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Use of Rice Rhizosphere Derived Plant Growth Promoting Rhizobacterial Inoculants and Chemical Fertilizers: Synergistic Impact on Allium Sativum L Growth and Development

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Abstract

This investigation focused on exploring the potential of Plant Growth-Promoting *Rhizobacteria* (PGPR) within the *rhizosphere* of rice fields located in the Tatypur region of Multan, Pakistan. Selected bacterial strains were identified through the analysis of the 16S ribosomal RNA gene sequence. Through phylogenetic analysis, two prominent bacterial strains, Achromobacter sp. FZ97 (GenBank accession KF848955.1) and *Stenotrophomonas maltophilia* strain AQN2 (GenBank accession HQ457015.1), were identified, characterised, and applied for plant growth promotion. The effect of these strains on the growth and yield of garlic was evaluated by a pot experiment. The experiment had 9 treatments, including 3 control groups (no treatment, 50% NPK, and 100% NPK). The other 6 treatments involved inoculating *Achromobacter* sp. or *Stenotrophomonas maltophilia* with or without NPK fertiliser. Vegetative and yield parameters were recorded in triplicate. These PGPR strains possess significant latent potential as biofertilisers, as evidenced by their application on garlic crops, which resulted in a marked increase in growth and productivity. This study suggests that the utilisation of biofertilisers in combination with a reduced amount of chemical fertilisers could be a practical strategy to alleviate the adverse environmental effects associated with the extreme use of chemical fertilisers. The identified strains can be used to develop novel biofertilisers for eco-friendly practices in Pakistan, and future research can explore their application on diverse crops to enhance growth and productivity.

Keywords: *Rhizobacteria*, Indole acetic acid, Nitrogen fixation, Biofertilizer, *Stenotrophomonas maltophilia, Stenotrophomonas maltophilia*

1. INTRODUCTION

The primary goal of agriculture is to meet the global food demand, which is increasingly challenging due to the rising population and the need for higher crop yields (Hemathilake and Gunathilake, 2022; Tian et al., 2021).

By 2050, farmers will need to produce 70% more food to feed a projected 9 billion people, as estimated by the Food and Agriculture Organisation (Rahman, 2016). Environmental stresses, such as contaminants, drought, and salinity, are limiting factors in crop production due to their impact on plant growth (Saddiq et al., 2021). Moreover, the prolonged use of chemical fertilisers poses significant environmental and health risks, including soil degradation, water pollution, and gas emissions (Hossain et al., 2022). Therefore, it is essential to explore sustainable alternatives to chemical fertilisers to maintain soil fertility and promote eco-friendly agricultural practices (Ejedegba, 2024).

Biofertilizers preserve soil micro- and macronutrients by dissolving phosphate or potassium, fixing nitrogen, producing antibiotics and releasing plant growth regulators (Singh et al., 2017). Plant Growth Promoting Rhizobacteria (PGPR) is directly engaged in enhanced nitrogen uptake, phytohormone (auxin) synthesis, mineral phosphorus solubilisation, and the formation of siderophores (Kharshandi et al., 2021) and, indirectly, in producing secondary compounds that inhibit soil pathogens (antibiotics and hydrogen cyanide) (Vejan et al., 2016). The association of rhizospheric microorganisms with plant growth-promoting properties is crucial for harnessing the potential of valuable soil microorganisms as farming inputs to improve crop production.

Microbial inoculants have several advantages over their chemical counterparts (Anirban and Dutta 2021). Phosphorus availability in plants is limited due to its insoluble state in soil, but phosphorus-solubilising microorganisms (PSM) can convert it into soluble forms (Zhu et al., 2024). Inoculation with P-solubilising bacteria enhances nodulation, increases biomass and yield, and improves nutrient uptake in plants like mungbean (Khan et al., 2022). Indole acetic acid (IAA), a fundamental phytohormone produced by plant growth-promoting rhizobacteria (PGPR), plays a crucial role in plant development (Kejela, 2024) by stimulating tropism responses, lateral bud development, and vascular bundle differentiation in various plant parts (Singh et al., 2020). Nitrogen-fixing biofertilizers, comprising microorganisms that convert atmospheric nitrogen into plant-accessible forms through biological nitrogen fixation (Amenaghawon et al., 2021), enhance soil nitrogen availability to crops, a crucial nutrient that plants typically absorb as nitrate or ammonia (Ramesh et al., 2023). Research reported the increase in the production of different crops by about 25%, and the use of chemical nitrogen and phosphorous fertilisers can be minimised by about 25-50% and 25%, correspondingly, by using biofertilisers (Aloo et al., 2020).

Bigatton et al. (2024) studied three PGPR strains, such as Bacillus velezensis RI3 and SC6 and Pseudomonas psychrophila P10 treatments, and found an increase in seed yield, larger seeds, and improved carbon allocation to seeds in peanuts. Zhang et al. (2024) studied and found the application of PGPR strains significantly enhanced the growth of tea, tobacco, and chilli pepper plants, increasing yields and improving tea flavour and appearance. El-Akhdar et al. (2024) highlights the combined application of integrating compost and PGPR. Azospirillum brasilense SWERI 111 and Azotobacter chroococcum OR512393 enhanced soil chemical properties, nutrient availability, and microbial activity, resulting in increased plant productivity and yields of wheat under varying NPK fertilisation levels. Microbial biofertilisers offer a sustainable solution to enhance crop productivity while reducing chemical fertiliser application (Shahwar et al., 2023). Objectives of the study include isolation of PGPR from the rhizosphere of rice and application of selected strains on the growth and yield of garlic with the combination of NPK. This study showcases a sustainable approach to garlic production by integrating PGPR, reducing reliance on chemical fertilisers while maintaining high yields.

