Compost enriched with ZnO and Zn-solubilising bacteria improves yield and Zn-fortification in flooded rice

Hassan Zeb,[1] Azhar Hussain,[2] Muhammad Naveed,[1] Allah Ditta,[3,4] Shakeel Ahmad,[5] Muhammad Usman Jamshaid,[5] Hafiz Tanvir Ahmad,[6] Muhammad Baqir Hussain,[5] Riffat Aziz,[3] Muhammad Sajjad Haider[7]

[1]Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad, Pakistan; [2]Department of Soil Science, University College of Agriculture and Environmental Sciences, the Islamia University of Bahawalpur, Bahawalpur, Pakistan; [3]Department of Environmental Sciences, Shaheed Benazir Bhutto University Sheringal, Khyber Pakhtunkhwa, Pakistan; [4]School of Biological Sciences, The University of Western Australia, Perth, WA, Australia; [5]Department of Soil and Environmental Sciences, Muhammad Nawaz Sharif University of Agriculture, Multan, Pakistan; [6]District Fertility Laboratory, Kasur, Pakistan; [7]Department of Forestry, College of Agriculture, University of Sargodha, Sargodha, Pakistan

Abstract

Zinc (Zn) is an essential element for humans, animals and plants, however, its deficiency has been widely reported around the world especially in flooded rice. Adequate amount of Zn is considered essential for optimum growth and development of rice. We hypothesised that management practices like Zn-mineral fertiliser, -compost, and -solubilising bacteria would improve Zn availability and uptake in flooded rice. A series of studies were conducted to find out the comparative efficacy of Zn-enriched composts (Zn-ECs) with Zn solubilising bacteria (ZnSB) *vs.* $ZnSO_4$ for improved growth, yield and Zn accumulation in rice. There were six treatments *viz.* control, ZnSB, ZnO (80% Zn), $ZnSO_4$ (33% Zn), Zn-$EC_{80:20}$ and Zn-$EC_{60:40}$. In all the treatments, Zn was applied at the rate of 5 kg ha^{-1} except the control. The treatment Zn-$EC_{60:40}$ resulted in the maximum Zn release in soil as compared to $ZnSO_4$ and all other treatments during incubation study. The treatment Zn-$EC_{60:40}$ significantly improved root dry weight, grain yield and 100-grain weight of rice by 15, 22 and 28%, respectively as compared to $ZnSO_4$. The same treatment resulted in the maximum increase in photosynthetic rate (11%), transpiration rate (21%), stomatal conductance (17%), chlorophyll contents (8%) and carbonic anhydrase activity (10%) while a decrease of 27% in electrolyte leakage was observed in comparison with $ZnSO_4$ application. Moreover, the maximum increase in grain quality parameters and Zn bioaccumulation was observed with the application of Zn-$EC_{60:40}$ in comparison with $ZnSO_4$ application and all other treatments. We conclude that Zn-$EC_{60:40}$ are not only an effective strategy to improve growth, physiology and yield parameters of rice, but also to improve the grain quality and Zn-bioaccumulation in rice compared to $ZnSO_4$.

Correspondence: Allah Ditta, Department of Environmental Sciences, Shaheed Benazir Bhutto University Sheringal, Dir (U), Khyber Pakhtunkhwa, 18000, Pakistan. E-mail: allah.ditta@sbbu.edu.pk; ad_abs@yahoo.com
Azhar Hussain, Department of Soil Science, University College of Agriculture and Environmental Sciences, The Islamia University of Bahawalpur, Bahawalpur-63100, Pakistan.
E-mail: azharhaseen@gmail.com

Key words: *Bacillus*; biofortification; rice; zinc solubilising bacteria; compost; ZnO; $ZnSO_4$.

Acknowledgements: the authors are thankful to Institute of Soil & Environmental Science, University of Agriculture, Faisalabad, Pakistan for provision of all research facilities required.

Received for publication: 23 June 2018.
Revision received: 30 August 2018.
Accepted for publication: 4 September 2018.

Italian Journal of Agronomy 2018; 13:1295
doi:10.4081/ija.2018.1295

Introduction

Rice is the staple food of more than 3 billion people around the world and the second most important cereal crop in Pakistan after wheat contributing about 0.7% of gross domestic product (GOP, 2013-14). Worldwide, more than 50% of the rice crop is prone to Zn deficiency (Rehman *et al.,* 2012), which is positively correlated with widespread human Zn deficiency. Malnutrition, especially in developing world due to consumption of food with insufficient vitamins, nutrient or mineral contents are a serious threat to human health (Wakeel *et al.,* 2018). It has been recognised as the third most deficient micronutrient after iron and iodine (Shivay *et al.,* 2015). Besides its role in human health, Zn is also crucial for optimum production of crop plants and has been recognised as an essential micronutrient. In plants, Zn is utilised for DNA replication, proteins and nucleic acids synthesis, structural and catalytic activities, membrane stability, energy transfer reactions (Gurmani *et al.,* 2012) and chlorophyll formation (Cakmak, 2009). Owing to the significance of Zn, optimum Zn nutrition is inevitable for normal human health and functioning and also to improving crop productivity. Therefore, the prevailing scenario warns the scientific community to find some sustainable and economical ways to combat Zn deficiency. Fortification of foods by the addition of deficient minerals or vitamins during food processing has been known to cure the deficiency of that particular element (Palmgren *et al.,* 2008). However, this approach is not affordable for the people residing in developing countries due to high costs involved. On the other hand, biofortification of crops has emerged as a promising approach in this regard. Production of food with higher concentration of Zn and consumption of that food by the people looks more sustainable and an economical approach as biofortified food in comparison to industrially fortified foods, is easily available and affordable for poor people.

