EFFECT OF *AZOTOBACTER* ALONG WITH PGPMs ON GROWTH AND BIOMASS OF TOMATO (*Solanum lycopersicum* L.)

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EFFECT OF *AZOTOBACTER* ALONG WITH PGPMs ON GROWTH AND BIOMASS OF TOMATO (*Solanum lycopersicum* L.)

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By

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CERTIFICATE

This is to certify that the thesis entitled **"EFFECT OF *AZOTOBACTER* ALONG WITH PGPMs ON GROWTH AND BIOMASS OF TOMATO (*Solanum lycopersicum* L.)"** submitted by **Mr. SRIKANTH, G. S.,** in partial fulfillment of the requirements for the award of the degree of **MASTER OF SCIENCE (Agriculture)** in **AGRICULTURAL MICROBIOLOGY** to the University of Agricultural Sciences, Raichur, is a record of bonafide research work carried out by her during the period of his study in this University under my guidance and supervision and that no part of the thesis has been submitted for the award of any other degree, diploma, associate ship, fellowship or other similar titles.

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Affectionately dedicated

to

My Parents,

Friends

and

Farming community

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LIST OF ABBREVIATIONS

Abbreviation		Expansion
µg	:	Microgram
BNF	:	Biological Nitrogen Fixation
CD	:	Critical Difference
CFU	:	Colony Forming Units
cm	:	Centimeter
nm	:	Nanometer
DAT	:	Days after transplanting
dS	:	Desi siemens
EC	:	Electrical conductivity
g	:	Gram
h	:	Hours
ha	:	Hectare
IAA	:	Indole Acetic Acid
kg	:	Kilogram
mg	:	Milligram
min	:	Minutes
ml	:	Milliliter
OC	:	Organic carbon
N	:	Nitrogen
P	:	Phosphorus
rpm	:	Revolutions per minute
sec	:	Seconds
SEm±	:	Standard error of mean
ANOVA	:	Analysis of Variance

LIST OF SYMBOLS

%	:	Per cent
µ	:	Micro
C	:	Celsius
cm	:	Centimetre
Fig.	:	Figure
g	:	Gram
kg	:	Kilogram
MPa	:	Mega Pascal
Sl.No.	:	Serial Number
º	:	Degree
viz.,	:	Namely
i.e.,	:	That is

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Introduction

I. INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is the second most cultivated vegetable in the world, after potato, belongs to family Solanaceae. It is self-pollinated crop and Peru-equator is the centre of origin. Tomato is one of the popular vegetables of great commercial value and is used in various forms. Studies have shown that high consumption of tomato is consistently correlated with a reduced risk of some types of cancer and may account for a low incidence of heart disease. It is grown for nutritive fruits which are a rich source of vitamin and minerals and it is typically a red and edible fruit.

It contains higher quantity of total sugar (2.5-4.5%), starch (0.6-1.2%) and minerals like potassium, calcium, sodium, magnesium, phosphorus, iron, *etc*. Apart from this, it also contains organic acids such as citric, malic and acetic acid which are known as health acids in fresh tomato fruit. Nearly 144 countries are involved in Tomato production. Among them major production countries are China, EU, India, US and Turkey, with China accounting for 31 per cent of the total world production followed by India. In India, it occupies an area of 791 thousand ha with production of 17398 thousand tons with an average yield of 22 t/ha (Anonymous, 2015).

Over the past two decades the agricultural policy in India has undergone a major change to meet the increased demand of food in the global competitive scenario through diversification and emphasis on sustainable production systems. The role of plant growth promoting bacteria (PGPB) have been extensively studied as biofertilizers to increase the yield of agronomically important crops such as wheat (Khalid *et al.,* 2004), corn (Mehnaz and Lazarovits, 2006) Beneficial effects of the introduction of specific microorganisms on plant growth have been reported for numerous crops, including tomato grown under green house in organic media (Guo *et al.,* 2004).

Plant growth promoting rhizobacteria (PGPR) can be defined as root colonizing bacteria (rhizobacteria) that exert beneficial effects on plant growth and development. PGPR are known to survive both in rhizosphere and phyllosphere (Krishnamurthy and Gnanamanickam, 1998). Fluorescent Pseudomonads are among the most effective rhizosphere bacteria because they produce plant growth promoting substance in addition to disease control (Dubeikovsky *et al.,* 1993). Such beneficial microorganism referred as

PGPR (plant growth promoting bacteria) which enhance plant growth through numerous direct mechanisms involving nitrogen fixation, phosphorus solubilization, HCN production and production of phytohormones such as auxins, cytokinins, gibberellins and lowering of ethylene concentration (Kloepper *et al.,* 1989) and indirectly by protecting the roots against infection by minor and major pathogens (Whipps, 2001).

Among plants growth regulators indole-3- acetic acid (IAA) is the most common natural auxin found in plants and its positive effect on root growth and morphology is believed to increase the access to more nutrient in the soil (Vessey, 2003). In plants, IAA induces cell division, extension and differentiation; stimulates seed and tuber germination; increases the rate of xylem and root development; controls processes of vegetative growth; initiates lateral and adventitious root formation; mediates responses to light, gravity; affects photosynthesis, pigment formation, biosynthesis of various metabolites, and resistance to stressful conditions (Spaepen and Vanderleyden, 2011). In recent years, considerable interest has been paid to rhizobacteria, which are aggressive root colonizers and produce siderophores. Siderophores provide an advantage in survival of both plants and bacteria (Leong, 1986).

Among many PGPRs, *Azotobacter chroococcum* is known to improve plant growth through nitrogen fixation or through production of plant growth regulators (El-Shourbagy *et al.,* 1979). It is an aerobic, free-living nitrogen fixing bacterium capable of synthesizing various plant growth promoting substances, which may augment its performance as an efficient inoculant for crop plants. Several studies have revealed the beneficial effects of these bacteria in the improvement of crop growth and yield (Abraham and Money, l994).

The synergistic effect on plant growth following inoculation with VA mycorrhizal fungi and phosphate solubilizing bacteria has been recorded by Barea *et al.* (1976). It was revealed that the synergistic effect might be due to plant growth substances produced by the bacteria rather than the soluble phosphate ion released. Bagyaraj and Menge (1978) recorded a synergistic interaction between *Azotobacter* and VA mycorrhizae on enhancing dry weight of tomato plants.

The present farming totally depends on the use of chemical fertilizers, pesticides and growth regulators for enhancing crop productivity. Gradually culminated in a

situation wherein, it is essential to reconsider the alternative to chemical agriculture as it is deteriorating the soil biological properties and nutrient cycling which in turn lowering the productivity. It is a well-documented fact that increased dependence on agro-chemicals including fertilizers has led to several ill effects on the environment.

Thus, gradual movement towards the organic farming is recommended as it is eco-friendly and helps for the sustaining the natural biota. It is a production system which avoids or largely excludes the use of synthetically produced fertilizers, pesticides, growth regulators and livestock feed additives. Considering the ill-effects of the chemical fertilizers and the beneficial aspects of the vermicompost and the microbial consortia, the present investigation was carried out to assess the combined effect of the efficient *Azotobacter* isolate in combination with PGPMs on the growth and yield of tomato with following objectives.

Objectives:

1. To isolate and characterize the efficient *Azotobacter* strains from rhizosphere soil of tomato.

2. To screen the *Azotobacter* isolates for their beneficial traits under *in vitro* conditions.

3. To evaluate the effect of efficient *Azotobacter* isolate in combination with PGPMs on the growth and biomass of tomato.

Review of Literature

II. REVIEW OF LITERATURE

Azotobacter is known for its nitrogen-fixing and plant growth promotional ability by the phytohormone production. It is one of very well-studied plant growth-promoting rhizobacteria, at lab scale to field. It has many direct and indirect mechanisms *viz*., production of siderophores, EPS, HCN, and IAA, *in vitro* N_2 fixation and P-solubilisation and many more. The present investigation aims for identification of *Azotobacter* isolates, selection of superior isolate and its effect on the growth and yield of tomato along with the PGPMs. The review pertaining to isolation and characterization, screening and PGPR activity on tomato have been documented in the present chapter.

2.1 To isolate *Azotobacter* isolates from rhizosphere soil of tomato grown in Hyderabad Karnataka region

The genus *Azotobacter*, belonging to family *Azotobacteriaceae,* represents the main group of heterotrophic non-symbiotic nitrogen-fixing bacteria principally inhabiting neutral or alkaline soils. The non-symbiotic free living *Azotobacter* is largely associated with nitrogen fixation in plant rhizosphere. It is also involved in various other metabolic pathways of plant growth promotion, antagonistic activity against plant pathogens in soil and activation of potential rhizospheric bio inoculants for enhancement of plant yield.

Beijerinck (1901) isolated *Azotobacter* sp., and described them as free living, heterotrophic, non-symbiotic nitrogen fixers and are dominant aerobes present in rhizosphere of soil.

Becking (1981) studied that ability of the members of the family *Azotobacteracae* to grow in a medium with no combined nitrogen helps in their selective isolation and reported organic carbon source and phosphate are usually the minimum nutrients required for the development of *Azotobacter* under natural conditions.

Haris (1981) studied and addressed the physical properties of differently textured soils in undisturbed and remolded state and their effect on N_2 fixation by different *Azotobacter* strains and the results showed that the maximum N_2 fixation by *Azotobacter* in coarse-textured (sandy clay loam) soils.

Gordon *et al.* (1983) isolated *Azotobacter vinelandii* strains from the wild type as methylalanine-resistant strains and these starins expressed up to 23% of the fully derepressed nitrogenase level when grown in medium containing excess ammonium. Mutant strains of *A. vinelandii* which might have potential for use as bacterial fertilizer were isolated.

Hartmann *et al.* (1983) compared the spects of tryptophan and IAA biosynthesis in wild-type strains of *A. brasilense* and *A. lipoferum* and describe the isolation and properties of mutants which excrete IAA without an exogenous supply of tryptophan. Under conditions of nitrogen fixation, the mutants excreted IAA up to 1 pg/mL, 10 times more than the wild type. However, none of the FT-resistant mutants of *Azospirillum lipoferum* Sp RG 20a excreted high levels of IAA.

Bacteria showing rapid growth on a nitrogen free medium and acetylene-reducing activity were isolated from maize roots collected from agricultural soils in Spain by Martinez-Toledo *et al.* (1985). The isolates were Gram-negative motile rods and were identified as *Azotobacter chroococcum.* Acetylene-reducing activity and microbial counts were determined on root segments from seven and 30-day-old plants.

Burgmann *et al.* (2003) reported that *Azotobacter* is a heterotroph bacterium of aerobic character having the capability of fixation of dinitrogen as non-symbiont and some strains have higher nitrogen fixing ability than others.

Tejera *et al.* (2005) isolated *Azotobacter* and *Azospirillum* strains from the sugarcane rhizosphere and characterized them as Gram negative rods and identified them as *Azotobacter chroococcum* and *Azospirillum brasilense* and documented that *Azotobacter* isolates do not have a particular affinity for sugarcane rhizospheres.

Suliasih and Widawati (2005) isolated nitrogen fixing bacteria *viz*., *Azotobacter chroococcum, Rhizobium* sp. and *Azospirillum* sp. from the soil samples of Wamena Biological Garden which is one of the mountain range-biota ex-situ conservation at Eastern part of Indonesia. Microbial population was estimated by plate count method.

Daniel *et al.* (2009) isolated and characterized *Azotobacter vinelandii* mutants impaired in Alkyl resorcinol (AR) synthesis and indicated that Alkyl resorcinol play a

structural role in the exine layer of the cysts, but they are not essential for either cyst formation or for desiccation resistance.

Sandeep *et al.* (2011) isolated and identified *A. chroococcum* from different agroclimatic zones of Karnataka. Seed germination studies of *Amaranthus gangeticus* inoculated with *A chroococcum* isolates was carried out and growth response of *A.gangeticus* to *A. chroococcum* isolated from different agroclimatic zones of Karnataka was studied under greenhouse conditions.

Diego *et al.* (2011) collected soil samples randomly from different vegetable organic cultures with neutral pH in different zones of Boyacá-Colombia with the purpose of isolating and characterizing free nitrogen fixing bacteria (FNFB) of the genus *Azotobacter*.

Ajay Kumar *et al.* (2012) collected soil samples from French bean rhizospheric soils and isolated, screened and characterized *Azotobacter* isolates for different plant growth promotion activities *viz.,* IAA production, ammonia production, ACC deaminase activity and phosphate solubilisation.

Akhter *et al.* (2012) conducted a research to isolate and characterize salinity tolerant *Azotobacter* sp. to be used as biofertilizer. In this study, soil samples were collected from different salinity affected areas in Bangladesh. The nitrogen fixation ability of the selected isolates was estimated ranging from 04.95 to 10.55 mg N/g substrate.

Murumkar *et al.* (2012) isolated *Azotobacter* isolates from soils of Maharashtra, documented that the isolates were biochemically positive for starch hydrolysis, H_2S production, catalase and oxidase test, but negative for gelatin liquefaction, used mannitol, glucose, sucrose and fructose as a carbon source by all isolates, while malate and citrate gave negative results and a significant variation was observed in nitrogenase activity (19.5 to 217.3 nmol C_2H_4 mg protein hr^{-1}).

Ponmurugan *et al.* (2012) isolated *Azotobacter* isolates from vegetable growing in soils of Namakkal district, Tamil Nadu, India for their multiple plant growth promoting activities in relation to indole acetic acid, ammonia, hydrogen cyanide, and siderophore production, phosphate solubilisation, heavy metal tolerance to zinc and mercury and

antifungal activity against three phytopathogenic organisms as *Aspergillus flavus*, *Cercospora* sp. and *Fusarium oxysporum*.

Zohreh *et al.* (2012) reported that great attention has been paid to the genus *Azotobacter* and their role in increasing the growth and health of plants. In their study, 30 samples of rhizospheric soil were taken to isolate *Azotobacter* from the rhizosphere of Gramineae in different regions of Iran.

Sahoo *et al.* (2013) discovered novel potential strains of *Azotobacter* species from rice fields, which notably increased crop growth and yield via their nitrogen-fixing and plant growth promotion activities. Acetylene reduction assay of Azotobacter spp. showed that *Azotobacter vinelandii* fixed higher amount of nitrogen (121.09 nmol $C_2H_4mg^{-1}$ bacteria h^{-1}). Likewise, its plant growth functions, *viz.* siderophore, hydrogen cyanide, salicylic acid, IAA, GA_3, zeatin, NH_3, phosphorus solubilisation, ACC deaminase and iron tolerance, were also higher.

Umesh (2013) isolated and characterized diazotrophic plant promoting rhizobacteria from rhizosphere of Parsa and Bara district of Nepal and reported that species of *Pseudomonas, Klebsiella, Azotobacter* and *Agrobacterium* were predominantly present in this region especially in the rhizosphere of rice plants.

Jadhav and Gangawane (2014) isolated *Azotobacter* isolates in the phyllosphere of crops grown in sewage irrigated fields and noted that higher number of *Azotobacter* on phyllosphere of tomato, groundnut, brinjal, followed by chilli, coriander, methi and cotton and recorded variation among the different seasons, during rainy season their number was maximum on tomato, during winter their number was maximum on brinjal, during summer number of *Azotobacter* appeared to be less when compared with other seasons.

Pathania *et al.* (2014) isolated diazotrophic bacteria from rhizosphere of wheat cropping system from central plain region of Punjab using cultural, morphological and biochemical techniques and identified diverse genera of *Pseudomonas* sp., *Bacillus* sp., *Azotobacter* sp., *Rhizobium* sp. based on their molecular characteristics.

Sandeep Upadhyay *et al.* (2015) isolated *Azotobacter* isolates from soil and reported a wide variability of these isolates in terms of their cell shapes, pigmentation, colony size, Gram reaction and catalase activity.

Jadhav and Dekhane (2016) carried out survey of *Azotobacter* isolates from sewage irrigated fields, studied the *Azotobacter* population in the rhizosphere of fifteen crop plants along with the soil samples during various seasons and also noted that the number of *Azotobacter* varied among the rhizosphere and soil of different crops and recorded this variation during the different seasons.

Rashi *et al.* (2016) PGPR were isolated from rhizosphere of Sesame plant grown in Haridwar region and three bacteria out of a total of 22 isolates were identified to belong to genus *Pseudomonas, Bacillus* and *Azotobacter*. All the three bacteria were found to exhibit a growth enhancing effect on Sesame plant reflected by enhanced growth of seedlings, increased shoot length and number of branches in plants treated with PGPR.

Scagliola *et al.* (2016) made a study in order to identify novel bacterial strains and their potentialities as biofertilizers, microorganisms from the rhizosphere of barley (*Hordeum vulgare* L.) and tomato (*Solanum lycopersicon* L.) plants were isolated, identified and characterized.

Sadik *et al.* (2016) worked on isolation, purification, and molecular identification of a nitrogen-fixing bacterium occurred in some rhizosphere soil of Taif region. Total ten bacterial isolates grown on the nitrogen-free specific medium (Waksman base No.77 Medium) were purified and separately used as hosts for bacteriophage isolation. The spot test and turbidity tests were used to detect the presence of the phage of interest in the prepared phage suspensions.

Ambesh *et al.* (2017) isolated and evaluated *Azotobacter* isolates from different crop rhizosphere from Terai zone of West Bengal and evaluated the influence of *Azotobacter* on the growth and phenology of chilli plants.

2.2 Screening the *Azotobacter* isolates for their beneficial traits under *in vitro* conditions

Vela and Cagle (1969) isolated and described a strain of *Azotobacter chroococcum* (designated *A. chroococcum* NTS) which produces cysts which are as resistant to desiccation and ultraviolet radiation as the typical cysts of *A. chroococcum* but which are mechanically very fragile.

Tchan and New (1986) reported that the colonies of *Azotobacter chroococcum* were generally 2 to 6 mm in diameter, opaque, entire, low convex, viscid, glistening and smooth and observed variant colony forms through desiccation in the quantity of extra cellular polysaccharides produced.

A study by Govedarica *et al.* (1993) worked on the production of growth substances by nine *Azotobacter chroococcum* strains isolated from soil showed that these strains have the ability to produce auxins, gibberellins, and phenols and in association with the tomato plant, increase the plant length, biomass and nitrogen content.

Thirty-eight *Azotobacter* spp. were isolated from Brazilian soils by Mottos and Oliveira (1997) and characterized for the following properties *viz.,* utilization of different carbon sources, desirable pigment production, melanin production, sodium dependent growth, sidrophore production, streptomycin resistance and antibiotic like substance production. Among the four strains, one was *A. armeniacus* and three were *A. paspali.*

Kader *et al.* (2002) observed that in addition to nitrogen fixation, *Azotobacter* produces thiamin, riboflavin, indole acetic acid and gibberellins. When *Azotobacter* is applied to seeds, seed germination is improved to a considerable extent, so also it controls plant diseases due to above substances produced by *Azotobacter.*

Edi Husen (2003) isolated and screened fourteen *Azotobacter* isolates for the plant growth promotional activities *in vitro* and the results showed that twelve isolates produced IAA ranging from 2.09 to 33.28 μmol ml^{-1}, ability to solubilize precipitated phosphate was positively exhibited by four isolates and seven isolates produced siderophore, none of the isolate produced nitrogenase activity and only one isolate did not exhibit any of the traits tested.

Aquilanti *et al.* (2004) isolated 196 Gram negative *Azotobacter* isolates from 35 soils, sampled in central Italy following three different methods of isolation and documented that screening of soil samples by means of soil paste-plate method combined with isolation on mannitol-agar as the best strategy in terms of reliability, whereas isolation on differential LG medium as extremely accurate, and identified these isolates by means of amplified ribosomal DNA restriction analysis.

Ahmad *et al.* (2005) isolated and screened twenty-one bacterial isolates (*Azotobacter* sp., 10 and *Pseudomnas* sp., 11) from different rhizospheric soils in and around the Aligarh city for their intrinsic ability to produce IAA in the presence of varying amounts of L-tryptophan and their effect on root elongation of germinating seeds of test plants.

Joseph *et al.* (2007) isolated 150 bacterial isolates belonging to the genera *Pseudomonas, Bacillus, Azotobacter,* and *Rhizobium* for their multiple plant growth promoting activities from different rhizospheric soils of chick pea in the vicinity of Allahabad. These test isolates were biochemically characterized and screened *in vitro* for their plant growth promoting traits like production of indoleacetic acid (IAA), ammonia (NH_3), hydrogen cyanide (HCN), siderophore and catalase.

Rajaee *et al.* (2007) studied the effects of different native strains of *Azotobacter chroococcum* on growth and yield of wheat under greenhouse conditions and reported that seeds of spring wheat (*Triticum aestivum* L.) inoculated with *Azotobacter chroococcum* strains were capable of producing IAA, HCN, siderophore and fixing molecular nitrogen.

Khin Mya Lwin *et al.* (2008) studied that the 12 isolates of *Azotobacter* sp. and 20 of *Pseudomonas* sp. were isolated from different rhizospheres of plants in Myanmar and screened with tryptophan containing medium for the productivity of IAA. The productivity range of IAA was 0.1 ppm to over 80 ppm. A low amount of IAA production (10.1 ppm) was recorded by *Azotobacter* strains and six isolates showed high level production of IAA (45-81.4 ppm) at 5 g / L of tryptophan.

Bhatia *et al.* (2009) determined the genetic diversity of twenty *Azotobacter chroococcum* isolates by RFLP (Restriction Fragment Length Polymorphism) analysis of the functional gene *nif*H. Genetic analysis of these isolates depicted a similarity coefficient of more than 80 per cent among them, suggesting that though the isolates were obtained from different cotton soils of India, still they have large commonality in the *nif*H gene and constituted a homogeneous *nif*H population.

