

# Application of Plant Growth Promoting Rhizobacteria for Growth Enhancement of *Tamarix Aphylla* in District Attock

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<https://doi.org/10.62345/jads.2025.14.2.122>

## Abstract

Microorganisms are essential for soil fertility. A study conducted in Attock, a subtropical broad-leaved evergreen forest, discovered that plant growth-promoting rhizobacteria (PGPR) from the rhizosphere of *Tamarix aphylla* are genetically diverse and possess various beneficial traits for plant growth. These traits include phosphate solubilization, indole acetic acid production, ammonia production, and siderophore production. The identified rhizobacterial isolates, specifically *Bacillus wiedmannii*, show promise as bioinoculants for sustainable agricultural practices. This study highlights the importance of soil diversity and microbial interactions in agricultural ecosystems, suggesting that concurrent screening of PGPR in the field can effectively aid in selecting candidates for biofertilizer development. Further research is necessary to understand the diversity present in the rhizosphere fully.

**Keywords:** Plant Growth, Promoting Rhizobacteria, Attock.

## Introduction

Invasive species are plants that grow outside their natural habitats, often displacing native species in the process (Milton, 2004). South Africa has long struggled with the encroachment of invasive alien species and the difficulties of implementing effective management strategies (McConnachie et al., 2011; Milton, 2004). Richardson and Van Wilgen (2004) emphasised that this invasion significantly threatens local ecology, biodiversity, and economic stability. One particularly concerning invasive species in both South Africa and the United States is *Tamarix* (Marlin et al., 2017).

*Tamarix* plants serve various functions, including erosion control, support for apiculture, windbreaks, shade provision, and ornamental uses (DiTomaso, 2000; Henderson, 2007;

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Gaskin, 2003). In South African gold mines, particularly at the AngloGold Ashanti (Vaal River) gold mine in the North West Province, *Tamarix* species are utilised for phytoremediation purposes. Since around 2005, only the native *T. usneoides* has been used for this purpose (Mayonde et al., 2015). This plant is known to alter the physical and chemical composition of its environment. Its notable effects include sediment entrapment and stream narrowing (Blackburn et al., 2009), reduced groundwater levels due to its extensive water consumption from deep roots (McDaniel et al., 2016), and increased soil salinity from the release of salts through its salt glands (Brotherson & Field, 1987). Additionally, salt accumulation can limit the types of plants that can thrive beneath its canopy, and its flammable litter can contribute to wildfires (Busch, 1995).

Managing invasive plant species in South Africa costs ZAR 6.5 billion annually. Under the Conservation of Agricultural Resources Act (CARA) (ACT 43 of 1983), exotic *Tamarix* species are classified as level 1b weeds (Newete et al., 2019). Regulation 15A of the Act explicitly prohibits the cultivation, maintenance, sale, or import of these Category 1 weeds, including exotic *Tamarix* species (Henderson, 2001).

In South Africa, the indigenous *T. usneoides* can hybridise with two alien *Tamarix* species, *T. ramosissima* and *T. chinensis* (Mayonde et al., 2015; Newete et al., 2019). The presence of native *T. usneoides* complicates potential biocontrol programs, as it can only be targeted if the proposed introduction of the *Tamarix* beetle occurs in South Africa, potentially undermining practical biocontrol efforts (Marlin et al., 2017). *Tamarix* trees are known for their rapid growth, reaching heights of 4 meters annually (Gary, 1965). Research by Warren and Turner (1975) and Mortenson et al. (2012) indicates their resilience to flooding, as they can endure complete submersion for up to 70 days and flooding of the root crown for up to 98 days. Even seedlings demonstrate flood tolerance, surviving inundation for about a month (Horton, 1960). The impact of *Tamarix* spp. on its habitat is substantial, notably narrowing river channels and contributing to increased instances of flooding (Mayonde et al., 2015; Brotherson & Field, 1987; Blackburn et al., 2009). With extensive roots reaching up to 30 meters, *Tamarix* can tap into subsurface water resources (Brotherson & Field, 1987). Being phreatophytes with a lengthy lifespan, *Tamarix* has the potential to significantly lower groundwater levels, particularly in regions such as the Western Cape, which have experienced recent droughts (Brotherson & Field, 1987; Le Maitre et al., 2015). Invasive *Tamarix* in South Africa is observed to consume twice the amount of water compared to other coexisting species (Le Maitre et al., 2015). The karoo biome and its riparian zones face notable threats due to *Tamarix*'s ability to infiltrate disturbed environments and regenerate after undergoing physical and mechanical management (Brotherson & Field, 1987; Newete et al., 2019).

