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INTEGRATING THE POTENTIAL OF *BACILLUS* SP. AZ6 AND ORGANIC WASTE FOR ZINC OXIDE BIO-ACTIVATION TO IMPROVE GROWTH, YIELD AND ZINC CONTENT OF MAIZE GRAINS

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Cereal crops are a major part of our daily dietary intake. However, they are reported to have poor zinc (Zn) content. Bio-activation of zinc oxide with zinc solubilizing bacteria (ZSB) is considered a relatively newer approach to enrich cereal grains with Zn. The present study was conducted to evaluate the role of four bio-activated ZnO (BOZ) formulations in improving the growth, yield and grain Zn concentration in maize. The BOZ formulations were prepared by mixing ZnO and ground orange peels inoculated with ZSB, *Bacillus* sp. AZ6. Four different BOZ formulations [BOZ-1 (9:1), BOZ-2 (8:2), BOZ-3 (7:3) and BOZ-4 (6:4)] were prepared by mixing ZnO in different ratios with inoculated ground orange peel. In a soil incubation study (72 days), the bioavailable Zn fraction was found to be higher in the soil treated with BOZ-4 formulation as compared to the soils treated with other formulations as well as zinc sulfate ($ZnSO_4$) and zinc oxide. The results of the pot study revealed the supremacy of BOZ-4 over other treatments in improving growth, yield and quality of maize. The BOZ-4 formulation resulted into 14, 20, 05, 11 and 27% increase in plant height, root length, 100-grain weight, grain yield and Zn concentration in grain, respectively, as compared to zinc sulfate treatment. Results suggested that bio-activation of ZnO by *Bacillus* sp. AZ6 and orange peels waste is an effective strategy to improve yield and nutritional quality of maize.
Keywords : *Bacillus* sp. AZ6, cereals, orange peels waste, Zn bio-activation, zinc solubilizing bacteria, zinc oxide

INTRODUCTION

Zinc (Zn) is most important micronutrient which required to complete the life cycle of plants as well as animals and human beings (McCall *et al.,* 2000; Sbartai *et al.,* 2011; Gurmani *et al.,* 2012; Zulfiqar *et al*., 2017). Due to Zn deficiency in soil, large number of scientist have documented that the number of physiological processes of plant negatively affected, yield losses as well as low grain Zn contents in cereals (rice, maize and wheat) due to less supply of Zn from zinc deficient soils (Imran *et al.,* 2014). In addition to the impacts on plants, Zn deficiency has also been reported as a cause of many health disorders in human beings and Zn is the fourth deficient micronutrient in humans after vitamin A, iron and iodine (Cakmak *et al.,* 2009; Shivay *et al.,* 2015). For example, about 37% of the total population in Pakistan is suffering from Zn malnutrition (Jamil *et al.,* 2015). In such developing countries, a large consumption of cereal based foods with small concentrations of Zn is considered one of the major reasons for insufficient Zn supply to human tissues (Cakmak and Kutman, 2018). The low concentration of Zn in cereals is primarily linked with the deficiency of available Zn in rhizoshere. Many soil factors including soil texture, pH, soil water content, organic matter and calcareousness of the soil are known to influence bioavailability of Zn in soil (Alloway, 2009). With the use of chemical fertilizers, biofertilizer and organic fertilizer can increase the bioavailable contents of Zn in soil (Imran *et al.,* 2014). Among the chemical fertilizers, zinc sulfate ($ZnSO_4$) is the most common Zn fertilizer in Pakistan. The major drawback of $ZnSO_4$ application is its poor fertilizer use efficiency because of which a major portion of Zn gets fixed quickly by reacting with soil matrix and becomes unavailable to the growing plants (Shivay *et al.,* 2008). Moreover, $ZnSO_4$ is considered costly among the farming community as it constitutes only 33% Zn. As compared to $ZnSO_4$, zinc oxide (ZnO) contains approximately 80% Zn and is available at lower price in the market. However, it is relatively lesser soluble source of Zn as compared to ZnSO4 (Alloway, 2008; Shivay *et al.,* 2008). Zinc oxide can be applied as a cost-effective source of Zn by improving its dissolution following some innovative approaches and by reducing the fixation of Zn in the soil.

