Investigation on effect of zinc solubilizing microorganisms on microbial population in soil of pigeonpea [*Cajanus cajan*] on vertisol

ABSTRACT:

The present study investigated on the effect of zinc solubilizing microorganisms on microbial population in soil of pigeonpea [Cajanus cajan] on vertisol. The field experiment was carried out in experimental field of department of soil science and agricultural chemistry at College of Agriculture, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani during kharif season 2018-19 and 2019-20 in factorial randomized block design with 3 replications. The pigeonpea variety BDN-711 was used for study. The studies revealed that, at flowering, pod formation and at harvesting stage significantly the maximum value of soil bacteria were recorded in *Pseudomonas striata* along with 30 kg $ZnSO_4$ ha^{-1} treated plots (64.00, 74.00 and 57.90 CFU x 10^{-7} g^{-1} of soil) during 2018-19; (76.11, 87.44 and 68.67 CFU x 10^{-7} g^{-1} of soil) during 2019-20, respectively. At flowering, pod formation and at harvesting stage significantly the highest fungal population was noticed in treatment receving *Trichoderma viride* alongwith $ZnSO_4$ 30 kg ha^{-1} (9.67, 8.00 and 8.33 CFU x 10^{-4} g^{-1} of soil) during 2018-19; (10.67, 9.78 and 8.89 CFU x 10^{-4} g^{-1} of soil) during 2019-20, respectively. Also, at flowering, pod formation and at harvesting stage the actinomycetes population was found to be increased with the application of different zinc solubilizing cultures which ranged from (27.28 to 32.64, 30.45 to 38.83 and 26.86 to 30.47 CFU X $10^{-5}g^{-1}$ of soil) during 2018-19; (31.64 to 38.67, 34.67 to 43.61 and 28.36 to 34.81 CFU X $10^{-5}g^{-1}$ of soil) during 2019-20.

Key words: Pigeonpea, microbial population, bacteria, actinomycetes, fungi

INTRODUCTION:

Pigeonpea (*Cajanus cajan* (L.) Millsp.) is a legume belongs to Leguminosae family that was domesticated 35,000 years ago in India. Around 2000 BC, the pigeonpea was domesticated in East Africa and subsequently imported to America. Pigeonpea is now cultivated all over the globe in tropical and subtropical climates. It is also known as red gram, arhar, or tur dal in India, is the country's second most significant pulse crop after gram (Ghadge *et al*., 2010). Pigeonpea may provide excellent economic yields when soil moisture is limited, and it also produces well in rain-fed and dryland agriculture (Joshi *et al*., 2001).

For centuries, several techniques have been used to remedy zinc deficiency. Thus, in order to meet the zinc requirements of plants, a large quantity of zinc fertilizer must be given to the soil. Zinc fertilizers have been used in the form of zinc sulphate or Zn-EDTA (Karak *et al*., 2005), but their use places economic and environmental strain on the environment, as they are converted to insoluble complex forms such as Zn(OH) at a pH of 7.7 and $Zn(OH)_2$ at a pH of 9.1; $ZnCO_3$ in calcium-rich alkali soils; $Zn_3(PO_4)_2$ in near-neutral alkali (Rattan and Shukla, 1991). These zinc forms are no longer accessible to growing plants. Crop rotation and intercropping have both been used to increase zinc absorption by plants (Zuo and Zhang, 2008). Additionally, traditional breeding techniques (Cakmak *et al*., 2010), transgenic procedures, and genetic engineering (Gustin *et al.,* 2009) were utilised to increase zinc absorption by plants. However, these prospects are expensive, time-consuming, and labor-intensive. To all of those viewpoints, the utilisation of zinc solubilizing microorganisms is a superior option.

Zinc solubilizers are capable of solubilizing zinc from insoluble complexes by a variety of mechanisms, one of which is acidification. These zinc-solubilizing bacteria create organic acids in the soil, which act as a sink for zinc cations. Additionally, the generation of organic acid lowers the pH of the surrounding soil (Alexander, 1997). Additionally, anions may chelate zinc and improve its solubility (Jones and Darrah, 1994). Other proposed mechanisms for zinc solubilization include siderophore synthesis (Saravanan *et al*., 2011), proton, oxidoreductive structures in the cell membrane, and chelated ligands (Wakatsuki, 1995; Chang *et al*., 2005). When inoculated with several PGPRs, higher growth and zinc content were observed. *Pseudomonas, Rhizobium* strains (Joshi *et al*., 2013; Naz *et al*., 2016), *Bacillus aryabhattai* strains (Ramesh *et al*., 2014), *Bacillus* sp. strains (Hussain *et al*., 2015), and Azospirillum are all PGPRs. On a laboratory scale, zinc solubilization has been seen in *Burkholderia cenocepacia* (Saravanan *et al.*, 2004), *Gluconacetobacter diazotrophicus, Pseudomonas striata, Pseudomonas fluorescence* (Pawar *et al.*, 2015), *Serratia liquefaciens,* and *Bacillus thuringiensis* (Abaid-Ullah *et al*., 2015). Scientists have characterised zinc-solubilizing microorganisms in *Zea mays* L. for increased nutrition and zinc absorption, which affects soybean and wheat development and production and also contributes to zinc biofortification (Khande *et al*., 2017). Vaid *et al*. (2014) observed that inoculating rice with zinc solubilizers boosted rice growth and grain zinc nutrition by 42.7 percent.

MATERIAL AND METHODS

Field Experiment and treatment details:

Field trials using pigeonpea variety BDN-711 were conducted in factorial randomised block design with three replications at the experimental field of the department of soil science and agricultural chemistry at college of agriculture, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani during the *kharif* seasons 2018-19 and 2019-20. The recommended pigeonpea package of practices was followed. The experimental treatments consist of two factors: one factor containing four laboratory pre-evaluated microbial cultures (*Pseudomonas striata, Bacillus megaterium, Trichoderma viride,* and Control), and another factor containing four graded dosages of $ZnSO_4$ (0 kg $ZnSO_4$ ha^{-1}, 10 kg $ZnSO_4$ ha^{-1}, 20 kg $ZnSO_4$ ha^{-1} and 30 kg $ZnSO_4$ ha^{-1}). Pigeonpea seeds were treated with zinc-solubilizing microbial cultures immediate before to sowing at a concentration of 100 ml per 10 kg of pigeonpea seed and zinc sulphate was applied in graded levels at the time of sowing with recommended dose of fertilizer. Three distinct media were used to isolate bacteria (nutrient agar media), fungi (Rose Bengal agar), and actinomycetes (Ken Knight media) from soil samples.

