Salinity Stress Response of Halotolerant *Bacillus Licheniformis* NJ04 and its Role in Improving the Growth Parameters of Chick Pea (*Cicer Arietinum L.*) in Salt-Stressed soil

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Abstract

Soil salinity possesses a significant challenge to crop productivity, especially in arid and semi-arid regions, as it is a major abiotic stress factor. This research delves into the response of the halotolerant bacteria *Bacillus licheniformis* NJ04 to salinity stress and its capacity to enhance the growth of chickpeas (*Cicer arietinum* L.) in salt-affected soil. To comprehensively understand the underlying mechanisms, stress response of *B. licheniformis* NJ04 was studied prior to exploring its effect on improving the growth of chickpeas. The findings of this study revealed that NJ04 strain displayed high resilience to salinity levels when subjected to salt stress and *B. licheniformis* NJ04 strain exhibited heightened production of osmo-protectants, highlighting its adaptive strategies to cope with salinity. Furthermore, studies on chickpea seeds sown in salt-stressed soil along with *B. licheniformis* NJ04 as liquid bioinoculant, mitigated the adverse impacts of salt stress on chickpea growth. *C. arietinum* plants were able to grow and show tolerance to NaCl salt in the soil up to 250 mM). The root length and shoot length of control plants (at 250mM salt stress) were found to be 10.82 ± 0.76 cm and 17.32 ± 0.48 cm respectively. Whereas, the root length and shoot length of test plants were 16.43 ± 0.43 cm and 19.56 ± 0.32 cm respectively. These findings demonstrate the ability of *B. licheniformis* NJ04 to thrive and sustain metabolic functions even in saline environments and hold significant implications for sustainable agricultural practices in regions plagued by soil salinity, offering a promising strategy to alleviate the detrimental effects on crop productivity.

Keywords: *Bacillus licheniformis* NJ04; *Cicer arietinum* L.; Halotolerant; Plant Growth Promoting bacteria

Introduction

Bacillus is a large genus of bacteria with a wide range of physiological traits that enable them to thrive-in and adapt to various habitats. One such trait is halotolerance, which is the ability of organisms to survive in salt-stressed conditions employing several mechanisms (Castaldi et al., 2023). When *Bacillus* spp. are put in high salt-stress, they exhibit mechanisms like regulating ion transporters and pumps, the influx and efflux

of ions like (Na+) and (K+) are regulated, therefore maintaining the homeostasis of ions and avoiding piling up of sodium ions (ZakaviAskari and Shahrooei, 2022). Production of osmo-protectants is another mechanism exhibited by *Bacillus* spp. to combat halotolerance. Compatible solutes like proline and glycine betaine aid to keep osmotic balance intact in the cells (Yasmin *et al.,* 2020). The bacterial cell wall integrity is maintained by modifying the structure of peptidoglycan or teichoic acid when exposed to high salt stress. Activation of regulatory genes like SigB regulon involves regulating osmotic stress responses. *Bacillus* spp. also exhibit improved DNA repair mechanisms when bacterial DNA is damaged under stress conditions. Production of chaperon proteins to assist the correction of folding in other proteins under stressed conditions (Ali et al., 2022a). Biofilm formation and metabolic adjustments are other important mechanisms observed in *Bacillus* spp. to withstand the harmful effects due to salt stress. Through these mechanisms *Bacillus* spp. maintains the cell integrity and continues to function normally. Such *Bacillus* spp. with plant growth promoting activity can be used as a biofertilizer in salt-stressed soil to improve the growth and yield in plants (Bhutani et al., 2022).

Chickpeas are the third most majorly produced pulse crop in the world. With an average output of 638 million metric tonnes between the years of 2006 and 2009, India is the world's top producer of chickpeas, producing 66% of the total. Growing chickpeas is eco-friendly and is a significant crop with great economical value for farmers. They are also exported internationally, which benefits the country's economy and trade on a worldwide scale (Ali et al., 2022b). Many countries of South Asia, Middle East and Africa use chickpeas as a common part of the food. Chickpeas are rich in fibres, vitamin B, proteins, iron, magnesium and phosphorus etc. They are also a crucial food source low in fat and supply necessary amino acids required. Belonging to the legume family, chickpeas work symbiotically with bacteria that have nitrogen fixing ability and increase the nitrogen availability in the soil. Chickpeas broaden the variety of foods in diets and fight malnutrition to promote a balanced, varied diet (Masmoudi *et al*., 2021).

