

Research Article

POTASSIUM AND ZINC SOLUBILIZING EFFICIENCY ASSESSMENT OF MICROORGANISMS FROM DIFFERENT RHIZOSPHERE SOILS

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Abstract- The amount of K released from potassium alumino silicate in a broth by the KSB isolates at 7, 15, 21 days after incubation (DAI) were increased with increase in incubation time and was maximum at 20 DAI (6.5 to 73.4 (µg/ml). Among the isolates KSB - 2 released maximum amount of K from potassium alumino silicate (73.4 (µg/ml). The KSB-2 significantly produced higher bacterial count (47.0 x 10 8 cfu ml⁻¹). The amount of polysaccharide production was observed on glucose minimal agar medium and visually scored. Compared to all, KSB-2 isolate was best performer (+++) followed by KSB-4 was a moderate producer. The amount of Zn available in broth at 4th, 8th and 16th day of incubation in ZnO and Zinc phosphate supplemented growth medium increased with increase in incubation time and was maximum at 16 DAI which was 3.98 to 36.62 µg/ml for ZnO wih pH change of 7.12 to 3.96 and 3.20 to 32.25 µg/ml for Zinc phosphate with pH change 7.0 to 4.9. The zinc solubilizing bacterium ZnSB-2 (36.62 µg/ml) showed the maximum value of available zinc in broth at 4th, 8th and 16th day of incubation in ZnO supplemented growth medium. The zinc solubilizing bacterium ZnSB-8 (32.25 µg/ml) showed the maximum value of available zinc in broth at 4th, 8th and 16th day of incubation in ZnO supplemented growth medium increase in incubation time and was maximum at 16 DAI which was 6.32 to 20.28 µg/ml for ZnO with pH change of 6.60 to 4.30. The zinc solubilizing fungi ZnSF-1 (20.28 µg/ml) showed the maximum value of available zinc in broth at 4th, 8th and 16th day of incubation in ZnO supplemented growth medium. ZnSF-1 significantly produced higher count (123 x 10 6 cfu ml -1) followed by ZnSF-2 (119.0 x 10 6 cfu ml -1). The bacterial isolate KSB-2 solubilized more potassium, ZnSF-8 solubilized more zinc phosphate and ZnSF-1 solubilized more zinc oxide in broth assay. The solubilization of insoluble source in liquid culture broth increased with increase in incubation time. The isolates produced exopolysaccharide and organic acids for

Key words- Potassium and zinc solubilizing bacteria, potassium alumino silicate.

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Introduction

Plant system required the different type of the necessary mineral nutrients for the growth and development of the plant. There are several essential elements required for various physiological processes that involve in plant growth and some of the elements are directly involved in plant metabolism. Plant requires specific elements for growth and for reproduction. Nitrogen, Phosphorus, Potassium (K) are mobile nutrients, while the others have varying degree of mobility. Some substrates are insoluble in the soil which is not available to the plant system. Microbes plant interaction play a very important role in the cycling of the minerals. Microbes produced the different type of the acid which helps in the release of the nutrient from the insoluble substrate. Soil has rich reserves of K, among which only 1-2 % can be directly absorbed by plants. About 90-98 % of the soil K exists in silicate minerals such as K-feldspar, mica, illite. Studies have shown that a variety of soil microbes can release soluble K from K-bearing minerals such as Kfeldspar, mica, and illite. These microbes release organic acids which guickly dissolve rock and chelate silicon ions, releasing K ions into the soil [1]. It has been shown that Bacillus mucilaginosus and Bacillus edaphicus can generate