Preparation of media

The agar-agar was boiled in 500 ml of distilled water in a beaker. In another beaker, approximately 500 ml of distilled water and all the chemical ingredients were added and mixed properly. All of these components have been carefully mixed, filtered, and diluted with distilled water to a level of 1000 ml. The corresponding media were distributed in two 500 mL conical flasks, plugged with non-absorbent cotton, threaded with paper, and autoclaved for 15 minutes at a pressure of 6.82 kg (15 lb). The dilution plate technique is a widely used method for isolating and identifying soil bacteria, fungus and actinomycetes.

Procedure (Dingra and Sinclair,1993)

1. 1 g soil sample was transferred to 10 ml of sterile distilled water in the test tube (1:10) and properly shaken.
2. 1 ml of suspension was transferred from this test tube to another tube containing 9 ml of sterile distilled water (1:100) and 1 ml of suspension was again transferred from this test tube to 9 ml of sterile distilled water (1:1000).
3. Likewise, the dilution procedure is repeated according to the requirements for bacteria 1: 10^7, fungal isolation 1: 10^4 and for actinomycetes 1:10^5.
4. The concerned diluted samples were poured at a rate of 1ml $plate^{-1}$.

5. The respective melted medium (cool to 45°C) was poured @ rate of 20 ml plate^{-1}.

RESULTS AND DISCUSSION:

Bacterial population in soil

The bacterial population of pigeonpea was increased significantly with zinc solubilizers as well as graded levels of zinc given in Table 1. At flowering, pod formation and at harvesting stage significantly the maximum value of soil bacteria were recorded in *Pseudomonas striata* treated plots (55.31, 62.00 and 50.17 CFU X 10^{-7} g^{-1} of soil) during 2018-19. Whereas, at flowering, pod formation and at harvesting stage significantly lower bacterial population per plot were noted in uninoculated control plots (35.06, 38.39 and 29.36 CFU X $10^{-7}g^{-1}$ of soil) during 2018-19.

Whereas, graded levels of zinc up to the 30 kg $ZnSO_4$ ha^{-1} increased bacterial population. At flowering, pod formation and harvesting stage significantly the highest bacterial population was noticed in $ZnSO_4$ 30 kg ha^{-1} treated plots (53.44, 61.00 and 47.31 CFU x 10^{-7} g^{-1} of soil) during 2018-19. However, the lower values of the bacterial population in soil was recorded in 0 kg $ZnSO_4$ ha^{-1} applied plots.

At flowering, pod formation and at harvesting stage significantly the highest bacterial population was noticed under *Pseudomonas striata* X $ZnSO_4$ 30 kg ha^{-1} (64.00, 74.00 and 57.90 CFU x 10^{-7} g^{-1} of soil) during 2018-19; (76.11, 87.44 and 68.67 CFU x 10^{-7} g^{-1} of soil) during 2019-20 and (64.00, 80.72 and 63.29 CFU x 10^{-7} g^{-1} of soil) and lower values of bacterial population was recorded in Zn_0 X S_0 uninoculated control (30.78, 32.00 and 26.11 CFU x 10^{-7} g^{-1} of soil) during 2018-19; (31.33, 33.67 and 29.45 CFU x 10^{-7} g^{-1} of soil) during 2019-20 and (30.78, 32.84 and 27.78 CFU x 10^{-7} g^{-1} of soil).

Results showed that the highest available Zn level in the treatment receiving *Pseudomonas striata* formulation could be attributed to the presence of comparatively more organic material as well as the higher bacterial population in this formulation. As soils dry, the demand for water increases, and microbial activity as well as intracellular enzyme activity slowed and thus decreases the bacterial population at harvest (Geisseler)

Table 1a. Interaction effect of zinc solubilizers and graded levels of zinc on periodical changes in bacterial population (cfu x 10^{-7} g^{-1} of soil) in soil

Treatments	Zn_0: $ZnSO_4$ 0 kg ha^{-1}	Zn_1: $ZnSO_4$ 10 kg ha^{-1}	Zn_2: $ZnSO_4$ 20 kg ha^{-1}	Zn_3: $ZnSO_4$ 30 kg ha^{-1}	Mean
Flowering (2018-19)					
S_0: Control	30.78	32.67	36.33	40.44	35.06
S_1: *Pseudomonas striata*	43.22	55.33	58.67	64.00	55.31
S_2: *Trichoderma viride*	44.45	46.33	50.62	54.00	48.85
S_3: *Bacillus megaterium*	43.89	49.67	51.89	55.33	50.19
Mean	40.58	46.00	49.38	53.44	
Interaction	**S**	**Zn**	**SXZn**		
SEm+	0.734	0.734	1.468		
CD at 5%	2.12	2.12	4.24		
Pod Formation (2018-19)					
S_0: Control	32.00	35.44	40.33	45.78	38.39
S_1: *Pseudomonas striata*	47.00	60.78	66.22	74.00	62.00
S_2: *Trichoderma viride*	47.22	50.33	57.00	60.89	53.86
S_3: *Bacillus megaterium*	47.44	54.89	59.00	63.33	56.17
Mean	43.42	50.36	55.64	61.00	

Interaction	**S**	**Zn**	**SXZn**		
SEm+	0.716	0.716	1.432		
CD at 5%	2.068	2.068	4.136		
At Harvest (2018-19)					
S_0: Control	26.11	27.56	30.33	33.44	29.36
S_1: *Pseudomonas striata*	39.67	50.44	52.67	57.90	50.17
S_2: *Trichoderma viride*	41.11	42.11	45.56	48.67	44.36
S_3: *Bacillus megaterium*	40.44	45.20	46.56	49.22	45.36
Mean	36.83	41.33	43.78	47.31	
Interaction	**S**	**Zn**	**SXZn**		
SEm+	0.728	0.728	1.456		
CD at 5%	2.103	2.103	4.206		
Flowering (2019-20)					
S_0: Control	31.33	35.22	40.33	46.45	38.33
S_1: *Pseudomonas striata*	48.67	62.67	68.11	76.11	63.89
S_2: *Trichoderma viride*	48.33	51.67	58.33	62.52	55.21
S_3: *Bacillus megaterium*	49.11	56.89	61.33	66.33	58.42
Mean	44.36	51.61	57.03	62.85	
Interaction	**S**	**Zn**	**SXZn**		
SEm+	0.81	0.81	1.62		
CD at 5%	2.339	2.339	4.678		
Pod Formation (2019-20)					
S_0: Control	33.67	37.56	44.89	53.78	42.47
S_1: *Pseudomonas striata*	52.89	69.44	76.11	87.44	71.47
S_2: *Trichoderma viride*	50.89	56.78	66.11	71.00	61.20
S_3: *Bacillus megaterium*	52.22	63.00	69.67	75.33	65.06
Mean	47.42	56.69	64.19	71.89	
Interaction	**S**	**Zn**	**SXZn**		
SEm+	0.963	0.963	1.927		
CD at 5%	2.782	2.782	5.564		
At Harvest (2019-20)					
S_0: Control	29.45	33.11	36.45	40.89	34.97
S_1: *Pseudomonas striata*	45.11	57.67	61.67	68.67	58.28
S_2: *Trichoderma viride*	45.33	47.11	52.67	56.78	50.47
S_3: *Bacillus megaterium*	45.45	52.00	55.56	59.56	53.14
Mean	41.33	47.47	51.58	56.47	
Interaction	**S**	**Zn**	**SXZn**		
SEm+	0.83	0.83	1.661		
CD at 5%	2.398	2.398	4.796		

et al. 2011). In wet soils, increased moisture can lead to the soluble organic matter in the soil, leading to an increase in the number of bacterial populations (Subhani *et al*., 2001). Our results are in agreement and might be supported by the findings of Kaur *et al*., (2017)

