



PGPR-based Bio stimulants: a sustainable approach to enhancing crop resilience under salinity

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ABSTRACT

Agricultural productivity is severely hampered by salinity stress, especially in areas where soil salinization is significant. The purpose of this study is to determine whether plant growth-promoting rhizobacteria (PGPR), which were isolated from the rhizosphere of *Rosmarinus officinalis* (rosemary), may improve *Brassica juncea* (mustard) resistance to salt. The characteristics of the isolates that promote plant growth, such as phosphate solubilisation, nitrogen fixation, and the synthesis of indole-3-acetic acid (IAA), were described. At sodium chloride (NaCl) concentrations ranging from 1% to 5%, the salt tolerance of several PGPR strains was evaluated. The effects of PGPR inoculation on mustard seedlings in saline environments (300 mM, 500 mM, 700 mM, and 900 mM NaCl) were assessed in pot experiments. The findings showed that PGPR considerably increased the growth of seedlings under salt stress, indicating that they could be used as biofertilizers in sustainable farming.

Keywords: PGPR, Rosemary, Mustard, Biofertilizer, Sustainability, Nutrient solubilization.

I. INTRODUCTION

One important abiotic stressor that severely restricts plant growth and agricultural productivity globally is soil salt. About 20% of irrigated fields and 6% of the entire land area are affected, and estimates show that this number is rising as a result of climate change and unsustainable farming methods. Excessive salt levels in the soil cause oxidative damage, osmotic stress, ion toxicity, and nutritional imbalance in plants, all of which lower crop production. In addition to osmotic and ionic stress, salinity stress usually causes an ionic imbalance, which can impair the selectivity of root plasma membranes and result in a potassium deficiency. [1]. Salt-affected soils can be managed in a number of ways, such as by flushing soils with salt crust at the surface, using chemicals like gypsum, organic matter, and acids, as well as biological methods. Deep tube wells can

also be used to lower groundwater levels and leach excess soluble salts using fresh irrigation water. Numerous environmental conditions, such as the type of vegetation cover, moisture content, soil texture, and nutrient availability, influence the number and types of microorganisms found in the soil [2]. PGPR are essential for biological activities such as the cycling of nutrients, the biological control of plant diseases, and the development of seedlings and plants via the production of various chemicals [3].

Microorganisms known as mycorrhiza fungi and plant growth-promoting rhizobacteria (PGPR) are crucial for preserving the soil's nutrient availability, which supports plant growth and boosts output, according to soil scientists. By enhancing soil fertility and increasing nutrient absorption, these microbes work with the rhizosphere or endosphere of plants to increase output [4]. Utilising biofertilizers reduces the high cost of purchasing chemical fertilisers and satisfies the demand for ecologically friendly crop production technologies worldwide. The use of PGPR and mycorrhizal fungi in the production of reasonably priced and ecologically friendly microbial biofertilizers is intended to enhance plant growth and yield [5]. The creation of organic acid was the main method by which insoluble phosphate compounds were changed into more soluble forms. PGPR activity in the rhizosphere affects rooting patterns and plant nutrition availability by changing the quantity and quality of root exudates [6].

Biofertilizers are cost-effective and environmentally benign, and they may be produced in big numbers on the farm if necessary. Up to 50 percent of the nitrogen is fixed, and crop output increases by 10 to 40 percent. When continuous applications of biofertilizer are performed, the ground can retain fertility for three to four years because parental inoculums are effective in sustaining plant growth and multiplication. Applying biofertilizers results in increased mineral and water intake, nitrogen fixation, root development, and vegetative growth [7]. Because they may be produced from natural sources, prevent damage, and aid in the development of healthy soil, biofertilizers are less detrimental to the environment than chemical fertilisers. They can also assist plants in reducing their reliance on precipitated chemical fertilisers [8] [9].

Because it inhibits the formation of clumps, salinity directly affects the structure of the soil, making it dense and impenetrable. It reduces the soil's ability to hold onto air and water. Plants may thus not receive enough moisture and oxygen to thrive. Plants are negatively impacted by high salt concentrations in soil solutions, which interfere with a number of physiological and biochemical functions and lower biomass and crop yields [10]. Through osmotic and ionic stress on the entire plant and particularly at the cellular level, salinity generally affects plant physiology [11]. To maintain agricultural sustainability, scientists around the world are now concentrating their efforts on using beneficial soil microbes instead of synthetic fertilisers and pesticides [12].

They reside in the rhizosphere and, when applied to seeds, plant surfaces, roots, or soil, their biological activity increases the soil's microbiota, promotes plant growth, and enhances nutrient accessibility. They are therefore ready-mades that rapidly improve the fertility of the soil [13].

