

Original Research Article

**Evaluation of Plant growth promoting and Salinity alleviating potential of Halophiles
isolated from marine water**

ABSTRACT

Aim: This study assesses the potential of Halophiles from marine water to promote plant growth and alleviate salinity effects. Salinity is a significant abiotic stress impacting plant growth and agricultural productivity globally. Halophiles are microorganisms that thrive in high saline environments, exhibiting unique adaptations to survive under extreme conditions.

Study Design: To test the hypothesis, Halophiles microorganisms were isolated from marine water samples, and their ability to tolerate high salinity was assessed using increasing salt concentrations. Subsequently, we assessed the plant growth promoting traits of the selected halophiles strains, including phosphate solubilization, siderophore production, indole-3-acetic acid (IAA) synthesis, and ammonia production.

Place and Duration of Study: Dr. D. Y. Patil Arts, Commerce and Science College, Pimpri, Pune- 411018 December 2022 to March 2023

Methodology: We evaluated plant promoting traits of Halophiles and also their ability to promote seed germination. In pot experiments, wheat plants were grown under controlled salinity conditions and inoculated with selected halophile strains on their roots. Key growth parameters, including shoot length and root length, were measured and contrasted with those of control plants that did not receive microbial inoculation.

Result: Three isolates (A1, B1, and C4) with plant growth promoting factors were tested for their ability to alleviate salinity in sterile soil. After inoculation and a 7-day incubation period, soil samples showed a reduction in salinity, indicating the isolates' potential for salinity alleviation. Our results demonstrated that the isolates possessed significant plant growth promoting traits and were able to alleviate salinity stress in the tested plant species.

Conclusion: This study demonstrates that halophiles from marine environments may help alleviate salinity stress in plants and enhance growth, suggesting their potential use in sustainable agriculture in saline regions. Further research is needed to investigate the mechanisms and assess these halophiles in field conditions.

Keywords: Halophiles, Marine water, Plant growth, Alleviate salinity, Abiotic stress

INTRODUCTION

Abiotic stressors like salt, drought, temperature, and others have had a negative impact on agriculture. These are significant obstacles to sustainable agriculture worldwide [1]. The main abiotic stress factors that are of great importance include light, temperature, drought, salt, soil, air, and water pollution. Plant growth hormones and metabolites are found to be significantly reduced by high nighttime temperatures. Additionally, temperature stress affects rice cultivars via changing antioxidant activity and ROS generation [2]. Soil salinization poses a significant danger to productivity, food security, and economic development by diminishing the net cultivable area [3]. Arid and semi-arid environments are more susceptible to salinization due to

elevated evaporation rates and diminished freshwater availability to leach salts [4]. By 2050, high salinity will have impacted 33% of irrigated agricultural land and about 20% of all farmed areas. More than half of the anticipated arable areas will be salinized [5]. Worldwide, salinity, alkalinity, and acidification damage about 23 million acres of land. An estimated 6.73 million hectares, or numerous states, in India are covered with salt-affected soil [6,7].

Saline soils (around 40 mM NaCl) at 25°C with 15% exchangeable sodium are defined as having high salt concentrations (i.e., electrical conductivity (EC) > 4 dS/m) [8]. All phases of plant development, including germination, vegetative growth, and reproductive development, are hampered by salinity. It increases ovule abortion, senescence of fertilized embryos, and programmed cell death in some tissue types [9]. Ion toxicity, low osmotic potential and nutritional deficiencies (N, Ca, K, P, Fe, and Zn) are all caused by salinity in the soil [10]. Many mitigating techniques are developed for sustainable agriculture since salt stress situations have detrimental effects on plant growth and cause significant losses. Microorganisms that stimulate plant development are organic strategies to counteract salt stress [11].

Halophiles are organisms that thrive in high-salt environments by regulating osmotic pressure to withstand the denaturing effects of salts. Halophiles are microorganisms found in all three domains of life: Archaea, Bacteria, and Eukarya. Examples include the alga *Dunaliella salina* and the fungus *Wallemia ichthyophaga*. These organisms exhibit diverse physiological types that enable them to thrive in various salt concentrations [12]. Plant growth-promoting rhizobacteria (PGPR) are known to stimulate plant growth in a number of ways [13]. One of the best ways to lessen the impact of salinity on plants is through PGPR. By creating biofilms, several microorganisms from different genera have been shown to improve plant growth and survival in saltwater environments [14]. The present study evaluated plant growth promoting characteristics of halophilic bacteria isolated from the marine water of coastal region of Arabian Sea, Mumbai.

MATERIALS AND METHODS

Collection of Sample

Water sample was collected in sterile bottle from the coastal region of Arabian Sea, Mumbai and stored at 4°C in the laboratory until used for isolation of the strain [15].

Enrichment and isolation of bacteria

Enrichment cultures for isolating halophilic microorganisms were conducted using nutrient broth with NaCl concentrations of 5%, 10%, and 15%. Media was autoclaved at 121 °C for 20 minutes and cooled. 1ml water sample was inoculated into each broth and incubated at 37°C for 48 hours. The enriched culture was serially diluted to 10⁻⁹, and the last five dilutions were plated on nutrient agar with corresponding NaCl concentrations, and then incubated at 37°C for one week. Various colonies, identified by color, were isolated and purified on nutrient agar and subsequently preserved on NA slants with the same NaCl concentration [16].