One of the ways to improve the dissolution of ZnO could be the use of zinc solubilizing bacteria (ZSB). During the recent years, a number of bacterial strains including *Pseudomonas aeruginosa* (CMG 823), *Bacillus* sp. ZSB-O-1, *Pseudomonas* sp. ZSB-S-2, *Bacillus cereus* ATCC 13061, *Bacillus sp.* KHBD-6, *Gluconaceto bacterdiazotrophicus* PAl5, Exiguo bacterium *Aurantiacum* strain MS-ZT10 and *Bacillus subtilis* (ZM63) have been reported for dissolution of ZnO in liquid and solid media (Fasim *et al.*, 2002; Saravanan *et al.*, 2004; Saravanan *et al.*, 2007; Sharma *et al.*, 2012; Imran *et al.*, 2014; Shaikh and Saraf, 2017; Mumtaz *et al.*, 2017). These microorganisms produce various organic acids which lower down the pH of the medium causing dissolution of ZnO. The application of zinc solubilizing bacteria have shown a probable to improve bioavailable Zn concentration in soil (Subramanian *et al.*, 2009; Imran *et al.*, 2014) and its accumulation in the grain of cereal (Imran *et al.*, 2014; Abaid-Ullah *et al.*, 2015; Shaikh and Saraf, 2017). Despite that zinc solubilizing bacteria have shown a good potential to improve bioavailable Zn in soils, there is no adequate information regarding the improvement of plant available Zn in the soils through the synergistic use of ZnO and ZSB in greenhouse or field plant studies. The synergistic use of ZnO and ZSB as a product might result into the dissolution of ZnO through its bio-activation which is a metabolic process in which a chemically reactive product is produced from relatively inactive precursor/material. It is hypothesized that development of such bio-activated ZnO can be excellent alternate to ZnSO in improving the bioavailable Zn soil and also minimize the reluctance of farmers of Pakistan for the use of biofertilizer due to extra cost involved in terms of labor and separate application.

In above context, the present experiment was conducted to prepare varying bio-activated ZnO formulations (BOZ) using ZnO and ground orange peel inoculated with a ZSB, *Bacillus* sp. AZ6. For this purpose, ground orange peel was inoculated with *Bacillus* sp. AZ6 and this material was mixed with ZnO at different rates to prepared different formulations. The produced BOZ formulations were also tested for their efficacy to improve plant available Zn content in soil as well as the growth, yield and grain quality of maize crop.

MATERIALS AND METHODS

***Bacterial strain and soil for experiment*:** The soil used in both experiments was collected from the Farm area of the Institute of Soil & Environmental Sciences (ISES), University of Agriculture Faisalabad (UAF), 63100-Pakistan. Before conducting the incubation and pot experiment the soil was analyzed for different physico-chemical properties. Particle size analysis was done using hydrometer method of Gee and Bauder (1986). Soil texture was sandy clay loam. Saturation percentage of the soil was determined by following the method reported by Sarfraz *et al.* (2017).

The saturation percentage of soil calculated by using the above formula was found to be 33%. The pH of the soil measured in a saturated soil paste using pH meter (Kent Eil 7015, UK) (US. Salinity Laboratory Staff, 1954) which was 7.7. Electrical conductivity of the saturated soil paste extract measured by conductivity meter (Jenway 4070, UK) was 1.44 dS m^{-1} (US Salinity Laboratory Staff, 1954). The method described by Moodie *et al.* (1959) was used to analyze soil organic matter (0.73%). Total nitrogen (0.05 %) was analyzed by Ginning and Hibbard’s method and kjeldhal’s apparatus was used for digestion (Jackson, 1962). Soil plant available phosphorus (P) content (5.35 mg kg^{-1}) was determined by sodium bicarbonate ($NaHCO_3$) extraction method (Watanabe and Olsen, 1965). Soil plant available potassium (K) was estimated through analysis on flame photometer (Jenway PFP7, UK) after extraction by ammonium acetate ($NH_4C_2H_3O_2$) (Simard, 1993). The soil available Zn (0.57 mg kg^{-1}) was determined by ammonium bicarbonate-diethylene triamine penta acetic acid (AB-DTPA) extraction (Soltanpour and Schwab, 1977).

