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In Vitro Evaluate the Impact of Salt Stress on the Germination and Growth of Cowpea Seedlings and Ameliorate with PGPR

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***Abstract*—The cowpea, a legume crop typically cultivated in arid and semi-arid regions, is an essential food source for both humans and livestock. The effectiveness and growth of plants are hindered by abiotic stresses, such as salt stress. The purpose of this research was to examine the *in vitro* effect of salt stress on seedling growth and germination potential of cowpea and the role of salt-tolerant plant growth-promoting bacteria to overcome the effect of salt stress and improve the growth of cowpea seedlings under different concentrations of NaCl. In this study, three bacterial cultures were used to overcome the effect of salt stress on cowpea. *Thalassobacillus devorans, Bacillus paralicheniformis,* and *Pseudalkalibacillus salsuginis* were used to overcome the effect of salt stress on cowpea.**

***IndexTerms*—Salt stress, PGPR, Salinity, Seedling, Soil, Concentration**

I. INTRODUCTION

Soil salinization is a major global issue that results in adverse effects on agriculture and several ecosystems. This intricate phenomenon is affected by various factors or their combinations, such as soil type, landscape characteristics, waterlogging, coastal flooding, elevated evapotranspiration rates, insufficient rainfall for soil flushing, land use patterns, unsustainable agricultural practices, and improper irrigation techniques (Kumar et al., 2023).

The strong nutritional content, environmental adaptability, and food security value of the cowpea (Vigna unguiculata) make it one of the most significant legume crops that are widely grown and consumed, particularly in Africa, Asia, and Latin America (Kwan et al., 2024). For millions of individuals, especially in the tropical and semi-tropical parts of the world, it is a significant source of plant-based proteins, carbohydrates, vitamins, and minerals (Abebe et al., 2022). The cowpea is an important crop in sustainable farming systems because, as a legume, it fixes nitrogen into the soil, making it a vital crop in regions that are vulnerable to poor soil fertility and erratic weather patterns (Mndzebele et al., 2022). Cowpea plants may suffer considerable morphological and phytological harm as a result of salinity. Cowpea plants' photosynthetic activity is decreased by salinity during the seedling stage, and an excess of Na+ in plant tissue can cause plant mortality. Salinity has more detrimental effects on crops in semi-arid and dry locations where cowpea farming is common, which may deter growers from increasing their cowpea production areas. Uses of plant growth-promoting rhizobacteria are an eco-friendly and reasonable technique to overcome the effects of salt stress. In this study, three salt-tolerant bacterial cultures were used and their effect on cowpea at different concentrations of NaCl.

II. MATERIALS AND METHODS

Three salt-tolerant bacterial cultures, *Thalassobacillus devorans, Bacillus paralicheniformis,* and *Pseudalkalibacillus salsuginis,* were used to overcome the effect of salt stress on cowpea. These three cultures were isolated and identified in the Botany department of C.C.S.U., Meerut, and submitted to NCBI. Their accession numbers were PP301450, PP237824, PP301453, respectively.

Solutions preparation

To prepare a 100ml solution of different molar concentrations of salt. Weighed 2.925g, 5.85g, 8.775g, and 11.7g salt for different concentrated molar solutions 50mM,100mM, 150mM, and 200 mM, respectively. Based on the different concentrations, place the NaCl salt in a 250ml volumetric flask. Add

100ml of distilled water to dissolve the salt completely and label the flask.

Prepare a soil sample of different concentrations of NaCl

After making saline solutions, add soil to this solution and make a saturated paste of saline soil. Leave it to dry. Dried soil was kept in small earthen pots and labelled according to salt concentration.

Seed Viability: -The viability of the seed accession is a measure of how many seeds are alive and could develop into plants which will reproduce themselves, given the appropriate conditions. The Tetrazolium chloride (TTC) approach, as developed by Verma and Majee (2013) was used to test the viability of legume seeds. Fifty lentil seeds were soaked in 0.1 percent TTC solution overnight. The staining of the embryo due to the reduction of tetrazolium chloride was used to test the viability of seeds. After this, five seeds of cowpea were sown in every pot. After 14 days, germination of cowpea seeds started.