Evaluation of Plant growth promoting activities of Halophiles

Phosphate solubilization

Sterile Pikovskaya's agar medium with 0.5% calcium tri phosphate was prepared and inoculated with overnight grown culture isolates. After incubation at 37°C for 7 days, a clear zone around the isolates indicated successful phosphate solubilization [17].

Production of ammonia

Loopful overnight grown cultures were inoculated in 5 ml sterile peptone water tubes and incubated at 37°C for 48 hours. After incubation, the addition of 0.5 ml of Nessler's reagent resulted in a yellow color, indicating positive ammonia production [18].

Production of IAA

Overnight grown cultures were inoculated in LB broth with 1% tryptophan and incubated for 48 hours at 37°C. Following centrifugation, 2ml of supernatant was mixed with 4ml of Salkowski reagent, resulting in a pink color that confirmed positive IAA production [19].

Siderophore production

Sterile CAS medium was prepared by mixing 35ml CAS reagent with 100ml Nutrient agar, both autoclaved separately, and allowed to solidify in sterile petri plates. Wells were created using a

sterile cork borer, and 50 μ L of overnight grown culture from each isolate was added to the wells. After 48 hours of incubation at 37°C, three isolates exhibited an orange zone around the wells, indicating positive siderophore production [20].

Seed germination

Red gram seeds were sterilized by surface sterilization, and then dipped in a bacterial suspension for one minute. Control seeds were uninoculated. The plates were autoclaved for sterility and contained tissue paper to maintain moisture. Both inoculated and control seeds were placed in the plates at room temperature and watered daily with distilled water. After 7 days, root and shoot lengths were measured using graph paper [21].

Analysis of soil samples for Electrical Conductivity

A total of four soil samples were collected from various regions of Maharashtra, including one from the Saltpan area of Mankhurd, Mumbai. The samples were placed in sterile polybags and sent to the laboratory for salinity analysis. 1g soil sample was dissolved in 100 ml distilled water, shaken at 120 rpm for 30 minutes, and then analyzed for conductivity to determine salinity [22].

Determination of Salinity Ameliorating Potential of isolates

Three isolates (A1, B1, and C4) were selected for salinity alleviation based on PGP factor evaluation. Fifty grams of sterile soil with known salinity were placed in five sterile petri plates, with one serving as a control with 25 ml of distilled water. Each of the remaining plates received 25 ml of overnight culture from the three isolates and was incubated at room temperature for seven days. Afterward, 1 gram of soil from each plate was sent to a laboratory for salinity analysis [21].

Pot Assay

Wheat seeds were surface sterilized with distilled water. Sterile soil sample of known salinity was taken in pot of same quantity (100gm) each. 10 seeds per pot were sowed. 30 ml of overnight grown bacterial culture was added to each pot. Pots were kept in sunlight and watered after every 24hrs for 14 days. Root and shoot length was calculated after 14 days [21].

RESULTS

Isolation of Halophiles

Fifteen marine water isolates demonstrated varying degrees of salt tolerance; all tolerated up to 5% salt, three grew at 10%, and five at 15% salt concentration. Detailed results are presented in the Table 1 and Fig 1.

Table 1: Number of isolates obtained at varying salt concentration

NaCl in (%)	Dilution	Colony Identification	Colony Obtained
5%	$10^{-5}, 10^{-8}, 10^{-9}$	A1,A2, A3,A4 ,A5,A6,A7	7
10%	$10^{-5}, 10^{-8}$	B1,B2 ,B3	3
15%	$10^{-3}, 10^{-4}, 10^{-9}$	C1, C2, C3, C4, C5	5

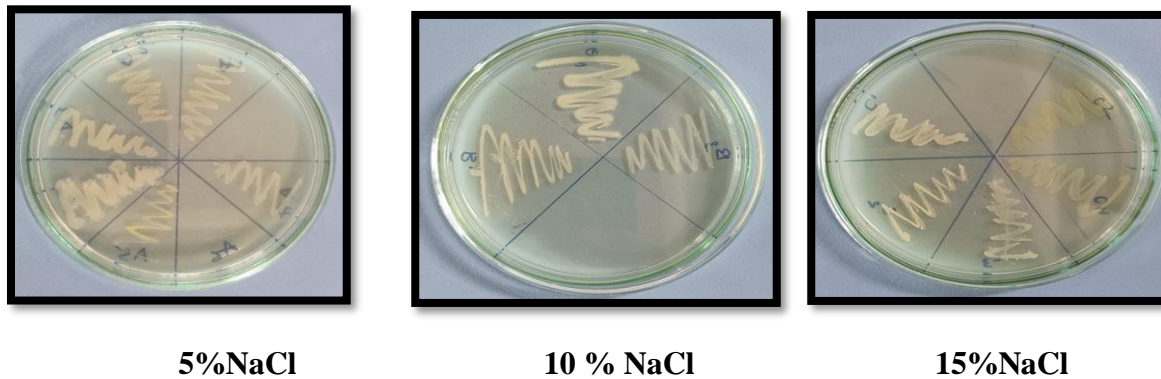


Fig.1: Pure culture plates for isolates

Plant Growth Promoting potential of isolates

A total of 15 isolates were identified at different salt concentrations, and they were screened for plant growth-promoting (PGP) traits, including phosphate solubilization, ammonia production, IAA production, and siderophore production. All isolates exhibiting PGP traits are listed in the accompanying Table 2 and Fig 2, 3, 4 and 5 represent detailed results.