Pre-isolated ZSB, *Bacillus* sp. AZ6; Accession # KT221633 (Hussain *et al.*, 2015) was collected from Environmental Sciences Laboratory ISES, UAF. The bacterial strain *Bacillus* sp. AZ6 possessed plant growth promoting traits viz. auxin production, 1-aminocyclopropane-1-carboxylate (ACC)-deamiase activity, siderophore production and production of different organic acids (Hussain *et al.*, 2015).

***Preparation of different Bio-activated Zn (BOZ) formulations*:** To prepare BOZ formulations, orange peels were collected from the local fruit market of Faisalabad, Pakistan. The collected orange peels were air dried, ground and placed in hot air oven at 80 °C for 60 min. This material was cooled at room temperature and inoculated with culture of *Bacillus* sp. AZ6 (0.5 optical density at 600 nm). The ground, dried peel (g) and culture of the bacterium (ml) were mixed in the ratio of 9:1 (w/v) and incubated for 72 h at 28±2 °C in an incubator (SANYO, Model MIR-253). Zinc oxide was crushed to 300-400 mm mesh size and mixed in different ratios with inoculated orange peels (w/w) to achieve different formulations [BOZ-1 (9:1), BOZ-2 (8:2), BOZ-3 (7:3) and

Table 1. Formulations of bio-activated Zn (BOZ)

BOZ-formulations	ZnO: ZSB inoculated ground orange peels (w/w)	Zn conc. (%)	Ground orange peels (%)	Application rate (kg ha^{-1})	Total Zn (kg ha^{-1})	pH
BOZ-1	9 : 1	72	10	6.80	4.91	5.1
BOZ-2	8 : 2	64	20	7.65	4.91	5.1
BOZ-3	7 : 3	56	30	8.74	4.91	4.9
BOZ-4	6 : 4	48	40	10.20	4.91	4.8

ZSB: zinc solubilizing bacterium

BOZ-4 (6:4)] of ZnO as shown in Table 1. This mixture was incubated for 72 h at 28±2 °C before application. This was done to achieve maximum chelation of Zn with organic complexes and to get optimal population of *Bacillus* sp. AZ6 in the formulations and partial dissolution of ZnO.

***Measurement of temporal release of Zn from BOZ formulations in soil*:** In order to measure temporal release of Zn in soil from different Zn sources, a soil incubation study was carried out in the wire house of the Environmental Sciences Laboratory, ISES, UAF. Soil was air dried, ground and passed through a 2 mm sieve. It was homogenized by thoroughly mixing and 5 kg soil was filled in each plastic pot (height 15 cm, diameter 12 cm, soil capacity 6 kg). The experiment comprised of eight treatments in triplicates and was laid down according to completely randomized design (CRD). The treatments were: T1: control (no Zn), T2: ZnO, T3: $ZnSO_4$, T4: BOZ-1, T5: BOZ-2, T6: BOZ-3, T7: BOZ-4 and T8: ZSB (Zinc Solubilizing Bacteria). Zinc from each of the above sources was applied at the rate of 4.9 kg ha^{-1}. Zinc was applied at the time of pot filling by uniformly mixing with soil matrix. To maintain the proper moisture at field capacity distilled water was used in all pots during the incubation period. The available Zn concentration in each type of soil was analyzed after every 12 days interval up to 72 days by AB-DTPA extraction method (Soltanpour and Schwab, 1977). For determining Zn content, 10 g air dried soil sample was suspended in 20 ml AB-DTPA extractant solution, suspension was shaked for 15 min at 200 rpm on reciprocal shaker. It was filtered through Whatman No. 42 filter paper. Zinc in the extract was directly measured using atomic absorption spectrometer (PerkinElmer $_{\text{for the better}}$, Aanalyst 100, Waltham, USA).

