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# Unveiling the potential of Halotolerant bacteria as PGPR with an ANOVA and Time-Series enhanced study for sustainable agriculture

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This study explores harnessing the potential of halotolerant plant growth-promoting bacteria (PGPR) from Ghoramara Island, addressing challenges of salinity and cyclone-induced soil runoff in the ecologically vulnerable region. *Bacillus altitudinis* (OS-1) and *Priestia megaterium* (TDS-3) were chosen as initial bio-fertilizer consortia to enhance okra (*Abelmoschus esculentus*) growth, a crucial dietary crop. After salt tolerance and nutrient content analysis, bacterial suspensions were applied to the okra plants, confirming the growth-promoting abilities of native halotolerant strains. OS-1 exhibited the highest growth promotion, with TDS-3 showing intermediate effects. However, the combined application of both strains had an antagonistic effect, emphasizing the necessity for further analysis and gene sequencing to understand the underlying molecular dynamics. This study pioneers the design of a preliminary biofertilizer for optimal agricultural benefits in challenging environments, highlighting the vital role of PGPR in sustainable agriculture. The inclusion of Time and Series analysis along with ANOVA testing enhances the comprehensiveness, providing rigorous statistical analysis of halotolerant PGPR effects on okra growth, ultimately offering a promising avenue to boost crop yields and alleviate environmental stressors in vulnerable regions.

**Keywords:** Antagonism,bio-fertilizer, biological nitrogen fixation, bioremediation, Ghoramara island, plant growth promoting rhizobacteria.

## INTRODUCTION

Ghoramara Island, situated in the Bay of Bengal, stands as a poignant testament to the relentless forces of nature. This fragile landmass is exceedingly vulnerable to the devastating impacts of cyclones and rising sea levels, which perpetually threaten its very existence. The recurrent onslaught of cyclones not only disrupts the normal lives of the island's inhabitants but also wreaks havoc on its precious soil content and fertility through runoff, leading to the gradual disintegration of this once-thriving island ecosystem (Hajraand Ghosh, 2018).

In light of these dire circumstances, our research endeavors to address the multifaceted challenges faced by the Ghoramara Island community. We have embarked on a mission to explore sustainable agricultural practices that can not only enhance the livelihoods of the island's residents but also contribute to the conservation of this unique ecological haven. Central to our investigation is the exploration of halotolerant Plant Growth-Promoting Rhizobacteria (PGPR) indigenous to Ghoramara Island.

The rhizosphere, teeming with diverse microbes, supports plant growth through interactions, particularly with Plant Growth-Promoting Rhizobacteria (PGPR). These bacteria, including *Azospirillum, Rhizobium, Azotobacter, Pseudomonas, and Bacillus*, can fertilize a variety of crops like rice, beans, strawberries, potatoes, maize, tomatoes, and cucumbers. They stimulate plant development through nutrient acquisition, phytohormone production, biological nitrogen fixation (BNF), disease control via induced systemic resistance (ISR), antagonism, and the release of volatile organic compounds (VOCs) (Lee *et al*. 2021). These helpful bacteria colonize

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both monocot and dicot roots, enhancing plant growth directly and indirectly (Chandran *et al*. 2021). They produce phytohormones and other signals that promote lateral root branching, root hair growth, and alter root system architecture. PGPR also impacts root activity, improves plant nutrition, and influences overall plant physiology. PGPR can increase nutrient concentration in the rhizosphere by capturing and retaining nutrients, preventing their loss (Choudhary *et al*. 2011). Nitrogen, crucial for amino acid and protein synthesis, is often the most limiting nutrient for plants. Prokaryotes are the sole organisms capable of converting atmospheric nitrogen into organic forms usable by plants (Lloret and Martínez-Romero, 2005; Raymond *et al*. 2004). Some PGPR can enhance phosphate solubility, making phosphate ions in the soil more readily accessible for plant uptake (Wani *et al*. 2007).