2. MATERIALS AND METHODS

2.1 Rhizospheric soil collection, isolation and purification

Rice rhizospheric soil was collected from the Tatypur area of Multan, Pakistan. Lab work was performed at the microbiology lab of the Department of Botany, The Women University Multan. Bacterial isolation was carried out using serial dilution, where 10 g of soil was mixed with 90 ml of 0.9% saline water and vortexed properly. Approximately 0.1 ml of the soil suspension was then serially diluted from 10^{-1} to 10^{-5} and poured onto nutrient agar plates (Srinivasan and Saranraj 2017). The plates were incubated in an inverted position at 35-37°C for 24-48 hours to observe bacterial colonies (Tiwari et al., 2021). Prominent single colonies were purified through repeated streaking on NA plates, followed by incubation at 37°C for 24 hours (Ashrafuzzaman et al., 2009). After purification, the isolated strains were screened for plant growth-promoting traits (Habib et al., 2016).

2.2 Plant growth promoted characteristics

Glucose Nitrogen-Free Mineral Medium (GNFMM) was used to evaluate nitrogen-fixing bacterial strains as described by Latt et al. (2018). Bacterial strains that grew on GNFMM were further screened for visual detection of nitrogen fixation using GNFMM with bromothymol blue (BTB). Isolates were streaked on GNFMM agar plates with a sterilised inoculation loop and incubated for 7 days. Strains showing a colour change from green to blue after 7 days of incubation were considered positive for nitrogen fixation (Latt et al., 2018). Indole production was estimated by the method of Shrivastava and Kumar (2011). Nutrient agar plates were prepared amended with tryptophan (100 µg/mL), and Salkowski reagent was

added to these plates. The appearance of pink colour zones indicated indole-positive test strains.

2.3 Molecular characteristics

The identification of selected bacterial isolates was achieved through the analysis of the 16S ribosomal RNA (16S rRNA) gene sequence. To amplify the 16S rRNA gene, polymerase chain reaction (PCR) was performed using the universal primers 27-F (5′-AGAGTTTGATC(AC)TGGCTAG-3′) and 1492-R (5′-CGG(CT)TACCTTGTTACGACTT-3′), which are complementary to the conserved regions at the 5′ and 3′ ends of the prokaryotic 16S rRNA gene, respectively. The PCR product was then sequenced at Macrogen, South Korea. The obtained sequences were compared with those available in the GenBank database, a comprehensive repository of genetic sequences maintained by the National Centre for Biotechnology Information (NCBI), to determine the identity of the bacterial isolates. The BLAST program, a powerful tool provided by NCBI, was used to perform this comparative analysis, following the methodology described by Patel and Saraf (2017). Furthermore, the sequences were used to construct a phylogenetic tree, which illustrates the evolutionary relationships among the bacterial isolates and their closest relatives. The phylogenetic tree provided a visual representation of these relationships, enabling the identification of the bacterial isolates and inference of their evolutionary history (Shakya et al., 2020). This comprehensive approach enabled the accurate recognition and identification of the selected bacterial isolates.

2.4 Experimental Design and Treatments

An experiment was conducted at the wire-net house of the Women's University Multan, Pakistan. A local garlic cultivar was used as a test crop. The experiment comprised a total of nine treatments with three replicates for each treatment, which included three control levels for comparative analysis: C0 (control with no treatment applied), C1 (application of 50% of the recommended dosage of NPK fertiliser, at a ratio of 1:1:1), and C2 (application of 100% of the recommended dosage of NPK fertiliser, at a ratio of 1:1:1). The remaining six treatments were experimentally designed to assess the efficacy of PGPR inoculations, either alone or in combination with NPK fertiliser, and consisted of T1 (inoculation with *Achromobacter sp*.), T2 (inoculation with Achromobacter sp. + 50% NPK fertiliser), T3 (inoculation with *Achromobacter sp.* + 100% NPK fertiliser), T4 (inoculation with *Stenotrophomonas maltophilia*), T5 (inoculation with *Stenotrophomonas maltophilia* + 50% NPK fertiliser), and T6 (inoculation with *Stenotrophomonas maltophilia* + 100% NPK fertiliser).

2.5 Data Collection

The effects of these treatments were evaluated for both vegetative parameters (including plant height, number of leaves, plant fresh weight, and plant dry weight) and yield parameters (including bulb fresh weight, bulb dry weight, and number of cloves per bulb) were meticulously recorded, with all measurements taken in triplicate to ensure statistical reliability.

2.6 Statistical analysis

The experiment was designed with three replicates for each treatment, and all measurements were duplicated to ensure accuracy and reliability. The results were presented graphically, showing mean values for clarity. A one-way analysis of variance (ANOVA) was performed to evaluate the effects of different treatments on garlic. To determine significant differences between treatment means, Duncan's multiple range test was used as a post-hoc analysis. All statistical analyses were conducted using SPSS software (version 27.0). Graphical representations of the data were created using Microsoft Excel, and the figures were prepared using Microsoft PowerPoint.

