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Studies on effect of PGPR on growth and yield of Tomato (*solanum lycopersicum* l.)

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Abstract— *The excessive dependence on chemical fertilizer has resulted in declining soil fertility, reduced microbial diversity, environmental pollution and increased production cost. The study aimed to evaluvate the efficiency uses of Azotobacter sp. and Pseudomonas sp. individually and in combination for promoting N-fixation, P-solubilization, siderophore production and phytohormone production like IAA and GA_3. The PGPR microorganism namely Azotobacter sp. and Pseudomonas sp. were isolated from the rhizosphere soil of different tomato cultivating field in cuddalore district. Among the all isolates Az-5 and Az-2 recorded highest nitrogenase activity and cellular nitrogen content. Among the isolated Pseudomonas strain Ps-4 exihibited the greatest phosphate solubilization of followed by Ps-2 demonstrated significant phosphate solubilization of . Azotobacter isolates Az-5 and Az-2, Pseudomonas isolates Ps-4 and Ps-2 subjected to evaluvation of plant growth promoting traits like siderophore, IAA and GA_3 production. Among the selected strain Az-5 and Ps-2 shows highest production of siderophore and phytohormone production. Azotobacter isolates Az-5 and Pseudomonas isolates Ps-2 were selected efficient strain used as the bioinoculant treatment in subsequent pot culture experiment. The result showed that dual inoculation of PGPR Az-5 and Ps-2 combined with 75% of the recommended NPK resulted maximum germination%, vigour index, No of fruits, fruit weight and fruit yield which is practically on par with 100% NPK. The study revealed that 25% of synthetic fertilizer have been reduced by using dual bioinoculant without reduction in yield.*

Keywords— ***Azotobacter, phytohormone production, Pseudomonas, siderophore production, synthetic fertilizer***

I. INTRODUCTION

Tomato (*Solanum lycopersicum* L) is a high-value vegetable crop and play significant role in Indian agriculture due to high nutritional value, medicinal and industrial value. The average productivity of tomato in Tamilnadu 1.2 – 1.5 million tonnes per year. Globally, India is a 2nd largest producer of tomato nearly 20-21 million tonnes per year. Nutrient requirement for tomato cultivation 200:250:250 kg NPK per hectare. However, excessive dependence on chemical fertilizers has resulted in declining soil fertility, reduced microbial diversity, environmental pollution, and increased production costs. Biofertilizers based on Plant Growth-Promoting Rhizobacteria (PGPR) offer a sustainable based microbial inoculant containing an efficient PGPR for enhancing tomato productivity. Beneficial bacterial strains capable of nitrogen fixation, phosphate solubilization and phytohormone production will be isolated from tomato rhizosphere soils (Sanjai *et al.,* 2026). Excessive and continues application of chemical fertilizer leads to soil degradation, decline in beneficial microbial activity and environmental pollution. PGPR have emerged as alternative source to chemical fertilizer. PGPR

colonize the beneficial rhizosphere microorganism which intract with plant roots through direct mechanism like N-fixation, P-solubilization and phytohormone production. Whereas indirect mechanism like siderophore production and defence mechanism against phytophathogens (Backer *et al.,* 2018).

Azotobacter is a free-living non-symbiotic bacterium which fix the atmospheric nitrogen in rhizosphere region of vegetable crops through the activity of nitrogenase enzyme complex. Azotobacter promotes the plant growth regulates like IAA,GA_3 and siderophore production. Which stimulate the nutrient absorption, seed germination and crop yield (Kumar *et al.,* 2023) (Prabudoss & Stella, D 2009).

Pseudomonas, a plant growth promoting rhizobacteria. Which produce low molecular weight organic acids that convert insoluble phosphate compounds into soluble ions. Pseudomonas indirectly act as a defence mechanism against soil-borne plant pathogens by producing antimicrobial metabolites (Sharma *et al.,* 2023). Pseudomonas synthesizes phytohormone such as Siderophore, IAA and GA_3 production. Pseudomonas produce chelated ferric iron during iron deficient condition (Rani *et al.,* 2024).

IAA is the principle source of auxin produced by the rhizobacteria which enhance root initiation, lateral root length and root hair development (Olanrewaju *et al.,* 2019). Likewise, GA_3 promotes the seed germination fruit development and nutrient use efficiency. In addition GA_3 promotes chlorophyll synthesis, biomass accumulation, leaf expansion, fruit development and stimulates the enzyme activities (Bottini *et al.,* 2004).

In this research study plant growth promoting rhizobacteria such as Azotobacter and Pseudomonas were utilized to produce the healthy nutrient rich Tomato. The efficient use of PGPR dual inoculum of Azotobacter and Pseudomonas are capable of enhancing tomato productivity through biologically based nutrient management.

II. MATERIAL AND METHODS

2.1 Collection of rhizosphere soil:

Samples of rhizosphere soil were taken from maize fields across various locations in Cuddalore district with particular emphasis on the rhizoplane, including soil particles firmly adhering to the root surface.Rhizosphere soil sample will be collected from the vegetative stage of tomato plant from different location. Nearly 100-200g of soil sample will be collected from the each sampling site. Sample code, location, date of collection and crop details will be labelled on each sample. collected soil smaple maintained at 4ºc refrigeretted for further studies (Dong *et al.,* 2019).

