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Prospective Zinc Solubilizing Microorganisms for Enhanced Growth and Nutrition in Maize (*Zea mays* L.)

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ABSTRACT

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Zinc (Zn) is one of the most essential micronutrients required for normal plant growth and development. Even though considerable quantity of inorganic Zn is applied in soil but significant quantity of it gets converted into unavailable forms. Zn solubilising microorganisms are the potential substitute for Zn supplement to plant from soil. Among the four isolates that were screened for Zn solubilization, fungal ones performed better than bacterial ones and *Aspergillus* sp. in particular, outperformed every other isolate in the test. It produced a clear halo zone of 22.7 mm on solid medium amended with ZnO. It also produced the biggest halo zone on ZnCO₃ amended media which was followed by *Penicillium* sp. and *Bacillus megaterium*. *Aspergillus* sp. also gave significant release of Zn in broth assay amended with ZnO and ZnCO₃ (88 and 62 ppm), respectively. The pH of the broth was acidic in all the cases ranging from 4.6 to 6.4 in ZnO and from 5.1 to 6.7 in ZnCO₃ amended media. A pot culture experiment with maize for 60 days was conducted which revealed that seed inoculation with *Aspergillus* sp. superiorly enhanced total dry weight of plant (63.21 g/plant) and N (2.42%), P (0.432%) and Zn (25.79 ppm) contents.

Introduction

Among micronutrients zinc (Zn) is one of the most crucial nutrient that is required in moderately less concentrations (5 to 100 mg/kg) in plants tissues for their optimum growth and development. Deficiency of this nutrient in plants has been reported to give rise to stunted growth, reduced integrity of cell membrane, less production of carbohydrates, repair of cell along with decreased synthesis of vital cell organelles such as cytochromes, nucleotides. It also leads to increased

susceptibility to abiotic stresses. Imbalanced use of zinc containing fertilizers create a problem for human beings too as it is known to impair the body absorption of other nutrients like copper and iron. It may also cause anomaly in reproductive health in males (Sharma *et al.*, 1990). Zn solubility is highly dependent on soil pH and soil moisture and this may be one of the reasons for its low availability in dry arid regions of India resulting in Zn deficient soils. Maize is grown in diverse climatic conditions in India from arid to humid regions. It is cultivated in about

8.26 Mha area with an yield of 19 Mt (Ministry of Agriculture, Government of India). Plenty of literature has cited that grain Zn content is inherently low particularly if crop is grown of Zn depleted soils. The main reason of this occurrence is due to low dissolution of Zn in soil. Conventional application of this nutrient to soil somewhat meets the plant demand as more than 90 percent of Zn gets converted to insoluble form depending on physicochemical reactions and type of soil on which it is applied within days of its application. Microorganisms are the prospective replacements that could cater to plant Zn requirement by solubilizing the complex and insoluble forms of Zn in soil. Several species within bacteria and fungi have been reported to solubilize Zn most of which belong to the genera of *Bacillus*, *Pseudomonas* and *Aspergillus* species. These organisms solubilize the metal via several biochemical pathways such as chelated ligands, production of keto-glutonic acids thereby reducing surrounding pH, extrusion of protons which are present on their membranes (Cakmak, 2008; Saravannan *et al.*, 2004). They are also known for their plant growth promoting traits such as production of regulatory hormones, vitamins, siderophores and antibiotics. In this study the ability to solubilize Zn in vitro of four microbes and their effect on growth enhancement of maize has been reported (Crane *et al.*, 1985; Hughes and Poole, 1981; Wakatsuki, 1995).

Materials and Methods

Microbial Cultures

The bacterial strains that were used in the experiment were procured from Agricultural Research Station, Parbhani, India which belong to *Bacillus* species namely, *Bacillus subtilis* and *Bacillus megaterium*. The fungal strains (*Aspergillus* sp. And *Penicillium* sp. identified on the basis of morphology) were

isolated from rhizospheric Zn deficient soils from college farm by serial dilution technique. Further purification was achieved by streak plate method. All four cultures were maintained on nutrient agar and potato dextrose agar media at 4⁰C.