Chickpeas are known for their drought resistance therefore can be grown even in dry regions. However, when chickpeas are grown in salt stressed land they are adversely affected and undergo reduced germination, stunted growth, damage of leaves and stem, reduced yield, ion toxicity, increase in susceptibility to diseases, reduced nutrient uptake, reduction in photosynthesis leading to yellowing of leaves(Yoo *et al*., 2019). To mitigate the negative effects methods like crop rotation, developing salt stressed seeds, soil leaching, adding organic matter like gypsum are utilised.

By investigating the interplay between microbial adaptation strategies and plant growth enhancement, this study aims to shed light on the multifaceted mechanisms through which *Bacillus licheniformis* NJ04 mitigates the adverse effects of salinity stress on Chickpea (Albdaiwi *et al*., 2019). Insights derived from this research could pave the way for innovative and eco-friendly strategies to bolster crop productivity in salt-stressed agricultural landscapes, offering a beacon of hope for farmers facing the issue of soil salinity. As such, a comprehensive understanding of the salinity stress response of halotolerant *B. licheniformis* NJ04 and its role in improving Chickpea growth represents a significant stride towards sustainable and resilient agriculture in an increasingly challenging environment (Hossain *et al*., 2023).

Materials and Methods

Salinity stress response of *Bacillus licheniformis* NJ04

The plant growth promoting rhizobacteria utilised in the study was previously isolated from the rhizosphere of Coffea arabica plant from Kodagu district, Karnataka and identified as halotolerant *B. licheniformis* NJ04 (Accession number OM780221) by gram staining, biochemical and molecular characterisation (James *et al*., 2023).

Test for proline production

Proline production by bacterial cells was accessed using a method described by Bates *et al*. (1973). First, overnight culture of plant growth promoting *B. licheniformis* NJ04 was inoculated and incubated (37°C for 24h) in LB broth, containing tryptone (10g per litre), yeast extract (5g per litre), NaCl (10g per litre), contains distilled water (1 litre). Bacterial cells were obtained (centrifugation at 10000 RPM at 4° C for 10 min) air dried and 1.2 ml of sulphosalicylic acid (3%) was added and centrifuged at 13000 RPM for 10 min at 4° C after centrifugation, 1 ml of supernatant was taken to which 1 ml of ninhydrin followed by 1 ml of glacial acetic acid were added. The contents in the tubes were incubated for 1 hour at 90° C. After an hour, the samples were cooled down rapidly by keeping them on ice cold water followed by addition of 2 ml toluene. The contents were vortexed for 2 min, and the absorbance of the upper phase (which exhibited a wine-red colour) was measured at 520 nm. To establish a standard curve, various concentrations of proline in mg/ml were employed, as per (Azeem *et al.*, 2022).

Test for glycine betaine production

The production of glycine betaine in bacterial cells under stressed conditions was analysed method. In LB broth medium (tryptone, yeast extract, NaCl, and distilled water) with the addition of various NaCl (w/v) concentrations (100 mM, 250 mM, and 500 mM and no stress), bacterial cultures were inoculated and incubated for 24 hours. After incubation, the contents were centrifuged at 10000 rpm at 4°C (10 min) to harvest the cultures. After adding 2N H_2SO_4 (w/v) (1:1 ratio) to further dilute the pellets, sterile distilled water was added. The sample (0.5 ml) was then collected and chilled in ice-cold water for an hour. Iodine mixture containing (0.2 ml) was added (15.7 g of iodine (w/v) and 20 g of potassium iodide (w/v) in 100 ml of distilled water), vortexed and incubated at 4°C (16 hours). Samples were then centrifuged (10,000 rpm for 15 min) and 9 ml of 1,2-dichloroethane (v/v) was added to the obtained pellets and mixed thoroughly by vortexing. The absorbance was measured at 365 nm, after 2 h and 30 min of incubation. The standard curve was made utilising glycine (RadhakrishnanKumar and Raveendran, 2021).

