Original Research Article

Evaluation of Plant Growth-Promoting and Salinity-Alleviating Potential of Halophilic Bacteria 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.

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-411018December 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: After inoculation of isolates and 7-day incubation period, soil samples showed a reduction in salinity in terms of electrical conductivity in which consortium (A1, B1 and C4) showed maximum reduction from 6.78 to 3.78 Millisiemens per cm, indicating the isolates' potential for salinity alleviation. Our results also demonstrated that the isolates possessed significant plant growth promoting traits.

Conclusion: This study demonstrates that halophiles from marine environments may help alleviate salinity stress inplants and enhance growth, suggesting their potential use in sustainable agriculture in saline regions

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].

Plant growth-promoting rhizobacteria (PGPR) are known to stimulate plant growth in a number of ways [12]. 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 [13].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 [14].

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. 1 ml 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 7 days. Various colonies, identified by color, were isolated and purified on nutrient agar and subsequently preserved on NA slants with the same NaCl concentration [15].

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 [16].

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 [17].

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 [18].

Siderophore production

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

Sterile cork borer, and 50µL 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 [19].

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 [20].

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. 1 g soil sample was dissolved in100 ml distilled water, shaken at 120 rpm for 30 minutes, and then analyzed for conductivity to determine salinity.

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 petriplates, 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 [20].

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 [20].

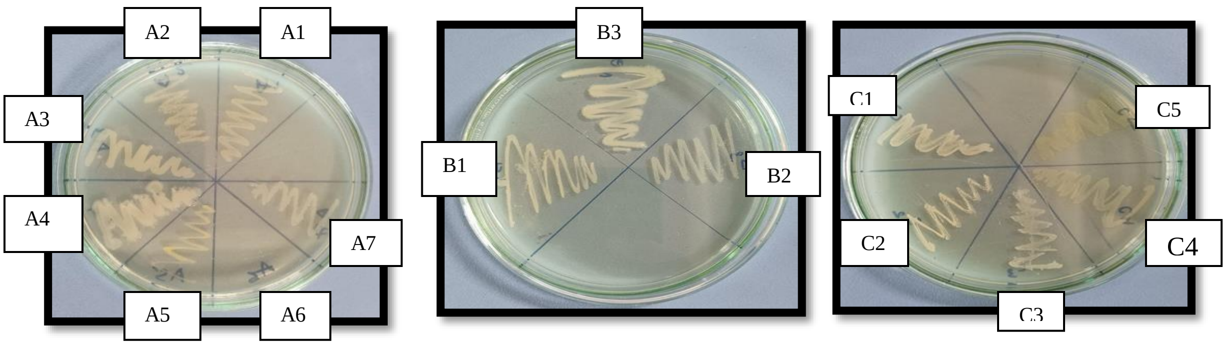
RESULTS

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 (%)	Number of colonies obtained	Colony labelled
5%	7	A1,A2,A3,A4,A5,A6,A7
10%	3	B1,B2 ,B3
15%	5	C1,C2,C3,C4,C5



5%NaCl **10 % NaCl** **15%NaCl**

Fig.1: Pure culture plates for isolates

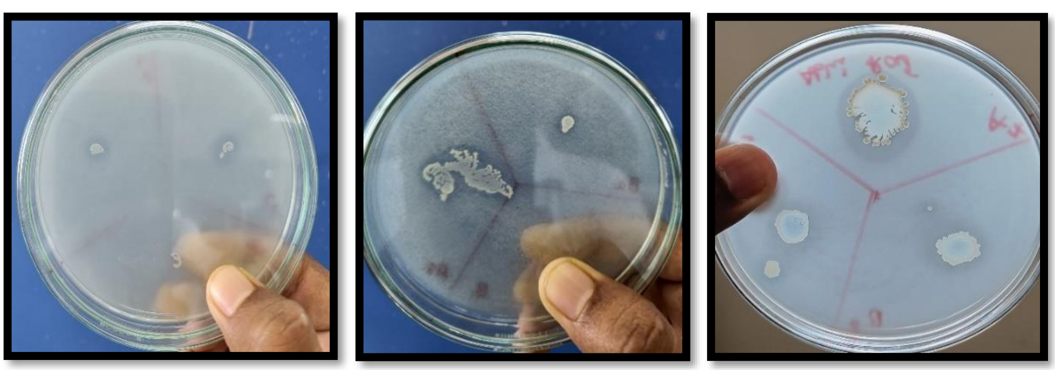
Plant Growth Promoting potential of isolates