Morphological Parameters

1) Germination Percentage: Germination is the process through which seeds germinate and grow into new plants. The difference between emerging and non-emerging seeds was used to determine seed germination.

$$\text{Germination percentage \%} = \frac{\text{No. of Germinated seeds}}{\text{Total number of seeds}} \times 100$$

2) Root length and shoot length: - The plants' root and shoot lengths were measured by thread and scale, respectively, and counting the lateral roots as well
3) Fresh weight and Dry weight: -Fresh weight of plants has been taken by weighing machine, and then let them dry at 80°C for at least 48 hours. When they are completely dried, weigh their dry weight and collect the data.

Biochemical Parameters

1) α Amylase activity

Alpha-amylase plays a critical role in transforming stored starch into useable sugars during seed germination, giving the growing plant the carbon skeletons and energy it needs for establishment and growth. This experiment was performed by Filner and Varner's (1967) method.

2) Phytase activity

During legume seed germination, phytase plays a critical role in facilitating energy requirements, nutrient availability, and metabolic activities by releasing phosphorus and minerals from phytic acid. This experiment was performed by Engelen (1994).

III. RESULTS AND DISCUSSION

1) Germination percentages

Cowpea (Vigna unguiculata), a legume crop known for its salt resistance yet comparatively susceptible to salinity, is greatly impacted by salt stress in terms of germination. In this study, germination percentages were significantly decreased as the concentration of salt increased (Table 1). Similar results were observed by Afonso et al. (2025). According to Murillo-Amador et al. (2006), high salt concentrations (such as NaCl levels above 50–100 mM) delay or prevent germination by decreasing the osmotic potential of the soil solution, which in turn reduces water uptake (imbibition). When treated with the salt-tolerant PGPR, the germination percentages significantly increased in comparison to the control (Fig. 1). In a recent study, Peng et al. (2021) chose a group of three bacterial strains (*Pseudomonas sp. P8, Peri bacillus sp. P10, and Streptomyces sp. X52*) to inoculate maize plant roots in soil that was affected by salt. The group's capacity to withstand salt resulted in faster plant growth.

S.N.	Treatments	50mM	100mM	150mM	200mM
1	Bacillus paralicheniformis	96	82	76	45
2	Thalassobacillus devorans	94	75	78	38
3	Pseudalkalibacillus salsuginis	92	85	74	40
4	Control	70	65	62	20

Table 1 shows the effect of salt stress on germination percentages of cowpea with PGPR.

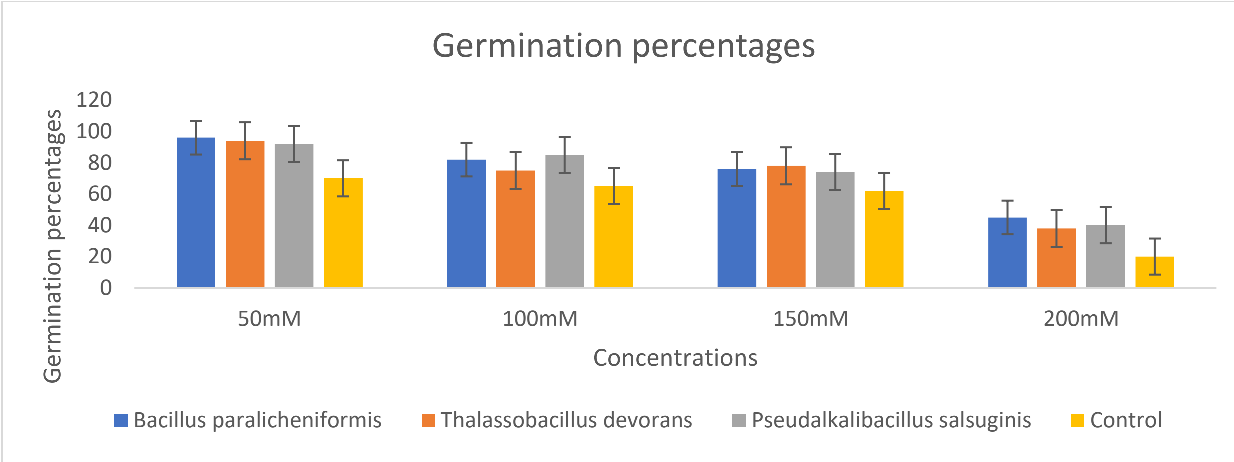

Fig.1 shows the effect of salt stress on germination percentages of cowpea with PGPR.