Abdel-Hamid *et al.* (2010) isolated *Azotobacter chroococcum* from some Egyptian soils and characterized them as ovoid to rod shaped; form cysts; Gram negative;

motile with peritrichous flagella; aerobic; catalase positive; producing insoluble pigment creamy changes to brown; hydrolyze starch; utilize citrate; produce H_2S and acetone.

Kanimozhi and Panneerselvam (2010) studied the nitrogen fixation ability of the 30 isolates of *Azotobacter* from paddy rhizosphere soil of Thanjavur district by using micro kjeldhal method. Among the 30 isolates tested, 28 isolates were able to fix nitrogen. The range of nitrogen fixing ability was from 3.3 to 15.6 mg N/g malate.

Ashraf *et al.* (2011) isolated twelve bacterial strains from rhizosphere of sugarcane and identified them as *Pseudomonas* and *Azotobacter*. Among these strains the presence of nifH gene was detected only in two *Azotobacter* strains which showed acetylene reduction activity of 1966 and 4210 nmol C_2H_4 per vial per 24 h, respectively and IAA of 4.49 mg l^{-1} to 0.2 and 0.1 mg l^{-1}, respectively.

Vikram Patil (2011) tested the IAA production potential of six bacterial isolates isolated from rhizosphere soils, in a medium supplemented with 0, 1, 2 and 5 mg per ml of tryptophan and recorded low amount of IAA production by *Azotobacter* isolates without tryptophan addition and reported that IAA production by *Azotobacter* increased with increase in tryptophan concentration from 1 to 5 mg ml^{-1}.

Mazinani *et al.* (2012) studied the effect of *Azotobacter* population on physicochemical characteristics of some soil samples in Iran and their role in increasing the growth and health of plants and reported a significant correlation between soil pH, total nitrogen and organic carbon with microbial population.

Murumkar *et al.* (2012) isolated *Azotobacter* isolates from soils of Maharashtra, documented that the isolates were biochemically positive for starch hydrolysis, H_2S production, catalase and oxidase test, but negative for gelatin liquefaction, used mannitol, glucose, sucrose and fructose as a carbon source by all isolates, while malate and citrate gave negative results and a significant variation was observed in nitrogenase activity (19.5 to 217.3 nmol C_2H_4 mg protein^{-1} hr^{-1}).

Adesh *et al.* (2014) characterized and screened sixteen *Azotobacter* isolates isolated from different rhizospheric soils collected from various districts of Uttar Pradesh for the production of IAA where the values ranged from 42.80 µg ml^{-1} to 82.00 µg ml^{-1}.

Dadook *et al.* (2014) isolated twelve *Azotobacter chroococcum* strains from rhizospheric soils of wheat, corn and asparagus and identified these based on morphological and biochemical characteristics, confirmed the *nifH* gene in all strains using PCR analysis and found Minimum Inhibitory Concentration (MIC) and optimum zinc concentration for growth of *Azotobacter i.e.*, 50 ppm and 20 ppm, respectively.

Kumar *et al.* (2014) studied the effect of *Azotobacter chroococcum* CL13 inoculation on growth and curcumin content of turmeric (*Curcuma longa* L.), and observed that the isolates produced significantly higher amount of IAA, NH_3, HCN and solubilised P during PGP trait analysis along with the plant growth parameters such as shoot height, shoot fresh biomass, rhizome fresh biomass enhanced in *A. chroococcum* CL13 inoculated turmeric than control (un-inoculated) and reported the concentration of curcumin significantly enhanced by 6 per cent in *A. chroococcum* CL13 inoculated turmeric.

Dragana *et al.* (2015) isolated a total of 50 bacterial isolates, of which 13 isolates belonged to the genera *Azotobacter.* They were further screened *in vitro* for plant growth promoting properties *viz.,* production of indole-3-acetic acid (IAA), siderophores, hydrogen cyanide (HCN), exopolysaccharides, phosphate solubilisation and antifungal activity (vs. *Helminthosporium* sp., *Macrophomina* sp., *Fusarium* sp.).

Twisha and Pratibha (2015) isolated a total of 67 bacteria and *in vitro* screening was done for different plant growth promoting traits such as phosphate solubilisation, zinc solubilisation, potassium solubilisation, nitrogen fixation, ACC deaminase activity, phytohormone production, HCN production, lytic enzymes production. In their study, transgenic *Gossypium hisrsutum* samples were collected in monsoon season from four different sampling sites of rhizospheric (rhizosphere, rhizoplane, endorhizosphere) and nonrhizospheric (bulk soil for comparison) soil from Agriculture farm, Cotton Research Centre, Surat, Gujarat.

Kasa *et al.* (2016) studied the molecular diversity of PGPR isolates of *Azotobacter* and *Trichoderma* and their beneficial activities and documented that 35 DNA bands out of 41 bands scored were 85.59 per cent polymorphic in *Azotobacter* and 29 bands out of 37 bands scored were 78.37 per cent polymorphic in *Trichoderma,* and the multiple beneficial activities of PGPR traits increase the plant growth and bio-control activity.

Monokoane *et al.* (2016) isolated, characterized and screened the growth promoting activity of free living diazotrophs from soils of Lesotho, classified strains into three groups as *Azospirillum, Azotobacter* and *Bacillus* sp., documented that strain NUL-2 (*Azospirillum* spp) exhibit higher nitrogenase activity of 190.20 $nmol^{-1}$ ml^{-1} h^{-1} and lower production of IAA (0.032 mg ml^{-1}) compared to strain MAF7-1 (0.448 mg ml^{-1}) from the group *Azotobacter* sp.

Sivasankari and Anandhraj (2016) isolated and screened *Azotobacter* sp. for the production of IAA and standardized at different tryptophan concentrations *i.e.,* 1-5 mg ml^{-1} and the results showed that *Azotobacter* sp. significantly produce higher quantity of IAA (52.80 mg ml^{-1}) at 5 mg ml^{-1} of tryptophan concentration on fifteenth day compared with others and a low amount of IAA (7.48 mg ml^{-1}) in control.

Krunal Modi *et al.* (2017) studied 6 strains of *Pseudomonas*, 4 strains of *Azotobacter* and 2 strains of *Bacillus* which were isolated from rice rhizospheric soil samples from different villages of Bardoli, Gujarat, India. Primary screenings were carried for their plant growth promoting activities such as IAA production, Phosphate solubilisation, siderophore production, N_2 fixation and protease production. Overall investigation revealed many potential PGPR candidates with significant results which can be utilized as potential biofertilizer for improving soil health and rejuvenation.

2.3 To evaluate the effect of microbial consortia on the growth and yield of tomato under pot culture conditions

Margaret *et al.* (1968) studied the *Azotobacter* culture and medium. Tomato seedling roots were treated with *Azotobacter* culture and with uninoculated medium. The *Azotobacter* culture significantly increased stem length and leaf size until five true leaves were formed, it also shortened the time between appearance of flower buds and petal fall, on the first truss by four days and on the second by six days.

Bagyaraj and Menge (1978) from their study reported that populations of rhizosphere microorganisms were significantly affected by mycorrhizal infection and inoculation with *Azotobacter*. Inoculation of tomato with either resulted in greater numbers of bacteria in the rhizosphere after 60 days compared to the non-inoculated

control. Inoculation with the *Azotobacter* + *Glomus* treatment resulted in a further (2-3-fold) increase in numbers of rhizosphere bacteria.

Kundu and Gaur (1980) observed synergistic interaction between *Azotobacter* and phosphate solubilizing bacteria when the two organisms were inoculated together in cotton. In the combined inoculation treatments, the population of both the organisms was enhanced in addition to increase in yield of cotton.

Sukhada Mohandas (1987) tested the response of tomato *(Lycopersicon esculentum* Mill) to inoculation with the vasicular arbuscular mycorrhizal (VAM) fungus *Glomus fasiculatum* and *Azotobacter vinelandii* singly and in combination in the field. It was found that *G. fasiculatum* as well as *A. vinelandii* significantly increased leaf area, shoot dry weight, nitrogen content phosphorus content and yield in respect to uninoculated control.

Abbass and Okon (1993) studied the effect of *Azotobacter paspali* on plant growth promotion concluded that treatment of seedling hypocotyls and roots of rapeseed (*Brassica campestris*), wheat (*Triticum aestivum***)** and tomato (*Lycopersicum esculentum*) with cultures of *Azotobacter paspali* changed plant growth and development and significantly increased weight of shoot and roots.

Begum (1998) studied the response of tomato crop to *Azotobacter* inoculation found that fruit yields were highest from seedlings raised from *Azotobacter* treated seeds given 150 kg N ha^{-1} into different splits at planting, and 30, 45 and 60 days later. Control plants (no seed treatment, no N fertilizer) yielded 13.39 t ha^{-1} and those dipped in *Azotobacter* inoculum 20.35-25.97 t ha^{-1}, depending on N rate.

Rodelas *et al.* (1999) evaluated that the effect of mixed inoculation with four different *Rhizobium / Azotobacter* and *Rhizobium/Azospirillum* strain combinations on the mineral composition of faba bean (*Vicia faba* L.) led to changes in total content, concentration and/or distribution of the mineral macro and micronutrients, K, P, Ca, Mg, Fe, B, Mn, Zn and Cu, when compared with plants inoculated with *Rhizobium* only. The effects varied to a great extent among the *Azotobacter* and *Azospirillum* strains selected for combined inoculation.

Sharma and Thakur (2001) studied the effect of *Azotobacter* on growth and yield of tomato and they observed that application of *Azotobacter* significantly increased plant height, number of branches and fruits per plant, fruit yield per plot, yield per hectare, nitrogen uptake at the flowering stage and root biomass. Among treatment combinations, the maximum yield per hectare was obtained when *Azotobacter* was applied in combination with 100 kg N ha^{-1}.

Chendrayan *et al.* (2003), observed the combined inoculants such as *Azospirillum lipoferum, Azotobacter chroococcum,* and *Pseudomonas fluorescens* enhanced yield of rice, ragi, bajra, black gram, red gram groundnut and sunflower as compared to individual inoculants.

El-Dsouky *et al.* (2003) isolated *Azotobacter, Azospirillum* and *Pseudomonas* from rhizosphere soils of different plants grown at Aswan area, Egypt, whereas *Bacillus polymyxa* strain no.37, a phosphate dissolving bacterium, was isolated from the rock phosphate of Sebaeia mine. *Azotobacter* strains no.5 and 11 induced the most pronounced effects in all plant growth parameters. The highest N per cent and total N content were found in plant shoots inoculated with these two *Azotobacter* strains.

Gajbhiye *et al.* (2003) studied the effect of *Azotobacter* and PSB on the growth and yield parameter of tomato and concluded that *Azotobacter* was more effective than phosphobacteria (PSB) in the improvement of plant height, number of primary branches $plant^{-1}$, number of fruits $plant^{-1}$, and weight of fruits $plant^{-1}$, fruit size and yield. *Azotobacter* in combination with the recommended fertilizer rate was superior in the enhancement of the aforementioned parameters of tomato.

Tiruvudainambi *et al.* (2004) conducted green-house experiments to determine efficacy of different biological control agents (*Pseudomonas fluorescens, Trichoderma viride* and *Azotobacter chroococcum*) and other methods (soil solarization and neem seed cake application) in managing damping off caused by *Pythium aphanidermatum* in tomato.

Rashmi *et al.* (2008) reported that application of 50 per cent N through *Azotobacter* and 50 per cent through inorganic fertilizers resulted in higher leaf yield (715 g), total soluble sugars (51.64 mg ml^{-1}) and total soluble protein (29.21 %).

Mahalakshmi and Reetha (2009) worked on PGP activities of bacterial isolates isolated from rhizosphere of tomato. Among 44 isolates, two from *Azotobacter* were selected and production of IAA, siderophore and antifungal activity against *R. solani* and *F. oxysporum* determined were 3.30 µg ml-1, 0.78 µg ml^{-1}, 23.30 per cent and 25.67 per cent, respectively.

Mahato *et al.* (2009) evaluated the response of bio-fertilizer and inorganic fertilizer on germination and growth of tomato plant. Nitrogen (N) was used as inorganic fertilizer and *Azotobacter* was used as bio-fertilizer. Three treatments were used for investigation i.e., control (T_1- only soil), Soil + N (T_2) and Soil + *Azotobacter* (T_3). The germination was observed higher in T_3 treatment in contrast to other treatments. The conclusion of the study was that the *Azotobacter* as bio-fertilizer reported better than inorganic fertilizer in relation to seed germination and all plant growth parameters described.

Rather *et al.* (2010) studied the effect of application of biofertilizers (*Rhizobium, Azotobacter* and phosphate solubilizing bacteria) on growth and yield of field pea (*Pisum sativum* L.). Co-inoculation of all the three bio-fertilizers showed significantly higher growth characters as compared to absolute control, increased plant height (45.26 cm), number of nodules/ plant (38.46) and yield of grain and straw of pea increased.

Kourosh and Mahdi (2011) investigated effects of plant growth-promoting rhizobacteria (PGPR) and Arbuscular Mycorrhiza Fungi (AMF) on lycopene, antioxidant activity and Total Soluble Solid (TSS) of tomato fruit. PGPR treatments were inoculated with *Pseudomonas putida*, *Azotobacter chroococcum* and the AMF treatment was inoculated with *Glomus mosseae*. Data showed lycopene, antioxidant activity and TSS increased when AMF added to PGPR treatments.

Sangeeta *et al.* (2011) studied that effects of dual inoculation of *A. chroococcum* as seed application and AM fungi as soil inoculant on cotton (*Gossypium hirsutum* L.) There was significant improvement in plant height, number of flowers and bolls formed, boll weight and seed cotton yield in most of the treatments as compared to uninoculated control. Synergistic effects of dual inoculation of all the *A. chroococcum* strains with AM fungi were observed for boll weight and seed cotton yield. A significant positive

correlation between population of *Azotobacter* sp. in rhizosphere of cotton with weight of bolls and seed cotton yield was observed.

Shwetha and Lakshman (2011) made a study to evaluate and assess the effect of co-inoculation with bacteria and arbuscular mycorrhizal (AM) fungi on the growth of sesame (*Sesamum indicum* L.) varieties. The two varieties of sesame TSES 1 and TSES 4 were inoculated with single, double and triple inoculations. The results revealed that triple inoculation of *Glomus fasciculatum* (Gf) + *Azotobacter chroococcum* (Ac) + Pseudomonas fluorescens (Pf) to TSES 1 and *Acaulospora laevis* (Al) + Ac + Pf to TSES 4 stimulated increased root colonization, plant growth, plant biomass, phosphorus content, and number of capsules significantly over the double and single inoculation treatments.

Trimurtulu *et al.* (2011) demonstrated the inoculation of mixed microbial consortium. Plant growth promoting rhizobacteria, Arbuscular mycorrhizal fungi performed the best in recording AM colonization, number of fruits per plant, fresh and dry fruit weights per plant in chilli crop.

Magda *et al.* (2012) isolated and identified indole acidic acid bacteria from saline soil, selected and identified the most active isolates. The use of the identified bacteria singly or in combination as biofertilizer of wheat plants grown under saline conditions was also carried out. Soaking wheat seeds in either *Azotobacter vinelandii* (AZ) or *Streptomyces* sp. (ST) or both culture filtrates (AZ+ST) increased significantly wheat germination. Moreover, soil inoculations with the bacterial cells of AZ, ST or AZ+ST increased the growth and development of wheat in normal and saline conditions.

Sahu (2012) studied that survival of *Azotobacter chroococcum*, *Acinetobacter* spp. and *Pseudomonas fluorescens* formulated employing FBD (Fluid bed drier) on talc-*based* carrier. Results found that consortium containing triple inoculants have given higher biomass, number of leaves, plant height, nitrogen content, phosphorus content, root dry weight, shoot dry weight and total dry weight in ragi compared to single and dual inoculation.

Kanchana *et al.* (2014) studied the interaction effect of combined inoculation of PGPR *viz., Azospirillum, Pseudomonas, Azotobacter* and *Bacillus* on growth and yield of parameters of chilli var. K1 and recorded maximum vigour index of 1460 and

germination percentage of 99.0 per cent, it also recorded that highest plant height of 78.65 cm and plant dry weight of 7.38 g plant^{-1} and the yield parameters such as a maximum of 28.00 number of fruits plant^{-1} and fruit yield of 1080.0 g plant^{-1}.

Ahirwar *et al.* (2015) isolated a total of 28 *Pseudomonas fluorescens* isolates from tomato plant rhizospheres and were screened. Among them, a strain *Pseudomonas fluorescens* SS5 showed the best plant growth-promoting activity as well as enhanced productivity in tomato crop. So, they studied the plant growth promoting characteristics and root colonization ability of *P. fluorescens* SS5, its effect on plant growth, physiology and fruit yield of tomato in pot culture and field study in tomato plants.

Jnawali *et al.* (2015) *Azotobacter* synthesize growth regulating substances like auxins, cytokinin and gibberellic acid, protect plants from phyto-pathogens, through improved nutrient uptake and biological nitrogen fixation thereby play an important role in soil fertility and sustainability exerting their beneficial effects on the crop growth and yield.

Jadhav and Dekhane (2016) made an investigation with the main objective to study the effect of nitrogen fixing *viz., Azotobacter* and *Azospirillum* with different levels of N (60%, 85% and 100%) on growth and yield of chilli (*Capsicum* spp. L.) cv. Acharya at ASPEE ARDF, Tansa (Maharashtra). All the growth characters like plant height (61.50cm) and number of branches/plant (25.25) was found higher with T_7-*Azospirillum* + 100%N + 100% PK, which was statistically at par with T_6 - *Azotobacter* + 100% N + 100% PK. Results showed that the inoculation with T_5-*Azospirillum* + 80% N along with 100% P & K recorded maximum fruit length (6.40 cm), fruit diameter (10.70 mm), number of fruits/plant (78.50) and fruit yield (10.35 t/ha).

Lubna (2016) from her study indicated the possibilities of using mycorrhiza*l* fungi associated with *Actinomycetes* isolates as bio-inoculums for growth promotion and biocontrol of fungal infections of tomato. Proline was enhanced in diseased plants and decreased by the presence of bio control agents. Inoculation with bio control agents enhanced seedling growth, biomass of shoot and root after 2 months after infection with *R. solani* LB4 which may be attributed to hormone (IAA) and lytic enzymes (Chitinase and β-1,3 Glucanase) production by the *Streptomyces* isolate or the antagonistic activity and enhancing phosphorus uptake by mycorrhizal fungi.

Moustaine *et al.* (2017) made a study evaluate and utilize the potential of rhizobacteria PGPR for plants tomato and characterize three PGPR, for their ability to produce metabolites IAA, solubilization of phosphorus, ammonia production, synthesis of enzymes (chitinase, cellulase and protease) and nitrogen fixation. The experiment was a completely randomized design with six replicates. There were four treatments *viz.,* T_1: (control; N0 - PGPR), T_2: (N0 + 2027- 2), T_3: (N0 + 2066-7) and T_4: (N0 + 2025 - 1). The results indicated that a remarkable increase in root growth, namely length, the diameter of the rod and the total chlorophyll.

Shashi *et al.* (2018) conducted an experiment to study the effect of biofertilizers (*Azotobacter, Azospirillum*, PSB and VAM) on growth and yield attributes of tomato. Significant difference was found for plant fresh weight, plant dry weight, stem diameter, fruits per plant and yield. Maximum plant height (121.0 cm) at final harvest and days to first harvest (69.67 cm) was observed with treatment T_2 (*Azospirillum* + 75 % N + recommended dose of P and K). Higher plant fresh weight (639.33 g) and dry (129.0 g) per plant was recorded with treatment having phosphorus solubilizing bacteria (PSB) and recommended dose of fertilizers.

Spandan *et al.* (2018) conducted an experiment with the aim to apply *Azotobacter* sp. and *Azospirillum* sp. as Plant Growth Promoting Rhizobacteria (PGPR) in Tomato (*Lycopersicon esculentum*). The Tomato seeds were sown in the nursery and transplanted into the pot and before sowing biofertilizer and chemical fertilizer were applied as single dose. Maximum growth of tomato was observed in treatment T_6 which comprised of 75% dose of NPK along with *Azotobacter* sp. and *Azospirillum* sp. The observed parameters in treatment T_6 were: germination (%) 90, plant height 51cm, Leaf area 59 cm^2, branches per plant 8.66 and leaf per branch 17.33 cm.

Van Oosten *et al.* (2018) evaluated the root inoculation effect of a strain of *Azotobacter* named *Azotobacter chrococcum* 76A on the tomato crop under both moderate (50 mM NaCl) and severe (100 mM NaCl) salt stresses. The expression profiles of *LEA* genes indicated that *A. chroococcum* 76A treated plants were more responsive to stress stimuli when compared to untreated controls.

Zahoor *et al.* (2018) carried out field experiment to study the effect of AZOPHOS and Inorganic fertilizers on growth and yield of Tomato. The treatments comprised of

three levels of biofertilizers (control, PSB, VAM) in combination with 4 levels of inorganic fertilizers (0, 25, 50 and 100 % RDF). Maximum plant height (207.86 cm), fruit yield (1.50 kg plant^{-1}), no. of Branches (11.18 plant^{-1}) and Nitrogen in fruit (0.32 %) were recorded in treatment *Azotobacter*-3 + PSB (PS6) + 100% NPK.