In Vitro Zinc Solubilization Assay

All four isolates were inoculated into Pikovaskaya media (g/L) specified by Saravanan *et al.*, containing dextrose: 10.0; (NH₄)₂SO₄: 1.0; KCl: 0.2; K₂HPO₄: 0.1; MgSO₄: 0.2; pH: 7.0 and insoluble Zn salts (ZnO and ZnCO₃: 0.1%; Agar: 15.0g) and autoclaved at 121⁰C for 20 min. Actively growing cultures of each strain were spot-inoculated with sterilized toothpick onto the agar plates and were incubated at 28⁰C for 3-5 days. The halo zone around colony was observed and recorded. Quantitative assay of zinc solubilization was studied in 150mL conical flasks containing 50mL of liquid Pikovaskaya medium. The broth was inoculated with 0.5 mL of overnight grown bacterial and fungal inoculums and incubated for 3-4 days in an incubator at 28 ± 2⁰C. After incubation, the culture broth was centrifuged and Zn concentration in supernatant was estimated using atomic absorption spectrophotometer.

Seed Inoculation

Seeds of maize of cultivable variety were firstly surface sterilized with 1% sodium hypochlorite for 5 min and then washed thoroughly three times with sterile distilled water. The seeds were dipped in liquid media containing inoculum of each isolate and air dried.

Pot Trial

A pot culture experiment was conducted in plastic pots (20 cm dia) of 4 kg capacity and

filled with 2.5 kg of sterile soil (pre sterilized for two consecutive days in autoclave) with three replications for each treatment. Maize seeds were treated with inoculants and were sown in pots at 5 cm depth under glasshouse condition. Pots were watered daily with sterile distilled water for 60 days.

The experimental setup consisted of 15 treatments namely, five treatments of isolates (two each of bacteria and fungi and an uninoculated control) and two nutrient sources of Zn as ZnO @ 12.5 kg/ha and 25 kg/ha along with recommended dose of fertilizer. Five plants per pot were sown.

Plant Growth Measurement

The crop was harvested after 60 days of sowing (DAS). Maize plants were carefully uprooted from each pot and plant growth parameters like, plant height, stem girth, and dry matter weight were recorded.

Nutrient Analyses

The plant samples were dried under shade and were ground finely in a mortar and pestle and 0.1g of powdered sample was taken in 150mL conical flask containing 10mL nitric acid and perchloric acid in the ratio 9:4. The flasks were placed on a hot plate and digested at 300⁰C until the entire material turned into colourless liquid avoiding charring. The colourless extract was collected in 100 mL volumetric flask and the volume was made to 100mL with distilled water. These samples were then used for estimation of zinc by AAS, potassium by flame photometer, nitrogen and phosphorus by Kjeldahl and Olsen methods respectively (Tandon, 2001).

Statistical Analysis

The data generated was subjected for analysis of variance as applicable two factorial CRD to

test differences among the treatment means as described by Gomez and Gomez, 1984.

Results and Discussion

Zinc Solubilization Activity

All four isolates used efficiently solubilized the insoluble Zn salt amended media, which were ZnCO₃ and ZnO, under *in vitro* conditions. The halo zone diameter was greater in ZnO amended medium than ZnCO₃. Size of the clear zone diameter ranged from 8.3 to 22.7 mm in ZnO and from 7.4 to 17.6mm in ZnCO₃ amended medium. Among the isolates, fungi showed more solubilization over bacterial ones and overall *Aspergillus* sp. had the highest zone of solubilization followed by *Penicillium* sp. And *Bacillus megaterium* in both ZnO and ZnCO₃ amended media. In ZnO amended media *Aspergillus* sp. showed a diameter of 22.7 mm followed by *Penicillium* sp. (18.5 mm) whereas in ZnCO₃ amended media *Aspergillus* sp. displayed a diameter of 17.6 mm followed by *Penicillium* sp. (14.9 mm), *B. megaterium* (10.7 mm) and lastly *B. subtilis* (7.4 mm). Quantitative assay of Zn solubilisation exhibited that *Aspergillus* sp., *Penicillium* sp. and *B. megaterium* were able to dissolve 88, 62, and 33 ppm, respectively from ZnO (Figure 1) in broth on seventh day of observation and were in accord to the observations made on solid medium. Hence, *Aspergillus* sp. And *B. megaterium* were found to be the major solubilizers on both plate and broth study but the fungal isolates were the dominant solubilizers in both cases. Among the treatments, significant reduction of pH was observed in the broth medias incorporated with ZnO (pH 4.6–6.4) (Figure 1) and ZnCO₃ (pH 5.1–6.7) but no significant correlation was observed between the pH and solubilization of Zn. Zn solubilization can be achieved via a variety of mechanisms by microorganisms, which include secretion or excretion of metabolites such as organic acids,