2.2 Isolation of Azotobacter from the rhizosphere soil sample

Serial dilutions were prepared up to 10^{-6} and 1 mL from 10^{-5} and 10^{-6} dilutions was spread on Burk's agar plates using the spread plate technique. All dilutions were plated in triplicate to ensure reliable isolation of purified Azotobacter colonies. For two to five days, the plates were incubated at 28 to 30°C, and the resulting colonies were purified and maintained on Burks slants at 4°C. These isolates were further used for biochemical analysis and evaluation of PGPR traits (Samal *et al.,* 2020).

2.3 Isolation of Pseudomonas from the rhizosphere soil sample

Soil sample from the rhizosphere is serially diluted and aliquots from 10^{-5} and 10^{-6} dilutions are taken for isolation using the dilution plate technique. For each dilution, three replicates are maintained to ensure accuracy and reproducibility. The samples are poured on solidified Pikovskaya medium, which contains tricalcium phosphate as insoluble phosphorus sources and incubated under optimum incubation conditions (48 hrs at 37° C). After the incubation period, colonies showing a clear zone around them are observed, indicating the solubilization of insoluble phosphorus present in the medium. These colonies are selected, subcultured onto Pikovskaya slants and preserved at 4°C for further biochemical and PGPR evaluation (Patel *et al.,* 2016).

2.4 Morphological and biochemical charecteristics of *Azotobacter sp.*. And *Pseudomonas sp.*.

2.4.1 Gram staining

Gram staining was carried out to determine the morphology and gram reaction of the *Azotobacter sp.* and *Pseudomonas sp.*. (Gram C, 1884). A thin bacterial culture smear was made on a clean glass slide from 48 hours old culture and allowed to air dry. The smear was heat fixed by passing through the flame. Crystal violet was applied for 1 minute, followed by iodine solution for 1 minute. Decolorization was carried out using 95% ethanol for 10-15 seconds and immediately washed with distilled water gently. Finally smear was counterstained with safranin foe 30 seconds subsequently washed with distilled water, then stained slide was observed under a coumpound microscope (100X) using an oil immersion.

2.4.2 Catalase test

The catalase test was performed following the procedure described by (Cappuccino & Sherman, 2011). A bacterial colony from 48 hours old culture transferred to a

clean glass slide using sterile inoculation loop. Few drops of prepared Hydrogen peroxide (H_2O_2) solution were added to the bacterial smear. Immediate formation of oxygen bubbles indicates the positive catalase test, whereas the absence of bubbles formation indicates a negative result.

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

MR-VP test was conducted according to the method described by the (Cappuccino & Welsh, 2017). A loopful of 48 hours fresh bacterial culture was selected and transferred into sterile MR-VP broth and incubated at 28±2 ºc for 48 hours. For the Methyl red (MR) test 5 drops of Methyl red indicator were added to the incubated broth. The indication of red colour shows positive reaction, whereas yellow or orange colour indicates a negative reaction.

For the Voges-Proskauer(VP) test, a few drops of α-naphthol solution (Barrit's Reagent A) and 40% Potassium hydroxide (Barrit's Reagent B) were added to the broth culture. The broth culture was shaked gently and allowed to stand for 15-30 minutes. The development of pink colour indicates a positive VP reaction. Whereas no colour changes was considered negative.

2.4.4 Citrate utilization test

The citrate utilization test was performed using simmons citrate agar according to the method of (Atlas RM, 2004). In this test slant simmons citrate agar were prepared in sterile culture tubes. The pure bacterial culture was inoculated in slant surface using sterile inoculating loop and incubated 28±2 ºc for 48 hours. A colour changes of the medium from green to prussion blue indicates a positive reaction. Whereas absence of colour changes indicates a negative result.

2.5 Screening of PGPR for plant growth promoting traits

The isolates of *Azotobacter sp.*. and *Pseudomonas sp.*. was screened for Nitrogen-fixation, Phosphate solubilization, Siderophore production and Phytohormone production such as IAA(Indole-3-acetic acid), GA3 (Gibberellic Acid).

2.5.1 Estimation of total nitrogen fixed by Azotobacter Isolates

The ability of nitrogen-fixation of the Azotobacter isolates was determined by (Humphries EC, 1956). In this method isolates were grown in 100ml of Nitrogen-free waksman's base medium No.77 broth in 250ml of Erlenmeyer flask at 28±2 ºc for 7 days. After 7 days the culture was mixed thoroughly to attain homogeneous suspension. Then 5ml of microbial culture was trasfered to a kjeldahl digestion tube along with 5ml of concentrated H_2SO_4 and 5ml of catalyst were added which consisting of 10:1 ratio of K_2SO_4 (Potassium sulfate) and $CUSO_4$ (copper sulfate). Digestion was continues until the solution became colourless. After cooling for few minutes at room temperature was transfered to a Micro-kjeldahl distillation unit. Then 10ml of 40% Sodium hydroxide (NaoH) was added to occur liberation of ammonia through steam distillation. The ammonia was distilled and collected in 20ml of 2% boric acid solution. The trapped ammonia was titrated against N/50 H_2SO_4 until the end point reaches. The amount of nitrogen fixed by isolate was calculated from the titre value and expressed amount of N present in the microbial medium using the following equatiom,

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).