Material and Methods

III. MATERIAL AND METHODS

The laboratory and pot experiments were carried out in the Department of Agricultural Microbiology, University of Agricultural Sciences, Raichur, which is situated in the North eastern dry zone (Zone-2) of Karnataka (at an altitude of 389.37 m above mean sea level) for the isolation and screening *Azotobacter* and its inoculation effect along with the PGPMs (*Pseudomonas* and *Mycorrhiza*) on growth and yield of tomato. The general procedures and special techniques adopted as well as the materials going to be used in this present study are detailed in this chapter.

3.1 Survey and collection of soil samples

A total of 40 tomato rhizosphere soil samples were collected from the tomato fields located in different locations of Raichur district of Karnataka for isolation of *Azotobacter*.

3.2 Characterization of soil samples for chemical properties

The samples were analyzed for their chemical properties like pH, EC, organic carbon, available N and P by following standard procedures as given below.

3.2.1 Determination of soil pH

Twenty-five gram of soil (field capacity) was taken in a clean dry 150 ml beaker and 50 ml distilled water was added. The contents were thoroughly stirred and pH of the suspension was measured with a digital pH meter (Piper, 1966).

3.2.2 Determination of Electrical conductivity (EC)

Electrical conductivity was determined by Conductometric method (Jackson, 1973).

3.2.3 Organic Carbon

The soil Organic Carbon was determined by using wet oxidation method of Walkey and Black (1934). Two gram of 2 mm sieved soil sample was ground in agate pestle and mortar and passed through 0.2 mm sieve. One g of this sample was transferred into 500 ml dry conical flask. To these flasks, ten ml of 0.1 N $K_2Cr_2O_7$ was added and

mixed well. By adding 20 ml of conc. H_2SO_4, these flasks were swirled for 2-3 min and allowed to stand for 30 min on an asbestos sheet. Two hundred ml of distilled water was poured to dilute the suspension. Ten ml of 85 per cent H_3PO_4 and one ml (10 drops) of diphenylamine indicator was added which gave violet color to the suspension. Contents of the flask were titrated against 0.5 N FAS solutions taken in burette till the color changed from violet through dark blue to bright green. A blank reading was also taken without soil. The per cent organic carbon in the soil was calculated by using the formula.

$$\text{Organic carbon in soil (\%)} = \frac{\text{N of FAS} \times (\text{BTV - STV}) \times 0.003 \times 100 \times 1\text{N } K_2Cr_2O_7}{\text{Weight of soil sample (g)}} \times 1.33$$

Where,

BTV = Blank Titre Value

STV = Sample Titre Value

3.2.4 Available nitrogen

The alkaline potassium permanganate method (Subbaiah and Asija, 1956) using Kjeldhal flask was adopted for the estimation of available nitrogen content in soil. Twenty gram of soil was taken in 800 ml dry Kjeldal flask to which, twenty ml of distilled water was added and swirled to moisten the soil. One ml of liquid paraffin was added to that flask together with a few glass beads. One hundred ml each of 0.32 per cent $KMnO_4$ and 2.5 per cent NaOH solution were added in Kjeldhal flask and immediately fit it up in distillation apparatus. Twenty ml of boric acid mixed indicator solution was pipetted out into a 250 ml conical flask and the delivery tube was dipped into the conical flask. Contents were distilled at a steady rate and liberated NH_3 was collected in a conical flask containing H_3BO_4 with mixed indicator. With the absorption of NH_3, the pinkish colour of the solution was turned to bluish green; the content was distilled till 100 ml of distillate was collected in the conical flask. Distillate was titrated against standard acid (0.05 N H_2SO_4) to the original color and blank titration (without soil) was carried out for final calculation. Available Nitrogen was estimated by using the formula,

$$\text{Per cent Nitrogen} = \frac{\text{Titre value} \times 0.014 \times \text{N of } H_2SO_4 \times \text{Volume made}}{\text{Weight of soil sample (g)}} \times 100$$

3.2.5 Available phosphorus

Available phosphorus content of the soil was extracted by using 0.5M $NaHCO_3$. The Phosphorus content was determined by chloromolybdic blue colour method using UV spectrophotometer. The intensity of blue colour was determined at 660 nm. The available phosphorus content was computed and expressed in kg /ha (Jackson, 1973) using the formula

$$\text{Available P (kg ha}^{-1}) = \frac{\text{Graph ppm} \times \text{Volume of extract} \times \text{Volume made} \times 2.24}{\text{Weight of soil} \times \text{Aliquot taken}}$$

3.2.6 Available Potassium (kg ha^{-1})

Available potassium was extracted with neutral normal ammonium acetate (pH 7.0). The quantity of potassium in the extractant was determined by flame photometry (Jackson, 1973). The available potassium (kg ha^{-1}) was calculated using the following formula.

3.3 Isolation of *Azotobacter* strains

The *Azotobacter* strains were isolated from tomato rhizosphere soil samples and purified by using serial dilution method and plating on Waksman No.77 media. Plates were incubated for 4-6 days at 30 °C. Small water droplets like glistening colonies which appeared on the medium were purified by repeated streaking on Waksman No.77 medium. The purified isolates were transferred to the slants of the same medium and stored for further studies.

3.4 Morphological characterization of efficient *Azotobacter* isolates

Bacterial isolates were examined for colony morphology, cell shape, gram reaction and cyst formation as per the standard procedures.

3.4.1 Shape

The cell shape was observed by simple staining. A smear of each isolates was made, air dried and stained with crystal violet for 30 sec. Stained smear was then washed, air dried and observed under oil immersion compound microscope.

3.4.2 Gram staining

Smear of *Azotobacter* isolates were made on the clean glass slides and treated with crystal violet solution for 1 min, the smear was gently rinsed off and iodine solution was applied for 1 min. This in turn was drained off and the smear was decolorized using ethyl alcohol (95 %). And next safranin was used as a counter stain for 10 sec. Then slide was gently rinsed off with water and blotted off.

3.4.3 Cyst formation and pigmentation

The *Azotobacter* isolates were grown on Waksman No.77 N free agar medium for seven days. All isolates of *Azotobacter* were subjected to react with Violamine stain (Winogradsky, 1949) for 45 sec, then washed and counterstained with dilute crystal violet (0.05 %) for 1 min. The cells which formed cysts will be confirmed as *Azotobacter*.

3.4.4 Cell motility

Cell motility was observed by hanging drop technique.

3.5 Biochemical characterization of efficient *Azotobacter* isolates

The biochemical tests for the identification of bacterial strains which were carried out in the present study are detailed below:

3.5.1 Starch hydrolysis

The ability of the isolates to hydrolyze starch was examined in the petriplates containing starch agar which were inoculated with test cultures and incubated at 30 °C for three days. After incubation the plates were flooded with Lugol's iodine solution and allowed to stand for 15-20 minutes. The clear zone around the colony was considered as positive for the test.

3.5.2 Catalase test

The nutrient agar slants were inoculated with test organisms and were incubated at 30 °C for 24 hours. After incubation the tubes were flooded with one ml of three per cent hydrogen peroxide and observed for production of gas bubbles. The occurrence of gas bubbles was scored positive for catalase activity.

3.5.3 Hydrogen sulphide production

The bacterial isolates were inoculated to test tubes containing 5 ml of sterile medium and were incubated at room temperature 28 °C. The test tubes were observed for H_2S production. The formation of black ring in the medium was taken as positive for H_2S production.

3.5.4 Gelatin liquefaction

To the pre-sterilized nutrient gelatin deep tubes, the test cultures were inoculated and tubes were incubated at 28 ± 2 °C for 24 hours. Following this, the tubes were kept in a refrigerator at 4 °C for 30 minutes. The tubes with cultures that remained liquefied were taken as positive and those that solidified on refrigeration were taken as negative for the test.

3.5.5 Ammonification

Pure culture of *Azotobacter* isolates were inoculated into tubes containing 10 ml peptone water in each tube and incubated for 48 h at 30 °C. After incubation, Nessler's reagent (0.5 ml) was added to each tube (Cappuccino and Sherman, 1992) and the tubes were observed for the development of yellow colour.

3.6 Screening of native *Azotobacter* isolates for quality parameters *in vitro*

3.6.1 Screening for *in vitro* nitrogen fixation quantitative estimation (Humpries *et al.*, 1956)

To 250 ml conical flasks, 100 ml of the medium (Waksman No.77) was dispensed for all flasks and autoclaved. One ml of 24 hr. old culture was inoculated to each flask. The flasks were incubated at 37 °C for seven days. After seven days of incubation, the culture was homogenized and 10 ml was digested with 5 ml of concentrated H_2SO_4 along with 0.2 g digestion catalyst mixture K_2SO_4: $CuSO_4$: Selenium (100:10:1). After cooling, volume was made up to 10 ml with distilled water. Later, 10 ml of aliquot was transferred to micro-kjeldhal distillation unit, for which 20 ml of 40 per cent NaOH was added and distilled. Ammonia evolved was trapped in 4 per cent boric acid mixed indicator (Bromo cresol green 0.066 g and methyl red 0.033 g in 100 ml methanol) till the solution turned

from pink to green. It was titrated against 0.05 N H_2SO_4 and total nitrogen content of the culture was determined and results were expressed as mg N fixed per g of malate.

$$\text{Per cent Nitrogen} = \frac{\text{Titre value} \times 0.014 \times \text{N of } H_2SO_4 \times \text{Volume made}}{\text{Volume of sample used}} \times 100$$

3.6.2 *In vitro* phosphate solubilisation by isolates

The *in vitro* phosphorus solubilisation ability of the *Azotobacter* isolates were studied on Pikovskaya′s agar plates. The pure culture of *Azotobacter* isolate was spread on agar plates containing Pikovskaya′s medium supplemented with phosphate and these plates were incubated at 28 ^{0}C for 7 days. Formation of halo zone was taken as positive for the solubilisation of phosphorus.

3.6.3 Siderophore assay (Schwyan and Neilands, 1987)

In order to screen the production of siderophores, the *Azotobacter* isolates were grown in CAS agar medium. The inoculated agar plates were incubated at 37 ^{0}C for 24 h. The observation was made for the change of medium colour from blue to reddish yellow to determine the siderophores production.

3.6.4 Acetic Acid (IAA) production

IAA production potential of *Azotobacter* isolates was tested in Ashby′s nitrogen free broth supplemented with 0.005 M concentration tryptophan at 28 °C. The concentration of IAA in the culture broth after 3 days of incubation was centrifuged at 5000 rpm for 5 min and determined by spectrophotometric method using Salkowaski's reagent as given below

One ml of the supernatant was mixed with 1 ml of Salkowaski's reagent (2 ml of 0.5 M $FeCl_3$ + 98 ml 35 % $HClO_4$) and the intensity of red colour developed within 30 min was checked at 530 nm using spectrophotometer. The concentration was determined using a standard curve prepared from standard solution of Indole acetic acid (Mali and Bodhankar, 2009).

3.7 Growth experiment conditions

3.7.1 Nursery preparation

Tomato (*Lycopersicum esculantum* L.) seeds were sown in plastic trays containing coco peat.

3.7.2 Inoculum preparation

Fifty ml of the efficient *Azotobacter* isolate TAZ-3 was mixed in 5 lt of water and the roots of the tomato seedlings were dipped in that solution for 30 minutes and then they were transplanted into the pots. In the same way, 100 gm of the biofertilizer *Pseudomonas* was mixed in 5 lt of water and the seedlings were root dipped for half an hour before transplanting. *Mycorrhiza* was applied to the pots directly after mixing with carrier material.

3.7.3 Transplanting

Twenty-one days old tomato seedlings grown in nursery was treated with the bioinoculants as per the treatments and transplanted.

3.7.4 Treatment Details (Table 1):

Treatments	Treatment details
T1	RDF
T2	75 % RDF + *Azotobacter* isolate (TAZ-3)
T3	75 % RDF + Reference *Azotobacter* strain
T4	75 % RDF + *Pseudomonas* sp.
T5	75 % RDF + *Mycorrhizae*
T6	75 % RDF + *Azotobacter* isolate (TAZ-3) + *Pseudomonas* sp.
T7	75 % RDF + *Azotobacter* isolate (TAZ-3) + *Mycorrhizae*
T8	75 % RDF + *Azotobacter* isolate (TAZ-3) + *Pseudomonas* sp. + *Mycorrhizae*
T9	75 % RDF + Ref. *Azotobacter* strain + *Pseudomonas* sp. + *Mycorrhizae*
Replications: 3	
Design: CRD	

3.7.5 Design and Experimental plan

The experiment was laid out in CRD with nine treatments replicated 3 times. A total No. of 27 pots (3 Replication × 9 Treatment) was maintained for the experiment and in each pot one plant was kept after thinning (Fig. 1).

T_1R_1	T_1R_2	T_1R_3
T_2R_1	T_2R_2	T_2R_3
T_3R_1	T_3R_2	T_3R_3
T_4R_1	T_4R_2	T_4R_3
T_5R_1	T_5R_2	T_5R_3
T_6R_1	T_6R_2	T_6R_3
T_7R_1	T_7R_2	T_7R_3
T_8R_1	T_8R_2	T_8R_3
T_9R_1	T_9R_2	T_9R_3

Fig. 1. Layout plan of pot experiment

3.7.6 Growth observations recorded at different intervals of time

3.7.6.1 Plant height

The plant height was recorded at 30 DAT, 60 DAT and 90 DAT.

3.7.6.2 Number of branches

The number of branches of the tomato plants was recorded at 30 DAT, 60 DAT and 90 DAT.

3.7.6.3 Total shoot biomass

The total fresh and dry shoot weight were recorded first and then the biomass was calculated at the harvest stage.

3.7.6.4 Total root biomass

The total fresh and dry shoot weight were recorded first and then the biomass was calculated at the harvest stage.

3.7.6.5 Chlorophyll content

The chlorophyll content of the leaves was measured using SPAD meter at 30 DAT, 60 DAT and 90 DAT.

3.7.7 AM fungi per cent colonization

Plant root samples will be collected at 90 DAT. The roots will be cut into 1cm long segments. The root segments will be placed in a 2.5% aqueous solution of KOH (w/v) and boiled in a water bath at 90 ºC for 15 min. Roots will be rinsed with distilled water and lightened in H_2O_2 (3ml of 20% NH_4OH in 30 ml H_2O_2) for 10- 45 minutes. The root segments will be rinsed thoroughly several times and acidified by soaking in 40-50 ml of 1% HCl for 3mins. Acidified roots will be stained in an acidic glycerol solution (500 ml glycerol, 450 ml distilled water and 50 ml 1% HCl) containing 0.05% tryphan blue. The tryphan blue solution will be poured off and the roots will be destained in acidic glycerol at room temperature **(**Phillips and Hayman 1970). The stained roots will be mounted on a glass slide and per cent infection will be calculated using the formula,

$$\text{Per cent root colonization} = \frac{\text{Number of root bits showing AM infection}}{\text{Number of root bits examined}} \times 100$$

3.7.8 Extramatrical spore count (Gerdemann and Nicolson, 1963)

The tomato rhizosphere soil will be collected at 90 DAT to estimate extrametrical spore count of AM fungi. The extramatrical chlamydospores produced by AM fungi will be isolated and counted by wet sieving and decanting technique. Fifty grams of air dried rhizosphere soil will be suspended and mixed well in water and allowed for 30 seconds. The supernatant will be passed through a set of sieves with mesh size 1000, 300, 250, 105 and 45 µm arranged in descending order with their mesh size. Spores collected from the last two sieves will be transformed to a nylon mesh having mesh size of 45 µm and counted under stereo microscope with 20X magnification.

3.7.9 Nitrogen estimation

Total nitrogen was determined by Kjeldahl's method as described by Jackson (1973). Powdered samples were digested with concentrated sulphuric acid in the presence of digestion mixture (K_2SO_4: $CuSO_4.5H_2O$: Se powder in the ratio 100:20:1). The nitrogen content in the aliquot of digested sample was determined by distillation under alkaline medium. The liberated ammonia was trapped in boric acid mixed indicator and was titrated against standard acid. The per cent nitrogen was calculated using the following formula,

$$\text{Nitrogen (\%)} = \frac{\text{TV (ml)} \times \text{N. of } H_2SO_4 \times \text{Volume of plant digest} \times 0.014}{\text{Aliquot taken} \times \text{Weight of plant sample (gm)}} \times 100$$

3.7.10 Phophorus estimation

The total phosphorus content of the plant tissues was estimated at 90 DAT stage of crop by Vanadomolybdate yellow colour method using colour intensity at 470 nm wave length on Spectronic-20 (Jackson, 1973). It was calculated by using the formula,

$$\text{Phosphorus (\%)} = \frac{\text{Graph ppm} \times \text{Volume of digest} \times \text{Volume made}}{10^6 \times \text{Aliquot taken} \times \text{Weight of plant sample}} \times 100$$

3.7.11 Statistical analysis

Randomized Block Design with three replications was followed for the analysis of data. The data was subjected to one-way Analysis of variance (ANOVA) of Snedecor and Cochran (1969).

Results and Discussion

IV. RESULTS AND DISCUSSION

Plant growth promoting rhizobacteria (PGPR) are those which promote the plant growth through various mechanisms *viz.,* nitrogen fixation, phosphorus solubilisation, HCN production and phytohormone secretion. *Azotobacter* is a free-living, aerobic, nitrogen fixing rhizobacteria. They not only fix nitrogen, but also produce various plant growth promoting substances such as auxins, cytokinins, gibberellins, etc., which improves the plant growth. They are usually associated with the non-leguminous crops as they don't form any nodules on the roots and also the fact that they are free-living. *Azotobacters are* normally found in the soils with moderate pH and are mesophilic in nature.

The present study was on the isolation of *Azotobacter* isolates from the Hyderabad-Karnataka region and identification of the efficient strains based on *in vitro* nitrogen fixing, growth promoting potentials and its effect on growth and yield of tomato under pot culture conditions as an isolate and as consortia along with other PGPMs.

4.1 Collection of soil samples for the isolation of *Azotobacter* isolates

The soil samples for the isolation of *Azotobacter* isolates were collected from different places in the Raichur district. The samples were collected from tomato growing fields.

4.2. Characterization of soil samples for their chemical properties

All the rhizospheric soil samples collected from different locations of Hyderabad Karnataka region were analyzed for their chemical properties like pH, EC, organic carbon, available N, P and K by standard procedures. Results of these analyses are mentioned in Table 2.

4.3 Isolation and purification of *Azotobacter* isolates

Thirty *Azotobacter* isolates were isolated from different soil samples collected from the fields where tomato was grown in Hyderabad Karnataka region by following the procedure as mentioned in 3. 3.

The *Azotobacter* strains were isolated and purified by using serial dilution

Table 2. Physiochemical properties of different soil samples collected from Raichur district

Sl. No.	Soil type	pH	EC ($dS\ m^{-1}$)	OC (%)	N (kg/ha)	P_2O_5 (kg/ha)	K_2O (kg/ha)	Isolate code
1	Red	7.46	0.18	0.51	150.37	31.92	205.20	**TAZ-1**
2	Black	7.75	0.52	0.49	166.34	58.35	220.80	**TAZ-2**
3	Red	7.35	1.03	0.40	171.57	30.66	205.20	**TAZ-3**
4	Black	7.70	0.33	0.39	155.81	27.80	174.00	**TAZ-4**
5	Black	7.50	1.01	0.35	162.16	43.85	190.80	**TAZ-5**
6	Black	7.63	0.65	0.46	203.91	48.57	240.19	**TAZ-6**
7	Black	8.03	0.28	0.39	194.43	33.46	187.50	**TAZ-7**
8	Black	7.78	0.65	0.47	213.24	51.75	200.31	**TAZ-8**
9	Black	7.98	0.40	0.41	199.44	47.34	182.50	**TAZ-9**
10	Black	7.60	0.95	0.43	219.05	34.19	225.08	**TAZ-10**
11	Black	7.72	0.63	0.40	220.17	37.21	227.09	**TAZ-11**
12	Red	7.52	0.53	0.38	200.18	44.49	178.37	**TAZ-12**
13	Red	7.67	1.02	0.39	175.61	40.21	199.19	**TAZ-13**
14	Red	8.02	0.35	0.44	186.52	45.39	200.06	**TAZ-14**

Conti…

15	Red	7.48	0.65	0.43	208.07	40.24	209.24	**TAZ-15**
16	Black	7.68	0.30	0.41	231.50	50.60	291.62	**TAZ-16**
17	Red	7.62	0.62	0.35	201.15	35.20	180.12	**TAZ-17**
18	Red	7.83	0.81	0.29	210.20	41.32	210.23	**TAZ-18**
19	Red	8.05	0.77	0.46	175.25	42.25	175.69	**TAZ-19**
20	Red	8.16	0.69	0.42	189.02	40.12	172.32	**TAZ-20**
21	Red	7.21	1.05	0.38	212.85	35.23	201.04	**TAZ-21**
22	Red	7.52	0.43	0.43	170.12	38.12	206.78	**TAZ-22**
23	Red	7.05	0.73	0.28	165.52	39.18	208.45	**TAZ-23**
24	Red	7.13	0.66	0.50	158.87	32.12	195.25	**TAZ-24**
25	Red	7.48	0.52	0.49	164.12	33.15	179.03	**TAZ-25**
26	Red	7.46	0.36	0.62	167.89	35.62	171.45	**TAZ-26**
27	Red	7.55	0.41	0.41	185.20	43.21	188.21	**TAZ-27**
28	Red	7.67	0.25	0.37	205.78	45.12	204.78	**TAZ-28**
29	Red	7.15	1.05	0.33	174.12	47.34	196.78	**TAZ-29**
30	Red	7.23	0.53	0.48	167.32	40.24	188.45	**TAZ-30**

method and plating on Waksman No.77 media. Plates were incubated for 4-6 days at 30 °C. Small water droplets like glistening colonies which appeared on the medium were purified by repeated streaking on Waksman No.77 medium. The purified isolates were transferred to the slants of the same medium and stored for further studies. Each isolate was identified according to specific characters described in Bergey′s Manual of Systematic Bacteriology (1994).