3. RESULT AND DISCUSSION

Collection, isolation of rice rhizosphere and PGP characterization is presented in Figure 1. Phylogenetic analysis of *Achromobacter* sp. strain FZ97 is presented in Figure 2 and *Stenotrophomonas maltophilia* strain AQN2 in Figure 3. Analysis of growth parameters and yield parameters is given in Figure 4, 5 and 6. Detailed analysis of result is given below.

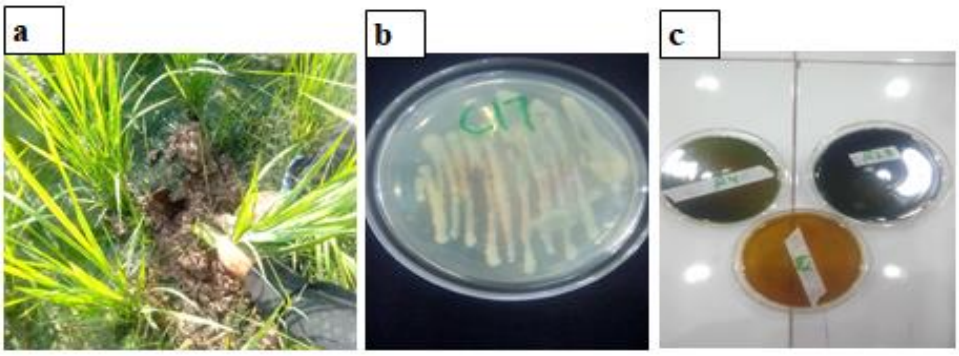

Figure 1 Illustrates the collection and isolation of rice rhizospheric soil sample collection b) indole acetic acid (appearance of pink colour showed presence of IAA) c) Nitrogen fixation (showing a color change from green to blue indicate nitrogen fixation).

3.1 Growth promoting characters

The basic group of phytohormones is auxin and indoleacetic acid is the most common acid in all auxin produced by rhizobacteria and has a major impact on plant physiology (Kesaulya et al., 2015). It stimulates cell proliferation, division and gene regulation (Kannahi and Kowsalya. 2013). Different species of PGPR produced varying amount of auxin. Culture condition, growth stage and substrate availability also influence it (Pantoja-Guerra et al., 2023). In current study, *Achromobacter sp*. showed positive result for indole production. Other restrictive nutrient for plant growth is Nitrogen (Shrivastav et al., 2020). The microorganisms that live on the rhizomes contribute to the release of phosphorus, potassium and nitrogen from the soil, which greatly influence root structure and growth (Xiong et al., 2021). Research has focused on nitrogen-fixing organisms because of concerns about greenhouse gas emissions from nitrogen fertilizers (Latt et al., 2018). In current study *Achromobacter* sp. and *Stenotrophomonas maltophilia* showed positive result for nitrogen fixing ability as color of media was changed from green to blue (Bhardwaj et al., 2017).

3.2 Molecular Identification:

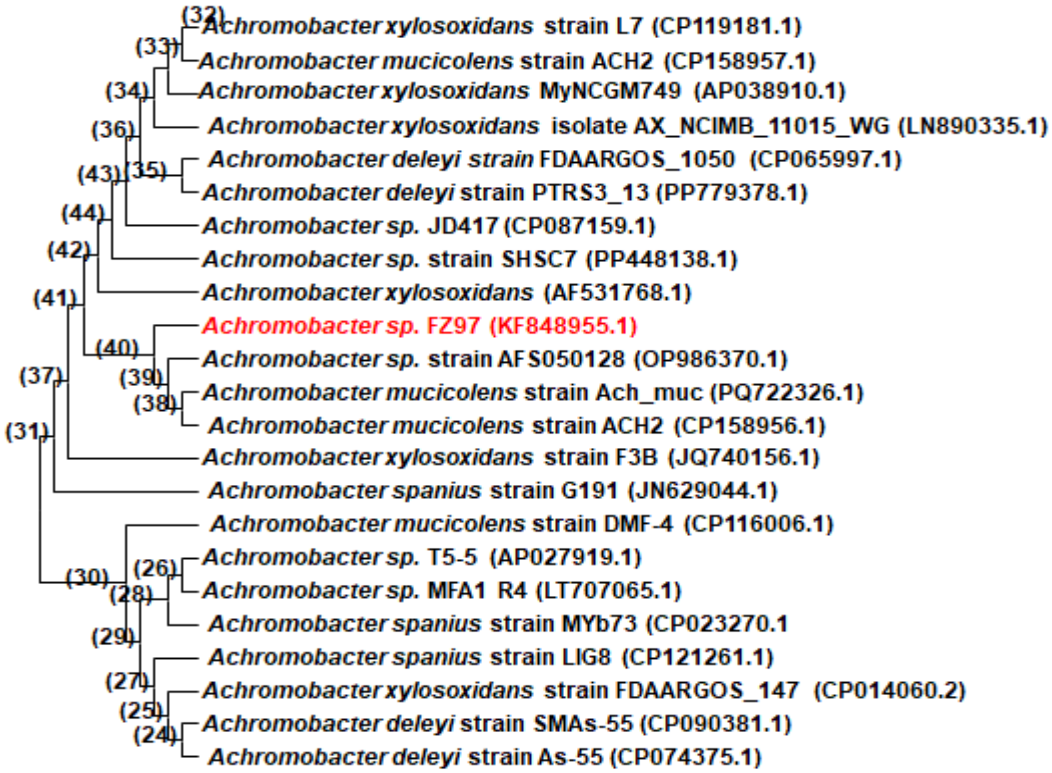

Figure 2: Phylogenetic analysis of *Achromobacter* sp. strain FZ97 under 16S ribosomal RNA