Table 2: PGP traits of the isolate

Sr. No.	Isolates	Phosphate solubilisation	Ammonia production	IAA production	Siderophore production
1	A1	+	+	+	+
2	A2	-	+	+	-
3	A3	-	+	+	-
4	A4	+	-	-	-
5	A5	-	+	-	-
6	A6	-	-	+	-
7	A7	+	+	+	-
8	B1	+	+	+	+
9	B2	-	+	+	-
10	B3	+	+	+	-
11	C1	+	-	+	-
12	C2	+	+	+	-
13	C3	+	+	+	-
14	C4	+	+	+	+
15	C5	-	-	+	-

+ : positive - : negative



5% NaCl

10% NaCl

15%NaCl

Fig.2. Zone of clearance around phosphate solubilisation isolates.

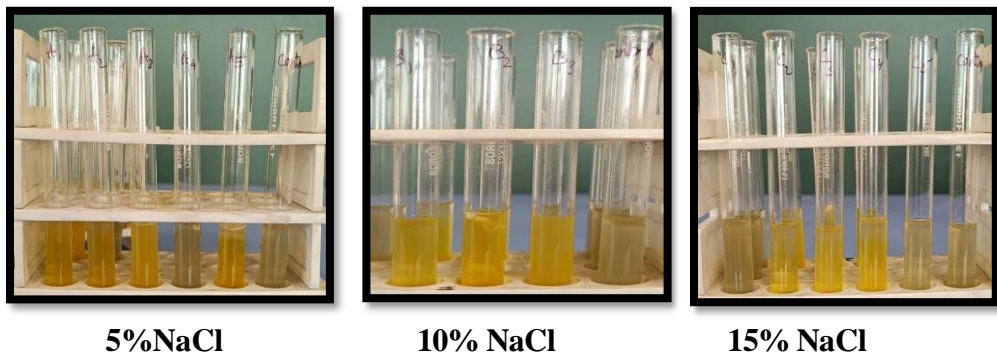


Fig.3: Development of yellow color by Ammonia producing isolates

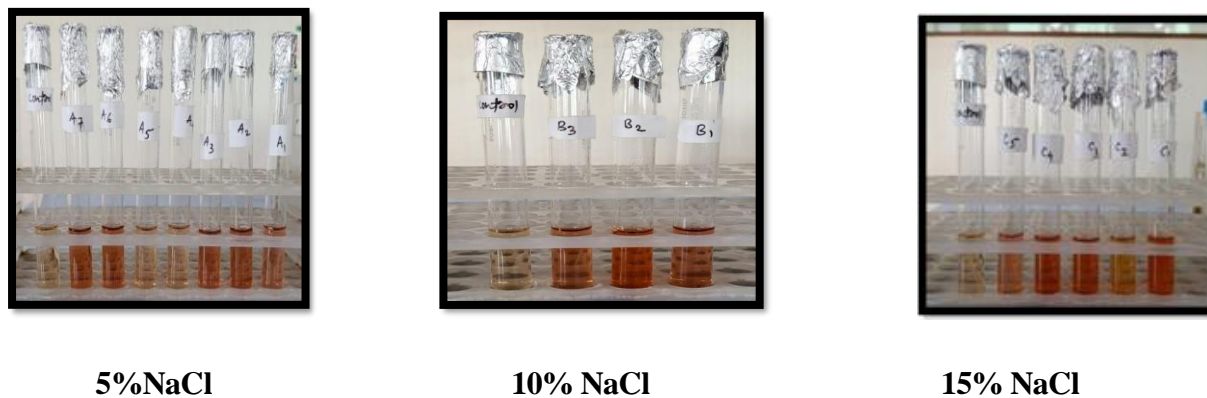


Fig.4 : Development of pink in tubes by IAA producing isolates.

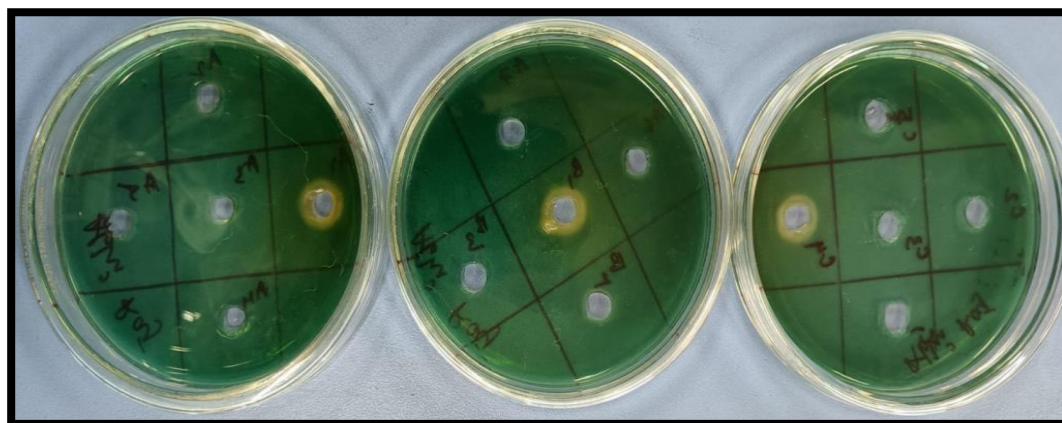


Fig.5: Isolates (A1, B1, C4) showing Siderophore producing isolates.

Seed germination

It appears that isolate A1 had the most significant impact on both shoot and root length, followed by the consortium and isolate Control. Isolate B1 and C4 had comparatively lower effects on the growth of red gram seedlings as shown in Table 3 and Fig 6 and 7.