***Pot experiment*:** A pot experiment was conducted in same wirehouse where incubation study was carried out to find out the comparative efficacy of different zinc sources in improving growth, yield and zinc concentration of maize grain. The soil was air dried, ground and, after passing through a 2 mm sieve, it was thoroughly mixed. Each pot (height 45 cm, diameter 25 cm, soil capacity 30 kg) was filled with 25 kg soil. The same set of treatments was tested in this pot study as described in the incubation trial. All the treatments were applied as soil application and mixed thoroughly with upper layer of soil at the time of pot filling except the treatment T8 (ZSB). In case of T8, seed inoculation with AZ6 was done to measure the response of the ZSB only. For this purpose, surface disinfected seeds of maize were inoculated with the broth culture mixed with 10% sugar solution and clay mixture, ground orange peel to clay ratio was used as 1:1 w/w (Baig *et al.,* 2012). The seeds were shaked well till fine coating appeared on seeds. Six seeds of maize cultivar-Syngenta NT662 were sown per pot. After the two weeks of plant growth, only two comparatively healthy plants were maintained per pot. nitrogen (N), phosphorus (P) and potassium (K) were applied @ 175, 160, 125 kg ha^{-1} using available fertilizers as urea, di-ammonium phosphate (DAP) and sulphate of potash (SOP), respectively. Nitrogen and P fertilizer were applied at the time of sowing, whereas, N was applied in two splits i.e. half at the time of sowing and remaining half after three weeks of sowing. The plants were irrigated with tap water as and when required. All the standard agronomic as well as insect and pest management strategies were adopted for this pot experiment. After ninty five days of sowing, the data regarding the growth and yield parameters was recorded.

Data collection

***Growth and yield parameters*:** Maize growth parameters including plant height, root length, shoot dry biomass, root dry biomass, cob length, cob diameter and plant girth were recorded at the stage of maturity after 95 days of sowing. Similarly, maize yield parameters including grain yield, stover yield, 100 grain weight, number of grains per line and number of lines per cob were recorded. With the help of meter rod, the plant height of two plants from each pot was measured then average was taken. The roots of the plants were excavated and root length of primary root was measured using a measuring tape. Both shoot and root samples were thoroughly washed with distilled water and then blotted dry with tissue papers before sun drying. Sun dried plants were placed in an oven at 72°C for 72 h until constant weight was achieved. The dry root and shoot biomass was recorded on balance. Plant girth was measured with use of Vernier Caliper in the middle of plant stem.

***Plant Zn analysis*:** After air drying, the collected grain, root and shoot samples were separately oven dried (65°C for 72 h) and finely ground in a grinder (IKA WERKE, MF 10 Basic, Staufen, Germany). Oven dried (1 gram) plant material (ground) was taken in the conical flask (digestion) and wet digested in a di-acid mixture (HNO_3:$HClO_4$ ratio of 2:1) (Jones and Case, 1990). The digestion was continued until colorless solution was obtained. The flasks were cooled down and then 2-4 ml of 70% perchloric acid ($HClO_4$) was added. The samples were heated again on digestion plate and allowed to evaporate to a small volume for some time. When the vapors were condensed, the plant digested material in the flask were add to a 50 ml volumetric flask and volume was made with the addition of distilled water (DW). Samples were diluted to 50 ml with distilled water, filtered using Whatman grade 42 filter papers. After digestion, atomic absorption spectrometer was used to measure Zn concentration of maize grain, root and shoots samples (PerkinElmer, Aanalyst 100, Waltham, USA).

***Statistical analysis*:** The recorded data from the experiment were subjected to analysis of variance (ANOVA) using computer software Statistix v. 8.1 (Analytical Software, USA). The treatment means were compared by Tukey's honest significant difference (HSD) test (Steel *et al.,* 1997) at 5% probability level.

