



Investigation on Effect of Zinc Solubilizing Microorganisms on Microbial Population in Soil of Pigeonpea [*Cajanus cajan*] on Vertisol

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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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, Vasanttrao 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 ZnSO₄ 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

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treatment receiving *Trichoderma viride* alongwith ZnSO₄ 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 10⁻⁵g⁻¹ of soil) during 2018-19; (31.64 to 38.67, 34.67 to 43.61 and 28.36 to 34.81 CFU X 10⁻⁵g⁻¹ of soil) during 2019-20.

Keywords: Pigeonpea; microbial population; bacteria; actinomycetes; fungi.

1. 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 [1]. Pigeonpea may provide excellent economic yields when soil moisture is limited, and it also produces well in rain-fed and dryland agriculture [2].

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 [3], 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; ZnCO₃ in calcium-rich alkali soils; Zn₃(PO₄)₂ in near-neutral alkali [4]. These zinc forms are no longer accessible to growing plants. Crop rotation and intercropping have both been used to increase zinc absorption by plants [5]. Additionally, traditional breeding techniques [6], transgenic procedures, and genetic engineering [7] 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 [8]. Additionally, anions may chelate zinc and

improve its solubility [9]. Other proposed mechanisms for zinc solubilization include siderophore synthesis [10], proton, oxidoreductive structures in the cell membrane, and chelated ligands [11, 12]. When inoculated with several PGPRs, higher growth and zinc content were observed. *Pseudomonas*, *Rhizobium* strains [13, 14], *Bacillus aryabhattai* strains [15], *Bacillus* sp. strains [16], and *Azospirillum* are all PGPRs. On a laboratory scale, zinc solubilization has been seen in *Burkholderia cenocepacia* [17], *Gluconacetobacter diazotrophicus*, *Pseudomonas striata*, *Pseudomonas fluorescence* [18], *Serratia liquefaciens*, and *Bacillus thuringiensis* [19]. 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 [20]. Vaid et al. [21] observed that inoculating rice with zinc solubilizers boosted rice growth and grain zinc nutrition by 42.7 percent.

2. MATERIAL AND METHODS

2.1 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, Vasant Rao 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 ZnSO₄ (0 kg ZnSO₄ ha⁻¹, 10 kg ZnSO₄ ha⁻¹, 20 kg ZnSO₄ ha⁻¹ and 30 kg ZnSO₄ ha⁻¹). Pigeonpea seeds were treated with zinc-

solubilizing microbial cultures immediately before 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.

2.2 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.

2.3 Procedure [22]

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: 10⁷, fungal isolation 1: 10⁴ and for actinomycetes 1:10⁵.
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.

3. RESULTS AND DISCUSSION:

3.1 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 ZnSO₄ ha⁻¹ increased bacterial population. At flowering, pod formation and harvesting stage significantly the highest bacterial population was noticed in ZnSO₄ 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 ZnSO₄ ha⁻¹ applied plots.

At flowering, pod formation and at harvesting stage significantly the highest bacterial population was noticed under *Pseudomonas striata* X ZnSO₄ 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 Zn₀ X S₀ 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 [23].

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 [24]. Our results are in agreement and might be supported by the findings of Kaur et al., [25].

observed that higher total bacterial population (150x10⁷ CFU g⁻¹ of soil) and PGPR (218x10⁵ 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 [26] who suggested that the microbial population of bacteria was recorded significantly higher value in treatment RDF + *Rhizobium* + *Bacillus megaterium*.

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 : <i>Pseudomonas striata</i>	55.31	62.00	50.17	63.89	71.47	58.28	55.31	66.74	54.22
S ₂ : <i>Trichoderma viride</i>	48.85	53.86	44.36	55.21	61.20	50.47	48.85	57.53	47.42
S ₃ : <i>Bacillus megaterium</i>	50.19	56.17	45.36	58.42	65.06	53.14	50.19	60.61	49.25
S.E.m.±	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 ZnSO₄ (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