In Pakistan, indigenous healers frequently utilise the ethnomedicinal herb *Tamarix aphylla* (from the Tamaricaceae family). This plant is abundant across the country, exhibiting antifungal properties and causing no adverse side effects (Marwat & Rehman, 2011). The bark of *Tamarix aphylla*, known for its astringent and tonic properties, is a popular remedy for various ailments, including hepatitis, eczema, and skin conditions such as candidiasis, syphilis, and scaly skin disorders (Panhwar & Abro, 2007). Local communities also use a poultice made from its bark powder for treating minor wounds.

On a global scale, *Tamarix aphylla* (L.) Karst., known as Khagal (Urdu) and Ghaz (Pashto) in Pakistan, is employed for medicinal purposes (Lefahal et al., 2010). *Tamarix aphylla* (Family, Tamaricaceae) is the largest and most commonly planted ornamental tree along roadsides in Pakistan (Marwat et al., 2008). Indigenous medical practitioners in the country prefer using various parts of the plant, including the bark, leaves, stem, and twigs, for a range of ailments due to their lack of adverse effects. The needle leaves are applied to injured body parts and then boiled in water, suggesting their potential in treating infectious disorders (Marwat et al.,

2008; Panhwar & Abro, 2007). Safe and cost-effective therapeutic compounds derived from plants may serve as promising alternatives for treating microbial diseases (Nisar et al., 2015). This study addresses the lack of previous research on the molecular characterisation of *Tamarix aphylla* roots and their rhizospheric soil. The objectives of this research are to: (1) isolate bacteria from the roots and rhizospheric soil of *Tamarix aphylla*, and (2) perform biochemical and molecular characterisation of these isolates, as well as evaluate the potential of plant growth-promoting rhizobacteria (PGPR) to improve seed germination and plant growth.

## Materials and Methods

The research will be conducted in the Attock district of Pakistan, focusing on its natural Scrub Forest area. The study site is located at specific latitude and longitude coordinates. The elevation of this region is approximately 458 meters above sea level. The mean annual temperature in the study area is 23°C with an average annual rainfall of 35 inches. The flora in this region primarily comprises *Acacia modesta*, *Dodonea visosa*, *Olea ferruginea*, and *Tamarix aphylla*.

### Survey and collection of rhizospheric soil and roots

In the tropical evergreen forest, it will be selected for the survey. At least 21 random soil samples (from seven tehsils) will be taken from rhizosphere of *Tamarix aphylla* at a depth of 0-15cm, 15cm-30cm and 30-45cm, respectively, and further mixed to make seven composite samples. Sample bags will be stored at 4°C in coolers during transit, and after reaching the lab, they will be preserved at 20 °C.

### Instrument used for data collection

Soil samples and relevant data will be collected with the assistance of a Soil Auger, Scissors, Zipper Bags, a Cutter, a Notebook, a Pen, an Ice Cooler, and a GPS device.

### Isolation of bacteria from rhizospheric soil of *Tamarix aphylla*

10g of soil in 100ml of water will be used to isolate bacteria from the rhizospheric soil of *Tamarix aphylla* through the serial dilution technique.

### Bacterial growth on L.B. (*Luria bertani*) agar medium

The Luria-Bertani agar medium is formulated using the following components: 1 litre of distilled water, 1 N NaOH, 10 grams of NaCl, 5 grams of yeast extract, 10 grams of tryptone, and 18 grams of agar (Miller, 1972). The pH will be maintained at 7.0. To ensure sterility, the medium will be autoclaved for 15 minutes at 121 degrees Celsius.

### Serial dilution

In autoclave-sterilised Falcon tubes with a 15 mL volume, serial dilutions of  $10^{-1}$  to  $10^{-9}$  will be made using sterilised distilled water. For dilution, 1 g of rhizospheric soil will be divided into 10 g and placed in a sterile Falcon tube with 100 ml of distilled water. The tube will then be centrifuged at 4000 rpm for 15 minutes. Following that, 50 L of supernatant will be used for consecutive dilutions of  $10^{-1}$  to  $10^{-9}$ .

### Streaking

The sterilised Luria-Bertani agar growth media will be poured into sterilised petri plates under a laminar flow hood air cabinet and left for 10 minutes to settle. Randomly, three serial dilutions will be selected. From the selected serial dilution, 50 µl will be spread on petri plates using a sterilised L-shaped spreader. After labelling, the plates will be kept in an incubator at 28°C for

24-48 hours. After 24 hours, a single bacterial colony will be taken for streaking and re-streaking to obtain pure cultures using a sterilised wire loop.

### **Preservation of bacteria isolated from soil**

A single pure colony of the bacterial isolate will be taken from an agar plate under a laminar flow hood air cabinet and suspended in a sterilised Eppendorf tube (1.5 ml) containing 80% glycerol stock, then preserved at -80 °C for future use.

