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Evaluation of different insoluble zinc sources along with zinc solubilizing bacteria on growth of wheat (*Tritium aestivum*) in an inceptisol having marginal Zn availability

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Abstract
The present study intended to evaluate the effect of insoluble Zn sources when applied with and without zinc solubilizing bacteria, on the growth and zinc content of the wheat crop. For this purpose, a pot experiment was designed and conducted during 2016-17 and 2017-18 using factorial completely randomized design (FCRD). Three zinc compounds ($ZnSO_4$ (Zn_1), ZnO (Zn_2), and $ZnCO_3$ (Zn_3)) with two distinct doses (1 mg kg^{-1} (D_1) and 2 mg kg^{-1} (D_1) Zn) were used in the experiment. In response to ZSB application, the data revealed an increase in wheat growth and, as a result, an increase in wheat yields also. Significant differences among the effects of sources and bacterial inoculations were noticed during the experiment. Performance of all the Zn sources including $ZnSO_4$ was found to improve when applied with ZSB. In contrast to the control, all isolates resulted in a nearly 1.5- to 2-fold increase in Zn content in grains and straw. In case of zinc solubilizing bacteria, inoculation of ZSB_2 showed best results as compared to other two sources. It was concluded that the application of $ZnSO_4$ @ 2 mg kg^{-1} Zn along with ZSB_2 was had the best zinc management strategy to conflict the zinc deficiency.

Keywords: Growth, yield, zinc solubilizing bacteria, Zn sources, pot experiment, Zn content

1. Introduction

Despite the massive increase in wheat production due to Green Revolution, the present average yield is nowhere near the potential yield (Liu *et al.*, 2017) [23]. One of the limiting factors being the cultivation of wheat in soils with severely low or marginal levels of Zn (Alloway, 2008) [2]. Over the years, various methods for increasing zinc availability in soil to plants have been used (Sharma *et al.*, 2015) [34]. Though it is a long shot, several approaches have been suggested to address the aforementioned problem, one of which is the use of zinc containing chemical fertilizers such as $ZnSO_4$, ZnO, $ZnCO_3$, $Zn_3(PO_4)_2$ and $ZnCl_2$ (Joy *et al.* 2017; Kumar *et al.*, 2017) [17, 21], use of organic farming (Bhattacharya *et al.*, 2006) [5], using transgenic approach (Bashir *et al.*, 2010) [4], and the use of microbial inoculants (PGPR) (Tariq *et al.*, 2007) [37]. The major problem with the use of chemical fertilizers are its quick fixation in soil. About 96% of the exogenously applied Zn fertilizers are converted into various unavailable forms depending upon the soil types and physicochemical reactions within a week of application (Gontia-Mishra *et al.*, (2017) [11]. Organic farming and transgenic approaches to addressing zinc deficiency have struggled due to their high production costs and implementation methods. Recently, some researchers have begun to look into the possibility of using microbial inoculants to convert the inaccessible form of zinc into the usable form. Use of these microbial inoculants in sustainable agriculture and soil regeneration is generating a lot of buzz. These microbial inoculants are referred as zinc solubilizers (Hussain *et al.*, 2015; Mumtaz *et al.*, 2017) [14, 27]. Among the bacterial genera *Bacillus*, *Acinetobacter*, *Pseudomonas*, and *Thiobacillus ferrooxidans*, *Enterobacter*, *Arthrobacter*, *Burkholderia*, *Exiguobacterium*, *Gluconacetobacter*, *Mycobacterium*, *Ralstonia*, *Stenotrophomonas*, *Sphingomonas* and *Xanthomonasare* have been reported as zinc solubilizers and shown to promote plant growth (Saravanan *et al*. 2007; Gandhi and Muralidharan, 2016) [32, 10].

Based on these facts a pot experiment was planned to be conducted in the rabi seasons of 2016 and 2017 with an objective to evaluate the effect of different insoluble zinc sources along with zinc solubilizing bacteria on growth parameters of wheat crop grown in an Inceptisol of Varanasi, having Zn availability just above critical value.