2) Shoot length

Like germination percentages, the shoot length of cowpea decreased as the concentration of salt increased, but with microbial treatment, it increased significantly (Table 2, Fig. 2). Treatment 3 showed the maximum shoot length at a 200mM concentration. Similar results were observed by Habib et al.2016 and Chaturvedi et al.2018).

S.N.	Treatments	50mM	100mM	150mM	200mM
1	Bacillus paralicheniformis	14.4	18.3	16.5	16.8
2	Thalassobacillus devorans	8.63	17.6	14.13	7.3
3	Pseudalkalibacillus salsuginis	15.66	10.53	15.76	19.16
4	Control	12.37	10.76	6.81	3.21

Table 2 shows the effect of salt stress on shoot length of cowpea with PGPR.

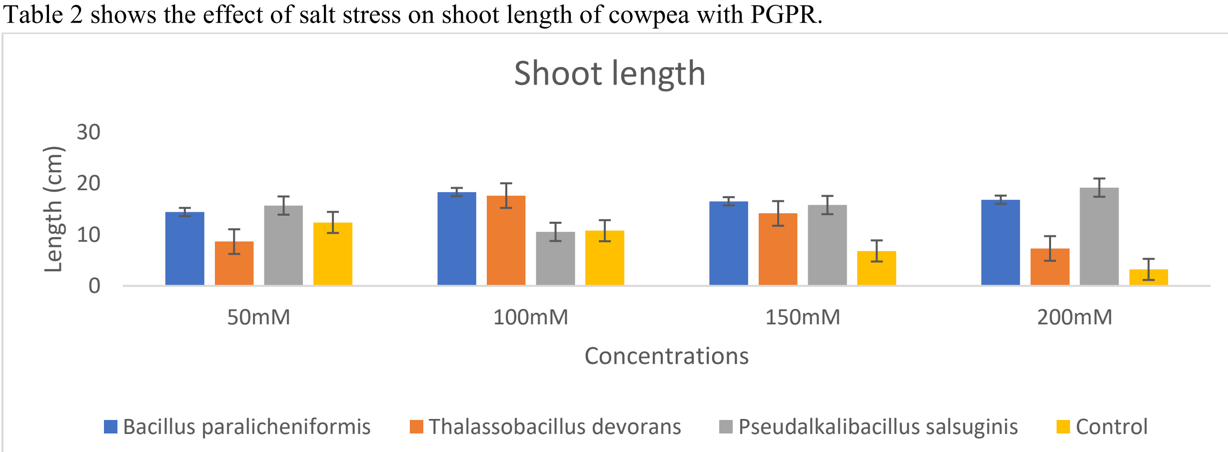

Fig.2 shows the effect of salt stress on shoot length of cowpea with PGPR.

3) Root Length

Due to the sensitivity of the root meristem and elongation zones to salt-induced osmotic and oxidative stress, the detrimental effects of salt on root length are frequently more noticeable than on shoot length. Treatment 3 showed maximum root length in all concentrations of NaCl (Table 3). Research on nine cowpea genotypes under 0, 50, 100, and 200 mM NaCl also showed that root length declined dramatically as the salt concentration increased, with genotypes varying in the degree of reduction, suggesting genotypic heterogeneity in salt tolerance (Gogile et al., 2013).