proton extrusion, or production of chelating agents [12, 13]. Also production of mineral acids such as sulphuric acid and carbonic acid may also facilitate the solubilisation of the nutrient in soil [8, 14]. From the given data it was revealed that zinc solubilization potential differed with each isolate. Reduction in pH of the supernatant and its acidification was observed for all four isolates. Solubilizing potential was also correlated with the amount of zinc that had been accumulated by plant. For this study Zn solubilization and fall in media pH could be due to production of organic acids, like 2-keto-gluconic acids. Zinc phosphate solubilization by *Pseudomonas fluorescens* was studied by Di Simine *et al.*, where they stated that gluconic acids produced in the culture medium mediated the solubilization of insoluble zinc salts. In the present investigation too, the pH in acidic range shown by all isolates supports the fact that Zn solubilization could be due to production of organic acids and higher the production of the same more is the available zinc content in the culture broth. Desai *et al.*, (2012) observed that higher availability of Zn is directly proportional to acidic pH of the culture broth. Similar results were also registered by Fasim *et al.*, (2002), Saravanan *et al.*, (2003) and Countinho *et al.*, (2012).

Plant Growth Promoting Activity of Bacterial Strains

Seed inoculation of maize with zinc solubilizing *isolates* significantly enhanced the plant growth at 30 DAS and after 60 DAS (Table 1). Varying nutrient levels also had a significant influence on plant height of maize at different crop growth periods. At 30 DAS maximum and significant increase was observed due to application of ZnO @ 25 kg/ha (48.53 cm) followed by ZnO @ 12.5 kg/ha (46.91 cm). ZnO @ 25 kg/ha application enhanced plant height over RDF by 8.3% at 30 DAS while ZnO @ 12.5 kg/ha increased it

over by 4.7%. At 60 DAS application of ZnO @ 25 kg/ha (132.33 cm) and ZnO @ 12.5 kg/ha (127.33 cm) registered significant gain in height over RDF (118.10 cm) by 12% and 7.7%, respectively. Inoculation also affected the height of maize plants with maximum significant gain being with *Aspergillus* (54.44 cm) and *Penicillium* (51.95 cm) over no inoculation (38.57 cm) by 41.4% and 34.7% respectively at 30 DAS. At 60 DAS inoculation with *Aspergillus* significantly increased the plant height by 18.4% followed by *Penicillium* and *B.megaterium* by 14.4% and 13.6% respectively, over no inoculation. The interaction effect between inoculants and nutrients was significant. The maximum plant height (55.57 cm) was measured due to inoculation with *Aspergillus* sp. + ZnO @ 25 kg/ha which was greater by 44.6% as compared to uninoculated control at 30 DAS. Between bacterial isolates maximum gain was observed by interaction of *B. megaterium* with ZnO @ 25 kg/ha (48.30 cm). Interaction effects of *Aspergillus* sp. with both nutrient levels except showed significant gain in height over RDF. Also all inoculants performed significantly well with both levels of ZnO. The best interaction effect at 60 DAS was observed with *Aspergillus* sp. + ZnO @ 25 kg/ha (143.33 cm) followed by both *Aspergillus* sp. and *Penicillium* sp. with ZnO @ 25 kg/ha which were at par with each other (139.33 cm). The varying nutrient levels significantly influenced the stem girth (Table 2). At 30 DAS the maximum and significant increase of 18.1 % over RDF (1.43cm) was recorded with the application of ZnO @ 25 kg/ha and by 10.4 % by ZnO @ 12.5 kg/ha. Effect was also significant with maximum increase of 4.3 % (2.39 cm) by application of ZnO @ 25 kg/ha over RDF (2.29cm) at 60 DAS. Zn solubilizers also significantly affected stem girth at 30 and 60 DAS. At 30 DAS the highest stem girth was resulted due to inoculation with *Aspergillus* sp. (1.78 cm) increasing it by 35.9% over no inoculation

(1.31 cm). At 60 DAS, inoculation with *Aspergillus* sp. enhanced the girth by 8.5% followed by *Penicillium* sp. (7.6%) and *B. megaterium* by 4.9% over no inoculation. Interaction effects, at 30 DAS were recorded significant due to all combinations of inoculants and nutrients with highest being with *Aspergillus* sp. + ZnO @ 25 kg/ha and *Aspergillus* sp. + ZnO @ 12.5 kg/ha. The increase due to both treatments was to the tune of 55.6% and 48.7% respectively, over RDF. An increase of 43.9% over RDF was recorded also due to *Penicillium* sp. + ZnO @ 25 kg/ha and of 48.7 % by *B. megaterium*+ ZnO@ 25kg/ha. Interaction effects, at 60 DAS, was maximum due to *Aspergillus* sp. + ZnO @ 25 kg/ha (2.46 cm) and *Penicillium* sp. + ZnO @ 25 kg/ha (2.42 cm) over RDF (2.13 cm) by 15.4% and 13.6%.

