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Nutrient Uptake and Biological Activity in Tomato by Zinc Solubilizing Bacterial (ZSB) Isolates

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A B S T R A C T

Zinc deficiency not only affects crop yields, but also nutritional quality and human health. Microbial transformation of unavailable forms of soil zinc to plant available zinc is an important approach contributing to plant zinc nutrition. Therefore, Nutrient uptake and biological activity in Tomato crop by zinc solubilizing bacterial isolates was tested under glass house condition. The study consisted of six treatments, each with five replications, including five different zinc solubilizing bacteria and uninoculated control, out of five bacteria *B. aryabhattai* was used as reference strain. The results revealed that tomato seedlings treated with *B. aryabhattai* and *Bacillus sp*. (PAN-TM1) substantially decreased rhizosphere pH and increased the macro and micro nutrient concentration in rhizosphere, plant and tomato fruit especially zinc concentration in the fruit (31.1 and 29.3 ppm respectively). Further biological activity *viz*., dehydrogenase, urease, acid and alkaline phosphatase, soil respiration and soil microbial biomass carbon was also found to be increased significantly with the *B. aryabhattai* and *Bacillus sp*.(PAN-TM1) treated seedlings as compared to uninoculated treatment. This assumes significance as the increased zinc concentration found in this study has large implications in terms of overcoming zinc malnutrition. ZSB isolates i.e. *B. aryabhattai* and *Bacillus sp*. (PAN-TM1) substantially influenced mobilization of zinc and its concentration in edible portion, which can be utilized as bio-inoculants for biofertilization and biofortification in the sustainable crop production practices.

Introduction

Micronutrients are important for the optimum growth and productivity of the plants. Though these elements are required in critical amounts, they are very important for plant development and for profitable crop production because they work 'behind the scene' as activators of many plant functions. Of the several micronutrients that increase plant growth and productivity zinc (Zn) plays a vital role. Zinc is one of the imperative micronutrient required relatively in small concentrations (5–100mg kg^{-1}) in tissues for healthy growth and reproduction of plants. Zn is an important component of enzymes that drive and increase the rate of many important metabolic reactions involved in crop growth

and development (Potarzycki and Grzebisz, 2009). Inadequate supply of Zn to the plant result in the cessation of physiological functions which lead to the development of visible symptoms of stress such as interveinal chlorosis, bronzing of chlorotic leaves, small and abnormally shaped leaves, stunting and rosetting. Zn deficiency significantly affects the root system including root development (Fageria *et al.,* 2002). The flowering and fruiting process were greatly reduced under severe Zn deficiency. Quality of harvested products, plant susceptibility to injury by high or light temperature intensity and to infection by fungal diseases can also increase by Zn deficiency (Cakmak, 2000).

The major reason for the widespread occurrence of Zn deficiency problems in crop plants is attributed to low solubility of Zn in soils rather than a low total amount of Zn. External addition of soluble Zn to alleviate deficiency results in the transformation of about 96- 99% to various fractions of unavailable forms and about 1- 49% is left as available fraction in the soil. So the water soluble Zn ($ZnSO_4.7H_2O$) advocated and applied in the soil cannot be detected beyond 15 days of period (Rattan and Shukla, 1991) and become unavailable which make the Zn nutrition to the plants critical. This requires a system that releases the required quantity of Zn that are converted to unavailable state and retained in the soil to available form. Microbes are potential alternate that could cater plant zinc requirement by solubilising the complex zinc in soil. Several genera of rhizobacteria belonging to *Pseudomonas* spp. and *Bacillus* spp. are reported to solubilise zinc. Microbes solubilize the metal forms by protons, chelated ligands, and oxidoreductive systems present on the cell surface and membranes. These bacteria also exhibit other traits beneficial to plants, such as production of phytohormones, antibiotics, siderophores, vitamins, antifungal substances, and hydrogen cyanide (Saravanan *et al.,* 2003). Thus microbial inoculants will be an alternative approach to overcome constraints due to synthetic fertilizer and to revive soil's fertility resulting in the intensive farming. Since zinc is a limiting factor in crop production, the study on zinc solubilization by bacteria has an immense importance in zinc nutrition to plants. Moreover, it is essential to find out a simple way to enhance zinc concentrations in foodsto solve malnutrition problem in our country. In view of above, the present investigation was undertaken in order to overcome the zinc deficiency in soils as well as to enhance zinc concentration in final produce by using zinc solubilizing bacteria (ZSB). Hence, this study was carried out to know the effect of ZSB on nutrient uptake and biological activity of tomato crop.

