediately to the main field.

2.5 Growth and Yield Parameters

Plant growth attributes such as plant height (cm) and the number of tillers per hill were observed and recorded at regular intervals of 30 DAT, 60 DAT, and 90 DAT. Yield attributes such as panicle length (cm), the number of grains per panicle, and grain yield were observed and recorded at the time of harvest.

2.6 Estimation of Available Zinc in Plants Using the ICP-MS

The plant samples were ground to obtain a homogenous portion for analysis. Exactly 0.25 g of ground plant samples were weighed into the digestion vessel and 7 ml of nitric acid and 0.5 ml of hydrogen peroxide were added carefully. Digestion vessel was covered and incubated for 10-15 minutes in the hood at room temperature. The digestion vessel containing homogenized plant sample material was then transferred into microwave digester. Digestion process was carried out for 80 minutes. The sealed pressure vessel was cooled to ambient temperature to reduce pressure inside the digestion vessel. After digestion and cooling, digestion vessel was removed from microwave digester and kept in a fume hood until brown fumes were no longer visible. This sample solution was filtered using a nylon membrane filter. The filtered sample was used for analysis by using the ICP-MS.

Table 1. Chemicals

Chemical	Grade
Nitric Acid	Supra pure, JT Baker
Hydrogen Peroxide	Supra pure, JT Baker
Water	Milli-Q
Stock solutions of Zn	Perkin Elmer, 1000 µg/ml

Table 2. Equipment

Instrument / Equipment	Make
ICP-MS	Perkin Elmer Nexion 350X
Weighing Balance	SARTORIUS-BSA 224S-CW
Microwave Digester	Titan MPS
Micro Pipettes- 0.2 ml, 1 ml	Eppendorf
Measuring cylinder- 10 ml	Rankem
Volumetric flask- 25 ml, 50 ml	Rankem

Table 3. Instrumental specifications

ICP-MS	Perkin Elmer Nexion 350X
Source	Plasma
ICP RF Power	1600 Watts
Argon Pressure	90-100 Psi
Helium Pressure	25 Psi
Plasma gas flow	18L /min
Nebulizer gas flow	0.92 L/min
Auxiliary gas flow	1.92 L/min
Pump Speed	20 rpm

2.7 Statistical Analysis of the Data

The data for parameters of growth and yield of paddy were subjected to one-way analysis of variance (ANOVA) of Snedecor and Cochran [9] and mean separations using Tukey's HSD at p<0.05.

3. RESULTS AND DISCUSSION

The field experiment was conducted during Jan-May 2018 to study the effect of zinc solubilizing bacteria on growth and yield of paddy and the details of the systematic study are unveiled below. Two efficient isolates of zinc solubilizing bacteria were evaluated under field conditions for their individual and interaction effect on growth and yield of paddy in comparison with control. The test inoculants from isolates of MZSB-6 and MZSB-8 were prepared according to the standard procedure and applied to the main field in the plots as per treatments. The uninoculated plot served as a control.

3.1 Plant Height

Significant differences in the plant height of rice were observed at 30, 60, 90 DAS and at harvest due to various inoculation treatments and fertilizer applications. On 30th DAT, the pooled data show that there was a significant difference between combined applications of the inoculants when compared to treatments with individual inoculation (Table 5). The combined inoculation in treatment T_8 (MZSB 6 + MZSB 8 + 75% RDF) showed maximum plant height of 31.2 cm followed by treatment T_2 (100% NPK +$ZnSO_4$) with 30.5 cm and these two treatments were significant with each other. The individual inoculation of zinc solubilizing bacterial inoculants in treatment T_3 (MZSB 6) showed a plant height of 23.5 cm whereas treatment T_4 (MZSB 8) recorded plant height of 24.3 cm and these two treatments were significant to each other. Individual inoculation of treatment T_5 (reference strain) recorded plant height of 25.1 cm. The control recorded lowest plant height of 20.2 cm. On 60th DAT, the combined inoculation in treatment T_8 showed significant plant height of 60.2 cm when compared to T_2 which recorded 57.2 cm. Combined inoculations were superior and significantly different when compared with individual inoculation. The individual inoculation in treatment T_3 showed a plant height of 48.5 cm and T_4 showed 49.2 cm those were non-significant to each other. Individual reference strain showed plant height 50.2 cm. The control recorded the lowest plant height of 45.2 cm. On 90th DAT, T_8 recorded plant height of 83.2 cm and T_2 recorded 81.5 cm and these two were non-significant to each other. Similarly, T_3 showed 70.3 cm of plant height and T_4 showed 71.2 cm of plant height which was non-significant to each other. However, combined inoculation was superior and significantly higher to individual inoculation. Treatment T_5 recorded a plant height of 72.3 cm. The control recorded lowest plant height of 68.2 cm. At harvest, T_2 and T_8 recorded plant height of 83.2 cm and 85.2 cm respectively which were significant to each other. Similar proceedings were followed for individual inoculation wherein T_3 and T_4 recorded plant height of 73.5 cm and 74.2 cm respectively. Treatment T_5 with individual inoculation reference strain showed plant height of 75.2 cm. The control recorded the lowest plant height of 70.2 cm.