Table 1: Effect of zinc solubilizers and graded level of zinc on periodical changes in bacterial population (cfu x 10^{-7} g^{-1} of soil) in soil

Treatments	Soil bacteria (cfu x 10^{-7} g^{-1} of soil)								
	2018-19			**2019-20**			**Pooled**		
	Flowering	Pod formation	At harvest	Flowering	Pod formation	At harvest	Flowering	Pod formation	At harvest
Zinc Solubilizers (S)									
S_0: Control	35.06	38.39	29.36	38.33	42.47	34.97	35.06	40.43	32.17
S_1: *Pseudomonas striata*	55.31	62.00	50.17	63.89	71.47	58.28	55.31	66.74	54.22
S_2: *Trichoderma viride*	48.85	53.86	44.36	55.21	61.20	50.47	48.85	57.53	47.42
S_3: *Bacillus megaterium*	50.19	56.17	45.36	58.42	65.06	53.14	50.19	60.61	49.25
S.Em.±	0.734	0.716	0.728	0.81	0.963	0.83	0.734	0.527	0.541
C.D. at 5 %	2.12	2.068	2.103	2.33	2.782	2.398	2.12	1.521	1.562
Levels of $ZnSO_4$ (Zn)									
Zn_0: $ZnSO_4$ 0 kg ha^{-1}	40.58	43.42	36.83	44.36	47.42	41.33	40.58	45.42	39.08
Zn_1: $ZnSO_4$ 10 kg ha^{-1}	46.00	50.36	41.33	51.61	56.69	47.47	46.00	53.53	44.40
Zn_2: $ZnSO_4$ 20 kg ha^{-1}	49.38	55.64	43.78	57.03	64.20	51.58	49.38	59.92	47.68
Zn_3: $ZnSO_4$ 30 kg ha^{-1}	53.44	61.00	47.31	62.85	71.89	56.47	53.44	66.44	51.89
SE ±	0.734	0.716	0.728	0.81	0.963	0.83	0.734	0.527	0.541
CD @ 5 %	2.12	2.068	2.103	2.339	2.782	2.398	2.12	1.521	1.562
Interaction (SXZn)									
SE ±	1.468	1.432	1.456	1.62	1.927	1.661	1.468	1.054	1.082
CD @ 5%	4.24	4.136	4.206	4.678	5.564	4.796	4.24	3.043	3.124
CV	5.37	4.72	5.96	5.2	5.56	5.85	5.37	3.24	4.09

observed that higher total bacterial population (150×10^7 CFU g^{-1} of soil) and PGPR (218×10^5 CFU g^{-1} of soil) population was observed in treatments when there was a combination of organic, inorganic and consortium biofertilizer. Also, these results corroborate the ideas of Kumar and Ismail (2017) who suggested that the microbial population of bacteria was recorded significantly higher value in treatment RDF + *Rhizobium* + *Bacillus megaterium.*

Fungi population in soil

Soil fungal population was also significantly affected due to the addition of different zinc solubilizing bacterial and fungal strains in treatments given in Table 2. At flowering, pod formation and at harvesting stage fungal population in rhizosphere soil was significantly increased with the application of different zinc solubilizing microbial cultures which ranged between (4.39 to 7.72, 3.81 to 6.42 and 3.92 to 6.39 CFU x 10^{-4} g^{-1} of soil) during 2018-19; (4.78 to 8.33, 4.11 to 7.47 and 3.78 to 6.89 CFU x 10^{-4} g^{-1} of soil) (4.58 to 7.78, 3.96 to 6.95 and 3.85 to 6.64 CFU x 10^{-4} g^{-1} of soil), respectively showing significantly higher fungal population in *Trichoderma viride* treated plots followed by *Bacillus megaterium* and *Pseudomonas striata*. Whereas, the significantly lower fungal population per plot were noted in uninoculated control plots.

Similarly, graded levels of zinc in the form of zinc sulphate also increased the fungal population with each incremental dose of zinc up to 30 kg $ZnSO_4$ ha^{-1}. At flowering, pod formation and at harvesting stage significantly the highest fungal population was noticed in $ZnSO_4$ 30 kg ha^{-1} treated plots (8.03, 6.97 and 7.08 CFU x 10^{-4} g^{-1} of soil) during 2018-19; (9.22, 8.31 and 7.64 CFU x 10^{-4} g^{-1} of soil) during 2019-20; and (CFU x 10^{-4} g^{-1} of soil) during pooled. However, the lower values of the fungal population in soil was recorded in (Zn_0) 0 kg ha^{-1} treated plots.

The interaction effect of zinc solubilizers and graded levels of zinc affecting the fungal population in pigeonpea grown soil is shown in Table 2a. At flowering, pod formation and at harvesting stage significantly the highest fungal population was noticed under in *Trichoderma viride* X $ZnSO_4$ 30 kg ha^{-1} (9.67, 8.00 and 8.33 CFU x 10^{-4} g^{-1} of soil) during 2018-19; (10.67, 9.78 and 8.89 CFU x 10^{-4} g^{-1} of soil) during 2019-20 and (10.17, 8.89 and 8.61 CFU x 10^{-4} g^{-1} of soil) and lower values of the fungal population was recorded in Zn_0 X S_0 uninoculated control (1.89,1.33 and 1.33 CFU x 10^{-4} g^{-1} of soil) during 2018-19; (1.56, 1.00 and 0.89 CFU x 10^{-4} g^{-1} of soil) during 2019-20 and (1.72, 1.17 and 1.11 CFU x 10^{-4} g^{-1} of soil).

