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Integrated Application of PGPR and Mycorrhizal Fungi for Improved Growth and Disease Suppression in Tomato

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***Abstract*— *The escalating cost of chemical fertilizers, along with their prolonged usage leading to depletion of natural soil nutrients and environmental pollution, has increased the importance and demand for biofertilizers. This study aimed to evaluate the efficacy of using Azospirillum sp., Pseudomonas and mycorrhizal fungi individually and in combination for promoting nitrogen fixation, phosphate solubilization, IAA production, siderophore production, suppression of pathogenic microorganisms and enhancement of plant growth. The PGPR microorganisms, namely Azospirillum sp., Pseudomonas, and mycorrhizal fungi were isolated from the rhizosphere soil of tomato cultivation fields in Cuddalore district. AZ-5 and AZ-2, two of the isolated Azospirillum sp. strains, exhibited the highest nitrogenase activity, with values of 225.75 nmol and 210.45 nmol, respectively. AZ-5 and AZ-2 had cellular nitrogen contents of 16.95 µg/g and 15.80 µg/g, respectively, which were greater than those of the other isolates. Among the isolated Pseudomonas strains, PS-4 demonstrated the greatest phosphate solubilization activity, registering 21.75 µg P/100 mL broth. This was followed by PS-2, demonstrated significant phosphate solubilization of 18.30 µg P/100 mL broth. AZ-5,2 and PS 4,2 also exhibited enhanced production of IAA and siderophores. Among the AM fungi, particularly Gigaspora sp., showed excellent phosphate mobilization activity of 24.10 mg/kg along with 74% root colonization, indicating its efficiency as a beneficial mycorrhizal fungus. In the controlled laboratory experiment using the petri plate method, the Pseudomonas isolate effectively inhibited the growth of Fusarium oxysporum with a growth inhibition zone measuring 41.21 mm. The efficient PGPR microorganisms, specifically Pseudomonas and Azospirillum sp., recorded superior nitrogen fixation, phosphate solubilization, IAA production, and siderophore production, in conjunction with the mycorrhizal fungus Gigaspora sp., which exhibited excellent phosphate mobilization activity, were applied both individually and in combination. These microbial treatments were prepared for seven experimental treatments under pot culture conditions. At the end of the experiment, the T6 treatment performed the best, recording 21.10 cm seedling length, 94.85% seed germination, and a vigour index of 2028.7, thereby producing healthy, disease-free, and high-quality tomato seedlings. Thus, it is confirmed that the integrated application of plant growth-promoting bacteria and beneficial mycorrhizal fungi leads to the production of high-quality tomato seedlings and improved seedling yield.**

Keywords*— *Azospirillum sp, Pseudomonas, Mycorrhiza, Consortium, Fusarium, Biocontrol.

I. INTRODUCTION

Agriculture is increasingly threatened by population growth, the ensuing climate change, and the escalating cost of inputs. These challenges are expected to be exacerbated by the deterioration of soil fertility, degradation of soil structure and water-related issues such as eutrophication brought by excessive leaching of nutrients (Wu & Ge, 2019). Furthermore, essential nutrients such as nitrogen, phosphorus, and potassium are derived from non-renewable resources. Among these, phosphorus is an important nutrient that plays a major role in plant growth, nucleic acid synthesis and ATP energy production (Bechtaoui *et al*., 2021). However, most of the phosphorus present in the soil exists in forms is not readily available for plant uptake, which is considered a major concern. The soil layer surrounding the roots of plants plays a major role in plant growth and nutrient availability. It is often referred to as the “second genome” of plants. The diverse types and large numbers of microorganisms present in the root region significantly influence plant growth and agricultural productivity (Berendsen *et al*., 2012). The root exudates released from root hairs contain sugars, amino acids, and organic acids that support the growth and activities of microorganisms. These substances provide a favourable environment for microbial multiplication. Due to this, the number and diversity of microorganisms are higher in the soil surrounding the roots (rhizosphere region) when compared to the soil away from the roots, where the microbial population is relatively lower (Reed & Glick, 2013). This type of plant–microorganism interaction has an influence on the quality, yield and growth of plants. Plant growth-promoting microorganisms enhance plant growth through both direct and indirect mechanisms. The direct mechanisms include nitrogen fixation and the solubilization of phosphorus and potassium. The indirect mechanisms include the production of disease-resistant substances and the production of siderophores (Colla, 2020). *Azospirillum sp.* is free living nitrogen fixing organism. Which fix the atmosphere nitrogen in rhizosphere region of vegetable crops. *Azospirillum sp.* produce phytohormone like IAA, an auxin hormone which promotes plant growth. The phytohormone improve plant tolerance to both biotic and abiotic stress (Sun *et al*., 2025). *Pseudomonas*, a plant growth-promoting bacteria, enhances plant growth through direct mechanisms such as phosphate solubilization and other beneficial processes, as well as indirect mechanisms like disease control. In particular, in tomato seedlings, it helps in control the fungal pathogen *Fusarium* through production antibiotic substances and enhancing the defense mechanism of the plant. They also activate and stimulate defense mechanisms. Through this process, the microorganism *Pseudomonas* play a major role in the biological control method, which is an important component of sustainable agriculture (Benchabane M, 2017). Not only bacteria, but also the fungal association known as VAM enhances plant growth under abiotic stress by facilitating the uptake of phosphorus, zinc, and certain other nutrients. It also absorbs water from regions that roots cannot reach, thereby promoting root growth, stem development, and the overall growth of the plant (Leuratti *et al*., 2025). Due to the high nutritional value of tomato, the demand from people and industries is naturally increasing. Furthermore, tomato seedlings are used as a model plant to study the interactions between plants and microorganisms (Quinet *et al*., 2019). Healthy tomato seedlings that are free from soil- and seed-borne diseases and enriched with adequate nutrients serve as an important foundation for improving tomato yield. In this research study, plant growth-promoting microorganisms such as *Azospirillum sp.* for nitrogen fixation, *Pseudomonas* for phosphate solubilization, *Fusarium* for inducing fungal disease interaction and VAM fungi for phosphorus mobilization were utilized in an attempt to produce healthy, nutrient-rich, and disease-free tomato seedlings.