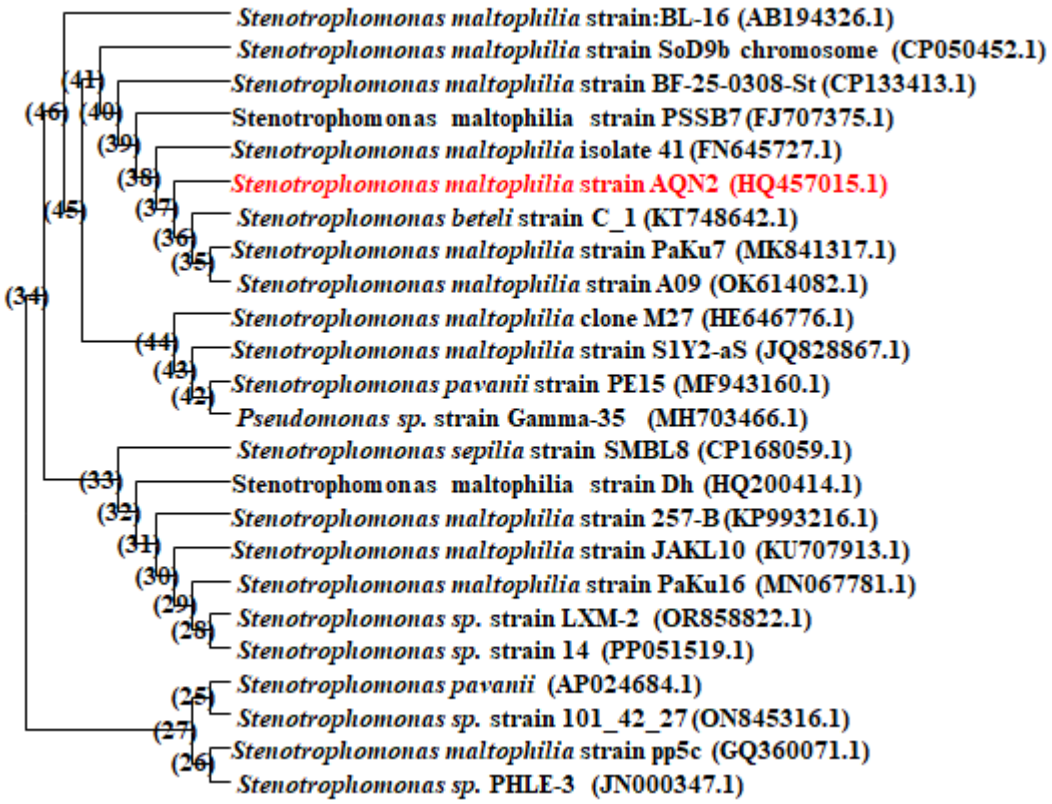

Figure 3: Phylogenetic analysis of *Stenotrophomonas maltophilia* strain AQN2 16S ribosomal under 16S ribosomal RNA

Strains phylogenetic analysis was performed from the resulted sequences. Blast program of NCBI's was used to perform the similarity index for the closest sequences. By using the Mega 12 software (Tamura et al., 2013), the phylogenetic trees were constructed according to nearest neighbor-joining method (Saitou and Nei 1987). The tree was analyzed by a bootstrap analysis obtained on 1000 replicates. The sequences were placed in Gen Bank under accession numbers KF848955 (FZ97) and HQ457015 (AQN2). The sequence similarity search using BLASTn resulted in 95.84% and 95.69% matching of 16S rRNA gene of strain *Achromobacter* sp. FZ97 with *Achromobacter spanius* strain MYb73 chromosome (CP023270.1) and *Achromobacter xylosoxidans* strain DN002 chromosome (CP045222.1), respectively The BLASTn and phylogenetic analysis of the 16S rRNA gene of the other strain discovered and named as *Stenotrophomonas maltophilia* strain AQN2 (accession number, HQ457015.1). The sequence similarity search using BLASTn resulted in 99.66% matching of 16S rRNA gene of *Stenotrophomonas maltophilia* strain AQN2 with *Stenotrophomonas maltophilia* strain PaKu7 16S ribosomal RNA gene (MK841317.1).

3.3 Effect of combined application of inoculants and chemical fertilizer on growth of *Allium Sativum* L.

Microbial communities associated with roots playing basic roles in nutrition and resistance of plants (Lemanceau et al., 2017). In our study *Achromobacter sp.* along with reduced quantity of NPK showed promising result as compared to full recommended dose of chemical fertilizer (C2) and showed an increase of 21.78% , 46.15%, 14.78%, 2,38% in plant height, number of leaves, plant fresh weight and plant dry weight respectively (Figure 4 and Figure 6B). Moreover strain alone showed enhanced result with increase of 28.44% , 12.5%, 29.56%, 79.32% in plant height, number of leaves, plant fresh weight and plant dry weight respectively as compared to untreated control (C0) (Figure 4 and Figure 6A).