To produce food with higher Zn contents, Zn bioavailability in soil must be improved in order to increase plant uptake. Although, most of the soils around the world contain adequate amount of total Zn but most of this is in unavailable form to the crop plants (Mandal and Hazara, 1997). Around 30% of the world and 70% of the agricultural soils in Pakistan are Zn deficient (Hamid and Ahmad, 2001; Cakmak, 2008). In Pakistan, the soils are calcareous in nature with low organic matter (0.4-0.7%) and high pH (7.5-8.4), which contributes towards Zn deficiency (Joy *et al.,* 2017). With a unit increase in soil pH, 100-times decrease in Zn availability has been documented (Havlin *et al.,* 2005). To ensure optimum Zn supply to plants, chemical fertilisers such as zinc sulphate ($ZnSO_4$) and chelated Zn are used, but these are not affordable for poor farmers due to low fertiliser use efficiency and high cost. It has been found that $ZnSO_4$ becomes unavailable to the crop plants within seven days of application due to its precipitation/adsorption with soil matrix (Rattan and Shukla, 1991). Therefore, it is imperative to improve Zn availability in the root zone through economical and sustainable use of organic and inorganic amendments.

Zinc oxide (ZnO) is an economical source of Zn as it contains (50-80% Zn) compared to $ZnSO_4$ (33% Zn). However, the main problem with ZnO application is its low solubility thereby, low bioavailability of Zn into the soil as compared to the readily soluble $ZnSO_4$. Moreover, the costs involved with $ZnSO_4$ application are more as compared to that of ZnO due to low Zn contents. It has been found that certain rhizobacteria with Zn solubilising activity, *e.g*. Zn-solubilising bacteria (ZnSB) have the potential to improve Zn availability to the crop plants via lowering the pH up to 0.47 units through the secretion of some organic acids and proton extrusion (Wu *et al.,* 2006; Hussain *et al*., 2015). There are also reports about the secretion of Zn chelating compounds and improvement in shoots and root growth of rice through the inoculation of ZnSB (Gontia-Mishra *et al.,* 2017; Othman *et al.,* 2017). Other mechanisms employed by plant growth promoting rhizobacteria with Zn-solubilising activity include synthesis of phytohormones, production of siderophores, phosphorus (P) solubilisation, nitrogen (N) fixation and tolerance against biotic and abiotic stresses (Compant *et al.,* 2005; Hussain *et al*., 2015; Gontia-Mishra *et al.,* 2017; Othman *et al.,* 2017).

Similarly, application of enriched compost has also been found an attractive option to increase availability of various nutrients like nitrogen, phosphorus and potassium (Ditta *et al.,* 2015, 2018; Farooq *et al.,* 2018). However, our study is based on the hypothesis that compost enriched with ZnO and ZnSB would be an effective biofortification strategy in order to alleviate Zn deficiency in crop plants. Addition of compost can also increase organic matter contents in the soil, thereby improving soil physiochemical and biological properties and ultimately the availability of Zn to the crop plants. To the best of our knowledge, we have first time utilised ZnO, a slow release Zn-fertiliser as Zn-enriched compost along with ZnSB. Based on the above hypothesis, a series of incubation and pot studies were conducted in order to investigate the biofortification potential of Zn-enriched compost with ZnSB using rice (*Oryza sativa* L.) as a test crop. The objectives of the studies conducted were as follows: i) to investigate Zn release pattern in soil amended with compost enriched with ZnO and ZnSB; and ii) to evaluate the potential of compost enriched with ZnO and ZnSB on growth, physiology, yield and Zn fortification of rice.

Materials and methods

Inoculum preparation

Pre-isolated and pre-characterised *Bacillus* sp. AZ6 (Accession number KT221633) having Zn-solubilising ability (13.55 µg Zn mL^{-1} broth media) was taken from the Environmental Sciences Laboratory, Institute of Soil & Environmental Science, University of Agriculture, Faisalabad, Pakistan (Hussain *et al.,* 2015). Broth culture of *Bacillus* sp. AZ6 was prepared using Bunt and Rovira medium containing 0.1% ZnO in a sterilised conical flask (Bunt and Rovira, 1955), incubated for 48 h at 28±1°C in a shaking incubator at 100 rpm. The final concentration of ZnSB in the broth culture was 10^7-10^8 colony-forming units (CFU) per mL.

Preparation of zinc-enriched compost

Standard procedures were followed for the preparation of Zn-enriched compost (Ditta *et al.,* 2015). In brief, fruit peels and vegetable waste were collected from the local vegetable market of Faisalabad-Pakistan, dried, ground and sieved (2 mm). Fresh inoculum *Bacillus* sp. AZ6 containing 10^7-10^8 CFU mL^{-1}, compost and ZnO were mixed in two combination ratios (Zn-$EC_{80:20}$ and Zn-$EC_{60:40}$). In Zn-$EC_{80:20}$, the ratio of ZnO and compost was 80:20 (w/w) while in Zn-$EC_{60:40}$, the ratio was 60:40 (w/w), respectively. In both types of formations, bacterial inoculation with ZnSB (*Bacillus* sp. AZ6) was done. The materials in each ratio were thoroughly mixed and composted in a locally fabricated composter having the capacity of 500 kg. During composting, moisture was maintained manually 40% v/w. After 07 days of composting, each ratio material was thoroughly cleaned in order to avoid any mixing/contamination with other ratio. After preparation, the pH of Zn-$EC_{80:20}$ and Zn-$EC_{60:40}$ was 4.9 and 4.7, respectively. The prepared Zn-enriched compost and simple compost were analysed following standard procedure (Ditta *et al.,* 2018) and the results are presented in Tables 1 and 2.