S.N.	Treatments	50mM	100mM	150mM	200mM
1	Bacillus paralicheniformis	1.06	1.53	0.73	1.16
2	Thalassobacillus devorans	1.03	1.06	2.63	0.66
3	Pseudalkalibacillus salsuginis	1.83	1.13	2.33	2.23
4	Control	0.96	1.86	1.22	2.22

Table 3 shows the effect of salt stress on root length of cowpea seedlings with PGPR.

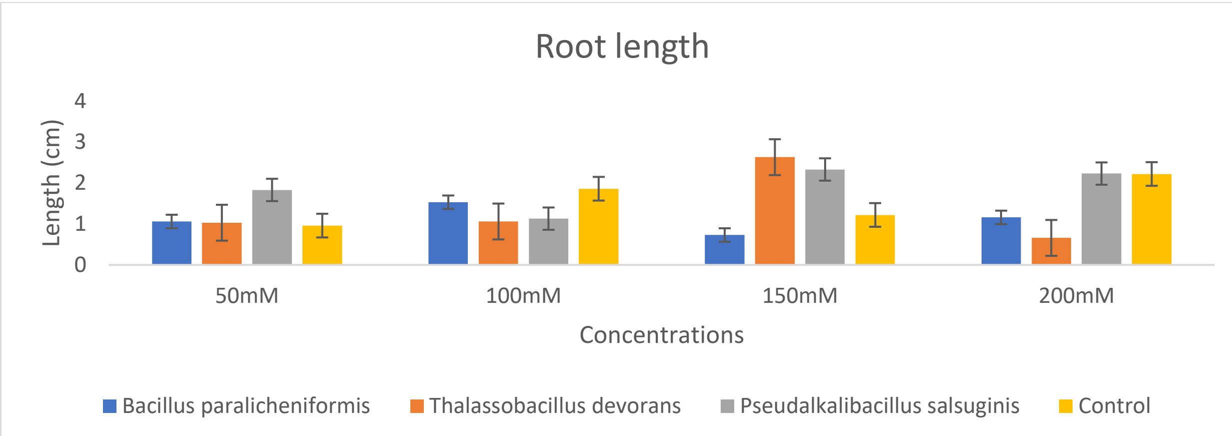

Fig.3 shows the effect of salt stress on root length of cowpea seedlings with PGPR.

4) Fresh weight and Dry weight

Fresh and dry weight of cowpea seedlings significantly declined as the concentration of salt increased, but with microbial treatment, fresh and dry weight increased in comparison to control (Table 4,5). According to Taher et al. (2022), fresh weight of cowpea shoots and roots continuously decreases as salinity rises because of osmotic stress, which restricts water absorption, and ionic toxicity, which damages tissues. PGPR helps to overcome the effect of osmotic stress and promote plant biomass. Similar results were observed by Manaf et al. (2015).

S.N.	Treatments	50mM	100mM	150mM	200mM
1	Bacillus paralicheniformis	0.395	0.653	0.627	0.619
2	Thalassobacillus devorans	0.465	0.623	0.591	0.724
3	Pseudalkalibacillus salsuginis	0.386	0.527	0.523	0.487
4	Control	0.318	0.379	0.22	0.316

Table 4 shows the effect of salt stress on fresh weight of cowpea seedlings with PGPR.