2. Materials and Methods

2.1 Bacterial isolates

Forty bacterial isolates were taken for the experiment from the Soil Microbiology Laboratory, Department of Soil Science and Agricultural Chemistry, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi. Based on the morphological characters and assayed *in vitro* for Zn solubilization on Bunt and Rovira media (Bunt and Rovira, 1955) [40] supplemented with ZnO (ZnO). Out of all, three bacterial isolates were selected as Zn solubilizers and tested in the pot for *in situ* Zn solubilizing activity. In addition to the bacterial inoculation, changes in the growth parameters as well as zinc status of the plants were also observed.

2.2 Plant Material and Treatment

Wheat variety (HUW 234) was used for the experiment which was obtained from the Department of Genetics and Plant Breeding, Banaras Hindu University, Varanasi, Uttar Pradesh, India. The selected wheat variety (HUW 234) is commonly grown in entire wheat area of the North Eastern Plains Zone of India. The experiment was steered in net house of the Department of Soil Science and Agricultural Chemistry, I. Ag. Sc., Banaras Hindu University, Varanasi in two succeeding *rabi* seasons (2016–17 and 2017–18). The experiment was laid out in FCRD with three factors *viz*., sources of Zn ($ZnSO_4$-Zn_1, ZnO-Zn_2, and $ZnCO_3$-Zn_3); two doses 1 mg kg^{-1}- D_1 and 2 mg kg^{-1}- D_2 Zn) and three bacterial isolates (ZSB_1, ZSB_2 and ZSB_3) treatments were replicated thrice. The recommended dose of fertilizers N, P, K (120:60:60) were mixed with soil in suitable quantities. Seeds were treated with selected bacterial isolates with the help of sticker solution (10% of Jaggery solution). Bacteria treated seeds of wheat were sown in each pot. After full emergence of first leaves, four healthy plants were maintained per pot.

2.3 Experimental soil

The experimental soil was collected from Agricultural Research Farm, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi. The initial soil properties are present in Table 1. After processing, the soil was filled in earthen pots of 10 Kg capacity which were lined with polythene sheet for checking the loss of water and nutrients from the pots.

Table 1: Physico-chemical properties of the initial soil

Particulars	Value	
	2016-17	2017-18
A. Mechanical separates		
Texture		
a) Sand (%)	63.20	60.15
b) Silt (%)	25.10	21.34
c) Clay (%)	15.44	16.20
Textural class	Sandy Clay Loam	
B. Physico-chemical characteristics		
pH	7.8	8.1
Electrical conductivity (dSm^{-1})	0.2	0.18
Bulk density	1.39	1.35
CEC	19.6	20.2
WHC (%)	42.35	40.22
Organic carbon (%)	0.40	0.38
Available N (mg/kg)	81.22	83.14
Available P (mg/kg)	8.24	7.56
Available K (mg/kg)	80.2	84.1
DTPA extractable-Zn (mg/kg)	0.68	0.71
C. Biological properties		
Soil microbial biomass carbon (mg C kg^{-1} soil)	92.6	90.1
Dehydrogenase activity (µg TPF g^{-1} soil day^{-1})	25.64	22.2
D. Soil microbial count		
Bacteria (cfu × 10^5 g^{-1} soil)	15.4	13.8
ZSB (cfu × 10^4 g^{-1} soil)	7.8	7.2

