



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

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SUMMARY

The use of Plant Growth Promoting Rhizobacteria (PGPR) has surfaced as an effective strategy to improve plant growth and soil fertility. This study examines the effects of PGPR on the growth of *Tamarix aphylla* in the Attock district of Pakistan. Bacterial strains were extracted from the rhizospheric soil of *Tamarix aphylla* and subsequently identified using biochemical and molecular characterisation techniques. The strains found—*Bacillus wiedmannii*, *Enterobacter mori*, and *Pseudomonas sihuiensis*—were put on *Tamarix aphylla* seedlings that were six months old, and their effects on the seedlings' height and collar diameter were studied. The findings demonstrated a notable enhancement in growth parameters, with *Enterobacter mori* showing the most substantial growth promotion. Biochemical assays validated the synthesis of substances that promote plant growth, such as Indole Acetic Acid (IAA) and the solubilisation of phosphate. The results emphasise the promise of PGPRs as biofertilizers for promoting sustainable reforestation and restoring ecosystems.

Keywords: Plant Growth Promoting Rhizobacteria (PGPR), Growth Enhancement, Sustainable Agriculture, Microbial Inoculation. Afforestation

INTRODUCTION

Tamarix species are acknowledged for their ecological and environmental importance, fulfilling multiple roles including erosion control, windbreaks, shade provision, ornamental landscaping, and supporting apiculture (Henderson, 2001). *Tamarix* species are used for phytoremediation in some places, like the AngloGold Ashanti (Vaal River) gold mine in South Africa, because they can grow in tough soil conditions and pick up pollutants (Buckham, 2011). However, *Tamarix* species do have some negative effects on the ecosystems around them. For example, they can change the composition of the soil, trap sediments, narrow river streams (Rupngam and Messiga, 2024), and significantly lower groundwater levels because of their deep roots (Iqbal et al., 2024). Furthermore, these species excrete salts via specialised salt glands, resulting in heightened soil salinity, which may restrict plant diversity beneath their canopy (Brotherson and Field, 1987). Their flammable litter significantly increases the occurrence of wildfires, presenting a challenge for land management (Busch, 1995).

The aggressive spread of specific *Tamarix* species has led to considerable economic and ecological management initiatives. The annual cost of managing

invasive plant species in South Africa is around ZAR 6.5 billion (De Lange and van Wilgen, 2010). The Conservation of Agricultural Resources Act (CARA) (ACT 43 of 1983) categorises exotic *Tamarix* species as Level 1B weeds, thereby prohibiting their cultivation, maintenance, sale, or import (Newete et al., 2019; Henderson, 2001a). Moreover, the hybridisation potential between the native *T. usneoides* and non-native species, like *T. ramosissima* and *T. chinensis*, adds complexity to biocontrol initiatives in the area (Mayonde et al., 2015; Newete et al., 2019). Bringing in biological control agents like the *Tamarix* beetle is not an easy idea, mainly because of the species' genetic diversity and how it interacts with other organisms in the ecosystem (Marlin et al., 2017).

Research indicates their ability to withstand extreme environmental conditions, including extended periods of submersion in floodwaters lasting from 70 to 98 days (Warren and Turner, 1975; Mortenson et al., 2012). It is amazing how resilient tamarix seedlings are; they can survive flooding for about a month, showing how well they can adapt to riparian ecosystems (Horton et al., 1960). Still, the fact that these things can narrow river channels and make flooding more likely makes people very worried about their unchecked growth (Mayonde et al., 2016; Brotherson and Field, 1987). With their deep roots that can reach up to 30 meters, these plants can get water from the ground, which has a big impact on groundwater reserves, especially in dry places like the Western Cape (Chamier et al., 2012). Comparative studies reveal that invasive *Tamarix* species utilise almost double the water compared to native species that coexist alongside them (Chamier et al., 20123), intensifying the challenges related to water scarcity.