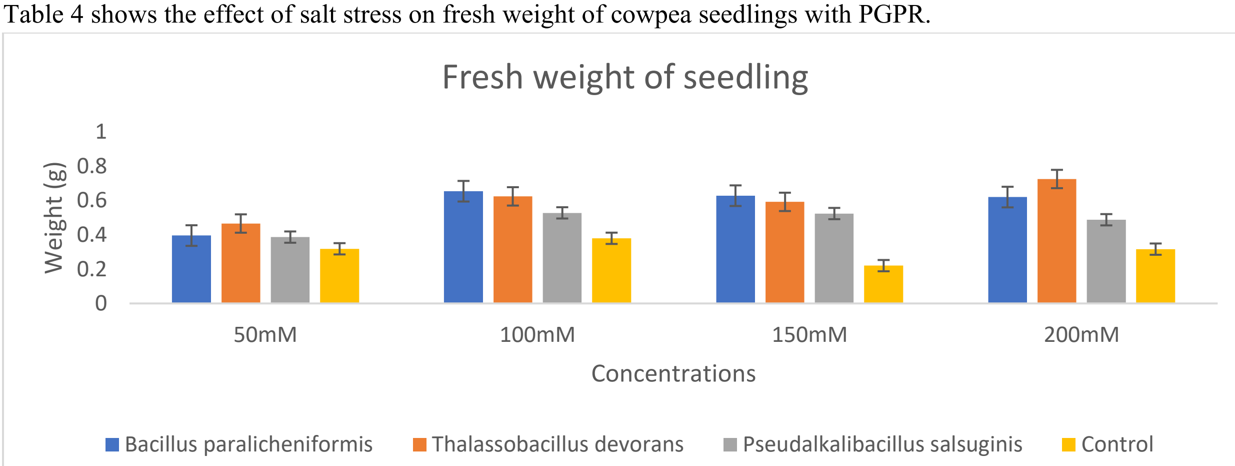

Fig.4 shows the effect of salt stress on fresh weight of cowpea seedlings with PGPR.

S.N.	Treatments	50mM	100mM	150mM	200mM
1	Bacillus paralicheniformis	0.41	0.421	0.351	0.348
2	Thalassobacillus devorans	0.52	0.381	0.297	0.546
3	Pseudalkalibacillus salsuginis	0.36	0.226	0.264	0.216
4	Control	0.063	0.156	0.121	0.112

Table 5 shows the effect of salt stress on dry weight of cowpea seedlings with PGPR.

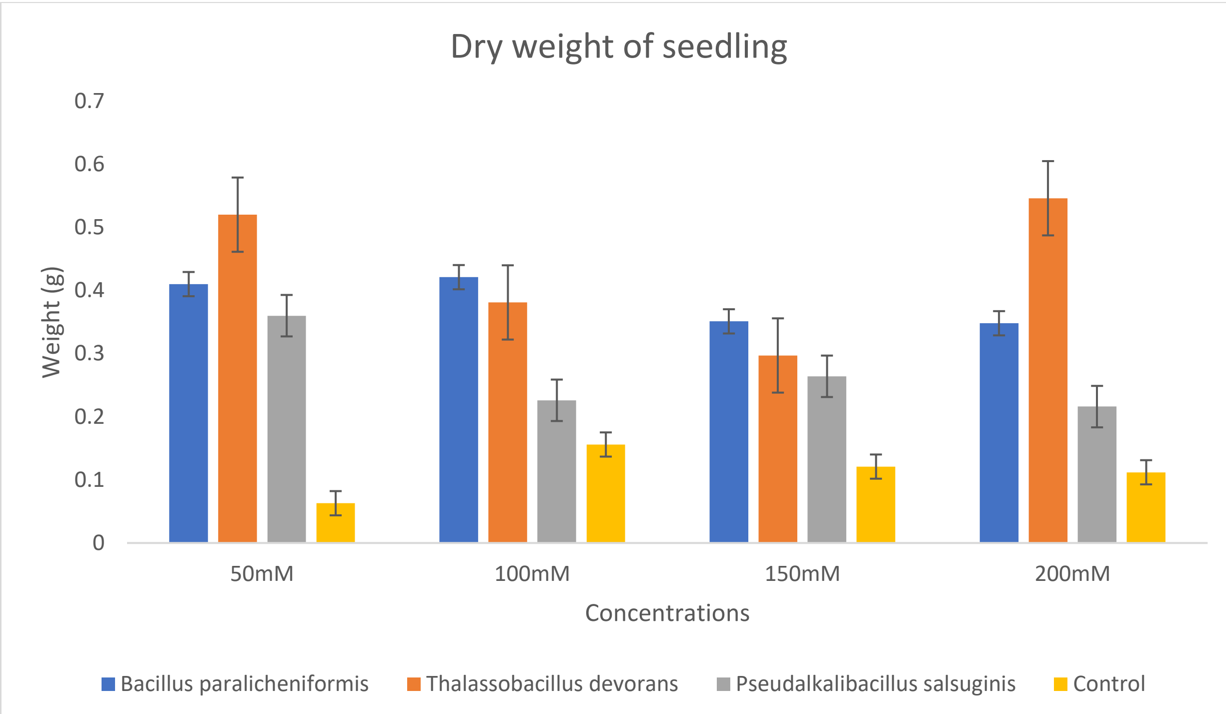

Fig.5 shows the effect of salt stress on dry weight of cowpea seedlings with PGPR.