Table 3: Table featuring the shoot and root length of Red gram seeds.

Isolates	Shoot length (cm)	Root Length (cm)
A1	8.0	12
B1	4.0	7.0
C4	5.5	6.0
Control	5.0	9.0
Consortium	7.0	9.0

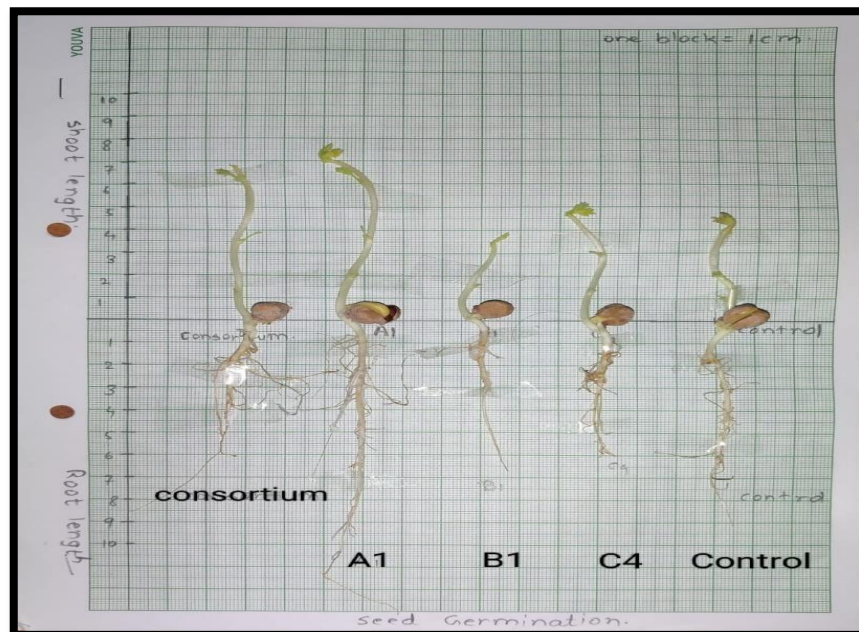


Fig. 6: Shoot and root length of Red gram seeds after 7 days.

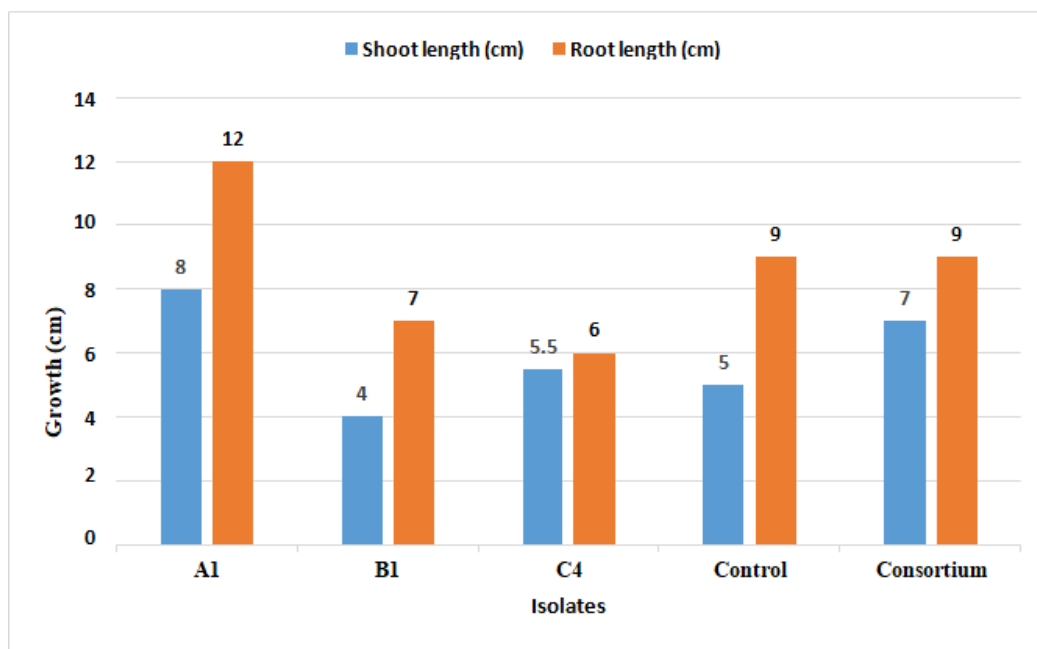


Fig.7: Graphical representation of Shoot and root length of Red gram seeds after 7 days

Analysis of soil samples for Electrical Conductivity

The results indicate the level of salinity in the respective soil samples. The Mankhurd soil sample shows significantly higher salinity compared to the other three samples collected from Kamsheth, Pali, and Pune. The higher electrical conductivity in the Mankhurd sample suggests elevated salt content, potentially due to its location in the Saltpan area as shown in Table 4.

Table 4: Report for soil Analysis

Sr. NO.	Sample Name	Salinity in terms of Electrical conductivity (1:5 solution) (MilliSiemens per cm)
1	KAMSHETH	0.052
2	PALI	0.101
3	PUNE	0.102
4	MANKHURD	14.90

Determination of Salinity Ameliorating Potential of isolates

The results indicate that the consortium of A1, B1, and C4 isolates demonstrated the most significant reduction in soil salinity compared to the control and individual isolates. The consortium showed the highest potential for salinity amelioration in the tested soil samples as shown in Table 5 and Fig 8.