### **Biochemical Characterization**

#### **Gram Staining**

The method of Vincent (1970) will be used for gram staining. A tiny Portion of Pure colony will be suspended into a drop of water on a slide using a sterile loop. A smear will be made, air dried and heat-fixed. After the following solutions (Appendix 4) will be used for staining:

1. Flooding for a minute with crystal violet solution, followed by five seconds of rinsing with tap water.
2. Flooding for one minute with Gram's Iodine Solution, then rinsing with tap water and draining.
3. Decolonisation with 95% alcohol (10 sec), then washed off with tap water and drained.
4. Flooding with Safranin solution (30 sec), washed with water, drained and observed under compound microscope with a drop of oil immersion.

#### **Catalase Test**

By adding a drop of H<sub>2</sub>O<sub>2</sub> to a 24-hour-old bacterial colony on a glass slide (30 per cent), it will be possible to determine whether catalase is present. The presence of the catalase enzyme is indicated by the production of gas bubbles (McFadden, 1980).

#### **P- Solubilization Index (SI)**

The isolated bacterial colonies with tricalcium phosphate will be cultured under sterile conditions for a week at 28 °C using autoclaved Pikovskaya's medium. The Solubilization Index (SI) will be calculated using the formula (Premono et al., 1996), represented by the equation:  $SI = (\text{Colony diameter} + \text{Halo zone diameter}) / \text{Colony diameter}$ .

### **Extraction of dna of bacteria isolated from rhizosperic soil of *Tamarix aphylla***

A 24-hour old bacterial colony isolated from soil of *Tamarix aphylla* will be grown on LB agar media and then pure single colony will be inoculated in L.B broth and kept on incubator shaker (150 rpm) for 24 hours at 37°C, DNA extraction mini kit Qigex dist teste will be used. Following steps will be followed for extraction of DNA.

### **DNA extraction procedure for the bacteria isolated from rhizospheric soil of *acacia modesta* extraction of dna through qiagen kit**

1. First of all buffer (AWL) will be prepared by mixing ethanol 250 ml.?
2. 1 ml of bacterial inoculate will be taken in Eppendorf and centrifuged.
3. Centrifugation will be done at 7500 rpm for 5 minutes at 11°C and more than 25°C.
4. Pellet will be obtained and supernatant will be discarded.
5. ATL buffer was placed in water bath for 5 minutes and 20 µl proteinase K.
6. Vortex for 10-15 seconds.
7. Kept in water bath for 1-hour AT 56 °C until completely lysed.
8. Add (200 µl) buffer Al (Lyses buffer).
9. Vortex for 15 second.
10. Incubate at 70°C for 10 minutes.

12. Add (200 µl) ethanol.
13. Vortex for 15 second.
14. Shifted in column tube.
15. Centrifugation at 8000 rpm for 1 minute at 11° C temperatures.
16. Discard the flow through and collect then collection tube.
17. Shifted in new collection tube.
18. Added (500µl) AW1 buffer (wash buffer),
19. Centrifuge for one minute at 8000 rpm.
20. Throw away the flow through and collecting tubes.
- Changed the column tube.
22. A (500 l) AW2 buffer was added.
23. Perform a 3-minute centrifugation at high power (1400 rpm).
24. Throw away the flow through and collecting tubes.
25. moved in the column tube.
26. Transfer to a new 1.5 ml microcentrifuge tube using a Qiagen spin column.
27. Gently introduce 100 µl of buffer AE.
28. Allow to incubate for one minute at room temperature.
29. Spin at 8000 RPM for one minute.
30. Discard the column and retrieve the DNA in an Eppendorf tube.

### **Gene Sequencing**

After DNA extraction PCR will be performed by using universal forward and reverse primers then sent to MacroGen Korea for identification of bacteria on the basis of 16S rRNA gene sequencing.