polysaccharide and carboxylic acids, such as tartaric acid and citric acid, to solubilize K compounds. Microorganisms play a key role in potassium (K) cycle. Some rhizobacteria are K solubilizing. Potassium solubilization by microbes enhances crop growth and yield. Heavy application of chemical fertilizers is one of the major causes of environmental pollution. Potassium solubilizing microbes are helpful to reduce these pollutions. About 96 to 99% of the exogenously applied Zn is converted into different insoluble forms depending upon the soil types, physicochemical reactions within 7 days of application [2]. The solubility of Zn is highly dependent upon soil pH and moisture and hence, arid and semi-arid areas of Indian agro-ecosystems are often zinc-deficient. Zinc content is present in various fixed forms such as hopeite (Zn₃(PO₄) 2.4H₂O), zincite (ZnO), wellemite (Zn₂SiO₄), franklinite (ZnFe₂O₄) and smithsonite (ZnCO₃). These fixed forms of Zn are sparingly soluble. Indian soil is deficient in available zinc content. Fixation of Zn in soils with pH >7.0 increases with increasing concentration of carbonates, thus become unavailable and can be reverted back to available form with Zn solubilizing microorganisms [3]. Zinc-solubilizing microorganisms can solubilize zinc from inorganic and organic pools of total soil zinc and can be utilized to

increase zinc availability to plants. *In vitro* and *in vivo* studies of fungi related to solubilization of insoluble form of zinc are also available. Many bacterial species of different genera are reported for zinc solubilization consists *Pseudomonas, Bacillus, Acinetobacter and Gluconacetobacter* [4]. Bacteria are known to immobilize metal by precipitation and adsorption. Dissolution ability of immobilized zinc to zinc carbonate, zinc phosphate and zinc oxide in adequate quantity is not common amongst the cultivable bacteria. *Pseudomonas Acinetobacter, Gluconacetobacter, Bacillus Thiobacillus thioxidans, Thiobacillus ferroxidans* and facultative thermophilic iron oxidizers have been identified for their ability to solubilize zinc [5].

Materials and Methods

The present investigation was carried out at the Department of Agricultural Microbiology & Bioenergy, College of Agriculture, Rajendranagar, Hyderabad for isolation, characterization of potassium and zinc solubilizing bacteria from different rhizospheric soil. The general laboratory techniques followed in the present study were those described by many researchers for preparation of media, sterilization, isolation and maintenance of bacterial cultures, with slight modifications wherever necessary [6-8].

Glassware

Petriplates, test tubes, microscopic slides, conical flasks of different capacities *i.e.*, 1000, 500, 250 ml, pipettes of 1.0, 2.2, 5.0, 10.0 ml beakers and measuring cylinders of 50, 100, 500 and 1000 ml, micropipettes of 0.5-10, 10-100, 100-1000 µl were used. All the glassware used was of Borosil made.

Cleaning of glassware

Glassware was first washed with a detergent, then cleaned with tap water and finally placed in the chromic acid solution prepared with following composition:

Potassium dichromate	:	60 g
Conc. H ₂ SO ₄	:	60 ml
Distilled water	:	1000 ml

The glassware was kept in the cleaning solution for 24 h and then thoroughly washed with running tap water before its final cleaning with distilled water and dried.

Sterilization of glassware

Glassware was wrapped in butter paper and sterilized in hot air oven at 160°C for 1 h before use. Media, distilled water, etc., were sterilized in an autoclave at 15 lb and 121°C for 20 min.

Precaution to avoid contamination

The inoculation works of microbial cultures were carried out under laminar air flow chamber. The laminar bench and air flow was disinfected by U.V lamp prior to commencement of work.

Equipment and Apparatus used

Hot air oven and autoclaves were used for sterilization of heat stable glassware and media respectively. BOD incubators were used for incubating cultures at different temperatures. Cultures were stored and maintained in a refrigerator. The pH was measured by using digital pH meter. Cyclomixer was used for homogenization during serial dilution. Plate mixer was used for spread plate technique. Centrifuge was used for making cell-free cultures. Hi-media zonal scale was used to measure zinc, potassium solubilization. Atomic Absorption Spectrophotometer (AAS) and flame photometer were used for detection of solubilization and release of potassium and zinc in liquid culture broths. Samples were weighed using a single pan electric balance. Compound electron microscope was used to observe the morphology of bacterial cultures.

Chemicals used

The chemicals used in the present investigation were of analytical and laboratory grades. The pH of the media was adjusted to the required level using 10 N NaOH and 10 N HCI. Formaldehyde 10 % solution was used to fumigate the Laminar air

flow chamber and biological oxygen demand (BOD) incubators for disinfection.