Materials and Methods

The glass house experiment was conducted in Microbiology Laboratory, Division of Soil Science and Agricultural Chemistry, ICAR-IIHR, Hesaraghatta, Bengaluru, Karnataka. The low zinc containing soil was collected from third block of IIHR, Hesaraghatta and the soil had the following characteristics. pH: 6.62, EC (dsm^{-1}): 0.535, OC%: 0.48, N(ppm): 77.76, P (ppm): 7.16, K (ppm): 134.4, Ca (ppm): 550.8, Mg (ppm): 100.3, Fe (ppm): 48.2, Cu (ppm): 2.31, Mn (ppm): 50.36, Zn (ppm):0.75.Further the soil was sieved to remove debris and sterilized for three consecutive days later on filled in to the pots of 30cm diameter at the rate of 20kg/pot. The seeds of tomato (ArkaRakshak) used in the study were collected from IIHR and were sown in protray containing cocopeat. After germination, 21 days old seedling with well root development and uniform growth were selected and transplanted to the pots. After 5 days of planting 20 gm of lignite based cultures containing population of 2.3 x 10^9cfu/g were added. The pots were uniformly watered regularly to maintain optimum

moisture content in the soil and the recommended dose of fertilizer for hybrid tomato (180:120:180 kg NPK per ha) was given. The plants were destructively sampled and soils were sampled from each pot at 45 days after planting (DAP). Soil and plant samples were analyzed for nutrient concentrations. Soil samples were analyzed for enzyme activities, organic carbon and biomass carbon and nutrient concentrations. One plant per pot was maintained throughout the experiment.

Treatments details

The pot culture experiment consisted of six treatments with five replication, which includes five different ZSB and uninoculated control, out of five bacteria *Bacillus aryabhattai* was used as reference strain.

These ZSB isolates were isolated from stone quarry dust powder and also the well characterized five PGPR bacteria *viz*., *Bacillus aryabhattai*, *Pseudomonas taiwenensis*, *Bacillus* sp. (PAN-TM1), *Enterobacter* sp.-2 and *Bacillus aerophilus* were received from the Culture Collection Centre, Microbiology Laboratory, Division of Soil Science and Agricultural Chemistry, ICAR- IIHR, Hesaraghatta, Bengaluru, Karnataka. These bacterial isolates were examined for their ability to solubilize zinc from insoluble Zinc source and their potential in enhancing the zinc content in tomato under pot culture studies. The details of treatments are as follows,

Estimation of nutrient content in rhizosphere soil, plant and fruit of tomato inoculated with ZSB

Soil nutrient analysis

The soil samples were collected at 45 days after planting were analyzed chemically for various characteristics. The soil pH, electrical conductivity (EC), Organic carbon (OC) and available potassium (K) was estimated by standard protocol developed by Jackson (1973). Available nitrogen was determined by macro distillation of the sample following alkaline permanganate method as suggested by Subbiah and Asija (1956). Available phosphorus was extracted with Bray's No.1 extractant (0.03 N NH_4F + 0.025 N HCl). The phosphorus in the extract was determined by chloro stannous reduced molybdo-phosphoric blue colour method in HCl acid medium. The intensity of blue colour was read at 660 nm using a spectrophotometer (Bray and Kurtz, 1945).