Test for EPS production

The synthesis of exopolysaccharide (EPS) was carried out using the method described in (Kumar *et al*., 2021). All of the salt-tolerant isolates were first cultivated in LB medium (tryptone- 10g (w/v), yeast extract- 5g (w/v), NaCl- 10g (w/v), distilled water- 1L), overnight. Following that, the cultures were inoculated in to tryptic soy broth (TSB) medium (pancreatic digest of casein, soytone- 17 g, glucose - 3 g, NaCl- 2.5g, K_2HPO_4 in 1 L of distilled water), which was treated with no stress and maximal stress (0.5 M NaCl). The flasks were incubated for 5 days at 30°C with 120 rpm stirring. Both the samples' supernatants were obtained by centrifuging them at 15,000 rpm at 4°C (for 20 min). EPS was removed from the supernatant by adding two times as much ice-cold absolute ethanol. The mixture was incubated at 4°C until all of the EPS was precipitated. The precipitated EPS was collected, lyophilized, and kept at room temperature.

Plant growth study on salt stressed soil

Inoculum preparation

B. licheniformis NJ04 was cultured in 100 ml of nutrient broth (peptone, 3 g (w/v), NaCl, 5 g (w/v), yeast extract, 3 g (w/v), and agar, 15 g in 1 L of distilled water). After 24 h, the cells were separated from the media by centrifuging the culture at 6000 rpm at 4 °C (for 10 min). The cells were carefully removed from the media without any cell loss and washed twice in sterile distilled water to remove the medium components. The obtained cells (10^4 CFU ml $^{-1}$) were resuspended in sterile distilled water (100 ml)(James *et al*., 2023).

Treatment of seeds and sowing

Chick pea seeds (MNK variety from University of Agricultural Sciences, Raichur, Karnataka, India) were used in the study. The seeds were coated with 1% (w/v) CMC after surface sterilisation with 0.1% (w/v) $HgCl_2$. These seeds were then coated in bioinoculant and left to soak for 30 min at 120 rpm. Seeds that were soaked in sterile distilled water without the bioinoculant served as control. Both control seeds and test seeds (coated with bioinoculant) were sown in a seedling tray with sterile soil in order to monitor seed germination and growth over a period of 14 days. On the 3rd day after sowing, the salt-stress was induced in the soil by adding NaCl in different concentrations (100mM, 250mM, 500mM and no stress). Root and shoot lengths were measured after 1st and 2nd week after sowing. These parameters were utilised to compare the growth rates of the sample plants and the control plants in soil stressed soil (El-Siddig, 2006).

Statistical analysis

Each test result was run in triplicate and presented as mean ± standard error. At $p<0.05$, the one-way ANOVA with DMRT (Duncan Multiple Range Test) was used to examine the significant difference.