Amongst PGPR, potential of *Stenotrophomonas sp* as a biocontrol and plant growth promoting has been demonstrated in previous studies which demonstrated that it can be used for scheming the variety of pathogenic fungi and, have huge prospective for biotechnology applications (Berg et al*.,* 2010). Moreover

Stenotrophomonas maltophilia strain AQN2 Showed increased in growth parameters as compared to untreated control (Figure 4 and Figure 6C). Our results are corresponding to previous reported result as Sharma et al., (2024) recognized *Stenotrophomonas maltophilia* BCM as a possible microbial contender for sustainable agriculture as confirmed by molecular, physiological, and phylogenetic characterization. Investigational confirmation also correlates with genomic insights to make clear the role of *Stenotrophomonas maltophilia* BCM as a latent biofertilizer and biocontrol driver. Similarly Kumar and Audipudi (2015) reported that strain S. *maltophilia* were isolated and characterized from the rich rhizospheric soils (chilli) and confirming their potential for plant growth. This genera of bacteria were usually have a high-quality phosphate solubilizations and used as biofertilizers, bio-remediation and as a pesticides (Suckstorff and Berg 2003).

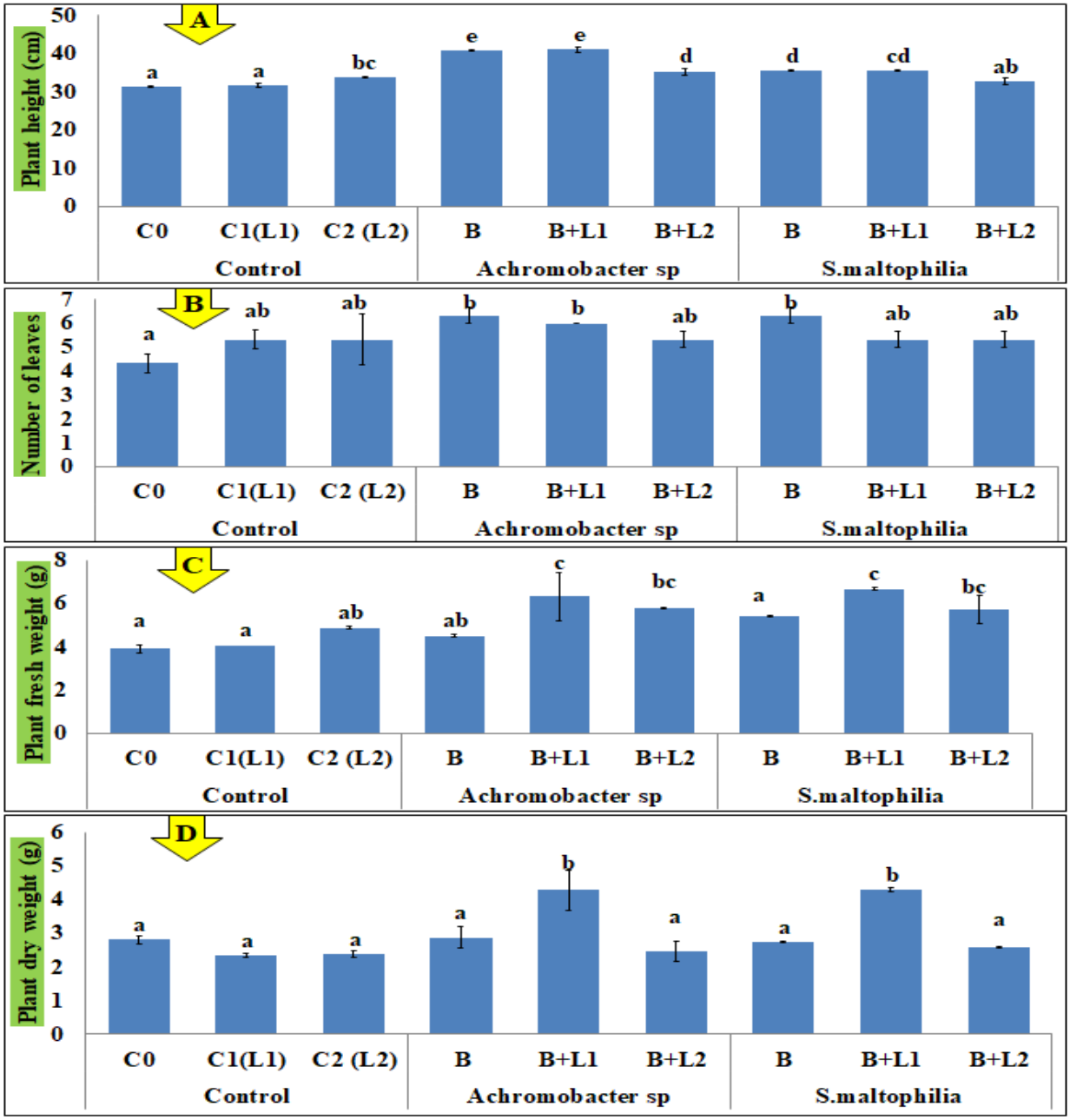

Figure 4: Effect of different treatments on growth attributes of *Allium sativum* L. A) Plant height B) number of leaves/plant C) plant fresh weight (g) D) plant dry weight (g) Where L1=50%NPK and L2=100%NPK. Values are the means ± standard error, at significance difference $p \leq 0.05$. Different superscripts a, b, c, ab cd, d shows statistically different values.