II. MATERIALS AND METHODS

2.1 Collection of rhizosphere soil samples

Rhizosphere soil samples of tomato plants were collected from various regions of Cuddalore district and the plant growth-promoting microorganisms such as *Azospirillum sp.*, *Pseudomonas*, and VAM fungi (Vesicular Arbuscular Mycorrhiza) were isolated and studied.

2.2 Isolation and Enumeration of *Azospirillum sp.* and *Pseudomonas*

One gram (1 g) of sample was mixed with 100 mL of sterile distilled water and kept in an incubator shaker at 100 rpm for 30 minutes. From this suspension, serial dilution was carried out up to 10^{-6} dilution using test tubes. Subsequently, 1 mL aliquots from the 10^{-5} and 10^{-6} dilutions were taken and inoculated into NFB medium for *Azospirillum sp.* and King’s B medium for *Pseudomonas*. A total of six plates were used, with three plates for each organism. Pure cultures of *Azospirillum sp.* and *Pseudomonas* were isolated using sub-streaking method. To confirm the isolates, several biochemical tests were performed.

2.3 Morphological and Biochemical Characteristics of *Azospirillum sp.*

2.3.1 Gram Staining

Gram staining was performed following the method of Gram C (1884) & boridge TJ (2001). A smear of the *Azospirillum sp.* culture was prepared on a clean glass slide using an inoculation loop. The smear was heat-fixed using a flame. The slide was then stained with crystal violet for 1 minute, followed by iodine solution for 1 minute. Decolorization was carried out using ethanol for 10–15 seconds and finally counterstained with safranin for 30 seconds. After each step, the slide was washed with distilled water. Finally, the stained slide was observed under a compound microscope using immersion oil.

2.3.2 Catalase Test

The catalase test was performed following the method described by (Cappuccino & Sherman, 2011). In this method, a smear of *Azospirillum sp.* culture was placed on a glass slide and a few drops of 3% hydrogen peroxide (H_2O_2) solution were added. The formation of bubbles indicated a positive catalase reaction, whereas the absence of bubbles indicated a negative result.

2.3.3 Methyl Red and Voges–Proskauer (MR–VP) Test

The MR–VP test was performed following the method described by Cappuccino & Welsh (2017) & Forbes *et al*., (2007). In this test, *Azospirillum sp.* culture was inoculated into MR–VP broth and incubated at 28°C for 48 hours. For the Methyl Red (MR) test, a few drops of methyl red indicator were added to the broth culture. The appearance of a red color indicated a positive result. For the Voges–Proskauer (VP) test, a few drops of alpha-naphthol and KOH were added to the broth culture. The development of a pink color indicated a positive result.

2.3.4 Citrate Utilization Test

The citrate utilization test was performed following the method of Atlas RM (2004) & Cheesbrough (2005). In this test, Simmons citrate agar was prepared in test tubes in a slant form. The *Azospirillum sp.* culture was streaked onto the slant surface and incubated at 28–30°C for 48 hours. A color change of the medium from green to blue was considered a positive result.

2.4 **Morphological** and Biochemical Characteristics of *Pseudomonas*

After 24 hours of incubation, the King's B medium plates inoculated with *Pseudomonas* were observed and the colony characteristics such as size, shape, elevation, and color were recorded. The motility, endospore formation, and Gram reaction of *Pseudomonas* were confirmed using a compound microscope. Biochemical tests such as gelatin liquefaction, starch hydrolysis, carbohydrate utilization, oxidase test, and catalase test were performed following the methods of Aneja KR (2022) & Ramya rai & Rav (2024).

2.5 **Isolation** of Pathogen

The pathogen *Fusarium oxysporum* was isolated from infected tomato plant leaves showing disease symptoms. The diseased microbial culture was obtained from the Department of Plant Pathology, Annamalai University.

2.6 **Isolation** and Enumeration of AM Fungi

AM fungi were isolated by the sieving and decanting method Gerdemann & Nicolson (1963). The spores were further purified using sucrose density gradient centrifugation and counted with the help of a stereo zoom microscope Boyno *et al*., (2023). The number of spores present in 100 g of soil was calculated.

2.7 **Identification** of AM Fungi

The identification of AM fungi was carried out with the help of taxonomic keys Goswami *et al*., (2018).

2.8 **Purification** and Mass Multiplication of AM Fungi

The isolated and identified AM fungi were mass multiplied by the soil-funnel method using tomato seedlings as trap plants (Schenck NC, 1982). The AM fungal spores harvested along with roots and soil were allowed to attain complete maturity before being used for tomato seedling application. It is noteworthy that the entire process was completed over a period of three months.

2.9 **Isolation** and Screening of Plant Growth Promoting Rhizobacteria for Plant Growth Promoting Traits

The isolated microorganisms were screened for plant growth promoting characteristics such as nitrogen fixation, phosphate solubilization, mobilization and plant hormone and siderophore production.