2.5.2 Estimation of Phosphate Solubilization

The ability of Phosphate solubilizing by the Pseudomonas isolates was determined by using Pikovskaya's broth described by (Nautiyal CS, 1999). In this methid 50ml of Pikovskaya broth was seperatly dispensed into 250ml of Erlenmeyer flask. The Pseudomonas culture was inoculated at each flask at a rate of 100µl and the flask was incubated at 30±2 ºc on a rotatory shaker at 150 rpm for 7 days. After 7 days the culture was centrifuged at 5000 rpm for 20 minutes to obtain a clear supernatant. The concentration of soluble phosphorous released into filtrate was estimated following the described by (Jackson ML, 1973).

2.5.3 Estimation of IAA(Indole-3-acetic acid)

The ability of producing IAA by the Azotobacter and Pseudomonas isolates determined by the Salkowsi calorimetric assay following the method of (Gordon & Weber. 1951) and Mayer (1958) and the modified procedure was described by (Bric *et al.,* 1991). In a sterile Erelenmeyer flask the bacterial isolate was inoculated into 50ml of yeast mannitol broth (YMB) supplemented with 1g/L of L-tryphtophan as a precursor and incubated at 30±2 ºc on a rotatory shaker at 150 rpm for 7 days. After 7 days the culture was centrifuged at 10000 rpm for 10 minutes at 4ºc to obtain supernatant. An 1 ml of aliquot supernatant mixed with 2 ml of Salkowski reagent (1 ml of 0.5 M $Fecl_2$ disolved in 49 ml of 35% of perchloric acid ($HCLO_{4)}$). The reaction mixture was incubated in the dark at 30º c for 30 minutes to allow the development of pink colour indicating the presence of IAA. Subsequently, the absorbance of IAA was measured 530 nm using a spectrophotometer and the

IAA concentration was determined by reference to standard calibration curve.

2.5.4 Estimation of siderophore production

The siderophore production ability of selected Azotobacter and Pseudomonas isolates was determined by the following procedure described by (Reeves *et al.,* 1983). In this method Azotobacter isolates were cultivated in Waksman's No 77 broth. Whereas Pseudomonas isolates were cultivated in King's B broth. For broth bacterial culture approximately 100 ml of respective media was supplied into 250 ml of Erelenmeyer flask and inoculated with 1 ml od actively growing bacterial culture. The inoculated flask was incubated at 30±2 ºc for 7 days. After 7 days the culture were centrifuged at 10000 rpm for 20 minutes at 4º c. The cell-free supernatant was collected ans used for siderophore estimation. For extraction of siderophore 20 ml of the culture supernatant was mixed with 20 ml of ethyl acetate. Hathway reagent was prepared by dissolving 1 ml of 0.1 m ferric chloride and 1 ml of 0.1 N hydroxchloric acid in 100 ml of distilled water followed by the addition of 1 ml of 0.1 m Potasssium Ferricyanide. This reagent used for the estimation of broth salicyte and catechol type siderophores.

2.5.4.1 Estimation of salicyte type siderophore

In this type, 5 ml of extracted assay sample was mixed with 5 ml of Hathway reagent. The absorbance was recorded at 560 nm using a UV- visible spectrophotometer and the concentration of salicyte type siderophore was calculated from a standard calibration curve.

2.5.4.2 Estimation of catechol type siderophore

Catechol type siderophore was estimated by mixing 5 ml of the extracted assay sample with 5 ml of Hathway reagent. The absorbance was recorded at 700 nm using a UV- visible spectrophotometer and the siderophore production was determined using standard calibration curve prepared with 2,3- dihydroxybenzoic acid (2,3 – DHBA). The equivalent value for 1 mole of DHBA was considered as 0.75. the result was expressed as ng/mL..

2.5.5 Estimation of Gibberelic acid (GA_3)

In this estimation, Azotobacter isolates cultivated in Waksman's No 77 broth. Whereas, Pseudomonas isolates were cultivated in King's B broth. 100 ml respective broth were suspended into 250 ml Erlenmeyer flask. Each flask was inoculated with 1 ml of a active bacterial culture and incubated at 28±2 ºc for 7 days at 150 rpm in incubater shaker. After 7 days culture were centrifuged at 8000 rpm for 10 minutes. 15 ml of cell free supernatant were transferred to test tube and add 2 ml of zinc acetate solution leave for 2 minutes. Then add 2 ml of potassium ferrocyanide solution and the mixture was centrifuged at 8000 rpm for 10 minutes. Then 5 ml of supernatant were mixed with 5 ml of 30% hydrochloric acid and incubated at 27 ºc for 3 hours. The absorbance were recorded at 254 nm using a UV- visible spectrophotometer. The concentration of GA_3 was determined from a standard curve and expressed as µg/ml of culture broth (Borrow *et al.,* 1955).

2.6 Pot culture experiment

Pot culture experiment conducted under greenhouse condition at Department of Agricultural Microbiology, Faculty of Agriculture, Annamalai university. To evaluate the effect of PGPR on the growth and yield of tomato (Solanum Lycopersicum) var. PKM 1. Pots are filled with 1:1 ration of land soil and sterilized sand. The tomato seeds are surface sterilized with 80% ethanol and 0.1% mercuric chloride, then wash the seeds with distilled water for 3 to 4 times to eliminate sterilizing agent (Vincent., 1970). Sterilized seeds are inoculated with bacterial inoculant as per treatment needs. Recommended dose fertilizer were applied according to experimental schedule. The experiment was arranged in a Randomized block design with 10 treatments and three replications.