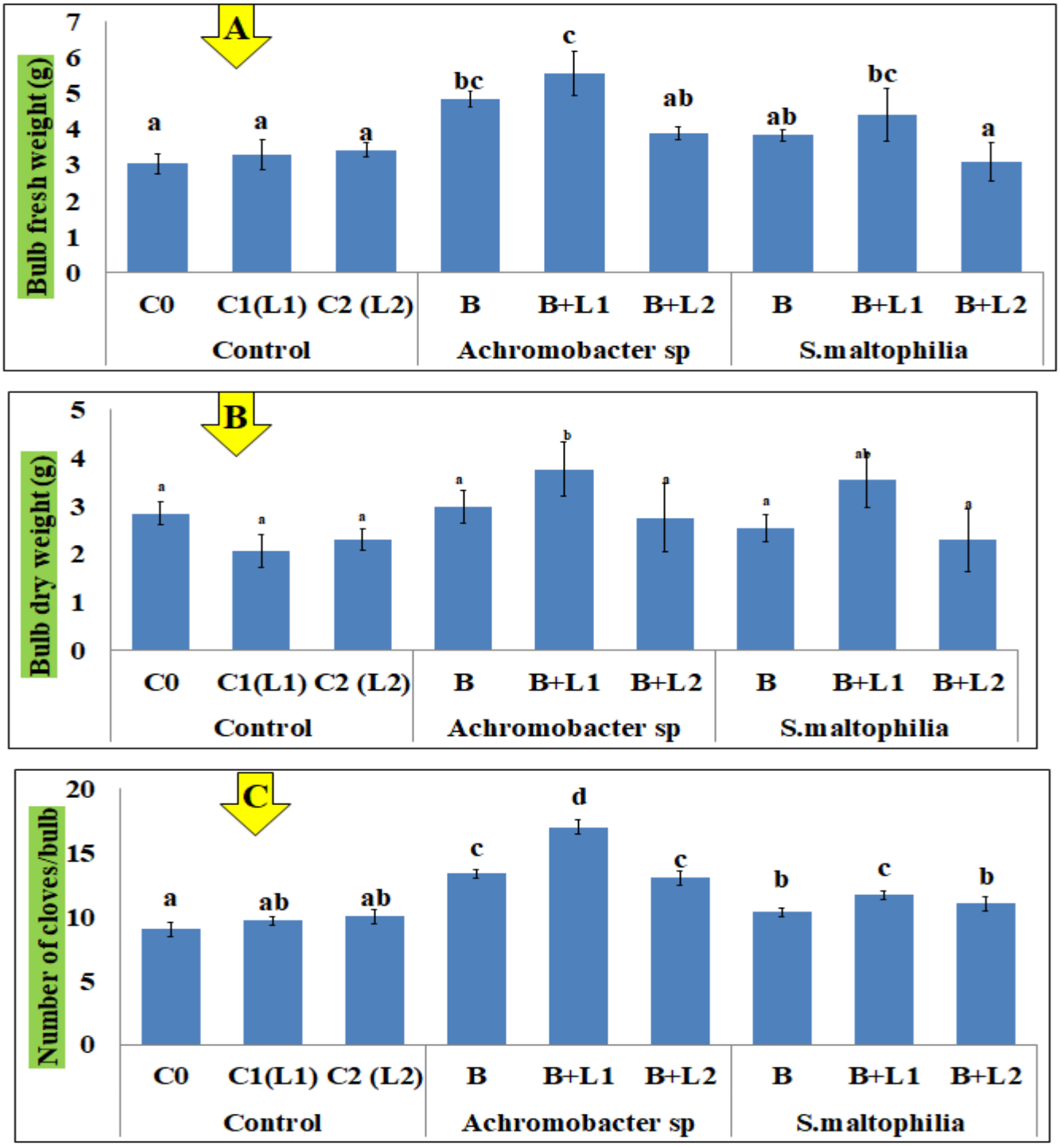

Figure 5: Effect of different treatments on yield attributes of *Allium sativum* L. A) Bulb fresh weight (g) B) Bulb dry weight C) Number of cloves/bulb Where L1=50%NPK and L2=100%NPK. Values are the means ± standard error, at significance difference $p \leq 0.05$. Different superscripts a, b, c, ab cd, d shows statistically different values.