Importance of Mustard (*Brassica juncea*)

A commercially significant oilseed crop, mustard is grown for its high nutritional content, potential medical uses, and industrial importance. It is abundant in antioxidants, vital minerals, and omega-3 fatty acids. However, soil salinity has a negative effect on mustard agriculture, resulting in lower yields, slowed growth, and decreased germination rates. A sustainable method of preserving productivity in saline-prone agricultural areas is to improve mustard's resistance to salt by microbial inoculation. Mustard cakes are mostly used for cow feed and manure [14].

Health and Environmental Benefits of Mustard

- **Health Benefits:** Glucosinolates, which are found in mustard seeds, may have anti-cancer effects. The omega-3 fatty acids promote cardiovascular health, and the substantial fibre content facilitates digestion.
- **Environmental Benefits:** By removing heavy metals from disturbed soils, mustard plants contribute to phytoremediation. Additionally, by suppressing weeds and enhancing soil health, they support sustainable farming.

Plant growth promotion and soil health

Because of their capacity to improve plant development and their potential as agents for plant stress management, plant growth-promoting rhizobacteria, or PGPR, have been the subject of increased attention for decades. Microorganisms that are good for plant crops naturally live in soil. The phrase "plant growth promoting rhizobacteria" refers to a broad category of bacterial species that, through a variety of mechanisms, have a significant influence on plant growth, yield, and disease resistance by forming a competitive and healthy connection with plant root systems [15]. By boosting the antioxidant activity, phenolic content, and photosynthetic pigments of economically significant crops, PGPR can also raise their nutritional levels.

Because of their biochemical flexibility in metabolising a variety of natural and xenobiotic substances, PGPR exhibit remarkable adaptability in a wide range of soil conditions [16]. Furthermore, the biological traits of an ideal PGPR, such as high rhizosphere competence, ease of mass multiplication, improvement of plant growth, development, and yield, pathogen suppression, broad spectrum of action, environmental friendliness, and compatibility with other rhizobacterial species, make them highly appropriate [17]. Since it is a prerequisite for their association in the rhizosphere, soil moisture is crucial for PGPR colonisation (though, of course, there are many other critical elements). The production of different biomolecules causes PGPR to associate with plant roots, which raises soil fertility. The health of the soil is then enhanced by the addition of these mineralised organic components. Additionally, they produce many phytohormones that have a significant effect on root structure [18]. Rhizobacteria have the capacity to increase the soil's nutritional content. All kinds of microbes require nitrogen as a food, and it is a crucial component of proteins, nucleic acids, and a few other important chemical substances. All of these processes enhance soil health, and a healthy soil body can store both macro and micronutrients, which helps to provide high yields and sustained productivity [19].

Nutrient Acquisition by PGPR

Generally speaking, soils with high levels of organic matter and dynamic microbial ecologies require less fertiliser than soils that are maintained conventionally. Research on Phyto-microbiomes is starting to identify particular interactions between microbes and plants that directly support plant nutrition. The generation of siderophores, HCN, nitrogen fixation, P-solubilization, and increased surface area accessible by plant roots are only a few of the ways that microbes that aid in plant nutrient acquisition (biofertilizers) work. Thus, there is a lot of promise for supplying crops with the nutrients they need by adjusting microbial activity [8]. Since fixing nitrogen from the atmosphere and turning it into ammonia requires a lot of energy, the bacterial cell must prioritise oxidative phosphorylation of carbon sources to produce ATP over glycogen production in order to increase nitrogen fixation. Nevertheless, despite increased dry matter and nodule number in inoculated bean plants, trials using *Rhizobium tropici* glycogen synthase deletion mutants have not survived in soil settings [20].

Growth and Yield of Mustard

Indian mustard, or *Brassica juncea*, is a major oilseed winter crop that is grown throughout the Northern Indian plains because of its substantial nutritional and economic value. Among the many species of oilseeds, it comes in third. The greatest oilseed production region is located in the North-West agro-climatic zone, where most soil and groundwater sources are severely saline (1.6–17%) and have sodicity difficulties [21]. Dry matter production, branching pattern, pod formation, plant height (root/shoot elongation), seedling growth, and seed germination are all significantly impacted by high salt irrigation water during pre-sowing and flower initiation [22]. Indian mustard (*Brassica juncea*), a winter oilseed crop, grows on northern Indian plains. Crop growth and productivity have decreased as a result of the recent increase in soil salinity. Applying rhizobacterial strains that stimulate plant development has been found to boost crop productivity in salty settings. Finding bacterial isolates from the rosemary rhizosphere that could help improve mustard growth while it is under salt stress was the aim of this study [23] [24].