2.10 Phosphate Solubilization

For the estimation of phosphate solubilization, 50 mL of Pikovskaya's broth were separately dispensed into three 250 mL Erlenmeyer flasks Nautiyal CS (1999). The isolated *Pseudomonas* culture was inoculated into each flask at the rate of 100 µl and the flasks were incubated for 7 days in a rotary shaker at 150 rpm and 30°C. After seven days of incubation, the broth culture was centrifuged at 5000 rpm for 20 minutes using a centrifuge machine. The phosphorus (P) released and dissolved in the liquid medium was estimated following the method described by Jackson (Jackson ML, 1973). Nitrogen fixation was carried out by the microorganism *Azospirillum sp.* The amount of nitrogen fixed was estimated by the Acetylene Reduction Assay (ARA) method. Each isolated *Azospirillum sp.* culture was grown in 100 mL of NFB medium in a 250 mL Erlenmeyer flask and incubated at 30°C for two days. The air present inside the Erlenmeyer flask was replaced with 10 % acetylene gas and the flask was incubated for 1 hour. After incubation, 1 mL of gas sample was analyzed using gas chromatography, and the concentration of ethylene gas produced was determined. The values were calculated using a standard calibration curve method Boddey *et al.*, (1987) & Reis *et al*., (2015). The nitrogen fixed by *Azospirillum sp.* was also estimated by the micro-Kjeldahl method described by (Humphries EC, 1956). In this method, *Azospirillum sp.* culture was grown in 100 mL of

NFB medium in a 250 mL Erlenmeyer flask at 30°C for 7 days. After seven days, 5 mL of the microbial culture along with 5 mL of medium, 5 mL of H_2SO_4, and 5 g of catalyst were added for nitrogen estimation. The mixture was combined with a catalyst mixture (K_2SO_4 : $CuSO_4$ in the ratio of 10:1) and digested until a clear solution was obtained. After cooling for a few minutes, the digested sample was transferred to a Micro-Kjeldahl distillation unit. Then, 10 mL of 40% NaOH was added and ammonia was liberated through steam distillation. The released ammonia was trapped in 20 mL of 2% boric acid solution. The ammonia absorbed in the boric acid solution was titrated against standard N/50 H_2SO_4 solution. From the titre value, the nitrogen content was calculated and expressed as the amount of nitrogen present in the microbial medium.

Total Nitrogen Content

Total Nitrogen (μg)} = r x 0.0028 x1000

Where: *r* = Titre value (mL of N/50 H_2SO_4 consumed), 0.00028 g nitrogen is equivalent to 1 mL of N/50 H_2SO_4 (Humphries EC, 1956).

2.11 Estimation of Phytohormone and Siderophore Production

L-tryptophan supplemented with broth was utilized for the estimation of IAA production Gordon & Weber (1951) and Mayer (1958). The Salkowski colorimetric assay method described by Bric was employed to evaluate the IAA production ability of *Pseudomonas* and *Azospirillum sp.* bacteria (Bric *et al.*, 1991). To assess the IAA production potential of both bacteria, 50 mL of YMB medium supplemented with 1 g/L L-tryptophan was prepared in 250 mL Erlenmeyer flasks. The cultures were incubated in an orbital shaker incubator at 150 rpm and 30°C for 7 days. After seven days, the broth culture was centrifuged at 10,000 rpm for 10 minutes at 4°C using a centrifuge. The supernatant obtained after centrifugation was collected for IAA estimation. To this supernatant, 2 mL of Salkowski reagent containing 1 mL of 0.5 mol/L $FeCl_3$ and 49 mL of 35% $HClO_4$ was added and mixed thoroughly. The reaction mixture was incubated at 30°C for 30 minutes. subsequently, the absorbance of IAA was measured at 530 nm using a spectrophotometer and the IAA concentration was determined by reference to standard calibration curve.

2.12 Estimation of Siderophore Production

The siderophore production potential of *Azospirillum sp.* and *Pseudomonas* isolated was estimated according to the method described by Reeves *et al.*, (1983). Distinct liquid media were prepared for siderophore production assays. Nitrogen-free bromothymol blue (NFB) broth was used for *Azospirillum sp.*, whereas minimal medium broth was employed for *Pseudomonas* isolates. For both bacterial cultures, 100 mL of the respective liquid medium was dispensed into 250 mL Erlenmeyer flasks and inoculated with 1 mL of bacterial culture. The flasks were incubated at 30°C for 7 days. After seven days, the broth cultures were centrifuged at 10,000 rpm for 20 minutes. The cell-free supernatant obtained after centrifugation was collected and used for siderophore estimation. For siderophore extraction, 20 mL of supernatant was mixed with 20 mL of ethyl acetate. Hathway reagent played an important role in siderophore estimation. The reagent was prepared by mixing 1 mL of 0.1 M ferric chloride and 1 mL of 0.1 N HCl in 100 mL of distilled water, followed by the addition of 1 mL of 0.1 M potassium ferricyanide. For the estimation of salicylate-type siderophore, 5 mL of assay medium was mixed with 5 mL of Hathway reagent. The absorbance was recorded at 560 nm using a spectrophotometer, and the siderophore concentration was determined by interpolation from a standard calibration curve.

2.13 Estimation of Catechol-Type Siderophore

For the estimation of catechol-type siderophore, 5 mL of assay medium was mixed with 5 mL of Hathway reagent. Absorbance was then recorded at 700 nm using a spectrophotometer, and the siderophore production was determined using standard calibration curve prepared using 2,3-dihydroxy benzoic acid (DHBA). In this method, the equivalent value for 1 mole of DHBA was considered as 0.75. The Catechol-Type siderophore production was finally expressed as ng/mL.