To assess the cultures with different potassium and zinc solubilizing capacity

Spectrophotometric\ Flame Photometer method for quantification of potassium solubilized by bacterial culture

A loopful of 48 h old grown bacterial cultures was inoculated into 25 ml Aleksandrov broth in 50 ml capacity flask containing insoluble potassium source. All the inoculated flasks were incubated at $28 \pm 2^{\circ}$ C for 7-21 days. The growth suspension was centrifuged at 7,000 rpm for 10 minutes in the centrifuge to separate the supernatant from the cell growth and insoluble potassium. One ml of the supernatant was taken in a 50 ml volumetric flask and the volume was made to 50 ml with distilled water and mixed thoroughly. The solution was fed to atomic absorption spectrometer to determine K content. Standard curve was prepared using various concentrations of 10 ppm KCl solution i.e., 0.5, 1.0 and 1.5 ppm. The amounts of potassium solubilized by the bacterial isolates were calculated from the standard curve.

Polysaccharide production by K solubilizing bacteria

All the efficient K solubilizing bacteria were tested for polysaccharide production by spotting 10 μ l of overnight culture on glucose minimal agar medium [9]. The plates were incubated at 28 ± 2°C for 24 to 48 h. The amount of polysaccharide produced on glucose minimal agar medium was observed visually and scored as no polysaccharide production (-), weak polysaccharide production (+), moderate polysaccharide production (++) and high polysaccharide production (+++) by the strains.

Qualitative assay for zinc solubilization

Zinc solubilizing ability of bacterial isolates were evaluated using zinc oxide, zinc phosphate and zinc sulphide in both plate and broth media assays.

Broth assay

Incubation of bacterial isolates was done separately in basal medium supplemented with 0.1% insoluble zinc compounds. For zinc solubilization assay laboratory grade ZnO, Zn₃(PO₄)₂ and ZnS (Sphalerite ore material) were used. Basal medium was prepared, splite into 50 ml aliquots in 100 ml erlenmeyer flasks and 0.1% of these chemicals were added, steam sterilized for 30 minutes in an autoclave for 3 consecutive days. The flasks were inoculated with 1 ml suspension of the test culture with a cell load of 10^7 cells ml⁻¹. Three flasks were maintained with an uninoculated control for each treatment. Experiments were done in triplicate. The samples were taken at 4, 8 and 16 day intervals after incubation, centrifuged to remove the debris and cells. Ten ml of the supernetant fed to Atomic Absorption Spectrophotometer (AAS) to determine the available zinc content.

Result and Discussion

Cultures with different potassium and zinc solubilizing capacity Quantification of potassium solubilization in broth by bacterial culture

The amount of K released from potassium alumino silicate in a broth by the KSB isolates were determined at 7, 15, 21 days after incubation (DAI) and the amount of K released increased with increase in incubation time by all strains. The significantly maximum release of potassium was observed at 20 DAI (6.5 to 73.4 (µg/ml). Among the isolates KSB - 2 released maximum amount of K from potassium alumino silicate (73.4 (µg/ml) followed by isolate KSB - 3 (31.6 (µg/ml) [Table-1]. Later the bacterial counts were taken, the KSB-2 significantly produced higher count (47.0 x 10⁶ cfu ml⁻¹) followed by KSB -1 (42.0 x 10⁶ cfu ml⁻¹) [Table-2]. The bacterial culture released the potassium from insoluble source due to production of organic acids, ESP and organic ligands in the presence of K rich minerals. The KSB-2 produced higher amount of exo-polysaccharide and released maximum amount of K. Similar results observed with Brindavathy and Gopalaswamy (2014) was quantitatively analyzed K dissolution rate of 14 bacterial isolates and concluded that the amount of K released from mineral K by all strains increased with increase in incubation time and was maximum at 21 DAI due to

International Journal of Agriculture Sciences ISSN: 0975-3710&E-ISSN: 0975-9107, Volume 10, Issue 6, 2018 production of exo-polysaccharide.