Incubation trial

An incubation trial was conducted to investigate Zn-release pattern in the soil with the application different Zn treatments (Table 1). Bulk surface soil samples (0-15 cm) were collected from the Institute of Soil and Environmental Sciences Research Area, University of Agriculture, Faisalabad. The soil samples were ground, sieved (2 mm) and subjected to pre-soil analysis for various physicochemical properties using standard methods. The soil was sandy clay loam in texture (Moodie *et al.,* 1959) with alkaline pH=7.6 (U.S. Salinity Laboratory Staff 1954) and ECe=1.59 dS m^{-1} (U.S. Salinity Laboratory Staff 1954), low in organic matter =0.71% (Moodie *et al.,* 1959). The nutrient contents of the soil were: total nitrogen =0.06% (Jackson, 1962), available phosphorus =5.79 mg kg^{-1} (Watanabe and Olsen 1965), extractable potassium =114 mg kg^{-1} (U.S. Salinity Laboratory Staff 1954) and available Zn 0.67 mg kg^{-1} (Soltanpour and Workman 1979). About 250 g soil was filled in 500 mL plastic beakers, respective treatment was thoroughly mixed and incubated at 28±1°C in an incubator (Model: WP25A) for 60 days. For the determination of Zn-release pattern, the soil samples were collected after every ten days. Standard methods were used for the determination of available Zn in soil (Soltanpour and Workman, 1979).

Pot experiment

After finding out the effect of different Zn-amendments/treatments on Zn release pattern in incubation study, a pot experiment was conducted using the same treatment as used during incubation study, in rain protected wire house of Institute of Soil & Environmental Sciences (ISES), University of Agriculture, Faisalabad, Pakistan. Ten kilograms of pre-analysed soil (incubation study) was filled in each pot (with 25 cm diameter and 23 cm of height). NPK Fertilisers were applied at 80, 60 and 45 mg kg^{-1} soil, using urea, diammonium phosphate (DAP) and sulphate of potash (SOP), respectively. Half of nitrogen and whole of phosphorus and potash were applied as basal dose; the remaining half of nitrogen was applied at flowering. The respective fertiliser dose in each pot was thoroughly mixed. After addition of fertilisers, six treatments were applied as mentioned in the incubation trial (Table 1). Each treatment was thoroughly mixed in respective pot and the pots were arranged according to the completely randomised design (CRD) in triplicate. Tap water was used for irrigation as and when required.

Viable seeds of rice (CV. Basmati-515) were used for nursery plantation on acid washed sand. After twenty days, five seedlings were transplanted in pots filled with pre-analysed soil as mentioned above. After 2 weeks of transplantation, each pot with three uniform plants was maintained by uprooting the rest of seedlings. For irrigation, tap water with the following characteristics like pH=7.86, EC=0.51 dS m^{-1} and SAR=1.17 (mmolc $L^{-1})^{1/2}$ with negligible amount of Zn. All the agronomic practices were carried out as and when needed.

Plant growth measurements

After four months of nursery transplantation at maturity, the plants were harvested and the data regarding growth, yield and quality parameters of rice were recorded. Physiological parameters were recorded during growth period. Plant height and root length were recorded with the help of measuring rod. After harvesting the plants, fresh shoot and root biomass and 100-grains weight was measured using a digital electrical balance. The soil from each pot was washed away to collect root samples. For shoot and root dry weight, fresh shoots and roots were sun dried and then placed in the oven at 72°C till constant weight was achieved using a digital electrical balance.

Physiological measurement

Data regarding gas exchange parameters, *i.e.* photosynthetic rate (A), transpiration rate (E) and stomatal conductance (gs) were measured by using CIRAS-3 (PP System, Amesbury, MA, USA) with PLC 3 universal leaf cuvette. These parameters were taken in the morning. Carbonic anhydrase (CA) activity was determined following the method of Dwivedi and Randhawa (1974). To assess membrane permeability, electrolyte leakage (%) was measured following the method described by Lutts *et al.* (1995). For chlorophyll contents, chlorophyll meter (SPAD-502, Konica Minolta, Japan) was used by taking three leaves from each plant. The readings were taken after calibration and average was calculated.

Quality measurements

The quality parameters of rice grains, *i.e.* crude protein, fibre and mineral contents were measured using standard methods. Nitrogen contents in the grains were determined following Kjeldahl method (Jackson 1962). The crude proteins were measured by multiplying nitrogen contents with conversion factor 6.25 (Shih *et al.,* 1999). Crude fibre of rice grain samples was determined by procedure mentioned in AACC (2000) Method No. 32-10. Each flour sample was tested for mineral content by following the procedure outlined in AACC (2000) method No. 08-01.

Zn analysis of in shoot, root and grain samples

The wet digestion procedure described by Jones and Case (1990) was used for the determination of Zn from plant root, shoot and grain samples by using Atomic Absorption Spectrophotometer (Model SP 2900).

Statistical analysis

Data regarding growth, physiological, yield and quality parameters were subjected to analysis of variance (ANOVA) using Statistix® v8.1 (Copy right 2005, Analytical Software, USA). Treatment means were compared by using least significant difference (LSD) test at α=0.05 (Steel *et al.,* 1997).

Results

Zinc release pattern in soil

All the treatments showed an increasing trend in Zn-release with increasing incubation time, maximum Zn-release was observed after 50 days of incubation that was reduced after 60 days of incubation (Figure 1). Zn-$EC_{60:40}$ caused maximum Zn release

Table 1. Treatment plan in incubation and pot studies.