RESULTS

***Temporal release of Zn in soil*:** The data regarding AB-DTPA extractable Zn content in soil fertilized with different Zn sources have been presented in Fig. 1. It clearly indicates that BOZ-2, BOZ-3, BOZ-4 formulations substantially improved Zn available content in the soil compared to $ZnSO_4$, the most common Zn fertilizer. However, almost similar Zn concentration was measured in the soil samples where BOZ-1 and $ZnSO_4$ were applied. Moreover, BOZ formulations improved soil Zn content more than ZSB alone.

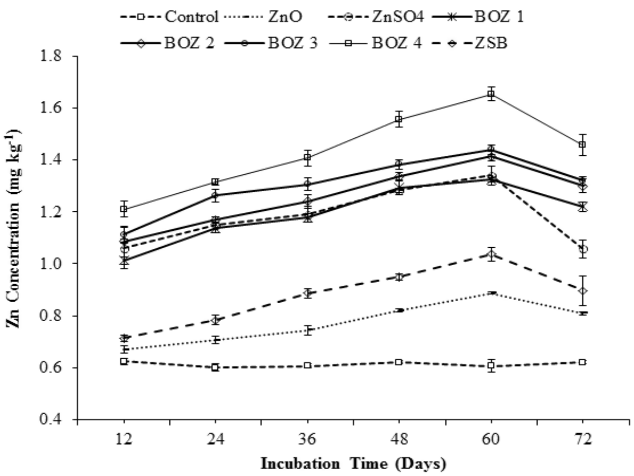

Figure 1. Effect of bio-activated zinc (BOZ) vs $ZnSO_4$ on temporal release of zinc in soil.

During the incubation period (72 days), soil plant available Zn content increased with an increase in incubation time in all Zn treatments except control. In all the treatments, plant available Zn content was found maximum after 60 days. However, a decrease in Zn content was observed after 72 days as compared to measured after 60 days of incubation. Under the set conditions, the maximum plant available Zn concentration at all the sampling times was recorded in the soil fertilized with BOZ-4 formulation. In the soil fertilized with BOZ-4 formulation, the plant available Zn concentration was about 26% higher than in the soil fertilized with $ZnSO_4$.

***Effect of different Zn sources on growth attributes of maize*:** Table 2 depicts the response of maize in terms of growth enhancement (plant height, length of primary roots, shoot dry biomass, root dry biomass, cob length, cob diameter and cob girth) to fertilization with different Zn sources. Overall inoculation with ZSB was found to promote growth as compared to un-inoculated control and produced similar response as the application of ZnO alone. However, all the plant growth parameters were significantly ($p<0.05$) improved by BOZ-3 and BOZ-4 application compared to plants in the control as well as the plants fertilized with ZnO. The highest increase in growth parameters was recorded with BOZ-4 formulation, but it was statistically ($p<0.05$) similar with BOZ-3 formulation, except length of primary roots. In detail, plant height of control plant was not improved by ZnO fertilization, however, BOZ formulations increased plant height up to 13-30%. The most effective BOZ formulation was BOZ-4 which improved plant height by 15% and 17% compared to $ZnSO_4$ and ZSB. Similarly, root length of plants was 36% higher in case of BOZ-4 fertilization compared to ZnO. Likewise, this treatment increased shoot dry biomass, root dry biomass, cob length, cob diameter and plant girth by 37%, 47%, 43%, 30% and 69%, respectively, as compared to the plants which were applied with ZnO. The BOZ-4 formulation also improved growth of maize compared to $ZnSO_4$, the most common Zn fertilizer.

***Effect of different sources of Zn on yield attributes of maize*:** Table 3 shows the impact of different Zn treatments on yield parameters (grain yield, stover yield, number of lines cob^{-1}, 100 grain weight and number of grains $line^{-1}$) of maize grown in pots. Like the growth attributes, the yield attributes of maize were also variably affected by different Zn sources.