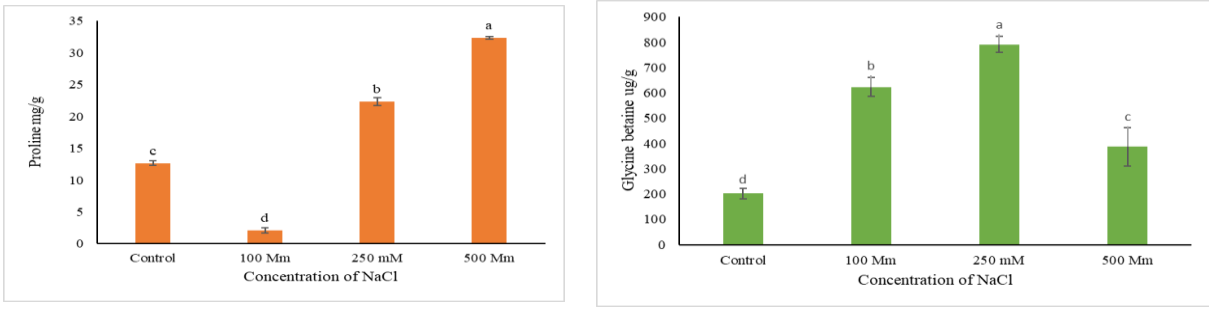
Results

Test for proline production

Proline is an amino acid that is essential for both bacteria and other organisms for stress adaptation and is generally referred to as an osmo-protectant. Bacteria produce proline under stress for osmotic stress regulation, protein stabilisation, Reactive Oxygen Species (ROS) scavenging, energy source and a signalling molecule. A study has reported that *Bacillus* spp. WU-9 produced high levels of proline under salt stress and the growth of *Capsicum annum* was improved when the strain was used as bioinoculant (Wang *et al*., 2018). Another study has reported *B. aryabhattai* to produce proline and stabilise membranes to improve the growth of Cowpea in NaCl stressed land (Abiala and Sahoo, 2022). *B. megaterium* in combination with Mycorrhizae and Brassinosteroids helped improve plant growth in sweet pepper by mitigating salt stress (HegaziEl-Shraiy and Ghoname, 2017). In the present study, *B. licheniformis* NJ04 produced higher levels of proline at 250 mM and 500 mM concentrations of NaCl; that is 22.32 and 32.31 mg/g of proline respectively (Figure 1A).

Test for glycine betaine production

In response to osmotic stress, bacteria produce glycine betaine to maintain cellular integrity and function under stressed environmental conditions. Glycine betaine aids organisms in adjusting to osmotic pressure fluctuations by reversing water loss and avoiding cell dehydration. Bacteria produce glycine betaine under stress for osmotic balance, water retention, protein stabilisation, membrane protection, Reactive Oxygen Species (ROS) scavenging and energy conservation. A study has reported that *B. amyloliquefaciens* along with glycine betaine improved growth in strawberry plants 30% more than control plants (Ntanos *et al*., 2021). Another study has reported that glycine betaine was an effective osmo-protectant in *B. subtilis* (Rath *et al*., 2020). A halotolerant *B.safensis* PM22 strain has reported to produce glycine betaine as an osmo-protectant and improved the growth of maize under salinity stress (Azeem *et al*., 2022). In the current study, *B. licheniformis* NJ04 produced the highest glycine betaine level at 250 mM concentration that is 791.225 μg/g (Figure 1B).



(A) (B)

Figure 1. (A) Test for proline production; (B) Test for glycine betaine production

Test for EPS production

Under stress, bacteria develop a complex matrix of polysaccharides, proteins, nucleic acids, and lipids called EPS (Extracellular Polymeric Substances) that surrounds the bacteria to provide protection. *B. pumilus* strain JPVS11 strain produced EPS to combat salt stress and improved the growth of rice improved soil health under salinity stress (Kumar et al., 2021). A study has reported that *Bacillus* spp. produced high amounts of EPS under salt stress and the growth of *Zea mays* was improved when the strain was used as bioinoculant (Misra and Chauhan, 2020). *B. tequilensis* UPMRB9 was reported to produce EPS as one of the osmo-protectants against salt stress and improves nutrient accumulation in rice varieties (Shultana et al., 2021). In the present study, *B. licheniformis* NJ04 produced 1.02 g of EPS from 100 ml of (10^4 CFU ml^{-1}) bacterial culture.

Plant growth study on salt stressed soil

MNK-1 variety seeds of *C. arietinum* were obtained from UAS, Raichur, Karnataka, India. The seeds coated with microbial inoculant and not coated (control) were sown in soil with salt stress (0, 100, 250, 500 mM of NaCl). The germination and growth parameters of plants were observed for the next 30 days. It was observed that plants from seeds coated with NJ04 strain showed enhanced overall growth than seeds without the bioinoculant coating. Effects like stunted growth, chlorosis, necrosis and reduced number of leaves were observed in plants grown in higher salt levels. In the control set of plants, it was observed that the plants were greener and healthier up-to 100 mM concentration. Whereas, at 250 and 500 mM, stunted growth and yellowing of leaves were observed. However, the plants germinated from seeds coated with NJ04 strain showed better growth up-to 250 mM compared to the control. Similar results were observed in week 2 except that at 500 mM, control plants could not survive the stress whereas the test plants remained healthy till 250 mM concentration (Figure 2).