Exchangeable Calcium and Magnesium were determined by complexometric titration method using appropriate indicators (Baruah and Barthakur, 1997). The method developed by Lindsay and Norwell (1978) using DTPA extractant (Diethylene triaminepenta acetic acid) was followed for the estimation of Zn, Cu, Mn and Fe. Ten grams soil was shaken with 20 ml of DTPA extractant for 2 hours for the extraction of micronutrient cations. Atomic absorption spectrophotometer was used for measuring the concentration.

Plant and fruit nutrient analyses

Dried plant and fruit samples were finely ground by using willey stainless steel millto amorphous powder and one gram was taken in 150mL conical flask containing 10 ml nitric acid (HNO_3) and perchloric acid ($HClO_4$) in 9:4 ratios. The flasks were placed on a hot plate and digested at 300°C until the entire plant material turned colorless. The extract was taken in 100 ml volumetric flask and the volume was made to 100 ml with distilled water. These samples were used for estimation of P, K, Ca, Mg and micronutrients Fe, Cu, Mn and Zn by using standard procedures. Phosphorous was quantified by forming

yellow color phosphovanadomolybdate complex using spectrophotometer at 430nm (Baruah and Barthakur, 1997). Potassium was estimated by feeding the diluted plant and fruit extract to flame photometer as described by Jackson (1973).

Total nitrogen content was estimated by taking About 1 gram of powdered plant and fruit samples were digested with concentrated sulphuric acid and digestion mixture (K_2SO_4: $CuSO_4.5H_2O$: Selenium in 100: 20: 1 proportion) till a green residue was obtained. The digested material was distilled by micro Kjeldhal distillation method. The liberated ammonia was trapped in boric acid and then nitrogen was estimated by titration against standard sulphuric acid (Piper, 1966). Similarly, micronutrients such as iron, copper, manganese, zinc, calcium and magnesium were estimated by atomic absorption spectrophotometer.

Biological activity in rhizosphere soil of tomato

The soil samples were collected by completely uprooting the plants at 45 days after planting (DAP) and used for the determination of enzyme activities.

Dehydrogenase activity

Moistened soil sample (20 g) was incubated with 0.2 g CaCO3and 2 ml 1% triphenyltetrazoilum chloride at 30^0C for 24 h. At theend of incubation period, soil sample was extracted with 25 ml methanol. The microbial activity produces H^+ ions, which reduces triphenyl tetrazoilum chloride into triphenyl tetrazoilum formazan, which is red in colour. Dehydrogenase activity, the index of microbial activity was determined by measuring the intensity of red colour at 485 nm and expressed as µg of TPF released/g soil/hour (Tate and Terry, 1980).

Phosphatase activity

Acid and alkaline phosphatase activity

Phosphatase activity of soil sample was determined by following the procedure of Eizavi and Tabatabai (1977). 1 gm of soil sample was placed in 50 ml Erlenmeyer flask to which 0.2 ml toluene followed by 4 ml of modified Universal buffer (pH 6.5) was added. 1 ml of para-nitrophenyl phosphate solution made in modified universal buffer was added to the flask and contents of the flasks were mixed by swirling for two minutes. The flasks were stoppered and incubated at 37^o C for one hour. After incubation, 1 ml of 0.5M $CaCl_2$ and 4 ml of 0.5M NaOH were added to the flask, swirled and filtered through Whatman No. 42 filter paper. The intensity of yellow colour developed was measured at 420nm against the reagent blank using spectrophotometer. Controls were also performed for each soil sample following the same procedure described above except that the paranitrophenyl phosphate solution was added after the addition of 0.5 M $CaCl_2$ and 0.5M NaOH and just before filtration. P-Nitro phenol content of the supernatant was calculated by referring to a calibration graph plotted from the results obtained with standards containing 0, 10, 20, 30, 40 and 50 µg of paranitrophenyl. The phosphatase activity in the soil samples were expressed as µg paranitrophenyl solution.

The alkaline phosphatase activity of the soil samples were determined by following the same procedure as that of acid phosphatase activity except that pH of modified universal buffer was adjusted to 11.