2.4 Pot experiment

Wheat variety (HUW 234) was used for the experiment which was obtained from Agriculture Farm, Banaras Hindu University, Varanasi, Uttar Pradesh, India. The selected wheat variety (HUW 234) is commonly grown in entire wheat area of the North Eastern Plains Zone of India. The experiment was conducted in net house of the Department of Soil Science and Agricultural Chemistry, I. Ag. Sc., Banaras Hindu University, Varanasi for two consecutive *rabi* seasons (2016–17 and 2017–18). The experiment was laid out in FCRD with three factors *viz*., sources of Zn ($ZnSO_4$-Zn_1, ZnO-Zn_2, and $ZnCO_3$-Zn_3); two doses 1 mg kg^{-1} -D_1 and 2 mg kg^{-1}-D_2 Zn) and three bacterial isolates (ZSB_1, ZSB_2 and ZSB_3) treatments were replicated thrice. The recommended dose of fertilizers N, P, K (120:60:60) were mixed with soil in suitable quantities. Wheat seed were treated with selected bacterial isolates with the help of sticker solution (10% of Jaggery solution). Bacteria treated seeds of wheat were sown in each pot. After full emergence of first leaves, four healthy plants were maintained per pot.

2.5 Analysis of growth attributes of wheat
Wheat growth parameters including plant height, number of leaves and chlorophyll content were recorded at 30, 60 and 90 DAS. Plant height was recorded with the help of meter scale and expressed in centimeter while the chlorophyll content of the leaves was measured by SPAD meter. The spike length of the plant was measured from base to top most tip of the spikelet and it was expressed in cm after computation.

2.6 Statistical analyses
The raw data generated during the whole experiment were subject to statistical analysis by following the Factorial Complete Randomized Design (FCRD) to draw the valid differences among the treatments.

3. Results
3.1 Chlorophyll content (SPAD)
Chlorophyll content (SPAD value) recorded during two years (2016-17 and 2017-18) of the pot experiment at three growth stages (30, 60, and 90 DAS) of wheat crop are presented in Table 2. The content increased up to 60 DAS and thereafter decreased. At 30 DAS, highest chlorophyll (36.24) was observed with application of Zn through $ZnSO_4$ during 2016-17. Between the two doses of Zn, higher SPAD value, (33.99) was recorded with application of zinc @ 2 mg kg^{-1} (Zn_2) while among the zinc solubilizing bacterial (ZSB) strains, highest solubilization was observed in case of ZSB_2 (33.30). In 2017-18, SPAD value (35.65) of plants receiving $ZnSO_4$ was higher than SPAD value of plants receiving other Zn sources. Application of Zn_2 resulted in 5.05% higher SPAD value than Zn_1. Highest chlorophyll (42.82), at 60 DAS was observed with application of Zn through the $ZnCO_3$ during 2016-17. Between the zinc doses, Zn_2 exhibited highest SPAD value (40.82) as compared to Zn_1 while in case of zinc solubilizing bacterial strains; ZSB $_2$ recorded the highest chlorophyll (41.17). In 2017-18, chlorophyll content (42.53) in plants receiving $ZnSO_4$ superior to other sources. Zn_2 caused higher SPAD value over Zn_1 which produced 4% lesser SPAD value than Zn_2. ZSB_2 resulted in higher chlorophyll (40.80) over ZSB_1 (40.01). At 90 DAS, highest chlorophyll (33.36) was observed with the application of Zn through $ZnCO_3$ during 2016-17. Among the dose and zinc solubilizing bacterial strains, Zn_2 and ZSB_2 recorded the highest SPAD value, i.e. 30.86 and 31.12, respectively. In 2017-18, the SPAD value (34.16) of plants receiving $ZnSO_4$ was superior to SPAD value of plants receiving other sources. Application of Zn_2 resulted in 10% higher content than Zn_1.

Table 2: Effect of zinc with zinc solubilizing bacteria on chlorophyll content of wheat