Table 2. Impact of different sources of zinc on growth parameters of maize

Treatments	Plant height (cm)	Root length (cm)	Shoot dry biomass (g pot^{-1})	Root dry biomass (g pot^{-1})	Cob length (cm)	Cob diameter (cm)	Plant girth (cm)
Control	129.0 c	29.5 e	117 c	19.6 d	9.3 c	9.8 b	5.0 e
ZnO	134.3 c	32.1 de	122 c	22.6 cd	11.7 bc	10.0 b	6.1 de
$ZnSO_4$	146.7 bc	36.5 bcd	132 bc	24.7 bc	14.0 ab	11.2 ab	7.7 bcd
BOZ-1	146.3 bc	36.2 bcd	143 abc	27.6 bc	14.0 ab	11.0 ab	7.8 bcd
BOZ-2	156.7 ab	37.1 bc	145 abc	29.6 ab	14.0 ab	11.7 ab	8.7 abc
BOZ-3	161.0 ab	40.3 ab	164 ab	30.3 ab	15.0 ab	12.3 ab	9.3 ab
BOZ-4	168.0 a	43.7 a	167 a	33.3 a	16.7 a	13.0 a	10.3 a
ZSB	143.7 bc	33.7 cde	122 c	23.8 cd	13.0 b	10.2 b	7.3 cd
HSD	20.24	4.89	34.52	5.63	3.47	2.69	1.91

Data are shown as mean of three replicates. Means sharing the same letter(s) within the column do not differ significantly (Tukey's test at $p \leq 0.05$)

Table 3. Impact of different sources of zinc on yield parameters of maize

Treatments	Grain yield (g pot^{-1})	Stover yield (g pot^{-1})	No. of line/cob	100 grain weight (g)	No. of grains $line^{-1}$
Control	131 c	26.2 c	8 c	20 dc	19 c
ZnO	134 c	29.2 bc	9 c	20 bc	20 bc
$ZnSO_4$	140 bc	35.9 ab	10 abc	21 abc	23 ab
BOZ-1	139 bc	40.6 a	11 ab	21 abc	22 abc
BOZ-2	141 bc	39.1 a	12 ab	22 ab	24 ab
BOZ-3	147 ab	41.7 a	12 a	22 ab	24 ab
BOZ-4	154 a	43.2 a	12 a	22 a	25 a
ZSB	135 c	34.7 abc	9 bc	20 abc	22 abc
HSD	11.84	9.54	2.45	1.88	4.04

Data are shown as mean of three replicates. Means sharing the same letter(s) within the column do not differ significantly (Tukey's test at $p \leq 0.05$)

The grain yield of maize was improved by only BOZ-3 and BOZ-4 forumulations as compared to control. However, all other Zn treatments did not significantly ($p<0.05$) increase the grain yield. The most effective Zn source was BOZ-4 formulation which resulted into 16% and 10% increase in grain yield as compared to the grain yield of the plants treated with ZnO and $ZnSO_4$, respectively. However, this increase was statistically similar with that of BOZ-3 formulation. The BOZ-4 formulation improved the stover yield, number of lines per cob and number of grains per line by 20%, 21%and 7%, respectively, as compared to $ZnSO_4$ fertilization. This increase in growth attributes by BOZ-4 formulation was more in case of ZnO fertilization than $ZnSO_4$.

***Soil and plant Zn concentration*:** The soil available Zn content after the crop harvest varied by fertilization of soil with different Zn sources (Table 4). AB-DTPA extractable Zn was increased in the soils fertilized with BOZ-2, BOZ-3 and BOZ-4 formulations, whereas, other Zn sources did not change the soil Zn content. With BOZ-4 formulation, the soil Zn content was about 22% higher than control plants.