### **Amplification of 16S-rRNA Gene**

In a PCR using Biometra equipment from Germany, amplification of the 16S rRNA gene will be carried out employing 25 µl of My Taq master mix, composed of the subsequent constituents: 16.5 µl of MQ H<sub>2</sub>O, 5 µl of 5 X My Taq reaction buffer, 1 µl of DNA template, 1 µl of 27F primer, 1 µl of 1492R primer (Turner et al., 1999), 0.25 µl of BSA, and 0.25 µl of My Taq Polymerase. The gene will undergo 35 amplification cycles in the thermocycler. The procedure commences with an initial denaturation at 95°C for 3 minutes, succeeded by 34 cycles of denaturation at 95°C for 20 seconds. This is followed by primer annealing at 60°C for 20 seconds and primer extension at 72°C for the remaining 45 seconds, concluding with a final extension at 72°C for three minutes. After electrophoresis on a 1.5 percent (w/v) agarose gel, the amplified PCR products will be purified utilizing the ISOLATE II PCR and Gel Kit (Bioline, Australia), following the manufacturer's instructions. The steps involve: (1) mixing the sample volume with the binding buffer CB, (2) placing the sample in an ISOLATE II PCR and Gel Column, centrifuging, and discarding the flow-through to bind the DNA, (3) washing the Silica membrane with 700 µl of CW wash buffer through centrifugation, repeating the washing for reducing salt carryover, (4) drying the Silica membrane with a 1-minute centrifugation at 11000 g to eliminate any residual ethanol, and (5) eluting the DNA from the silica membrane by adding 15 to 30 µl of elution buffer C followed by incubation at room temperature for a minute and a subsequent centrifugation at 11000 g for a minute. The final DNA obtained has a purified structure, achieved through centrifugation at 11000 g.

### **Inoculation of bacteria on seedlings of *tamarix aphylla***

The seedlings will be six months old and following treatments of inoculation will be applied. There will be 6 treatments and three replications and five plants in each replication. Total will be 180 plants and design will be RCBD.

To= control  
 T1= PGPR 1  
 T2= PGPR2  
 T3= PGPR3  
 T4= PGPR 1+2  
 T5 PGPR1+3  
 T6= PGPR2+3

Various growth parameters will be assessed, such as seedling height, root-to-shoot ratio, leaf count, leaf length, branch count, and collar length. Furthermore, we will analyze the content of total ash, calcium, phosphorus, nitrous-free extract, ether extract, crude protein, crude fiber, and total ash.

### Statistical analysis

SPSS software 16.0 will be used.

## Results

### Isolation of bacteria from rhizospheric soil of *tamarix aphylla*

A detailed survey was carried out in Attock, having coordinates mentioned in Table 1. The bacteria isolated from *Tamarix aphylla* was named as TS (Tamarix soil) Table 2 with location.

**Table 1: Climate and Geographic location of the research area**

| Sr. No. | Total Area (HA) | Forest Type                                      | Site | Altitude (m) | Co-ordinates              |
|---------|-----------------|--------------------------------------------------|------|--------------|---------------------------|
| 1       | 685,700         | Sub tropical broad leaved<br>evergreen<br>forest |      | 355m         | 33.7660° N,<br>72.3609° E |

**Table 2: Bacteria isolated from rhizospheric soil of *Tamarix aphylla***

| Sr. No. | Bacterial ID | Site              | Forest Tree            | Location            |
|---------|--------------|-------------------|------------------------|---------------------|
| 1       | TS           | Kala Chitta Range | <i>Tamarix aphylla</i> | Attock, Kala Chitta |

### Biochemical characterization of isolated bacteria from rhizospheric soil of *tamarix aphylla*

For biochemical characterization, bacteria isolated from rhizospheric soil of *Tamarix aphylla* tree having bacterial strains TS was tested, following four tests were carried out; showed in Table 3.

#### Cytochrome Oxidase Test

Bacteria isolated from rhizospheric soil having strain (TS) was tested for oxidase test, the strain showed oxidase positive, given in Table 3.

#### Catalase Test

Isolated bacteria were tested and showed catalase positive test for bacterial strain as show in Table 3.

#### Indole Acetic Acid (IAA)

Indole acetic acid test was carried out for isolated bacteria. Isolated bacteria from Kala Chitta rhizospheric soil having strains TS showed negative test, given in Table 3. Optical density was measured at 530 nm with the help of spectrophotometer, given in Table 3.



**Phosphate Solubilization Index (SI) Test**

Isolated bacteria were checked for P-Solubilization test. All the bacterial strains showed a clear halo-zone formation, the test was considered P-Solubilization positive, given in Table 3.

**Table 3: Biochemical characterization of isolated bacteria from rhizospheric soil of *juniperus excelsa* tree specie**

| Sr. No. | Characterization         | TS |
|---------|--------------------------|----|
| 1       | Oxidase reaction         | +  |
| 2       | Catalase reaction        | +  |
| 3       | Indole acetic acid (IAA) | -  |
| 4       | P-Solubilization         | +  |

**Table 4: Table Showing optical density of IAA production of isolated bacterial strain at 530 nm**

| Sr. No. | Bacteria Isolated | Optical Density at 530 nm |
|---------|-------------------|---------------------------|
| 1       | TS                | 0.917                     |

**Molecular characterization of isolated bacteria**

**Quantification of DNA**

The bacteria isolated strains TS has the following DNA quantification, given in Table 4.