Table-1 Solubilization of potassium alumina silicate (0.2%) by the potassium	
solubilizing bacterial (KSB) isolates on release of potassium	

Isolates	Potas	sium release	(ua/ml)
			(PS) III/
	7 DAI	15 DAI	20 DAI
Control	6.0	10.4	12.3
KSB-1	8.0	12.8	28.9
KSB-2	26.0	42.5	73.4
KSB-3	12.0	21.2	31.6
KSB-4	6.50	11.3	26.8
C.D.	0.676	0.742	0.777
SE(m)	0.212	0.232	0.244
SE(d)	0.299	0.329	0.344
C.V.	3.161	2.049	1.219
	KSB-1 KSB-2 KSB-3 KSB-4 C.D. SE(m) SE(d)	Control 6.0 KSB-1 8.0 KSB-2 26.0 KSB-3 12.0 KSB-4 6.50 C.D. 0.676 SE(m) 0.212 SE(d) 0.299	Control 6.0 10.4 KSB-1 8.0 12.8 KSB-2 26.0 42.5 KSB-3 12.0 21.2 KSB-4 6.50 11.3 C.D. 0.676 0.742 SE(m) 0.212 0.232 SE(d) 0.299 0.329

KSB - Potassium Solubilizing Bacteria, DAI – Days after Incubation , SE – Standard Error, C.V. – Coefficient of Variance

Similarly, Archana *et al.* (2012) isolated and evaluated thirty rhizobacterial isolates genera *Bacillus* and *Pseudomonas* for their plant growth promotional activity. The amount of potassium released into the liquid medium by potassium solubilizing isolates ranged from 2.41 to 44.49µg/ml. Among the isolates, KSB 33 released maximum amount of K (44.49 µg/ml). Most of the isolates were capable of producing plant growth promoting substances, and the amounts of IAA and GA produced by them ranged from 1.10 to 16.50 and 0.60 to 3.29 µg/25 ml broths respectively.

Polysaccharide production by K solubilizing bacteria

All the efficient K solubilizing bacteria were tested for polysaccharide production by spotting 10 µl of overnight culture on glucose minimal agar medium (Sambrook et al., 1989). The plates were incubated at 28 ± 2°C for 24 to 48 h. The amount of polysaccharide produced on glucose minimal agar medium was observed visually and scored as no polysaccharide production (-), weak polysaccharide production (+) by KSB - 4, moderate polysaccharide production (++) by KSB - 1, KSB - 3 and high polysaccharide production (+++) by KSB- 2 the strains [Table-2]. The isolate KSB-2 produce high polysaccharide production (+++) resulting more solubilization of insoluble potassium source. Efficient K solubilizing bacteria were tested for polysaccharide production by spotting 10 µl of overnight culture on glucose minimal agar medium [10]. The results obtained showed wide variability in production of polysaccharide by K solubilizing bacteria. Bacillus sp. Strain KSB 11 and KSB 42 produced higher amount of polysaccharides followed by KSB14, KSB16, KSB18, KSB33, KSB35 and KSB47.The results agree with studied of polysaccharide production by potassium bio-dissoluting isolates and found the the isolates were good producer of exopolysaccharide [11].

Table-2 Bacterial count of KSB isolates in broth culture (10 ⁶ cfu ml ⁻¹)								
Isolates	Polysaccharide							
	0 hour	7 th days	15 th days	20th days	production			
Control	48.0	20.0	10.0	6.0				
KSB-1	42.0	12.0	7.0	3.0	++			
KSB-2	+++							
KSB-3	25.0	10.0	5.0	3.0	++			
KSB-4	36.0	11.0	6.0	2.0	+			
K	ny forming unit							

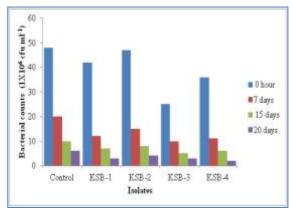
Study the quantitative assay for zinc solubilization