Table 5: Analysis report of soil salinity

Sr. No.	Isolates	Salinity in terms of Electrical conductivity (1:5solution) (MilliSiemens per cm)
1	A1	6.55
2	B1	4.95
3	C4	5.76
4	Control	6.78
5	Consortium	3.78

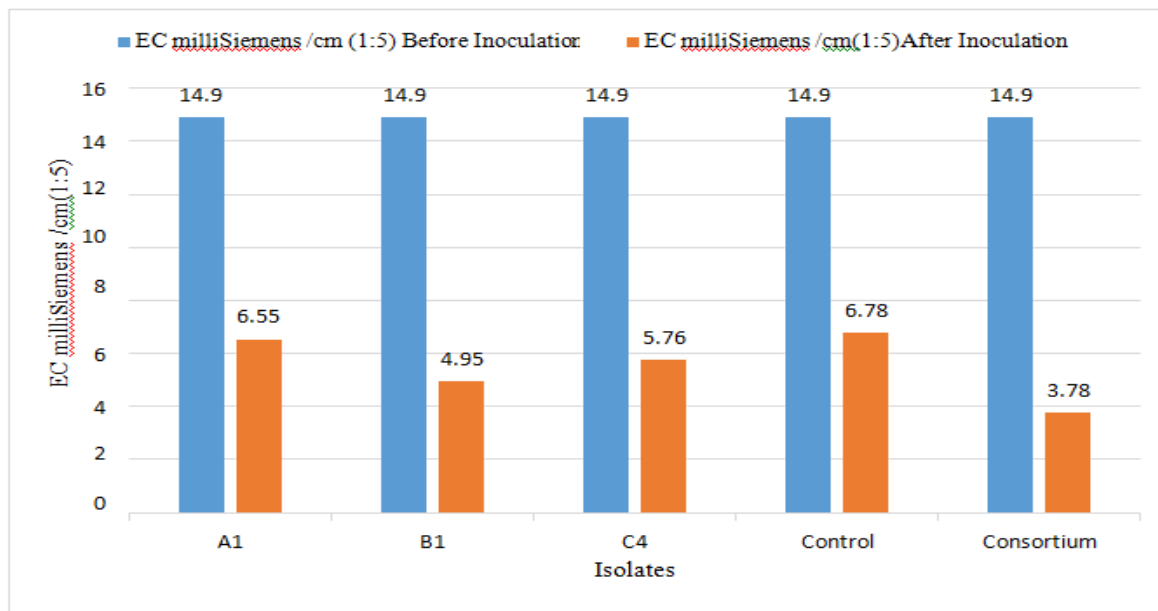


Fig. 8: Graphical representation of Salinity reduction in terms of Electrical conductivity (1:5 solution) (MilliSiemens per cm)

Pot Assay

These results indicate the growth-promoting effects of the tested isolates and consortium on the wheat plants. The isolates and the consortium showed improvement in shoot and root length compared to the control. However, the specific effects on plant growth may vary depending on other factors and experimental conditions as shown in Table 6 and Fig. 9 and 10.

Table 6: Table featuring the shoot and root length of wheat seeds after 14 days.

Isolates	Shoot length(cm)	Root Length (cm)
A1	15.0	7.00
B1	16.5	7.00
C4	17.0	6.00
Control	13.0	5.00
Consortium	15.0	7.00