Effect of Sodium Chloride stress on Mustard seeds

Mustard and rapeseed are the most important edible oil crops in the world, accounting for 50.74% of total oilseed production, however they are the third most important edible oil source behind soybean and palm. The detrimental effects of salinity are thought to be caused by osmotic stress, ionic imbalance, and oxidative stress [25]. These salts hinder crop establishment and seed germination. Both the osmotic effect and/or certain ion toxicities to radicle emerging or seedling development can be blamed for the effects of salt stress on seed germination. A crucial phase in crop production, seedling establishment is heavily reliant on the physiological and biochemical characteristics of the seed. High-vigour seeds are required to supply vital nutrients for seedling establishment and to allow them to photosynthesise on their own, resulting in a quick and healthy establishment of seedlings [26]. The most practical parameters for choosing salt tolerance are germination and seedling traits. Additionally, key factors for cultivar selection are germination rate, germination percentage,

and seedling growth. In order to achieve greater yields and control salt, it is necessary to screen genotypes at the seedling stage in saline settings in order to find tolerant genotypes for improved germination and early seedling establishment [27].

Role of Plant Growth-Promoting Rhizobacteria (PGPR)

A varied population of bacteria known as rhizobacteria that promote plant growth (PGPR) live in the rhizosphere soil, close to the root surfaces, and in association with roots. They either directly or indirectly improve the health of the plant and soil. However, they also speed up certain nutrients' absorption and mineralisation, for example. In recent decades, it has been demonstrated that a variety of bacteria, including species of *Azospirillum*, *Azotobacter*, *Pseudomonas*, *Enterobacter*, *Arthrobacter*, and *Bacillus*, aid in the development of plants. Beneficial rhizobacteria could reduce fertiliser use and support sustainable agriculture as part of an all-encompassing management system [28]. PGPR are essential for biological activities such the cycling of nutrients, the biological control of plant diseases, and the development of seedlings and plants via the production of various chemicals [3].

Rhizobacteria promoting plant growth in salt-tolerant environments (ST-PGPR)

A vast variety of microorganisms from various bacterial, fungal, and archaeal groups can be found in soil. Some microorganisms are now well known for their innate ability to support plant growth and withstand varying salt concentrations. Agriculture greatly benefits from these salt-tolerant plant-beneficial microorganisms. They have demonstrated the ability to increase crop yields in semiarid and arid areas. The most well-reported genera for increasing the productivity of a variety of crops in salty environments are *Pseudomonas*, *Bacillus*, *Enterobacter*, *Agrobacterium*, *Streptomyces*, *Klebsiella*, and *Ochromobacter* [29].

Investigated the genetic diversity of the wheat rhizosphere-derived ST-PGPR. They discovered that the majority of the isolates, which are members of the genus *Bacillus*, could withstand up to 8% NaCl. Taoyuan, China's paddy rhizosphere yielded a variety of salt-tolerant microorganisms. 162 of the 305 bacterial strains they identified were examined for salt tolerance at concentrations of up to 150 g/l NaCl [4].

Features of an Ideal PGPR A rhizobacterium is regarded as a supposed PGPR if it possesses some features that promote plant growth and can also boost plant growth after inoculation. The following traits should be present in a perfect PGPR strain [30].

- i. They ought to be environmentally friendly and highly rhizosphere capable.
- ii. After inoculation, PGPR should significantly colonize the plant roots.
- iii. They need to encourage plant development.
- iv. The wide spectrum should expose it.
- v. It needs to get along with other rhizosphere bacteria.

- vi. Heat, desiccation, radiation, and oxidants are examples of physicochemical conditions that PGPR should be able to withstand.

The purpose of this study is to determine if PGPR, which was isolated from *Rosmarinus officinalis* rhizosphere, can effectively stimulate the growth of *Brassica juncea* seedlings under salt stress. Isolating and characterising PGPR strains, testing their salt tolerance, and determining how they affect mustard seedling growth at different salinity levels are the study's goals.

II. Materials and Methods

2.1 Sample Collection and Isolation Soil samples were collected from the rhizosphere of *Rosmarinus officinalis* at Mohali, Chandigarh. Rhizobacteria were isolated using serial dilution techniques and cultured on nutrient agar, Luria Bertani agar, and Pseudomonas agar media. Pure cultures were obtained through repeated sub-culturing.