Treatment	Amount of each treatment applied for Zn @ 2.5 mg kg^{-1} soil	Amount of NPK and/compost applied
Control	-	Without any treatment
ZnSB	-	Only recommended NPK
ZnO (80% Zn)	3.125 mg kg^{-1}	Recommended NPK
$ZnSO_4$ (33% Zn)	7.576 mg kg^{-1}	Recommended NPK
Zn-$EC_{80:20}$	3.859 mg kg^{-1}	Recommended NPK using simple compost (200 mg kg^{-1} soil) and chemical fertilisers
Zn-$EC_{60:40}$	5.131 mg kg^{-1}	Recommended NPK using simple compost (200 mg kg^{-1} soil) and chemical fertilisers

Where in Zn-EC80:20, the ratio of ZnO and compost was 80:20 (w/w) and in Zn-EC60:40, the ratio was 60:40 (w/w), respectively and both were inoculated with ZnSB. ZnSB, zinc-solubilising bacteria.

(1.65 ppm), which was 24% higher in comparison to the application of $ZnSO_4$ after 50 days of incubation.

Growth and yield parameters of rice

Growth parameters like plant height, root length, fresh and dry biomass of root and shoot were maximally increased with the application of Zn-$EC_{60:40}$ in comparison to all other treatments (Table 3). Zn-$EC_{60:40}$ significantly improved plant height, root length, fresh root biomass, dry root biomass, fresh shoot biomass, dry shoot biomass, grain yield and 100-grain weight by 8.6, 23, 15, 15, 18, 15, 22 and 28%, respectively as compared to that with the application of $ZnSO_4$. Zn-$EC_{80:20}$ showed statistically non-significant effect regarding most of growth parameters investigated in comparison to that with the application of $ZnSO_4$.

Physiological parameters of rice

Regarding physiological parameters, significant differences in pots receiving the Zn-enriched compost with ZnSB in the form of Zn-$EC_{80:20}$ and Zn-$EC_{60:40}$ were observed in comparison to other treatments (Table 4). In comparison to $ZnSO_4$, the maximum increase in photosynthetic rate (11%), transpiration rate (21%), stomatal conductance (17%), chlorophyll contents (8%) and carbonic anhydrase activity (10%) was observed with the application of Zn-$EC_{60:40}$. The same treatment resulted in minimum value of electrolyte leakage (27%) as compared to that with the application of $ZnSO_4$.

Grain quality parameters of rice

After harvesting, the rice grains were investigated for any effect of the application of Zn-enriched composts on grain quality parameters such as mineral contents, crude protein, grain moisture, grain dry matter and fibre contents. Among the Zn-enriched composts, Zn-$EC_{60:40}$ significantly improved the various grain quality parameters studies as compared all other treatments applied. It improved mineral contents, crude protein and fibre contents by 12, 13 and 23%, respectively in comparison with the application of $ZnSO_4$. Similarly, Zn-$EC_{60:40}$ improved grain dry matter by reducing the moisture percentage (17%) as compared to $ZnSO_4$ (Table 5). Zn-$EC_{80:20}$ also showed better grain quality as compared to $ZnSO_4$. Inoculation with ZnSB also promoted yield parameters significantly as compared to control (no Zn).

Zinc accumulation in rice

A similar trend was observed in case of Zn accumulation in various parts of rice. Among all the applied treatments, Zn-$EC_{60:40}$ was far better in improving Zn accumulation in shoot, root and grain parts of rice. It improved Zn accumulation in the shoot, root and grains of rice by 18, 16 and 11% in comparison with the application of $ZnSO_4$ (Table 6). Inoculation with Zn-solubilising bacteria also improved grain quality parameters in significant manner, compared to control (no Zn).

Table 2. The chemical analysis of zinc-enriched composts.

Parameters	Simple compost	Zn-$EC_{80:20}$	Zn-$EC_{60:40}$
pH	4.8±0.1	4.9±0.1	4.7±0.1
Carbon (g kg^{-1})	255±6.6	61±2.5	125±4.6*
Nitrogen (g kg^{-1})	22.1±0.3	4.5±0.1	9.1±0.1
Total P (g kg^{-1})	3.7±0.9	0.84±0.2	1.7±0.4
Olson P (mg kg^{-1})	450±20	53±3.5	112±8.3
C:N	11.53±0.18	13.56±0.12	13.74±0.15
Zn (mg kg^{-1})	185±3.7	38.6±0.34	25.2±0.23

*Shows the standard error of means (SEM) where n=4.

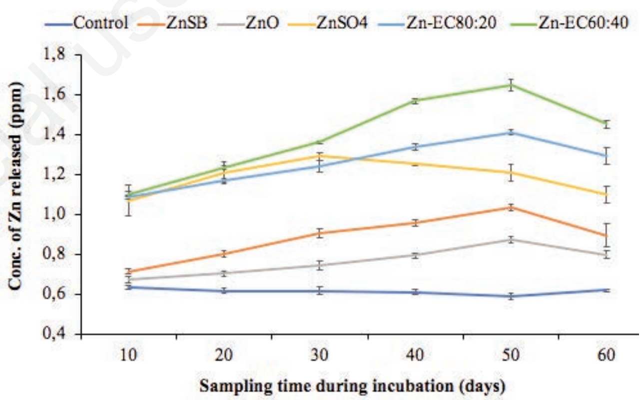

Figure 1. Zinc (Zn)-release in soil with time under different Zn-amendments. Where in Zn-$EC_{80:20}$, the ratio of ZnO and compost was 80:20 (w/w) and in Zn-$EC_{60:40}$, the ratio was 60:40 (w/w), respectively and both were inoculated with ZnSB; Error bars show standard error (n=3). *ZnSB, sinc-solubilising bacteria.

Table 3. Effect of different zinc amendments on growth and yield parameters of rice.