Tamarix aphylla is recognised in Pakistan for its important ethnomedicinal value and is extensively utilised by local communities for its healing properties. This species, part of the Tamaricaceae family, is widely distributed throughout the country and is recognised for its antifungal properties, which have no documented adverse effects (Shah et al., 2013). The bark is used by traditional healers for its astringent and tonic properties, addressing ailments including hepatitis, eczema, and syphilis, as well as a range of skin disorders (Panhwar and Abro, 2007). The powdered bark is frequently used as a poultice to facilitate wound healing. *Tamarix aphylla*, referred to as "khagal" in Urdu and "ghaz" in Pashto, is recognised as the most widely planted roadside ornamental tree in Pakistan (Alshehri et al., 2021). Different components of the plant, such as leaves, stems, and twigs, are employed in traditional medicine, especially for the treatment of infectious diseases (Marwat et al., 2011; Panhwar and Abro, 2007). The exploration of economical plant-based therapeutic agents has intensified, with investigations indicating that *Tamarix aphylla* may represent a promising option for the treatment of microbial diseases (Alshehri et al., 2022).

Tamarix aphylla is recognised for its medicinal and ecological significance, yet its potential for growth enhancement through the application of plant growth-promoting rhizobacteria (PGPR) remains underexplored.. Beneficial microorganisms known as PGPR colonise plant roots and promote growth by enhancing nutrient availability, producing plant hormones like auxins and gibberellins, and improving soil structure. It has also been seen that PGPR makes plants more resistant to a number of biotic and abiotic problems, such as drought, soil salinity, and pathogen attacks. In light of growing issues related to soil degradation and water scarcity,

utilising PGPR to promote the growth of *Tamarix aphylla* offers a sustainable and environmentally friendly strategy for afforestation and land reclamation initiatives. According to this study, the effect of PGPR on the growth of *Tamarix aphylla* in the Attock district, an area with dry conditions and low soil fertility, will be looked at. The point of this study is to find out how well PGPR work as biofertilizers to help plants grow in damaged areas by checking important growth factors like seedling height and collar diameter. The results of this study will help us learn more about how to use microbial inoculants in reforestation projects. They may also lead to the development of long-lasting, microbial-based ways to make plants grow better in semi-arid areas.

MATERIALS AND METHODS

The research was conducted in the Attock district of Pakistan, focusing on its natural scrub forest area. The study site was located at specific latitude and longitude coordinates. The elevation of this region was approximately 458 meters above sea level. The mean annual temperature in the study area was 40°C with an average annual rainfall of 1500 millimetres. The flora in this region primarily was comprised of *Acacia modesta*, *Dodonea visosa*, *Olea ferruginea*, and *Tamarix aphylla*.

SURVEY AND COLLECTION OF RHIZOSPHERIC SOIL AND ROOTS

In the present study, the subtropical evergreen forest of district Attock was selected for a detailed survey. *There were at least 21 random soil samples from seven tehsils taken from the rhizosphere of Tamarix aphylla at depths of 0–15 cm and 15–30 cm. We then mixed these samples to create seven composite samples.* During the journey, we stored the sample bags in coolers at 4°C, and once we arrived at the laboratory, we further preserved the samples at the same temperature.

INSTRUMENT USED FOR DATA COLLECTION

Soil samples were collected using essential field equipment, including a soil auger for precise sampling, zipper bags for secure storage, and a notebook and pen for recording important details such as sampling locations and environmental conditions. Additionally, a GPS device was used to accurately document the coordinates of each sampling site, ensuring precise spatial reference. To maintain the integrity of the samples, an ice cooler was utilised for transportation, keeping the samples at an optimal temperature to prevent microbial alterations before laboratory analysis. This systematic approach ensured the reliability and accuracy of the collected soil samples for further examination.