The present data showed that the total fungi population decreased over a period of time as plant growth progressed and at harvesting reached the lowest values. This may be attributed to the lack of soil nutrients and moisture during plant growth during the maturing period. Soil microorganism’s metabolism and survival are strongly influenced by the availability of water, which is necessary for microbial survival and development (Uhlirova *et al.*, 2005). As a result, low water availability can stop microbial activity by minimizing the capacity of soil moisture and enzyme activity (Wall and Heiskanen, 2003). Our results are in agreement and might be supported by the findings of Kumar and Ismail (2017) showed that the microbial population of fungi were noted significantly highest in treatment RDF + *Rhizobium* + *Trichoderma viride* and was found at par with RDF + *Rhizobium* + *Trichoderma harzianum*. It was also suggested by studies of Sable *et al.*, (2016) that the population of actinomycetes and fungi were noted significantly higher in RDF + *Rhizobium* + *Pseudomonas striata*.

Table 2: Effect of zinc solubilizers and graded level of zinc on periodical changes in fungal population (cfu x 10^{-4} g^{-1} of soil) in soil

Treatments	Soil fungi (cfu x 10^{-4} g^{-1} of soil)								
	2018-19			**2019-20**			**Pooled**		
	Flowering	Pod formation	At harvest	Flowering	Pod formation	At harvest	Flowering	Pod formation	At harvest
Zinc Solubilizers (S)									
S_0: Control	4.39	3.81	3.92	4.78	4.11	3.78	4.58	3.96	3.85
S_1: *Pseudomonas striata*	6.00	5.42	5.17	6.53	5.70	5.22	6.27	5.56	5.19
S_2: *Trichoderma viride*	7.22	6.42	6.39	8.33	7.47	6.89	7.78	6.95	6.64
S_3: *Bacillus megaterium*	6.78	6.09	5.61	7.75	6.92	6.42	7.26	6.50	6.01
S.Em.±	0.078	0.097	0.095	0.152	0.106	0.111	0.078	0.072	0.091
C.D. at 5 %	0.225	0.28	0.274	0.438	0.306	0.321	0.226	0.208	0.263
Levels of $ZnSO_4$ (Zn)									
Zn_0: $ZnSO_4$ 0 kg ha^{-1}	3.78	3.28	3.39	4.41	3.78	3.47	4.10	3.53	3.43
Zn_1: $ZnSO_4$ 10 kg ha^{-1}	5.50	4.97	4.45	5.78	5.00	4.64	5.64	4.99	4.54
Zn_2: $ZnSO_4$ 20 kg ha^{-1}	7.08	6.50	6.17	7.97	7.11	6.56	7.53	6.81	6.36
Zn_3: $ZnSO_4$ 30 kg ha^{-1}	8.03	6.97	7.08	9.22	8.31	7.64	8.63	7.64	7.36
SE ±	0.078	0.097	0.095	0.152	0.106	0.111	0.078	0.072	0.091
CD @ 5 %	0.225	0.28	0.274	0.438	0.306	0.321	0.226	0.208	0.263
Interaction (SXZn)									
SE ±	0.156	0.194	0.19	0.303	0.212	0.222	0.156	0.144	0.182
CD @ 5%	0.451	0.559	0.547	0.876	0.611	0.642	0.451	0.416	0.525
CV	4.43	6.17	6.23	7.67	6.06	6.91	4.18	4.35	5.81

Table 2a. Interaction effect of zinc solubilizers and graded levels of zinc on periodical changes in fungal population (cfu x 10^{-4} g^{-1} of soil) in soil

Treatments	Zn_0: $ZnSO_4$ 0 kg ha^{-1}	Zn_1: $ZnSO_4$ 10 kg ha^{-1}	Zn_2: $ZnSO_4$ 20 kg ha^{-1}	Zn_3: $ZnSO_4$ 30 kg ha^{-1}	**Mean**
Flowering (2018-19)					
S_0: Control	1.89	3.89	5.22	6.56	4.39
S_1: *Pseudomonas striata*	4.11	5.89	6.78	7.22	6.00
S_2: *Trichoderma viride*	4.67	6.22	8.33	9.67	7.22
S_3: *Bacillus megaterium*	4.44	6.00	8.00	8.67	6.78
Mean	3.78	5.50	7.08	8.03	
Interaction	**S**	**Zn**	**SXZn**		
SEm+	0.078	0.078	0.156		
CD at 5%	0.225	0.225	0.451		
Pod formation (2018-19)					
S_0: Control	1.33	3.45	4.78	5.67	3.81
S_1: *Pseudomonas striata*	3.67	5.33	6.11	6.56	5.42
S_2: *Trichoderma viride*	4.33	5.56	7.78	8.00	6.42
S_3: *Bacillus megaterium*	3.78	5.56	7.33	7.67	6.09
Mean	3.28	4.97	6.50	6.97	
Interaction	**S**	**Zn**	**SXZn**		
SEm+	0.097	0.097	0.194		
CD at 5%	0.28	0.28	0.559		
At harvest (2018-19)					
S_0: Control	1.33	3.33	5.67	5.33	3.92
S_1: *Pseudomonas striata*	4.00	3.56	5.78	7.33	5.17
S_2: *Trichoderma viride*	4.67	5.56	7.00	8.33	6.39
S_3: *Bacillus megaterium*	3.56	5.33	6.22	7.33	5.61
Mean	3.39	4.45	6.17	7.08	
Interaction	**S**	**Zn**	**SXZn**		
SEm+	0.095	0.095	0.19		
CD at 5%	0.274	0.274	0.547		
Flowering (2019-20)					
S_0: Control	1.56	4.00	6.44	7.11	4.78
S_1: *Pseudomonas striata*	4.78	4.45	7.67	9.22	6.53
S_2: *Trichoderma viride*	6.10	7.34	9.22	10.67	8.33
S_3: *Bacillus megaterium*	5.22	7.33	8.56	9.89	7.75
Mean	4.41	5.78	7.97	9.22	
Interaction	**S**	**Zn**	**SXZn**		
SEm+	0.152	0.152	0.303		
CD at 5%	0.438	0.438	0.876		
Pod formation (2019-20)					
S_0: Control	1.00	3.44	5.78	6.22	4.11
S_1: *Pseudomonas striata*	4.11	3.66	6.78	8.22	5.70
S_2: *Trichoderma viride*	5.44	6.44	8.22	9.78	7.47
S_3: *Bacillus megaterium*	4.56	6.45	7.67	9.00	6.92
Mean	3.78	5.00	7.11	8.31	
Interaction	**S**	**Zn**	**SXZn**		

SEm+	0.106	0.106	0.212		
CD at 5%	0.306	0.306	0.611		
At harvest (2019-20)					
S_0: Control	0.89	3.22	5.33	5.66	3.78
S_1: *Pseudomonas striata*	3.78	3.22	6.22	7.67	5.22
S_2: *Trichoderma viride*	5.11	6.00	7.56	8.89	6.89
S_3: *Bacillus megaterium*	4.11	6.11	7.11	8.33	6.42
Mean	3.47	4.64	6.56	7.64	
Interaction	**S**	**Zn**	**SXZn**		
SEm+	0.111	0.111	0.222		
CD at 5%	0.321	0.321	0.642		