Treatments	PH (cm)	RL (cm)	FRB (g pot^{-1})	DRB (g pot^{-1})	FSB (g pot^{-1})	DSB (g pot^{-1})	GY (g pot^{-1})	HGW (g)
Control	146.7^{e}*	13.2^{d}	28.7^{d}	15.5^{d}	46.6^{d}	21.4^{d}	5.00^{d}	1.5^{d}
ZnSB	162.7^{c}	14.9^{cd}	31.6^{cd}	17.7^{bcd}	52.2^{bc}	23.7^{cd}	5.95^{c}	1.7^{cd}
ZnO	150.6^{de}	14.2^{d}	30.7^{cd}	16.3^{cd}	49.0^{cd}	22.8^{d}	5.69^{c}	1.6^{d}
$ZnSO_4$	183.2^{c}	16.7^{bc}	34.2^{bc}	19.0^{bc}	54.7^{b}	25.9^{bc}	6.74^{b}	1.8^{bc}
Zn-$EC_{80:20}$	179.2^{c}	18.3^{b}	35.7^{ab}	20.0^{ab}	61.4^{a}	27.4^{ab}	7.26^{b}	2.0^{b}
Zn-$EC_{60:40}$	198.9^{b}	20.5^{a}	39.3^{a}	21.8^{a}	64.7^{a}	29.9^{a}	8.23^{a}	2.3^{a}
LSD values	6.86	1.81	3.65	2.71	5.21	2.84	0.54**	0.17

Data are shown as mean of three replicates. PH, plant height; RL, root length; FRB, fresh root biomass; DRB, dry root biomass; FSB, fresh shoot biomass; DSB, dry shoot biomass; GY, grain yield; HGW, 100 grain weight In Zn-$EC_{80:20}$, the ratio of ZnO and compost was 80:20 (w/w) and in Zn-$EC_{60:40}$, the ratio was 60:40 (w/w), respectively and both were inoculated with ZnSB. Means followed by same letter(s) within the column are not significantly different according to least significance difference (LSD) test at P≤0.05.

Discussion

Different strategies to improve Zn deficiency in humans, such as Zn supplementation, food fortification and food diversification have been found impractical and rather uneconomical in developing countries (Bouis *et al.,* 2011). There is a dire need to find out an economical, sustainable and environment friendly approach to counter Zn deficiency in comparison to classical approaches discussed earlier. In this study, we have used various management strategies such as Zn-enriched compost with ZnSB to improve growth, yield and Zn-biofortification of rice in comparison with traditionally used chemical amendment *i.e.* $ZnSO_4$. Prior to employ this strategy under pot/field conditions, an incubation study was conducted to evaluate the efficacy of Zn-enriched compost with ZnSB compared with traditionally applied $ZnSO_4$, in improving Zn-release in soil under controlled conditions for 60 days. In the present study, the maximum Zn release was noted with the application of Zn-$EC_{60:40}$ which contained ZnO and compost in 60:40 (w/w), significantly during all the sampling times compared to all other treatments applied. ZnO is an economical but a slow release fertiliser in comparison with $ZnSO_4$. For proper mineralisation in the presence of ZnSB and also the indigenous microbes, the ratio of combination is very important. In Zn-$EC_{60:40}$, the ratio of ZnO and compost was 60:40 (w/w) while it was 80:20 (w/w) in case of Zn-$EC_{80:20}$. Recently, it has been confirmed that different ratios of combination have direct influence on phosphorus release from rock phosphate (Ditta *et al.,* 2015, 2018; Farooq *et al.,* 2018). Moreover, application of plant growth promoting rhizobacteria has also a significant affect in improving the solubility of material under consideration (Saravanan *et al.,* 2004; Ditta *et al.,* 2015). Like in case of rock phosphate solubilisation, phosphate-solubilising microorganisms played a significant role in improving the solubility of rock phosphate (Ditta *et al.,* 2015, 2018; Farooq *et al.,* 2018). In the present study, *Bacillus* sp. AZ6 was used in both combination ratios during incubation study and it significantly has improved Zn release from Zn-enriched composts (1.29-1.45 ppm) in comparison to the alone application of ZnO (0.80 ppm) on the 60th day. Earlier, *Pseudomonas sp.* (ZSB-S-2) with Zn solubilising activity significantly improved Zn-release from ZnO which was suggested to be due to the production of organic acids as the pH of the broth culture was shifted to acidic after 15 days of inoculation (Saravanan *et al.,* 2004). Moreover, the decrease in pH of alkaline soils results in increased availability of Zn as one-unit increase in pH decreases the Zn availability by 100 units (Havlin *et al.,* 2005; Goteti *et al.,* 2013). Organic amendments serve as a carbon source for microbes, which ultimately decrease the pH of microclimate due to the production of organic acids during composting (Chakraborty *et al.,* 2011). In the present study, *Bacillus* sp. AZ6 possessed higher organic acid production ability as well as siderophores production (Hussain *et al.,* 2015) that might involve in Zn release from added amendments due to lowering of soil solution pH. Our results are consistent with Imran *et al.* (2014), who observed increase bioavailability of Zn with the application of composted organic amendments.

Better growth and yield parameters are ensured by the provision of balanced nutrient to the crops. In the present study, Zn-$EC_{60:40}$ resulted in the maximum increase in growth and yield parameters of rice. It might be due to the provision of macro- and micro-nutrients with the application composted organic amendment. Moreover, there was a slow and steady release of Zn as clear from the Zn release pattern in incubation study. Proper Zn supply is helpful in various enzymes activation, protein synthesis and metabolism of carbohydrates (Mousavi *et al.,* 2013). Similarly, the *Bacillus* sp. AZ6 possessed the ACC-deaminase and P-solubilising activities which ultimately reduced any abiotic stress and improved P-availability to the plants. With the provision of phosphorus, root growth was improved to explore more unit area, thereby increasing the nutrient availability and ultimately improved the growth and yield parameters of rice in the present study. Earlier, it was found that organic acids released with microbial inoculation resulted in better nutrient uptake, which ultimately increased the growth and yield parameters of potato (Belimov *et al.,* 2015). Likewise, in our study, the increase in grain yield was 22%, which might be due to the application of bacterial strain *Bacillus* sp. AZ6 having the ability to produce organic acids, siderophore, ACC-deaminase and P-solubilising activities.