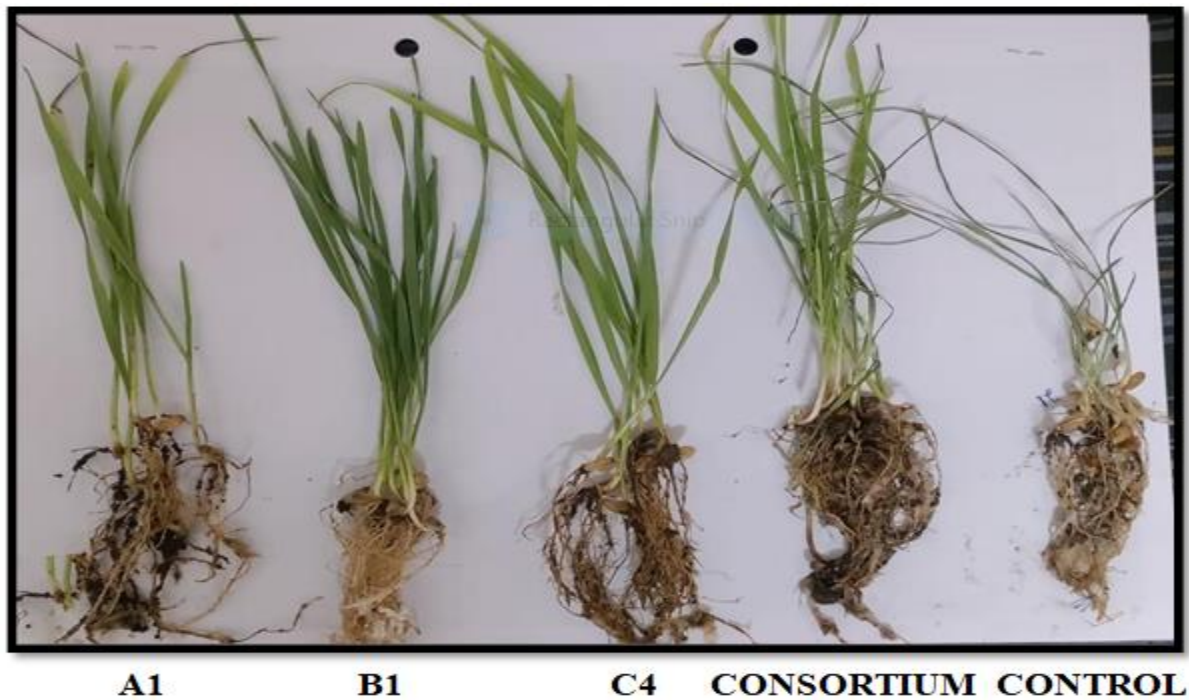


Fig. 9: Root and shoot length of the plants in response to salt stresses (NaCl) inoculated with different bacterial isolates.

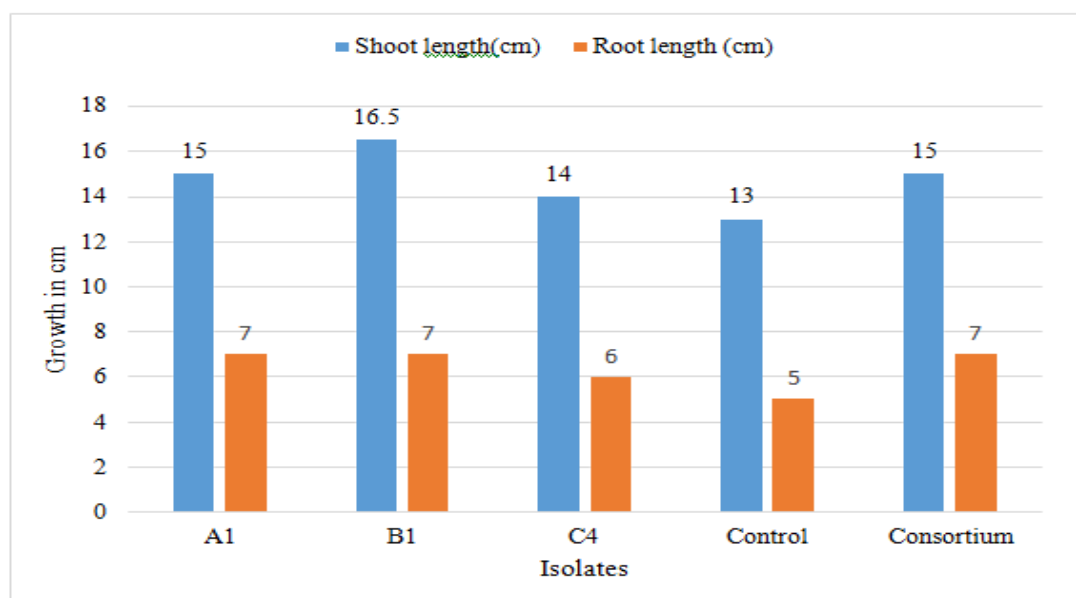


Fig. 10 Graphical representation shoots and root length of wheat seeds after 14days inoculated with different bacterial isolates.

DISCUSSION

In developing nations and heavily exploited agricultural areas, soil salinization frequently occurs, limiting plant growth and agricultural productivity by as much as 50% [23]. In this study, 15 bacterial strains were isolated from a marine water sample collected from the Arabian Sea near Mumbai. Several plant growth-promoting activities, which include phosphate solubilization, ammonia production, indole-3-acetic acid (IAA) production, siderophore production, pot assays, and seed germination [24], were investigated.

The formation of clear zones indicated the solubilization of phosphates by the isolates. This suggests that P-solubilization occurs through the secretion of substances during growth that can solubilize phosphate or organophosphate. Results of phosphorous solubilization vary based on the type of metabolin, its release rate, and its spread on the medium. Thus, the observational method for P solubilizing zones is only suitable for qualitative assays [25].

Siderophores are produced in iron-deficient environments by bacteria to sequester and solubilize iron in the extracellular space. The isolates studied were unable to produce siderophores, likely due to sufficient iron availability in their environments [26]. Additionally, research indicated that

in halophiles, there is a reduced need for iron and histidine, along with decreased siderophore synthesis under high salinity conditions [27].

Ammonia production by halophiles is a vital plant growth-promoting activity studied in this project. Ammonia serves as a nitrogen source usable by plants or convertible to nitrate by nitrifying bacteria. The production of ammonia enhances nitrogen availability, positively affecting plant growth. Quantifying ammonia from isolated halophiles will evaluate their potential as nitrogen suppliers in saline environments [28].

Indole-3-acetic acid (IAA) is a plant hormone that promotes growth and development, affecting root elongation, lateral root formation, and nutrient uptake. Halophiles from marine water can produce IAA, suggesting they may enhance plant growth via hormonal regulation, especially under saline conditions. IAA production evaluation can be conducted through colorimetric or chromatographic methods [29].

Pot assays and seed germination tests are crucial for assessing the plant growth-promoting potential of halophiles. Pot assays involve inoculating halophiles onto potted plants to evaluate their impact on growth parameters like shoot/root biomass, chlorophyll content, and nutrient uptake. Seed germination tests measure halophiles' effects on seed germination and seedling vigor, offering early insights into their growth-promoting abilities [21].