The effect of varying nutrient levels on dry matter yield was significant (Table 3). Maximum and significant increase of yield was obtained by the application of ZnO @ 25 kg/ha (63.73 g/plant) over RDF (61.21 g/plant) by 4.1% followed by application of ZnO @ 12.5 kg/ha (63.01 g/plant) over the same by 2.9%. All inoculants had a significant effect on dry matter yield with maximum input by *Aspergillus* sp. (63.21 g/plant) by 3.2% followed by *Penicillium* sp (63.19 g/plant) by 3.1% over no inoculation (61.83 g/plant), respectively. The interaction effect on dry matter yield ranged from 60.50 g/plant to 64.67 g/plant. Significantly maximum yield was obtained on inoculation of *Aspergillus* sp. + ZnO @ 25 kg/ha followed by significant effects of *Penicillium* sp. + ZnO @ 25 kg/ha with increase of 6.2% over RDF.

An increase in overall growth can be attributed to the synthesis and secretion of growth promoting substances by inoculants that carry out stem expansion, increased chlorophyll content and photosynthesis rate (Burd *et al.*, 2000; Panhwar *et al.*, 2011).

Rudresh *et al.*, (2005) recorded the highest plant height of 34.6 cm in treatment, which received combined inoculation of *Rhizobium*, PSB and *T. harzianum* with rock phosphate over control in chickpea, Rafi *et al.*, (2012) reported dual inoculation with *Azospirillum* strain A2 and PSB isolates resulted in maximum shoot height of foxtail millet (cv. Chitra) over control. Wu *et al.*, (2005) observed co-inoculation with *P. chlororaphis* and *A. pascens* amendment with RP resulted in the highest plant height in walnut seedling, a significant increment in plant height (45%) and shoot length (19%) over control was observed by Viruel *et al.*, (2014) in maize treated with *Pseudomonas tolaasii* IEXb with 50 kg P per ha applied as TSP under pot and field trial. Srinivasan *et al.*, (2012) reported that *Aspergillus* sp. PSFNRH-2 recorded the highest stem girth (2.63 cm), which was significantly higher than that recorded by all other fungal isolates (0.80–2.20 cm) including the reference strain, *A. awamori* (2.30 cm) but was on par with the SSP control (2.70 cm) in sorghum. Mfilinge *et al.*, (2014) reported that *Rhizobium* inoculation with 30 kg/ha P application increased plant girth by 1.3% 6 WAP in field experiment and 5.1% and 11.67% in green house for 3 WAP and 6 WAP respectively in bush bean. Akhtar *et al.*, (2014) reported that integrated effect of *Rhizobium* and *Bacillus* spp. on the growth of maize (*Zea Mays* L.) with recommended dose of fertilizer (120-60 kg NP/ha) increased stem diameter (15.43mm) over control. Mehrvarz *et al.*, (2008) found significant increase in chlorophyll content of leaves of barley due to positive effect of phosphorous with microbes. Also he found that fungal inoculation was more effective in increasing chlorophyll content over bacterial inoculants due to antagonistic effects on it by chemical fertilizer. Panhwar *et al.*, (2011) recorded highest chlorophyll content (29.30) was obtained in treatments with P at 60 kg per ha inoculated with PSB16 (*Bacillus* sp.)