Carbon assimilation rate/photosynthetic rate determine the growth and productivity of a crop. Regarding physiological parameters, the maximum values were recorded with the application of Zn-$EC_{60:40}$ in comparison with the other treatments applied. Earlier, it has been found that increased supply of Zn to the crop plants improved various metabolic processes like photosynthesis, protein synthesis, pollen formation, membrane stability, DNA replication, and energy transfer reactions (Gurmani *et al.,* 2012). Recently, it was found that crude protein contents kernel and bran of rice were significantly increased with the application of Zn (Ghasal *et al.,* 2016). Moreover, Zn has been involved in the proper plant functioning and its deficiency decreases carbonic anhydrase (CA) activity. Carbonic anhydrases are Zn metalloenzymes, involved in a wide variety of physiolog-

Table 4. Effect of different zinc amendments on physiological parameters of rice.

Treatment	A (μmol CO_2 m^{-2} s^{-1})	E (mmol H_2O m^{-2} s^{-1})	gs (mmol m^{-2} s^{-1})	CC (SPAD value)	EL (%)	CAA [μmol (CO_2) kg^{-1} (leaf f.m.) s^{-1}]
Control	17.8^{d}	5.9^{e}	108.0^{d}	41.0^{d}	59.0^{a}	259.3^{f}
ZnSB	19.6^{cd}	6.7^{cd}	120.3^{cd}	44.2^{c}	56.0^{a}	288.7^{d}
ZnO	19.3^{cd}	6.4^{de}	117.3^{cd}	43.4^{cd}	57.33^{a}	272.7^{e}
$ZnSO_4$	21.3^{b}	7.2^{bc}	129.7^{bc}	45.1^{bc}	52.0^{b}	304.7^{c}
Zn-$EC_{80:20}$	21.5^{b}	7.4^{b}	147.6^{ab}	46.8^{ab}	47.0^{c}	321.0^{b}
Zn-$EC_{60:40}$	23.7^{a}	8.7^{a}	152.3^{a}	48.6^{a}	38.0^{d}	336.3^{a}
LSD value	1.55	0.67	19.00	2.50	3.58	8.36

Data are shown as mean of three replicates where: A, photosynthetic rate; E, transpiration rate; gs, stomatal conductance; CC, chlorophyll contents; EL, electrolyte leakage; CAA, carbonic anhydrase activity. In Zn-EC80:20, the ratio of ZnO and compost was 80:20 (w/w) and in Zn-EC60:40, the ratio was 60:40 (w/w), respectively and both were inoculated with ZnSB. [a-d]Means followed by same letter(s) within a column are not significantly different according to least significance difference (LSD) test at $P \leq 0.05$.

ical processes like photosynthesis (Chang *et al.,* 2005; Sly and Hu, 1995). In the present study, carbonic anhydrase activity of rice plants was maximised with the application of Zn-$EC_{60:40}$ in comparison to all other treatments applied.

The ultimate goal of the application of different Zn amendments is to increase its concentration in dietary parts *i.e.* grains in case of rice. With the application of Zn-$EC_{60:40}$, the maximum Zn accumulation was noted in root shoot and grains of rice as compared to that with the application of $ZnSO_4$. It has been reported that the addition of organic fertilisers caused an increase in the amount of soil available Zn that ultimately taken up by the crop (Sidhu and Sharma, 2010). Moreover, slow and steady release of Zn from enriched compost might have increased the accumulation of Zn in different plant parts of rice. This premise is supported by the Zn release pattern investigated in our incubation study. The increase in Zn bioaccumulation may also be due to the presence of nutrients in soil organic matter, improvement in soil physicochemical and biological properties of soil (Marschner, 2012). Similar to our results, increased bioaccumulation of Zn in rice, wheat and barley was observed with the application of rhizobacteria in comparison with un-inoculated control (Sadaghiani *et al.,* 2008).

Table 5. Effect of different zinc amendments on grain quality parameters of rice.

Treatments	Mineral contents (%)	Crude protein (%)	Crude fibre (%)
Control	1.12^{c}	9.77^{d}	1.79^{e}
ZnSB	1.23^{c}	10.53^{cd}	1.97^{d}
ZnO	1.17^{c}	10.09^{d}	1.88^{de}
$ZnSO_4$	1.37^{b}	11.21^{bc}	2.21^{c}
Zn-$EC_{80:20}$	1.43^{ab}	11.68^{b}	2.38^{b}
Zn-$EC_{60:40}$	1.53^{a}	12.66^{a}	2.72^{a}
LSD values	0.12	0.95	0.15

Data are shown as mean of three replicates. In Zn-EC80:20, the ratio of ZnO and compost was 80:20 (w/w) and in Zn-$EC_{60:40,}$ the ratio was 60:40 (w/w), respectively and both were inoculated with ZnSB Means followed by same letter(s) within the column are not significantly different according to least significance difference (LSD) test at P≤0.05.

Table 6. Effect of different zinc amendments on zinc-concentration in rice.