4.4 Morphological characterization of efficient *Azotobacter* isolates

Bacterial isolates were examined for colony morphology, cell shape, gram reaction and cyst formation as per the standard procedures. Results of these tests are depicted in Table 3 (Plate 1).

4.4.1 Shape

The cell shape was observed by simple staining. All the cells were round, some were flat and some were raised.

4.4.2 Gram staining

All the isolates were Gram negative.

4.4.3 Cyst formation and pigmentation:

The cysts were observed in every isolate and the colour of the colonies was brown but varied in intensity from light to medium and dark.

4.4.4 Cell motility

The cells of every isolate which were obtained during this study were found to be motile in all the cases.

All the *Azotobacter* isolates showed morphological characteristics identical to those internationally reported in Bergey's Manual of Systemic Bacteriology (2005). The morphological characters which were studied in the present study were found to be in relation to that of Sandeep Upadhyay *et al.* (2015) and Sahoo *et al.* (2013).

Table 3. Morphological characteristics of *Azotobacter* isolates studied *in vitro*

Isolates of *Azotobacter*	Gram's reaction	Cysts formation	Pigmentation	Colony Morphology
TAZ-1	Negative	Present	Dark brown	Moderate, flat and slimy
TAZ-2	Negative	Present	Light brown	Good growth, raised and slimy
TAZ-3	Negative	Present	Dark brown	Good growth, raised and entirely slimy
TAZ-4	Negative	Present	Light brown	Moderate, raised and slimy
TAZ-5	Negative	Present	Light brown	Moderate, flat and entirely slimy
TAZ-6	Negative	Present	Light brown	Moderate, raised and slimy
TAZ-7	Negative	Present	Light brown	Moderate, raised and wrinkled
TAZ-8	Negative	Present	Dark brown	Moderate, raised and entirely slimy
TAZ-9	Negative	Present	Light brown	Moderate, raised and entirely slimy
TAZ-10	Negative	Present	Dark brown	Moderate, raised and entirely slimy
TAZ-11	Negative	Present	Dark brown	Moderate, raised and entirely slimy
TAZ-12	Negative	Present	Dark brown	Good growth, flat and wrinkled
TAZ-13	Negative	Present	Pale brown	Good growth, raised and slimy
TAZ-14	Negative	Present	Dark brown	Good growth, flat and slimy
TAZ-15	Negative	Present	Light brown	Moderate growth, raised and slimy

Conti…

TAZ-16	Negative	Present	Dark brown	Moderate, raised and entirely slimy
TAZ-17	Negative	Present	Dark brown	Good growth, flat and wrinkled
TAZ-18	Negative	Present	Pale brown	Moderate growth, raised and slimy
TAZ-19	Negative	Present	Light brown	Moderate growth, raised and slimy
TAZ-20	Negative	Present	Light brown	Good growth, flat and slimy
TAZ-21	Negative	Present	Dark brown	Moderate, raised and wrinkled
TAZ-22	Negative	Present	Pale brown	Moderate, raised and entirely slimy
TAZ-23	Negative	Present	Dark brown	Moderate, flat and entirely slimy
TAZ-24	Negative	Present	Light brown	Moderate, raised and entirely slimy
TAZ-25	Negative	Present	Dark brown	Moderate, raised and entirely slimy
TAZ-26	Negative	Present	Dark brown	Good growth, raised and wrinkled
TAZ-27	Negative	Present	Pale brown	Good growth, raised and slimy
TAZ-28	Negative	Present	Dark brown	Good growth, flat and slimy
TAZ-29	Negative	Present	Dark brown	Moderate, raised and wrinkled
TAZ-30	Negative	Present	Pale brown	Moderate, flat and entirely slimy

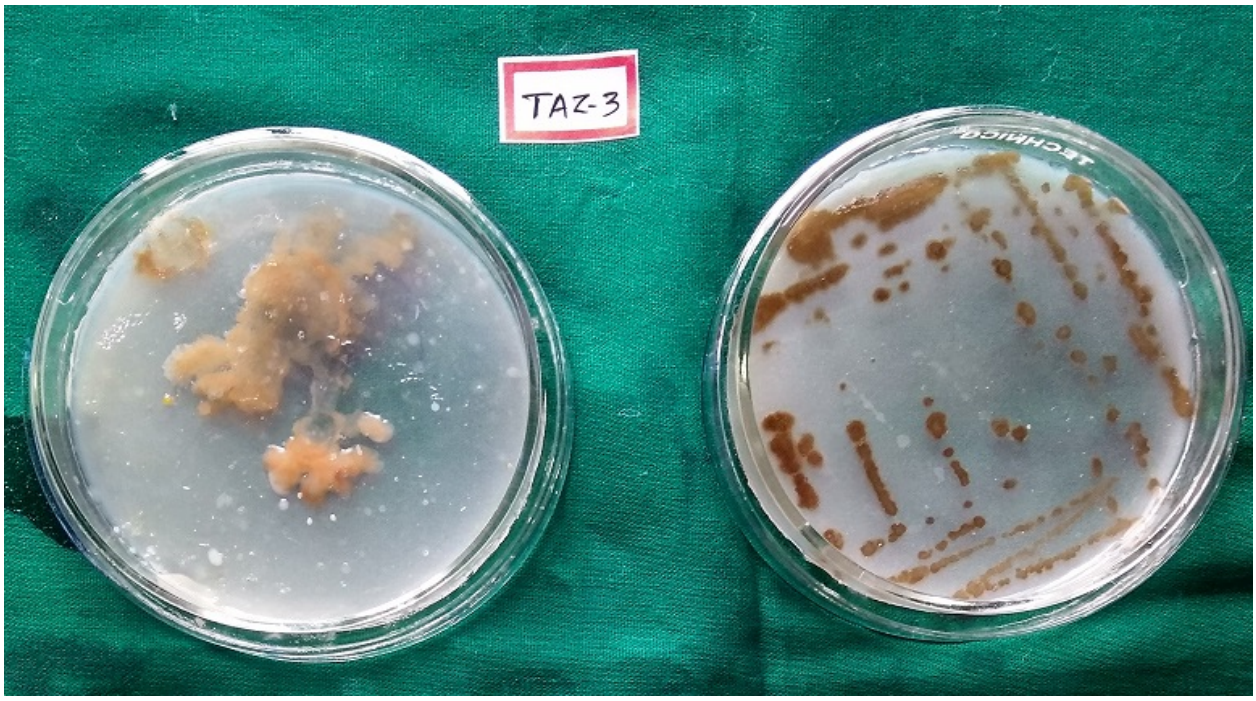


Plate 1. Growth of *Azotobacter* isolates on Waksmann No. 77 plates

4.5 Biochemical characterization of efficient *Azotobacter* isolates

The biochemical tests for the identification of bacterial strains which were carried out in the present study are detailed in the Table 4.

4.5.1 Starch hydrolysis

Except for seven isolates *viz.,* TAZ-3, TAZ-5, TAZ-6, TAZ-12, TAZ-19, TAZ-22 and TAZ-29, all the other twenty three isolates showed negative result for the starch hydrolysis (Plate 2).

For this test, the cultures were streaked on the starch agar plates and incubated at 37 ^{o}C for 24 h. After the incubation the plates were flooded with iodine solution and observed for the formation of blue-black colour due to the formation of starch iodine complex. Clear zone around the colony indicates that the starch has degraded due to the production of amylase and in this study, there was a clear zone around the colonies after the addition of iodine and reported as positive for the test.

4.5.2 Catalase test

Among the thirty isolates, eighteen isolates showed positive result and twelve isolates showed negative result. The isolates which showed negative result were TAZ-7, TAZ-8, TAZ-11, TAZ-14, TAZ-17, TAZ-21, TAZ-22, TAZ-23, TAZ-24, TAZ-27, TAZ-28 and TAZ-30 (Plate 3).

To check this, nutrient agar tubes were inoculated with overnight grown test organism and were incubated at 30 ^{o}C for 24 h. After the incubation the individual tubes inoculated with *Azotobacter* isolates were flooded with 3 per cent hydrogen peroxide and observed for the formation of gas bubble. The tubes inoculated with *Azotobacter* that developed bubbles were scored as positive for the test.

4.5.3 Nitrate reduction test

All the thirty isolates showed negative result for nitrate reduction test (Plate 4).

In this test, the *Azotobacter* isolates were inoculated to the pre sterilized nitrate broth and incubated at 37 ^{o}C for 48 h. After that, in a porcelain dish, 3 drops of reagent and 1 drop of sulphuric acid was mixed with 1 drop of culture and observed for the blue

Table 4. Biochemical characteristics of native *Azotobacter* isolates under *in vitro* conditions

Sl. No.	Isolate code	CT	SH	GL	NR	A
1	**TAZ-1**	+	-	-	-	-
2	**TAZ-2**	+	-	-	-	+
3	**TAZ-3**	+	+	-	-	+
4	**TAZ-4**	+	-	-	-	-
5	**TAZ-5**	+	+	-	-	+
6	**TAZ-6**	+	+	-	-	+
7	**TAZ-7**	-	-	-	-	+
8	**TAZ-8**	-	-	-	-	-
9	**TAZ-9**	+	-	-	-	+
10	**TAZ-10**	+	-	-	-	-
11	**TAZ-11**	-	-	-	-	+
12	**TAZ-12**	+	+	+	-	+
13	**TAZ-13**	+	-	+	-	+
14	**TAZ-14**	-	-	-	-	+
15	**TAZ-15**	+	-	-	-	+
16	**TAZ-16**	+	-	-	-	-
17	**TAZ-17**	-	-	-	-	+
18	**TAZ-18**	+	-	+	-	+
19	**TAZ-19**	+	+	-	-	-
20	**TAZ-20**	+	-	+	-	+
21	**TAZ-21**	-	-	+	-	+
22	**TAZ-22**	-	+	-	-	+
23	**TAZ-23**	-	-	-	-	+
24	**TAZ-24**	-	-	-	-	+
25	**TAZ-25**	+	-	-	-	+
26	**TAZ-26**	+	-	+	-	-
27	**TAZ-27**	-	-	-	-	+
28	**TAZ-28**	-	-	-	-	+
29	**TAZ-29**	+	+	-	-	-
30	**TAZ-30**	-	-	+	-	+

CT – Catalase test

SH – Starch Hydrolysis test

GL – Gelatin Liquefaction

NR – Nitrate Reductase test

A – Ammonification



Plate 2. Clear zone formation around the *Azotobacter* colony indicating positive result for starch hydrolysis test

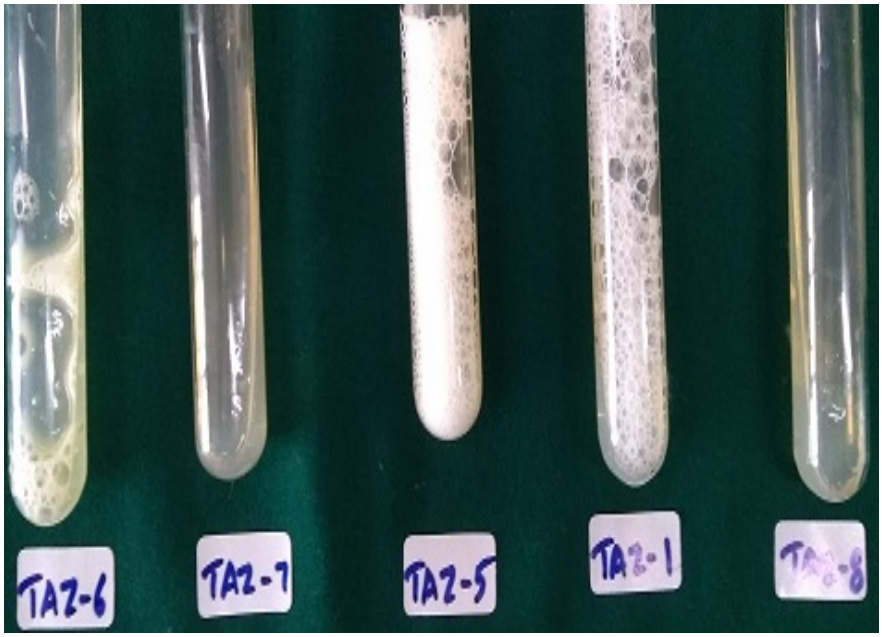


Plate 3. Foam production by *Azotobacter* isolates indicating positive test result for Catalase test.

coloration. The blue colour development was not seen in any of the *Azotobacter* isolate. Hence recorded negative for the test.

4.5.4 Gelatin liquefaction

Only seven isolates showed positive result *viz.,* TAZ-12, TAZ-13, TAZ-18, TAZ-20, TAZ-21, TAZ-26 and TAZ-30 remaining twenty three isolates showed negative test result (Plate 5).

In this study, the test cultures were inoculated into the pre-sterilized nutrient gelatin deep tubes and were incubated at 28 ^{o}C for 48 h. After the incubation, the tubes were kept in refrigerator at 4 ^{o}C for 30 min. The tubes with the culture that remained soilidified was taken as negative for the test due to the property of gelatin is to remain solid below 22 ^{o}C while the degraded form of gelatin *i.e.* amino acids and peptides is to remain liquid below 22 ^{o}C were taken negative (Tejera *et al.,* 2005).

4.5.5 Ammonification

Out of the thirty isolates, twenty two isolates showed positive result for this test and the remaining eight isolates showed negative result. The isolates which were observed to be negative were TAZ-1, TAZ-4, TAZ-8, TAZ-10, TAZ-16, TAZ-19, TAZ-26 and TAZ-29 (Plate 6).

This test was conducted by inoculating the pure culture of *Azotobacter* isolates into tubes containing 10 ml peptone water in each tube and incubated for 48 h at 30 ^{o}C. After incubation, Nessler's reagent (0.5 ml) was added to each tube and the tubes were observed for the development of yellow colour. Those tubes which developed yellow colour were taken as positive.

All the above biochemical test results were found to be conclusive with study made by Akhter *et al.* (2012) who, isolated and studied the biochemical characters of fifteen *Azotobacter* isolates and found that they were positive for catalase, oxidase and starch hydrolysis tests and also ten isolates were positive for nitrate reductase test. Also, the results were supported by Patel *et al.* (2013).

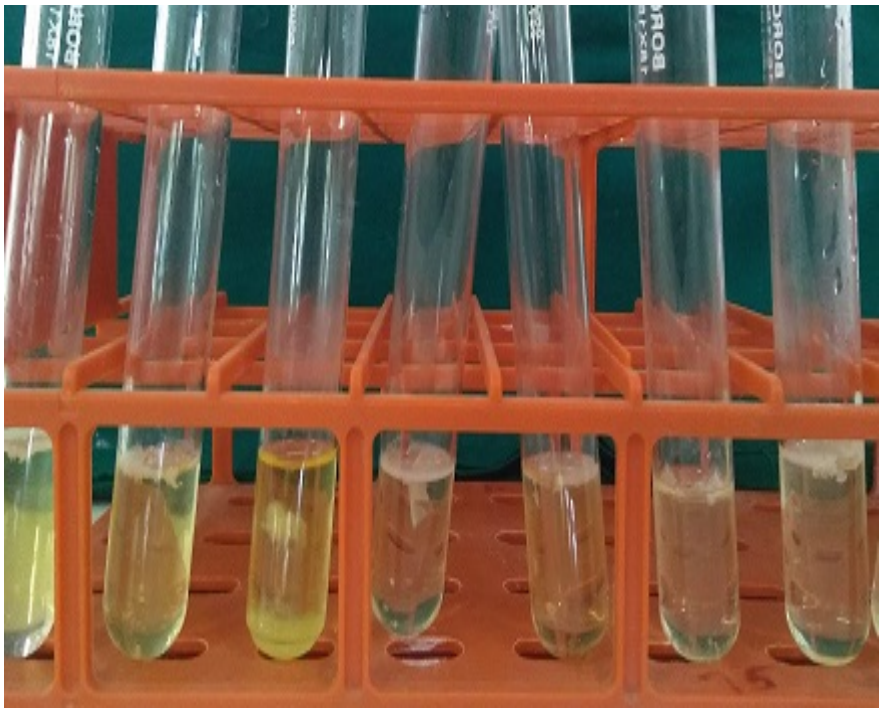


Plate 4. No blue colour formation by the *Azotobacter* isolates indicating negative result for nitrate reduction.

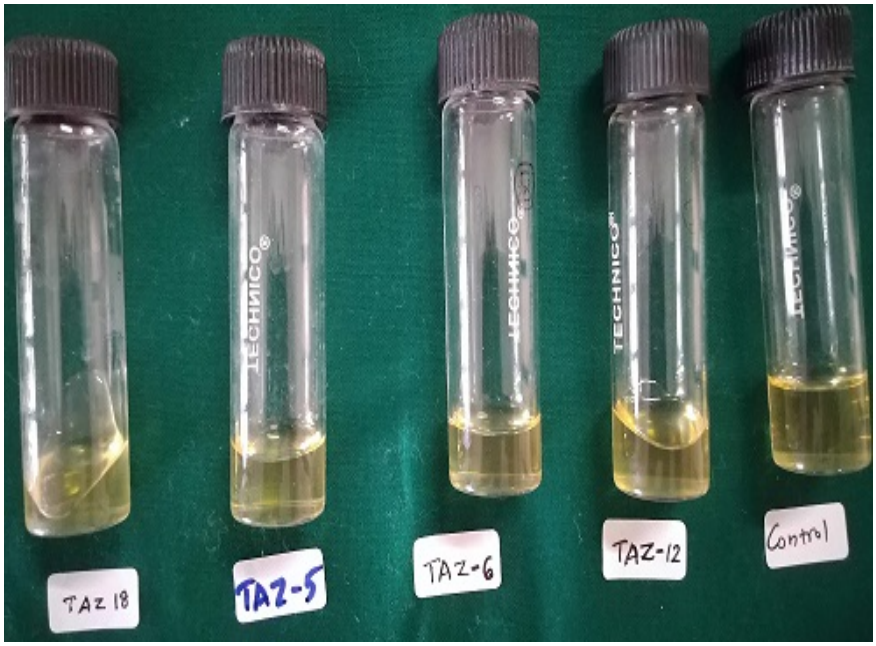


Plate 5. Gelatin liquefaction test- solid tubes indicate negative result and liquid tubes indicate positive result

4.6 Screening for efficient isolates of *Azotobacter*

Based on the biochemical and molecular characters, ten isolates were selected from thirty isolates for the screening of efficient *Azotobacter* isolate. The screening was carried out according to the studies done by Saida *et al.* (2015), Krunal Modi *et al.* (2017) and Edi Husen (2003).

4.6.1 *In vitro* nitrogen fixation

The nitrogen fixing ability ranged from 9.67 to 18.87 µg of N /mg of C source. Among the ten isolates selected, the highest nitrogen fixing ability was shown by TAZ-3 (18.87 µg of N / mg of C source), followed by TAZ-12 (16.92 µg of N / mg of C source). The isolate TAZ-19 recorded the lowest nitrogen fixing ability of 9.67 µg of N /mg of C source. The isolate TAZ-5 showed the nitrogen fixing ability of 16.31 µg of N /mg of C source which was on par with TAZ-12. The native isolate TAZ-3 showed higher nitrogen fixing potential than the reference *Azotobacter* strain whose nitrogen fixing potential was found to be 16.75 µg of N / mg of C source (Table 5) (Fig. 2).

The results obtained were found to be in line with the results obtained by Rajaee *et al.* (2007), Krunal Modi *et al.* (2017) who studied four isolates of *Azotobacter* and Kanimozhi and Panneerselvam (2010) studied the nitrogen fixation ability of the 30 isolates of *Azotobacter* from paddy rhizosphere soil.

4.6.2 *In vitro* phosphate solubilisation

Five isolates showed positive result *viz*., TAZ-3, TAZ-5, TAZ-12, TAZ-18 and TAZ-19 and the other five isolates showed negative result for *in vitro* phosphorus solubilisation. The isolates which showed positive result formed a zone of solubilisation on inoculating to the petriplates with Pikovskaya′s media which were incubated at 35 °C for 4 to 5 days (Table 5) (Plate 7).

These results obtained from the current study were in co-relation with the study done by Ponmurugan *et al.* (2012) who isolated 10 *Azotobacter* isolates from the rhizosphere soils and were also in relation with Twisha and Pratibha (2015). Edi Husen (2003) also reported the P solubilisation by the *Azotobacter* isolate.