3.2 Total Number of Tillers per Hill

Significant differences in the number of tillers per hill of rice were observed at 30, 60, 90 DAS and at harvest due to various inoculation treatments and fertilizer application. On 30th DAT, the pooled data insisted that there was a significant difference between combined application of inoculants when compared to treatments with individual inoculation (Table 6). The combined inoculation in treatment T_8 (MZSB 6 + MZSB 8 + 75% RDF) showed maximum number of tillers per hill (6.12) followed by treatment T_2 (100% NPK + $ZnSO_4$) with 5.25 tillers per hill and these two treatments were significant with each other. The individual inoculation of zinc solubilizing bacterial inoculants in treatment T_3 (MZSB 6) showed 4.01 whereas treatment T_4 (MZSB 8) recorded 4.21 tillers per hill and these two treatments were nonsignificant to each other. Individual inoculation of treatment T_5 (reference strain) recorded 4.25 number tillers per hill. The control recorded 4.00 tillers per hill. On 60th DAT, the combined inoculation in treatment T_8 showed 8.25 tillers per hill when compared to T_2 which recorded 7.51 tillers per hill. Combined inoculations were superior and significantly different when compared with individual inoculation. The individual inoculation in treatment T_3 showed 5.01 tillers per hill and T_4 showed 5.12 which were non-significant to each other. Individual reference strain showed 5.25 tillers per hill (Fig. 1). The control recorded lowest number of tillers per hill (4.56). On 90th DAT, T_8 recorded 11.2 and T_2 recorded 9.21 tillers per hill and these two were non-significant to each other. Similarly, T_3 showed 6.0 and T_4 showed 6.12 tillers per hill which were non-significant to each other. However, combined inoculation was superior and significantly higher to individual inoculation. Treatment T_5 recorded 6.35 tillers per hill. The control recorded 5.24 tillers per hill. At harvest, T_2 and T_8 recorded 10.2 and 12.5 tillers per hill respectively which were significant to each other. Similar proceedings were followed for individual inoculation wherein T_3 and T_4 recorded 6.01 and 6.18 tillers per hill respectively as observed at harvest. Treatment T_5 with individual inoculation of reference strain showed 6.85 tillers per hill. The control recorded the lowest number of tillers per hill (6.21).

These two bacteria were studied previously for Zn and P solubilization, siderophore, acid production, and IAA production. Thus, these characters are responsible for the increase in the growth of the plants. Several workers have reported the beneficial effects of different strains of *Burkholderia, Acinetobacter, Bacillus, Enterobacter, Alcaligenes, Arthrobacter, Azospirillium, Azotobacter, Beijerinckia, Erwinia, Flavobacterium, Pseudomonas, Rhizobium* and *Serratia* and identified them as prominent PGPR's [10].

3.3 Yield Parameters

3.3.1 Panicle length

The combined inoculation in T_8 (MZSB 6 + MZSB 8 + 75% RDF) recorded 21.4 cm of panicle length and T_2 (100% RDF + $ZnSO_4$) which showed 20.4 cm of panicle length (Table 6). The treatments were significant to each other. However, T_2 and T_8 were significant to single inoculation in T_3 (MZSB 6) which recorded 17.1 cm of panicle length and T_4 (MZSB 8) which showed 17.2 cm of panicle length. Similarly, single inoculation in T_6 (MZSB 6 + 75% RDF) recorded 19.1 cm and T_7 (MZSB 8 + 75% RDF) recorded 19.2 cm of panicle length which was significantly lower to T_7 and T_8. The data pertaining to the panicle length showed that the application of MZSB 6 and MZSB 8 along with 75% RDF significantly increased the length of panicle at harvest of the crop. The control recorded significantly lower (15.2 cm) panicle length compared to reference strain while it recorded readings of 17.5 cm of panicle length.