5) α Amylase enzyme

In this study, alpha amylase activity declined as the concentration of NaCl increased. Alpha-amylase activity in cowpea seeds and seedlings often declines under salt stress, which impedes early growth and delays or reduces germination. The primary causes of this reduction are ionic toxicity, which affects enzyme synthesis and function, and osmotic stress, which limits water uptake. According to Atta et al. (2023), salt stress slows down the breakdown of starch into the sugars needed for seedling growth by delaying water absorption, which in turn lowers alpha-amylase activity. With microbial treatment, alpha amylase activity increased significantly as compared to control. PGPR produce or stimulate plant hormones, increase antioxidant enzyme activity, and help in osmotic adjustment that upregulate alpha-amylase synthesis during germination, counteracting salt-induced suppression of enzyme activity (Ilangumaran et al. 2017; Kumawat et al. 2023). Treatment 1 showed maximum alpha amylase activity at all salt concentrations.

S.N.	Treatments	50mM	100mM	150mM	200mM
1	*Bacillus paralicheniformis*	0.064	0.034	0.038	0.049
2	*Thalassobacillus devorans*	0.065	0.072	0.041	0.018
3	*Pseudalkalibacillus salsuginis*	0.057	0.036	0.042	0.034
4	Control	0.038	0.022	0.019	0.012

Table 6 shows the effect of salt stress on alpha amylase activity of cowpea seedlings with PGPR.

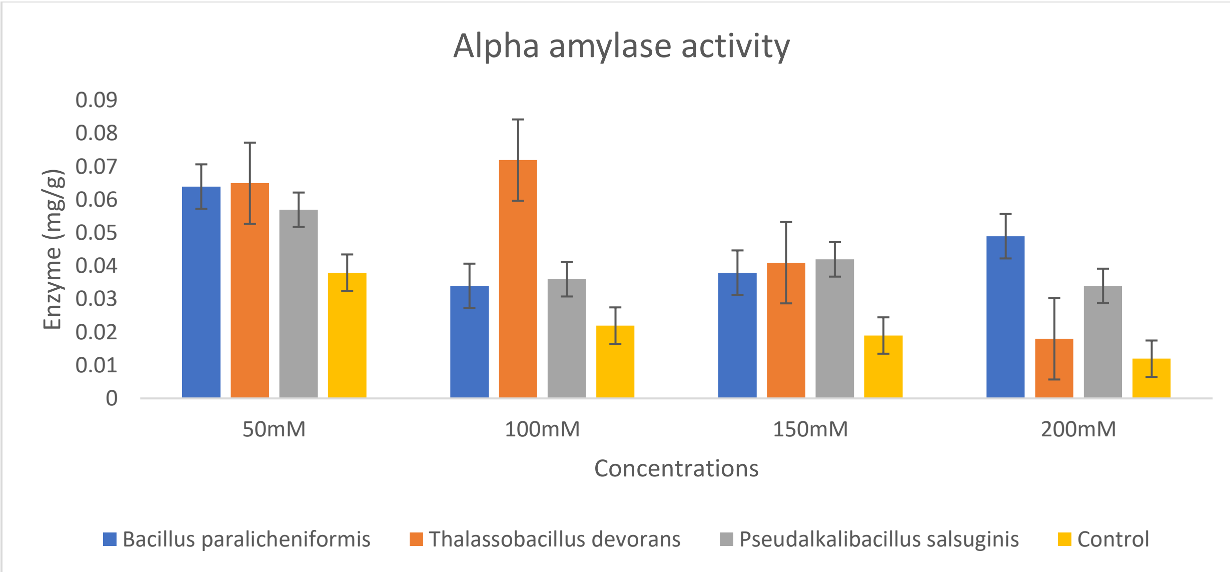

Fig.6 shows the effect of salt stress on alpha amylase activity of cowpea seedlings with PGPR.