Actinomycetes population in soil

A perusal of the data presented in Table 3 indicates the effect of different zinc solubilizing microbial inoculants on soil actinomycetes. At flowering, pod formation and at harvesting stage the actinomycetes population was found to be increased with the application of different zinc solubilizing cultures which ranged from (27.28 to 32.64, 30.45 to 38.83 and 26.86 to 30.47 CFU X $10^{-5}g^{-1}$ of soil) during 2018-19; (31.64 to 38.67, 34.67 to 43.61 and 28.36 to 34.81 CFU X $10^{-5}g^{-1}$ of soil) during 2019-20; and (29.46 to 35.65, 32.56 to 41.22 and 27.61 to 32.64 CFU X $10^{-5}g^{-1}$ of soil) during pooled mean showing significantly higher actinomycetes population in *Pseudomonas striata* treated plots and it was at par *Bacillus megaterium* only at flowering and pod formation stage during 2019-20. Whereas, at flowering, pod formation and harvesting stage significantly lower actinomycetes population per plot were noted in uninoculated control plots (27.28, 30.45 and 26.86 CFU X $10^{-5}g^{-1}$ of soil) during 2018-19; (31.64, 34.67 and 28.36 CFU X $10^{-5}g^{-1}$ of soil) during 2019-20; and (29.46 32.56, 27.61 CFU X $10^{-5}g^{-1}$ of soil) during pooled mean.

Similarly, applied graded levels of zinc in the form of zinc sulphate also increase the actinomycetes population with each incremental dose of zinc up to 30 kg $ZnSO_4$ ha^{-1}. At flowering, pod formation and harvesting stage significantly the highest actinomycetes population was noticed in $ZnSO_4$ 30 kg ha^{-1} treated plots (32.64, 38.83 and 30.47 CFU x 10^{-4} g^{-1} of soil) during 2018-19; (38.67, 43.61 and 34.81 CFU x 10^{-4} g^{-1} of soil) during 2019-20; and (35.65, 41.22 and 32.64 CFU x 10^{-4} g^{-1} of soil) during pooled mean. However, the lower values of actinomycetes population in soil was recorded in (Zn_0) 0 kg $ZnSO_4$ ha^{-1}treated plots.

The interaction effect of zinc solubilizers and graded levels of zinc affecting the actinomycetes population in pigeonpea grown soil were shown in Table 3a showing significantly the highest actinomycetes population was noticed under *Pseudomonas striata* X $ZnSO_4$ except at pod formation during 2018-19 and at flowering, harvesting stage during 2019-20. However, the lower values of actinomycetes population were recorded in uninoculated 0 kg $ZnSO_4$ ha^{-1} applied plots.

Our investigations showed that the population of actinomycetes increased during plant growth over a relatively long period, reaching the maximum values at the second sampling period (Pod forming stage), then slightly decreased at harvest as the soil becomes dry. These findings are consistent with those of previous studies by Ghorbani-Nasrabadi *et al.* (2013), who enumerated the soil actinomycetes and analysed the impact of moisture on the population of actinomycetes and found that the count of actinomycetes in dry soils decreased markedly. Organic matter, salinity, relative moisture, temperature, pH and vegetation are important factors that regulate actinomycetes abundance in soil (McCarthy and Williams, 1992). The organic matter content may be one of the most significant factors affecting the level of the actinomycete population of soil. Our results are in agreement and might be

Table 3: Effect of zinc solubilizers and graded level of zinc on periodical changes in actinomycetes population ($cfu \times 10^{-5}\ g^{-1}$ of soil) in soil

Treatments	Soil actinomycetes ($cfu \times 10^{-5}\ g^{-1}$ of soil)								
	2018-19			**2019-20**			**Pooled**		
	Flowering	Pod formation	At harvest	Flowering	Pod formation	At harvest	Flowering	Pod formation	At harvest
Zinc Solubilizers (S)									
S_0: Control	27.28	30.45	26.86	31.64	34.67	28.36	29.46	32.56	27.61
S_1: *Pseudomonas striata*	32.64	38.83	30.47	38.67	43.61	34.81	35.65	41.22	32.64
S_2: *Trichoderma viride*	29.89	34.95	28.21	34.97	40.17	32.89	32.43	37.56	30.55
S_3: *Bacillus megaterium*	30.47	35.97	28.42	36.58	42.97	34.67	33.53	39.47	31.54
S.Em.±	0.398	0.755	0.505	0.85	0.831	0.486	0.481	0.549	0.356
C.D. at 5 %	1.149	2.18	1.46	2.454	2.4	1.403	1.388	1.585	1.029
Levels of $ZnSO_4$ (Zn)									
Zn_0: $ZnSO_4$ 0 kg ha^{-1}	25.81	28.92	22.53	29.33	33.53	27.83	27.57	31.22	25.18
Zn_1: $ZnSO_4$ 10 kg ha^{-1}	28.86	32.75	27.86	34.84	39.31	31.42	31.85	36.03	29.64
Zn_2: $ZnSO_4$ 20 kg ha^{-1}	32.45	37.64	30.44	38.25	43.06	35.28	35.35	40.35	32.86
Zn_3: $ZnSO_4$ 30 kg ha^{-1}	33.17	40.89	33.13	39.45	45.53	36.20	36.31	43.21	34.66
SE ±	0.398	0.755	0.505	0.85	0.831	0.486	0.481	0.549	0.356
CD @ 5 %	1.149	2.18	1.46	2.454	2.4	1.403	1.388	1.585	1.029
Interaction (SXZn)									
SE ±	0.795	1.51	1.011	1.7	1.662	0.972	0.961	1.098	0.713
CD @ 5%	2.297	NS	2.919	NS	4.8	NS	2.776	3.169	2.058
CV	4.58	7.46	6.15	8.3	7.13	5.15	5.08	5.04	4.03

supported by the findings of Kumar and Ismail (2017) showed that the microbial population of actinomycetes and fungi were noted significantly highest in treatment RDF + *Rhizobium* + *Trichoderma viride* and was found at par with RDF + *Rhizobium* + *Trichoderma harzianum*. It was also suggested by studies of Sable *et al*., (2016) that the population of actinomycetes and fungi were noted significantly higher in RDF + *Rhizobium* + *Pseudomonas striata*. In the case of actinomycetes population treatment RDF + *Rhizobium* + *Pseudomonas fluorescens* shows the highest population. However, Supanekar (1999) reported that actinomycetal population was higher at pH range 7.51 to 8.00 in salt-affected soils than other pH ranges. As compared to normal soils, the actinomycetal population of salt-affected soils was less.