2.14 In Vitro Antifungal Activity

The antagonistic activity of *Pseudomonas* against *Fusarium oxysporum* was evaluated using well plate diffusion method described by Vincent JM (1947). *Fusarium oxysporum* and *Pseudomonas* were co-cultured on Potato Dextrose Agar (PDA) medium under dual-culture conditions. After incubation for 3 to 7 days, the zone of inhibition was measured and recorded.

2.15 Pot Culture Experiment

The pot culture experiment on tomato plants was conducted in the greenhouse of the Department of Microbiology, Faculty of Agriculture, Annamalai University, from December to January. The experiment was carried out using a Randomized Block Design (RBD) with seven different treatments. For this study, efficient *Azospirillum sp.* isolates showing high nitrogen fixation ability and efficient *Pseudomonas* isolates exhibiting phosphate solubilization, IAA production, and siderophore production were selected and prepared as separate liquid cultures. VAM fungi exhibiting efficient phosphate solubilization ability were prepared as separate inoculum cultures. Similarly, *Fusarium oxysporum*, the pathogenic fungus used for laboratory-level disease induction, was also prepared as a separate culture inoculum. The experiment was then arranged in a Randomized Block Design (RBD) with the following treatments: T_1 – *Pseudomonas,* T_2 – *Azospirillum sp.*, T_3 – VAM Fungi, T_4 – *Fusarium oxysporum*, T_5 – PGPR + AM Fungi + *Fusarium oxysporum,* T_6 – Consortium (PGPR + AM Fungi) , T_7 – Control. These treatments were subjected to experimental evaluation.

To determine the seed germination percentage, the method described by (Zhao *et al*., 2023) was followed by recording the number of seeds germinated daily. The seed germination percentage was calculated using the following formula:

$$GP = \frac{Number\ of\ total\ germinated\ seeds}{Total\ number\ of\ seeds\ tested} x100$$

The Seedling Vigour Index (SVI) was calculated using the seed germination percentage and the mean total seedling length. According to the method described by (Manjeet *et al*., 2020), the Seedling Vigour Index was calculated as:

Seedling Vigour Index = $G(\%)$ × Mean total seedling length The mean total seedling length was calculated as:

Mean total seedling length = Mean root length + Mean shoot length

III. RESULT AND DISCUSSION

Chemical and Microscopic Characteristics of *Azospirillum sp.* Microorganisms

3.1 Microscopic Characteristics

The *Azospirillum sp.* microorganism was identified as Gram-negative by Gram staining test. Based on microscopic observation, the shape and arrangement of the cells were studied. The cells appeared as rod-shaped and slightly curved rods. Cells were observed either singly or arranged in clusters. A tail-like structure called a flagellum was observed at the posterior end of each microorganism. This flagellum was confirmed to help in the movement of the microorganism and in colonizing specific sites. The Gram reaction and microscopic features observed in this study were consistent with the characteristic features of *Azospirillum sp.* microorganisms. The microscopic observations obtained in present study were in agreement with the findings reported by (Sulaiman *et al*., 2019).

3.2 Biochemical Characteristics of *Azospirillum sp.* Microorganisms

All five isolated *Azospirillum sp.* microorganisms showed positive results in the catalase test, indicating the presence of the catalase enzyme. The catalase enzyme is biochemically significant because it breaks down hydrogen peroxide (H_2O_2) into water (H_2O) and oxygen (O_2). This characteristic is considered significant in the identification and biochemical characterization of *Azospirillum sp.* microorganisms. The detailed biochemical characteristics of all isolates are presented in Table 1.

Azospirillum sp. isolates Az-1 and Az-2 showed positive results for the MR (Methyl Red) test, indicating their association with the butanediol fermentation process. The isolates Az-3, Az-4, and Az-5 exhibited positive results in the MR test. Among them, the microorganism Az-4 showed a negative result in the VP (Voges–Proskauer) test. Based on these findings, the isolates were confirmed to possess mixed acid fermentation characteristics. Except for Az-4, all the isolates showed positive citrate utilization test. This authenticated the utilization of citrate through the following enzymatic reaction:

Citrate → Pyruvate + Oxaloacetate

As a result of this reaction, the pH of the medium increased, causing the medium color to change from green to blue, indicating a positive result. The biochemical test results observed in this study were in agreement with the findings reported by (Rabara *et al*., 2023). Therefore, the isolates were confirmed as *Azospirillum sp.*

Table 1. Biochemical characterization of Azospirillum sp. isolates

S. No.	Isolate Code	Gram Staining	Catalase Test	Methyl Red (MR)	Voges-Proskauer (VP)	Citrate Utilization
1	Az-1	-ve	+	-	+	+
2	Az-2	-ve	+	-	+	+
3	Az-3	-ve	+	+	+	-
4	Az-4	-ve	+	+	-	+
5	Az-5	-ve	+	+	+	+

3.3 Microscopic Characteristics of *Pseudomonas*

Each *Pseudomonas* isolate formed flat, irregular, and shoe-shaped colonies on agar plate. The colonies exhibited smooth surface and translucent appearance. One of the characteristic features of *Pseudomonas sp.* is the siderophore production. This characteristic was confirmed by the fluorescence exhibited by the microorganisms. The yellowish-green colored colonies observed on the agar surface indicated the presence of *Pseudomonas sp.* and further confirmed siderophore production. These observations were in agreement with the previous findings (Reddy & Sudaramoorthi, 2026). Therefore, the isolate was identified as *Pseudomonas sp.*

3.4 Biochemical Characteristics of *Pseudomonas*

All the isolate yielded negative results upon Gram staining test, indicating their classification as Gram-negative bacteria. It was further observed that none of the showed endospores formation. The purified isolates tested negative for the Indole, MR (Methyl Red), and VP (Voges–Proskauer) biochemical assays. These findings confirmed the absence of mixed acid fermentation or butanediol fermentation biochemical pathways in the tested microorganisms. consequently, the microorganisms were determined to lack the enzymatic for these fermentative processes . A comprehensive summary of the biochemical characteristics is given in Table 2.