The week wise changes in root length, shoot length and morphology of both control and test plants at different salt concentrations of NaCl in soil is represented in Table 1. A work by Abd_Allah et al., in 2018 has shown that halotolerant *B. fortis* strain SSB21 has induced salt tolerance in *Capsicum annum* by altering its gene expression and by production of osmolytes (Abd_Allah et al., 2018). Another work on endophytic bacteria *B. subtilis* BERA71 shows improved salt tolerance in chickpea plants by modulating different mechanisms in plants (Yasin et al., 2018). In this work, on week 2 the root length and shoot length of control plants were 10.82 ± 0.76 cm and 17.32 ± 0.48 cm respectively at 250 mM whereas, the root length and shoot length of test plants were 16.43 ± 0.43 cm and 19.56 ± 0.32 cm respectively. Therefore, *Bacillus licheniformis* NJ04 can be used to improve the growth of chickpea and tolerate salt tolerance up to 250 mM.

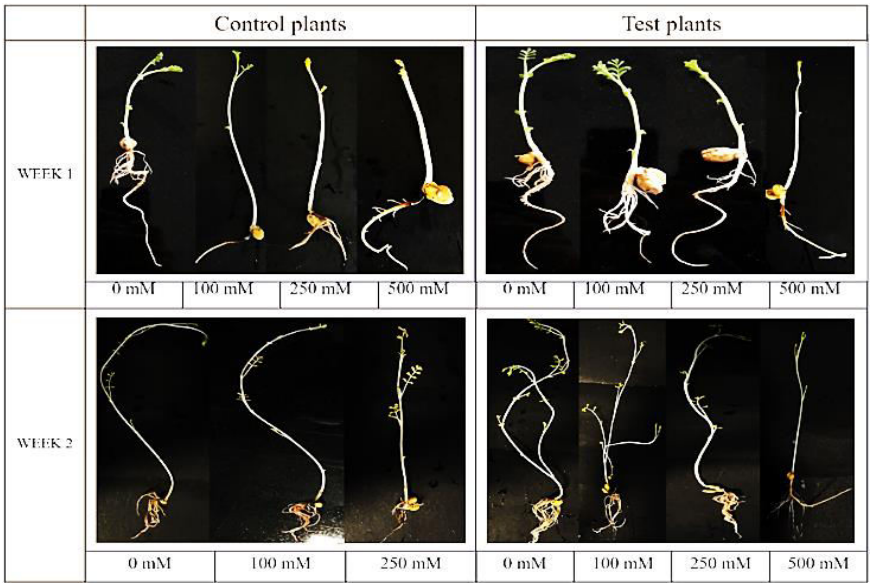


Figure 2. Plant Growth study of *Cicer arietinum*

Table 1: Root length, shoot length and morphology of Chickpea (control and test plants) at different salt concentrations of NaCl

	Control Plants				
		0 mM	100 mM	250 mM	500 mM
Root length in cms	Week 1	9.86 ± 0.75 ^{e,f}	9.23 ± 0.53 ^{e,f}	7.32 ± 0.34 ^{f,g}	5.47 ± 0.67^{g}
	Week 2	14.43 ± 0.34 ^{b,c}	14.00 ± 0.62 ^{b,c,d}	10.82 ± 0.76 ^{d,e}	-
Shoot length in cms	Week 1	7.45 ± 0.24 ^{g,h}	8.14 ± 0.18 ^{f,g}	7.62 ± 0.9 ^{f,g}	5.12 ± 0.87^{g,h}
	Week 2	20.32 ± 0.94 ^{c,d}	21.32 ± 0.27 ^{c,d}	17.32 ± 0.48 ^{d,e}	-
Morphological changes	Week 1	Normal growth	Reduced leaf size	Chlorosis	Chlorosis and Necrosis
	Week 2	Normal growth	Normal growth	Reduced stem size	Complete death of plant
	Test Plants (Seeds coated with *B. licheniformis* NJ04)				
		0 mM	100 mM	250 mM	500 mM
Root length in cms	Week 1	14.43 ± 0.65 ^{b,c}	14.32 ± 0.87 ^{b,c}	14.21 ± 0.76 ^{b,c}	6.32 ± 0.54 ^{f,g}
	Week 2	20.21± 0.28 ^{a}	19.00 ± 0.54 ^{a}	16.43 ± 0.43 ^{a,b}	8.24 ± 0.71 ^{e,f}
Shoot length	Week 1	9.32 ± 0.42 ^{f,g}	8.87 ± 0.11 ^{f,g}	7.32 ± 0.44 ^{g,h}	6.32 ± 0.80^{g,h}

in cms	Week 2	32.54 ± 043[a]	28.56 ± 0.87[a,b]	19.56 ± 0.32[c, d]	11.42 ± 0.34[e, f]
Morphological changes	Week 1	Enhanced root growth	Enhanced growth	Normal growth	Reduced root length and Chlorosis
	Week 2	Enhanced growth	Normal growth	Reduced stem size and count	Reduced overall growth and chlorosis