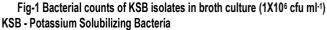
Zinc solubilizing ability of bacterial isolates was evaluated using zinc oxide, zinc phosphate and zinc sulphide in broth assays. The amount of Zn available in broth were determined at 4th, 8th and 16th day of incubation in ZnO and ZnP supplemented growth medium increased with increase in incubation time and was maximum at 16 DAI which is 3.98 to 36.62 µg/ml for ZnO with pH change of 7.12 to 3.96 and 3.20 to 32.25 µg/ml for ZnP with pH change 7.0 to 4.9. The zinc solubilizing bacterium ZnSB-2 (36.62 µg/ml) showed the maximum value of available zinc in broth at 4th, 8th and 16th day of incubation in ZnO supplemented growth medium followed by ZnSB-7 (32.46 µg/ml). The isolate ZnSB-8 (32.25

 μ g/ml) showed the maximum value of available zinc in broth at 4th [Fig-2], 8th [Fig-3] and 16th [Fig-4] day of incubation in ZnP supplemented growth medium followed by ZnSB-2 (30.26 μ g/ml) [Table-4, 5 & 6]. ZnSB - 5 significantly produced higher bacterial count (255 x 10⁶ cfu ml⁻¹) followed by ZnSB-2 (245.0 x 10⁶ cfu ml⁻¹) [Table-6] & [Fig-5].

The amount of Zn available in broth for ZnSF were determined at 4th, 8th and 16th day of incubation in ZnO supplemented growth medium increased with increase in incubation time and was maximum at 16 DAI which is 6.32 to 20.28 µg/ml for ZnO with pH change of 6.8 to 4.30. The zinc solubilizing fungi ZnSF-1 (20.28) µg/ml) showed the maximum value of available zinc in broth at 4th, 8th and 16th day of incubation in ZnO supplemented growth medium followed by ZnSF-2 (18.68 µg/ml). [Table-3, 4 & 5]. The ZnSF-1 significantly recorded higher bacterial count (123 x 10⁶ cfu ml⁻¹) followed by ZnSF-2 (119.0 x 10⁶ cfu ml⁻¹) [Table-6] & [Fig-6]. The variation in the ability of solubilizing given zinc sources could be due to metabolic activity of a given strain which is in agreement with observations of different research. There are different mechanisms of solubilization which have been identified including proton excretion, production of organic acids and other chelating metabolites. Organic acid production by microbial strains has been reported to be a major mechanism of solubilization [12]. The zinc solubilization in our studies could be due to production of organic acids, like gluconic acids that is augmented by the fall in pH of culture media noted in all cases.

Thus, the solubilization potential varies among different cultures. The solubilization might be due to production of acids by the culture, since the pH of the culture broth has been shifted for ZnO form 7.12 to 3.96 and for ZnP form 7.0 to 4.9 after 16 days of inoculation. Studies were assayed 15 selected bacterial isolates for determining their zinc solubilizing capacity in broth culture *in vitro*. They found significantly increased the availability of zinc in tris mineral salt liquid medium supplemented with zinc oxide and zinc carbonate at 16 days of incubation.





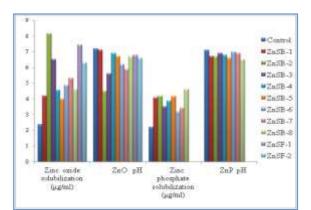


Fig-2 Soluble zinc content in Tris-minimal liquid broth supplemented with zinc oxide and zinc phosphate 0.1% after 4 days of incubation ZnSF – Zinc Solubilizing Fungi, ZnSB – Zinc Solubilizing Bacteria, ZnP – Zinc Phosphate

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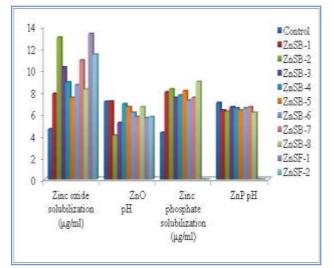


Fig-3 Soluble zinc content in Tris-minimal liquid broth supplemented with zinc oxide and zinc phosphate (0.1%) after 8 days of incubation