All the microorganisms designated Ps-1 through Ps-5 demonstrated positive reactions in the citrate utilization test, oxidase test and catalase test. These results confirmed the presence of citrate-utilizing enzymes activity, cytochrome-c oxidase, and catalase activity within the isolates. The biochemical profile of these isolates was consistent with the established characteristics of genus *Pseudomonas*. Furthermore, the results were in agreement with the findings reported by Labhasthrar *et al*., (2019). Therefore, the isolated microorganisms were conclusively identified as *Pseudomonas sp*.

Table 2. Biochemical characterization of Pseudomonas isolates

S. No.	Isolate Code	Gram Staining	Spore Formation	Indole Production	Methyl Red (MR)	Voges-Proskauer (VP)	Citrate Utilization	Oxidase Test	Catalase Test
1	Ps-1	-ve	-	-	-	-	+	+	+
2	Ps-2	-ve	-	-	-	-	+	+	+
3	Ps-3	-ve	-	-	-	-	+	+	+
4	Ps-4	-ve	-	-	-	-	+	+	+
5	Ps-5	-ve	-	-	-	-	+	+	+

3.5 Isolation of Nitrogen Fixing Plant Growth Promoting Rhizobacteria (PGPR)

The isolation of nitrogen fixing microorganisms facilitates the identification of highly efficient microorganisms through quantification of nitrogenase activity and cellular nitrogen content. The in vitro nitrogenase activity and cellular nitrogen content of *Azospirillum sp.* are given in Table 3. The nitrogenase activity of the five *Azospirillum sp.* isolates (Az-1 to Az-5) were recorded as follows Az-1 – 145.20 nmol, Az-2 – 210.45 nmol, Az-3 – 198.60 nmol, Az-4 – 170.30 nmol, Az-5 – 225.75 nmol (C_2H_4 μg^{-1} protein h^{-1}). Among these isolates, Az-5 exhibited the highest nitrogenase activity with (225.75 nmol C_2H_4 μg^{-1} protein h^{-1}) followed by Az-2 (210.45 nmol C_2H_4 μg^{-1} protein h^{-1}).

The cellular nitrogen content varied among the five microorganisms Az-1 – 11.35, Az-2 – 15.80, Az-3 – 14.90, Az-4 – 13.40, Az-5 – 16.95 μg/g cell weight. With respect to cellular nitrogen content, Az-5 recorded highest value of 16.95 μg/g cell weight, followed by Az-2 with 15.80 μg/g cell weight. The results of present study were consistent with those findings reported by (Naqqash *et al*., 2022), wherein the nitrogen-fixing efficiency of TN23 and TN09 isolates ranged from 138 to 143 nmol μg^{-1} protein h^{-1}.These findings corroborate the reported values, thereby validating the results of present study. The cellular nitrogen content recorded in the present study was also in accordance with values reported by (Hassain *et al*., 2014). where the cellular nitrogen content ranged from 10.33 to 13.11 μg/g. Thus, our research findings were further substantiated.

Table 3: Invitro nitrogenase activity and cell nitrogen content of Azospirillum sp. sp. obtained from rhizosphere soils of tomato

S. No.	Isolate code	Nitrogenase activity (nmol C_2H_4 mg^{-1} protein h^{-1})	Cellular nitrogen content (mg g^{-1} cell weight)
1	Az-1	145.20	11.35
2	Az-2	210.45	15.80
3	Az-3	198.60	14.90
4	Az-4	170.30	13.40
5	Az-5	225.75	16.95
SED		2.10	0.18
CD (p=0.05)		4.25	0.40

Table 4: Phosphate solubilizing potential of Pseudomonas isolates

s.no	Isolate code	Phosphate solubilization (mg P/100 mL broth)	Solubilization index	Final pH of medium
1	Ps-1	12.45	2.10	5.8
2	Ps-2	18.30	2.85	5.2
3	Ps-3	15.60	2.40	5.5
4	Ps-4	21.75	3.10	5.0
5	Ps-5	10.20	1.95	6.0
SED		0.85	0.12	0.08
CD (p=0.05)		1.75	0.25	0.15

3.6 Assessment of Phosphorus Solubilization potential of *Pseudomonas* isolates

The phosphorus solubilization potential of *Pseudomonas* isolates was evaluated based on soluble phosphorus content, final pH of the medium, and solubilization index (SI). The quantitative values for phosphorus solubilization, solubilization index and final pH of the medium are summarized in Table 4. The phosphorus solubilization values of *Pseudomonas* isolates PS-1 through PS-5 were recorded as 12.45, 18.30, 15.60, 21.75, and

12.20 µg P/100 mL broth respectively. Among these isolates, PS-4 exhibited the highest phosphorus solubilization capacity (21.75 µg P/100 mL broth), followed by PS-2 (18.30 µg P/100 mL broth), while PS-5 recorded the lowest value (12.20 µg P/100 mL broth) . The solubilization index (SI) values were as follows: PS-1 – 2.10, PS-2 – 2.85, PS-3 – 2.40, PS-4 – 3.10, and PS-5 – 1.95. PS-4 and PS-2 recorded highest SI values of 3.10 and 2.85 respectively, PS-5 exhibited the lowest value of 1.95. It is noteworthy that the final pH of the medium and phosphorus solubilization were inversely correlated. Accordingly, the final pH values of the growth medium for isolates PS-1 to PS-5 were recorded as follows: PS-1 – 5.8, PS-2 – 5.2, PS-3 – 5.5, PS-4 – 5.0, and PS-5 – 6.0. Notably, PS-4 and PS-2 recorded

lower pH values of 5.0 and 5.2, indicating higher degree of acidic nature. The production of organic acids is considered a primary mechanism underlying phosphorus solubilization. These findings are consistent with those reports of (Xu & Chen, 2025) who demonstrated the phosphorus solubilization capability of *Pseudomonas* isolated reached 432.36 µg/L and 364 µg/L respectively attributed to production of organic acids and the consequent reduction in pH. Thus, our experimental results were clearly confirmed by their findings.