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 : <i>Pseudomonas striata</i>	43.22	55.33	58.67	64.00	55.31
S ₂ : <i>Trichoderma viride</i>	44.45	46.33	50.62	54.00	48.85
S ₃ : <i>Bacillus megaterium</i>	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 : <i>Pseudomonas striata</i>	47.00	60.78	66.22	74.00	62.00
S ₂ : <i>Trichoderma viride</i>	47.22	50.33	57.00	60.89	53.86
S ₃ : <i>Bacillus megaterium</i>	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 : <i>Pseudomonas striata</i>	39.67	50.44	52.67	57.90	50.17
S ₂ : <i>Trichoderma viride</i>	41.11	42.11	45.56	48.67	44.36
S ₃ : <i>Bacillus megaterium</i>	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 : <i>Pseudomonas striata</i>	48.67	62.67	68.11	76.11	63.89

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
S ₂ : <i>Trichoderma viride</i>	48.33	51.67	58.33	62.52	55.21
S ₃ : <i>Bacillus megaterium</i>	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 : <i>Pseudomonas striata</i>	52.89	69.44	76.11	87.44	71.47
S ₂ : <i>Trichoderma viride</i>	50.89	56.78	66.11	71.00	61.20
S ₃ : <i>Bacillus megaterium</i>	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 : <i>Pseudomonas striata</i>	45.11	57.67	61.67	68.67	58.28
S ₂ : <i>Trichoderma viride</i>	45.33	47.11	52.67	56.78	50.47
S ₃ : <i>Bacillus megaterium</i>	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		

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 : <i>Pseudomonas striata</i>	6.00	5.42	5.17	6.53	5.70	5.22	6.27	5.56	5.19
S ₂ : <i>Trichoderma viride</i>	7.22	6.42	6.39	8.33	7.47	6.89	7.78	6.95	<u>6.64</u>
S ₃ : <i>Bacillus megaterium</i>	6.78	6.09	5.61	7.75	6.92	6.42	7.26	6.50	6.01
S.E.m.±	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 ZnSO₄ (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	<u>7.36</u>
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 : <i>Pseudomonas striata</i>	4.11	5.89	6.78	7.22	6.00
S ₂ : <i>Trichoderma viride</i>	4.67	6.22	8.33	9.67	7.22
S ₃ : <i>Bacillus megaterium</i>	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 : <i>Pseudomonas striata</i>	3.67	5.33	6.11	6.56	5.42
S ₂ : <i>Trichoderma viride</i>	4.33	5.56	7.78	8.00	6.42
S ₃ : <i>Bacillus megaterium</i>	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 : <i>Pseudomonas striata</i>	4.00	3.56	5.78	7.33	5.17
S ₂ : <i>Trichoderma viride</i>	4.67	5.56	7.00	8.33	6.39
S ₃ : <i>Bacillus megaterium</i>	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 : <i>Pseudomonas striata</i>	4.78	4.45	7.67	9.22	6.53

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
S ₂ : <i>Trichoderma viride</i>	6.10	7.34	9.22	10.67	8.33
S ₃ : <i>Bacillus megaterium</i>	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 : <i>Pseudomonas striata</i>	4.11	3.66	6.78	8.22	5.70
S ₂ : <i>Trichoderma viride</i>	5.44	6.44	8.22	9.78	7.47
S ₃ : <i>Bacillus megaterium</i>	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 : <i>Pseudomonas striata</i>	3.78	3.22	6.22	7.67	5.22
S ₂ : <i>Trichoderma viride</i>	5.11	6.00	7.56	8.89	6.89
S ₃ : <i>Bacillus megaterium</i>	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		

Table 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

Treatments	Soil actinomycetes (cfu x 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 : <i>Pseudomonas striata</i>	32.64	38.83	30.47	38.67	43.61	34.81	35.65	41.22	32.64
S ₂ : <i>Trichoderma viride</i>	29.89	34.95	28.21	34.97	40.17	32.89	32.43	37.56	30.55
S ₃ : <i>Bacillus megaterium</i>	30.47	35.97	28.42	36.58	42.97	34.67	33.53	39.47	31.54
S.E.m.±	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 ZnSO₄ (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