Table 4. Impact of different sources of zinc on chemical parameters of maize

Treatment	Zn in shoot (µg g^{-1})	Zn in root (µg g^{-1})	Zn in grain (µg g^{-1})	Available Zn in soil mg kg^{-1}
Control	15.4 e	25.8 c	40.7 c	0.51 c
ZnO	17.0 de	27.2 bc	42.0 bc	0.53 bc
$ZnSO_4$	20.6 abc	31.3 bc	44.3 bc	0.63 abc
BOZ-1	19.6 bc	34.1 ab	44.6 bc	0.66 ab
BOZ-2	20.9 ab	34.4 ab	46.7 b	0.66 ab
BOZ-3	21.2 ab	35.1 ab	53.7 a	0.69 a
BOZ-4	22.9 a	40.4 a	56.3 a	0.71 a
ZSB	18.1 cd	29.7 bc	43.0 bc	0.62 bc
HSD	2.60	8.25	5.54	0.143

Data are shown as mean of three replicates. Means sharing the same letter(s) within the column do not differ significantly (Tukey's test at $p \leq 0.05$)

Plants fertilized with BOZ-4 forumlation also had high Zn content compared to control. Zn contents in root, shoot and grain were improved up to 29%, 11% and 27%, respectively, as compared to the plants treated with $ZnSO_4$ and 56%, 49% and 39%, respectively, as compared to control (no application of Zn) plants. In addition, data showed that alone application of zinc oxide and ZSB (T8) had non-significant impact on Zn concentration in root, shoot and grains of maize as compared to control.

DISCUSSION

Zinc is the most important nutrient required for optimum growth and development plants as well as growth and development of human beings and animals. Several researchers have correlated the drastic yield losses in cereals (maize, rice and wheat) due to less supply of Zn from zinc deficient soils (Maqsood *et al.*, 2009; Imran *et al.*, 2014).

Furthermore, Zn deficiency in cereals is due to less availability of Zn in soil. Cereals are used as food in the developing countries including Pakistan. Human faced many disordered due to dependence on Zn deficient cereals for daily calorie intake in the developing countries (Cakmak *et al.*, 2009). To overcome zinc malnutrition, there are several ways which help in increasing the dietary Zn intakes (Cakmak, 2009). One of such ways is the exploitation of soil microorganisms that can mobilize unavailable zinc in the soil and increase Zn assimilation, plant growth and yield (Roesti *et al.*, 2006; Rana *et al.*, 2012). In the present work, four BOZ formulations were prepared by mixing powdered ZnO and ground orange peel waste already inoculated with ZSB, *Bacillus* sp. AZ6. The effectiveness of products to improve the growth, yield and grain Zn content of maize was compared with ZnO and $ZnSO_4$ fertilizers.

Fertilization with BOZ formulations increased AB-DTPA extractable Zn in soil as compared to ZnO (Fig.1). The highest available Zn content was recorded in the soil treated with BOZ-4 formulation. Despite that a few researchers have previously reported an increase in plant available Zn content in soils by the inoculation of different ZSB (Imran *et al.,* 2014; Whiting *et al.,* 2001; Mumtaz *et al.,* 2017), however, this study is novel in the sense that a product comprising of ZnO, ZSB and orange peel waste have been tested for increasing soil Zn bioavailability. This increase in soil Zn contents in response to the application of BOZ formulations might be due to Zn solubilizing activity of *Bacillus* sp. AZ6 which has already been reported as an efficient ZSB (Hussain *et al.,* 2015: Nazir *et al.,* 2016). This strain might have solubilized ZnO during one-week incubation before soil application, slowly releasing Zn in soil from ZnO after soil application. The solubilization of ZnO by the strain *Bacillus* sp. AZ6 might be due to a decrease in pH thanks to the production of diffenent organic acids including ferulic acid, gallic acid, chlorggenic acid, cinamic acid, caffeic acid and syrirgic acid by this bacterial strain (Hussain *et al.,* 2015). The ground orange peel waste in product was primarily involved not only in supporting the growth and survival of ZSB but also in maintaining the acidic nature of the products suitable for ZnO dissolution. The pH of the product was recorded as 4.8. The highest available Zn level in the soil receiving BOZ-4 formulation might be associated with the presence of relatively more orange peel waste content, higher bacterial population and lower pH.