3.3.2 Total number of seeds per panicle

The maximum of a total number of grains per panicle was observed in T_8 (220), which received the treatment combination of MZSB 6 and MZSB 8 along with 75% RDF (Table 6). Followed by T_7 (215) that was treated with RDF (100% NPK) along with $ZnSO_4$. The minimum was observed in T_1 (150) which was control. Treatments namely T_3 showed 173 seeds per panicle, T_4 recorded 178 seeds per panicle and T_5 showed 180 seeds per panicle in which the inoculant was reference strain. There was no significant difference between them. The observations recorded in T_6 and T_7 were 210 and 215 respectively which received individual inoculants (MZSB 6 and MZSB 8, respectively) along with 75% RDF. But there was a significant difference observed in the treatments than the control.

3.3.3 Grain yield

The maximum grain yield was observed in T_8 which yielded 5245 kg/ha which received a dual application of MZSB6 and MZSB8 along with 75% RDF (Table 6). The minimum grain yield was observed in T_1 (3215 kg/ha) which is control. The grain yield increased in all the treatments over control. The individual inoculations in treatment T_3 (MZSB 6) yielded 3985 Kg/ha and T_4 (MZSB 8) yielded 4025 Kg/ha and were non-significant to each other (Fig. 2). However, combined inoculations were significantly superior to their individual inoculations. The individual inoculation of zinc solubilizing bacteria in T_6 (MZSB 6 + 75% RDF) yielded 4753 Kg/ha and T_7 (MZSB 8 + 75% RDF) which yielded 4865 Kg/ha. The treatments were non-significant to each other. The individual inoculation of reference strain in T_5 yielded 4125 Kg/ha which was significantly lower than the combined inoculations of MZSB 6 and MZSB 8 along with 75% RDF. T_2 which received RDF (100% NPK) and inorganic zinc amendment *i.e.* $ZnSO_4$ recorded 5132 Kg/ha which was nonsignificant to the T_8 which received dual inoculants along with 75% RDF. The control recorded a significantly lower grain yield of 3215 Kg/ha compared to all the treatments.

3.3.4 Straw yield

The maximum straw yield was observed in T_8 (5717 kg/ha) which received a dual application of MZSB 6 and MZSB 8 along with 75% RDF (Table 6). The minimum grain yield was observed in T_1 (3504 kg/ha) which is control. The straw yield increased in all the treatments over control. The individual inoculations in treatment T_3 (MZSB 6) yielded 4344 kg/ha and T_4 (MZSB 8) yielded 4387 kg/ha and were non-significant to each other (Fig. 2). However, combined inoculations were significantly superior to their individual inoculations. The individual inoculation of zinc solubilizing bacteria in T_6 (MZSB 6 + 75% RDF) yielded 5181 Kg/ha and T_7 (MZSB 8 + 75% RDF) which yielded 5303 Kg/ha. The treatments were non-significant to each other. The individual inoculation of reference strain in T_5 yielded 4496 Kg/ha which was significantly lower than the combined inoculations of MZSB 6 and MZSB 8 along with 75% RDF. T_2 which received RDF (100% NPK) and inorganic zinc amendment *i.e.* $ZnSO_4$ recorded 5594 Kg/ha which was significantly inferior to the T_8 which received dual inoculants along with 75% RDF. The control recorded a significantly lower straw yield of 3504 Kg/ha compared to all the treatments.

3.4 Estimation of Available Zinc in Plants Using the ICP-MS

Available zinc in plants was highest of 46.18 mg/kg in the treatment T_8 which had dual bacterial culture of both MZSB 6, MZSB 8 along with the 75% of RDF followed by T_2 having 100% NPK and when compared to reference (T_5) which had 27.46 mg/kg of available zinc and the minimum available zinc was noted in control (Table 7; Fig. 3).

The results agreed with the observations of other workers. Vaid et al. [6] reported that the effect of 160 *Burkholderia sp.* SG1 (BC), *Acinetobacter sp*. SG2 (AX) and *Acinetobacter sp*. SG3 (AB) isolated from rice fields deficit in Zn on the growth parameters and Zn nutrition of rice plants was significantly high and found that the co-inoculation of rice seedlings with isolated *Burkholderia* and *Acinetobacter* strains significantly increased the number of productive tillers plant^{-1}. Similarly, Mohite [11] reported that inoculation of wheat seedlings with rhizosphere soil isolates significantly increased the plant height, root length and chlorophyll content over the control. In our study, we observed that bacterial inoculations were effective in enhancing the Zn uptake in plants.