Table 3a. Interaction effect of zinc solubilizers and graded levels of zinc on periodical changes in actinomycetes population (cfu x 10⁵ 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 : <i>Pseudomonas striata</i>	28.89	31.89	34.56	35.22	32.64
S ₂ : <i>Trichoderma viride</i>	25.89	29.22	32.23	32.22	29.89
S ₃ : <i>Bacillus megaterium</i>	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 : <i>Pseudomonas striata</i>	25.67	28.67	31.89	35.67	30.47
S ₂ : <i>Trichoderma viride</i>	23.22	26.56	28.78	34.29	28.21
S ₃ : <i>Bacillus megaterium</i>	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 : <i>Pseudomonas striata</i>	36.67	40.33	45.44	52.00	43.61
S ₂ : <i>Trichoderma viride</i>	34.45	41.34	42.22	42.67	40.17
S ₃ : <i>Bacillus megaterium</i>	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		

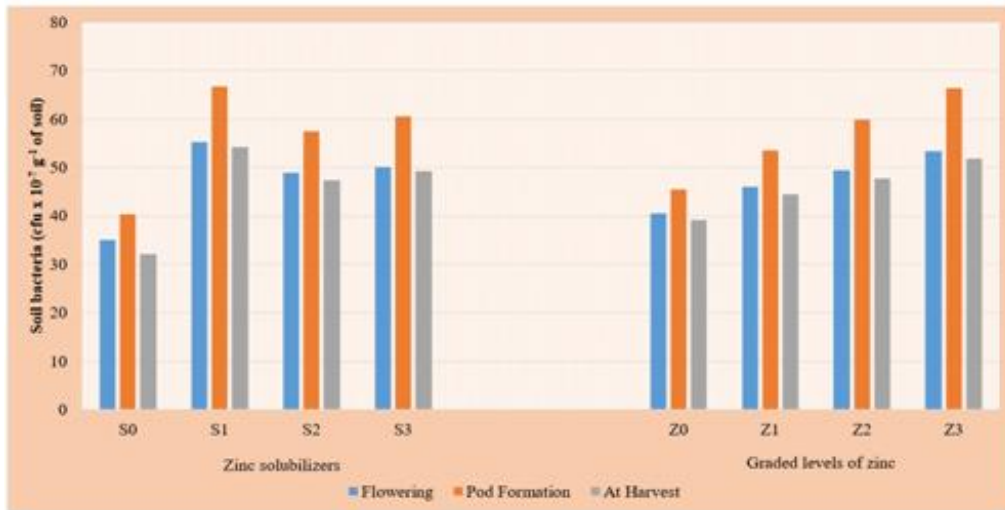


Fig. 1: Effect of zinc solubilizers and graded level of zinc on periodical changes in bacterial population ($cfu \times 10^7 g^{-1}$ of soil) in soil

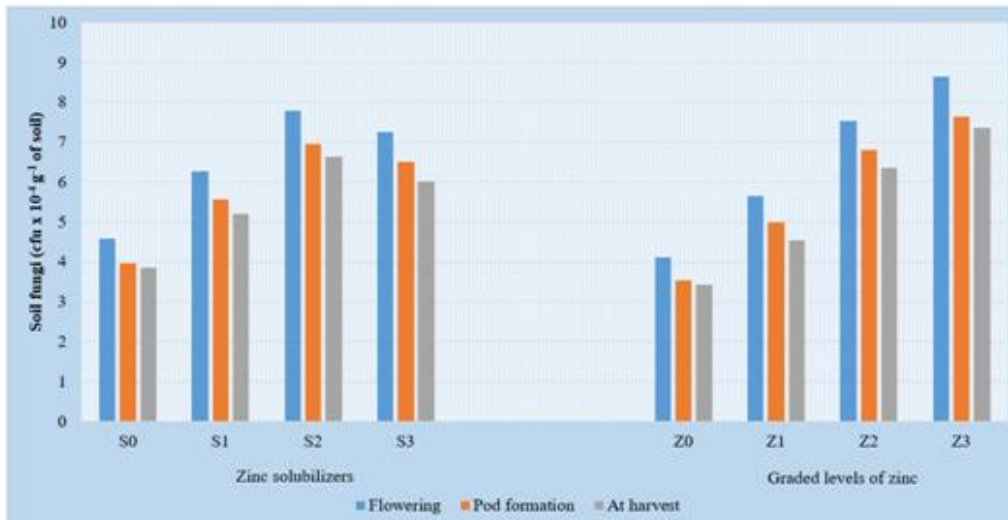


Fig. 2: Effect of zinc solubilizers and graded level of zinc on periodical changes in fungal population ($cfu \times 10^4 g^{-1}$ of soil) in soil