T_1 - Control

T_2 - 100% NPK

T_3 - 75% NPK

T_4 - 50% NPK

T_5 - 75% NPK + *Azotobacter sp.*.

T_6 - 75% NPK + *Pseudomonas sp.*.

T_7 - 50% NPK + *Azotobacter sp.*.

T_8 - 50% NPK + *Pseudomonas sp.*.

T_9 - 75% NPK + *Azotobacter sp.*. + *Pseudomonas sp.*.

T_{10} - 50% NPK + *Azotobacter sp.*. + *Pseudomonas sp.*.

2.7 Evaluvation of growth and yield parameters

The effect of PGPR on the growth and yield of tomato under pot culture was assessed by the observations at different treatments.

2.7.1 Plant height (cm)

Plant height was measured from the soil surface to the apical point using a measuring scale.

2.7.2 Germination percentage

Seed germination percentage was calculated according to the method of ISTA, (2022) using following

formula

Germination (%)=Total number of seeds sown

Number of germinated seeds×100

2.7.3 Seedling vigour index

Seedling vigour index was measured according to the procedure proposed by Abdul-Baki & Anderson, 1973) using the following formula

Seedling Vigour Index (SVI)=Germination (%)×Mean Sho ot Length (cm)

2.7.4 Number of fruits per plant

Total number of fruits produced by the each plants was recorded at a harvest and mean number of fruits per plant was calculated for each treatment.

2.7.5 Fruit weight (g/fruit)

Fruit weight were examined by weighting fruit at harvest timefrom randomly selected plants from each treatment.

2.7.6 Fruit yield (t/ha)

Fruit yield was determined by weighting the total weight of fruits harvested from the randomly selected plants from each treatment. The yield was expressed as g/plant and then converted into t/ha.

III. RESULT AND DISCUSSION

2.8 Microscopic charecteristics of *Azotobacter sp.*.

The Azotobater culture were identified as Gram Negative by gram staining test. All isolates were observed Gram Negative, rod shaped and slightly curved rods, ocassionally cells were observed single or arranged in small clusters. The presence of flagelluym was confirmed due to the high pleomorphic and active motile. The gram reaction and microscopic features observed in this study were closely matched with the standard description of *Azotobacter sp.*. the microscopic observation in the present study have been similier morphological feature reported by Holt *et al*. (1994).

2.9 Biochemical charecteristics of *Azotobacter sp.*.

All five isolates were Gram Negative and showed positive reaction for catalase and citrate utilization test. The result indicating the presence of catalase enzyme and ability of utilize citrate as a carbon source. The detailed biochemical charecterictics of all isolates (Az-1 – Az-5) are presented in Table.1. Azotobacter isolates Az -3 and Az-5 showed negative result for the MR (Methyl red) test indicating their association with thr butanediol fermentation process. The isolates Az-1, Az-2 and Az-4 exibited positive result in MR test. All the isolates showed the positive result in VP test. The biochemical charecteristics observed in the present study were similer aggrement with the findings reported by Page (1986). Therefore, the isolates were confirmed as *Azotobacter sp.*.

Table 1. Biochemical characterization of Azotobacter 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	+	-	+	+

2.10 Microscopic charecteristics of *Pseudomonas sp.*.

Each pseudomonas isolates were exhibited Gram Negative reaction, formed flat, irregular and shoe-shaped colonies on agar plate. Microscopic examination revealed the presence of functional flagella that promotes the effective root colonization. The pseudomonas charecteristics was examined by UV light. Under UV light colonies exhibited yellowish to green fluroscence due to the production of flouroscent siderophore. These observations were in aggrement with the previous findings (Reddy & Sudaramoorthi, 2026). Therefore the isolates was identified as *Pseudomonas sp.*.

2.11 Biochemical charecteristics of *Pseudomonas sp.*.

All the isolates shows the negative reaction by gram staining test therefore, indicating Gram negative bacteria. All the isolates shows negative reaction for endosore formation MR-VP test. The result indicates that isolates does not mixed acid or butanediol fermentation pathways. The comprehensive summary of the biochemical charecteristics is given in Table 2. All the pseudomonas isolates demonstrated positive reaction in catalase and citrate utilization test. These result confirmed thet the

isolates are ability to decompose hydrogen peroxide (H_2O_2) into water and oxygen due to the positive catalase reaction. Positive citrate utilization indicate that the citrate is the sole carbon source for the microorganisms. Furthermore, the result agreement with the findings reported by (Garrity *et al.,* 2005). Based on the morphological and biochemical charecteristics the isolates were confirmed as *Pseudomonas sp.*.