3.7 Production of Siderophore and IAA by Plant Growth-Promoting Microorganisms

Among the plant growth-promoting microorganisms, *Azospirillum sp.* isolates AZ-2 and AZ-5, and *Pseudomonas* isolates PS-2 and PS-4, which demonstrated superior performance, were selected for siderophore and IAA production studies. Siderophore and IAA production were detected at varying levels in all selected *Azospirillum sp.* and *Pseudomonas* isolates. The quantitative data on siderophore and IAA production are summarized in Table 5. Among the *Pseudomonas* isolates, PS-2 showed superior performance with the production of 3.10 µg mL⁻¹ catechol-type siderophore, 4.60 µg mL⁻¹ salicylate-type siderophore, and 58.20 µg/25 mL broth of IAA. PS-4 recorded the second highest values with 2.35 µg mL⁻¹ catechol-type siderophore production and 42.50 µg/25 mL broth. Among the *Azospirillum sp.* isolates, AZ-5 recorded the highest production, yielding

2.40 µg mL⁻¹ catechol-type siderophore, 4.10 µg mL⁻¹ salicylate-type siderophore, and 38.75 µg/25 mL broth of IAA. The isolate AZ-2 recorded 1.85 µg mL⁻¹ catechol-type siderophore,

3.20 µg mL⁻¹ salicylate-type siderophore and 31.40 µg/25 mL broth of IAA. collectively, isolates PS-2 and AZ-5 demonstrated highest production of siderophore and IAA, thereby confirming their efficiency as potential plant growth promoting microorganisms through enhanced iron acquisition and plant hormone biosynthesis. The findings of the present study are consistent with those observations of (Lenin G, 2012) who demonstrated that *Pseudomonas fluorescens* exhibited high levels of siderophore production, while *Azospirillum lipoferum* produced comparatively higher levels of IAA. These previously reported corroborate the results of present study. Three different arbuscular mycorrhizal fungi (AMF) were subjected to investigation in order to evaluate their phosphorus mobilization ability and rhizosphere soil colonization percentage. The plant-available phosphorus and the percentage of rhizosphere colonization are presented in the table 6. The plant-available phosphorus content ranged from 16.30 to 24.10 mg/kg of soil. Among these isolates *Gigaspora sp.* recorded the highest plant available phosphorus content (24.10 mg/kg of soil) and ranked first, followed by *Glomus sp.* with a value of 22.75 mg/kg of soil in second place. *Acaulospora sp.* recorded the lowest value of 16.30 mg/kg of soil. The root colonization percentages were recorded as follows: *Gigaspora sp.* – 74%, *Glomus* sp. – 70%, and *Acaulospora sp.* – 55%. The final results of our research were in agreement with the findings of the research article by (Parvin *et al.*, 2020). which reported that the fungi *Gigaspora margarita* and *Acaulospora laevis* showed efficient root colonization and phosphorus mobilization, thereby increasing plant yield by 143% and 125%, respectively. Thus, our research findings were further validated.

Table 5: Siderophore and IAA production by Azospirillum sp. and Pseudomonas isolates

Isolate	**Siderophore (Catechol type, µg mL⁻¹)**	**Siderophore (Salicylate type, µg mL⁻¹)**	**IAA production (µg 25 mL⁻¹ broth)**
Ps-4	3.10	4.60	58.20
Ps-2	2.35	3.80	42.50
Az-2	1.85	3.20	31.40
Az-5	2.40	4.10	38.75
SED	0.10	0.12	1.25
CD (p=0.05)	0.22	0.25	2.65

Table 6: Phosphorus mobilizing potential of mycorrhiza species

S. No.	**Mycorrhizal species**	**Available P (mg kg⁻¹ soil)**	**Root colonization (%)**
1	*Glomus sp.*	22.75	70
2	*Acaulospora sp.*	16.30	55
3	*Gigaspora sp.*	24.10	74
SED		1.05	2.10
CD (p=0.05)		2.30	4.50

3.8 Evaluation of Biological Control Agents Against Plant Pathogens Under Laboratory Conditions

Under laboratory conditions, the microorganism *Pseudomonas* effectively controlled the plant pathogen *Fusarium oxysporum* in Petri plates. The agar well diffusion method was employed to assess antifungal activity of *Pseudomonas* microorganism against the test pathogen. The *Pseudomonas* isolate inhibited the mycelial growth inhibition zone of 41.7 ± 2 mm against *Fusarium oxysporum* on agar plates. These results confirmed that *Pseudomonas* possesses significant biocontrol potential against plant pathogenic fungi. These results were consistent with the findings of the research article by Chinmay *et al.*, (2024) which reported that *Pseudomonas fluorescens* effectively controlled *Fusarium oxysporum* with a growth inhibition value of 36.3 ± 4 mm. Therefore, the results of present study is confirmed by previous studies chinmay *et al.*, (2024)

3.9 Effect of Plant Growth Promoting Rhizobacteria (PGPR) and Arbuscular Mycorrhizal (AM) Fungi on Seedling Growth

Microorganisms exhibiting superior plant growth promoting traits such as nitrogen fixation, phosphorus solubilization, IAA production, siderophore production, and antagonistic activity against *Fusarium oxysporum* were selected for the evaluation. Among them, *Pseudomonas*, *Azospirillum sp.*, and AM fungi were used under controlled conditions to evaluate their effect on the growth of tomato seedlings. The observations recorded at the initiation of the experiment and after 30 days are presented in Table 7.