Conclusion

Bacillus licheniformis NJ04 exhibited a robust tolerance to high salinity levels, showcasing its adaptability to saline environments. This inherent resilience was reflected in its capacity to thrive and maintain critical metabolic activities under salt stress conditions. Inoculation of Chickpea seeds with *B. licheniformis* NJ04 demonstrated a tangible positive impact on chickpea growth parameters under salt stress. The test plants exhibited increased root and shoot lengths, and improved morphological traits compared to non-inoculated controls with significant differences ($p<0.05$). This indicates the potential of *B. licheniformis* NJ04 to alleviate the detrimental effects of salt stress on crop growth and collectively emphasise the promising role of *B. licheniformis* NJ04 as a bioinoculant for enhancing chickpea productivity in salt-stressed soils. The study can be further extended by understanding the stress mechanisms in plants by analysing osmo-protectants produced by plants as well as molecular analysis of their genes associated with stress tolerance. The use of halotolerant bacteria like the NJ04 strain presents a sustainable and environmentally friendly approach to address the challenges posed by soil salinity in agriculture. Ultimately, this study paves way for broader applications of halotolerant microorganisms in crop cultivation practices, offering a tangible solution for farmers in salt-affected regions and contributing to the overall goal of ensuring global food security in the face of changing environmental conditions.

Authors' Contributions

NJ: Execution of wet lab works, original draft preparation, editing and proofreading
MU: Research supervision, conceptualization, proofreading

Acknowledgements

The authors thank the Department of Life Sciences, CHRIST (Deemed to be University), Bangalore for providing necessary facilities for the successful completion of this research work.