2.2 Morphological and Biochemical Characterization The isolates were subjected to Gram staining and biochemical tests, including catalase, oxidase, methyl red, and indole tests. Morphological observations recorded colony shape, size, and pigmentation.

2.3 Bacterial Morphology and Behaviour

- The isolated bacteria displayed varied morphological characteristics, with some forming mucoid colonies and others exhibiting pigmentation.
- Motility tests confirmed that certain strains exhibited swarming behaviour, aiding in root colonization.
- The isolates demonstrated resistance to high salt concentrations, confirming their adaptability to saline environments.

2.4 Salt Tolerance and Pot Experiment PGPR isolates were tested for salt tolerance in liquid media containing increasing NaCl concentrations. Strains were tested for their ability to tolerate increasing concentrations of NaCl (1%, 2%, 3%, 4%, and 5%) in nutrient broth. Bacterial growth was observed after 48 hours of incubation at 28°C. Growth was monitored at 600 nm spectrophotometrically.

2.5 Pot Experiment for Mustard Growth Under Salt Stress *Brassica juncea* seeds were grown in pots containing soil treated with different concentrations of NaCl (300mM, 500mM, 700mM, and 900mM). The control group was grown without NaCl treatment. Growth parameters such as plant height, leaf size, root weight, and seedling vigour were recorded.

2.6 Application of PGPR Biofertilizer A rhizobacterial consortium was prepared using yeast extract mannitol broth. The bacterial slurry was applied to the mustard plants to assess their effect on plant growth under salt stress conditions.

III. RESULTS AND DISCUSSION

3.1 Isolation of rhizobacteria

The pseudomonas agar plates, nutrient agar plates and Luria Bertani plates inoculated with soil sample of Mohali, Chandigarh region showed bacterial growth after two days incubation at 28°C (Fig 3.1).

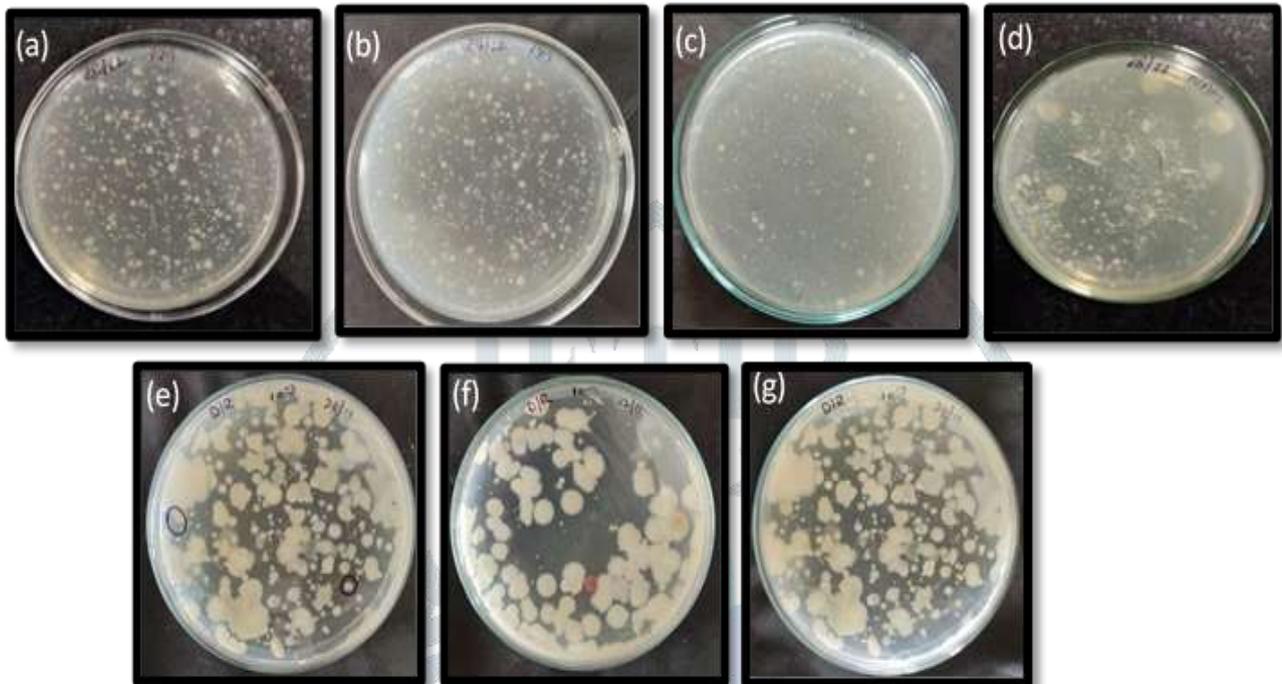
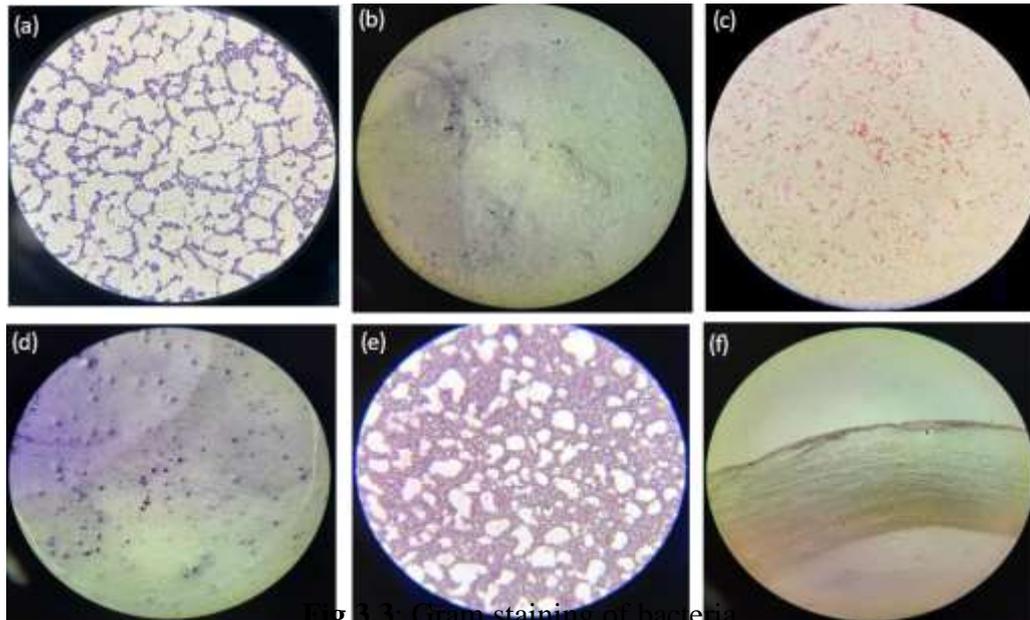