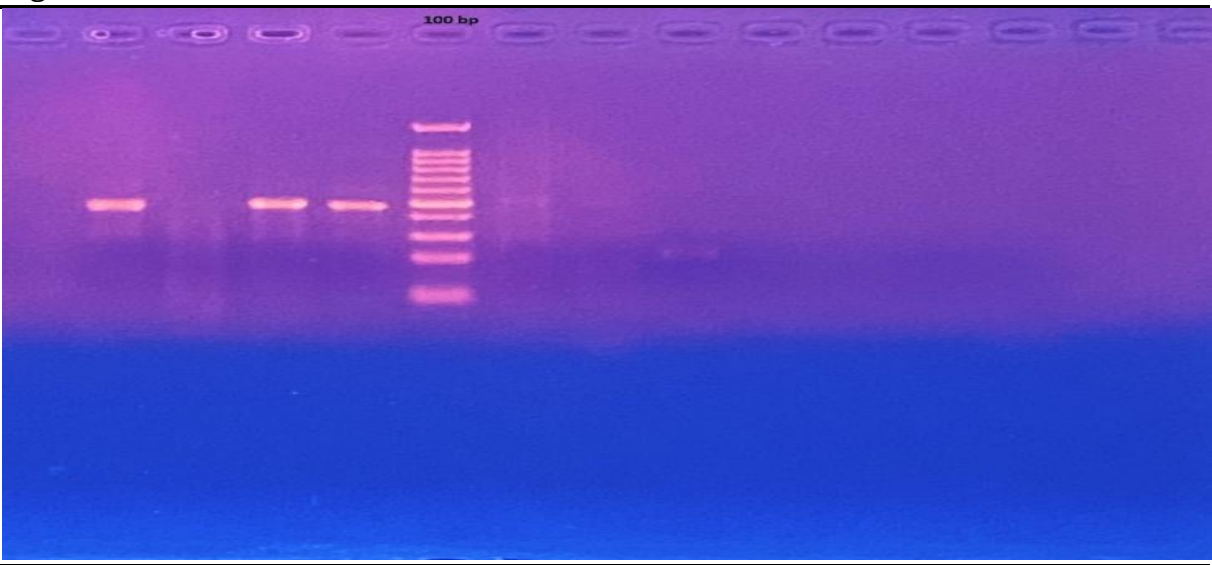
**Table 5: Showing DNA quantification of bacteria isolated**

| S. No. | Bacterial ID | ng/ $\mu$ L | 260/280 |
|--------|--------------|-------------|---------|
| 1      | TS           | 1353        | 2.15    |

**PCR Gel Electrophoresis**

After DNA extraction from the sample isolated from rhizospheric soil of *Tamarix aphylla* through DNA extraction procedure, bacterial strains TS was sent to Macrogen Korea for getting gene sequencing, given in the figure 1.

**Figure 1: DNA of bacteria isolated**



Identification of Isolated Bacteria Through Gene Sequencing

>1st\_BASE\_5164998\_TS\_Primer  
NNNNNNNNCTTCNCGCTCAGTGTACAGACCAGAAAGTCGCCTTCGCCACT  
GGTGTTCCTCCATATCTCTACGCATTTACCGCTACACATGGAATTCCACTTTCCT  
CTTCTGCACTCAAGTCTCCCAGTTTCCAATGACCCTCCACGGTTGAGCCGTGGGC  
TTTCACATCAGACTTAAGAAACCACCTGCGCGCGCTTTACGCCCAATAATTCCGG  
ATAACGCTTGCCACCTACGTATTACCGCGGCTGCTGGCACGTAGTTAGCCGTGGC  
TTTCTGGTTAGGTACCGTCAAGGTGCCAGCTTATTCAACTAGCACTTGTTCCTCCC  
TAACAACAGAGTTTTACGACCCGAAAGCCTTCATCACTCACGCGGCGTTGCTCCG  
TCAGACTTTCGTCCATTGCGGAAGATTCCCTACTGCTGCCCCCATAGGA

Identified strain TS from rhizospheric soil of *Tamarix aphylla* was carried out by 16-S rRNA sequence analysis. The comparison of sequence shown in (Table 6), the bacteria having strain TS showed 99.07 %homology with *Bacillus wiedmannii*.

Table 6: Alignment of 16-S rRNA and phylogenic tree of bacterial isolated strains

| ID | Specie                     | Homology |
|----|----------------------------|----------|
| TS | <i>Bacillus wiedmannii</i> | 99.07    |

Discussion

Historically, plants have played a vital role in supplying high-quality fodder for livestock. Today, however, many countries are experiencing a shortage of this essential resource, resulting in an increased demand for tree-based fodder. This strategy not only improves livestock health but also supports ecosystem restoration and yields high-quality timber. Trees offer numerous benefits, contributing significantly to ecological services. They supply valuable pharmaceutical compounds, protein-rich fodder, fuelwood for local communities, and essential minerals (Anuragi et al., 2023).