Table 2. Biochemical characterization of Pseudomonas sp.. isolates

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

2.12 In vitro Nitrogenase activity and cellular nitrogen content of *Azotobacter sp.*.

The Nitrogen Fixing ability of a microorganism estimated by the determining nitrogenase activity and estimating cellular nitrogen content. The in vitro nitrogenase activity and cellular nitrogen content of *Azotobacter sp.*. are given in Table 3. The nitrogenase activity of the five Azotobacter isolates (Az-1 to Az-5) were recorded as follow 158.45, 218.70, 205.35, 182.60 and 236.80 nmol C_2H_4 mg^{-1} protein h^{-1}. Among the isolation Az-5 shows the highest nitrogenase value 256.80 followed by Az-2 (218.70 nmol C_2H_4 mg^{-1} protein h^{-1}). similarly the cellular nitrogen content value among the five isolates ranged from 12.40 to 16.95 mg g^{-1} cell weight. Among the all isolates Az-5 shows the highest cellular nitrogen content 16.95 mg g^{-1} followed by Az-2 15.80 mg g^{-1}. Az-5 shows the highest nitrogenase activity and cellular nitrogen content. So isolate Az-5 confrims the greatest efficiency of nitrogen fixation. The result of present study were consistent with those findings reported by Bhattacharyya & Jha (2012) who demonstrated the Azotobacter strain exibit the higher nitrogenase activity and cellular nitrogen content are considered effective PGPR.

Table 3: Invitro nitrogenase activity and cell nitrogen content of Azotobacter 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	158.45	12.40
2	Az-2	218.70	15.80
3	Az-3	205.35	14.65
4	Az-4	182.60	13.20
5	Az-5	236.80	16.95
SED		13.71	0.82
CD (p=0.05)		38.06	2.28

2.13 Assessment of phosphate solubilization potential of Pseudomonas isolates

The phosphate solubilizing ability of the Pseudomonas isolates (Ps-1 – Ps-5) was evaluated based on soluble phosphorous content in the culture broth, solubilization index(SI) and final PH of the medium. The result was summarized in Table 4. Among the five isolates Ps-4 shows the highest phosphorous solubilization 20.58 µg P/100 mL broth followed by Ps-2 with 17.20 µg P/100 mL broth.whereas Ps-5 recorded lowest phosphorous solubilization of 10.36 µg P/100 mL broth. The solubilization index whereas follow values ranged from 1.96 to 3.14 similar to phosphate solubilization. Solubilization index Ps-4 shows maximum value 3.14 followed by Ps-2 (2.80). Ps-5 showed comparatively low value 1.96. The final PH of the medium was decreased in all isolates after incubation, that result acidification of the medium during microbial growth. The final PH value of the growth medium for isolates Ps-1 to Ps-5 were recorded as follow; Ps-1 – 5.7, Ps-2 – 5.1, Ps-3 – 5.4, Ps-4 – 4.9, Ps-5 – 5.9. The lower PH indicating the secretion of acids. The production of organic acids is a primary mechanism

underlaying phosphorous solubilization. These findings are consistent with (Xu & Chen, 2025), more recently demonstrated that the phosphate solubilization capability of Pseudomonas isolates.

Table 4: Phosphate solublizing potential of Psedumonas sp. obtained from rhizosphere soils of tomato

S. No.	Isolate code	Phosphate solubilization (mg P/100 ml broth)	Solubilization index	Final pH of medium
1	Ps-1	11.89	2.08	5.7
2	Ps-2	17.20	2.80	5.1
3	Ps-3	14.21	2.14	5.4
4	Ps-4	20.58	3.14	4.9
5	Ps-5	10.36	1.96	5.9
SED		1.83	0.23	0.18
CD (p=0.05)		5.08	0.64	0.50

2.14 Production of siderophore, IAA(Indole-3-acetic acid) and GA_3(Gibberelic acid) by plant growth promoting microorganisms

Among the all isolates Azotobacter isolates Az-2 and Az-5, Pseudomonas isolates Ps-2 and Ps-4 exibited superior plant growth promoting isolates were selected for estimation of siderophore, IAA(Indole-3-acetic acid) and GA_3(Gibberelic acid) production. The quantitative data of isolates on siderophore, IAA and GA_3 production are summarized on Table 5. Among the selected Azotobacter isolates Az-5 recorded yielding of (2.55 µg mL^{-1}) of catechol type siderophore, (4.32 µg mL^{-1}) of salicylate type siderophore, (42.80 µg/25 mL broth) of IAA and (26.5 µg/25 mL broth) of GA_3 production. The isolate Az-2 showed (1.95 µg mL^{-1}) of catechol type siderophore, (3.45 µg mL^{-1}) of salicylate type siderophore, (35.20 µg/25 mL broth) of IAA and (21.60 µg/25 mL broth) of GA_3 production. Among the Pseudomonas isolates Ps-2 recorded the highest production of catechol type siderophore (3.25 µg mL^{-1}), salicylate type siderophore (4.85 µg mL^{-1}), IAA production of (61.40 µg/25 mL broth) and GA_3 production of (34.85 µg/25 mL broth). The isolates Ps-4 recorded (2.68 µg mL^{-1}) of catechol type siderophore, (4.18 µg mL^{-1}) of salicylate type siderophore, (48.65 µg/25 mL broth) of IAA and (28.40 µg/25 mL broth) of GA_3 production.

Overall the present study shows Azotobacter isolates Az-3 and Pseudomonas isolates Ps-2 demonstrated higher production of siderophore, IAA and GA_3 compared to that remaining isolates. The findings of the present study are consistent with demonstrating the *Pseudomonas sp.* are effective producer of siderophore and phytohormones including IAA and GA_3 (Kloepper *et al.,* 1980)(Ahmad *et al.,* 2008). Similarly, *Azotobacter sp.* reported ability to produce efficient quantities of IAA and GA_3 (Brown & Burlingham, 1968)(Hayat *et al.,* 2010).