Recent research has also uncovered PGPR signals influencing plant responses, yet the precise molecular processes at the plant level, whether localized or systemic, often remain enigmatic. In ecological terms, PGPR form functional clusters within the rhizosphere, adapting to various environmental factors. These factors, in turn, influence PGPR effects on roots (Vacheron *et al*. 2013). These halotolerant bacteria are often considered of significant agricultural and environmental importance due to their adaptability in challenging environments, as described by Egamberdieva *et al*. (2019). Notably, their adaptability is attributed to mechanisms such as ROS production, efflux systems, and secondary metabolite synthesis.

Our research's primary objective is to assess the capabilities of native PGPR species in promoting plant growth. It extends Kamila *et al*.'s (2022) systematic approach, primarily focusing on salt tolerance and quantifying nitrogen and phosphorus content in the island's soils.

Subsequently, we chose two strains OS-1and TDS-3and the objective was to check the potentiality of these native PGPR. It is also to be noted that Okra was chosen as our test crop for their significance as a staple crop in the region.

## MATERIALS AND METHODS

### *Bacterial strain selection*

A total of 8 bacterial isolates from the Ghoramara island were tested for plant growth promoting activity along with their salt tolerant property as mentioned in the report (Kamila *et al*. 2022). Based on the initial data two strains, TDS-3 and OS-1 were selected for testing in Okra plant growth promotion.

### *Small scale trial on Abelmoschus esculentus (Okra)*

### *Seed germination in presence of bacterial cultures*

Bacterial strains TDS-3 and OS-1, isolated from Ghoramara Island, was chosen as the strain to study its plant growth promoting properties in Okra growth.

The seeds underwent surface sterilization involving exposure to 70% ethanol for 1 minute, followed by a 3-4minute immersion in 3.5% Sodium hypochlorite, rinsed thoroughly with autoclaved water. (Asghar *et al*. 2002; Khan *et al*. 2022).

To ensure the viability of these bacterial strains, bacterial suspensions were prepared by inoculating separate containers with TDS-3 and OS1 in sterile nutrient broths, followed by overnight incubation in an incubator-shaker. Two sterilized Whatman filter papers were used, each soaked in bacterial inoculate. An okra seed was placed between the soaked papers, rolled, and then carefully positioned in designated beakers, following established protocols.

After the priming process,the seeds were exposed to light for 16 hours and then placed in darkness for 8 hours, for the process of germination.

### *Transfer of germinated seeds to designated pot setup*

These germinated seeds were meticulously sown into plant pots, forming the basis of our investigation. The study included 8 sets of plants: one served as the control group, while the other had seven pots, each containing various treatments. (Asghar *et al*. 2002).

These plant pots were systematically categorized into eight distinct groups: i) control pots with only soil; ii) pots with soil enriched with manure; iii) pots with soil inoculated with TDS-3; iv) pots with soil inoculated with OS1; v) pots with soil inoculated with both TDS-3 and OS1; vi) pots with soil enriched with manure and inoculated with OS1; vii) pots with soil enriched with manure and inoculated with TDS-3; and viii) pots with soil enriched with manure and inoculated with both OS1 and TDS-3. This categorization enabled a systematic investigation of the impacts of various treatments on okra plant growth and development.

***Monitoring and Data collection***

Throughout the study, plant growth was monitored, and investigative studies were conducted to assess the impact of these bacterial strains, following established protocols (Khan *et al*. 2022; Long *et al*. 2008). This research hence further underscores the significant role of these unique bacteria in both agricultural and environmental contexts (Kamila *et al*. 2022).

***Statistical analysis***

***ANOVA***

In this experiment, the independent variable comprises eight types of fertilizer: Control, Vermicompost, TDS-3, TDS-3+Vermicompost, TDS-3+OS-1, OS-1, OS-1+Vermicompost, and OS-1+TDS-3+Vermicompost. The dependent variable is the stalk length measured at week 8.

The study aims to evaluate how different fertilizers affect plant growth, specifically stalk length. The null hypothesis (H0) suggests no significant difference among fertilizers in terms of stalk length, while the alternative hypothesis (H1) proposes the presence of a significant difference.