3.4 Effect of combined application of inoculants and chemical fertilizer on yield of *Allium Sativum* L

Achromobacter species have a broad allocation all over soil environments and belongs to aerobic gram-negative species of bacteria (Jha and Kumar 2009). For identifying *Achromobacter* species, analyses based on phenotypic profile have been established to differentiate them from other comparable Gram-negative, aerobic species (Wittmann et al., 2014). 16Sr RNA biomarker gene is used in identifying diverse isolates (Gomila et al., 2014). There are many PGPR that belongs to *Achromobacter* sp produced different plant hormones (Mareque et al., 2015). Diazotrophic endophytic *Achromobacter* sp. fixed Nitrogen more competently than rhizospheric organisms (Prabhat and Kumar 2009). A. *xylosoxidans* and A. *marplatensis* were exhibited many growth stimulating characters (Wedhastri et al., 2013) In present study the *Achromobacter* sp. strain FZ97 alone showed increase in bulb fresh weight (59.38%), bulb dry weight (4.87%) and number of cloves (48.14%) than the un-inoculated control (Figure 5, 6) and with reduced amount of NPK (L1 50%NPK) increase in bulb fresh weight (63.14%), bulb dry weight (63.95%) and number of cloves (70%) as compared to full recommended application of NPK (L2 100%) (Figure 5, 6). Vazquez et al., (2024) demonstrated in their experiment that inoculation with beneficial rhizobacterium *Achromobacter sp.* 5B1 enhanced biomass production, restored root growth, and increased auxin responses. Jha and Kumar (2009) determined that *Achromobacter xylosoxidans* WM234C-3, with promising plant growth-promoting traits, including nitrogen fixation, indole acetic acid production, and phosphorus solubilization. Inoculation with WM234C-3 significantly enhanced rice plant growth, increasing root/shoot length, fresh weight, and chlorophyll content. Rashad et al., (2022) explored the potential of strain of *Achromobacter sp.,* was isolated from fenugreek seeds, producing growth-promoting compounds and antifungal molecules that inhibited Rhizoctonia solani growth by 43.75%. Greenhouse and field trials showed that the bacterium improved plant growth, physiology, and yield while effectively controlling root rot disease, highlighting its potential as a biocontrol agent. Abdel-Rahman et al., (2017) identified *Achromobacter sp.* EMCC1936 as a potent plant-growth-promoting rhizobacteria (PGPR). This strain produced beneficial compounds like indole acetic acid and gibberellin, solubilized rock phosphate, and showed non-pathogenic characteristics. The isolate's potential as a biofertilizer for various crops was highlighted due to its beneficial properties and ability to increase soil enzymatic activity. Corsini et al., (2018) showed *Achromobacter sp.* strain N2, isolated from contaminated soil, exhibits plant growth-promoting traits and arsenic transformation abilities. This strain is resistant to various metals and improved seed germination, seedling height, and enhanced arsenic uptake. *Achromobacter marplatensis* has concerned in various biotic actions positively in the soil which improve plant growth as an effect of nutrient absorption in the soil plus producing a variety of plant growth regulators (Ahemad and Kibret 2014).

Stenotrophomonas maltophilia strain AQN2 alone showed increased in bulb dry weight 10.5% and number of cloves as compared to uninoculated control (Fig 6 D) and with application of NPK 50% increased 53.77% and 16.66% in bulb dry weight and number of cloves respectively as compared to full dose of NPK 100%. Fariman et al., (2022) that *S. maltophilia* isolated from local rice fields in peninsular Malaysia are used in seed treatment and foliar spray increases yield components and growth support parameters such as shoot height, root length, number of tillers per hill, and shoot and root dry weight. Singh and Jha (2017) reported that inoculation of the diverse *S. maltophilia* in wheat in salinity stress considerably improves the development, ionic balance and biochemical parameters. The S. *maltophilia* was tested positive to produce phyto hormone IAA and phosphate solubilization that increase the plant growth under unfavorable circumstance like salt. The effect on plant growth *S. maltophilia* in wheat might be credited to the ACC-deaminase activity seedlings when exposed to salinity stress, which consecutively reduces the ethylene synthesis (Penrose and Glick 2003).

Liaquat et al (2020) reported that of *S. maltophilia* isolated from the soil samples from Nanjing mining area of china possesses noteworthy tolerance to cadmium and are probable for bioremediation (Piotrowska-Seget et al., 2005). Hassan and Bano (2016) documented that the S. *maltophilia* can be used to retrieve saline or saline sodic soil by growing organic matter, declining the electron conductivity and sodium content, lessening Na accretion in leaves, modulating the level plant hormones, increase uptake of uptake of K, Ca, N-NO3 and P and a skirmishing osmotic and oxidative stress. In overall result strain *Achromobacter* sp. strain FZ97 with the application of reduced amount of NPK (1:1:1) promote plant garlic growth and produced high yield than the remaining treatments.