ISOLATION OF BACTERIA FROM RHIZOSPHERIC SOIL OF *Tamarix aphylla*

To isolate bacteria from the rhizospheric soil of *Tamarix aphylla*, a precise and standardised serial dilution plate technique was employed. Initially, 1 gram of soil sample was carefully measured and suspended in 100 mL of sterile distilled water in a conical flask. The mixture was thoroughly shaken to ensure uniform dispersion of microbial cells within the solution. Serial dilutions were then prepared by transferring a specific volume of the soil suspension into successive test tubes containing

sterilised distilled water, thereby reducing the bacterial concentration in a stepwise manner. These serially diluted samples were then plated onto nutrient-rich agar media under aseptic conditions using the spread plate technique. The Petri dishes were kept at the right temperature to help the bacteria grow, which let different bacterial colonies form over a certain amount of time. To get pure bacterial cultures, the isolated colonies were streaked over and over on fresh agar plates to clean them even more. This method made sure that different types of bacteria found in the rhizosphere of *Tamarix aphylla* were successfully separated. These bacteria were then studied for their biochemical and plant-growth-promoting properties.

Bacterial Growth on Luria-Bertani (LB) Agar Medium

The preparation of Luria-Bertani (LB) agar medium was carried out following a standardized formulation to support bacterial growth under controlled laboratory conditions. The medium was prepared by dissolving specific components in 1 litre of distilled water, including 10 grams of sodium chloride (NaCl) to maintain osmotic balance, 5 grams of yeast extract as a rich source of vitamins and growth factors, 10 grams of tryptone to provide essential peptides and amino acids for bacterial metabolism, and 18 grams of agar to solidify the medium. Additionally, 1 N sodium hydroxide (NaOH) was used to adjust the pH of the medium to a neutral level of 7.0, ensuring optimal conditions for bacterial growth. To eliminate any potential contaminants, the prepared medium was subjected to autoclaving at 121°C for 15 minutes under high-pressure steam sterilisation. This step ensured the complete eradication of unwanted microbial presence, making the medium sterile and suitable for culturing bacterial isolates. Once autoclaved, the medium was allowed to cool slightly before being poured into sterilised Petri plates under aseptic conditions inside a laminar flow hood. After solidification, the plates were ready for inoculation, enabling bacterial colonies to grow efficiently for further study and characterisation.

SERIAL DILUTION PLATE METHOD

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

STREAKING

The sterilised Luria Bertani agar growth media was poured in sterilised petri plates under the laminar flow hood air cabinet and left for 10 min to settle down. Randomly, three serial dilutions were selected for spreading 50 µl from the selected serial dilutions that were spread in petri plates using sterilised L-shaped spreaders. After labelling, Petri plates were kept in an incubator at 28°C for 24-48 hours. After 24 hours, a single bacterial colony was picked for streaking and re-streaking to get pure cultures using a sterilised wire loop.

PRESEVATION OF BACTERIA ISOLATED FROM SOIL

A single pure colony of bacterial isolate was taken from the agar plate under the laminar flow hood air cabinet and was suspended in a sterilised Eppendorf tube (5 ml) containing 80% glycerol stock and preserved at -80°C for future use.

BIOCHEMICAL CHARACTERIZATION

Gram Staining

We used Vincent's (1970) method for gram staining. A tiny portion of pure colony was suspended into a drop of water on a slide using a sterile loop; a smear was made, air-dried, and heat-fixed. We then used the following solutions (Appendix 4) for staining.

- The process involves flooding for a minute with crystal violet solution, followed by five seconds of rinsing with tap water.
- Flooding for one minute with Gram's Iodine Solution, then rinsing with tap water and draining.
- The process involved decolonising with 95% alcohol for 10 seconds, followed by washing it off with tap water and draining.
- Flooding with Safranin solution (30 sec), washed with water, drained, and observed under a compound microscope with a drop of oil immersion.

Catalase Test

By adding a drop of H₂O₂ to a 24-hour-old bacterial colony on a glass slide (30 percent), it was possible to determine whether catalase is present or not. The production of gas bubbles indicated the presence of the catalase enzyme (McFadden, 1980).

P- Solubilization Index (SI)

Three groups of bacteria were separated with tricalcium phosphate and then grown in Pikovskaya's medium (Pikovskaya, 1948) in a clean room at 28°C for one week. The formula (Premono et al., 1996) was used to find the Solubilisation Index (SI), which is given by $SI = (\text{Colony diameter} + \text{Halo zone diameter}) / \text{Colony diameter}$.