Table 3a. Interaction effect of zinc solubilizers and graded levels of zinc on periodical changes in actinomycetes population (cfu x 10^{-5} g^{-1} of soil) in soil

Treatments	Zn_0: $ZnSO_4$ 0 kg ha^{-1}	Zn_1: $ZnSO_4$ 10 kg ha^{-1}	Zn_2: $ZnSO_4$ 20 kg ha^{-1}	Zn_3: $ZnSO_4$ 30 kg ha^{-1}	**Mean**
Flowering (2018-19)					
S_0: Control	23.67	23.89	29.33	32.22	27.28
S_1: *Pseudomonas striata*	28.89	31.89	34.56	35.22	32.64
S_2: *Trichoderma viride*	25.89	29.22	32.23	32.22	29.89
S_3: *Bacillus megaterium*	24.78	30.45	33.67	33.00	30.47
Mean	25.81	28.86	32.45	33.17	
Interaction	**S**	**Zn**	**SXZn**		
SEm+	0.398	0.398	0.795		
CD at 5%	1.149	1.149	2.297		
At harvest (2018-19)					
S_0: Control	21.22	26.33	29.22	30.67	26.86
S_1: *Pseudomonas striata*	25.67	28.67	31.89	35.67	30.47
S_2: *Trichoderma viride*	23.22	26.56	28.78	34.29	28.21
S_3: *Bacillus megaterium*	20.00	29.89	31.89	31.89	28.42
Mean	22.53	27.86	30.44	33.13	
Interaction	**S**	**Zn**	**SXZn**		
SEm+	0.505	0.505	1.011		
CD at 5%	1.46	1.46	2.919		
Pod formation (2019-20)					
S_0: Control	28.89	30.56	38.89	40.34	34.67
S_1: *Pseudomonas striata*	36.67	40.33	45.44	52.00	43.61
S_2: *Trichoderma viride*	34.45	41.34	42.22	42.67	40.17
S_3: *Bacillus megaterium*	34.11	45.00	45.67	47.11	42.98
Mean	33.53	39.31	43.06	45.53	
Interaction	**S**	**Zn**	**SXZn**		
SEm+	0.831	0.831	1.662		
CD at 5%	2.4	2.4	4.8		

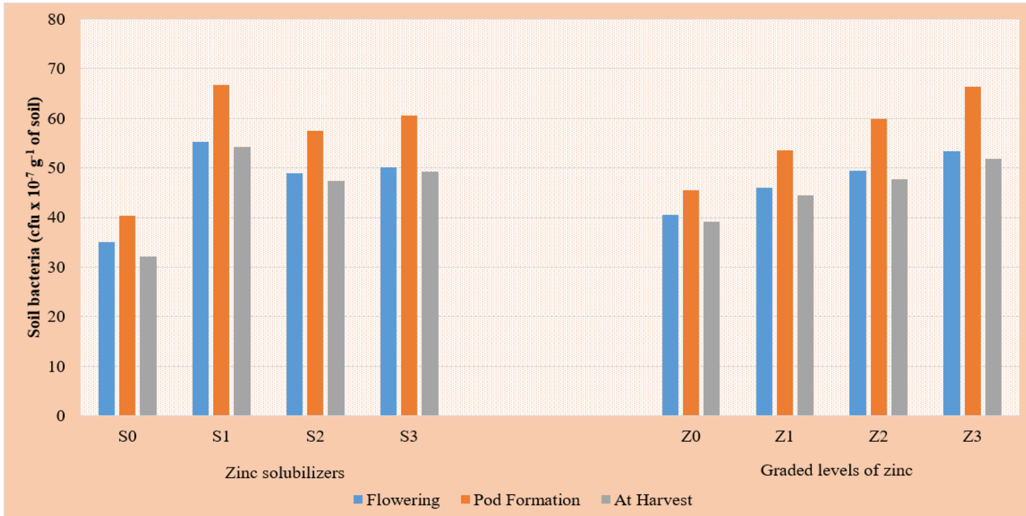

Fig. 1: Effect of zinc solubilizers and graded level of zinc on periodical changes in bacterial population (cfu x 10^{-7} g^{-1} of soil) in soil

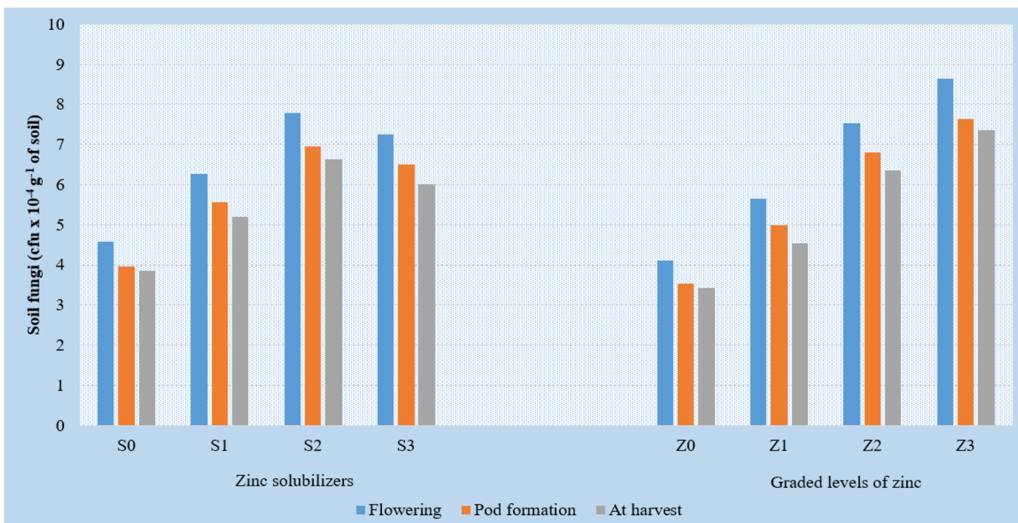

Fig. 2: Effect of zinc solubilizers and graded level of zinc on periodical changes in fungal population (cfu x 10^{-4} g^{-1} of soil) in soil