Total 15 isolates from different salt concentrations, 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 solublisation	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**

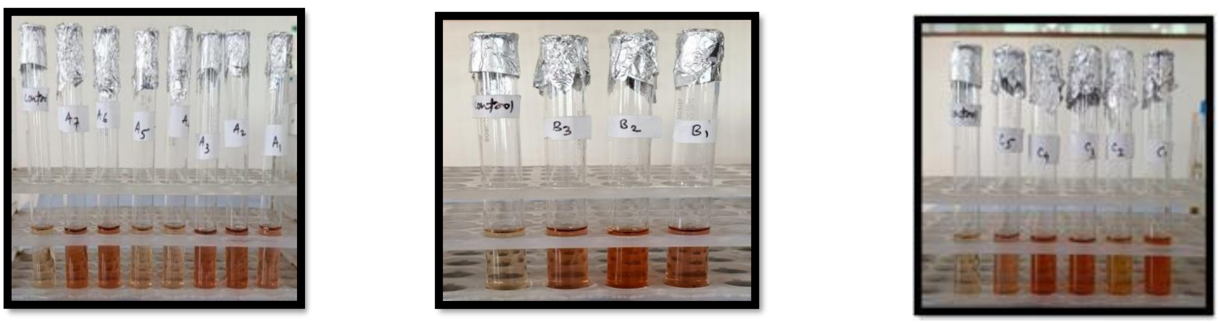


5% NaCl **10% NaCl** **15%NaCl**

Fig.2. Zone of clearance around phosphate solubilizing isolates

5%NaCl **10%NaCl** **15%NaCl**

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



5%NaCl **10%NaCl** **15%NaCl**

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

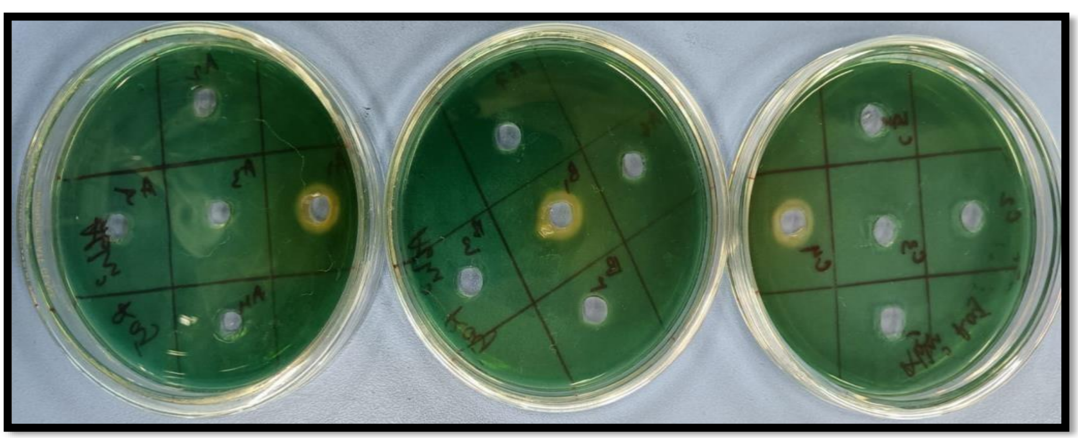


Fig.5:Isolates (A1,B1,C4) showing Siderophore producing isolates.