compared to non-inoculated treatments. Gupta and Gangwar (2012) in chickpea reported highest chlorophyll content (6.20mg/g fresh leaves) was observed with 1.0 kg AM/ha as soil application + Rhizobium + PSB +RDF. Abbas *et al.*, (2013) also recorded higher chlorophyll content in maize with coinoculation between PGPR and reduced doses of nitrogen and phosphorous over chemical control. Sharma and Banik (2014) reported in maize plants grown with 100% recommended dose of fertilizer (RDF) [N: P₂O₅: K₂O) = 150:60:60 kg/ha¹] + AM + *Azospirillum* (T15) produced maximum chlorophyll over uninoculated control. Saxena *et al.*, (2015) also recorded high chlorophyll content in maize on co inoculation with TCP over control. The increase in dry matter yield could be due to PGPR effect of inoculated microbe leading to high uptake of nutrients, increased photosynthesis, and increased growth of root and shoot organs, siderophore and phytohormone production, as well as to their capacity to colonize the root system and interact positively with the plant (Viruel *et al.*, 2011). It could be attributed to the increased vegetative growth possibly as a result of effective utilization of nutrients absorbed through extensive root system and prolific shoot development on account of improved nourishment Kumawat *et al.*, (2009). Vikram *et al.*, (2008) in chickpea reported highest root dry matter by PSBV-5, PSBV-9 and PSBV-13 (all of which recorded 0.59 g) while highest shoot and total dry matter was recorded by PSBV-14 (6.41 and 6.97 g, respectively) with recommended dose of P in the form of MRP in comparison with SSP control and RP control. Kumawat *et al.*, (2009) in mung bean reported that application of vermicompost, seed inoculation with PSB and 40 kg P₂O₅/ha significantly increased dry matter yield over control. Panhwar (2011) reported a significantly higher dry matter (21.48 g) in treatments with 60 kg P₂O₅ per ha inoculated with PSB16, while the response in the control

treatment was very low in aerobic rice. Messele and Pant (2012) recorded that inoculation of *Sinorhizobium ciceri* + *Pseudomonas* sp. with 18/20 kg NP ha⁻¹ as urea and DAP increased dry matter 181.40% respectively over uninoculated control at mid flowering stage in chickpea. Umesha *et al.*, (2013) in a field experiment of maize reported that treatment (T13) having recommended dose of NPK + *Azotobacter chroococcum* + *Bacillus megaterium* + *Pseudomonas fluorescence* + enriched compost gave the highest total dry matter production at harvest (375.80 g) over uninoculated control.

Nutrient content (%)

N content

Among various varying levels of nutrients higher dose of ZnO i.e., @ 25 kg/ha showed maximum N content increases by 15.2% in maize (Table 4). The significant increase was also observed with lower level of ZnO application @ 12.5 kg/ha (2.02%) over RDF by 9.7%. Inoculation of different microorganisms also showed a significant increase in N content of maize. Among the inoculants, fungus *Aspergillus* showed maximum increase in N content (2.42%) which is about 69.23% more over uninoculated control. *Penicillium* also contributed to a higher N content (2.23%) by 55.9% more over uninoculated control. *Bacillus megaterium* and *B. subtilis* also showed significant results. In general, the trend was found that higher dose of nutrient level with inoculants provided more N content in maize. Variation among interactions in N content of maize varied widely from 1.27% to 2.37%. Maximum N content perceived by interaction of *Aspergillus* sp. with the treatment of ZnO @ 25 kg/ha. All inoculants with RDF showed an increase in N content of maize by 56% to 60.6% when compared to RDF with no inoculation.

Table.1 Influence of Zn solubilizers and nutrient levels on Plant height (cm) at 30 and 60 DAS

Nutrient Isolate	RDF	ZnO	ZnO	Average	RDF	ZnO	ZnO	Average
		(12.5 kg/ha)	(25 kg/ha)			(12.5 kg/ha)	(25 kg/ha)	
30 DAS				60 DAS				
No inoculation	38.43	38.33	39.10	38.62	112.83	118.33	117.33	116.16
<i>B. megaterium</i>	42.00	41.60	47.30	43.63	118.00	128.67	130.00	125.55
<i>B. subtilis</i>	45.23	47.20	48.30	46.91	118.33	126.67	131.67	126.11
<i>Aspergillus</i> sp.	50.53	54.63	55.57	53.57	121.33	139.33	143.33	134.66
<i>Penicillium</i> sp.	47.70	52.80	52.40	50.96	120.00	123.67	139.33	127.66
Average	44.78	46.91	48.53	46.74	118.10	127.33	132.33	125.92
	Nutrient		Isolate	Nutrient X Isolate	Nutrient	Isolate		Nutrient X Isolate
S.Em±	0.34		0.34	0.58	0.41	0.41		0.92
CD at 5%	0.96		0.96	2.15	1.17	1.17		2.61

Table.2 Impact of Zn and P solubilizing microbes and varying nutrient levels on stem girth (cm) at 30 and 60 DAS