The use of medicinal plants has been intertwined with human civilisation since ancient times. Among these plants, *Juniperus excelsa* stands out as one of the most important and dominant tree species in many arid and semi-arid areas. Commonly known as Greek juniper, this significant member of the Cupressaceae family holds great importance as a medicinal plant. This comprehensive study aims to provide insights and an up-to-date summary of the traditional medicinal values of the *Tamarix aphylla* tree species. Additionally, it explores beneficial bacteria known as Plant Growth Promoting Rhizobacteria (PGPR) in the rhizospheric soil of the *Tamarix aphylla* tree species.

Plant Growth Promoting Rhizobacteria (PGPR) are a group of microorganisms that live in the rhizospheric soil of various plant species. They promote plant growth through several processes, including the production of plant hormones (such as auxins and gibberellins), mineral solubilization, siderophore production, phytohormone synthesis, enhanced nutrient uptake, increased leaf foliage, higher protein content in leaves, and functioning as biofertilizers. Additionally, PGPR protect plants from the adverse effects of oxidative stress by producing antioxidant enzymes that neutralise reactive oxygen species. These microorganisms and beneficial bacteria play an essential role in preserving soil ecology and fertility (Nagendran et al., 2021). Plant Growth Promoting Rhizobacteria (PGPR) offers cross-protection by enhancing plant defence mechanisms, reducing pathogen resistance through induced systemic resistance, and mitigating abiotic stress by altering phytohormone metabolism (Khan et al., 2020). These rhizobacteria play a vital role in protecting plants, improving soil health and promoting growth. Some well-known strains of beneficial bacteria include *Azospirillum*, *Pseudomonas*, *Serratia*, *Bacillus*, and *Rhizobium* species. These rhizospheric bacteria are



renowned for their symbiotic relationships with leguminous forage, promoting plant growth and soil fertility (Ijaz et al., 2019).

Today, the use of specific rhizobacteria as biofertilizers is considered crucial for ensuring high productivity and sustainability in the agricultural sector (Hakim et al., 2021). Most rhizospheric bacteria interact favorably with plants, significantly boosting their growth and survival (Bizos et al., 2020; Hakim et al., 2021).

Identification on the basis of phylogenetic analysis of 16S rRNA gene showed that isolates belong to *Bacillus wiedmannii* (Swami et al., 2016). Many rhizospheric bacteria from this genus have been identified as plant growth-promoting bacteria (Nagargade et al., 2018; Basu et al., 2021).

In this study, the objective was to research eco-friendly and cultural bacteria to create a prospective microbial consortium as bio-inoculants, to boost soil health, plant productivity and nutritional content. The isolated bacterial strain (*Bacillus wiedmannii*) is considered a novel species and has the capability to boost plant growth (Sarkar et al., 2021). Biochemical characterisation for all three isolated strains showed significant improvement of positive indole acetic acid (IAA), P solubilization, positive catalase and oxidase results.

Novel bacteria *Bacillus wiedmannii* was a novel bacteria, its a psychrotolerant, cytotoxic bacillus. Its origin was the USA.

## Conclusion

This study highlights the promising role of plant growth-promoting rhizobacteria (PGPR), particularly *Bacillus wiedmannii*, isolated from the rhizosphere of *Tamarix aphylla*, in enhancing plant growth and soil health in the subtropical forests of District Attock. The biochemical and molecular characterisation confirmed the presence of beneficial traits such as phosphate solubilization, catalase and oxidase activity, positioning this strain as a potential biofertilizer for sustainable forestry and agriculture. By fostering better seedling growth and improving nutrient uptake, these rhizobacteria offer an eco-friendly solution for boosting plant productivity while preserving ecological balance. This research lays the foundation for future field-scale applications and bio-inoculant development, supporting the broader goals of environmental restoration and sustainable land management.