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

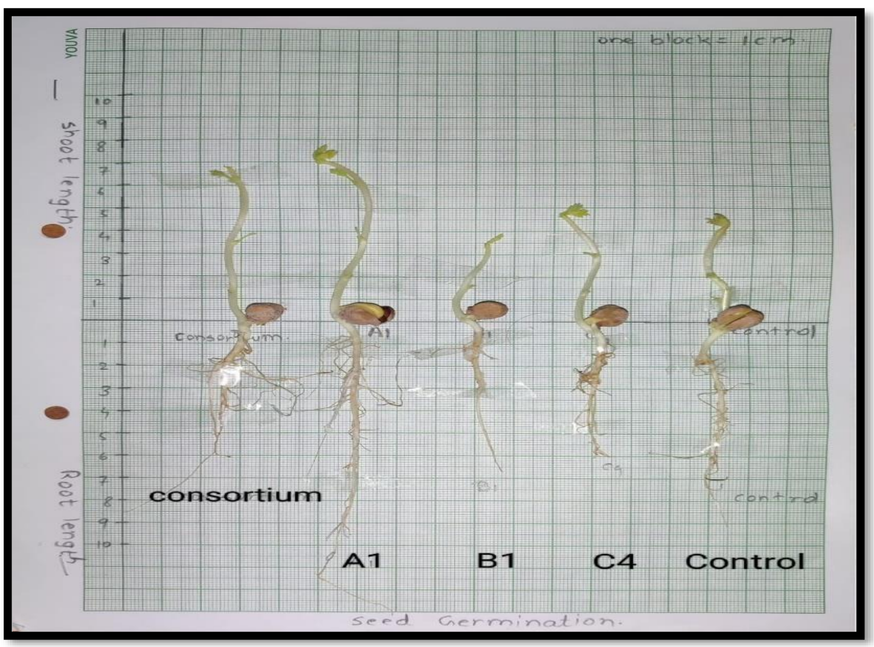


Fig.6 : Shoot and root length of Red gram seeds after 7 days.