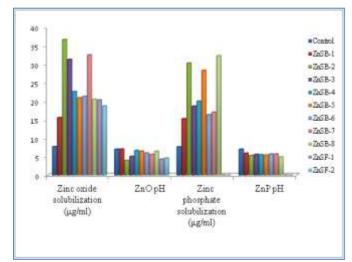


Fig-4 Soluble zinc content in Tris-minimal liquid broth supplemented with zinc oxide and zinc phosphate (0.1%) after 16 days of incubation ZnSF – Zinc Solubilizing Fungi, ZnSB – Zinc Solubilizing Bacteria, ZnP – Zinc Phosphate

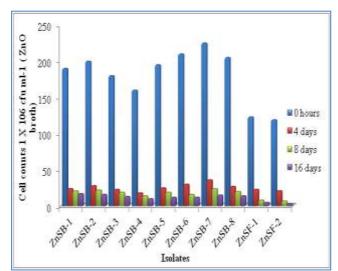


Fig-5 Cell count of isolates in culture broth 1 X 10⁶ cfu ml⁻¹ (ZnO 0.10% broth) ZnSF – Zinc Solubilizing Fungi, ZnSB – Zinc Solubilizing Bacteria

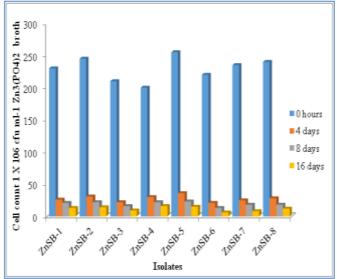


Fig-6 Cell count of ZnSB isolates in culture broth 1 X $10^6\,cfu\ ml^{-1}\ Zn_3(PO4)_2$ 0.10% broth

ZnSB – Zinc Solubilizing Bacteria

Table-3 Solublization of zinc in Tris-minimal liquid broth supplemented with Zinc
oxide (ZnO 0.1%) and Zinc phosphate Zn ₃ (PO ₄) ₂ 0.1% after 4 days of incubation

	Zincs	olubilizat	ion (4 days) (µg/ml)	,
Isolate	Zinc oxide	рН	Zinc phosphate	рН
Control	2.36	7.20	2.21	7.10
ZnSB-1	4.20	7.10	4.08	6.70
ZnSB-2	8.16	4.50	4.20	6.70
ZnSB-3	6.50	5.60	3.52	6.90
ZnSB-4	4.56	6.90	3.88	6.80
ZnSB-5	3.98	6.70	4.16	6.60
ZnSB-6	4.86	6.20	3.20	7.00
ZnSB-7	5.32	5.90	3.42	6.90
ZnSB-8	4.62	6.70	4.62	6.50
ZnSF-1	7.46	6.80	-	
ZnSF-2	6.32	6.60	-	
C.D.	0.120		0.087	
SE(m)	0.040		0.029	
SE(d)	0.057		0.041	
C.V.	1.406		1.355	
ZnSB – Zinc – Standard	: Solubilizing B Error	acteria	C.D Crit C.V. – Coef	

Table-4 Solublization of zinc in Tris-minimal liquid broth supplemented with Zinc oxide (ZnO 0.1%) and Zinc phosphate Zn₃(PO₄)₂ 0.1% after 8 days of incubation.

	Zinc solubilization (4 days) (µg/ml)					
Isolate	Zinc oxide	рН	Zinc phosphate	рН		
Control	2.36	7.20	2.21	7.10		
ZnSB-1	4.20	7.10	4.08	6.70		
ZnSB-2	8.16	4.50	4.20	6.70		
ZnSB-3	6.50	5.60	3.52	6.90		
ZnSB-4	4.56	6.90	3.88	6.80		
ZnSB-5	3.98	6.70	4.16	6.60		
ZnSB-6	4.86	6.20	3.20	7.00		
ZnSB-7	5.32	5.90	3.42	6.90		
ZnSB-8	4.62	6.70	4.62	6.50		
ZnSF-1	7.46	6.80	-			
ZnSF-2	6.32	6.60	-			
C.D.	0.120		0.087			
SE(m)	0.040		0.029			
SE(d)	0.057		0.041			
C.V.	1.406		1.355			
ZnSB – Z	Zinc Solubilizing	C.D- Critical difference				
SE – Standa	rd Error		C.V – Coefficient of Variance			