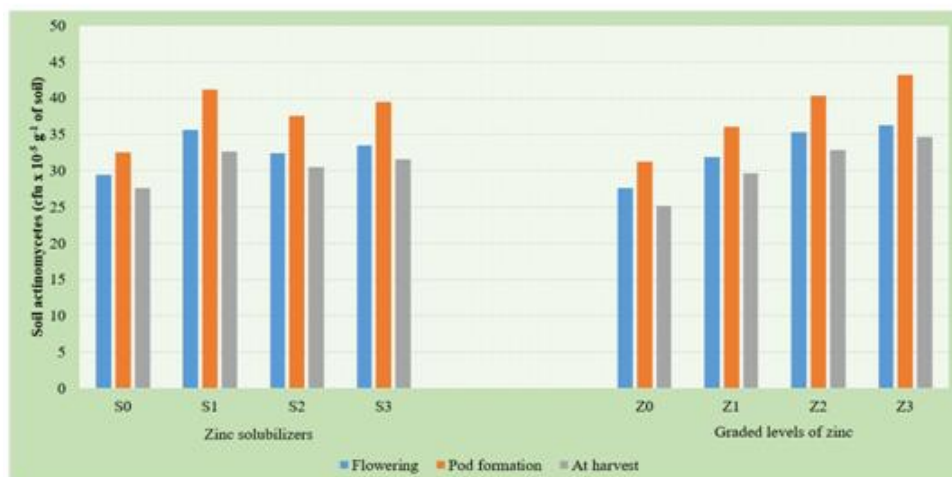


Fig. 3: Effect of zinc solubilizers and graded level of zinc on periodical changes in actinomycetes population ($cfu \times 10^5 g^{-1}$ of soil) in soil