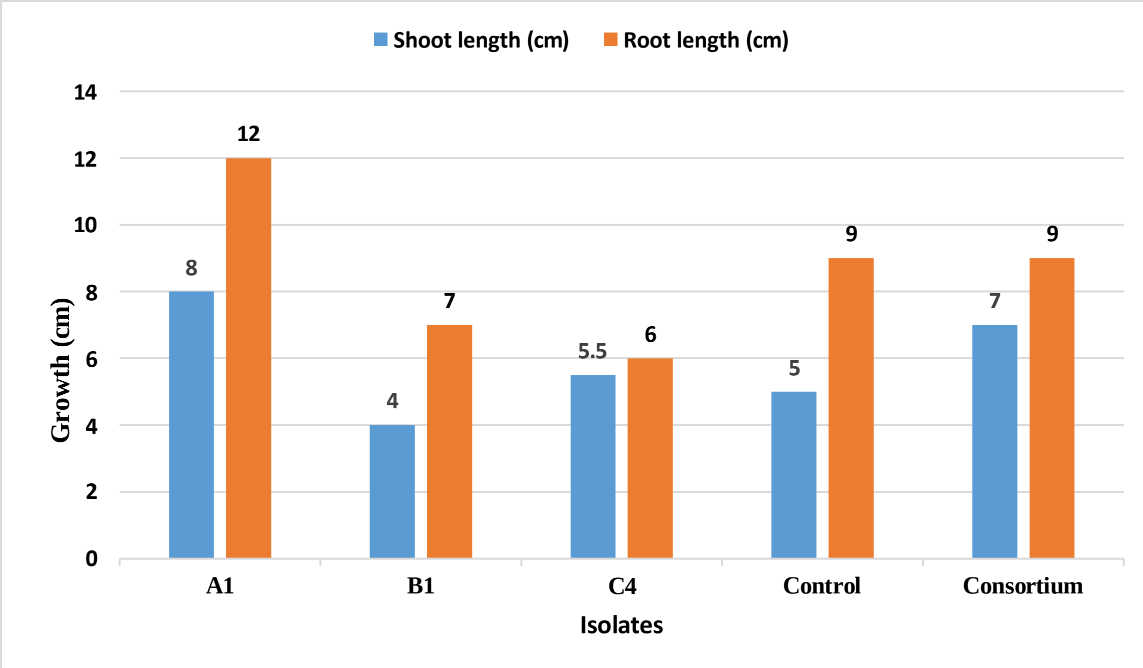


Fig.7: Graphical representation off shoot and root length of Red gram seeds after 7 days

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 interms of Electrical conductivity (1:5solution)(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 (1:5 solution) (MilliSiemens per cm)
1	A1	6.55
2	B1	4.95
3	C4	5.76
4	Control	6.78
5	Consortium	3.78

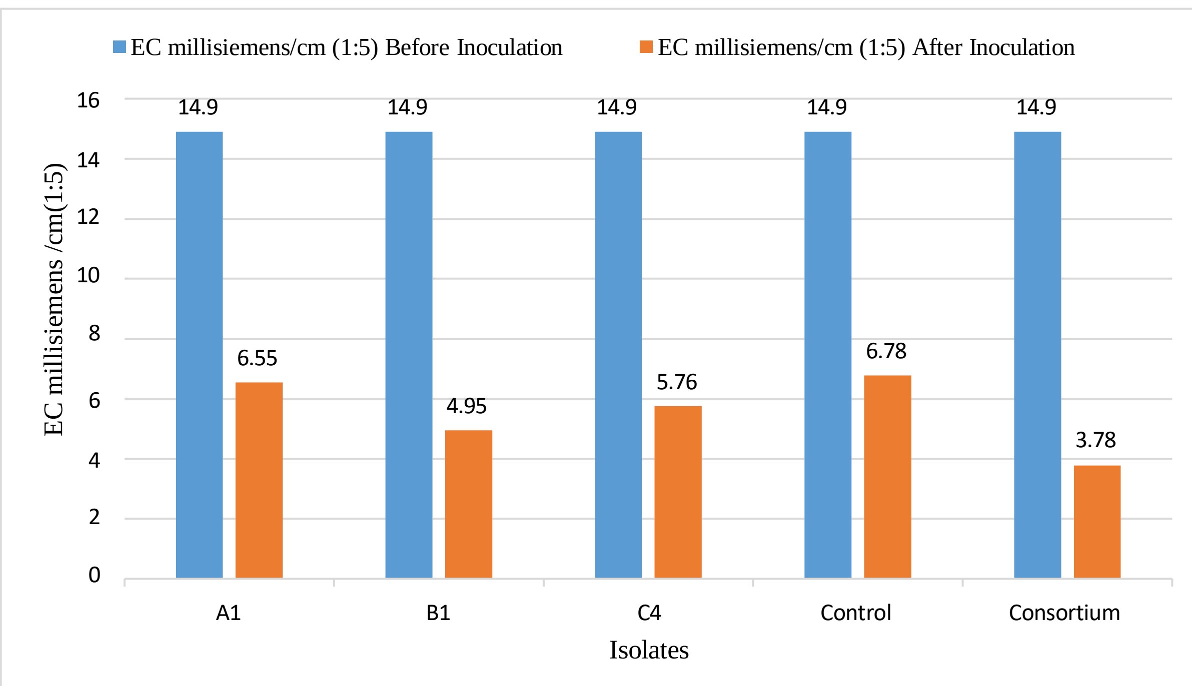


Fig.8: Graphical representation of Salinity reduction in terms of Electrical conductivity (1:5 solution) (Millisiemens per cm)

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 and10.