EXTRACTION OF DNA OF BACTERIA ISOLATED FROM RHIZOSPHERIC SOIL OF *Tamarix aphylla*

A 24-hour-old bacterial colony isolated from the soil of *Tamarix aphylla* was grown on Lauria Bertani (LB) agar media, and then a pure single colony was inoculated in LB broth and kept on an incubator shaker (150 rpm) for 24 hours at 37°C; a DNA extraction mini kit from Qiagen was used. We followed these steps to extract the DNA.

DNA EXTRACTION PROCEDURE FOR THE BACTERIA ISOLATED FROM RHIZOSPHERIC SOIL OF *Acacia modesta* EXTRACTION OF DNA THROUGH QIAGEN KIT

The buffer (AWL) was first prepared by mixing 250 mL of ethanol. Subsequently, 1 mL of bacterial inoculate was taken in an Eppendorf tube and centrifuged at 7500

rpm for 5 minutes at a temperature between 11°C and 25°C. We obtained the pellet after centrifugation and discarded the supernatant. The ATL buffer was then placed in a water bath for 5 minutes, followed by the addition of 20 µL of proteinase K. The mixture was vortexed for 10-15 seconds and incubated in a water bath at 56°C for 1 hour until complete lysis occurred. Afterward, 200 µL of buffer AL (lysis buffer) was added, and the sample was vortexed for 15 seconds. The sample was then incubated at 70°C for 10 minutes before adding 200 µL of ethanol, followed by another 15-second vortex. We transferred the mixture to a column tube and centrifuged it at 8000 rpm for 1 minute at 11°C. We discarded the flow-through and replaced the collection tube with a new one. Subsequently, 500 µL of AW1 buffer (wash buffer) was added, and the sample was centrifuged for 1 minute at 8000 rpm. The flow-through was discarded again, and the collection tube was changed. Then, 500 µL of AW2 buffer was added, and centrifugation was performed at high power (1400 rpm) for 3 minutes. The flow-through and collection tubes were discarded, and the column tube was transferred to a new 1.5 mL microcentrifuge tube using a Qiagen spin column. Afterward, 100 µL of buffer AE was gently introduced, and the sample was incubated at room temperature for 1 minute. Finally, we spun the sample at 8000 rpm for 1 minute, discarded the column, and retrieved the DNA in an Eppendorf tube.

Gene Sequencing

After DNA is extracted, PCR will be done using universal forward and reverse primers. The samples will then be sent to MacroGen Korea so that 16S rRNA gene sequencing can be used to identify the bacteria.

Implification 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 H₂O, 5 µl of 5 X My Taq reaction buffer, 1 µl of DNA template, 1 µl of 27F primer (Lane et al., 1991), 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 utilising 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 grams.

INOCULATION OF BACTERIA ON SEEDLINGS OF *TAMARIX APHYLLA* L.

The seedlings used in the experiment were six months old, and various inoculation treatments were applied. A total of six treatments were implemented, with three replications for each treatment and five plants per replication, resulting in a total of 180 plants. The experiment was designed using a Randomised Complete Block Design (RCBD). The treatments included a control group (T0) without inoculation, while the remaining groups received different combinations of Plant Growth Promoting Rhizobacteria (PGPR). The treatments were as follows: T1 (PGPR 1), T2 (PGPR 2), T3 (PGPR 3), T4 (PGPR 1+2), T5 (PGPR 1+3), and T6 (PGPR 2+3). Growth parameters assessed in the study included seedling height and collar diameter to evaluate the impact of PGPR on plant development.

STATISTICAL ANALYSIS

The data collected from the experiment was systematically organised and analysed using Microsoft Excel and SPSS 16.0 software. Excel was used for data entry, initial processing, and graphical representation, while SPSS 16.0 was employed for statistical analysis, including mean comparisons, variance analysis, and correlation studies. This method made sure that the results were correctly interpreted, which let a full evaluation of the impacts of various treatments on seedling growth parameters be made.

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* were named TS (Tamarix soil) and Table 2 with location.