## References

- Ahmed, A. U. (2006). *Bangladesh climate change impacts and vulnerability*. Climate Change Cell, Department of Environment.
- Anuragi, A., Sisodia, D. S., & Pachori, R. B. (2023). Classification of focal and non-focal EEG signals using optimal geometrical features derived from a second-order difference plot of FBSE-EWT rhythms. *Artificial Intelligence in Medicine*, 139, 1025–1042. <https://doi.org/10.1016/j.artmed.2023.102500>
- Basu, A., Prasad, P., Das, S. N., Kalam, S., Sayyed, R. Z., Reddy, M. S., & El Enshasy, H. (2021). Plant growth promoting rhizobacteria (PGPR) as green bioinoculants: Recent developments, constraints, and prospects. *Sustainability*, 13(3), 1140. <https://doi.org/10.3390/su13031140>
- Bizos, G., Papatheodorou, E. M., Chatzistathis, T., Ntalli, N., Aschonitis, V. G., & Monokrousos, N. (2020). The role of microbial inoculants on plant protection, growth stimulation, and crop productivity of the olive tree (*Olea europaea* L.). *Plants*, 9(6), 743. <https://doi.org/10.3390/plants9060743>
- Blackburn, T. M., Lockwood, J. L., & Cassey, P. (2009). *Avian invasions: The ecology and evolution of exotic birds*. Oxford University Press.
- Brotherson, J. D., & Field, D. (1987). *Tamarix*: Impacts of a successful weed. *Rangelands Archives*, 9(3), 178–182.
- Busch, D. E. (1995). Effects of fire on southwestern riparian plant community structure. *The Southwestern Naturalist*, 40(3), 259–267.

- DiTomaso, J. M. (2000). Invasive weeds in rangelands: Species, impacts, and management. *Weed Science*, 48(2), 255–265. [https://doi.org/10.1614/0043-1745\(2000\)048\[0255:TWIRSI\]2.0.CO;2](https://doi.org/10.1614/0043-1745(2000)048[0255:TWIRSI]2.0.CO;2)
- Gary, H. L. (1965). *Some sprouting characteristics of five-stamen tamarisk* (Vol. 39). Rocky Mountain Forest and Range Experiment Station, Forest Service, U.S. Department of Agriculture.
- Gaskin, J. (2003). Molecular phylogenetic investigation of US invasive *Tamarix*. *Systematic Botany*, 28(1), 86–95. <https://doi.org/10.1043/0363-6445-28.1.86>
- Hakim, S., Naqqash, T., Nawaz, M. S., Laraib, I., Siddique, M. J., Zia, R., & Imran, A. (2021). Rhizosphere engineering with plant growth-promoting microorganisms for agriculture and ecological sustainability. *Frontiers in Sustainable Food Systems*, 5, 171–177. <https://doi.org/10.3389/fsufs.2021.598421>
- Henderson, L. (2001). Alien weeds and invasive plants. *Biological Invasions*, 9(20), 2971–2992. <https://doi.org/10.1007/s10530-007-9300-2>
- Henderson, L. (2007). Invasive, naturalized and casual alien plants in southern Africa: A summary based on the Southern African Plant Invaders Atlas (SAPIA). *Bothalia*, 37(2), 215–248.
- Horton, J. S. (1960). *Seed germination and seedling establishment of phreatophyte species* (No. 48). Rocky Mountain Forest and Range Experiment Station, Forest Service, U.S. Department of Agriculture.
- Ijaz, B., Zhao, N., Kong, J., & Hua, J. (2019). Fiber quality improvement in upland cotton (*Gossypium hirsutum* L.): Quantitative trait loci mapping and marker-assisted selection application. *Frontiers in Plant Science*, 10, 1585–1596. <https://doi.org/10.3389/fpls.2019.01585>
- Khan, M., Khan, H., Khan, S., & Nawaz, M. (2020). Epidemiological and clinical characteristics of coronavirus disease (COVID-19) cases at a screening clinic during the early outbreak period: A single-centre study. *Journal of Medical Microbiology*, 69(8), 1114–1123. <https://doi.org/10.1099/jmm.0.001272>
- Le Maitre, D. C., Gush, M. B., & Dzikiti, S. (2015). Impacts of invading alien plant species on water flows at stand and catchment scales. *South African Journal of Botany*, 25(2), 888–897. <https://doi.org/10.1016/j.sajb.2015.03.002>
- Lefahal, M., Benahmed, M., Louaar, S., Zallagui, A., Duddeck, H., & Akkal, S. (2010). Antimicrobial activity of *Tamarix gallica* L. extracts and isolated flavonoids. *Advances in Natural and Applied Sciences*, 10(6), 289–293.
- Macfaddin, J. F. (1976). *Biochemical tests for identification of medical bacteria* (2nd ed.). Williams & Wilkins.
- Marlin, D., Newete, S. W., Mayonde, S. G., Smit, E. R., & Byrne, M. J. (2017). Invasive *Tamarix* (Tamaricaceae) in South Africa: Current research and the potential for biological control. *Biological Invasions*, 19(7), 2971–2992. <https://doi.org/10.1007/s10530-017-1477-5>
- Marwat, K. B., Khan, M. A., Nawaz, A., & Amin, A. (2008). *Parthenium hysterophorus* L.—A potential source of bioherbicide. *Pakistan Journal of Botany*, 40(5), 1933–1942.
- Marwat, S. K., & Rehman, F. U. (2011). Medicinal folk recipes used as traditional phytotherapies in district Dera Ismail Khan, KPK, Pakistan. *Pakistan Journal of Botany*, 43(8), 1453–1462.
- Mayonde, S. G., Cron, G. V., Gaskin, J. F., & Byrne, M. J. (2015). Evidence of *Tamarix* hybrids in South Africa, as inferred by nuclear ITS and plastid trnS–trnG DNA sequences. *South African Journal of Botany*, 96(2), 122–131. <https://doi.org/10.1016/j.sajb.2014.11.008>
- McConnachie, M. M., Cowling, R. M., Van Wilgen, B. W., & McConnachie, D. A. (2011). Evaluating the cost-effectiveness of invasive alien plant clearing: A case study from South Africa. *Biological Conservation*, 155(3), 128–135. <https://doi.org/10.1016/j.biocon.2011.01.009>
- McDaniel, V., Skaggs, R. W., & Negm, L. M. (2016). Injury and recovery of maize roots affected by flooding. *Applied Engineering in Agriculture*, 32(5), 627–638.
- McFadden, D. (1980). Econometric models for probabilistic choice among products. *Journal of Business*, 90(12), 128–137.
- Miller, R. L. (1972). Ultrafine-grained microstructures and mechanical properties of alloy steels. *Metallurgical and Materials Transactions B*, 3(20), 905–912.
- Milton, S. J. (2004). Grasses as invasive alien plants in South Africa: Working for water. *South African Journal of Science*, 100(1), 69–75.