Soil urease activity

Urease activity was determined by the following procedure described by Tabatabai and Bremner (1972). One gram of soil sample

was taken in a 50 ml volumetric flask to which 0.20 ml toluene and 9 ml THAM buffer was added and the flasks were swirled for few minutes to mix the contents. To the flask, 1 ml of 0.2M urea solution was added and swirled again. Then the flask was stoppered and placed in an incubator, at 37^{o} C. After 2 hours, approximately 35 ml of Ag_2SO_4 solution was added and was filtered. 20 ml of aliquot was taken in 100 ml distillation flask and NH_4-N released with 0.2 g MgO was determined using Kjeldahl distillation apparatus. Urease activity was expressed as µg NH_4-N released gram^{-1} of compost hour^{-1}.

Soil respiration and substrate induced soil respiration

Fifty gram sieved soil was taken (55% WHC) in a beaker and placed it in a jar containing 25ml NaOH (0.05 M) and made it air tight immediately by the lid. 2-5 jars with NaOH (0.05 M) without soil sample used as control. The jars were incubated for 24 hours at 25^{0}C. Same method was used to determine the substrate induced soil respiration. In this case 0.5% glucose mixed with the soil sample and incubated for 4-6 hours at 22^{0}C. After 24 hours jars were opened and took out the beaker and washed external surface of the beaker with CO_2 - free water to bring the NaOH solution completely in to the jar then added 5ml of barium chloride solution (0.5M) and some drop of phenolphthalein indicator and then titrated against HCl (0.05M) under continuous stirring until the colour changes from pink to colourless. Then the rate of the respiration was calculated and expressed as CO_2 –C mg/kg soil/hour.

Soil microbial biomass carbon

Microbial biomass C (Biomass C) was determined by the chloroform fumigation–extraction method, with 0.5 M K_2SO_4 as extractant. The organic C of extracts was estimated by oxidation with potassium dichromate (Vance *et al.*, 1987). The difference in C content of the fumigated and unfumigated extracts was converted to microbial biomass C (expressed in mg kg^{-1} of dry soil) by applying a factor (Kc) of 0.45 (Jenkinson, 1988).

Results and Discussion

Effect of zinc solubilizing bacterial isolates on rhizosphere nutrient content of tomato at 45 DAP

There was a decline in the rhizosphere pH ranged from 5.95 to 6.52 with the inoculation of ZSB strains compared to uninoculated control (Table 1). Among the different ZSB inoculated treatments, *B. aryabhattai* acidify the rhizosphere solution to a greater extent and declined pH (5.95) as compared to uninoculated control (6.52).The electric conductivity (EC) was found to be non-significant among the different ZSB inoculated treatments. The organic carbon content in the rhizosphere was found to be significant, the highest was observed in *B. aryabhattai* (0.79 %) inoculated treatment followed by *Enterobacter* sp.-2 (0.77%) inoculated plants. Significant differences of major nutrients were observed among the different treatments inoculated with ZSB isolates. Significantly highest N, P and K were recorded in the plants inoculated with *B. aryabhattai* (144.1, 18.9 and 120.2 ppm respectively) followed by *Bacillus* sp. (PAN-TM1) (140.9, 18.4 and 107.5 ppm respectively). The highest micro nutrient content *viz.*, calcium, iron, zinc were observed in the treatments inoculated with *B. aryabhattai* (974.1, 180.2 and 4.27 ppm respectively) which was significantly higher compare to all the other treatments, whereas the least micro nutrients was recorded in the rhizosphere of uninoculated control. The other micro nutrients *viz.*, magnesium, copper and

manganese content in rhizosphere of tomato revealed non-significant differences among the treatments as influenced by the zinc solubilizing bacterial isolates. However, the maximum magnesium (114.5ppm) content observed in the plants inoculated with *B. aryabhattai* whereas copper (3.6ppm) and manganese (160.6ppm) content in the plants inoculated with *Bacillus* sp. (PAN-TM1).