Treatments	Shoots ($\mu g\ g^{-1}$)	Roots ($\mu g\ g^{-1}$)	Grains ($\mu g\ g^{-1}$)
Control	9.4^{d}	26.2^{d}	19.4^{d}
ZnSB	10.4^{cd}	30.1^{bc}	20.6^{c}
ZnO	9.9^{d}	27.9^{cd}	19.9^{cd}
$ZnSO_4$	11.4^{bc}	32.9^{b}	21.4^{b}
Zn-$EC_{80:20}$	11.8^{b}	33.2^{b}	21.8^{b}
Zn-$EC_{60:40}$	13.5^{a}	38.0^{a}	22.7^{a}
LSD value	1.07	3.50	0.67

Data are shown as mean of three replicates. In Zn-$EC_{80:20}$, the ratio of ZnO and compost was 80:20 (w/w) and in Zn-$EC_{60:40}$, the ratio was 60:40 (w/w), respectively and both were inoculated with ZnSB Means followed by same letter(s) within the column are not significantly different according to least significance difference (LSD) test at P≤0.05.

Conclusions

The results showed that composting and inoculation of ZnO with ZnSB improve availability of Zn and had a positive effect on growth, physiology and yield of rice in comparison to $ZnSO_4$. In the pot experiment, Zn-enriched compost with ZnSB (Zn-$EC_{60:40}$) in comparison with $ZnSO_4$, significantly improved growth, physiology and yield parameters of rice through a slow and steady release of Zn from ZnO as found in incubation study. Moreover, the same treatment improved the quality of rice grains produced and also bioaccumulation of Zn in various parts of rice. This approach could be practiced under Zn deficient condition after its confirmation under field conditions at various places.

References

AACC, 2000. Approved Methods of American Association of Cereal Chemists, 10th (ed.), USA.

Belimov AA, Dodd IC, Safronova VI, Shaposhnikov AI, Azarova TS, Makarova NM, Davies WJ, Tikhonovich, IA, 2015. Rhizobacteria that produce auxins and contain 1-amino-cyclopropane-1-carboxylic acid deaminase decrease amino acid concentrations in the rhizosphere and improve growth and yield of well-watered and water-limited potato (Solanum tuberosum). Ann. Appl. Biol. 167:11-25.

Bouis HE, Hotz C, McClafferty B, Meenakshi J, Pfeiffer WH, 2011. Biofortification: a new tool to reduce micronutrient malnutrition. Food Nutr. Bull. 32:31-40.

Bunt JS, Rovira AD, 1955. Microbiological studies of some subantartic soils. Eur. J. Soil Sci. 6:119-28.

Cakmak I, 2008. Enrichment of cereal grains with zinc: agronomic or genetic biofortification? Plant Soil. 302:1-17.

Cakmak I, 2009. Agronomic approaches in biofortification of food crops with micronutrients. The proceedings of the international plant nutrient colloquium XVI UC Davis, USA.

Chakraborty A, Chakrabarti K, Chakraborty A, Ghosh S, 2011. Effect of long-term fertilizers and manure application on microbial biomass and microbial activity of a tropical agricultural soil. Biol. Fertil. Soils. 47:227-33.

Chang HB, Lin CW, Huang HJ, 2005. Zinc induced cell death in rice (Oryza sativa L.) roots. Plant Growth Regul. 46:261-6.

Compant S, Duffy B, Nowak J, Clément C, Barka EA, 2005. Use of plant growth-promoting bacteria for biocontrol of plant diseases: principles, mechanisms of action, and future prospects. Appl. Environ. Microbiol. 71:4951-9.

Ditta A, Arshad M, Zahir ZA, Jamil A, 2015. Comparative efficacy of rock phosphate enriched organic fertilizer vs. mineral phosphatic fertilizer for nodulation, growth and yield of lentil. Int. J. Agric. Biol. 17:589-95.

Ditta A, Muhammad J, Imtiaz M, Mehmood S, Qian Z, Tu S, 2018. Application of rock phosphate enriched composts increases nodulation, growth and yield of chickpea. Int. J. Recycl. Org. Waste Agric. 7:33-40.

Dwivedi RS, Randhawa NS, 1974. Evaluation of rapid test for hidden hunger of zinc in plants. Plant Soil. 40:45-51.

Farooq N, Kanwal S, Ditta A, Hussain A, Naveed M, Jamshaid MU, Iqbal M, 2018. Comparative efficacy of KCl blended composts vs. sole application of KCl or K_2SO_4 in improving K nutrition, photosynthetic capacity and growth of maize. Soil Environ. 37:68-74.

Ghasal PC, Shivay YS, Pooniya V, Kumar P, Verma RK, 2016. Zinc fertilization enhances growth and quality parameters of aromatic rice (Oryza sativa L.) varieties. Ind. J. Plant Physiol. 21:323-32.

Gontia-Mishra I, Sapre S, Tiwari S, 2017. Zinc solubilizing bacteria from the rhizosphere of rice as prospective modulator of zinc biofortification in rice. Rhizosphere 3:185-90.

GOP, 2013-2014. Economics Survey of Pakistan, Ministry of Food, Agriculture and Livestock. Government of Pakistan, Islamabad, Pakistan.

Goteti PK, Emmanuel LDA, Desai S, Shaik MHA, 2013. Prospective zinc solubilising bacteria for enhanced nutrient uptake and growth promotion in maize (Zea mays L.). Int. J. Microbiol. Article ID 869697:1-7.

Gurmani AR, Khan SU, Andaleep R, Waseem K, Khan A, 2012. Soil application of zinc improves growth and yield of tomato. Int. J. Agric. Biol. 14:91-6.

Hamid A, Ahmad N, 2001. Integrated plant nutrition system: Development and rural poverty alleviation. Regional Workshop on Integrated Plant Nutrition System (IPNS), Development and Rural Poverty Alleviation, FADINAP, 18-20 September, Bangkok, Thailand.