International Journal of Agriculture Sciences ISSN: 0975-3710&E-ISSN: 0975-9107, Volume 10, Issue 6, 2018 Table-5 Solublization of zinc in Tris-minimal liquid broth supplemented with Zinc oxide (ZnO 0.1%) and Zinc phosphate Zn₃(PO₄)₂ 0.1% after 16 days of incubation

		Zinc solubiliz	zation (16 days) (μg/ml)	
Isolate	Zinc oxide	рН	Zinc phosphate	pH
Control	7.70	7.00	7.64	7.00
ZnSB-1	15.52	7.02	15.23	5.90
ZnSB-2	36.62	3.96	30.26	5.30
ZnSB-3	31.24	5.04	18.63	5.60
ZnSB-4	22.56	6.66	19.96	5.50
ZnSB-5	20.88	6.46	28.30	5.40
ZnSB-6	21.25	5.96	16.32	5.70
ZnSB-7	32.46	5.60	16.98	5.70
ZnSB-8	20.46	6.42	32.25	4.90
ZnSF-1	20.28	4.30	-	
ZnSF-2	18.68	4.60	-	
C.D.	0.146		0.087	
SE(m)	0.049		0.029	
SE(d)	0.069		0.041	
C.V.	0.363		0.244	
	ZnSB – Zinc Solubiliz	zing Bacteria	C.D - Critical difference	
	SE – Standard Error		C.V – Coefficient of Variance	

Table-6 Cell count of isolates in culture broth 1 X 10⁶ cfu ml⁻¹ (ZnO/Zn₃(PO₄)₂ 0.10%)

ZnO 0 hours 190.0 200.0 180.0 160.0 195.0 210 225.0 205.0 123.0 1 4 days 24.0 28.0 23.0 18.0 25.0 30 36.0 27.0 23.0 23.0 23.0 14.0 19.0 16 24.0 20.0 8.0	Cell count 1 X 10 ^s cfu ml ⁻¹ (ZnO/ Zn ₃ (PO ₄) ₂ ,0.10%)											
4 days 24.0 28.0 23.0 18.0 25.0 30 36.0 27.0 23.0 23.0 8 days 21.0 22.0 19.0 14.0 19.0 16 24.0 20.0 8.0 16 days 17.0 16.0 13.0 10.0 12.0 12 15.0 14.0 5.0 Zn ₃ (PO ₄₎₂ 0 hours 23.0.0 245.0 210.0 200.0 255.0 220 235.0 240.0 - <th>Sources</th> <th>Time</th> <th>ZnSB-1</th> <th>ZnSB-2</th> <th>ZnSB-3</th> <th>ZnSB-4</th> <th>ZnSB-5</th> <th>ZnSB-6</th> <th>ZnSB-7</th> <th>ZnSB-8</th> <th>ZnSF-1</th> <th>ZnSF-2</th>	Sources	Time	ZnSB-1	ZnSB-2	ZnSB-3	ZnSB-4	ZnSB-5	ZnSB-6	ZnSB-7	ZnSB-8	ZnSF-1	ZnSF-2
8 days 21.0 22.0 19.0 14.0 19.0 16 24.0 20.0 8.0 16 days 17.0 16.0 13.0 10.0 12.0 12 15.0 14.0 5.0 Zn ₃ (PO ₄) ₂ 0 hours 230.0 245.0 210.0 200.0 255.0 220 235.0 240.0 -	ZnO	0 hours	190.0	200.0	180.0	160.0	195.0	210	225.0	205.0	123.0	119.0
16 days 17.0 16.0 13.0 10.0 12.0 12 15.0 14.0 5.0 Zn ₃ (PO ₄) ₂ 0 hours 230.0 245.0 210.0 200.0 255.0 220 235.0 240.0 -		4 days	24.0	28.0	23.0	18.0	25.0	30	36.0	27.0	23.0	21.0
Zn ₃ (PO ₄₎₂ 0 hours 230.0 245.0 210.0 200.0 255.0 220 235.0 240.0 -		8 days	21.0	22.0	19.0	14.0	19.0	16	24.0	20.0	8.0	7.0
· · · · · · · · · · · · · · · · · · ·		16 days	17.0	16.0	13.0	10.0	12.0	12	15.0	14.0	5.0	3.0
4 days 26.0 31.0 22.0 30.0 36.0 21 25.0 28.0 -	Zn ₃ (PO ₄) ₂	0 hours	230.0	245.0	210.0	200.0	255.0	220	235.0	240.0	-	-
		4 days	26.0	31.0	22.0	30.0	36.0	21	25.0	28.0	-	-
8 days 21.0 22.0 16.0 22.0 23.0 13 18.0 -		8 days	21.0	22.0	16.0	22.0	23.0	13	18.0	18.0	-	-
16 days 13.0 14.0 9.0 16.0 15.0 6 8.0 12.0 -		16 days	13.0	14.0	9.0	16.0	15.0	6	8.0	12.0	-	-