Fig 3.1: (a) and (b) Pseudomonas Medium, (c) and (d) Nutrient agar medium, (e), (f) and (g) Luria Bertani medium

3.1.1 Pure culture

Streak Plate Method - After 24 hours of incubation pure colonies were observed (Fig 3.2).



Fig 3.2: Pure culture of isolates in LB medium**3.1.2 Microphotograph showing stained bacterial cells****Fig 3.3:** Gram staining of bacteria**Table 3.1** Morphological characteristics of bacterial isolates

S. No.	isolates	taining	Colour	hape
1	PGPR 1	Positive	Purple	Rod
2	PGPR 2	Positive	Purple	Cocci
3	PGPR 3	Negative	Pink	Rod
4	PGPR 4	Positive	Purple	Cocci
5	PGPR 5	Negative	Pink	Rod
6	PGPR 6	Positive	Purple	Cocci

3.2 Biochemical test

The selected bacterial isolates were positive for methyl red, catalase test, oxidase test and MacConkey agar test.

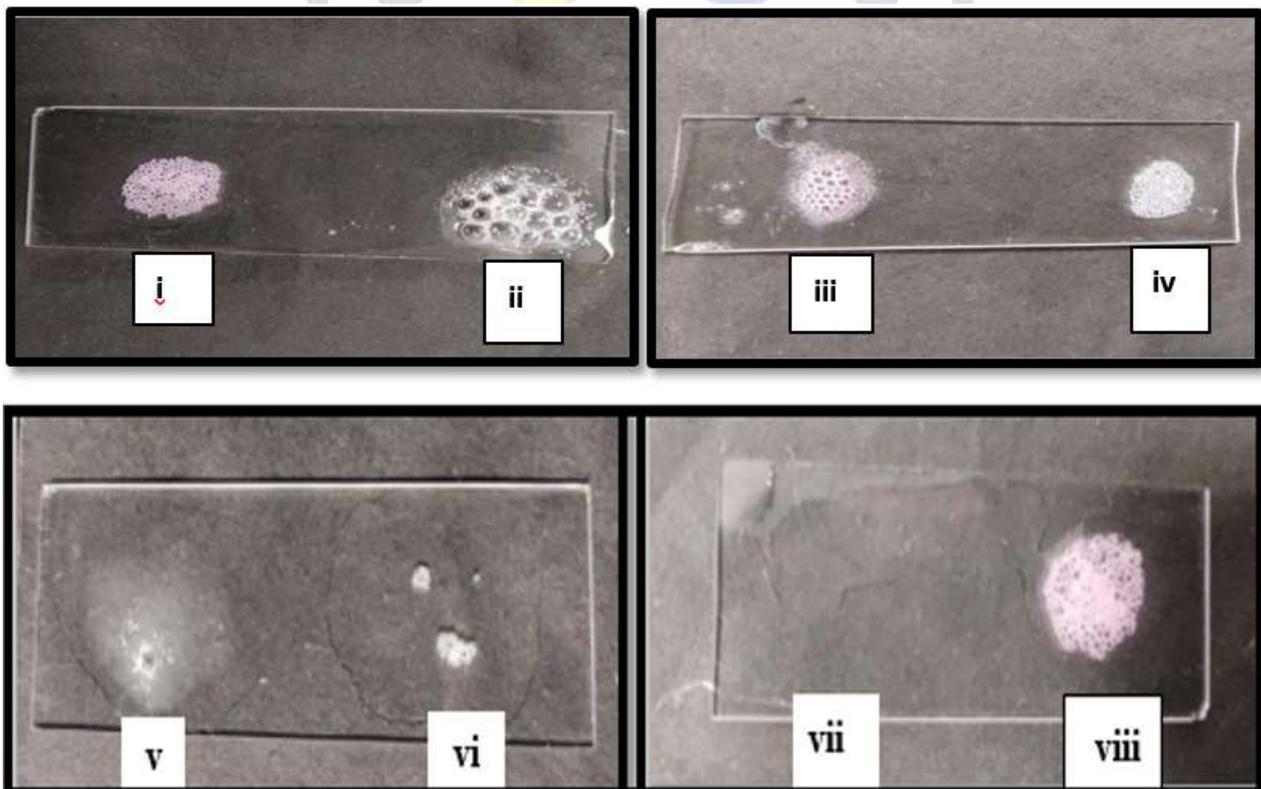
There is no conclusive result obtained indole test.

Table 3.2 Biochemical characteristics of bacterial isolates.

S. No.	Isolates	Catalase test	Oxidase test	Methyl Red test	Indole test	MacConkey Test
1	PGPR 1	Positive	Positive	Positive	Negative	Positive