Effect of zinc solubilizing bacterial isolates on plant nutrient concentration of tomato at 45 DAP

Major and micro nutrients content of tomato plant at 45 days after planting was observed upon the inoculation of zinc solubilizing bacterial isolates. Significant differences of major plant nutrients concentration *viz.*, N, P and K were observed among the plants inoculated with zinc solubilizing bacterial isolates (Table 2). The treatment inoculated with *B. aryabhattai* recorded higher per cent of N and P (2.45 and 0.65%) as compared to other treatment. The potassium concentration was recorded more or less similar in *Bacillus* sp. (PAN-TM1) (5.3%) and *B. aryabhattai* (5.2%) inoculated plants.

Among the minor plant nutrients concentration of tomato at 45 days after planting, significant differences were observed in calcium, copper, manganese and zinc concentration of tomato plants among the various treatments.

The highest concentration of calcium, copper and zinc was recorded in the treatment inoculated with *B. aryabhattai* (6.86%, 19.1 and 56.3 ppm respectively) as compared to other treatments, whereas *Bacillus* sp. (PAN-TM1) inoculated treatment recorded highest Mn content (85.1ppm). The highest magnesium and iron concentration in plants were noticed in *B. aryabhattai* treated plants (0.396% and 373.6ppm).

Effect of zinc solubilizing bacterial isolates on fruit nutrient concentration of tomato at harvest

The results on the fruit nutrient concentration of tomato at harvest upon inoculation of efficient ZSB isolates reveals that, there was a significant difference in the major nutrients concentration in the tomato fruit (Table 3). The treatment inoculated with *B. aryabhattai* recorded significantly higher nitrogen, phosphorus and potassium (2.36, 0.34 and 3.58% respectively) which are statistically on par with the treatment *Bacillus* sp. (PAN-TM1) (2.28, 0.32 and 3.41% respectively). Among the micro nutrients, the significant concentration of calcium, magnesium, iron and zinc was recorded in the treatment inoculated with *B. aryabhattai* (3.89%, 0.233%, 134.1ppm and 31.1 ppm respectively), followed by the treatment *Bacillus* sp. (PAN-TM1) (3.82%, 0.232%, 103.1ppm and 29.3ppm respectively). The higher concentration of copper and manganese content in tomato fruits were noticed in *B. aryabhattai* (10.1 and 46.1 ppm respectively) inoculated treatment at harvest, which is non-significantly different.

Effect of zinc solubilizing bacterial isolates on biological activity in tomato rhizosphere at 45 days after planting

The effect of ZSB isolates on soil enzymes activity in the tomato rhizosphere after 45 days of planting significantly increase the dehydrogenase, phosphatase and urease activity (Table 4). Highest dehydrogenase, alkaline phosphatase and urease activity was observed in the treatment inoculated with *B. aryabhattai* (355.2 µg of TPF, 68.9µg of PNP and 24.80 µg NH_4-N released/g soil/hour) followed by the treatment inoculated with *Bacillus* sp. (PAN-TM1) (298.5 µg of TPF, 66.3µg of PNP and 21.4 µg NH_4-N released/g soil/hour).

Table.1 Effect of zinc solubilizing bacterial isolates on rhizosphere nutrient content of tomato at 45DAP

Treatments	pH	EC (dsm^{-1})	OC (%)	N (ppm)	P (ppm)	K (ppm)	Ca (ppm)	Mg (ppm)	Fe (ppm)	Cu (ppm)	Mn (ppm)	Zn (ppm)
B. aryabhattai	5.95	0.734	0.79	144.1	18.9	120.2	974.1	114.5	180.2	3.1	153.7	4.27
P. taiwenensis	6.05	0.693	0.72	116.6	16.5	102.5	910.9	113.5	161.6	3.3	152.3	3.85
Bacillus sp. (PAN-TM1)	6.10	0.663	0.77	140.9	18.4	107.5	956.7	113.5	113.5	3.6	160.6	3.95
Enterobacter sp.-2	6.16	0.594	0.57	92.3	14.1	82.6	763.5	102.6	101.8	3.5	149.3	2.65
Bacillus aerophilus	6.34	0.596	0.66	106.9	13.8	90.1	724.3	99.8	87.3	3.6	146.2	2.6
Un inoculated	6.52	0.577	0.49	68.04	11.9	70.3	654.2	88.5	55.6	3.3	143.1	2.2
S.Em±	0.44	0.04	0.04	7.95	1.08	6.65	57.27	7.27	7.47	0.24	10.62	0.22
CD at 5%	NS	NS	0.13	23.39	3.19	19.55	168.39	NS	21.9	NS	NS	0.65