Table 4. Plant height of transplanted rice as influenced by the application of microbial inoculants

Treatment	Plant height (cm)			
	30 DAT	60 DAT	90 DAT	At harvest
T_1-Control	20.2	45.2	68.2	70.2
T_2- RDF (100 % NPK) + $ZnSO_4$	30.5	57.2	81.5	83.2
T_3- MZSB 6	23.5	48.5	70.3	73.5
T_4- MZSB 8	24.3	49.2	71.2	74.2
T_5- Reference strain	25.1	50.2	72.3	75.2
T_6- MZSB 6 + 75 % RDF	27.6	55.1	79.5	81.5
T_7-MZSB 8 + 75 % RDF	28.5	56.3	80.2	82.1
T_8- MZSB 6 + MZSB 8 + 75 % RDF	31.2	60.2	83.2	85.2
SEM (±)	0.24	1.07	0.59	0.75
C.D. at 5 %	0.72	3.21	1.78	2.25

Table 5. Number of tillers per hill of transplanted rice as influenced by the application of microbial inoculants

Treatment	Number of tillers/hill			
	30 DAT	60 DAT	90 DAT	At harvest
T_1 - Control	4.00	4.56	5.24	6.21
T_2 - RDF (100 % NPK) + $ZnSO_4$	5.25	7.51	9.21	10.2
T_3 - MZSB 6	4.01	5.01	6.00	6.01
T_4 - MZSB 8	4.21	5.12	6.12	6.18
T_5 - Reference strain	4.25	5.25	6.35	6.85
T_6 - MZSB 6 + 75 % RDF	5.05	7.02	8.12	9.15
T_7 - MZSB 8 + 75 % RDF	5.21	7.12	8.25	9.81
T_8 - MZSB 6 + MZSB 8 + 75 % RDF	6.12	8.25	11.2	12.5
SEM (±)	0.30	0.27	0.67	0.81
C.D. at 5 %	0.92	0.81	2.01	2.45

Table 6. Yield parameters of transplanted rice as influenced by the application of bacterial inoculants

Treatment	Yield parameters			
	Panicle length (cm)	No. of seeds/ panicle	Grain yield (kg/ha)	Straw yield (kg/ha)
T_1 - Control	15.2	150	3215	3504
T_2 - RDF (100 % NPK) + $ZnSO_4$	20.4	210	5132	5594
T_3 - MZSB 6	17.1	173	3985	4344
T_4 - MZSB 8	17.2	178	4025	4387
T_5 - Reference strain	17.5	180	4125	4496
T_6 - MZSB 6 + 75 % RDF	19.1	210	4753	5181
T_7 - MZSB 8 + 75 % RDF	19.2	215	4865	5303
T_8 -MZSB 6 + MZSB 8 + 75 % RDF	21.4	220	5245	5717
SEM (±)	0.34	3.73	38.3	41.6
C.D. at 5 %	1.02	11.2	115	125

Table 7. Estimation of available zinc in plants using the ICP-MS

Treatment	Available Zn (mg/kg)
T_1 -Control	27.08
T_2 - RDF (100 % NPK) + $ZnSO_4$	31.39
T_3- MZSB 6	27.46
T_4- MZSB 8	27.89
T_5-Reference strain	28.03
T_6- MZSB 6 + 75 % RDF	29.46
T_7- MZSB 8 + 75 % RDF	30.06
T_8- MZSB 6 + MZSB 8 + 75 % RDF	46.18
SEM (±)	0.299
C.D. at 5 %	0.915