- Mortenson, W. B., Demers, L., Fuhrer, M. J., Jutai, J. W., Lenker, J., & DeRuyter, F. (2012). How assistive technology use by individuals with disabilities impacts their caregivers: A systematic review of the research evidence. *American Journal of Physical Medicine & Rehabilitation*, 91(11), 984–998.
- Nagendran, G. A., Singh, H., Raj, R. J. S., & Muthukumaran, N. (2021, February). Input assistive keyboards for people with disabilities: A survey. In *2021 Third International Conference on Intelligent Communication Technologies and Virtual Mobile Networks (ICICV)* (pp. 829–832). IEEE.
- Newete, S. W., Mayonde, S. G., & Byrne, M. J. (2019). Distribution and abundance of invasive *Tamarix* genotypes in South Africa. *Weed Research*, 59(2), 191–200. <https://doi.org/10.1111/wre.12352>
- Nisar, N., Li, L., Lu, S., Khin, N. C., & Pogson, B. J. (2015). Carotenoid metabolism in plants. *Molecular Plant*, 8(1), 68–82. <https://doi.org/10.1016/j.molp.2014.12.007>
- Panhwar, A. Q., & Abro, A. A. (2007). Ethnobotanical studies of Mahal Kohistan (Khirthar National Park). *Pakistan Journal of Botany*, 39(7), 2301–2315.
- Premono, M., Moawad, A. M., & Vlek, P. L. G. (1996). Effect of phosphate-solubilizing *Pseudomonas putida* on the growth of maize and its survival in the rhizosphere. *Indonesian Journal of Crop Science*, 11(1), 13–23.
- Richardson, D. M., & Van Wilgen, B. W. (2004). Invasive alien plants in South Africa: How well do we understand the ecological impacts? *South African Journal of Science*, 100(1), 45–52.
- Sarkar, D. J., Sarkar, S. D., Das, B. K., Sahoo, B. K., Das, A., Nag, S. K., & Samanta, S. (2021). Occurrence, fate and removal of microplastics as heavy metal vector in natural wastewater treatment wetland system. *Water Research*, 192, 116–128. <https://doi.org/10.1016/j.watres.2021.116128>
- Swami, A., Kumar, R., Kumar, N., & Kumar, M. (2016). Phylogenetic analysis of 16S rRNA gene showed that bacterial isolates belong to *Bacillus wiedmannii*. *International Journal of Current Microbiology and Applied Sciences*, 5(10), 652–660.
- Turner, D. P., Cohen, W. B., Kennedy, R. E., Fassnacht, K. S., & Briggs, J. M. (1999). Relationships between leaf area index and Landsat TM spectral vegetation indices across three temperate zone sites. *Remote Sensing of Environment*, 70(1), 52–68.
- Vincent, J. M. (1970). *The cultivation, isolation and maintenance of rhizobia*. In *A manual for the practical study of the root-nodule bacteria* (Vol. 23, pp. 1–13). IBP Handbook No. 15. Blackwell Scientific Publications.
- Warren, D. K., & Turner, R. M. (1975). Saltcedar (*Tamarix chinensis*) seed production, seedling establishment, and response to inundation. *Journal of the Arizona Academy of Science*, 10(3), 135–144.