The study indicates that the consortium of A1, B1, and C4 isolates shows the highest potential for alleviating salinity compared to individual isolates and the control group [30]. This suggests that the interactions among these halophiles enhance their effectiveness in reducing soil salinity. The findings highlight the role of microbial consortia in managing salinity stress in agriculture, implying that multiple strains can improve salinity management efficiency. Future research should investigate the mechanisms behind the salinity amelioration of this consortium and its practical applications in agricultural contexts.

CONCLUSION

In conclusion, the evaluation of plant growth promoting factors and the alleviation of salinity using halophiles indicates promising results for sustainable agricultural practices. They produce plant growth-promoting factors such as phytohormones, enzymes, vitamins, and osmoprotectants, which enhance nutrient availability, root development, and stress tolerance.

The alleviation of salinity stress by halophiles is essential for boosting plant growth in saline soils. They restore osmotic balance, mitigate ion toxicity, and enhance nutrient uptake, thus improving plant-microbe interactions and salt tolerance. Studies indicate their effectiveness in improving growth and alleviating salinity stress in various crops, which is crucial for sustainable agriculture in salt-affected areas where traditional farming suffers from salinity.

Further research is essential to fully understand the mechanisms of halophiles in agriculture, focusing on strain selection, inoculum preparation, and application methods. Long-term effects and ecological impacts also require investigation. Utilizing halophiles for salinity alleviation presents a promising strategy to enhance crop productivity and sustainability in salt-affected soils, potentially leading to innovative and environmentally friendly solutions to salinity stress in agriculture.

REFERENCES

1. Gupta S, Pandey S. ACC Deaminase Producing Bacteria With Multifarious Plant Growth Promoting Traits Alleviates Salinity Stress in French Bean (*Phaseolus vulgaris*) Plants. *Front Microbiol.* 2019 Jul 9;10:1506. doi: 10.3389/fmicb.2019.01506. PMID: 31338077; PMCID: PMC6629829.
2. Al-Zahrani HS, Alharby HF, Fahad S. Antioxidative Defense System, Hormones, and Metabolite Accumulation in Different Plant Parts of Two Contrasting Rice Cultivars as Influenced by Plant Growth Regulators Under Heat Stress. *Front Plant Sci.* 2022 May 27;13:911846. doi: 10.3389/fpls.2022.911846. PMID: 35712584; PMCID: PMC9196032.

3. Sagar A, Rai S, Ilyas N, Sayyed RZ, Al-Turki AI, El Enshasy HA, Simarmata T. Halotolerant Rhizobacteria for Salinity-Stress Mitigation: Diversity, Mechanisms and Molecular Approaches. *Sustainability* **2022**, *14*, 490. <https://doi.org/10.3390/su14010490>
4. Fazeli-Nasab B, Sayyed RZ. “Plant growth-promoting rhizobacteria and salinity stress: a journey into the Soil,” in *Plant Growth Promoting Rhizobacteria for Sustainable Stress Management*, eds Sayyed R. Z., Arora N. K., Reddy M. S. (Singapore: Springer;).2019; 12: 21–34.
5. Shrivastava P, Kumar R. Soil salinity: A serious environmental issue and plant growth promoting bacteria as one of the tools for its alleviation. *Saudi J Biol Sci.* 2015 Mar;22(2):123-31. doi: 10.1016/j.sjbs.2014.12.001. Epub 2014 Dec 9. PMID: 25737642; PMCID: PMC4336437.
6. Sharma SB, Sayyed RZ, Sonawane M, Trivedi MH, Thivakaran GA. Neurospora sp. SR8, a novel phosphate solubiliser from rhizosphere soil of Sorghum in Kachchh, Gujarat, India. *Indian J Exp Biol.* 2016 Oct;54(10):644-649. PMID: 30084564.
7. Gopalakrishnan T, Kumar L. Modeling and Mapping of Soil Salinity and its Impact on Paddy Lands in Jaffna Peninsula, Sri Lanka. *Sustainability* **2020**, *12*, 8317. <https://doi.org/10.3390/su12208317>
8. Thomas SJ, Pierzynski GM. Chemistry of phosphorus in soils. *Chem. Proc. Soil.* 2008; **8**: 11–17. <https://doi.org/10.2136/sssabookser8.c2>
9. Kamran M, Parveen A, Ahmar S, Malik Z, Hussain S, Chattha MS, et al. An Overview of Hazardous Impacts of Soil Salinity in Crops, Tolerance Mechanisms, and Amelioration through Selenium Supplementation. *Int J Mol Sci.* 2019 Dec 24;21(1):148. doi: 10.3390/ijms21010148. PMID: 31878296; PMCID: PMC6981449.
10. Su J, Qiu Y, Yang X, Li S, Hu Z. Dose-Effect Relationship of Water Salinity Levels on Osmotic Regulators, Nutrient Uptake, and Growth of Transplanting Vetiver [*Vetiveria zizanioides* (L.) Nash]. *Plants* (Basel). 2021 Mar 16;10(3):562. doi: 10.3390/plants10030562. PMID: 33809717; PMCID: PMC8002220.