Nutrient Isolate	RDF	ZnO	ZnO	Average	RDF	ZnO	ZnO	Average
		(12.5 kg/ha)	(25 kg/ha)			(12.5 kg/ha)	(25 kg/ha)	
30 DAS				60 DAS				
No inoculation	1.23	1.33	1.28	1.28	2.13	2.22	2.34	2.23
<i>B. megaterium</i>	1.44	1.55	1.83	1.60	2.31	2.37	2.36	2.34
<i>B. subtilis</i>	1.43	1.55	1.69	1.55	2.26	2.32	2.35	2.31
<i>Aspergillus</i> sp.	1.58	1.83	1.89	1.76	2.38	2.42	2.47	2.43
<i>Penicillium</i> sp.	1.47	1.63	1.77	1.62	2.37	2.45	2.46	2.42
Average	1.43	1.58	1.69		2.29	2.36	2.39	
	Nutrient		Isolate	Nutrient X Isolate	Nutrient	Isolate		Nutrient X Isolate
S.Em±	0.01		0.01	0.02	0.01	0.01		0.02
CD at 5%	0.03		0.03	0.06	0.02	0.02		0.05

Table.3 Effect of nutrient sources and P and Zn solubilizers on dry matter yield (g/plant) of maize

Nutrient \ Isolate	RDF	ZnO (12.5 kg/ha)	ZnO (25 kg/ha)	Average
No inoculation	60.500	61.167	62.113	61.256
<i>B. megaterium</i>	61.243	63.997	63.587	62.934
<i>B. subtilis</i>	61.247	63.580	63.157	62.651
<i>Aspergillus</i> sp.	61.373	63.590	64.670	63.216
<i>Penicillium</i> sp.	61.707	64.297	63.587	63.191
Average	61.214	63.017	63.731	62.650
	Nutrient	Isolate		Nutrient X Isolate
S.Em±	0.069	0.069		0.154
CD at 5%	0.196	0.196		0.439

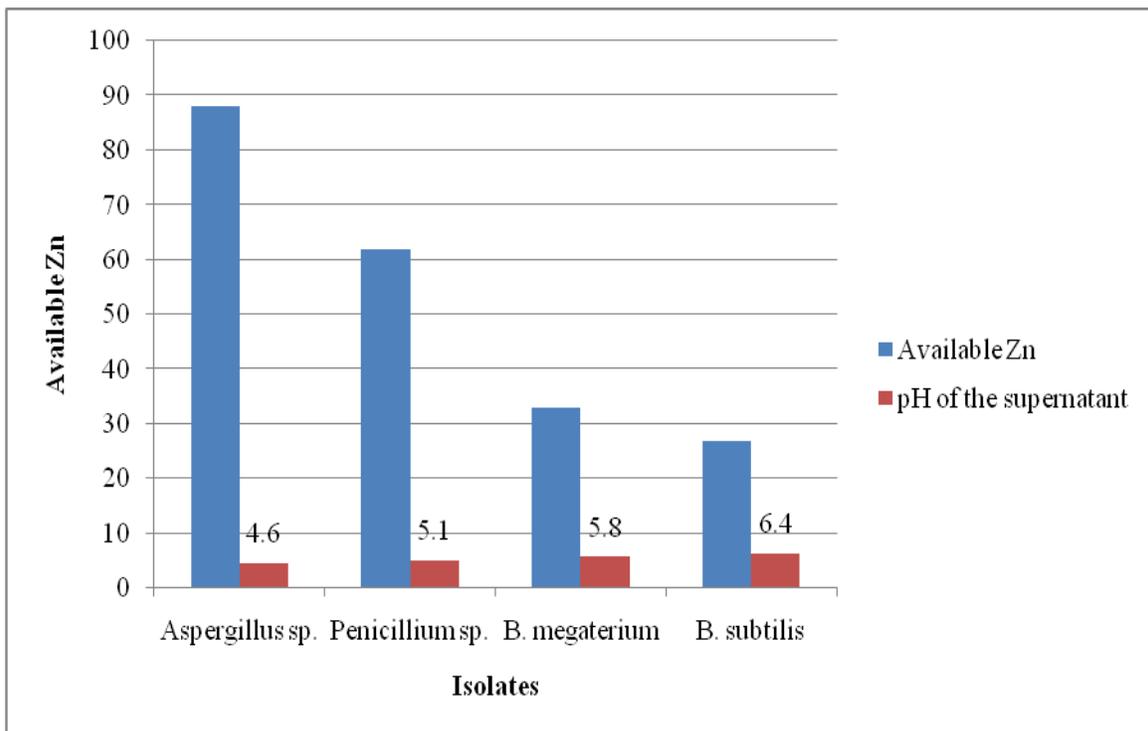
Table.4 Influence of different inoculants and nutrient levels on N and P contents (%) in maize after harvest