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

Sr. No.	Total Area (Ha)	Forest Type	Altitude (m)
1	685,700	Sub tropical broad leaved evergreen forest	355m 33.7660° N, 72.3609° E

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

Sr. No.	Bacterial ID	Site	Forest Tree	Location
1	TS1, TS2, TS3	Kala Chitta Range	<i>Tamarix aphylla</i>	Attock, Ghazi Barotha

BIOCHEMICAL CHARACTERIZATION OF ISOLATED BACTERIA FROM RHIZOSHERIC SOIL OF TAMARIX APHYLLA

To learn more about the biochemical traits of bacteria found in the rhizospheric soil of *Tamarix aphylla*, TS1, TS2, and TS3 species were put through a full set of biochemical tests. These tests sought to determine how these isolates work and whether they could aid plant growth by interacting with roots. The characterisation comprised the Cytochrome Oxidase Test, Catalase Test, Indole Acetic Acid (IAA) Production Test, and Phosphate Solubilisation Index (SI) Test, as detailed in Table 3.

Cytochrome Oxidase Test

The cytochrome oxidase test was performed to identify the presence of the cytochrome c oxidase enzyme, essential for the bacterial electron transport chain involved in aerobic respiration. The procedure required the application of an oxidase reagent to bacterial colonies, with the rapid development of a deep purple or blue colour indicating a positive outcome. All three bacterial strains (TS1, TS2, and TS3) exhibited oxidase positivity, which confirms their aerobic characteristics and their capacity to utilise oxygen as a terminal electron acceptor during respiration.

Catalase Test

The catalase test was conducted to assess the ability of the bacterial isolates to produce the catalase enzyme, which facilitates the breakdown of hydrogen peroxide (H_2O_2) into water and oxygen. This enzymatic reaction serves as a protective mechanism for bacteria against oxidative stress. When a drop of hydrogen peroxide was added to bacterial cultures, bubbles appeared right away, which meant that the catalase reaction was working. The test results indicated that all three bacterial strains (TS1, TS2, and TS3) exhibited catalase positivity, highlighting their ability to neutralise reactive oxygen species and thrive in oxygen-rich environments.

Indole Acetic Acid (IAA)

The Indole Acetic Acid (IAA) Production Test was done to see how well the bacterial strains could make auxins, which are a type of plant growth hormone. The bacteria that were isolated from the rhizosphere of *Tamarix aphylla* in the Kala Chitta region were grown in a medium that had extra tryptophan in it, which is needed to make IAA. Following the incubation period, the optical density (OD) of the medium was assessed at 530 nm with the aid of a spectrophotometer. The test results showed that none of the three bacterial strains (TS1, TS2, or TS3) produced IAA. This means that they do not make any auxins at all under the conditions that were tested. The recorded optical density values are presented in Table 4.

Phosphate Solubilization Index (SI) Test

The Phosphate Solubilisation Index (SI) Test was conducted to evaluate the capacity of the bacterial strains to solubilise inorganic phosphate, a crucial characteristic for enhancing plant nutrient absorption. The bacterial isolates were cultivated on Pikovskaya's agar medium, where the presence of a clear halo zone surrounding the bacterial colonies indicated phosphate solubilisation activity. The findings indicated that all three bacterial strains (TS1, TS2, TS3) exhibited positive results for phosphate

solubilisation, highlighting their potential to increase soil phosphorus availability and promote plant growth.

The biochemical tests yield valuable information regarding the metabolic and functional abilities of the isolated bacterial strains. The positive oxidase and catalase results show that these bacteria do well in places with lots of oxygen, which shows that they are aerobic. Additionally, the positive phosphate solubilisation test underscores their potential as biofertilizers, as they enhance nutrient availability in the soil. Nevertheless, the negative IAA production test indicates that these isolates might facilitate plant growth through alternative mechanisms beyond auxin biosynthesis, including phosphate solubilisation or the enhancement of stress resistance. The biochemical test results have been summarised, and they are displayed in Tables 3 and 4.

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

Sr. No.	Characterization	TS1 TS2, TS3
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 strains at 530 nm.