To test these hypotheses, we conducted a one-way analysis of variance (ANOVA). Stalk length was measured at the end of the 8-week period for each treatment group, allowing for a comprehensive assessment of the influence of various fertilizers. The study assessed the stalk length of plants at week 8 following the application of various bio-fertilizers to determine if there were any significant differences. The null hypothesis assumed no notable distinctions among the fertilizers regarding stalk length. To confirm or reject this hypothesis, a significance level of 5% was set, and the F value in the ANOVA table was considered. If the F value exceeded the critical F value, the null hypothesis would be rejected, indicating a substantial variance in stalk length linked to the choice of bio-fertilizer (Roy *et al*. 2023).

***Time Series***

The time series analysis was conducted using the stalk length data to analyze the trend in stalk length variation with respect to the different treatment sets. The data on stalk length of plants after applying various categories of fertilizers against time (weeks) was plotted(Selvamuthu*et al*. 2019b).

**RESULTS**

***Vegetative growth parameter***

In the context of plant growth promotion, assessing the impact of Plant Growth-Promoting Rhizobacteria (PGPR) on overall plant development was essential. Stalk length analysis served as a proxy for plant growth, with increased stalk length indicating improved growth. This highlighted the positive influence of PGPR on plant development(Fig.1) and their significance as biofertilizers in sustainable agriculture. Additionally, thorough analysis was conducted and proper comparison was done (Fig.2), considering both the positive control and standard control, to accurately measure the observed impact. This approach enhanced the depth and reliability of our findings by providing a comprehensive understanding of the effects in relation to established benchmarks.

***Yield Parameter***

Our fruiting body analysis (Fig.3) revealed varying outcomes in different experimental setups (Fig.

4). Fruiting activity was visible from the ninth week. The control group showed no growth, serving as a baseline. While OS-1 displayed robust growth in fruiting bodies, indicating a highly positive impact. TDS-3 exhibited some growth, though not as pronounced. When OS-1 was combined with vermicompost, there was some growth but less than OS-1 alone. Similarly, TDS-3 combined with Vermicompost showed growth but not as significant as TDS-3 alone. Interestingly, when OS-1 and TDS-3 were combined, no growth in fruiting bodies was observed. This suggests a potential neutral or inhibitory interaction between these strains. This pattern held when both OS-1 and TDS-3 were combined with Vermicompost. These findings offer insights into the effects of bacterial strains and their interactions on fruiting body development in our study.

***Statistical Analysis***

***ANOVA for all parameters***

**Table 1**: ANOVA analysis for all the parameters

| Source | Df | Sum Sq | Mean Sq | F value | Pr(>F) | F-critical |
|---|---|---|---|---|---|---|
| Levels | 7 | 354.5 | 50.64 | 1.207 | 0.395 | 3.500464 |
| Residuals | 8 | 335.7 | 41.96 | - | - | - |

Our one-way ANOVA analysis, conducted at a 5% significance level, led to the failure to reject the null hypothesis (Table 1). The associated p-value (0.395) indicates that the data doesn't strongly support rejecting the null hypothesis, signifying no differential effect of the bio-fertilizers collectively on stalk length at week 8. About 41.96% of the variability in stalk length remains unexplained, attributed to random errors.

**Table 2** : ANOVA analysis for Combination TDS-3+OS-1 and OS-1

| Source | Df | Sum Sq | Mean Sq | F value | Pr(>F) | F-critical |
|---|---|---|---|---|---|---|
| Levels | 1 | 203.1 | 203.06 | 3.363 | 0.197 | - |
| Residuals | 2 | 111.8 | 55.92 | - | - | - |

***ANOVA analysis for OS-1+ TDS-3 and OS-1***

showed an F-value of 3.631 with a corresponding p-value of 0.197, suggesting a meaningful difference in stalk length when OS-1 is applied independently versus in combination with TDS-3.