Treatment	Chlorophyll (SPAD)					
	2016-17			2017-18		
	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS
Zn_0	31.90	37.76	26.32	32.22	37.48	27.09
Zn_1	36.24	39.84	28.21	35.65	42.53	34.16
Zn_2	32.03	39.63	30.21	32.31	39.18	28.80
Zn_3	33.86	42.82	33.36	33.89	39.48	30.80
Sem±	0.17	0.18	0.29	0.16	0.16	0.31
CD (0.05)	0.48	0.50	0.81	0.47	0.46	0.88
D_1	33.02	39.21	28.18	32.69	38.92	28.83
D_2	33.99	40.82	30.86	34.34	40.42	31.60
Sem±	0.12	0.13	0.20	0.12	0.12	0.22
CD (0.05)	0.34	0.36	0.57	0.33	0.33	0.62
B_0	31.72	38.45	27.58	31.90	38.09	28.36
B_1	33.74	40.26	30.18	33.63	40.01	31.00
B_2	35.27	41.17	31.12	34.67	40.80	31.70
B_3	33.30	40.17	29.21	33.87	39.77	29.81
Sem±	0.17	0.18	0.29	0.16	0.16	0.31
CD (0.05)	0.48	0.50	0.81	0.47	0.46	0.88

Where Zn_0 = no zinc, Zn_1=$ZnSo_4$, Zn_2= ZnO, Zn_3= $ZnCO_3$, D_1 and D_2= Doses of Zn, B_0= no bacteria, B_1, B_2, B_3= Treatment with bacterial strain 1,2 and 3 respectively

3.2 Plant height
The data pertaining to plant height of wheat during the two-year pot experiment (2016-17 and 2017-18) at three growth stages (30, 60, and 90 DAS) are presented in Table 3. The highest plant height (19.15 cm) at 30 DAS was observed with the application of Zn through $ZnSO_4$ during 2016-17. Application of Zn @ 2 mg kg^{-1} (Zn_2) resulted in higher plant height (18.67 cm) over-application of Zn @ 1 mg kg^{-1} (Zn_1). Among the zinc solubilizing bacterial strains (ZSB), the highest increment was observed in the case of ZSB_2 (18.86 cm). In 2017-18, plants receiving $ZnSO_4$ showed the highest plant height (21.41 cm) over plant receiving other sources. The application of Zn_2 (dose) exhibited 11.41% higher plant height than Zn_1. The highest plant height (45.43 cm) was observed at 60 DAS when Zn was applied through $ZnSO_4$ in 2016-17. Comparing the two doses of Zn (Zn_1 and Zn_2) pointed out that Zn_2 resulted in 5% higher effect on plant height. Among the zinc solubilizing bacteria, highest plant height (44.43 cm) was noted in ZSB_1. In 2017-18, the highest plant height (45.54 cm) was noted when Zn was applied through $ZnSO_4$. At 90 DAS, $ZnSO_4$ @ 2 mg kg^{-1} treated plants showed the highest plant height during both the years (2016-17 and 2017-18). Among the ZSB treated plants, highest plant height (48.57cm) was observed with ZSB_1. In 2017-18, ZSB_2 showed the highest plant height (48.76 cm).

Table 3: Effect of zinc with zinc solubilizing bacteria on plant height of wheat

Treatment	Plant height (cm)					
	2016-17			2017-18		
	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS
Zn_0	18.18	38.24	43.77	17.44	38.83	43.57
Zn_1	19.15	45.43	50.87	21.41	45.54	50.52
Zn_2	18.38	41.24	48.27	20.57	41.05	47.32
Zn_3	18.61	42.47	48.50	21.35	40.76	46.79
SEm±	0.11	0.15	0.32	0.27	0.17	0.18
CD (0.05)	0.32	0.43	0.90	0.77	0.48	0.52
D_1	18.49	40.87	46.74	19.10	40.59	45.87
D_2	18.67	42.82	48.97	21.28	42.49	48.23
SEm±	0.08	0.11	0.22	0.19	0.12	0.13
CD (0.05)	0.22	0.30	0.64	0.55	0.34	0.37
B_0	18.27	39.07	46.63	18.83	38.97	44.85
B_1	18.58	44.43	48.57	20.78	42.44	47.55
B_2	18.86	42.52	48.33	21.13	43.41	48.76
B_3	18.60	41.36	47.89	20.03	41.36	47.04
SEm±	0.11	0.15	0.32	0.27	0.17	0.18
CD (0.05)	0.32	0.43	0.90	0.77	0.48	0.52