2	PGPR 2	Positive	Positive	Positive	Negative	Positive
3	PGPR 3	Positive	Positive	Positive	Negative	Positive
4	PGPR 4	Positive	Positive	Positive	Negative	Negative
5	PGPR 5	Negative	Positive	Positive	Negative	Positive
6	PGPR 6	Positive	Positive	Positive	Negative	Negative

3.2.1 Catalase test

The catalase test was performed on 5 isolates. Bubbles were formed indicating the production of the enzyme, catalase. One is negative (Table 3.2).

**Fig 3.4** Catalase test

3.2.2 Oxidase assay

The oxidase test showed colour change in the oxidase disc from white to purple conforming the presence of cytochrome oxidase, an enzyme sometimes called indophenol oxidase (Table 3.2).

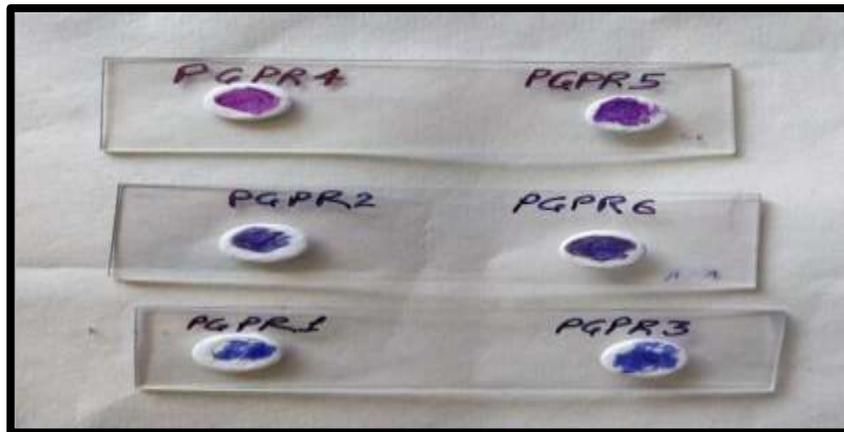


Fig 3.5 Oxidase test

3.2.3 Methyl red test

The methyl red test had shown colour change from yellow to red indicating all the isolates are positive. This confirmed that all the six bacteria have the ability to metabolize glucose to pyruvic acid, which is further metabolized through the 'mixed acid pathway to produce the stable acid (Table 3.2).