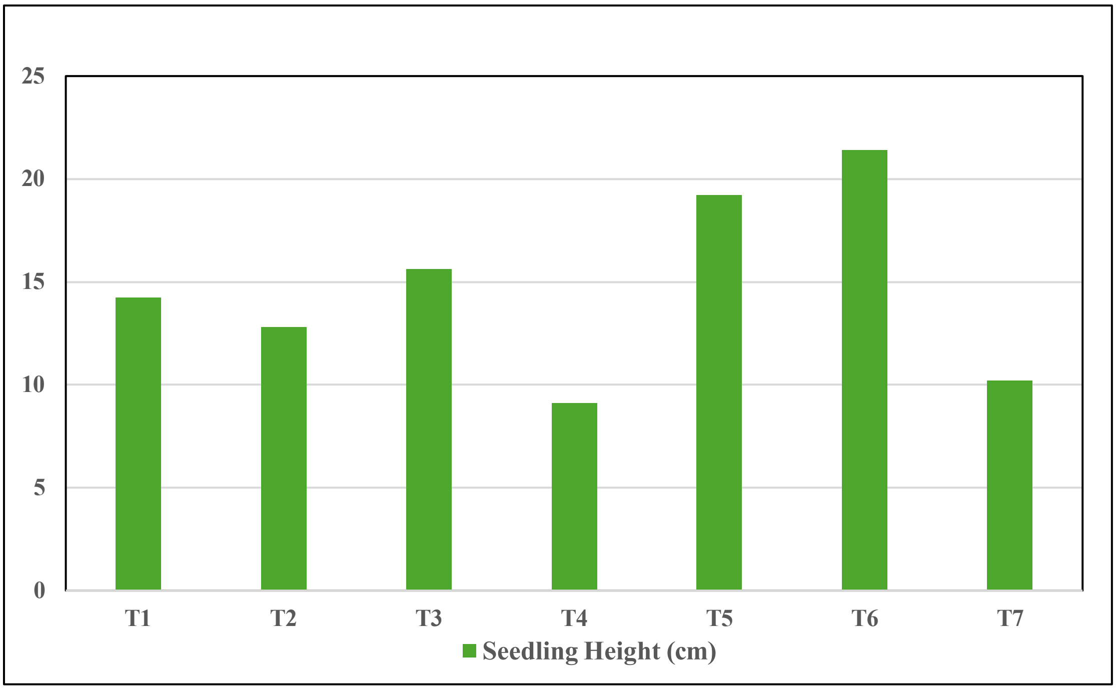
Seven different treatments were imposed as follows:

T1 – *Pseudomonas*, **T2** – *Azospirillum sp.* **T3** – Mycorrhiza, **T4** – *Fusarium oxysporum,* **T5** – Consortium (T1 + T2 + T3) + *Fusarium oxysporum* **T6** – Consortium alone, T7 – Control