Table.2 Effect of zinc solubilizing bacterial isolates on plant nutrient concentration of tomato (45 DAP)

Treatments	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Fe (ppm)	Cu (ppm)	Mn (ppm)	Zn (ppm)
B. aryabhattai	2.45	0.65	5.2	6.86	0.396	373.6	19.1	76.6	56.3
P. taiwenensis	2.13	0.58	4.4	4.62	0.371	327.4	14.3	77.5	48.1
Bacillus sp.(PAN-TM1)	2.44	0.62	5.3	5.80	0.375	353.3	17.2	85.1	52.5
Enterobacter sp.-2	2.32	0.45	4.4	4.76	0.353	342.2	15.3	69.6	44.3
Bacillus aerophilus	1.97	0.42	3.9	4.91	0.343	335.1	15.1	74.6	42.3
Un inoculated	1.57	0.38	2.6	4.19	0.311	298.4	13.2	45.3	37.1
S.Em±	0.14	0.03	0.29	0.35	0.02	23.66	1.10	5.02	3.19
CD at 5%	0.43	0.10	0.87	1.04	NS	NS	3.25	14.7	9.39

Table.3 Effect of zinc solubilizing bacterial isolates on fruit nutrient concentration of tomato at harvest

Treatments	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Fe (ppm)	Cu (ppm)	Mn (ppm)	Zn (ppm)
B. aryabhattai	2.36	0.34	3.58	3.89	0.233	134.1	10.1	46.1	31.1
P. taiwenensis	1.95	0.26	3.36	3.15	0.206	98.3	08.3	46.2	26.2
***Bacillus* sp. (PAN-TM1)**	2.28	0.32	3.41	3.82	0.232	103.1	09.5	45.3	29.3
***Enterobacter* sp.-2**	2.17	0.24	2.88	2.80	0.161	108.2	08.6	41.4	24.2
Bacillus aerophilus	1.95	0.21	2.68	2.64	0.181	96.1	08.5	44.4	20.3
Un inoculated	1.31	0.18	2.14	2.16	0.152	85.2	08.1	35.2	17.1
S.Em±	0.13	0.01	0.20	0.21	0.01	6.87	0.62	2.92	1.68
CD at 5%	0.40	0.05	0.60	0.62	0.04	20.2	NS	NS	4.95

Table.4 Effect of zinc solubilizing bacterial isolates on soil enzyme activity in tomato rhizosphere (45 days after planting)

Treatments	Dehydrogenase (µg of TPF released /g soil/hour)	Acid phosphatase (µg of PNP released /g soil/hour)	Alkaline phosphatase (µg PNP released /g soil/hour)	Urease (µg NH_4-N/g soil/hour)
B. aryabhattai	355.2	52.39	68.9	24.80
P. taiwenensis	219.5	50.07	64.8	19.24
***Bacillus* sp. (PAN-TM1)**	298.5	50.42	66.3	21.40
***Enterobacter* sp.-2**	257.4	50.07	58.8	18.33
Bacillus aerophilus	216.4	50.60	53.4	17.64
Un inoculated	186.6	43. 73	47.6	15.98
S.Em±	18.3	3.48	4.09	1.35
CD at 5%	53.9	NS	12.04	3.99

Table.5 Effect of zinc solubilizing bacterial isolates on soil respiration and soil microbial biomass carbon in tomato rhizosphere (45 days after planting)