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

A1 B1 C4 CONSORTIUM CONTROL

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

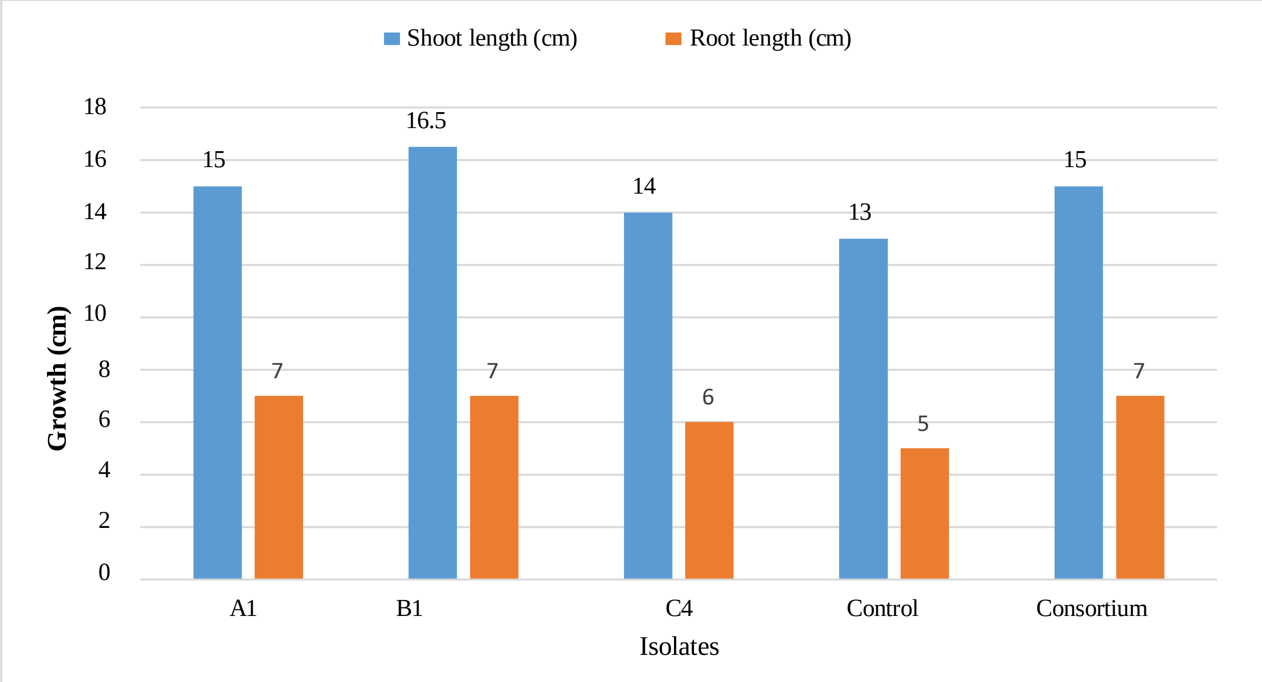


Fig.10 Graphical representation shoot and root length of wheat seeds after 14 days inoculated with different bacterial isolates.

DISCUSSION

In developing nations and heavily exploited agricultural areas, soil salinization frequently occurs, limiting plant growth and agricultural productivity by as much as 50% [21]. 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 [22], 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 [23].

The isolates studied were unable to produce siderophores, likely due to sufficient iron availability in their environments [24].Quantifying ammonia from isolated halophiles evaluated their potential as nitrogen suppliers in saline environments [25]. 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 [26].

Pot assays involved inoculating halophiles onto potted plants to evaluate their impact on growth parameters like shoot/root biomass, chlorophyll content, and nutrient uptake. Seed germination tests measured halophiles' effects on seed germination and seedling vigor, offering early insights into their growth-promoting abilities [20].

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. After inoculation of isolates and 7-day incubation period, soil samples showed a reduction in salinity in terms of electrical conductivity in which consortium showed maximum reduction from 6.78 to 3.78 Millisiemens per cm, indicating the isolates' potential for salinity alleviation. [27]. 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.

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, andosmoprotectants,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.

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