Sr. No.	Bacteria Isolated	Optical Density at 530 nm
1	TS1	0.917
2	TS2	0.915
3	TS3	0.916

MOLECULAR CHARACTERIZATION OF ISOLATED BACTERIA

Quantification of DNA

The isolated bacterial strains TS1, TS2, and TS3 had the following DNA quantification, given in Table 5.

Table 5: Showing DNA quantification of bacteria isolated.

S. No.	Bacterial ID	ng/ μ L	260/280
1	TS1	1353	2.15
2	TS2	1354	2.14
3	TS3	1353	2.16

PCR Gel Electrophoresis

After DNA extraction from the sample isolated from rhizospheric soil of *Tamarix aphylla* through the DNA extraction procedure, bacterial strains TS1, TS2, and TS3 were sent to Macrogen Korea for gene sequencing, as given in figure 1.

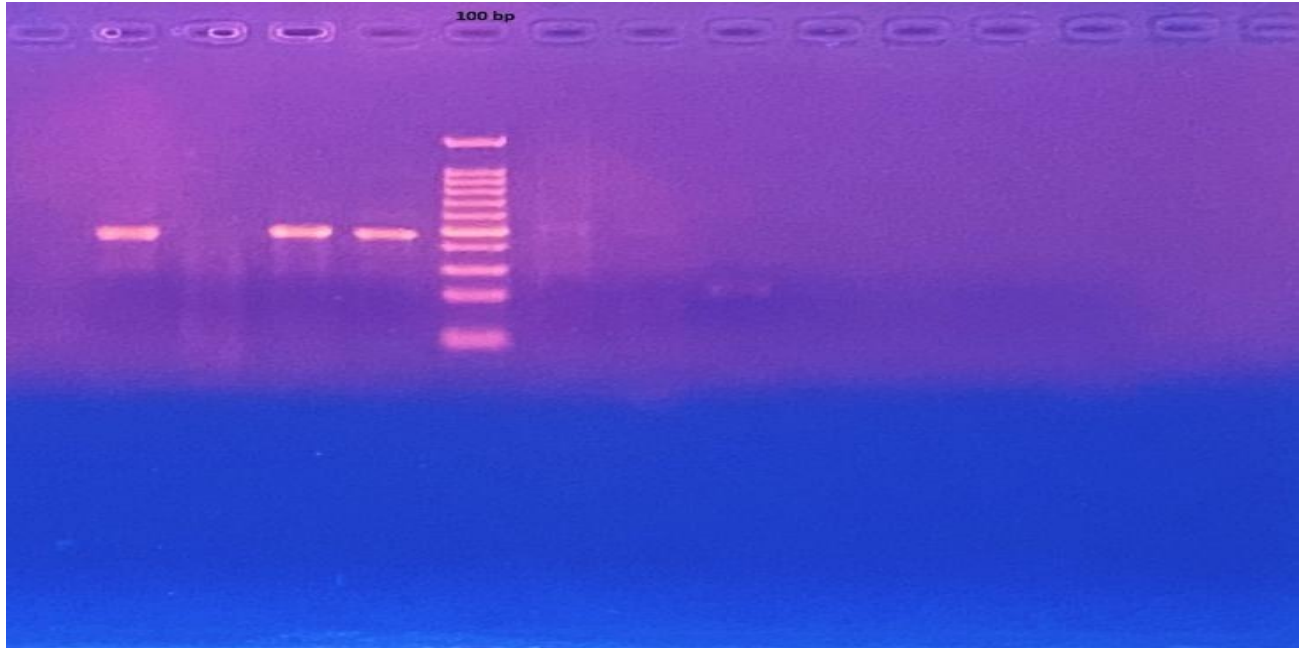


Figure 1: DNA of isolated bacterial strains TS1.TS2, TS3.