Fig 4.6: Methyl red test

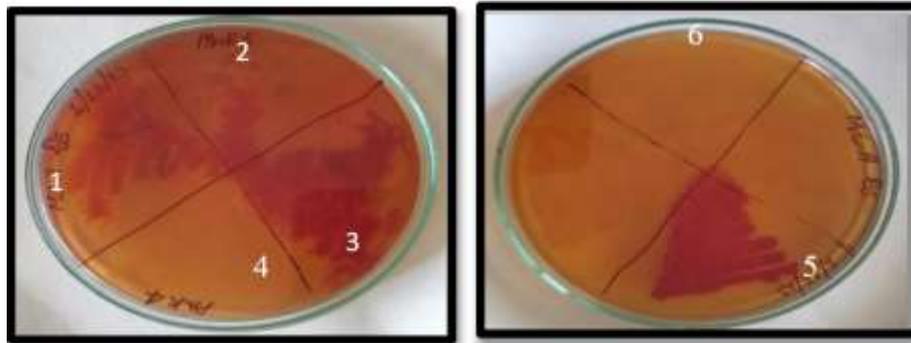
3.2.4 Indole Test

The indole test is a biochemical test performed on bacterial species to detect the ability of an organism to degrade the amino acid tryptophan and produce indole. The test is based on the presence of an enzyme system called "tryptophanase" that converts tryptophan into indole (Table 3.2).



Fig 3.7: Indole test**3.2.5 Mac-Conkey Agar test**

The MacConkey agar test is one of the widely used culture media for identifying enteric organisms. MacConkey agar test is not only helpful in identifying enteric organisms, but also in isolating pathogens from foods and determining the presence of coliforms in water (Table 3.2).

**Fig 3.8:** Mac Conkey agar test**3.3 Salt Tolerance of PGPR bacteria**

Plant growth promoting rhizobacteria have evolved several mechanisms to cope with salinity stress. Five isolates obtained from the Rhizospheric soil of rosemary plants on Luria Bertani medium and tolerate 1%, 2%, 3%, 4%, and 5% NaCl were evaluated for their ability to reduce salinity [31]. Five isolates PGPR1, PGPR2, PGPR3, PGPR4, and PGPR5 were found to be capable of tolerating salt stress up to 5% NaCl. Salt-tolerant plant growth promoting rhizobacteria (ST-PGPR) have evolved several mechanisms to cope with salinity stress [32]. Research on ST-PGPR also indicates their vast potential in remediation and productivity enhancement of agro-ecosystems suffering from problems of salinity [29]. The LB agar plate inoculated with PGPR strains and showed growth after 48 hrs incubation at 28°C.

Control

1%



2%



3%



4 %



5 %



3.4 Effect of NaCl in *Brassica juncea* seedlings

The findings showed that salinity has a major impact on mustard germination characteristics. Mustard cultivar germination was negatively impacted by salt stress. The most practical parameters for choosing salt tolerance are germination and seedling traits. Crucial factors for cultivar selection include germination rate, germination percentage, and seedling growth. As the saline levels rose, it was discovered that plant height steadily decreased. At greater salinity levels, *B. juncea* displayed varying plant heights. This finding suggested that at greater salt levels, salinity had an impact on plant height [33].

Salt's effects on mustard (*Brassica juncea*) were observed by examining its impacts on physiological traits such as germination %, root length, fresh and dried weight of the mustard root, and seedling vigour index I (cm) and II (mg) [34].

Percentage of germination: Mustard seedlings were affected by salt when exposed to different doses of salt (300 mM, 500 mM, 700 mM, and 900 mM). The percentage of seeds that germinated was decreased by that

salt. The decline was negligible at 300 mM, a lower concentration. However, the germination percentage dramatically dropped at higher concentrations (500 mM, 700 mM, and 900 mM).



Fig 3.9 Effect of NaCl stress (300mM, 500mM, 700mM, 900mM) on *Brassica juncea* seedlings in 30 days.