Table 5. Nitrogen fixing, siderophore production and phosphorous solubilisation potential of native *Azotobacter* isolates *in vitro*

Azotobacter Isolates	mg N per g of C utilized	Siderophore production	Phosphorus solubilisation
TAZ-2	13.72	+	-
TAZ-3	18.87	+	+
TAZ-5	16.31	+	+
TAZ-6	15.63	+	-
TAZ-9	12.46	+	-
TAZ-10	11.28	+	-
TAZ-12	16.92	+	+
TAZ-16	14.28	+	-
TAZ-18	10.32	+	+
TAZ-19	9.67	+	+
Ref. *Azotobacter* strain	16.75	+	+
S.Em±	**0.09**	-	-
C.D. at 1%	**0.25**	-	-

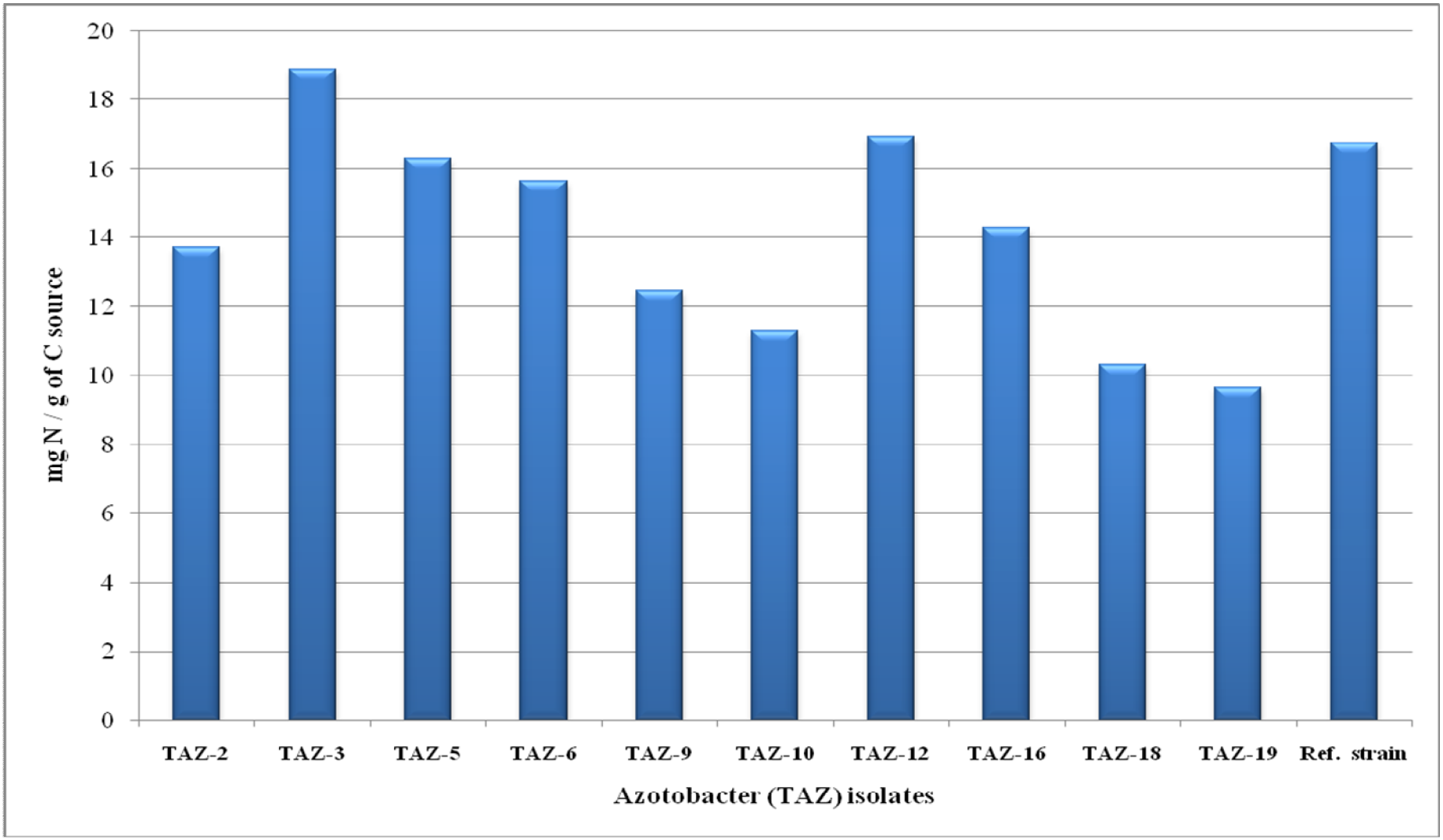


Fig 2. N_2 fixing potential of *Azotobacter* isolates under *in vitro* condition

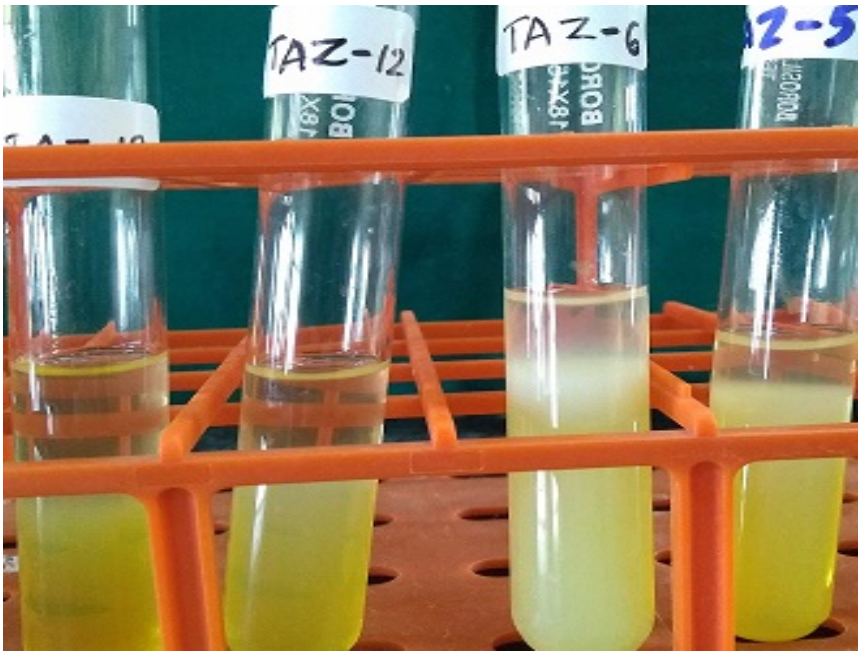


Plate 6. Formation of yellow colour indicates positive result of *Azotobacter* isolates for Ammonification test



Plate 7. Hallow zone development on Pikovskaya's plate indicating the P-solubilisation potential of *Azotobacter* isolate TAZ-3.

4.6.3 Siderophore production

All the ten isolates showed the formation of siderophores (Table 5).

On the Chrome Azurol S (CAS) blue media all the ten isolates produced the orange colonies, indicating the activity of iron chelaters, the colonial zone varied with the isolate. This character of iron chelation is an important factor for the *Azotobacter* isolates, as it deprives the pathogen from the available iron in the surrounding (Muthuselvan and Balagurunathan, 2013; Jenifer *et al.,* 2013).

Dragana *et al.* (2015) also reported the siderophore production during the screening of six *Azotobacter* isolates for the PGPR activities. The present study was in line with the above mentioned study.

4.6.4 Indole Acetic Acid (IAA) production

The production of IAA from the isolates varied from 12.28 to 29.01 µg per 50 ml of medium. The highest IAA production was seen in the isolate TAZ-3 (29.01 µg per 50 ml of broth) followed by TAZ-5 (24.35 µg per 50 ml of broth). The native isolate TAZ-3 showed higher IAA production than the reference *Azotobacter* strain whose IAA production was found to be 25.81 µg per 50 ml of broth. The lowest amount of IAA was obtained from the isolate TAZ-19 (12.28 µg per 50 ml of broth) (Table 6) (Fig. 3) (Plate 8).

Ahmad *et al.* (2005), made a research on the production of IAA by ten *Azotobacter* isolates and reported that the highest IAA was produced by the strain AZS_6. Similar studies were made by Ashraf *et al.* (2011), Saida *et al.* (2015) and Vikram (2011).

4.7 Pot culture experiment

Based on the screening characters, the isolate TAZ-3 was selected over the other nine isolates due to its efficiency over the other isolates and was used for the pot culture experiment. In addition to this, the *Pseudomonas* sp. and mycorrhizal cultures were provided by the Department of Agricultural Microbiology, College of Agriculture, Raichur. All the inoculants were prepared according to the standard procedures. The seedlings of tomato were treated by root dip method and were later transplanted in the pots. The uninoculated pot was taken as control. The inoculants were treated separately as

Table 6. Indole acetic acid production by native *Azotobacter* isolates under *invitro* conditions

***Azotobacter* Isolates**	**IAA (µg per 50 ml of broth)**
TAZ-2	19.67
TAZ-3	29.01
TAZ-5	24.35
TAZ-6	23.51
TAZ-9	17.05
TAZ-10	15.70
TAZ-12	21.20
TAZ-16	17.66
TAZ-18	12.26
TAZ-19	12.28
Ref. *Azotobacter* strain	25.81
S.Em±	**0.78**
C.D. at 1%	**2.31**

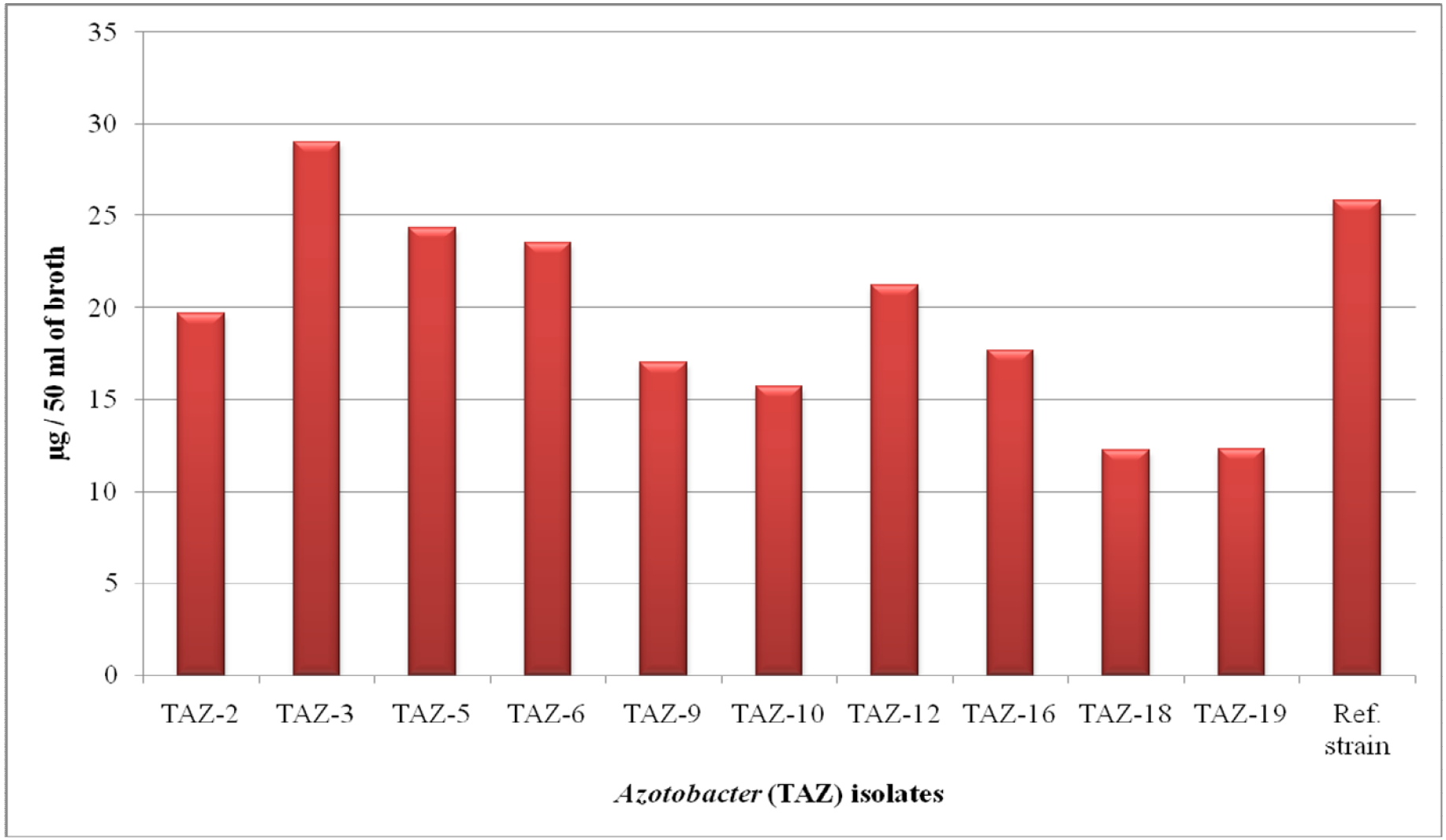


Fig 3. **IAA production by *Azotobacter* isolates under *in vitro* condition**

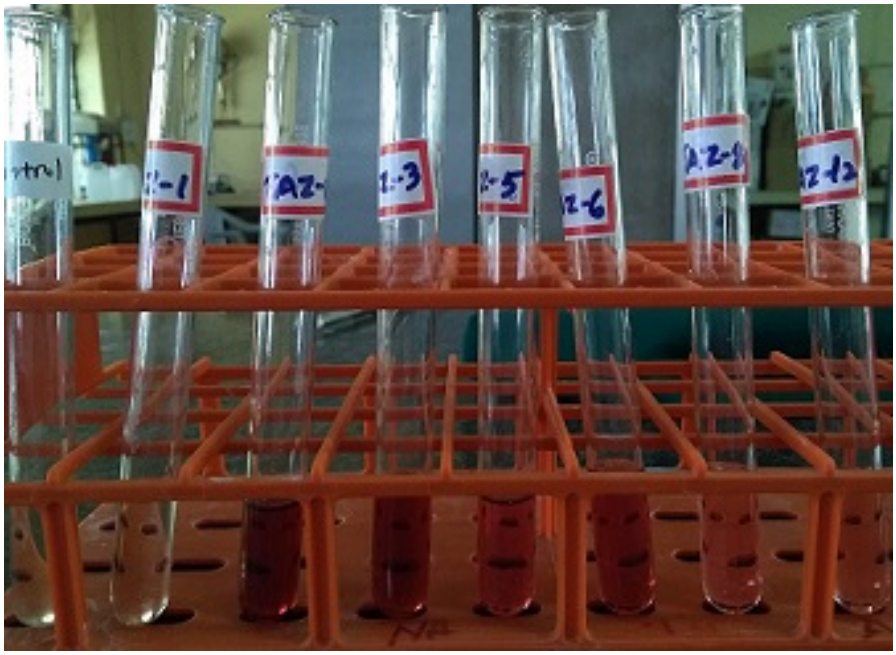


Plate 8. Development of red colour in Ashby's N-free medium supplemented with tryptophan by the *Azotobacter* isolates indicating positive result for IAA production.

well as in combination with one another. For all the treatments 75 per cent RDF was applied in common except for that of control to which 100 per cent RDF was applied. The general view of the plants in pots is shown in the plate 9.

4.7.1 Effect of efficient *Azotobacter* isolate in combination with PGPMs on the height of the tomato plants.

The plant height was recorded at 30 DAT, 60 DAT and 90 DAT stages. Among all the treatments, T_8 (*Azotobacter* + *Pseudomonas* sp. + Mycorrhiza + 75 % RDF) showed more growth compared to all other treatments significantly.

At each interval, the treatment T_8 (*Azotobacter* (TAZ-3) + *Pseudomonas* sp. + Mycorrhiza + 75% RDF) showed the highest height of 34.42 cm, 63.91 cm and 74.39 cm and was significant over the treatment T_9 (75 % RDF + Ref. *Azotobacter* isolate + *Pseudomonas* sp. + Mycorrhiza*)* (33.49 cm, 62.48 cm and 72.78 cm) at 30 DAT, 60 DAT and 90 DAT respectively. The control with 100% RDF (T_1) was recorded with a height of 32.80 cm, 61.22 cm and 71.53 cm at 30 DAT, 60 DAT and 90 DAT respectively which was on par with T_9. The lowest plant height was seen in T_4 (75 % RDF + *Pseudomonas* sp.) (28.00 cm, 52.57 cm and 63.38 cm) (Table 7) (Fig. 4) (Plate 10).

Treating roots of tomato seedlings with *Azotobacter* cultures accelerates plant growth due to the auxins, gibberellin-like substances and cytokinin like substances. And also, the increase in the nitrogen content helps in the increase of plant height, number of branches per plant and also in the fruit production.

This was concluded by Rosario and Barea (1975) in their study on tomato. Similar results were obtained by Sabannanavar *et al.* (2008) as they stated that inoculation of AM fungi + *Azotobacter chroococcum* + *Pseudomonas fluorescens,* to DS 1 and E 8 varieties of sesamum increased AMF colonization, plant growth, *i.e.* shoot, root length, fresh and dry weight of shoot and root, phosphorus uptake and number of capsules significantly over the dual and single inoculation treatments. Also, the results obtained by the current study were in accordance with Jadhav and Gangawane (2014).

Table 7. Influence of PGPMs on the height of tomato plants under pot culture conditions

Treatment	Plant height (cm)		
	30 DAT	**60 DAT**	**90 DAT**
T_1 - 100 % RDF	32.80	61.22	71.53
T_2 - 75 % RDF + *Azotobacter* isolate (TAZ-3)	30.83	58.32	67.63
T_3 - 75 % RDF + Reference *Azotobacter* strain	29.07	56.09	66.04
T_4 - 75 % RDF + *Pseudomonas* sp.	28.00	52.57	63.38
T_5- 75 % RDF + Mycorrhiza	28.76	54.71	64.50
T_6 - 75 % RDF + *Azotobacter* isolate (TAZ-3) + *Pseudomonas* sp.	31.98	59.83	69.85
T_7 - 75 % RDF + *Azotobacter* isolate (TAZ-3) + Mycorrhiza	31.13	58.91	68.38
T_8 - 75 % RDF + *Azotobacter* isolate (TAZ-3) + *Pseudomonas* sp. + Mycorrhiza	34.42	63.91	74.39
T_9 - 75 % RDF + Ref. *Azotobacter* strain + *Pseudomonas* sp. + Mycorrhiza	33.49	62.48	72.78
S.Em±	**0.18**	**0.34**	**0.39**
C.D. at 1%	**0.53**	**1.01**	**1.18**

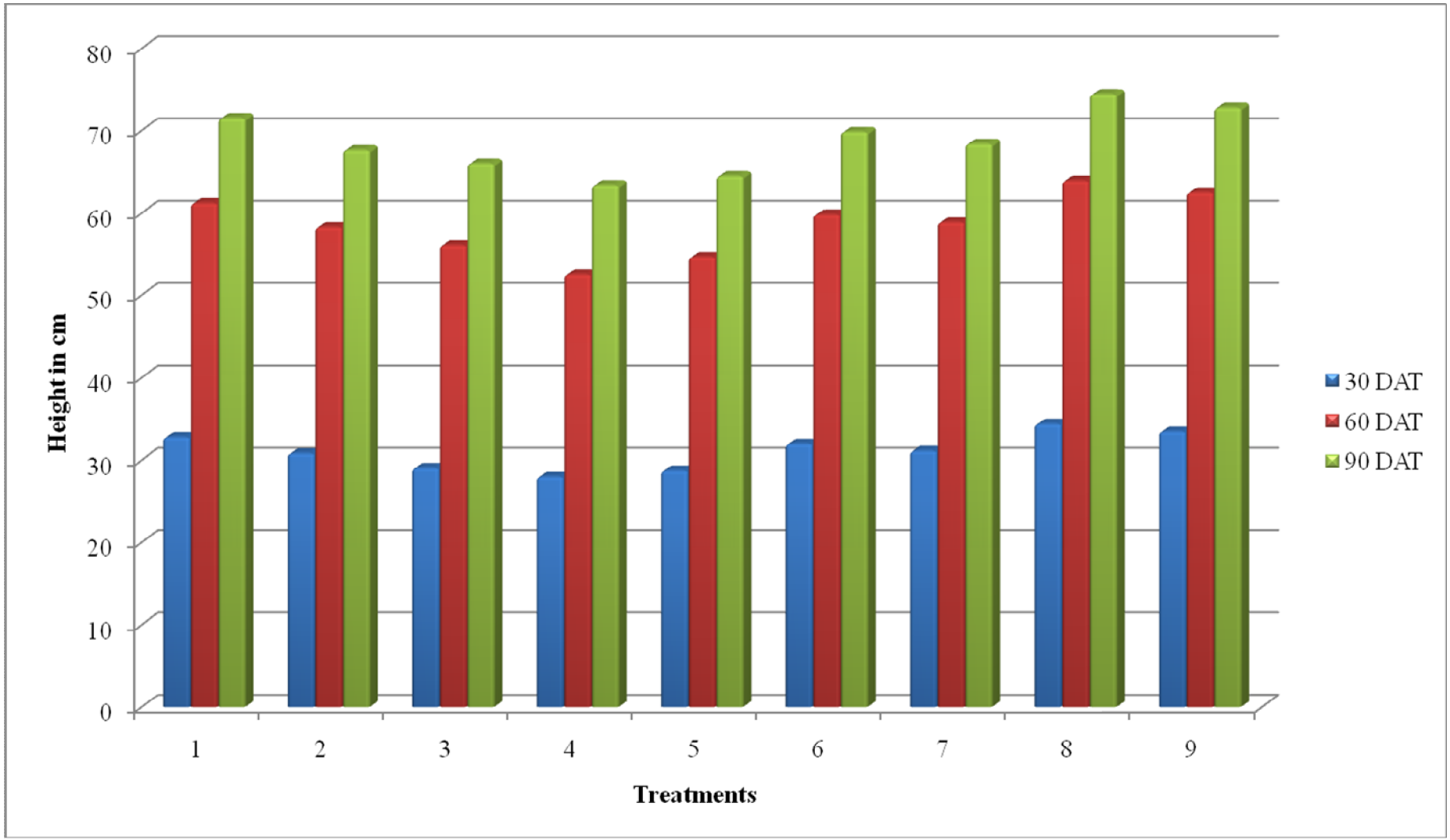


Fig 4. Effect of PGPMs on the height of tomato plants



Plate 9. General view of the pot culture plants

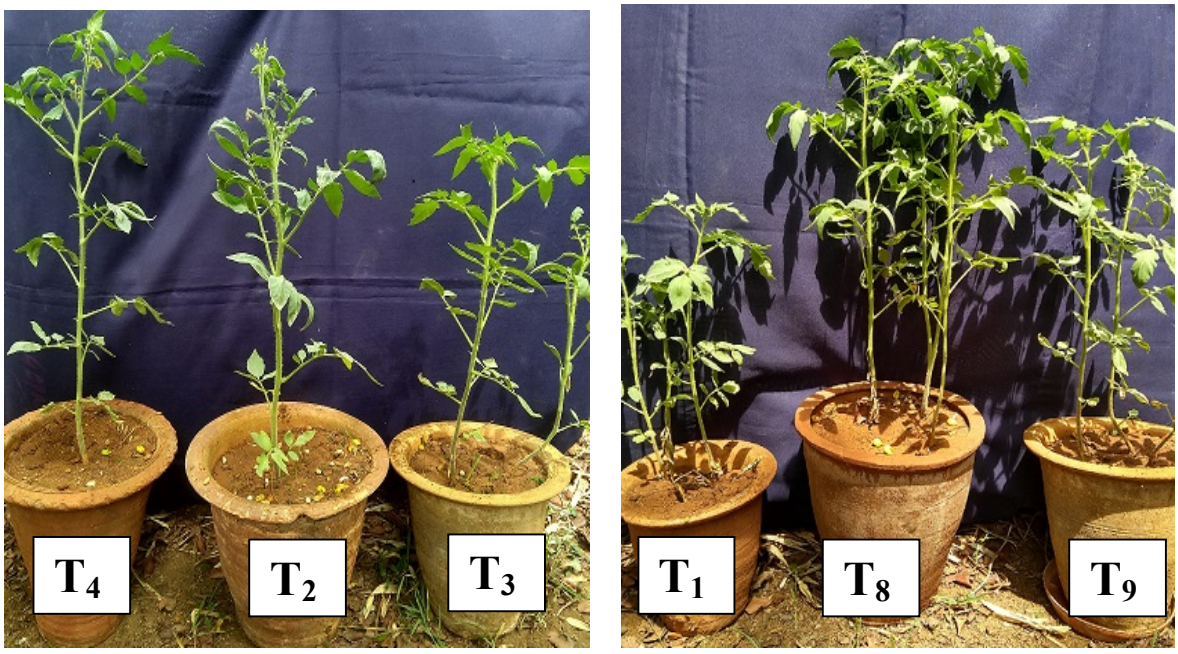


Plate 10. Effect of different treatments on the tomato plants

4.7.2 Effect of efficient *Azotobacter* isolate in combination with PGPMs on the number of branches tomato.

Similar to the plant height, the branches were counted and noted at 30 DAT, 60 DAT and 90 DAT stages (Table 8) (Fig. 5).