The results of the pot experiment also depicted that BOZ formulations were quite effective in improving growth and yield attributes of maize (Table 2 and Table 3). Overall yield of maize was about 17% higher in the soils treated with BOZ-4 fertilization as compared to ZnO treatment. Zinc oxide application alone did not have any significant impact on growth and yield attributes of maize because it is an insoluble and non-available source of Zn. Despite that the indigenous microorganisms might have solubilized this source upto some extent, but the concentration of available Zn was too small to significantly increase the growth and yield of maize. Several previous studies also reports increase in growth and yield attributes of different agricultural crops inoculated with Zn solubilizing bacterial strains (Mehta *et al.,* 2015; Shaikh and Saraf, 2017; Gontia-Mishra *et al.,* 2017; Mumtaz *et al.,* 2017, Javed *et al.,* 2018). This promotion in the growth and yield parameters in response to BOZ formulations might be linked either with an improved uptake of Zn in plants due to an increase in bioavailable content of Zn in soil by the application of BOZ formulations or with plant growth promoting traits including auxin production, ACC-deaminase activitity, siderophore production and phosphate solubilization possessed by this strain (Hussain *et al.,* 2015). These plant growth promoting traits might also have improved the root growth which subsequently produced higher yields due ot better acquisition of water and nutrients from soil (Kamilova *et al.,* 2006; Hussain *et al.,* 2015; Javed *et al.,* 2018).

All BOZ formulations resulted in an increase in AB-DTPA extractable Zn, root Zn, shoot Zn and grain Zn of maize as compared to those of with ZnO fertilization. However, BOZ-4 was the most effective treatment. Relatively more Zn in the plant parts receiving the BOZ-4 formulation could be due to the higher activity of the plant growth promoting ZSB, *Bacilus* sp. AZ6, due to more content of orange peel waste. The more growth and activity of AZ6 might have resulted in relatively higher release in Zn bioavailable for the plant to be uptaken. Javed *et al.* (2018) also concluded that the *Bacillus* spp. have been well documented for their potential to solubilize insoluble Zn source. Several bacterial species have ability to solubilize insoluble zinc compounds (ZnO, $ZnCO_3$, ZnS) in liquid medium (Saravanan *et al.,* 2007) and in soil (Tariq *et al.,* 2007). Yilmaz *et al.* (1997) also reported improved in grain Zn concentration by the exogenous application of Zn in alkaline calcareous soils. The reason of this high response in our case is the application of Zn and ZSB in combined form. The improved grain Zn might also be due to enzymatic activities in response to application of BOZ-4 formulation. Similar increase in dry matter accumulation and Zn acquisition through the inoculation of plant growth promoting rhizobacteria has also been reported by others (Eleiwa *et al.,* 2012; Rana *et al.,* 2012; Minaxi *et al.,* 2012). Bio-activation of ZnO is economic as compared to market available resources $ZnSO_4$. Nazir *et al.* 2016 also concluded that the farmers of poor community can get maximum benefit from the economical bio-activated ZnO by their limited resources and more nutrition in maize grain.

***Conclusion*:** On the basis of results of incubation and pot experiment, it can be concluded that application of bio-activated Zn formulations based on ZnO, zinc solubilizing strain AZ6 and orange peels based organic amendment significantly improved the growth, yield and Zn content of maize grain under pot conditions. However, the BOZ-4 formulation containing relatively higher organic matter and microbial population in combination with ZnO was found as more effective formulation as compared to other bio-activated zinc (BOZ) formulations. Hence, the use of orange peels waste as organic material enriched with cheap Zn source (ZnO) and Zn solubilizing bacteria (*Bacillus* sp. AZ6) in proper formulation could be a novel approach for improving the growth, yield and quality of agricultural crops under Zn deficient conditions. Moreover, it is cost effective, less time consuming and environment friendly approach resulting into recycling of the organic waste. However, there is need to evaluate the potential of optimize BOZ formulations to enhance the growth, yield and nutritional quality of maize under field experiments.

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