Table 3.3 30 days observation of fresh mustard plants

Parameters	Control	300mM NaCl	500mM NaCl	700mM NaCl	900mM NaCl
Leaf Size	1 cm	0.7 cm	0.7 cm	0.5 cm	0.4 cm
Leaf Diameter	1.3 cm	1.2 cm	1.2 cm	0.9 cm	0.7 cm
Plant height	10 cm	7 cm	6.5 cm	4 cm	3.5 cm
Weight of Root	0.08 gm	0.08 gm	0.05 gm	0.04 gm	0.04 gm
Weight of Leaf	0.04 gm	0.025 gm	0.023 gm	0.015 gm	0.015 gm

Table 3.4 30 days observation of dry mustard plants

Parameters	Control	300mM NaCl	500mM NaCl	700mM NaCl	900mM NaCl
Leaf Size	0.8 cm	0.6 cm	0.6 cm	0.4 cm	0.3 cm
Leaf Diameter	1 cm	0.9 cm	0.9 cm	0.7 cm	0.7 cm
Plant height	9 cm	6 cm	5.5 cm	4 cm	3 cm
Weight of Root	0.06 gm	0.06 gm	0.04 gm	0.03 gm	0.03 gm

Weight of Leaf	0.070 gm	0.032 gm	0.028 gm	0.024	0.020
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Length of seedling: Progressively increased salt concentrations reduced seedling lengths. Various salt concentrations significantly reduced root, and whole seedling length.

Weight of seedlings (fresh and dry): Different salt concentrations had a detrimental effect on the fresh weight of the roots and entire seedlings. Under various salt treatments, the fresh weight of the roots and the entire seedling decreased considerably. Salt reduced the dry weight of both entire seedlings and roots. At several salt treatments (500 mM, 700 mM, and 900 mM), a considerable decrease in root dry weight was observed; however, at the lowest dosage (300 mM), this reduction was negligible. Different salt treatments greatly reduced the dry weight of root and entire seedlings [35].

Another prevalent abiotic stressor that has a detrimental impact on plant growth and output is salinity. Five isolates of bacteria were found in the rosemary rhizosphere. Our study's findings demonstrated that the rhizobacterial isolates could withstand salinity, indicating that they might live in saline environments. Media containing 5% NaCl could support the growth of rhizobacterial isolates. The growth, yield, chlorophyll, and ionic levels of mustard plants cultivated in salt-stressed pots were all adversely impacted. At every salinity level, rhizobial inoculation markedly enhanced every attribute [36]. At varying salinity levels, each rhizobium isolate reacted differently. Furthermore, the rhizobia consortium outperformed their application alone. salt reduced mustard plant height, cob weight, cob length, and weight, with the effect being greater at higher salt levels, according to data (Tables 3.3 and 3.4). All salinity levels saw an improvement in plant growth and yield following inoculation with various rhizobia strains. But when rhizobia strains were applied together (a consortium) under salinity stress, maize growth and yield improved more than when the strains were applied separately.

In comparison to the corresponding un-inoculated control plant, the rhizobial consortium (PGPR1, PGPR2, PGPR3, PGPR4, and PGPR5) increased the plant height (34%), cob weight (25%), cob length (67%), and weight (49%), at salinity levels. When compared to an uninoculated, unstressed control, the results showed that salinity stress dramatically reduced the relative water content, crude protein, chlorophyll "a" and "b," and carotenoids concentrations of mustard plants. At all salinity levels, rhizobial inoculation markedly enhanced these qualities [37].

IV. CONCLUSION

According to the study, *Brassica juncea's* growth and stress tolerance were markedly enhanced by salt-tolerant PGPR isolates from *Rosmarinus officinalis*. The use of PGPR strengthens plant antioxidant defences, controls ion homeostasis, and increases nutrient solubilisation. To improve mustard production in saline-prone areas, future research should concentrate on field tests and the creation of commercial biofertilizers. The results demonstrate how PGPR strains may be used in environmentally benign ways to improve crop productivity, reduce phytopathogens, and promote plant health. This method mitigates the health and environmental risks associated with chemical-based pest management techniques by utilising the beneficial microorganisms' and

plants' natural interactions as a substitute for manufactured pesticides. The study also shows how certain PGPR strains may effectively produce antimicrobial chemicals, induce systemic resistance, and promote nutrient intake. These many advantages highlight the need for more study into PGPR-based biopesticide formulation optimisation, field use, and commercial scalability. Long-term effects on soil microbiomes and crop yield, strain-specific processes, and synergistic effects with other biocontrol agents should be the main topics of future research.

Biopesticides mediated by PGPR offer a viable and sustainable answer to today's agricultural problems. By incorporating them into traditional farming methods, they can help ensure global food security, encourage environmental sustainability, and drastically lessen reliance on chemical pesticides.

V. CONFLICT OF INTEREST

There was no conflict of Interest.

VI. AUTHOR'S CONTRIBUTION

Experimentation was done by Ruchi and Abhishek. The manuscript preparation was done by Ruchi and Gurinder. The proof reading was done by Dr. Puneet and infrastructure was provided by Harpreet kaur.

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