At 30 DAT, all treatments which were inoculated with PGPR isolates performed significantly, the number of branches per plant was found to significantly more in the treatment T_8 (*Azotobacter* (TAZ-3) + *Pseudomonas* sp. + Mycorrhiza + 75% RDF) with (6.33 branches per plant) compared to T_1-control (with 100% RDF) (6.00 branches / plant) and followed by T_9 (75 % RDF + Ref. *Azotobacter* isolate + *Pseudomonas* sp. + Mycorrhiza) and T_7 (75 % RDF + *Azotobacter* isolate (TAZ-3) + Mycorrhiza) with (5.67 branches / plant) and (4.67 branches / plant) respectively.

At 60 DAT, number of branches per plant significantly increased in the treatment T_8 (*Azotobacter* (TAZ-3) + *Pseudomonas* sp. + Mycorrhiza + 75% RDF) with (8.67 branches per plant) compared to T_1-control (with 100% RDF) (8.33 branches / plant) and followed by T_9 (75 % RDF + Ref. *Azotobacter* isolate + *Pseudomonas* sp. + Mycorrhiza) and T_7 (75 % RDF + *Azotobacter* isolate (TAZ-3) + Mycorrhiza) with (8.00 branches / plant) and (7.67 branches / plant) respectively.

Finally, 90 DAT, the highest number of branches per plant were observed in the treatment T_8 (*Azotobacter* (TAZ-3) + *Pseudomonas* sp. + Mycorrhiza + 75% RDF) with 10.00 branches per plant as compared with T_1-control (100% RDF) (9.33 branches / plant) and followed by T_9 (75 % RDF + Ref. *Azotobacter* isolate + *Pseudomonas* sp. + Mycorrhiza) and T_7 (75 % RDF + *Azotobacter* isolate (TAZ-3) + Mycorrhiza) with (9.67 branches / plant) and (8.67 branches / plant) respectively. T_9 was on par with the treatment T_1 and T_8 and T_9 were significantly higher when compared to the treatments T_6 (75 % RDF + *Azotobacter* isolate (TAZ-3) + *Pseudomonas* sp.) (8.33 branches / plant) and T_7 which were inoculated with only two PGPMs. The increase in number of branches in the consortium was may be due to the more nitrogen uptake, more production of plant growth promoting substances like IAA, auxins *etc*., increased phosphorus uptake and many other factors contributed by the PGPRs.

This was supported by the study made by Sharma and Thakur (2001), who stated that better vegetative growth may be because of the direct effect of biofertilizers in

Table 8. Influence of PGPMs on the number of branches of tomato plants under pot culture conditions

Treatment	Branches per plant		
	30 DAT	60 DAT	90 DAT
T_1 - 100 % RDF	6.00	8.33	9.33
T_2 - 75 % RDF + *Azotobacter* isolate (TAZ-3)	3.67	6.00	7.33
T_3 - 75 % RDF + Reference *Azotobacter* strain	3.33	5.67	7.00
T_4 - 75 % RDF + *Pseudomonas* sp.	3.33	5.33	6.67
T_5- 75 % RDF + Mycorrhiza	4.00	6.33	7.67
T_6 - 75 % RDF + *Azotobacter* isolate (TAZ-3) + *Pseudomonas* sp.	4.33	7.33	8.33
T_7 - 75 % RDF + *Azotobacter* isolate (TAZ-3) + Mycorrhiza	4.67	7.67	8.67
T_8 - 75 % RDF + *Azotobacter* isolate (TAZ-3) + *Pseudomonas* sp. + Mycorrhiza	6.33	8.67	10.00
T_9 - 75 % RDF + Ref. *Azotobacter* strain + *Pseudomonas* sp. + Mycorrhiza	5.67	8.00	9.67
S.Em±	**0.40**	**0.52**	**0.56**
C.D. at 1%	**1.20**	**1.56**	**1.66**

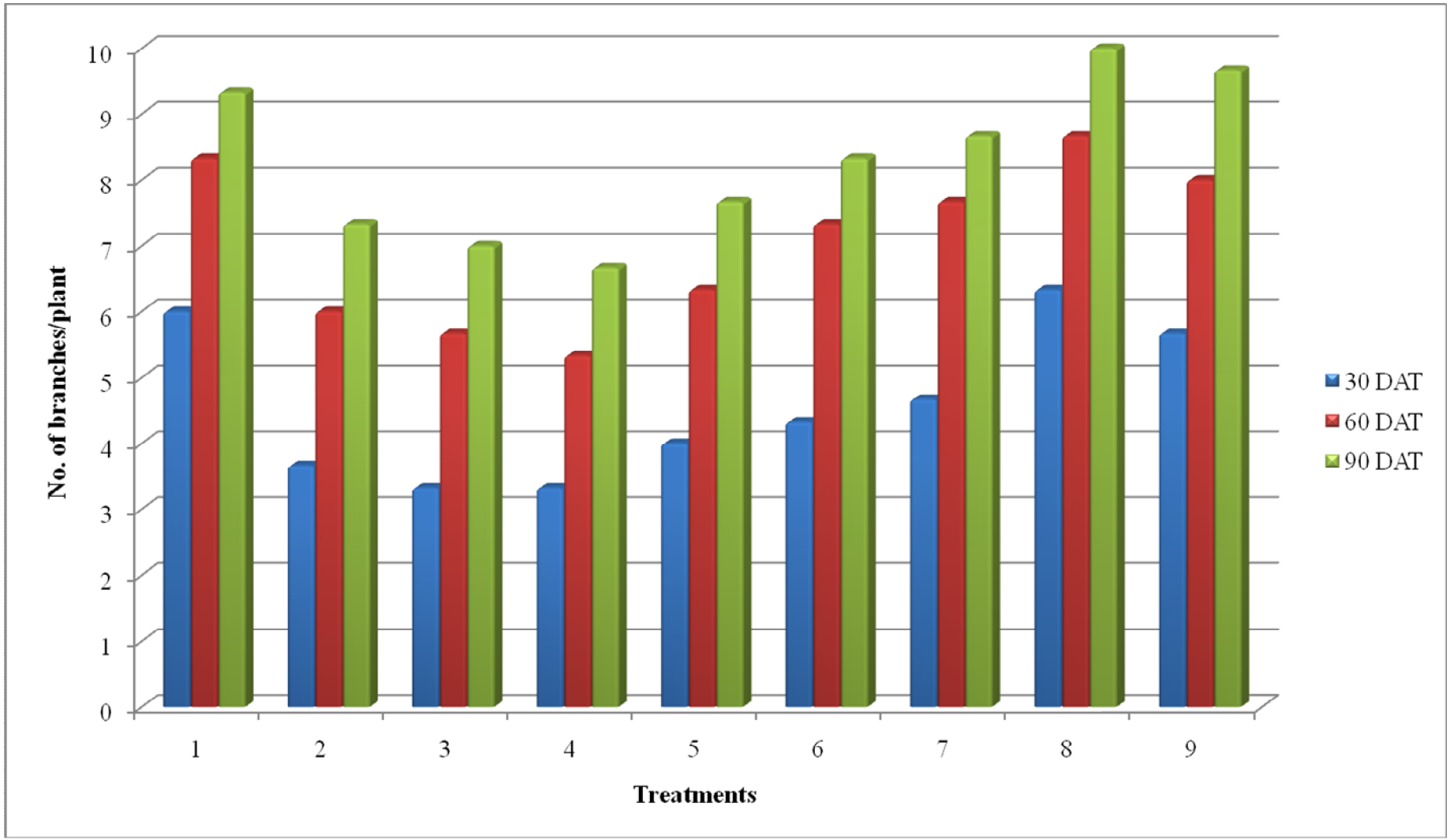


Fig 5. Effect of PGPMs on the branch number of tomato plants

improving soil fertility status by bacterial activity which results in increased uptake of nitrogen and other nutrients. The results were also similar to that of Spandan *et al.* (2018), Pathak *et al.* (1975) and Wang *et al.* (1995).

4.7.3 Effect of efficient *Azotobacter* isolate in combination with PGPMs on the shoot biomass of tomato.

The shoot biomass was calculated by recording the fresh weight and dry weight of the shoots at 90 DAT. As in case of height and number of branches, the shoot biomass was also influenced and improved by the inoculation of PGPRs.

The maximum shoot fresh weight at 90 DAT was recorded in the treatment T_8 (*Azotobacter* (TAZ-3) + *Pseudomonas* sp. + Mycorrhiza + 75% RDF) with 68.85 g per plant which was significantly higher as compared to T_1-Control (100% RDF) with 62.16 g per plant. The treatment T_9 (75 % RDF + Ref. *Azotobacter* isolate + *Pseudomonas* sp. + Mycorrhiza) was on par with the RDF with a fresh shoot weight of 63.77 g per plant. The lowest shoot fresh weight was seen in the treatment T_4 (75 % RDF + *Pseudomonas* sp.) with a weight of 43.43 g per plant.

Similarly, the highest shoot dry weight at 90 DAT was also seen in the treatment T_8 (*Azotobacter* (TAZ-3) + *Pseudomonas* sp. + Mycorrhiza + 75% RDF) with 19.42 g per plant as compared to T_1-Control (100% RDF) with 18.60 g per plant. That was followed by T_9 (75 % RDF + Ref. *Azotobacter* isolate + *Pseudomonas* sp. + Mycorrhiza) with a dry shoot weight of 19.03 g per plant. The lowest shoot dry weight was seen in the treatment T_4 (75 % RDF + *Pseudomonas* sp.) with a weight of 13.76 g per plant.

The biomass was highest in the treatment inoculated by the consortium of the PGPRs i.e., T_8 (*Azotobacter* (TAZ-3) + *Pseudomonas* sp. + Mycorrhiza + 75% RDF) with 71.78 %. This treatment was significantly high over the T_1-control (100% RDF) with 70.06 % and all other treatments. T_8 was proceeded by T_9 (75 % RDF + Ref. *Azotobacter* isolate + *Pseudomonas* sp. + Mycorrhiza) with 70.15 % which was on par with T_1 (control) and the lowest shoot biomass was seen in T_4 (75 % RDF + *Pseudomonas* sp.) with 68.30 % (Table 9) (Fig. 6). More shoot biomass was observed in the treatment with consortium (T_8) and this was may be due to the synergistic effect of bacterium and the

Table 9. Influence of PGPRs on shoot biomass (90 DAT) of tomato plants under pot culture conditions

Treatments	Fresh shoot weight(g)	Dry shoot weight(g)	Shoot biomass (%)
T_1 - 100 % RDF	62.16	18.60	70.06
T_2 - 75 % RDF + *Azotobacter* isolate (TAZ-3)	47.74	14.67	69.26
T_3 - 75 % RDF + Reference *Azotobacter* strain	45.95	14.35	68.76
T_4 - 75 % RDF + *Pseudomonas* sp.	43.43	13.76	68.30
T_5- 75 % RDF + Mycorrhiza	58.02	17.36	70.06
T_6 - 75 % RDF + *Azotobacter* isolate (TAZ-3) + *Pseudomonas* sp.	52.92	16.04	69.67
T_7 - 75 % RDF + *Azotobacter* isolate (TAZ-3) + Mycorrhiza	54.83	16.39	70.11
T_8 - 75 % RDF + *Azotobacter* isolate (TAZ-3) + *Pseudomonas* sp. + Mycorrhiza	68.85	19.42	71.78
T_9 - 75 % RDF + Ref. *Azotobacter* strain + *Pseudomonas* sp. + Mycorrhiza	63.77	19.03	70.15
S.Em±	**0.81**	**0.10**	**0.39**
C.D. at 1%	**2.41**	**0.30**	**1.16**

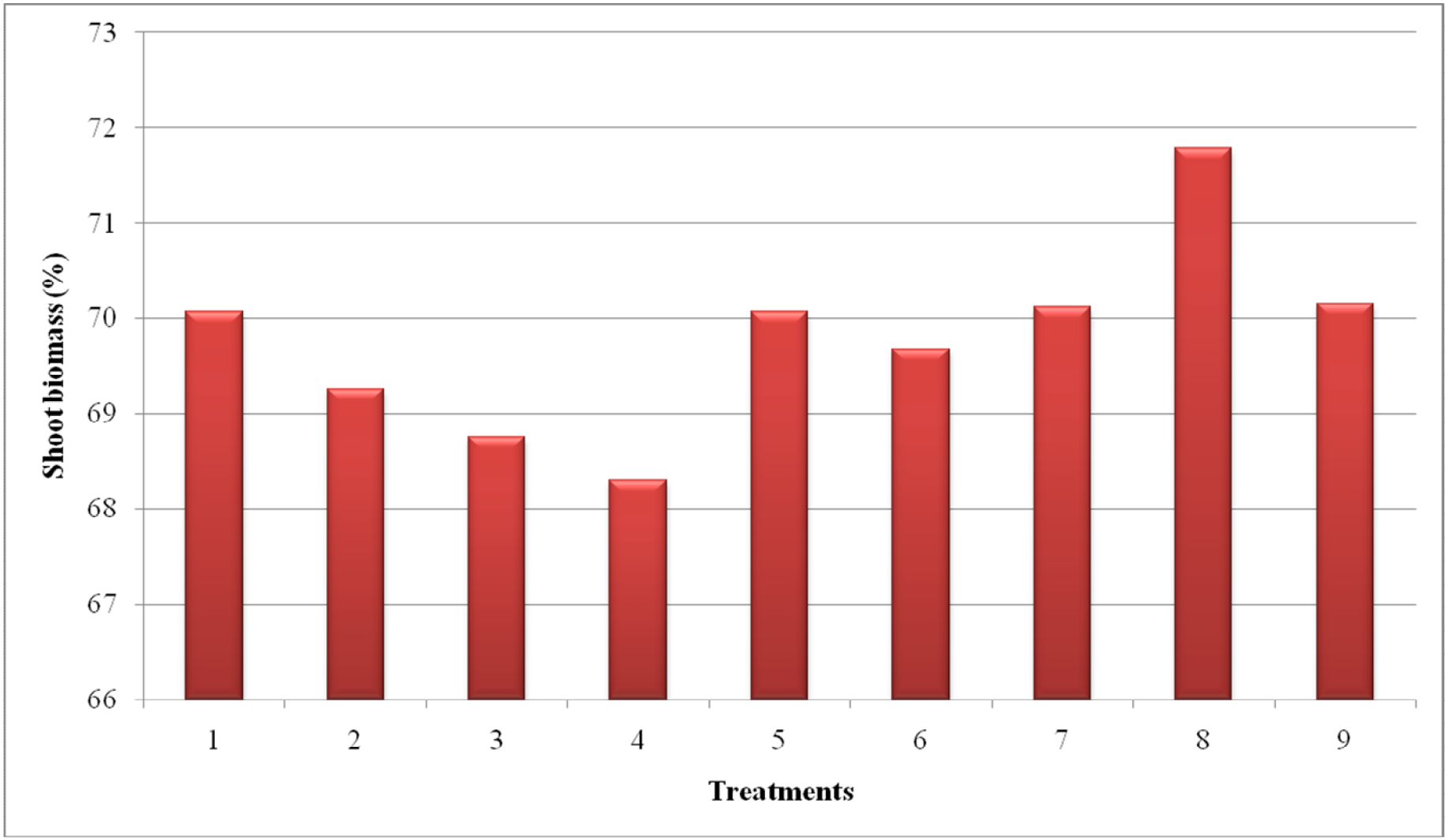


Fig 6. Effect of PGPMs on the shoot biomass of tomato plants (90 DAT)

AM fungi which increased the nitrogen content and uptake, more root colonisation, more phosphorous availability and other beneficial factors attributed by the PGPRs.

These results were strongly backed up by the studies of Sukhada (1987) who, through his experiment found that VAM fungi and *Azotobacter* increased shoot dry weight, leaf area, nitrogen content, phosphorus content and yield in respect to uninoculated control. The VAM fungi alone didn't show much increase but in combination with *Azotobacter*, there were additional effects on shoot dry weight, leaf area, phosphorus content and yield. The improvement on vegetative growth of the plant due to the combined application of AM fungi, *Azotobacter* and *Pseudomonas* isolates has been further reported by Kim *et al.* (1998), Bagyaraj and Menge (1978) and Prathibha (1993).

4.7.4 Effect of efficient *Azotobacter* isolate in combination with PGPMs on the root biomass of tomato.

The root biomass was also taken at 90 DAT just like the shoot biomass. The highest root fresh weight at 90 DAT was recorded in the treatment T_8 (*Azotobacter* (TAZ-3) + *Pseudomonas* sp. + Mycorrhiza + 75% RDF) with 10.11 g per plant as compared to T_1-Control (100% RDF) with 9.10 g per plant. The treatment T_9 (75 % RDF + Ref. *Azotobacter* isolate + *Pseudomonas* sp. + Mycorrhiza) had the next best root fresh weight of 9.63 g per plant. The lowest root fresh weight was seen in the treatment T_4 (75 % RDF + *Pseudomonas* sp.) with a weight of 5.81 g per plant.

In the same way, the highest root dry weight at 90 DAT was also seen in the treatment T_8 (*Azotobacter* (TAZ-3) + *Pseudomonas* sp. + Mycorrhiza + 75% RDF) with 4.20 g per plant as compared to T_1-Control (100% RDF) with 4.10 g per plant. That was followed by T_9 (75 % RDF + Ref. *Azotobacter* isolate + *Pseudomonas* sp. + Mycorrhiza) with a dry shoot weight of 4.08 g per plant. The lowest root dry weight was seen in the treatment T_4 (75 % RDF + *Pseudomonas* sp.) with a weight of 3.05 g per plant.