Havlin J, Beaton JD, Tisdale SL, Nelson WL, 2005. Soil fertility and fertilizers: An introduction to nutrient management. Pearson Prentice Hall, Upper Saddle River eNJ NJ, USA.

Hussain A, Arshad M, Zahir ZA, Asghar M, 2015. Prospects of zinc solubilizing bacteria for enhancing growth of maize. Pak. J. Agric. Sci. 52:915-22.

Imran M, Arshad M, Khalid A, Kanwal S, Crowley DE, 2014. Perspectives of rhizosphere microflora for improving Zn bioavailability and acquisition by higher plants. Int. J. Agric. Biol. 16:653-62.

Jackson ML, 1962. Soil chemical analysis. Prentice Hall, Inc., Englwood Cliff, New York, USA.

Jones JB, Case VW, 1990. Sampling, handling, and analyzing plant tissue samples. In: Westerman RL (Eds.), Soil Testing and Plant Analysis, SSSA Book Ser. 3. SSSA, Madison, WI, USA. pp 389-427.

Joy EJM, Ahmad W, Zia MH, Kumssa DB, Young SD, Ander EL, Watts MJ, Stein AJ, Broadley MR, 2017. Valuing increased zinc (Zn) fertiliser-use in Pakistan. Plant Soil. 411:139-50.

Lutts S, Kinet JM Bouharmont J, 1995. Changes in plant response to NaCl during development of rice (Oryza sativa L.) varieties differing in salinity resistance. J. Exp. Bot. 46:1843-52.

Mandal B, Hazra GC, 1997. Zinc adsorption in soils as influenced by different soil management practices. Soil Sci. 162:713-21.

Marschner P, 2012. Mineral Nutrition of Higher Plants, third ed. Academic Press, London, UK.

Moodie CD, Smith HW, McCreery RA, 1959. Laboratory Manual for Soil Fertility. Department of Agronomy, State College of Washington Pullman, Washington, USA, pp 1-75.

Mousavi SR, Galavi M. Rezaei M, 2013. Zinc (Zn) importance for crop production - A review. Int. J. Plant Prod. 4:64-8.

Othman NMI, Othman R, Saud HM, Wahab PEM, 2017. Effects of root colonization by zinc-solubilizing bacteria on rice plant (Oryza sativa MR219) growth. Agric. Nat. Resour. 51:532-7.

Palmgren MG, Clemens S, Williams LE, Krämer U, Borg S, Schjørring JK, Sanders D, 2008. Zinc biofortification of cereals: problems and solutions. Trends Plant Sci. 13:464-73.

Rattan R, Shukla L, 1991. Influence of different Zn carriers on the utilization of micronutrients by rice. J. Indian Soc. Soil Sci. 39:808-10.

Rehman H, Aziz T, Farooq M, Wakeel A, Rengel Z, 2012. Zinc nutrition in rice production systems: a review. Plant Soil. 361:203-26.

Sadaghiani MR, Barin M, Jalili F, 2008. The effect of PGPR inoculation on the growth of wheat. In: 6th International Meeting on Soil Fertility Land Management and Agroclimatology. Oct 29-1 Nov 2008, Kusadasi, Aydin, Turkey, pp 891-898.

Saravanan VS, Subramoniam SR, Raj SA, 2004. Assessing in vitro solubilization potential of different zinc solubilizing bacterial (ZSB) isolates. Braz. J. Microbiol. 35:121-5.

Shih FF, Champagne ET, Daigle K, Zarins Z, 1999. Use of enzymes in the processing of protein products from rice bran and rice flour. Nahrung Food. 43:14-8.

Shivay YS, Prasad R, Singh RK, Pal M, 2015. Relative efficiency of zinc-coated urea and soil and foliar application of zinc sulphate on yield, nitrogen, phosphorus, potassium, zinc and iron biofortification in grains and uptake by basmati rice (Oryza sativa L.). J. Agric. Sci. 7:9752-60.

Sidhu GS, Sharma BD, 2010. Diethylenetriaminepentaacetic acid-extractable micronutrients status in soil under a rice - wheat system and their relationship with soil properties in different agroclimatic zones of indo-gangetic plains of India. Commun. Soil Sci. Plant Anal. 41:29-51.

Sly WS, Hu PY, 1995. Human Carbonic Anhydrases and Carbonic Anhydrase Deficiencies. Ann. Rev. Biochem. 64:375-401.

Soltanpour PN, Schwab AP, 1977. A new soil test for simultaneous extraction of macro and micronutrients in alkaline soils. Commun. Soil Sci. Plant Anal. 8:109-207.

Steel RGD, Torrie JH, Dickey D, 1997. Principle and procedure of Statistical Analysis. (2nd Ed.). McGraw Hill Book Co. Inc., New York, USA.

US Salinity Laboratory Staff, 1954. Diagnosis and improvement of saline and alkali soils. USDA Hand b. No. 60, Washington, DC, USA.

Wakeel A, Farooq M, Bashir K, Ozturk L, 2018. Micronutrient Malnutrition and Biofortification: Recent Advances and Future Perspectives. In: Hossain MA, Kamiya T, Burritt D, Tran LP, Fujiwara T (Eds.), Plant Micronutrient Use Efficiency. Academic Press, Cambridge, MA, USA, pp. 225-243.

Watanabe FS, Olsen SR, 1965. Test of an ascorbic acid method for determining phosphorus in water and $NaHCO_3$ extracts. Soil Sci. Soc. Am. J. 29:677-8.

Wu S, Cheung K, Luo Y, Wong M, 2006. Effects of inoculation of plant growth-promoting rhizobacteria on metal uptake by Brassica juncea. Environ. Pollut. 140:124-35.