Table 5: Siderophore and IAA production by Azotobacter and pseudomonas isolates

Isolate	Siderophore (Catechol type, µg ml^{-1})	Siderophore (Salicylate type, µg ml^{-1})	IAA production (µg 25 ml^{-1} broth)	GA_3 production (µg/25 mL broth)
Az-5	2.55	4.32	42.80	26.5
Az-2	1.95	3.45	35.20	21.60
Ps-4	2.68	4.18	48.65	28.40
Ps-2	3.25	4.85	61.40	34.85
SED	**0.22**	**0.26**	**3.89**	**2.02**
CD (p=0.05)	**0.71**	**0.85**	**12.38**	**6.44**

2.15 Effect of PGPR on plant growth and yield of tomato

From the above study shows Azotobacter isolates (Az-3) and Pseudomonas isolates (Ps-2) recorded higher production of siderophore and phytohormone includes IAA and GA_3. Ten different treatment were imposed as follow;

T_1 - Control

T_2 - 100% NPK

T_3 - 75% NPK

T_4 - 50% NPK

T_5 - 75% NPK + *Azotobacter sp.*. (AZ-5)

T_6 - 75% NPK + *Pseudomonas sp.*. (PS-2)

T_7 - 50% NPK + *Azotobacter sp.*. (AZ-5)

T_8 - 50% NPK + *Pseudomonas sp.*. (PS-2)

T_9 - 75% NPK + *Azotobacter sp.*. (AZ-5) + *Pseudomonas sp.*. (PS-2)

T_{10} - 50% NPK + *Azotobacter sp.*. (AZ-5) + *Pseudomonas sp.*. (PS-2)

Among the 10 treatments tested, the germination percentage of (93.96%) and vigour index (1767) was recorded in the treatment T_9 - 75% NPK + *Azotobacter sp.*. (AZ-5) + *Pseudomonas sp.*. (PS-2) on par with the treatment T_2 - 100% NPK recorded (89.01%) of germination percentage and (1602) vigour index. Minimum germination percentage (68.11%) and vigour index (748) was recorded in T_1 – Control. The observation recorded and presented in Table 6.

The effect of PGPR inoculum on the No of fruits, fruit weight and fruit yield of tomato variety PKM 1 was evaluated at harvest time and presented in Table7. Among the all treatment observed the No of fruits, fruit weight and fruit yield were maximum observed in T_9 - 75% NPK + *Azotobacter sp.*. (AZ-5) + *Pseudomonas sp.*. (PS-2) recorded (32 per plant) for No of fruits, (69.12 g/plant) for fruit weight, (31.00 t/ha) for fruit yield on par with the T_2 - 100% NPK recorded (31 per plant) for No of fruits, (66.40 g/plant) for fruit weight, (31.06 t/ha) for fruit yield.

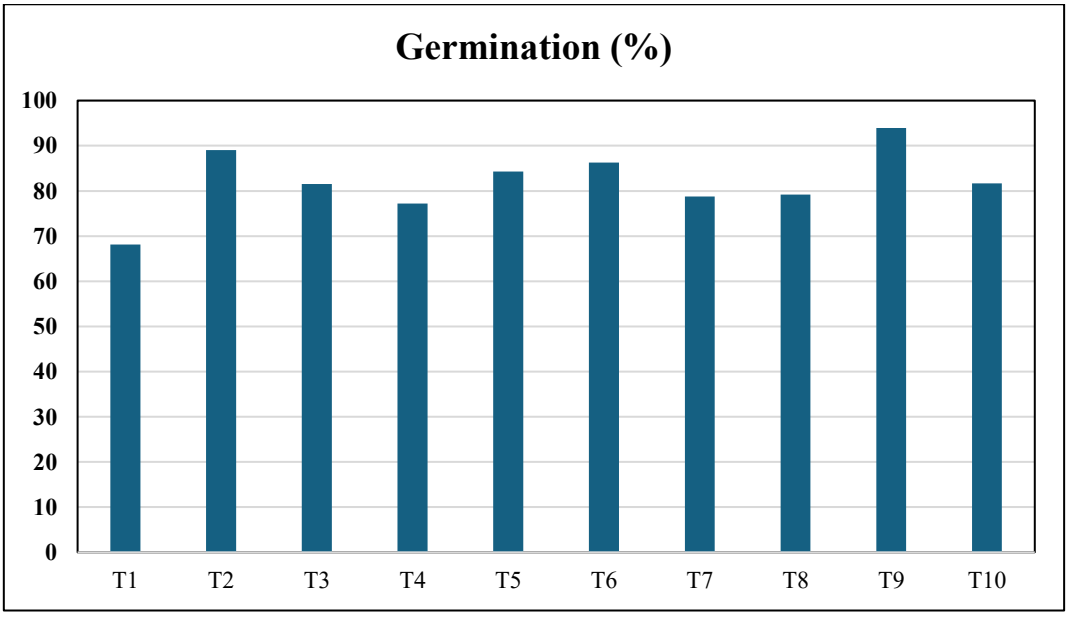
Therefore the result showed that the inoculation of dual culture of PGPR (*Azotobacter sp.* and *Pseudomonas sp.*) with the graded level of NPK increased thr germination %, vigour index, No of fruits, fruit weight and fruit yield. **These study revealed that 25% of synthetic chemical fertilizer can be reduced by using bioinoculant.**

Table-6. Effect of plant Growth Promoting Rhizobacterial (PGPR) inoculation on the germination percentage and vigour index of Tomato variety PKM-1 under pot culture condition.