So accordingly, the root biomass was highest in the treatment inoculated by the consortium of the PGPMs *i.e.,* T_8 (*Azotobacter* (TAZ-3) + *Pseudomonas* sp. + Mycorrhiza + 75% RDF) with 58.05 %. This treatment was significantly high over the T_1-control (100% RDF). The next highest biomass was seen in the treatment T_9 (75 % RDF + Ref. *Azotobacter* isolate + *Pseudomonas* sp. + Mycorrhiza) with 56.60 % and the

Table 10. Influence of PGPRs on root biomass (90 DAT) of tomato plants under pot culture conditions

Treatments	Fresh root weight(g)	Dry root weight(g)	Root biomass (%)
T_1 - 100 % RDF	9.10	4.10	54.97
T_2 - 75 % RDF + *Azotobacter* isolate (TAZ-3)	6.95	3.33	50.66
T_3 - 75 % RDF + Reference *Azotobacter* strain	6.43	3.25	49.47
T_4 - 75 % RDF + *Pseudomonas* sp.	5.81	3.05	47.55
T_5- 75 % RDF + Mycorrhiza	8.58	3.84	54.44
T_6 - 75 % RDF + *Azotobacter* isolate (TAZ-3) + *Pseudomonas* sp.	7.77	3.65	53.03
T_7 - 75 % RDF + *Azotobacter* isolate (TAZ-3) + Mycorrhiza	8.13	3.76	53.74
T_8 - 75 % RDF + *Azotobacter* isolate (TAZ-3) + *Pseudomonas* sp. + Mycorrhiza	10.11	4.20	58.05
T_9 - 75 % RDF + Ref. *Azotobacter* strain + *Pseudomonas* sp. + Mycorrhiza	9.63	4.08	56.60
S.Em±	**0.12**	**0.02**	**0.41**
C.D. at 1%	**0.35**	**0.07**	**1.21**

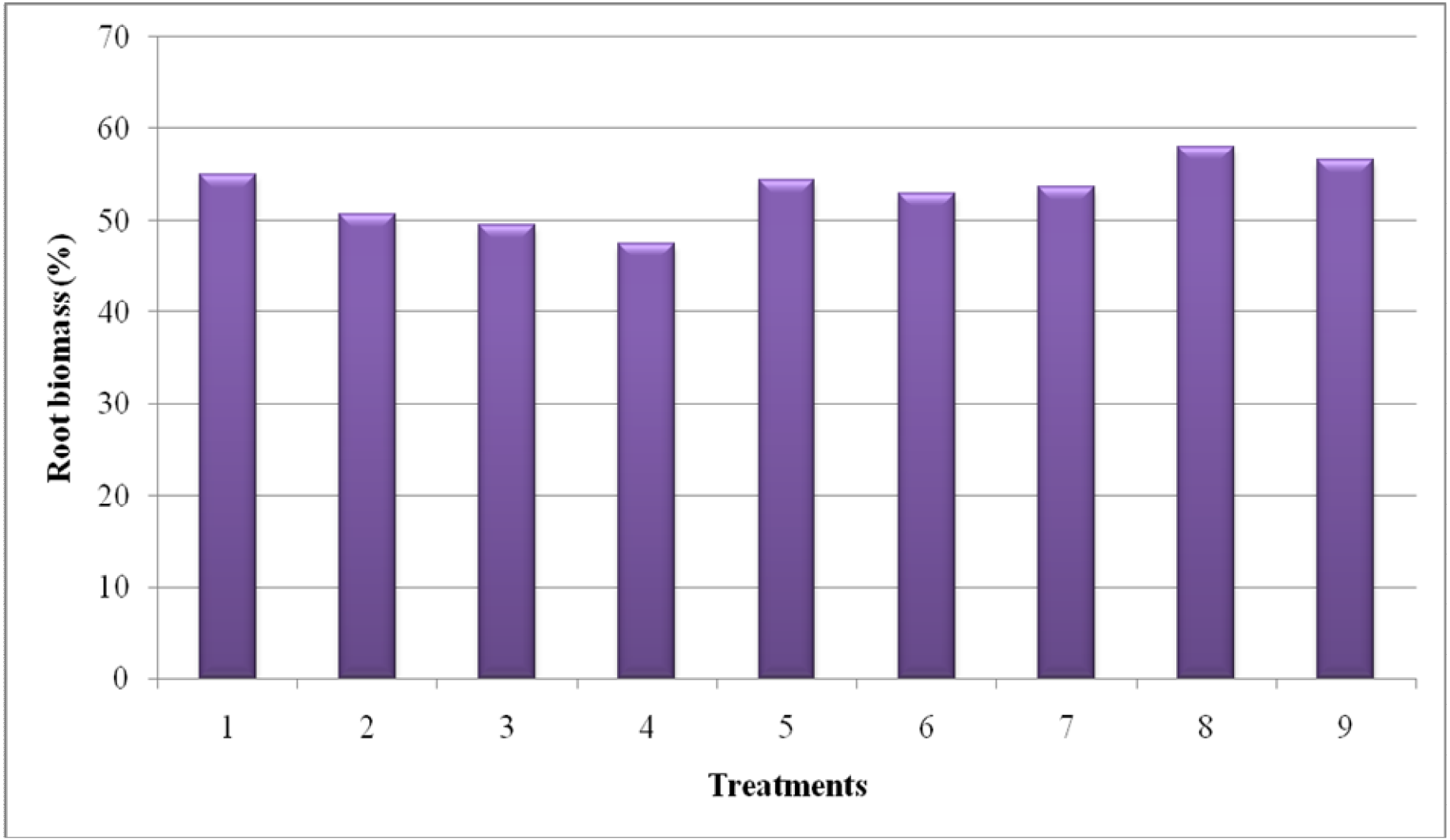


Fig 7. Effect of PGPMs on the root biomass of tomato plants (90 DAT)

lowest shoot biomass was seen in T_4 (75 % RDF + *Pseudomonas* sp.) with 47.55 % (Table 10) (Fig. 7). The possible reason for increased biomass in the combination was may be due to the production of auxins and root colonisation by the PGPMs. Colonization of plant roots by AM fungi significantly influences the mycorrhizosphere microorganisms, including PGPR which helps in the better absorption of mineral salts from the soil.

These results obtained from current study were similar to the research done by Mahdi Zare *et al.* (2011) who investigated the effect of *Azotobacter* chroococum, *Pseudomonas putida* and AM fungi, wrote that *Azotobacter* chroococum was more effective than *Pseudomonas putida* and the combined inoculation produced the higher results than the control or individual inoculants. The results were also conclusive and found correlated with Michael James *et al.* (2018), Sharma and Thakur (2001) and Sabannavar and Lakshman (2011).

4.7.5 Effect of efficient *Azotobacter* isolate in combination with PGPMs on the chlorophyll content tomato.

The chlorophyll content of the leaves was recorded by using SPAD meter and was taken at 30 DAT, 60 DAT and at 90 DAT.

At 30 DAT, all the treatments which were inoculated with PGPMs performed significantly. The chlorophyll content was found to be more in the treatment T_8 (*Azotobacter* (TAZ-3) + *Pseudomonas* sp. + Mycorrhiza + 75% RDF) with a SPAD reading of 38.00 units as compared to T_1-Control (100% RDF) with 36.70 SPAD units and was followed by T_9 (75 % RDF + Ref. *Azotobacter* isolate + *Pseudomonas* sp. + Mycorrhiza) and T_7 (75 % RDF + *Azotobacter* isolate + Mycorrhiza) with 37.30 and 34.20 SPAD units respectively. The lowest chlorophyll content was seen in the treatment T_4 (75 % RDF + *Pseudomonas* sp.) with 29.87 SPAD units.

The chlorophyll content increased significantly at 60 DAT. The highest chlorophyll content was seen in the treatment T_8 (*Azotobacter* (TAZ-3) + *Pseudomonas* sp. + Mycorrhiza + 75% RDF) with 53.43 SPAD units and it was significant when compared to T_1-control (100% RDF) which recorded 50.64 SPAD units. T_8 was followed by T_9 (75 % RDF + Ref. *Azotobacter* isolate + *Pseudomonas* sp. + Mycorrhiza) and T_7 (75 % RDF + *Azotobacter* isolate (TAZ-3) + Mycorrhiza) with 51.33 and 46.33 SPAD

units respectively and T_9 was significant over T_7. The lowest chlorophyll content was seen in the treatment T_4 (75 % RDF + *Pseudomonas* sp.) with 40.57 SPAD units. As mentioned earlier, beneficial effects reported for PGPMs include increase in a number of parameters such as germination rate, root and shoot growth (length and/or weight), yield, leaf area, chlorophyll content, nutrients and protein uptake. Hence more chlorophyll was seen in the treatment T_8.

And at 90 DAT, the chlorophyll content was seen low and reduced significantly. Even then, the highest chlorophyll content was seen in the treatment T_8 (*Azotobacter* (TAZ-3) + *Pseudomonas* sp. + Mycorrhiza + 75% RDF) with 24.96 SPAD units and it was more. That was followed by T_9 (75 % RDF + Ref. *Azotobacter* isolate + *Pseudomonas* sp. + Mycorrhiza) and T_7 (75 % RDF + *Azotobacter* isolate (TAZ-3) + Mycorrhiza) with 24.10 and 21.10 SPAD units respectively. The chlorophyll content in the treatment T_1-Control (100 % RDF) was noted to be 23.83 SPAD units. The lowest chlorophyll content was seen in the treatment T_4 (75 % RDF + *Pseudomonas* sp.) with 18.78 SPAD units (Table 11) (Fig. 8). The decrease in the chlorophyll content at 90 DAT was may be due to the fact that the depletion of nutrients and most of the nutrients will be concentrated in the fruits during harvest.

The results of the present study were found to be similar with Gmaa (2015), who evaluated the effect of application of biofertilizers (nitrobin, phosphorin and potassumage) on tomato and concluded that the nitrobin biofertilizer enhanced the chlorophyll content and growth characters, *i.e.,* plant height, leaves number, plant fresh weight of tomato especially when combined with phosphorin and potassumage as compared with control or without biofertilizer treatments. The chlorophyll content presented in this study was in line with the experiments done by Kabir *et al.* (2013) who wrote that there was a significant increase the shoot dry weight, root dry weight, chlorophyll content of the tomato plants under greenhouse inoculated with bacterial strains than the uninoculated control.

4.8 Per cent AM root colonization (PRC) and spore count

The percent root colonization and spore count were determined at 90 DAT. The highest root colonisation was seen in the treatment T_8 (*Azotobacter* (TAZ-3) + *Pseudomonas* sp. + Mycorrhiza + 75% RDF) with 86.80 percent was significantly high

Table 11. Influence of PGPMs on the chlorophyll content of tomato plants under pot culture conditions

Treatment	Chlorophyll content (SPAD units)		
	30 DAT	**60 DAT**	**90 DAT**
T_1 - 100 % RDF	36.70	50.64	23.83
T_2 - 75 % RDF + *Azotobacter* isolate (TAZ-3)	32.50	43.23	21.01
T_3 - 75 % RDF + Reference *Azotobacter* strain	31.47	41.67	19.73
T_4 - 75 % RDF + *Pseudomonas* sp.	29.80	40.57	18.78
T_5- 75 % RDF + Mycorrhiza	30.37	41.50	19.06
T_6 - 75 % RDF + *Azotobacter* isolate (TAZ-3) + *Pseudomonas* sp.	33.10	45.50	20.59
T_7 - 75 % RDF + *Azotobacter* isolate (TAZ-3) + Mycorrhiza	34.20	46.63	21.16
T_8 - 75 % RDF + *Azotobacter* isolate (TAZ-3) + *Pseudomonas* sp. + Mycorrhiza	38.00	53.43	24.96
T_9 - 75 % RDF + Ref. *Azotobacter* strain + *Pseudomonas* sp. + Mycorrhiza	37.30	51.33	24.10
S.Em±	**0.83**	**0.80**	**0.60**
C.D. at 1%	**2.49**	**2.40**	**1.80**

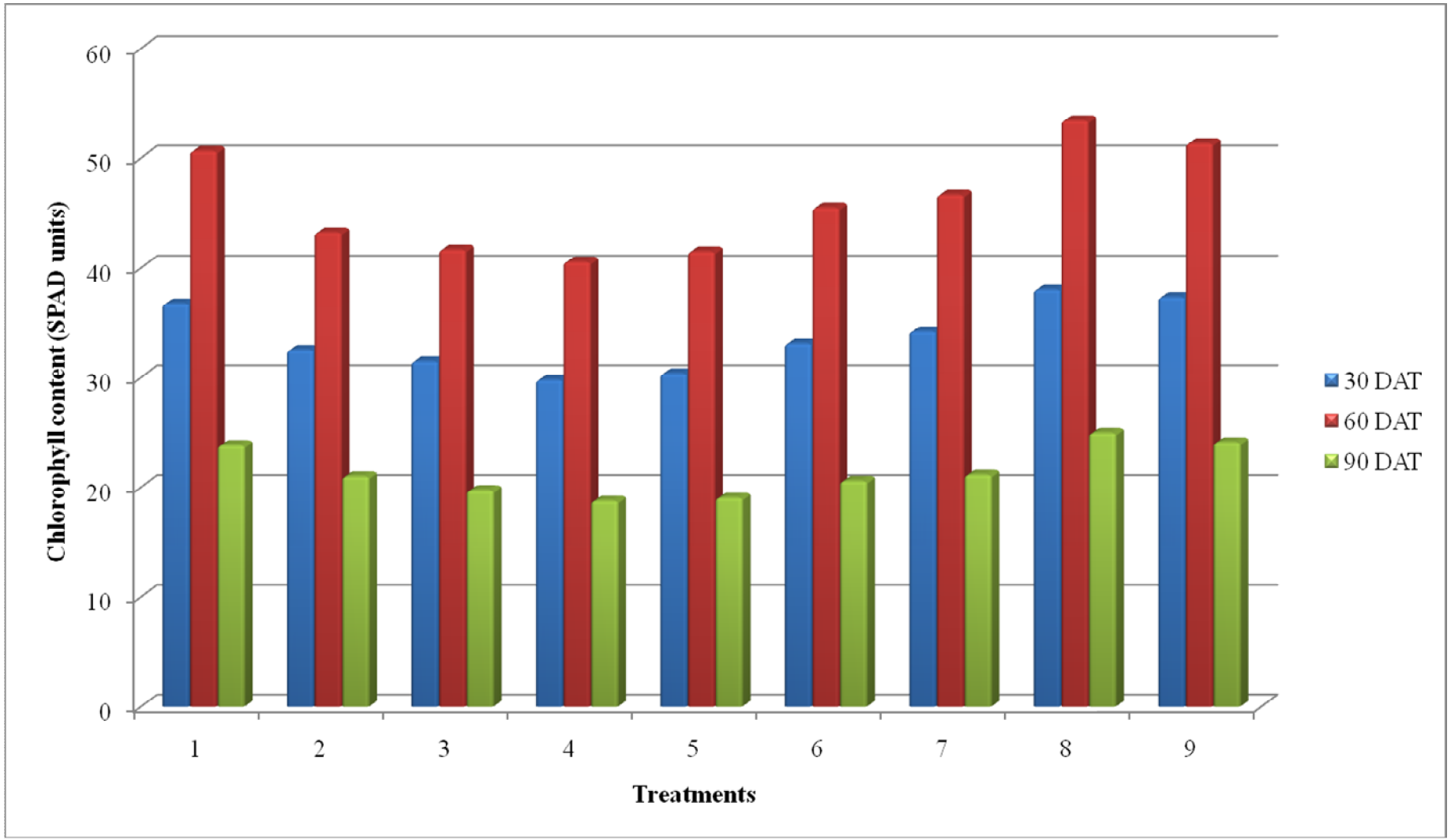


Fig 8. Effect of PGPMs on the chlorophyll content of tomato

over T_9 (75 % RDF + Ref. *Azotobacter* isolate + *Pseudomonas* sp. + Mycorrhiza) with 84.91 percent. The treatment T_5 (75 % RDF + Mycorrhiza) showed the colonisation percent to be 79.44. The treatments T_2 (75 % RDF + *Azotobacter* isolate (TAZ-3) and T_3 (75 % RDF + Ref. *Azotobacter* isolate) were on par with the values of 69.34 percent and 68.26 percent respectively.

Spore count was seen significantly more in the treatment T_8 with 111.98 and was followed by T_9 with 107.07. Lowest spore count was seen in the treatment T_4 (75 % RDF + *Pseudomonas* sp.) with 53.06 (Table 12) (Fig. 9 & 10). This was may be due to the inoculation of mycorrhiza and the synergistic effects of *Azotobacter* along with mycorrhiza which increased the root colonisation and spore count of mycorrhiza in inoculated treatments rather than in control and uninoculated treatments.

The values for mycorrhizal fungus spore density associated with different plants at different sites have varied greatly in previous reports. Seasonality, edaphic factors, host-dependence, age of the host plants, the sporulation abilities of arbuscular mycorrhizal fungi and the dormancy and the distribution pattern of arbuscular mycorrhizal fungal spores in the soils, have been reported previously by (Walker *et al*., 1982; Sylvia, 1986; Koske, 1987).

4.9 Nitrogen content (%)

The total nitrogen uptake was measured at 90 DAT and the treatment T_8 (*Azotobacter* (TAZ-3) + *Pseudomonas* sp. + Mycorrhiza + 75% RDF) showed highest uptake of 5.17 per cent which was on par with T_9 (75 % RDF + Ref. *Azotobacter* isolate + *Pseudomonas* sp. + Mycorrhiza) with 5.05 per cent, but both T_8 and T_9 were significantly higher when compared to other treatments. The lowest nitrogen uptake was seen in the treatment T_4 (75 % RDF + *Pseudomonas* sp.) with 2.92 per cent (Table 13) (Fig. 11).

The increased uptake of nitrogen in the treatment T_8 was may be due to the combined effect of *Azotobacter* along with Mycorrhiza and *Pseudomonas.* Mycorrhiza, not only solubilise P but also fix nitrogen to a little extent. Also, the increased population of *Azotobacter* may have been helped in higher nitrogen uptake.

Table 12. Mycorrhizal spore count and percent root colonization in roots of tomato (90 DAT)

Treatment	Mycorrhizal spore count	Per cent Root colonization
T_1 - 100 % RDF	61.78	58.03
T_2 - 75 % RDF + *Azotobacter* isolate (TAZ-3)	68.20	69.34
T_3 - 75 % RDF + Reference *Azotobacter* strain	66.34	68.26
T_4 - 75 % RDF + *Pseudomonas* sp.	53.06	50.75
T_5- 75 % RDF + Mycorrhiza	93.31	79.44
T_6 - 75 % RDF + *Azotobacter* isolate (TAZ-3) + *Pseudomonas* sp.	72.20	61.07
T_7 - 75 % RDF + *Azotobacter* isolate (TAZ-3) + Mycorrhiza	104.01	82.28
T_8 - 75 % RDF + *Azotobacter* isolate (TAZ-3) + *Pseudomonas* sp. + Mycorrhiza	111.98	91.39
T_9 - 75 % RDF + Ref. *Azotobacter* strain + *Pseudomonas* sp. + Mycorrhiza	107.07	89.50
S.Em±	**0.73**	**0.46**
C.D. at 1%	**2.19**	**1.38**

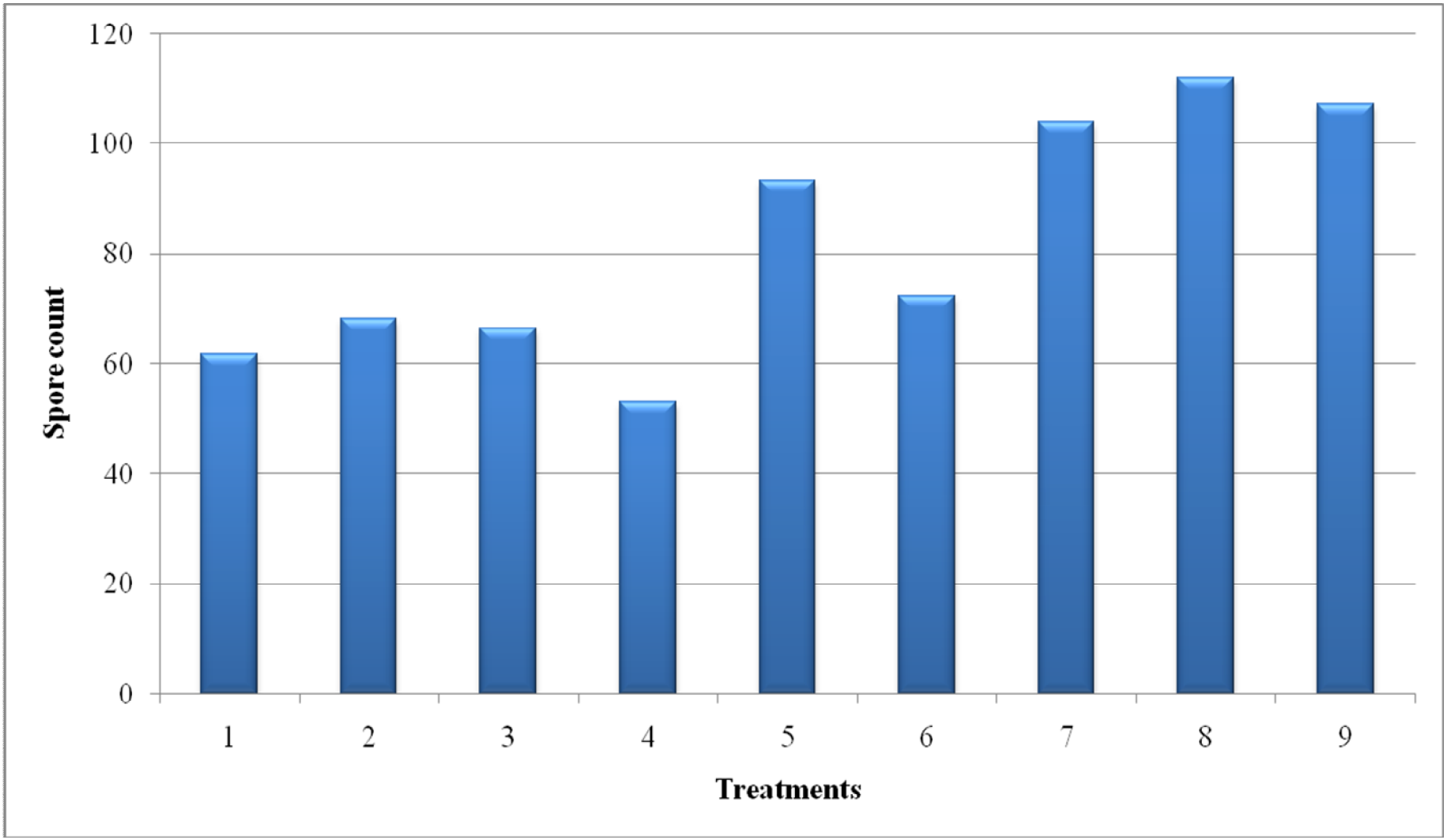


Fig 9. Effect of PGPMs on the Mycorrhizal spore count of tomato (90 DAT)