Treatment	Soil respiration (CO_2-C mg /kg soil/hour)	Soil microbial biomass carbon(µg/g soil)
B. aryabhattai	12.5	1982.3
P. taiwenensis	10.8	1830.1
***Bacillus* sp.(PAN-TM1)**	16.1	1830.1
***Enterobacter* sp.-2**	9.7	1613.9
Bacillus aerophilus	10.8	1882.2
Un inoculated	2.6	1229.5
S.Em±	0.78	121.18
CD at 5%	2.31	356.28

Treatment details	
T_1	*B. aryabhattai* + 100% N and K, 75% P
T_2	*P. taiwenensis*+100% N and K, 75% P
T_3	*Bacillus*sp. (PAN-TM1) +100% N and K, 75% P
T_4	*Enterobacter* sp.-2 +100% N and K, 75% P
T_5	*Bacillus aerophilus* + 100% N and K, 75% P
T_6	Un inoculated Control + 100%NPK

There was no significant difference noticed in acid phosphatase activity among different zinc solubilizing bacterial isolates, however, the highest activity recorded in *B. aryabhattai* (52.39 µg of PNP released/g soil/hour) treatment.

Effect of zinc solubilizing bacterial isolates on soil respiration and soil microbial biomass carbon in tomato rhizosphere after 45 DAP

Significant differences in soil respiration and soil microbial biomass carbon was observed in rhizosphere of Tomato as influenced by the ZSB isolates after 45 days of planting (Table 5). Maximum soil respiration was recorded in the treatment inoculated with *Bacillus sp.* (PAN-TM1) (16.1 CO_2-C mg/kg soil/hour) followed by *B. aryabhattai* (12.5 CO_2-C mg/kg soil/hour), whereas treatment inoculated with *B. aryabhattai* (1982.3 µg/g soil) recorded the highest soil microbial biomass carbon. The least soil respiration and soil microbial biomass carbon was recorded in the uninoculated control.

Zinc is an essential nutrient for not only improving crop productivity, but also to alleviate malnutrition in human populations and provide nutritional security. Zn deficiency has become a serious problem affecting nearly half of the world's population (Cakmak, 2008). This is actually due to fixation of major portion of available form of Zn caused by chemical reactions which leads to the crops grown in Zn deficient soils. Many Indian soils exhibit the deficiency of Zn with the content much below the critical level of 1.5ppm (Tiwari and Dwivedi, 1994). It is expected to increase from 42% in 1970 to 63% by 2025 due to continuous depletion of soil fertility (Singh, 2009). To overcome zinc malnutrition, there are many interventions that help in increasing the dietary zinc intakes. One such intervention is through the exploitation of soil microorganisms that can mobilize unavailable zinc, increase zinc assimilation, plant growth and yield. Pot culture screening experiments are very much essential for analyzing the effects of microbial bio inoculants on various plant growth parameters as well as on nutrient uptake before evaluating their efficacy under field conditions. In the present study, five zinc solubilizing bacterial isolates were inoculated individually Tomato plants improved nutrient concentration in rhizosphere soil, plant and fruit of Tomato and also soil enzymes activitiy.

B. aryabhattai inoculated treatment reduced the soil pH their by significantly increased the major and minor nutrients in the soil. Similar decrease in the rhizospheric pH with the inoculation of *Bacillus cereus* W9 and *Bacillus* sp. was previously reported by Yu *et al.,* (2011). This might be attributed to excretion of organic anions is often associated with proton extrusion, leading to a substantial lowering of rhizosphere pH (Neumann and Romheld, 2002). In addition to the change in rhizospheric pH, production of organic acids can also directly facilitate the mobilization of nutrients by reducing sorption of nutrients by altering the surface charge characteristics of soil colloids, desorption of nutrients from sorption sites (Jones, 1998), which helps in the uptake of macro and micro nutrients by the plants and leads to accumulation of nutrients in different parts of the plants (Dahaji *et al.*, 2012 and Desai *et al.*, 2012). Similarly, inoculation of maize with *Pseudomonas* and *Bacillus* significantly

increased the nutrient content of N, P, K, Fe, Cu, Mn, and Zn content in maize leaves by applying PGPR (Puente *et al.*, 2004). Thus ZSB strains proved to have a favorable effect on the availability of major and minor nutrients in the rhizosphere soil which helps in uptake by plants.