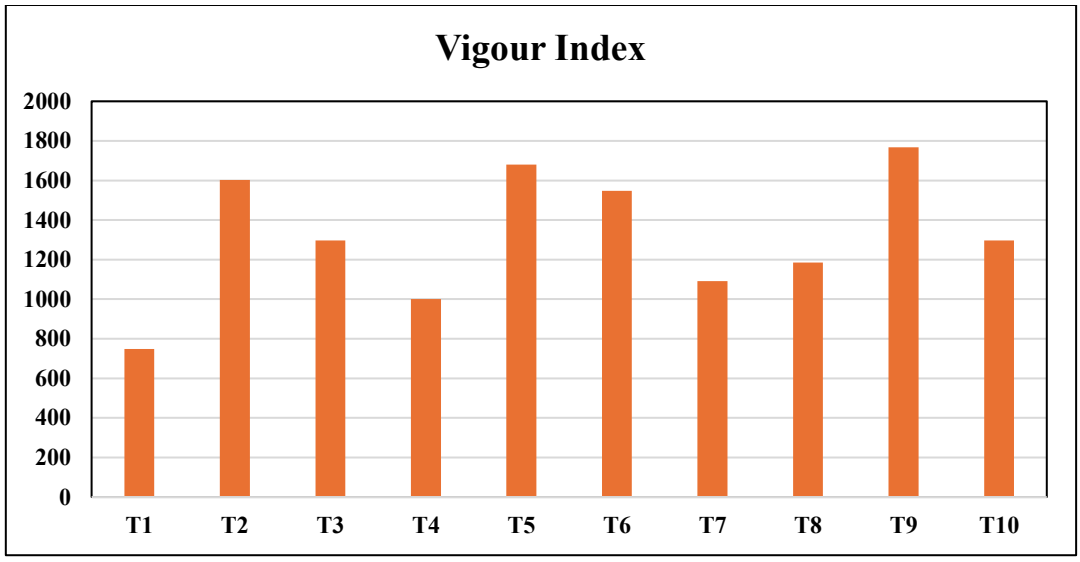
S.No	Treatments	Germination (%)	Vigour Index
1.	T_1 – Control	68.11	748
2.	T_2 - 100% NPK	89.01	1602
3.	T_3 - 75% NPK	81.52	1296
4.	T_4 - 50% NPK	77.23	1001
5.	T_5 - 75% NPK + *Azotobacter sp.*. (AZ-5)	84.28	1680
6.	T_6 - 75% NPK + *Pseudomonas sp.*. (PS-2)	86.28	1548
7.	T_7 - 50% NPK + *Azotobacter sp.*. (AZ-5)	78.79	1092
8.	T_8 - 50% NPK + *Pseudomonas sp.*. (PS-2)	79.21	1185
9.	T_9 - 75% NPK + *Azotobacter sp.*. (AZ-5) + *Pseudomonas sp.*. (PS-2)	93.96	1767
10.	T_{10} - 50% NPK + *Azotobacter sp.*. (AZ-5) + *Pseudomonas sp.*. (PS-2)	81.68	1296
	S. Ed	**2.23**	**103.34**
	CD(p=0.05)	**5.06**	**233.76**

Table – 7. Effect of Plant Growth Promoting Rhizobacterial (PGPR) inoculation at graded levels of NPK on the fruit weight and fruit yield Tomato variety PKM 1 under pot culture conditions