Where Zn_0 = no zinc, Zn_1=$ZnSo_4$, Zn_2= ZnO, Zn_3= $ZnCO_3$, D_1 and D_2= Doses of Zn, B_0= no bacteria, B_1, B_2, B_3= Treatment with bacterial strain 1,2 and 3 respectively

3.3 Number of leaves

The data related to the effect of different treatments on the number of leaves of wheat crop at different growth stages during 2016-17 and 2017-18 are presented in Table 4. At 30 DAS, application of Zn through ZnO and $ZnCO_3$ was at par in its effect on the no. of leaves in first year. Whereas, significantly higher number of leaves (3.81) was recorded with Zn_2 (2 mg kg^{-1}). In case of zinc solubilizing bacterial strains, ZSB_2 followed by ZSB_3 caused highest number of leaves (3.84 and 3.78, respectively) over uninoculated control (3.47). In second year of pot experiment, application of Zn through ZnO showed highest leaves (3.67). Zn_2 significantly increase the no. of leaves in comparison to Zn_1. Inoculation with bacterial strains ZSB_2, ZSB_1 and ZSB_3 significantly increased the number of leaves as compared to un-inoculated control. Application of ZSB_2 showed 10% higher leaves than ZSB_0. Results revealed that application Zn through $ZnSO_4$ showed highest number of leaves (10.09) followed by ZnO (9.80) at 60 DAS in 2016-17. Higher number of leaves was recorded with Zn_2 which was ~5% higher in its effect on number of leaves than Zn_1. Among the ZSB, Highest increase in plant leaves was exhibited by ZSB_2 (10.50) followed by ZSB_1 (9.64). During the second year of pot experiment, plants receiving $ZnSO_4$ showed highest number of leaves (10.01). Significantly higher (9.54) leaves were recorded with application of Zn_2. The ZSB_2 caused highest no. of leaves (10.48) and showed its significant superiority over the other bacterial strains. ZSB_2 caused an increment of 30% in number of leaves over ZSB_0. Among the zinc sources, the highest number of leaves (9.98) at 90 DAS was recorded with $ZnSO_4$ during 2016-17. Application of ZnO and $ZnCO_3$ @ Zn_2 exhibited 9.96 and 9.53 leaves, respectively. Between the two doses (Zn_1 and Zn_2) of Zn, Zn_2 produced significantly higher number of leaves (9.74) which was ~4% higher than the effect caused by Zn_1. Among the ZSB, ZSB_2 produced the highest number of leaves (10.66) followed by ZSB_1 (9.58) inoculated plants. In the second year (2017-18) of pot experiment, the plants receiving ZnO produced highest number of leaves (10.47). While in the case of doses, the application of Zn_2 resulted in significantly higher number of leaves (9.94) over-application of Zn_1 (9.92). Among the ZSB, plants inoculated with ZSB_2 produced the highest number of leaves (11.10) which was 26.27% higher than the effect caused by uninoculated control.

Table 4: Effect of zinc with zinc solubilizing bacteria on no. of leaves of wheat

Treatment	No. of Leaves					
	2016-17			2017-18		
	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS
Zn_0	3.55	8.47	8.78	3.48	8.14	8.60
Zn_1	3.66	10.09	9.98	3.63	10.01	10.02
Zn_2	3.83	9.80	9.96	3.67	9.85	10.47
Zn_3	3.82	9.49	9.53	3.59	9.38	10.65
SEm±	0.04	0.11	0.10	0.08	0.10	0.06
CD (0.05)	0.13	0.30	0.27	0.22	0.30	0.17
D_1	3.62	9.24	9.39	3.61	9.15	9.94
D_2	3.81	9.68	9.74	3.57	9.54	9.92
SEm±	0.03	0.08	0.07	0.06	0.07	0.04
CD (0.05)	0.09	0.21	0.19	0.16	0.21	0.12
B_0	3.47	8.31	8.52	3.44	8.07	8.79
B_1	3.77	9.64	9.58	3.69	9.55	10.10
B_2	3.84	10.50	10.66	3.80	10.48	11.10
B_3	3.78	9.40	9.49	3.43	9.28	9.74
SEm±	0.04	0.11	0.10	0.08	0.10	0.06
CD (0.05)	0.13	0.30	0.27	0.22	0.30	0.17