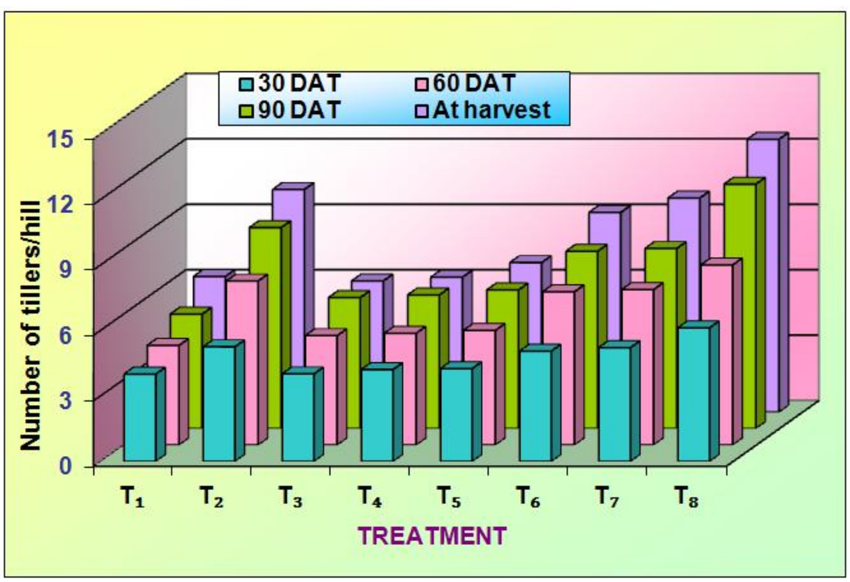

Fig. 1. Number of tillers per hill of transplanted rice as influenced by the application of microbial inoculants

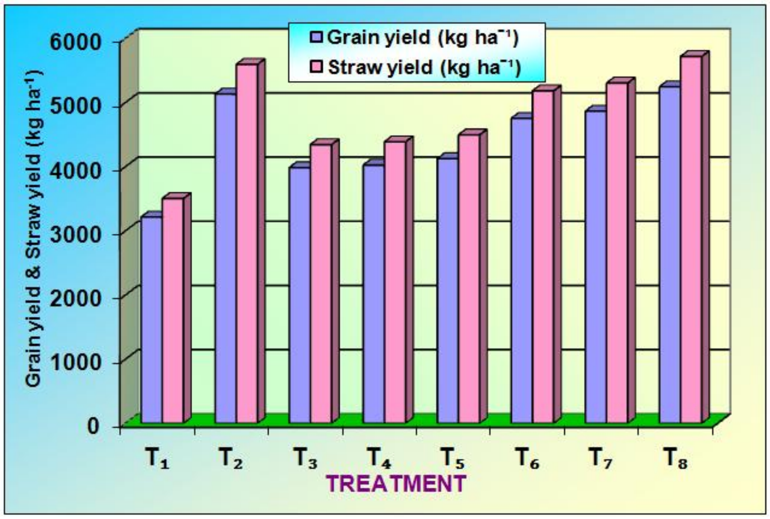

Fig. 2. Grain yield and straw yield of transplanted rice as influenced by the application of bacterial inoculants

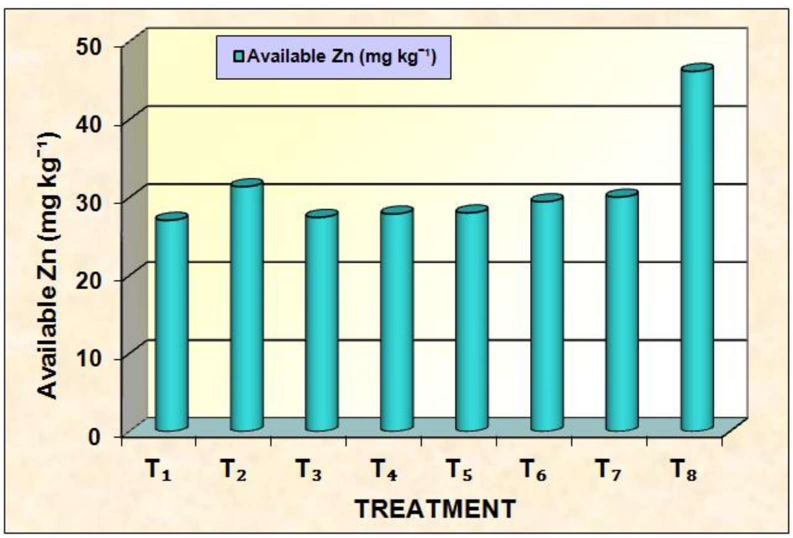

Fig. 3. Estimation of available zinc in plants using ICP-MS

4. CONCLUSION

Results from field experiment clearly indicated that the strains had positively influenced plant growth attributes *viz.,* plant height and number of tillers as well as yield parameters such as panicle length, number of grains per panicle, grain and straw yield. The results from the study evidently proved the advantage of combining MZSB 6 and MZSB 8 along with 75% RDF. Therefore, this study indicated that the growth and yield of rice would be improved by the application of zinc solubilizing bacteria along with the nutrient management by reducing at least 25% of the recommended dosage of chemical fertilizers.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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