6) Phytase enzyme

Phytase activity declined as the concentrations of salt increased. According to Sommer et al. (2024), phytase activity was decreased at greater salinities, which was associated with delayed metabolic progress and impeded seedling growth. It initially increased or maintained moderate levels under mild salt stress. Phytase activity was increased with microbial treatments. In this study, treatment 2 showed maximum phytase activity. According to EI Ifa et al. (2024), phytase-producing PGPR enhances barley growth under phosphate-limited and saline conditions.

S.N.	Treatments	50mM	100mM	150mM	200mM
1	Bacillus paralicheniformis	0.113	0.094	0.056	0.032
2	Thalassobacillus devorans	0.119	0.087	0.063	0.036
3	Pseudalkalibacillus salsuginis	0.22	0.145	0.112	0.28
4	Control	0.094	0.042	0.028	0.011

Table 7 shows the effect of salt stress on phytase enzyme activity of cowpea seedlings with PGPR.

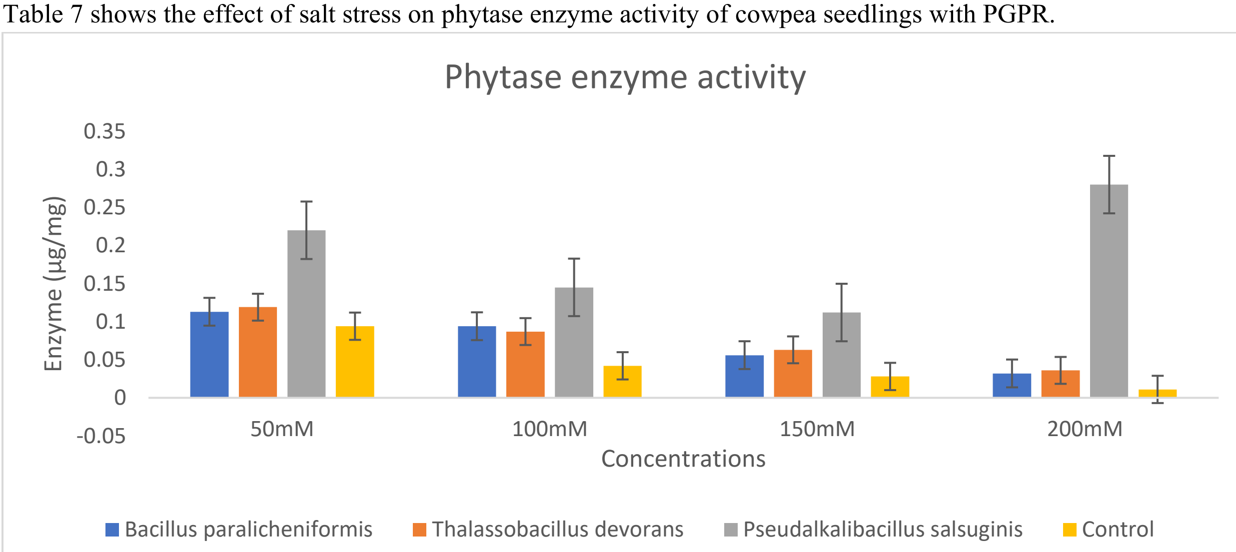

Fig.7 shows the effect of salt stress on phytase enzyme activity of cowpea seedlings with

IV. CONCLUSION

In this study, we observed that increased concentration of salt declines the overall growth of cowpea. Bacterial cultures *Thalassobacillus devorans, Bacillus paralicheniformis,* and Pseudalkalibacillus salsuginis can overcome the effect of salt stress at a higher level (200mM). These cultures are halophilic bacteria can be used as an effective biofertilizer to improve cowpea growth under salt stress. It's an eco-friendly and reasonable technique to overcome the effect of salt stress.

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