Nutrient \ Isolate	RDF	N			P			Average
		ZnO (12.5 kg/ha)	ZnO (25 kg/ha)	Average	RDF	ZnO (12.5 kg/ha)	ZnO (25 kg/ha)	
No inoculation	1.27	1.43	1.43	1.37	0.307	0.321	0.319	0.315
<i>B. megaterium</i>	1.98	2.06	2.07	2.03	0.427	0.431	0.432	0.429
<i>B. subtilis</i>	1.93	2.04	2.05	2.00	0.424	0.424	0.416	0.425
<i>Aspergillus</i> sp.	2.04	2.35	2.69	2.36	0.426	0.435	0.437	0.432
<i>Penicillium</i> sp.	1.99	2.19	2.37	2.18	0.435	0.432	0.435	0.431
Average	1.84	2.02	2.12		0.404	0.409	0.408	
	Nutrient		Isolate	Nutrient X Isolate	Nutrient	Isolate		Nutrient X Isolate
S.Em±	0.02		0.02	0.05	0.002	0.002		0.004
CD at 5%	0.06		0.06	0.13	0.007	0.007		0.012

Table.5 Influence of different inoculants and nutrient levels on K and Zn contents (% and ppm) in maize after harvest

Isolate \ Nutrient	RDF	ZnO		Average	RDF	ZnO		Average
		(12.5 kg/ha)	(25 kg/ha)			(12.5 kg/ha)	(25 kg/ha)	
	K				Zn			
No inoculation	1.25	1.23	1.24	1.24	14.88	24.95	24.46	21.43
<i>B. megaterium</i>	1.35	1.54	1.55	1.48	19.62	26.10	26.02	23.91
<i>B. subtilis</i>	1.35	1.51	1.53	1.46	20.38	24.61	26.04	23.72
<i>Aspergillus</i> sp.	1.45	1.55	1.62	1.54	23.43	27.24	26.72	25.79
<i>Penicillium</i> sp.	1.43	1.54	1.57	1.50	22.10	24.76	26.19	24.30
Average	1.36	1.48	1.50		20.08	25.53	25.89	
	Nutrient		Isolate	Nutrient X Isolate	Nutrient	Isolate		Nutrient X Isolate
S.Em±	0.01		0.01	0.04	0.34	0.34		0.76
CD at 5%	0.02		0.02	0.07	0.97	0.97		2.16

Fig.1 Available zinc (ppm) released by bacteria in broth medium containing zinc oxide



P content

Among the different levels of nutrient applied ZnO @ 25 kg/ha shows maximum P content (0.409%) in maize. This was followed by ZnO @ 12.5 kg/ha application (0.408%). Inoculation of different strains of Zn solubilizers had a profound increase in P% content over uninoculated treatments by 36.2 to 37.1 per cent (Table 4). Maximum average P content was found by inoculation with *Aspergillus* (0.432%). The bacterial inoculants *B. megaterium* and *B. subtilis* performed better than no inoculation in P content by 34 to 36 per cent. Interaction among nutrient levels and inoculants showed a positive response on P content in maize. Maximum P content was found between *Aspergillus* + ZnO @ 25 kg/ha (0.437%) whereas, *Penicillium* + ZnO @ 25 kg/ha and *B. megaterium* + ZnO @ 25 kg/ha performed suitably well.

K content

Influence of different nutrient levels had significant effect on K content in maize being maximum with 10.2% increase with application of ZnO @ 25 kg/ha over RDF (Table 5). It was closely followed by application of ZnO @ 12.5 kg/ha with significant increase of 8.8% over RDF. Influence of incorporation of inoculants also provided a good K content in maize. Maximum K content was observed by inoculation of *Aspergillus* sp. with an increase of 24.1% over uninoculated control. Inoculation of *Penicillium* and *B. megaterium* contributed 1.50 and 1.48 per cent K content which was 20.9% and 19.3% more over no inoculation. Interaction effect of nutrient levels and inoculants was found to be significant over their respective controls. Profound effect was observed by interaction of *Aspergillus* sp. + ZnO @ 25 kg/ha with an increase of 29.6% over RDF followed by

Penicillium sp. + ZnO @ 25kg/ha and *B. megaterium* sp. + ZnO @ 25 kg/ha with an increase of 25.6 and 24 per cent, respectively over RDF.