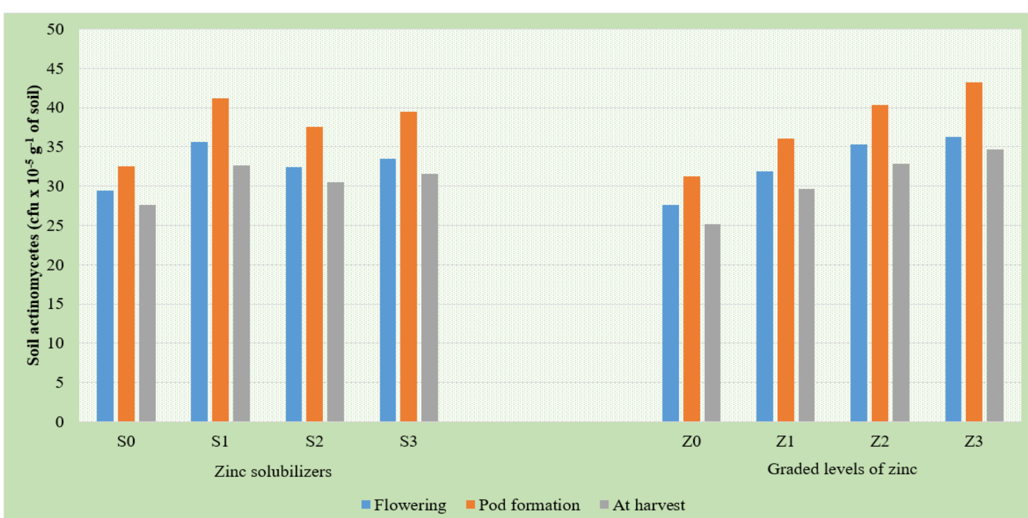

Fig. 3: Effect of zinc solubilizers and graded level of zinc on periodical changes in actinomycetes population (cfu x 10^{-5} g^{-1} of soil) in soil

Conclusion:

The bacterial and actinomycetes population in the soil after harvest of pigeonpea was significantly increased with inoculation of *Pseudomonas striata* along with RDF. Fungi population significantly increased with inoculation of *Trichoderma viride* along with RDF. Similarly graded levels of zinc in the form of zinc sulphate also increased the bacterial and actinomycetes population with each incremental dose, and was recorded maximum in 30 kg $ZnSO_4$ ha^{-1}.

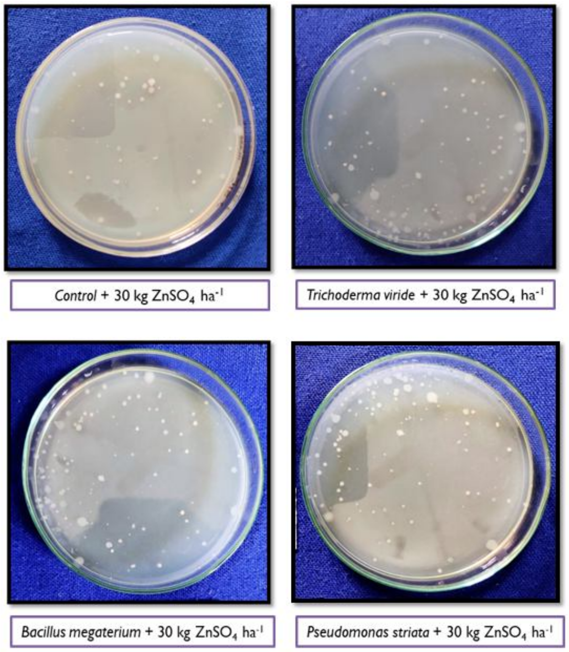

Plate 1: Bacterial population in soil

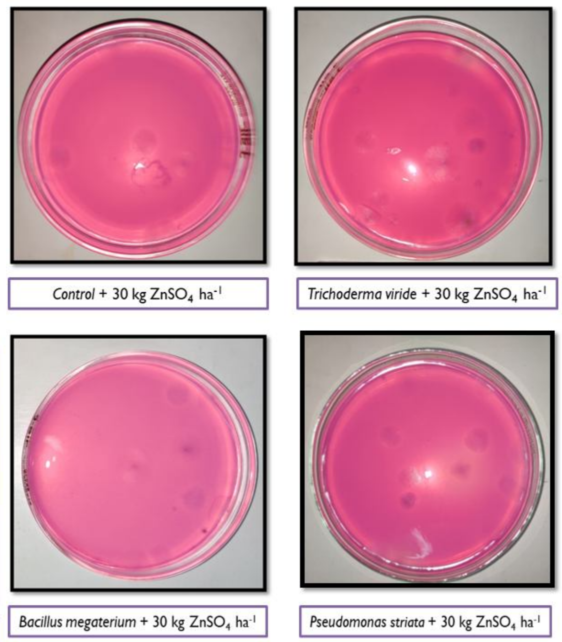

Plate 2: Fungal population in soil

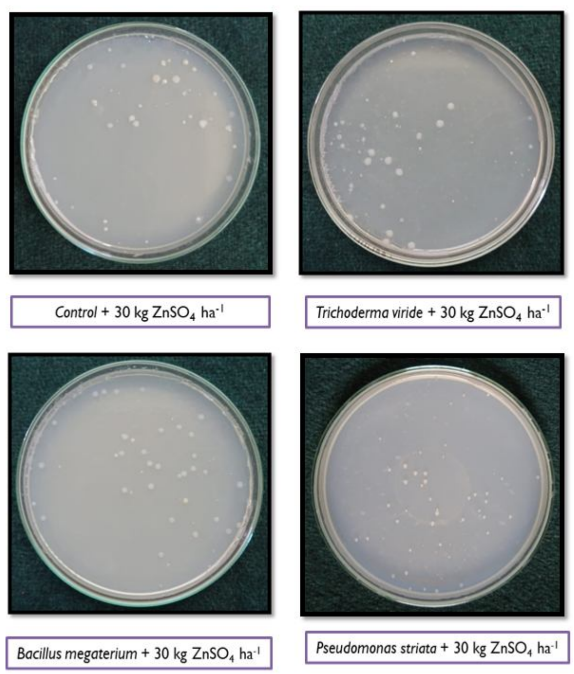

Plate 3: Actinomycetes population in soil

LITERATURE CITED

Abaid-Ullah, M., Nadeem, M., Hassan, M., Ganter, J., Muhammad, B., Nawaz, K., Shah, A. S. and Hafeez, F. Y. (2015). Plant growth promoting rhizobacteria: an alternate way to improve yield and quality of wheat (*Triticum aestivum*). *International Journal of Agriculture and Biology*, **17**(1): 51-60

Alexander, M. (1997). Introduction to soil microbiology. *John Wiley and sons, New York. Asia, P.E.A.*, **125**(5): 331

Cakmak, I., Pfeiffer, W. H. and McClafferty, B. (2010). Biofortification of durum wheat with zinc and iron. *Cereal Chemistry*, **87**(1): 10-20.

Chang, H. B., Lin, C. W. and Huang, H. J. (2005). Zinc-induced cell death in rice (Oryza sativa L.) roots. Plant Growth Regulation, 46(3): 261-266.