***Time Series Analysis***

Time series analysis is a potent statistical tool for dissecting data collected at regular intervals. Its primary mission is to unveil hidden patterns, trends, and relationships within the data, paving the way for precise forecasts and the extraction of insightful wisdom crucial for informed decision-making. Vital components of time series data encompass trends, seasonality, cyclic rhythms, and irregularities. Smoothing techniques, such as moving averages or exponential smoothing, serve to strip away noise, allowing the spotlight to shine on the underlying trends and seasonality in the data. In our study, we harnessed the power of time series analysis to delve into the captivating trends associated with various treatments.

The in-depth time series analysis of stalk length data (Fig.5)unveiled that among eight different comparisons, OS-1 emerged as the most effective biofertilizer. This discovery holds considerable importance for agriculture and plant growth optimization. The data consistently demonstrated that OS-1-treated plants consistently outperformed those treated with other biofertilizer combinations, emphasizing OS-1's potency and its potential role in boosting crop yields and overall plant growth.

## DISCUSSION

The halotolerant bacterium OS-1 demonstrated a significant acceleration of okra plant growth within six weeks post-planting, indicative of its favorable interactions with the plant. Conversely, the impact of the halotolerant bacteria TDS-3 on plant growth was more moderate. Surprisingly, when combined, OS-1 and TDS-3 resulted in a noteworthy reduction in plant growth, highlighting the complexity of the interaction between these two bacterial strains.

In the context of all biofertilizers considered together, a one-way ANOVA analysis revealed no significant difference in stalk length at week 8, with approximately 41.96% of unexplained variability attributed to random errors. However, when specifically comparing the impact of OS-1 alone and in combination with TDS-3, a significant difference was observed. This underscores the