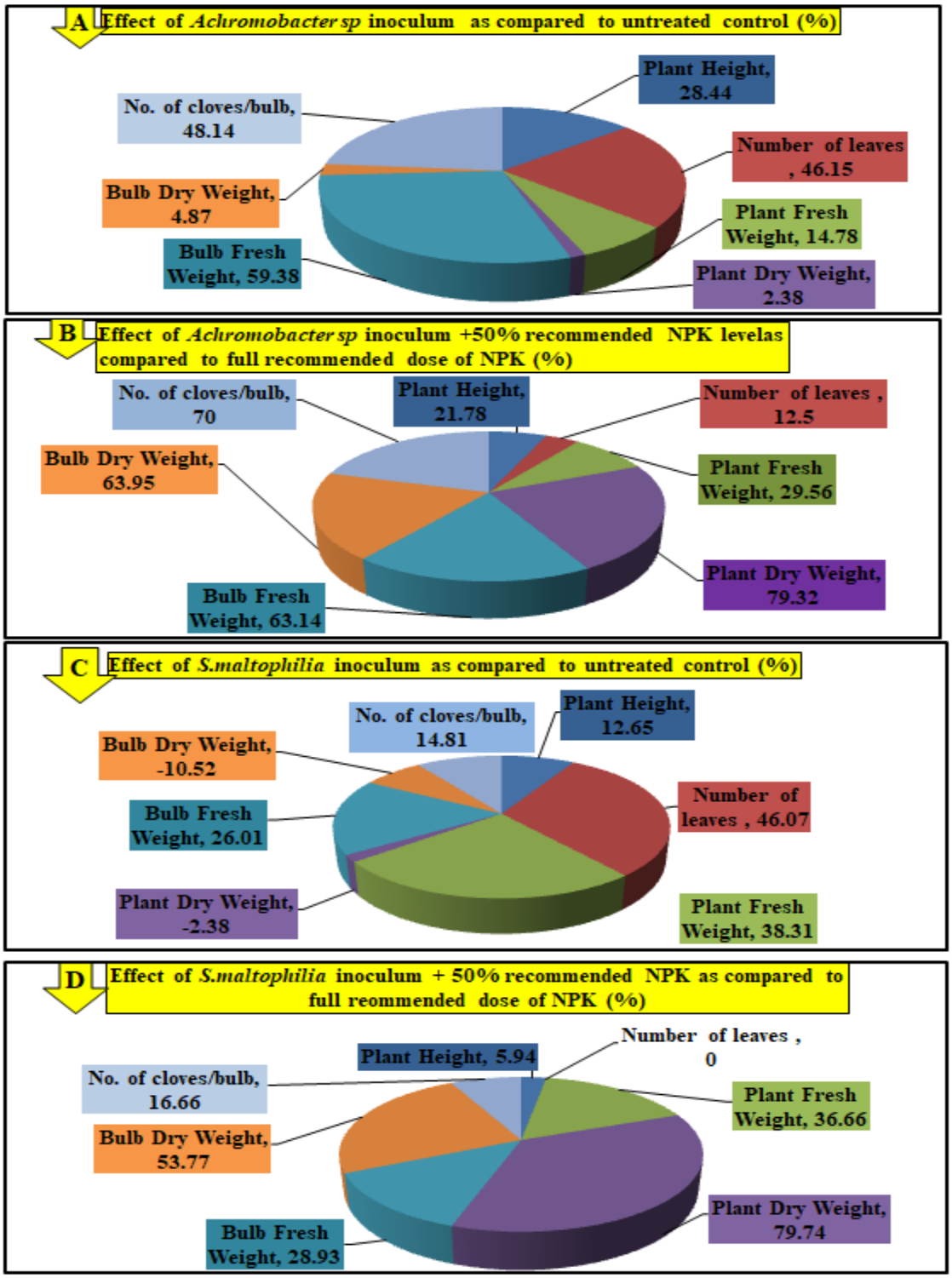

Figure 6: Effect of different treatments on % increase of growth and yield parameters of *Allium Sativum* L. A) Effect of *Achromobacter sp* inoculum (%) as compared to untreated control, B) Effect of *Achromobacter sp* inoculum + NPK 50% as compared to full rcommended dose of NPK, C) Effect of *S.maltophilia* inoculum as compared to untreated control D) Effect of *S.maltophilia* inoculum + NPK 50% as compared to full rcommended dose of NPK

4. CONCLUSION

This study highlights the crucial role of Plant Growth-Promoting Rhizobacteria (PGPR) in the context of sustainable agricultural practices thereby contribute a viable and eco-friendly alternative to the conventional use of chemical fertilizers. The two prominent bacterial strains identified and characterized through this research, namely *Achromobacter sp. FZ97 and Stenotrophomonas maltophilia strain AQN2,* possess considerable potential for the development of novel biofertilizers. These biofertilizers can be strategically utilized to promote and facilitate the adoption of eco-friendly agricultural practices within Pakistan, contributing to a more sustainable agricultural sector. The findings of this study indicate that combining biofertilizers with a decreased quantity of synthetic fertilizers may serve as an effective approach to mitigate the negative ecological impacts resulting from the overuse of chemical-based fertilizer. Furthermore, future research endeavors can build upon the foundation established by this study by exploring the application of these PGPR strains on a diverse array of crops, with the ultimate goal of enhancing their growth and productivity, and thereby broadening the scope of their beneficial impact on agricultural sustainability.

Disclosure Statement

The authors of the article entitled "**Use of Rice Rhizosphere-Derived PGPR Inoculants and Chemical Fertilizers: Synergistic Impact on *Allium Sativum* L Growth and Development"** hereby declare that there are no conflicts of interest. The authors affirm that all data and information contained within this manuscript are original, and this work has not been submitted concurrently to any other publication. Moreover, all authors have made substantial contributions to the preparation of this manuscript.

Data Availability Statement

The datasets generated and/or analyzed during the present study are available from the corresponding author upon reasonable request. Data sharing is in compliance with the policies and ethical guidelines stipulated by the International Journal of Agricultural Research and Review.

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