ZnSB -Zinc Solubilizing Bacteria

Conclusion

Zinc and potassium are considered as the important minerals nutrients for the growth and development of the plant. These nutrients are present in the insoluble form which is not available to the plant. In present investigation four potassium solubilizing, eight zinc solubilizing bacteria and two zinc solubilizing fungal were tested for their solubilizing efficiency. The significant k solubilizing was shown by the KSB-2 at the 20 DAI. KSB produced highest bacterial count. ZnSB-8 produced and released the highest amount of the available zinc at 4th, 8th and 16th day of incubation in Zinc phosphate supplemented growth medium and ZnSF-1 sowed the maximum amount of released zinc. The present study encourages the authors to use the isolates at the field level.

Application of research: The present study has the scope of using the zinc and potassium solubilizing microorganism at the field level. The best isolates can be used to make the formulations and can be apply in the zinc and potassium deficient field.

Research category- Agriculture, Microbiology

Abbreviations-

DAI- Days after incubation KSB- Potassium Solubilizing Bacteria ZnSB- Zinc Solubilizing Bacteria ZnSF- Zinc Solubilizing Fungus ZnO- Zinc Oxide AAS- Atomic Absorption Spectrophotometer BOD- Biological Oxygen Demand cfu- Colony Forming Unit

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References

- [1] Friedrich S., Platonova N.P., Karavaiko G.I., Stichel E. and Glombitza F. (1991) *Engineering in Life Sciences*, 11(3), 187-196.
- [2] Venkatakrishnan S.S., Sudalayandy R.S. and Savariappan A.R. (2003) *Brazilian Journal of Microbiology*, 34, 121-125.
- [3] Shahab S. and Ahmed N. (2008) African Journal of Biotechnology, 7(10), 1543-1549.
- [4] Gadd G. M. (2007) Mycological Research, 111, 3-49.
- [5] Saravanan V.S., Madhaiyan M. and Thangaraju M. (2007) Chemosphere, 66, 1794–1798.
- [6] Cappuccino J.G. and Sherman N. (1992) The Benjamin/Comings Publishing Company, Inc. California.
- [7] Nene and Thapliyal (1993) Fungicides in Plant Disease Control, Oxford and IBH Publishing House, New Delhi. 163.

- [8] Aneja K.R. (2001) Experiments in Microbiology, Plant Pathology and Tissue culture. Viswaprakasham, New Delhi, 471.
- [9] Sambrook J., Fritsch E.F. and Maniatis T. (1989) "Molecular cloning", Cold spring Harbor laboratory press, Cold Spring Harbor, NewYork.
- [10] Archana D.S., Nandish M.S., Savalagi V.P. and Alagawadi A.R. (2013) Bioinfolet-A Quarterly Journal of Life Sciences, 10(1b), pp.248-257.
- [11] Brindavathy R. and Gopalaswamy G. (2014) *Trends in Biosciences*, 7(22), 3651-3659.
- [12] Fasim F., Ahmed N., Parsons R. and Gadd G.M. (2002) FEMS Microbiology Letters, 213, 1-6.