IDENTIFICATION OF ISOLATED BACTERIA THROUGH GENE SEQUENCING

>1st_BASE_5164998_TS1_Primer

NNNNCNNNCTTCNCGCTCAGTGTCAGTTACAGACCAGAAAGTCGCCTTCG
CCACTGGTGTTCCTCCATATCTCTACGCATTTACCGCTACACATGGAATT
CCACTTTCCTCTTCTGCACTCAAGTCTCCAGTTTCCAATGACCCTCCACG
GTTGAGCCGTGGGCTTTCACATCAGACTTAAGAAACCACCTGCGCGCGCT
TTACGCCCAATAATTCCGGATAACGCTTGCCACCTACGTATTACCGCGGCT
GCTGGCACGTAGTTAGCCGTGGCTTTCTGGTTAGGTACCGTCAAGGTGCC
AGCTTATTCAACTAGCACTTGTTCTTCCCTAACAACAGAGTTTTACGACCC
GAAAGCCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATTG
CGGAAGATTCCCTACTGCTGCCCCCATAGGA

>2nd_BASE_5164998_TS2_Primer

NNNNCNNNCTTCNCGCTCAGTGTCAGTTACAGACCAGAAAGTCGCCTTCG
CCACTGGTGTTCCTCCATATCTCTACGCATTTACCGCTACACATGGAATT
CCACTTTCCTCTTCTGCACTCAAGTCTCCAGTTTCCAATGACCCTCCACG
GTTGAGCCGTGGGCTTTCACATCAGACTTAAGAAACCACCTGCGCGCGCT
TTACGCCCAATAATTCCGGATAACGCTTGCCACCTACGTATTACCGCGGCT
GCTGGCACGTAGTTAGCCGTGGCTTTCTGGTTAGGTACCGTCAAGGTGCC
AGCTTATTCAACTAGCACTTGTTCTTCCCTAACAACAGAGTTTTACGACCC
GAAAGCCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATTG
CGGAAGATTCCCTACTGCTGCCCCCATAGGA

>3rd_BASE_5164998_TS3_Primer

After looking at the 16-S rRNA sequence, strains TS1, TS2, and TS3 from *Bacillus wiedmannii*, *Enterobacter mori*, and *Pseudomonas sihuiensis* were found. These

strains have 99.07%, 99.76%, and 99.55% of the same sequence as shown in Table No. 1.6. There are three strains, TS1 through TS3, that these sequences belong to. They are *Bacillus wiedmannii*, *Enterobacter mori*, and *Pseudomonas sihuiensis*.

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

ID	Species	Homology
TS1	<i>Bacillus wiedmannii</i>	99.07%
TS2	<i>Enterobacter mori</i>	99.76%
TS3	<i>Pseudomonas sihuiensis</i>	99.55%

INOCULATION OF BACTERIA ON SEEDLINGS OF *TAMARIX APHYLLA*

It was an experiment of 8 weeks in which bacterial strains *Bacillus wiedmannii*, *Enterobacter mori*, and *Pseudomonas sihuiensis* were applied to six-month-old seedlings of *Tamarix aphylla*. C1=control, T1=*Tamix aphylla* seedlings inoculated with *Bacillus wiedmannii*, T2=*Tamix aphylla* seedlings inoculated with *Enterobacter mori*, T3=*Tamix aphylla* seedlings inoculated with *Pseudomonas sihuiensis*. The growth parameter seedling height was a maximum of 4 cm in T2, followed by T2 at 3.8 cm, and then the lowest height was received in T3 at 3.3 cm. We also recorded the maximum growth parameter collar diameter in T1 at 0.5 mm, followed by 0.4 mm in T2 and 0.3 mm in T3.