Dingra, D. D. and Sinclair, J. B. (1993). *Basic Plant Pathology Methods*. CBS Pub. New Delhi, 128-135.

Geisseler, D., Horwath, W. and Scow, K. (2011). Soil moisture and plant residue addition interact in their effect on extracellular enzyme activity. *Pedobiologia*, **54**: 71-78

Ghadge, P. N., Shewalkar, S. V. and Wankhede, D. B. (2010). Effect of processing methods on qualities of instant whole legume: Pigeonpea (*Cajanus cajan* L.). Agricultural Engineering International: CIGR Journal Manuscript FP 08 004. Vol. X.

Ghorbani-Nasrabadi, R., Greiner, R., Alikhani, H. A., Hamedi, J. and Yakhchali, B. (2013). Distribution of actinomycetes in different soil ecosystems and effect of media composition on extracellular phosphatase activity. Journal of Soil Science and Plant Nutrition, 13(1): 223-236.

Joshi, P. K., Rao, P. P., Gowda, C. L. L., Jones, R. B., Silim, S. N., Saxena, K. B. and Kumar, J. (2001). The world chickpea and pigeon pea economies facts, trends, and outlook. International Crops Research Institute for the Semi-Arid Tropics.

Gustin, J. L., Loureiro, M. E., Kim, D., Na, G., Tikhonova, M. and Salt, D. E. (2009). MTP1 dependent Zn sequestration into shoot vacuoles suggests dual roles in Zn tolerance and accumulation in Zn hyperaccumulating plants. *The Plant Journal*, **57**(6): 1116-1127.

Hussain, A., Arshad, M., Zahir, Z. A. and Asghar, M. (2015). Prospects of zinc *solubilizing bacteria for enhancing growth of maize.* Pakistan Journal of Agricultural Sciences, **52**(4): 915-922.

Jones, D. L. and Darrah, P. R. (1994). Role of root derived organic acids in the mobilization of nutrients from the rhizosphere. *Plant and Soil*, **166**(2): 247-257.

Joshi, D., Negi, G., Vaid, S. and Sharma, A. (2013). Enhancement of wheat growth *Journal of Agriculture, Environment and Biotechnology*, **6**(3): 363-370.

Karak, T., Singh, U. K., Das, S., Das, D. K. and Kuzyakov, Y. (2005). Comparative efficacy of $ZnSO_4$ and Zn-EDTA application for fertilization of rice (*Oryza sativa* L.). *Archives of Agronomy and Soil Science*, **51**(3): 253-264.

Rattan, R. K. and Shukla, L. M. (1991). Influence of different Zn carriers on the utilization of micronutrients by rice. *Journal of the Indian Society of Soil Science*, **39**(4): 808-810.

Kumar, B. K. and Ismail, S. (2017). Influence of different microbial inoculants on biological health of soil and economics in Soybean crop grown on Vertisol. Journal of Pharmacognosy and Phytochemistry, 6(3): 198-201.

Kaur, H., Gosal, S. K. and Walia, S. S. (2017). Synergistic effect of organic, inorganic and biofertilizers on soil microbial activities in rhizospheric soil of green pea. Annual Research and Review in Biology, 1-11.

Khande, R., Sharma, S. K., Ramesh, A. and Sharma, M. P. (2017). Zinc solubilizing *Bacillus* strains that modulate growth, yield and zinc biofortification of soybean and wheat. *Rhizosphere*, **4**: 126-138.

McCarthy, A. J. and Williams, S. T. (1992). Actinomycetes as agents of biodegradation in the environment-a review. Gene, 115(1-2): 189-192.

Naz, I., Ahmad, H., Khokhar, S. N., Khan, K. and Shah, A. H. (2016). Impact of zinc solubilizing bacteria on zinc contents of wheat. *American-Eurasian Journal of Agricultural and Environmental Sciences*, **16**: 449-454.

Pawar, A., Ismail, S., Mundhe, S. and Patil, V. D. (2015). Solubilization of insoluble zinc compounds by different microbial isolates in vitro condition. *International Journal of Tropical Agriculture*, **33**(2): 865-869.

Peoples, M. B., Herridge, D. F. and Ladha, J. K. (1995). Biological nitrogen fixate

Ramesh, A., Sharma, S. K., Sharma, M. P., Yadav, N. and Joshi, O. P. (2014). Inoculation of zinc solubilizing *Bacillus aryabhattai* strains for improved growth, mobilization and biofortification of zinc in soybean and wheat cultivated in Vertisols of central India. *Applied Soil Ecology*, **73**: 87-96.

Sable, P., Ismail, S. and Pawar, A. (2016). Effect of zinc solubilizing microorganisms in enhancing enzyme activity and nutrient availability in groundnut grown on vertisol. International Journal of Agricultural Science, 8(49): 2099-2102.

Saravanan, V. S., Kumar, M. R. and Sa, T. M. (2011). Microbial zinc solubilization and their role on plants. In Maheshwari D. (eds) *Bacteria in Agrobiology: Plant Nutrient Management, Springer, Berlin, Heidelber*g pp. 47-63.

Saravanan, V. S., Subramoniam, S. R. and Raj, S. A. (2004). Assessing in vitro solubilization potential of different zinc solubilizing bacterial (ZSB) isolates. *Brazilian Journal of Microbiology*, **35**(1-2): 121-125.

Subhani, A., Changyong, H., Zhengmiao, Y., Min, L. and El-Ghamry, A. (2001). Impact of soil environment and agronomic practices on microbial/dehydrogenase enzyme activity in soil. A review. Pakistan Journal of Biological Sciences, 4(3): 333-338.

Supanekar, S. V. (1999). Impact of soil salinity on microflora and ground water pollution in Sangli district. (Doctoral Dissertation). Shivaji University, Kolhapur.

Uhlirova, E., Elhottova, D., Triska, J. and Santruckova H. (2005). Physiology and microbial community structure in soil at extreme water content. Folia Microbiologica, 50(2): 161-166.

Vaid, S. K., Kumar, B., Sharma, A., Shukla, A. K. and Srivastava, P. C. (2014). Effect of Zn solubilizing bacteria on growth promotion and Zn nutrition of rice. *Journal of Soil Science and Plant Nutrition*, **14**(4): 889-910.

Wakatsuki, T. (1995). Metal oxidoreduction by microbial cells. *Journal of Industrial Microbiology*, **14**(2): 169-177.Chang *et al*., 2005.

Wall, A. and Heiskanen, J. (2003). Water-retention characteristics and related physical properties of soil on afforested agricultural land in Finland. Forest Ecology and Management, 186(1-3): 21-32.

Zuo, Y. and Zhang, F. (2008). Iron and zinc biofortification of dicot plants by intercropping with gramineous species. A review. *Agronomy for Sustainable Development*, **29**: 63-71.