Inoculation of ZSB strains significantly increased macro and micro nutrient concentration in tomato fruit. *B. aryabhattai* inoculated treatment recorded highest major and minor nutrient concentration, especially Zn concentration in tomato fruit (31.1ppm). Similarly inoculation of *B. aryabhattai* to soyabean and wheat crop significantly increased the zinc concentration in soyabean seed and wheat grain between 49.70 and 61.25 mg/kg (Ramesh *et al.,* 2014). Mader *et al.,* (2011) also reported a substantial increase in Mn and Zn concentration in rice grains due to application of combination of natural mycorrhiza consortia and fluorescent *Pseudomonas* strain R62 + R81. Roesti *et al.,* (2006) and Mader *et al.,* (2010) had reported that inoculation of *Pseudomonas synxantha* HHRE81 (R81) and *P. jessenii* LHRE62 (R62) increased zinc concentration in seeds of wheat and black gram. In another study, Tariq *et al.,* (2007) demonstrated the efficiency of a commercial PGPR consortium acting as zinc solubilizer that increased Zn concentration to an extent of 157%. The results are mainly due to higher enzyme activities, microbial biomass-C, significant drop in rhizosphere pH, and redistribution among native zinc pools resulting in increased zinc availability for crop acquisition. Many reports indicated that the zinc concentration is too low to meet the daily human requirement in regions solely dependent on cereal based diet (Cakmak, 2008). In our present study the zinc concentration in tomato fruit is the above the minimum requirements needed for daily human requirements. The increased zinc concentration found in this study has large implications in terms of overcoming zinc malnutrition of the rural Indian population, wherein, zinc malnutrition is wide spread.

Soil enzyme activities have been used as an early and sensitive indicator to soil perturbations like, tillage, addition of organic manure, crop rotation and microbial inoculation and are reflection of ecosystem functioning (Sharma *et al.,* 2010). In this regard, efforts were made to determine changes in rhizosphere enzyme activities pertinent to zinc cycling as a consequence of inoculation with ZSB strains to Tomato crop. Significant increase in the dehydrogenase activity, phosphatase activity, urease activity, soil respiration and microbial biomass carbon in *B. aryabhattai* inoculated treatment as compared to uninoculated control. The results are mainly due to the availability of a high quantity of biodegradable substrates, provide simple sugars for the soil microbial population and are explicitly related to soil functions such as nutrient cycling (Stott *et al.,* 2010). The study revealed an increase in microbial respiration with inoculation in the rhizosphere soils indicates the oxidative capacity of soil microorganisms influenced both by the energy sources that there are in the soil and the number of microorganisms in the soil (Bastida *et al.,* 2008). Inoculation with *B. aryabhattai* strains also significantly increased microbial biomass-C in the rhizosphere of tomato making it a useful index for diagnosing early changes in soil C stabilization and nutrient dynamics following perturbation (Joergensen and Emmerling, 2006).

From the present study we can clearly conclude that among the different ZSB isolates, *B. aryabhattai* and *Bacillus* sp. (PAN-TM1) strains were found to be the most promising zinc solubilizing bacterial isolates based on polyphasic approaches. Inoculation of these isolates decreased rhizosphere pH and increased biological activity in the rhizosphere of tomato crop. This intense microbial activity in rhizosphere resulted in a depletion of organically complexed, bound zinc and calcium carbonate bound zinc thus increased in the concentration of zinc and other nutrients. As a result, better uptake and accumulation of nutrients in different parts of the tomato crop was observed. Hence, these ZSB isolates can be

promoted as potential bio-inoculants to mitigate zinc deficiency in soils after proper field evaluation and validation.

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