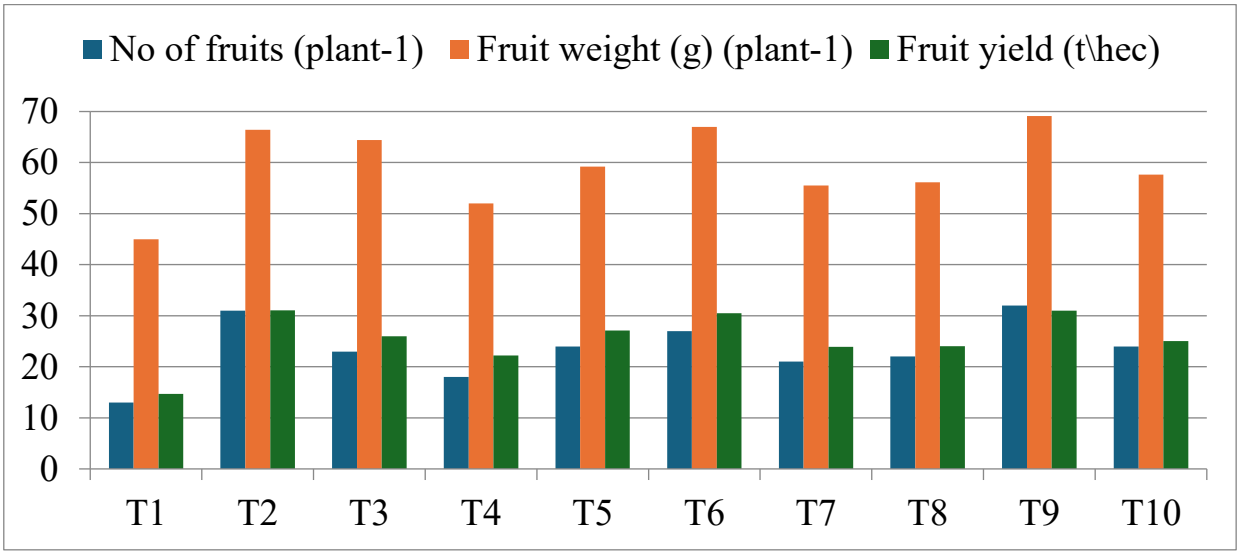
S.No	Treatments	No of fruits (plant^{-1})	Fruit weight (g) (plant^{-1})	Fruit yield (t\hec)
1.	T_1 – Control	13	45.00	14.67
2.	T_2 - 100% NPK	31	66.40	31.06
3.	T_3 - 75% NPK	23	64.41	26.00
4.	T_4 - 50% NPK	18	52.00	22.23
5.	T_5 - 75% NPK + *Azotobacter sp.*. (AZ-5)	24	59.20	27.11
6.	T_6 - 75% NPK + *Pseudomonas sp.*. (PS-2)	27	67.01	30.50
7.	T_7 - 50% NPK + *Azotobacter sp.*. (AZ-5)	21	55.50	23.88
8.	T_8 - 50% NPK + *Pseudomonas sp.*. (PS-2)	22	56.11	24.01
9.	T_9 - 75% NPK + *Azotobacter sp.*. (AZ-5) + *Pseudomonas sp.*. (PS-2)	32	69.12	31.00
10.	T_{10} - 50% NPK + *Azotobacter sp.*. (AZ-5) + *Pseudomonas sp.*. (PS-2)	24	57.66	25.01
	S. Ed	**1.79**	**2.39**	**1.57**
	CD(p=0.05)	**4.06**	**5.42**	**3.55**



Bar graph 1. Effect of different treatments (T_1-T_{10}) on Germination %



Bar graph 2. Effect of different treatments (T_1-T_{10}) on Vigour index



Bar graph 3. Effect of different treatments (T_1-T_{10}) on No of fruits, Fruit weight and fruit yield

Fig 1. Azotobacter sp.

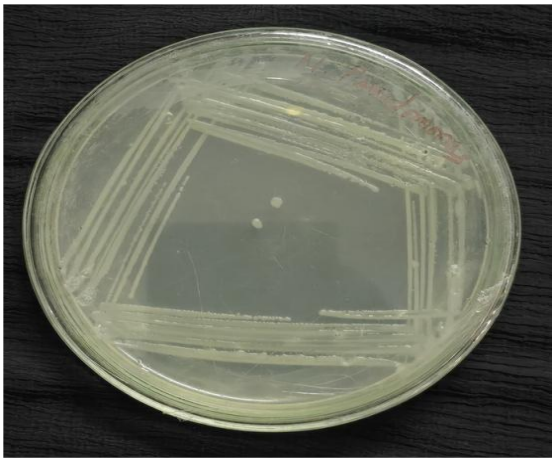


Fig 2. Pseudomonas sp.

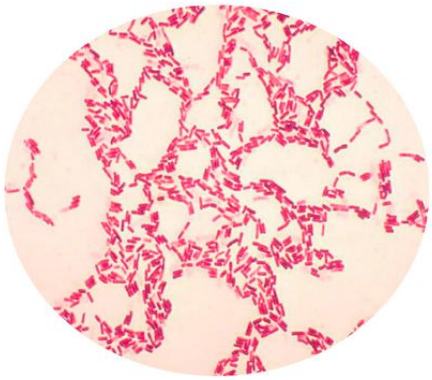


Fig 3. Microscopic view of Azotobacter sp.

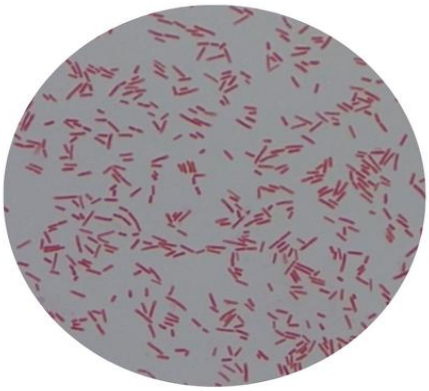


Fig 4. Microscopic view of Pseudomonas sp.

Fig 5. Evaluation of microbial consortium on tomato plant growth under pot culture experiment

IV. CONCLUSION

The findings of the present investigation demonstrate that the application of Plant Growth-Promoting Rhizobacteria (PGPR) significantly improved the growth and productivity of tomato (*Solanum lycopersicum* L.). Excessive and continues application of chemical fertilizer leads to soil degradation, decline in beneficial microbial activity and environmental pollution. The beneficial effects of PGPR are attributed to their ability to fix atmospheric nitrogen, solubilize insoluble phosphate, produce plant growth regulators, synthesize siderophores, and improve the availability of essential nutrients in the rhizosphere. Therefore, Reducing the excessive use of chemical fertilizers by promoting beneficial rhizobacteria can improve crop productivity, conserve soil health, reduce environmental pollution, and contribute to the development of sustainable agricultural.

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