11. Sabagh EL, Hossain A, Barutçular C, Iqbal MA, Islam MS, et al. Consequences of salinity stress on the quality of crops and its mitigation strategies for sustainable crop production: an outlook of arid and semi-arid regions. *Environ. Clim. Plant Veg. Growth*.2020 doi: 10.1007/978-3-030-49732-3_20
12. Aljohny BO. Halophilic bacterium—A review of new studies. *Biosci. Biotechnol. Res. Asia* 2015;**12**(3), 2061–2069.
13. Kalam S, Basu A, Ahmad I, Sayyed RZ, El-Enshasy HA, Dailin DJ, Suriani NL. Recent Understanding of Soil Acidobacteria and Their Ecological Significance: A Critical Review. *Front Microbiol*. 2020 Oct 30;11:580024. doi: 10.3389/fmicb.2020.580024. PMID: 33193209; PMCID: PMC7661733.
14. Nasab BF, Sayyed RZ, Mojahed LS, Rahmani AF, Ghafari M, Antonius S, et al. *Biofilm Production: A Strategic Mechanism for Survival of Microbes Under Stress Conditions*. *Biocat. Agric. Biol*.2022; 42, 102375.
15. Irshad A, Ahmad I, Kim SB. Culturable diversity of halophilic bacteria in foreshore soils. *Brazilian journal of microbiology : [publication of the Brazilian Society for Microbiology]*. 2014; 45(2), 563–571. <https://doi.org/10.1590/s1517-83822014005000050>
16. Kumar S, Karan R, Kapoor S, et al. Screening and isolation of halophilic bacteria producing industrially important enzymes. *Brazilian journal of microbiology : [publication of the Brazilian Society for Microbiology]*. 2012; 43(4), 1595–1603. <https://doi.org/10.1590/S1517-838220120004000044>
17. Ibarra-Galeana JA, Castro-Martínez C, Fierro-Coronado RA, et al. Characterization of phosphate-solubilizing bacteria exhibiting the potential for growth promotion and phosphorus nutrition improvement in maize (*Zea mays* L.) in calcareous soils of Sinaloa, Mexico. *Ann Microbiol* 2017; **67**, 801–811. <https://doi.org/10.1007/s13213-017-1308-9>

18. Tran TTH, Thai TL, Nguyen TH, Nguyen XC, Characterization and identification of nitrogen fixing bacteria isolated from agricultural soil , Vietnam Journal of Science ,Technology and Engineering 2018 ;60(3):48-54
19. Gang S, Sharma S, Saraf M, Buck M, Schumacher J, Analysis of Indole-3-acetic Acid (IAA) Production in *Klebsiella* by LC-MS/MS and the Salkowski Method. Bio Protoc 2019 ;9(9):e3230.
20. Milagres AM , Machuca A, Napoleão D , Detection of siderophores production from several fungi and bacteria by a modification of chrome azurol S (CAS) agar plate assay, J Microbiol Methods 1999;37(1):1-6.
21. Kapadia C, Patel N, Rana A, Vaidya H, Alfarraj S, Ansari MJ, et al. Evaluation of Plant Growth-Promoting and Salinity Ameliorating Potential of Halophilic Bacteria Isolated From Saline Soil. *Frontiers in plant science*, 2022; 13, 946217. <https://doi.org/10.3389/fpls.2022.946217>
22. Machado Siqueira G , Dafonte Dafonte J, Bueno Lema J, Valcárcel Armesto M, França e Silva ÊF. Using soil apparent electrical conductivity to optimize sampling of soil penetration resistance and to improve the estimations of spatial patterns of soil compaction. *TheScientificWorldJournal*, 2014, 269480. <https://doi.org/10.1155/2014/269480>
23. Li H, La S, Zhang X, Gao L, Tian Y. Salt-induced recruitment of specific root-associated bacterial consortium capable of enhancing plant adaptability to salt stress. *ISME J.* 2021, 15, 2865–2882.
24. Lebrazi S, Niehaus K, Bednarz H, Fadil M, Chraibi M, Fikri-Benbrahim K. Screening and optimization of indole-3-acetic acid production and phosphate solubilization by rhizobacterial strains isolated from *Acacia cyanophylla* root nodules and their effects on its plant growth. *Journal, genetic engineering & biotechnology*, 2020; 18(1), 71.
25. Zh, F, Qu L, Hong X, Sun X. Isolation and characterization of a phosphate- solubilizing halophilic bacterium *Kushneria* sp. YCWA18 from Daqiao Saltern on the coast of Yellow Sea of China. *Evid. Based Complement. Altern. Med.* 2011. <https://doi.org/10.1155/2011/615032>

26. Page MGP. The Role of Iron and Siderophores in Infection, and the Development of Siderophore Antibiotics. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America*, 2019; 69(Suppl 7), S529–S537.
27. Anbumalar S, Ashokumar P, Devi SA, Venkatachalam P. Analysis of potentiality of halobacterium in promoting plant growth. *Int. J. Basic Appl. Res.* 2018 <http://www.pragatipublication.com>
28. Sridhar D, Alheswairini SS, Barasarathi J, Enshasy HAE, Lalitha S, Mir SH, et al. Halophilic rhizobacteria promote growth, physiology and salinity tolerance in *Sesamum indicum* L. grown under salt stress. *Frontiers in microbiology*, 2025 16, 1590854. <https://doi.org/10.3389/fmicb.2025.1590854>
29. Meinzer M, Ahmad N, Nielsen BL. Halophilic Plant-Associated Bacteria with Plant-Growth-Promoting Potential. *Microorganisms*, 2023; 11(12), 2910. <https://doi.org/10.3390/microorganisms11122910>
30. Oliveira ALM, Stoffels M, Schmid M, Reis VM, Baldani JI, Hartmann A. Colonization of sugarcane plantlets by mixed inoculations with diazotrophic bacteria. *European journal of soil biology* 2009; 45(1): 106-113.