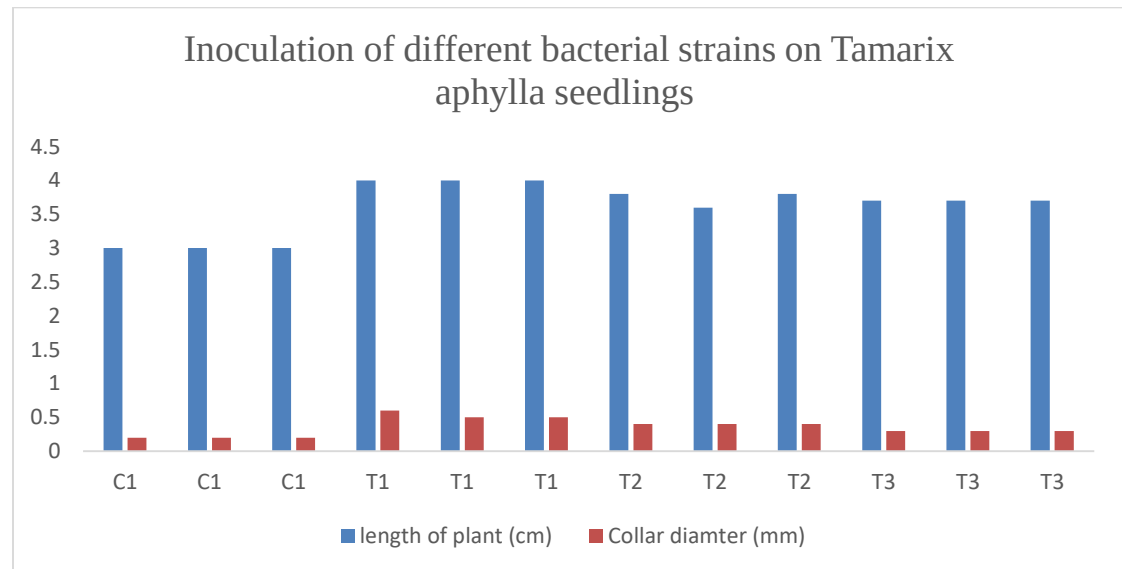


Figure 2: Bacterial strains inoculation on *Tamarix aphylla* seedlings. There was significant relationship between T1, T2 and T3.

DISCUSSION

Throughout history, plants have been crucial in providing quality fodder for livestock. However, in today's world, many countries are facing a shortage of fodder, leading to a high demand for tree-based fodder. This approach can enhance livestock health, aid in ecosystem restoration, and provide high-quality timber. Trees offer multiple benefits and play a vital role in ecological services. They provide valuable

pharmaceutical compounds, abundant fodder with high levels of protein, fuelwood for local communities, and essential minerals (Nyberg et al., 2020).

The study focuses on the *Tamarix aphylla* tree species. It also looks at Plant Growth Promoting Rhizobacteria (PGPR), which are beneficial bacteria, in the rhizosphere 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 solubilisation, siderophore production, phytohormone synthesis, enhanced nutrient uptake, increased leaf foliage, higher protein content in leaves, and their functioning as biofertilizers. Additionally, PGPR protects plants against 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 (De Andrade et al., 2023). 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 (Chieb and Gachomo et al., 2023). 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 (Fahde et al., 2023).

Using certain rhizobacteria as biofertilizers is important for making sure that agriculture is productive and lasts for a long time (Hakim et al., 2021). Most rhizospheric bacteria interact favourably and significantly with plants (L., 2021). Most rhizospheric bacteria interact favourably with plants, significantly boosting their growth and survival (Kumar et al., 2021). Identification on the basis of phylonia (De Araújo et al., 2020). Many rhizospheric bacteria from this genus have been identified as plant growth-promoting bacteria (Tsotetsi et al., 2022; 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 solubilisation, and positive catalase and oxidase results. Novel bacteria *Bacillus wiedmannii* was a novel bacteria; it was a psychrotolerant, cytotoxic bacillus, and its origin was the USA.

CONCLUSION

When bacteria strains like *Bacillus wiedmannii*, *Enterobacter mori*, and *Pseudomonas sihuiensis* were grown by themselves, they made the seedlings taller and their collars wider, which suggests that they could be used as biofertilizers. T. B., *Enterobacter mori*, and *Pseudomonas sihuiensis* all made seedlings taller and their collars wider, which suggests they could be used as biofertilizers. The positive biochemical

characterisations, including IAA production and phosphate solubilisation, further validate their role in promoting plant health. The use of PGPR offers an eco-friendly and sustainable approach to improving soil fertility and plant productivity, making it a viable strategy for afforestation and agricultural applications. Future research should explore field applications and long-term ecological impacts of PGPR to further validate their effectiveness in diverse environmental conditions.

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