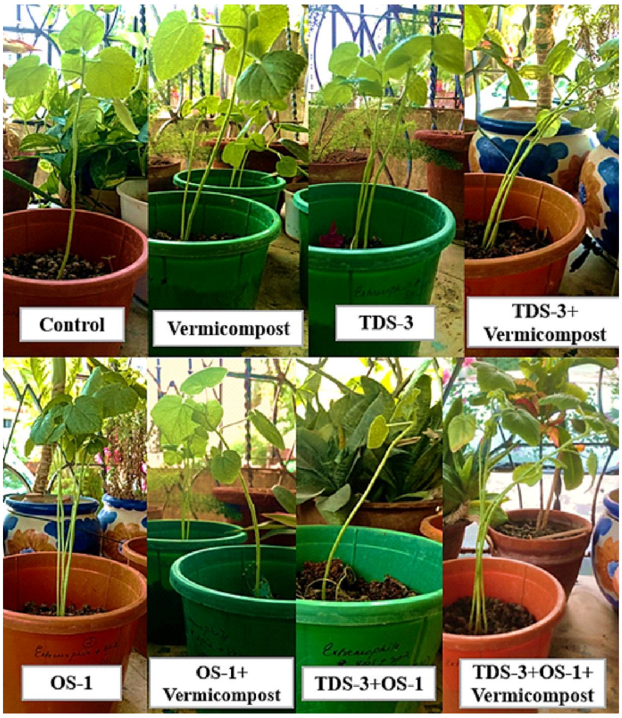


**Fig. 1**: The representative images for different treatment setups of the Okra plants

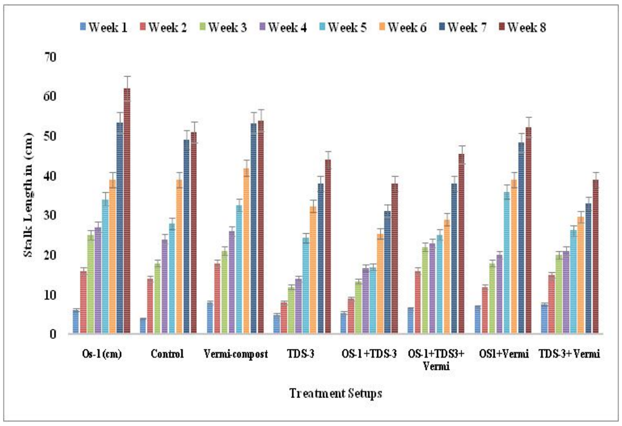


**Fig.2**: Stalk Length variation over a period of 8 weeks for the experimental set ups.

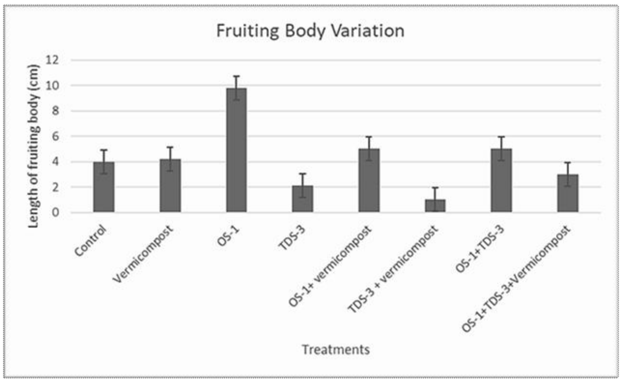


**Fig. 3**: Analysis of the length of the Yield parameters of different treatments.


**Fig.4**: Fruiting observed in the different Plant setups.

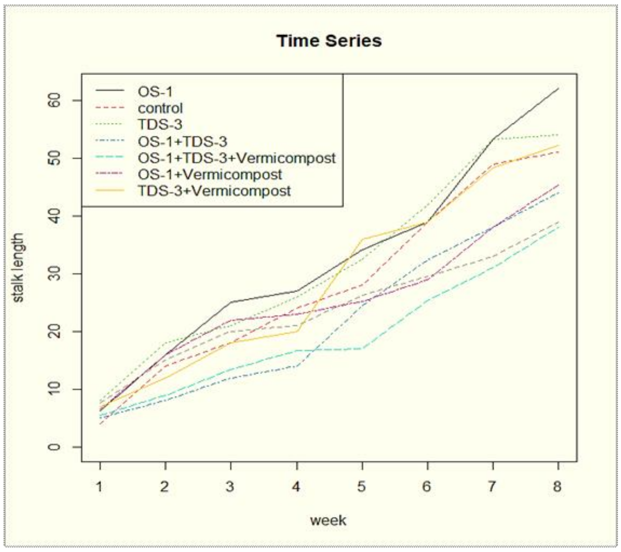


**Fig. 5** : Time series analysis of stalk length data

importance of individualized treatments for optimizing plant growth. Although no significant difference emerged when considering all biofertilizers collectively, a closer examination of OS-1 and its combination with TDS-3 unveiled a meaningful influence on stalk length. This emphasizes the critical role of tailored bio-fertilizer choices in agricultural research to maximize plant growth outcomes.

In contrast to a study by Al Ali *et al.*(2022), which focused on isolating bacteria from non-agricultural soils in the Al-Ahsa region, the current research delves into the intricate interactions between halotolerant bacteria and Okra plants. Specifically, emphasis is placed on the halotolerant bacterium OS-1 and its interactions with TDS-3. While they identified *Bacillus baekryungensis* (DPM17) with multifaceted bio-stimulating characteristics, this study takes a distinctive approach by exploring the combined effects of OS-1 and TDS-3 on okra plant growth. The unexpected reduction in plant growth observed when these two bacterial strains are combined underscores the complexity of plant-microbe interactions. Additionally, a time series analysis consistently showcasing the superior performance of OS-1 in promoting stalk length over time provides a dynamic perspective on plant growth dynamics, offering insights often overlooked in static analyses. This work contributes to understanding the impact of extremophilic bacteria on plant growth and underscores the importance of tailored bio-fertilizer choices and multidisciplinary collaboration for optimal outcomes in agricultural research. Beyond scientific inquiry, the research project aligns with a common commitment to improving food security and livelihoods, demonstrating a dedication to addressing unique environmental challenges on Ghoramara Island.

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## DECLARATION

Conflict of interest: Authors declare no conflict of interest.

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