Where Zn_0 = no zinc, Zn_1=$ZnSo_4$, Zn_2= ZnO, Zn_3= $ZnCO_3$, D_1 and D_2= Doses of Zn, B_0= no bacteria, B_1, B_2, B_3= Treatment with bacterial strain 1,2 and 3 respectively

3.4 Spike length

The data about the spike length (cm) of wheat during 2016-17 and 2017-18 are presented in table 5. During the 1st year of the pot experiment, the highest spike length (8.10 cm) was observed with the application of Zn through $ZnCO_3$ followed by ZnO (7.98 cm) and $ZnSO_4$ (7.80 cm). Similarly, the highest increase in spike length was observed due to the application of Zn @ 2mg kg^{-1} (Zn_2). In the case of plants inoculated with ZSB, ZSB_3 inoculated plants showed the highest spike length (8.08 cm). In the second year of the pot experiment, the application of Zn through $ZnSO_4$ and ZnO caused the significant increase in spike length (8.26 cm and 7.81 cm, respectively). Zn_2 produced the significantly higher spike length (8.04 cm) which was ~5% higher than the effect caused by Zn_1. Among the zinc solubilizing bacterial strains, highest spike length (8.04 cm) was observed in plants inoculated with ZSB_2 which was 19.16% higher than the effect caused by uninoculated control.

Table 5: Effect of zinc with zinc solubilizing bacteria on spike length of wheat

Treatment	Spike length (cm)	
	2016-17	2017-18
Zn_0	7.73	7.59
Zn_1	7.80	8.26
Zn_2	7.98	7.81
Zn_3	8.10	7.80
Sem	0.14	0.10
CD	0.41	0.28
D1	7.83	7.69
D2	7.97	8.04
Sem	0.10	0.07
CD	0.29	0.20
B0	7.77	7.20
B1	7.85	7.92
B2	7.90	8.58
B3	8.08	7.75
Sem	0.14	0.10
CD	0.41	0.28

Where Zn_0 = no zinc, Zn_1=$ZnSo_4$, Zn_2= ZnO, Zn_3= $ZnCO_3$, D_1 and D_2= Doses of Zn, B_0= no bacteria, B_1, B_2, B_3= Treatment with bacterial strain 1,2 and 3 respectively

4. Discussion

The increment in SPAD value may be due to the action of zinc solubilizing bacteria on different insoluble zinc sources resulting in enhanced supply and uptake of zinc to the plant. Zinc plays a very important role in the enhancement of chlorophyll content in the plant because it acts as a structural and catalytic component of proteins and enzymes responsible for pigment biosynthesis (Samreen *et al*., 2013) [30]. Similarly, Othman *et al.* (2017) [28] reported that inocubation with zinc-solubilizing bacterium (TM56) at 0.2 mg/L $ZnSO_4$ produced the highest chlorophyll content (12.34 mg/cm^2) followed by plants inoculated with (TM9) with a chlorophyll content of 10.97 mg/cm^2 at 0.2 mg/L $ZnSO_4$. Similar findings were also reported by Sarathambal *et al*. (2009) [31] and Majumdar and Chakraborty (2015) [24].