Zn content (ppm)

Effect of varying nutrient levels showed significant results of Zn content over RDF being maximum increase of 29.0% with an application of ZnO @ 25kg/ha followed by application of ZnO @ 12.5 kg/ha with 27.1% increase over RDF (Table 5). Incorporation of microbial inoculants significantly improved the Zn content in the maize plant compared with uninoculated control. Inoculation of *Aspergillus* sp. showed significantly greatest impact on Zn content by 20.3% over no inoculation followed by *Penicillium* sp. with increase of 13.3 per cent over RDF. Comparable results were obtained on inoculation with both bacterial inoculants. In general, significantly more Zn content was observed with inoculants at both level of ZnO. Significant interaction effects between *Aspergillus* sp. + ZnO @ 25 kg/ha showed maximum Zn content in maize by 83% over RDF followed by inoculation of *Penicillium* sp. with the same with 76% increase over RDF.

The present study indicated that microbial inoculation of maize with *Zn solubilizers* significantly enhanced the N, K and P content in maize plants. This enhanced uptake of these major nutrients when compared to uninoculated plants could be explained on the basis that the unavailable forms of these nutrients were solubilized and made available near the root region of soil by applying these plant growth promoting isolates. Plants inoculated with these nutrient solubilizing microbes usually had more nitrogen content than that of uninoculated plants (Punte *et al.*, 2004). This is further reinforced by experiments conducted by Murty and Ladha

(1988) who revealed that *Azospirillum* inoculation increased ammonium and phosphate uptake in rice. Even K^+ concentration was more in treatments other than control. Results showed that all fungal treated plants showed significant improvement in their Zn content over bacterial and control ones in maize. The enhancement of macro and Zn uptake by plants by inoculation with these isolates may be due to their effect on growth and development of lateral roots (Rolfe *et al.*, 1997), increased root volume and weight, and nutrient uptake (Canbolat *et al.*, 2006). Studies done by Goldstein and Liu, 1997 demonstrated that phosphate and potash solubilizing bacteria may enhance mineral uptake in plants. This was confirmed in the study due to higher percentage of macro nutrients content evaluated in maize was significantly and/or relatively increased in inoculated plants. On observing the both Zn levels of treatments it can be remarked that higher dosage ZnO recorded more Zn content in plants but it was less compared to the interaction effect of it with various isolates. This may be due to the fact that presence of readily available Zn source in soil itself is not sufficient for uptake, but also the mobility of mineral element itself is required. This difficulty was overcome on inoculation of an isolate which helped in its migration to plant roots and hence, in its increased uptake. On the basis of the performance of these four isolates with different dosage of nutrient sources it was confirmed that *Aspergillus* sp. was the best in terms of Zn solubilization and its uptake in plants. Also the fungal species performed comparatively better than the bacteria ones which can be correlated to more acid production. Kumawat *et al.*, (2009) reported that application of vermicompost at 2t/ha, seed inoculation with PSB and 40 kg P_2O_5 /ha significantly increased the N, P and K concentration in seed, straw and their total uptake in mung bean. Kumar *et al.*, (2013)

reported increased N, P, K content and uptake in mung bean due to PSB inoculation with SSP over uninoculated control. The enhancement in nutrient content and uptake by inoculation with insoluble sources may be due to the production of low molecular weight, organic acid and subsequent release of Zn from insoluble compounds by reducing sorption of Zn by altering the surface charge of soil colloids Jones (1998). It may also be due to the fact that initiation of development of lateral roots and increased root weight Rolfe *et al.*, (1997), Canbolat *et al.*, (2006). Increased Zn content and uptake by plants due to incorporation of inoculants at various P sources were also reported by Whiting *et al.*, (2001), Tariq *et al.*, (2007) in wheat, Joshi *et al.*, (2013) in wheat, Goteti *et al.*, (2013) in maize and Ramesh *et al.*, (2014) in soybean and wheat.

In our study with Zn solubilizing isolates and its effect on inoculation upon maize for plant growth promoting activities it revealed that inoculation with such beneficial microorganisms is an efficient method for enhancing growth of maize and help in its nourishment over no inoculation. *Aspergillus* sp. could be effectively used as bio input for improving the plant growth and yield. Moreover all these four isolates can be used as a substitute and/or with integration to chemical fertilizers to correct the nutrient deficiencies in crops depending on situation for increased productivity and better plant nutrition in a sustainable manner.

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