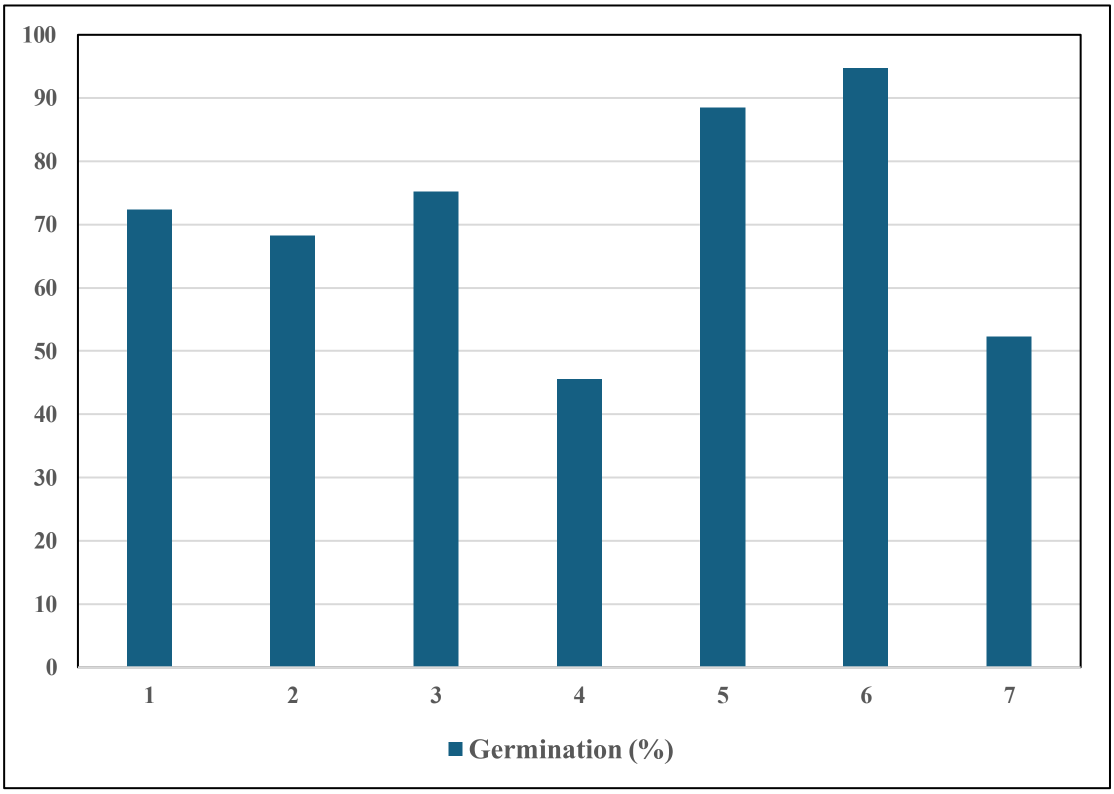
The values recorded for seedling height, seed germination percentage, and vigour index for treatments T1 to T3 were as follows: T1 – 14.25 cm seedling height, 72.40% seed germination, 1031.4 vigour index (VI) ,T2 – 12.80 cm seedling height, 68.30% seed germination, 874.2 vigour index (VI) ,T3 – 15.62 cm seedling height, 75.20% seed germination, 1173.1 vigour index (VI) The values were recorded as follows: T4 – 9.15 cm, 45.60%, 612.3 VI ,T5 – 29.20 cm, 88.50%, 1785.6 VI ,T6 – 21.40 cm, 94.80%, 2028.7 VI ,T7 – 10.80 cm, 52.70%, 533.5 VI . Among these treatments, T6 exhibited superior performance with seedling height of 21.40 cm, 94.80% seed germination, and a vigour index (VI) of 2028.7. This was followed by T5 (microbial Consortium + *Fusarium oxysporum*), which recorded 19.20 cm seedling growth, 88.50% seed germination, and a vigour index of 1785.6, ranking next to T6. These results confirmed that the T5 treatment effectively controlled *Fusarium* disease and promoted healthy tomato seedling growth. In contrast, T4 (*Fusarium oxysporum*) showed only 9.15 cm seedling length, 46.60% seed germination, and a vigour index of 612.1, indicating that the fungal pathogen severely affected the growth and vigour of tomato seedlings. Thus, the consortium containing *Pseudomonas*, *Azospirillum sp.*, and mycorrhizal fungi played an important role in enhancing plant growth through phosphate solubilization, IAA production, and siderophore production. It was clearly demonstrated that these microorganisms contribute significantly to improve soil structure and soil fertility by reducing the use of chemical fertilizers. They also play an important role in enhancing plant tolerance against biotic and abiotic stresses and help in sustaining long-term soil productivity and crop health. The results obtained in our study were in agreement with the findings of researchers zeng *et al.*, (2025) & widawati *et al.*, (2018) who reported that plant growth-promoting microorganisms such as PGPR and AM fungi, when used as biofertilizers, enhanced seed germination percentage, shoot length (15.67 cm), and vigour index (2127.20). Their study also confirmed that these beneficial microorganisms stimulate root and shoot growth, suppress plant pathogens, and facilitate the production of healthy and high-quality tomato seedlings.

Table 7: Effect of plant growth promoting rhizobacteria inoculation of seedling height, Germination % and vigour index

Treatment	Seedling Height (cm)	Germination (%)	Vigour Index
T1 – *Pseudomonas*	14.25	72.40	1031.4
T2 – *Azospirillum sp.*	12.80	68.30	874.2
T3 – Mycorrhiza	15.60	75.20	1173.1
T4 – *Fusarium oxysporum*	9.10	45.60	612.3
T5 – Consortium + *f. oxysporum*	19.20	88.50	1785.6
T6 – Consortium alone	21.40	94.80	2028.7
T7 – Control	10.20	52.30	533.5
SED	1.35	4.20	18.5
CD (p = 0.05)	2.80	8.95	38.4



Bar graph 1. Effect of different treatments (T1-T7) on seedling height (cm)



Bar graph 2. Effect of different treatments (T1-T7) on germination %

Bar graph 3. Effect of different treatments (T1-T7) on vigour index

Fig 1. Evaluation of microbial consortium on tomato plant growth under pot culture experiment

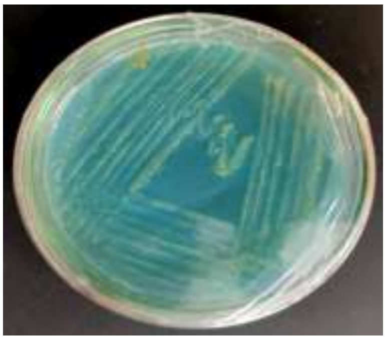


Fig2.Azospirillum sp. sp.

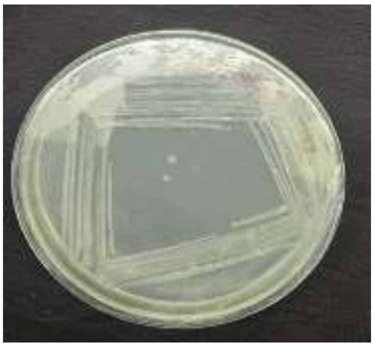


Fig3.Pseudomonas sp.

Fig4.Fusarium sp

IV. CONCLUSION

Excessive use of chemical fertilizers and fungicides increases soil acidity, leading to root scorching, unavailability of essential micronutrients, toxic effects, and dissolution of heavy metals in the soil. Similarly, the overuse of fungicides adversely affects beneficial mycorrhizal fungi, which are important for water absorption and nutrient uptake. The beneficial functions of mycorrhiza, such as supplying nutrients to plants and providing resistance against harsh environmental conditions like drought and salinity stress, are also greatly reduced. "From the results of our study, it is evident that reducing the use of chemical fertilizers and chemical fungicides, while properly utilizing natural biofertilizers and antagonistic microorganisms, can effectively enhance plant growth and control plant pathogens.

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