Various sources and doses produced similar variation in plant height recorded at 30, 60 and 90 DAS during both years of pot experiment. In case of zinc solubilizing bacteria, the trend was not similar at different stages. Application of $ZnSO_4$ @ Zn_2 and ZSB_2 significantly increased the plant height in wheat at 30 DAS in first year and 30, 60 and 90 DAS in second year. In first year at 60 and 90 DAS highest height was observed in plants treated with $ZnSO_4$ @ Zn_2 and ZSB_1. This finding shows that application of zinc with zinc solubilizing bacteria causes significant effect on plant height. Similarly, Goteti *et al.* (2013) [12] reported that seed bacterization with zinc solubilizing bacteria resulted in increased plant height. Ramesh *et al*. (2014) [29] reported that inoculation with zinc solubilizing bacteria *Bacillus aryabhattai* significantly increases the plant height. The increase in plant height may be attributed to the adequate supply of zinc which could meet the enhanced zinc demand by the action of zinc solubilizing bacteria on different zinc sources. Zinc solubilizing bacteria increase the plant growth by making the nutrient available through N_2 fixation, phosphate solubilization, Zn solubilization, siderophores and phytohormones production and antagonistic effect on plant pathogens (Iqbal *et al*., 2010; Wang *et al*., 2014 and Gandhi and Muralidharan 2016) [16, 39, 10]. Kamran *et al.* (2017) also observed that inoculation of zinc solubilizing bacteria *P. agglomerans* in jute plant significantly increased the plant height as compared to uninoculated control. Othman *et al.* (2017) [28] reported that Inoculation of plant with Zn solubilizing bacterium *Acinetobacter* sp. (TM56) at 0.2 mg/L $ZnSO_4$ produced the significantly higher plant height. Gandhi and Muralidharan (2016) [10] reported that application of zinc solubilizing bacterial strains (AGM_3 + AGM_9) with insoluble source of zinc significantly enhanced the growth and yield of rice crop over control under pot experiment. This might be due to the production of different types of growth hormones by ZSB.

Zinc solubilizing bacterial strains ZSB_2 and ZSB_1 significantly increased the number of leaves in wheat under both the years of pot experiment at 30, 60 and 90 DAS. Among the sources, plants receiving Zn through ZnO recorded highest number of leaves at 30 DAS which was at par with $ZnCO_3$ treated plants and both were higher than the control in both the years. At 60 and 90 DAS, $ZnSO_4$ treated plants recorded highest number of leaves. Results showed that zinc solubilizing bacterial isolates with zinc sources significantly increased the number of leaves in wheat. This increament in number of leaves may be attributed to the solubilization of nutrients by zinc solubilizing bacteria. Improvement in different plant parametrs by seed bacterization with zinc solubilizing bacteria has been reported by Goteti *et al.* (2013) [12].

The spike length of wheat was significantly higher in the plants receiving zinc through $ZnCO_3$ and $ZnSO_4$ during 2016-17 and 2017-18, respectively. However, the similar trend was observed in case of doses where Zn_2 produced the highest spike length in both the years of pot experiment. Plants inoculated with ZSB_3 the highest spike length in 1st year of pot experiment. But in 2nd year of pot experiment, plants inoculated with ZSB_2 recorded highest spike length. Results of our study showed that inoculation of ZSB along with different zinc sources enhanced spike length of wheat under net house condition. The increase in spike length may be associated with the more availability of other nutrients in the rhizosphere due to zinc (Tarique *et al*., 2016) [37]. Kenbaev and Sack (2002) [18] also reported that increase in zinc availability to the plant increases the number of spikes m^{-2}. Dewal and Pareek (2004) [8] also reported that zinc application improved spike length. Similar findings were reported by Asad and Rafique (2000) [3] and Curtin *et al*, (2008) [6].

5. Conclusion

The result demonstrated that the growth attributes *viz.* chlorophyll content (SPAD), plant height, number of leaves and spike length of wheat crop were enhanced by the application of zinc with zinc solubilizing bacteria over uninoculated control. Hence**,** on the basis of above findings it was concluded that the application of Zn @ 2mg kg^{-1} through $ZnSO_4$ along with bacterial isolate ZSB_2 exhibited the best Zn management strategy for improving growth of wheat in inceptisol having marginal Zn availability.

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