Conflict of Interests

Authors do not have any conflict of interests to declare

References

1. Abd_Allah EF, Alqarawi AA, Hashem A, et al. (2018) Endophytic bacterium Bacillus subtilis (BERA 71) improves salt tolerance in chickpea plants by regulating the plant defense mechanisms. *J. Plant Interact.* 13(1): 37-44.
2. Abiala MA and Sahoo L (2022) Bacillus aryabhattai enhanced proline content, stabilized membrane and improved growth of cowpea under NaCl-induced salinity stress. *J. Appl. Microbiol.* 133(3): 1520-1533.
3. Albdaiwi RN, Khyami-Horani H, Ayad JY, et al. (2019) Isolation and Characterization of Halotolerant Plant Growth Promoting Rhizobacteria From Durum Wheat (subsp.) Cultivated in Saline Areas of the Dead Sea Region. *Front. Microbiol.* 10: 1639.
4. Ali B, Hafeez A, Ahmad S, et al. (2022a) PM25 ameliorates oxidative damage of salinity stress in maize regulating growth, leaf pigments, antioxidant defense system, and stress responsive gene expression. *Front. Plant Sci.* 13: 921668.
5. Ali B, Wang X, Saleem MH, et al. (2022b) PM35 Reinforces Photosynthetic Efficiency, Antioxidant Defense, Expression of Stress-Responsive Genes, and Ameliorates the Effects of Salinity Stress in Maize. *Life* 12(2).
6. Azeem MA, Shah FH, Ullah A, et al. (2022) Biochemical Characterization of Halotolerant PM22 and Its Potential to Enhance Growth of Maize under Salinity Stress. *Plants* 11(13).
7. Bhutani N, Maheshwari R, Sharma N, et al. (2022) Characterization of halo-tolerant plant growth promoting endophytic Bacillus licheniformis MHN 12. *J. Genet. Eng. Biotechnol.* 20(1): 113.
8. Castaldi S, Valkov VT, Ricca E, et al. (2023) Use of halotolerant Bacillus amyloliquefaciens RHF6 as a bio-based strategy for alleviating salinity stress in Lotus japonicus cv Gifu. *Microbiol. Res.* 268: 127274.
9. Hegazi AM, El-Shraiy AM and Ghoname AA (2017) Mitigation of salt stress negative effects on sweet pepper using arbuscular mycorrhizal fungi (AMF), Bacillus megaterium and brassinosteroids (BRs). *Gesunde Pflanzen* 69(2): 91-102.
10. Hossain MS, Frith C, Bhattacharyya SS, et al. (2023) Isolation and Characterization of Bacterial Endophytes from Small Nodules of Field-Grown Peanut. *Microorganisms* 11(8).
11. James N, Umesh M, Sarojini S, et al. (2023) Unravelling the potential plant growth activity of halotolerant Bacillus licheniformis NJ04 isolated from soil and its possible use as a green bioinoculant on Solanum lycopersicum L. *Environ. Res.* 216: 114620.
12. Kumar A, Singh S, Mukherjee A, et al. (2021) Salt-tolerant plant growth-promoting Bacillus pumilus strain JPVS11 to enhance plant growth attributes of rice and improve soil health under salinity stress. *Microbiol. Res.* 242: 126616.
13. Masmoudi F, Tounsi S, Dunlap CA and Trigui M (2021) Endophytic halotolerant Bacillus velezensis FMH2 alleviates salt stress on tomato plants by improving plant growth and altering physiological and antioxidant responses. *Plant Physiol. Biochem.* 165: 217-227.
14. Misra S and Chauhan PS (2020) ACC deaminase-producing rhizosphere competent spp. mitigate salt stress and promote growth by modulating ethylene metabolism. *3 Biotech* 10(3): 119.
15. Ntanos E, Kekelis P, Assimakopoulou A, et al. (2021) Amelioration Effects against Salinity Stress in Strawberry by Bentonite–Zeolite Mixture, Glycine Betaine, and Bacillus amyloliquefaciens in Terms of Plant Growth, Nutrient Content, Soil Properties, Yield, and Fruit Quality Characteristics. *NATO Adv. Sci. Inst. Ser. E Appl. Sci.* 11(19): 8796.
16. Radhakrishnan EK, Kumar A and Raveendran A (2021) *Biocontrol Mechanisms of Endophytic Microorganisms.* Academic Press.
17. Rath H, Sappa PK, Hoffmann T, et al. (2020) Impact of high salinity and the compatible solute glycine betaine on gene expression of Bacillus subtilis. *Environ. Microbiol.* 22(8): 3266-3286.

18. Shultana R, Kee Zuan AT, Yusop MR, et al. (2021) Bacillus tequilensis strain 'UPMRB9' improves biochemical attributes and nutrient accumulation in different rice varieties under salinity stress. *PLoS One* 16(12): e0260869.
19. Wang W, Wu Z, He Y, et al. (2018) Plant growth promotion and alleviation of salinity stress in Capsicum annuum L. by Bacillus isolated from saline soil in Xinjiang. *Ecotoxicol. Environ. Saf.* 164: 520-529.
20. Yasin NA, Akram W, Khan WU, et al. (2018) Halotolerant plant-growth promoting rhizobacteria modulate gene expression and osmolyte production to improve salinity tolerance and growth in Capsicum annum L. *Environ. Sci. Pollut. Res. Int.* 25(23): 23236-23250.
21. Yasmin H, Naeem S, Bakhtawar M, et al. (2020) Halotolerant rhizobacteria Pseudomonas pseudoalcaligenes and Bacillus subtilis mediate systemic tolerance in hydroponically grown soybean (Glycine max L.) against salinity stress. *PLoS One* 15(4): e0231348.
22. Yoo S-J, Weon H-Y, Song J and Sang MK (2019) Induced Tolerance to Salinity Stress by Halotolerant Bacteria H19-1 and H20-5 in Tomato Plants. *J. Microbiol. Biotechnol.* 29(7): 1124-1136.
23. Zakavi M, Askari H and Shahrooei M (2022) Maize growth response to different Bacillus strains isolated from a salt-marshland area under salinity stress. *BMC Plant Biol.* 22(1): 367.