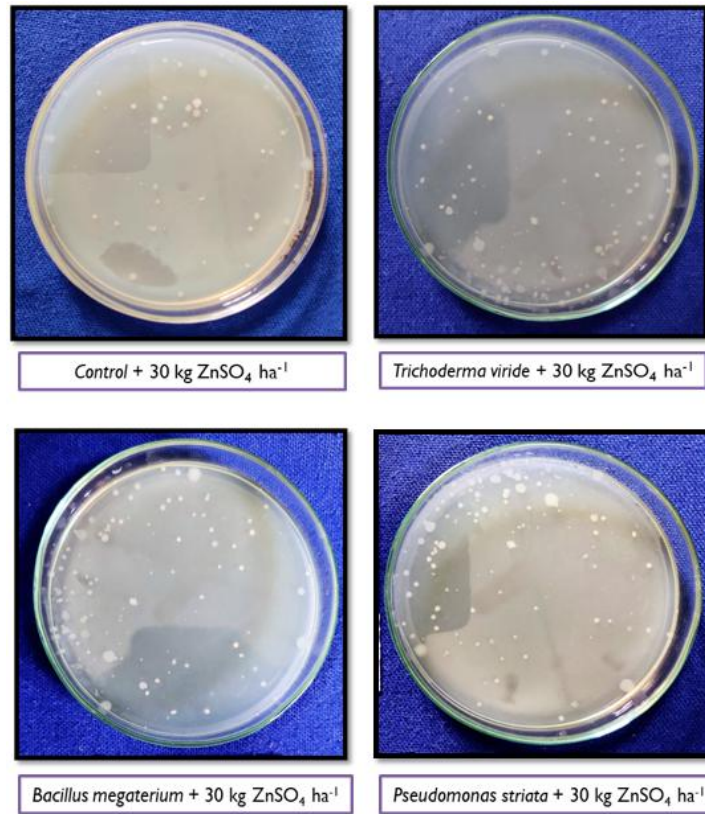


Plate 1: Bacterial population in soil

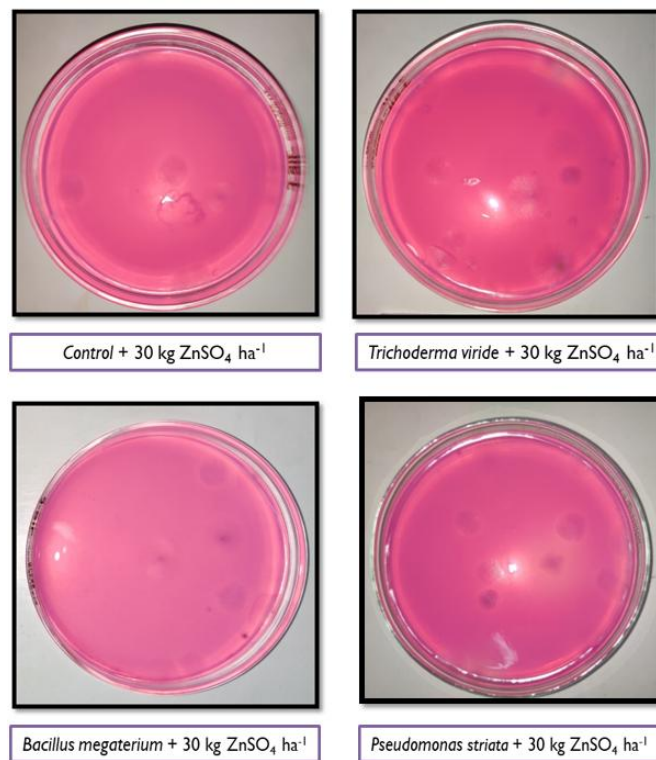


Plate 2: Fungal population in soil

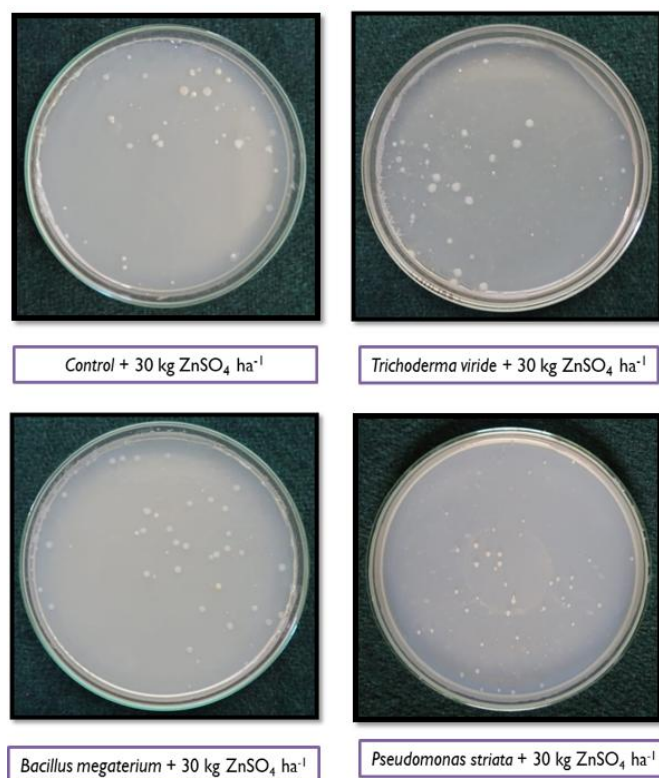


Plate 3: Actinomycetes population in soil

3.2 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 ZnSO₄ ha⁻¹. At flowering, pod formation and at harvesting stage significantly the highest fungal population was noticed in ZnSO₄ 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 (Zn₀) 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 ZnSO₄ 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 Zn₀ X S₀ 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 [27]. As a result, low water availability can stop microbial activity by minimizing the capacity of soil moisture and enzyme activity [28]. Our results are in agreement and might be supported by the findings of Kumar and Ismail [26] 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.*, [29] that the population of actinomycetes and fungi were noted significantly higher in RDF + *Rhizobium* + *Pseudomonas striata*.

3.3 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 ZnSO₄ ha⁻¹. At flowering, pod formation and harvesting stage significantly the highest actinomycetes population was noticed in ZnSO₄ 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 (Zn₀) 0 kg ZnSO₄ 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 ZnSO₄ 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 ZnSO₄ 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.* [30], 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 [31]. 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 supported by the findings of Kumar and Ismail [26] 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.*, [29] 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 [32] 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.

4. 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 ZnSO₄ ha⁻¹.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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