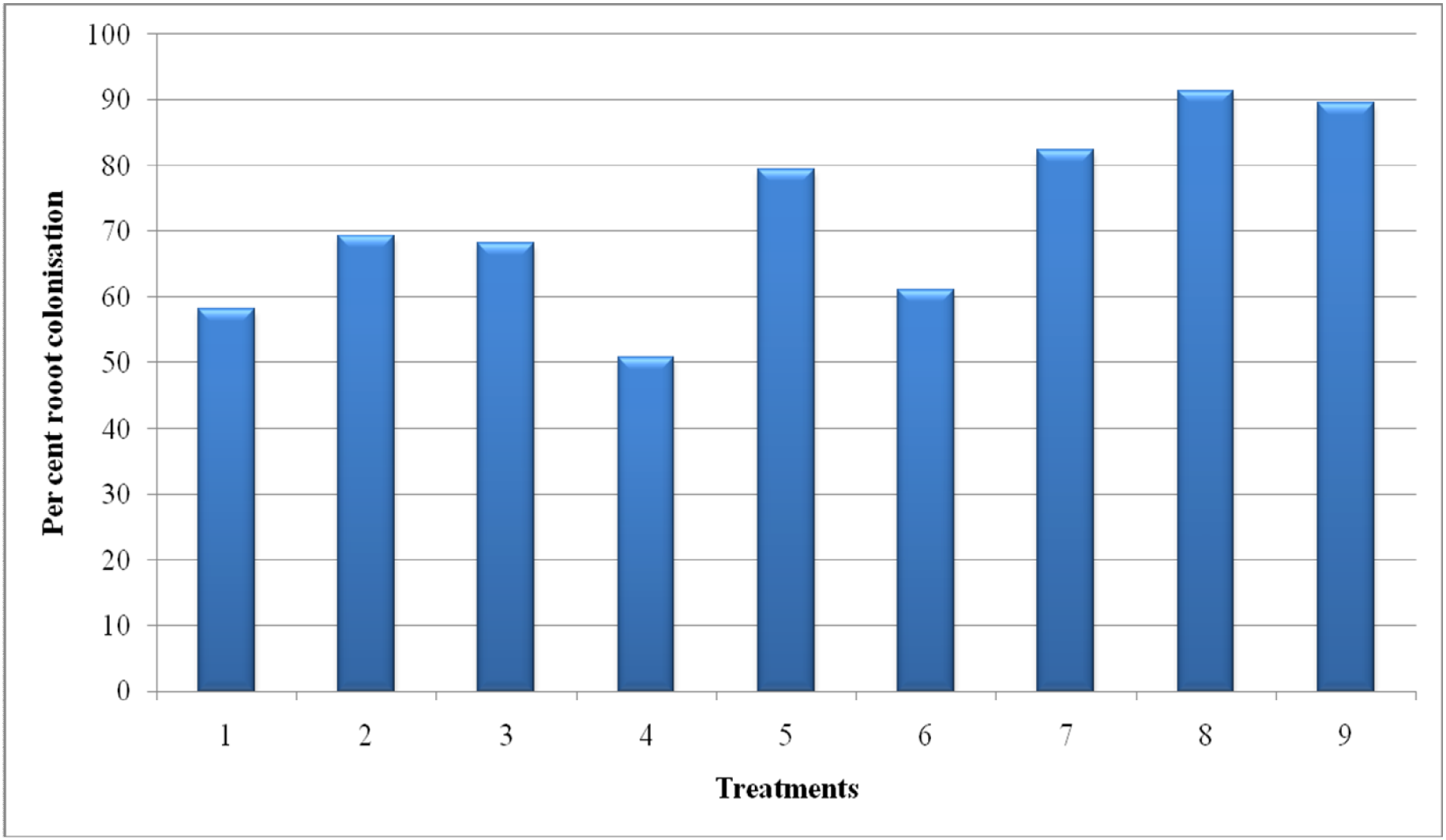


Fig 10. Effect of PGPMs on the percent root colonisation by Mycorrhiza (90 DAT)

Table 13. Influence of PGPMs on the nitrogen content of tomato plants under pot culture conditions

Treatment	N content (%)
T_1 - 100 % RDF	3.83
T_2 - 75 % RDF + *Azotobacter* isolate (TAZ-3)	3.75
T_3 - 75 % RDF + Reference *Azotobacter* strain	3.55
T_4 - 75 % RDF + *Pseudomonas* sp.	2.92
T_5- 75 % RDF + Mycorrhiza	3.06
T_6 - 75 % RDF + *Azotobacter* isolate (TAZ-3) + *Pseudomonas* sp.	4.09
T_7 - 75 % RDF + *Azotobacter* isolate (TAZ-3) + Mycorrhiza	4.86
T_8 - 75 % RDF + *Azotobacter* isolate (TAZ-3) + *Pseudomonas* sp. + Mycorrhiza	5.17
T_9 - 75 % RDF + Ref. *Azotobacter* strain + *Pseudomonas* sp. + Mycorrhiza	5.05
SEm±	**0.03**
CD 1%	**0.10**

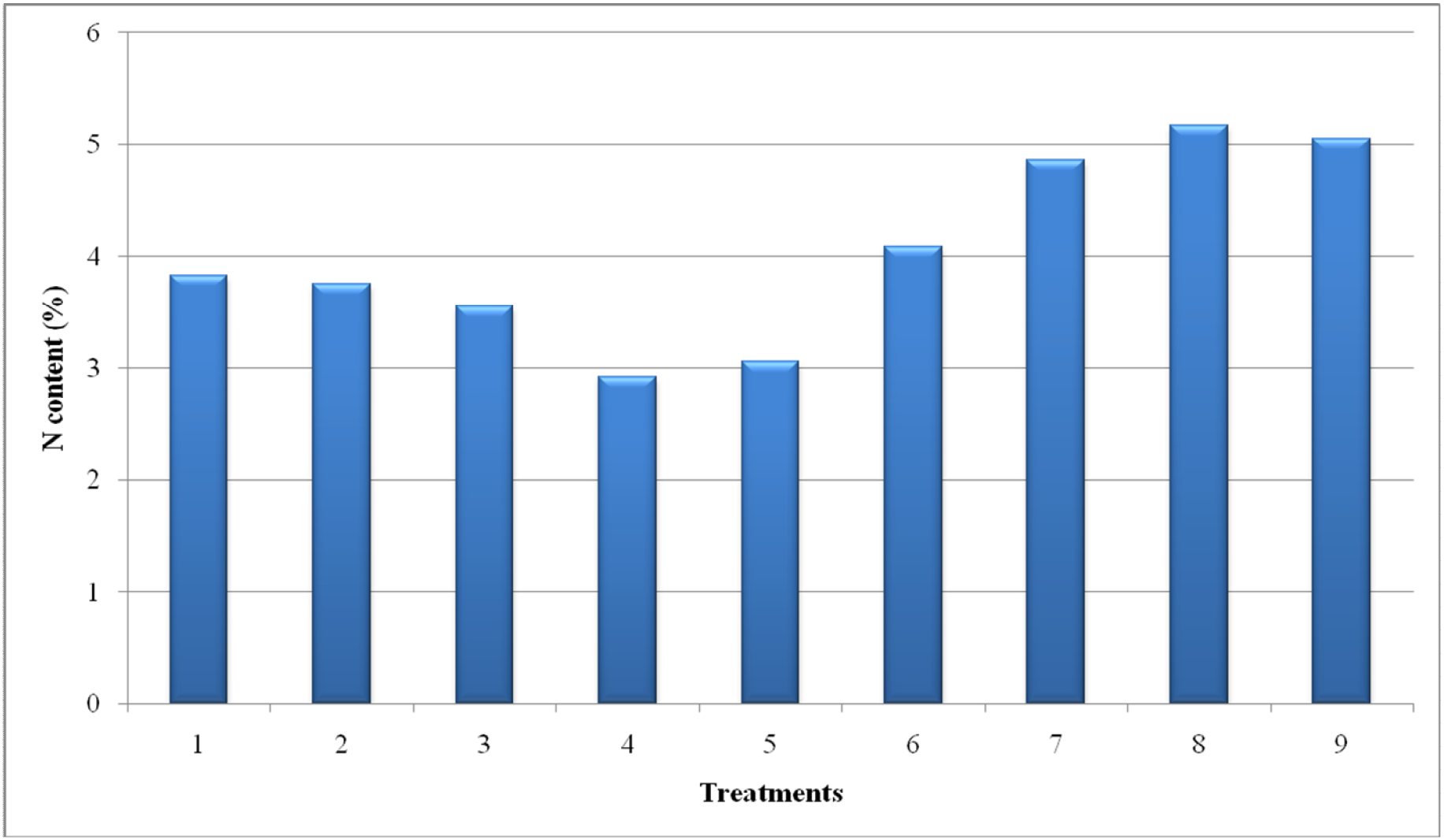


Fig 11. Effect of PGPMs on the nitrogen content of tomato plants (90 DAT)

Bagyaraj and Menge, 1978, reported that mycorrhizal colonization may alter roots physiologically, and may result in a change in the microbial population. This led to the increased uptake of nitrogen and phosphorus by tomato plants. Sukhada Mohandas (1987) also, reported that Inoculation with *G. fasiculatum* alone brought about substantial increase in growth, nitrogen content, phosphorus content and yield. Combined inoculation further improved the growth, phosphorus content and yield.

4.10 Phosphorus content (%)

Similar to the nitrogen uptake, the phosphorus uptake was also measured at 90 DAT. The treatment T_8 (*Azotobacter* (TAZ-3) + *Pseudomonas* sp. + Mycorrhiza + 75% RDF) showed highest P uptake with 0.99 per cent and was significantly higher than all the other treatments. It was followed by T_9 (75 % RDF + Ref. *Azotobacter* isolate + *Pseudomonas* sp. + Mycorrhiza) with 0.94 per cent. The treatment T_1 (100% RDF) recorded the phosphorus uptake of 0.52 per cent. The lowest phosphorus uptake was seen in the treatment T_3 (75 % RDF + Reference *Azotobacter* strain) with 0.50 per cent (Table 14) (Fig. 12).

The increase in phosphorus uptake was because of the inoculation of *Pseudomonas* sp. and mycorrhiza, both of them which are capable of solubilising phosphorus and increase the uptake of phosphorus by the plants and also, the synergistic effect of *Azotobacter* and mycorrhiza, which increases the population of phosphate-solubilising microorganisms in the rhizosphere due to the production of growth promoting substances.

These results were supported by Kim *et al.* (1998) who, concluded that the inoculum containing both *Enterobacter agglomerans* and *Glomus etunicatum* led to a significant increase in total P and N uptake and plant growth. *E. agglomerans* and mycorrhizae might convert phosphate into available forms and thus enhance plant P uptake. Also, the present study was in line with Barea *et al.* (1976) and Bagyaraj and Menge (1978) who reported that synergistic effect of *Azotobacter* and mycorrhiza enhanced the P content and the plant growth significantly when inoculated together.

Table 14. Influence of PGPMs on the phosphorous content of tomato plants under pot culture conditions

Treatment	P content (%)
T_1 - 100 % RDF	0.52
T_2 - 75 % RDF + *Azotobacter* isolate (TAZ-3)	0.56
T_3 - 75 % RDF + Reference *Azotobacter* strain	0.50
T_4 - 75 % RDF + *Pseudomonas* sp.	0.65
T_5- 75 % RDF + Mycorrhiza	0.71
T_6 - 75 % RDF + *Azotobacter* isolate (TAZ-3) + *Pseudomonas* sp.	0.86
T_7 - 75 % RDF + *Azotobacter* isolate (TAZ-3) + Mycorrhiza	0.91
T_8 - 75 % RDF + *Azotobacter* isolate (TAZ-3) + *Pseudomonas* sp. + Mycorrhiza	0.99
T_9 - 75 % RDF + Ref. *Azotobacter* strain + *Pseudomonas* sp. + Mycorrhiza	0.94
SEm±	**0.01**
CD 1%	**0.03**

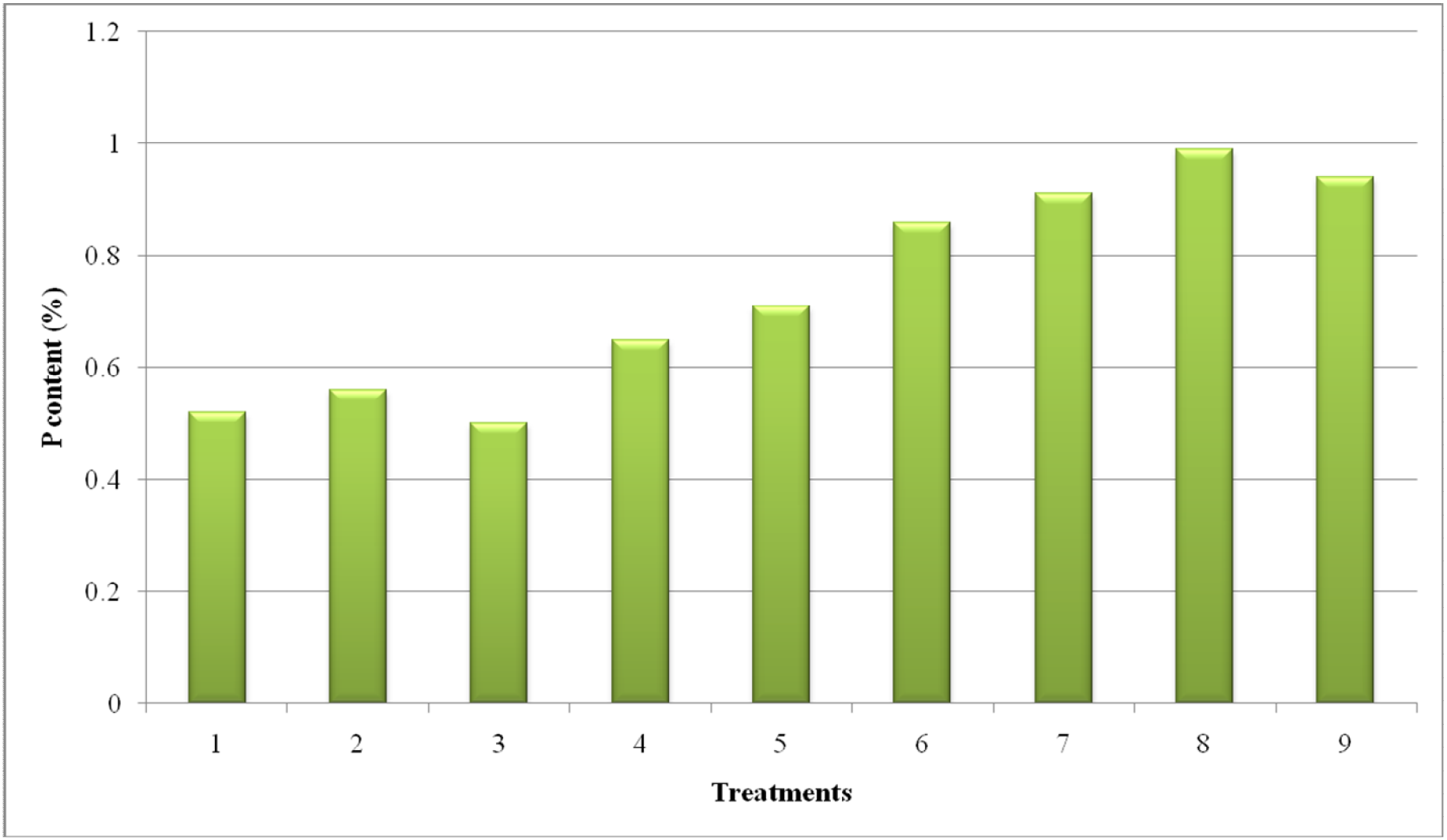


Fig 12. Effect of PGPMs on the phosphorous content of tomato plants (90 DAT)

Summary and Conclusions

V. SUMMARY AND CONCLUSIONS

The present investigation was carried out to isolate and screen the *Azotobacter* isolates from the tomato rhizosphere and rhizoplane samples collected from different locations of Raichur district of Karnataka and to study their influence along the PGPMs (plant growth promoting microorganisms) on the growth and yield of tomato. A pot culture experiment was carried out in green house of Department of Agricultural Microbiology, College of Agriculture, UAS Raichur, Karnataka, during the year 2018-19.

A total of 30 *Azotobacter* isolates were isolated from the soil samples collected from the different places of Raichur district. They were characterized based on morphological and biochemical characteristics. All the isolates were Gram negative and showed cyst formation. The pigmentation was dark brown in some isolates and it was light brown in some isolates. All the isolates were concluded as *Azotobacter* spp. based on the molecular and biochemical characterization.

Based on the molecular and biochemical characters, ten isolates were selected for the screening of beneficial traits *in vitro.* All the isolates showed nitrogen fixing ability and among them TAZ-3 was the highest with 18.87 mg N per g of C source and the lowest was seen in the isolate TAZ-19 with 9.67 mg N per g of C source. Also, all the isolates produced siderophores including reference strain and only five strains (TAZ-3, TAZ-5, TAZ-12, TAZ-18 and TAZ-19) showed phosphorus solubilisation *in vitro.*

The IAA production was seen highest in the isolate TAZ-3 of 29.01 µg / 50 ml of the broth which was highest over all the isolates and reference strain (25.91 µg / 50 ml of the broth), while the lowest IAA production was seen in the isolate TAZ-18 with 12.26 µg / 50 ml of the broth.

The effect of efficient *Azotobacter* isolate along with the *Pseudomonas* and mycorrhiza strains in combination on the growth and yield of tomato was studied. The height was maximum (90 DAT) in the treatment T_8 (*Azotobacter* (TAZ-3) + *Pseudomonas* sp. + Mycorrhiza + 75% RDF) with 74.39 cm and the lowest was seen in T_4 with 63.38 cm.

Number of branches (at 90 DAT) was more in the treatment T_8 with 10.00 branches per plant and was followed by the treatment T_9 with 9.67 branches per plant. The lowest number of branches was seen in T_4 with 6.67 branches per plant.

The highest shoot and root biomass was seen in the treatment with combination of all the organisms T_8 with 71.78 per cent and 58.05 per cent respectively at 90 DAT. It was followed by T_7.

The chlorophyll content was found to be increased and recorded highest in the treatment T_8 and lowest with T_4 with. The mycorrhizal root colonization and spore count was found to be more in T_8 among all the treatments with 91.39 percent and 111.98 spores respectively.

The highest nitrogen content and phosphorous content was also seen significantly higher in the treatment T_8 with 5.17 per cent and 0.99 per cent respectively.

CONCLUSIONS:

By the results obtained from this present study, the treatment T_8 with the combination of native *Azotobacter* isolate (TAZ-3) + *Pseudomonas* sp. + Mycorrhiza along with 75 per cent RDF proved to increase the growth of the tomato when compared to the other treatments applied.

The consortia provides more nutrients, increase the nitrogen supply and also other plant growth promoting substances are produced more when compared to individual inoculations. Thus this treatment proved to be superior over the others for the growth and improvement of dry matter in tomato.

By this study we can conclude that biofertilizers can be used for the better growth and to increase growth and also we can minimize the use of chemical fertilizers, even though we cannot completely avoid using them.

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Appendices

APPENDIX I

Composition of different growth media used

1. Pikovskaya's medium

Glucose	10 g
$Ca_3 (PO_4)_2$	2.5 g
$NH_4 SO_4$	0.5 g
KCl	0.2 g
$MgSO_4.7 H_2O$	0.1 g
$MnSO_4$	Trace
$FeSO_4$	Trace
Yeast extract	0.5 g
Agar	15 g
Distilled water	1000 ml

2. Gelatin Agar

Peptone	5.00 g
Beef extract	3.00 g
Gelatin	120.00 g
Distilled water	1000 ml
Adjust pH to 6.8-7.0	

3. Iodine solution (For starch hydrolysis)

Iodine	0.50 g
Potassium iodide	1.00 g
Distilled water	1000 ml

4. Nitrate broth

Peptone	0.5 g
Beef extract	0.3 g

Potassium nitrate	0.5 g
Sodium chloride	0.5g
Distilled water	1000 ml
pH	6.8 – 7.2

5. Nutrient Agar

Peptone	5.00 g
Beef extract	3.00 g
Sodium chloride	5.00 g
Agar	15.00 g
Distilled water	1000 ml
pH	6.8 – 7.2

6. Waksman No. 77 medium

Mannitol	10 g
$CaCO_3$	5.0 g
K_2HPO_4	0.5 g
$MgSO_4.\ 7H_2O$	0.2 g
NaCl	0.2 g
$MnSO_4.\ 4H_2O$	Trace
$FeCl_3$	Trace
N-free washed agar	20 g
pH	7.0
Distilled water	1000 ml