Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2018; 7(4): 2130-2137
Received: 14-05-2018
Accepted: 18-06-2018

Spandan Reddy
Agriculture Microbiologist (M.Sc. Ag. Microbiology) SHIATS, Allahabad, Uttar Pradesh, India

Ajay Kumar Singh
Department of Food Process Engineering, SHUATS, Allahabad, Uttar Pradesh, India

Harison Masih,
Department of Industrial Microbiology, SHUATS, Allahabad, Uttar Pradesh, India

Jane C Benjamin
Department of Industrial Microbiology, SHUATS, Allahabad, Uttar Pradesh, India

Surendra Kumar Ojha
Agriculture Microbiologist (M.Sc. Ag. Microbiology) SHIATS, Allahabad, Uttar Pradesh, India

Pramod W Ramteke
Department of Biological Sciences, Sam Higginbottom University of Agriculture Technology & Sciences, Allahabad, Uttar Pradesh, India

Ankit Singla
Regional Centre of Organic Farming, Ministry of Agriculture & Farmers Welfare, Bhubaneswar, Govt. of India

Correspondence
Ajay Kumar Singh
Department of Food Process Engineering, SHUATS, Allahabad, Uttar Pradesh, India

Effect of *Azotobacter* sp and *Azospirillum* sp on vegetative growth of Tomato (*Lycopersicon esculentum*)

Spandan Reddy, Ajay Kumar Singh, Harison Masih, Jane C Benjamin, Surendra Kumar Ojha, Pramod W Ramteke and Ankit Singla

Abstract
The use of chemical fertilizers and pesticides has caused serious health disease. Biofertilizer are the formulation of living microorganisms which are capable of fixing atmospheric N_2 in the soil and thereby increasing the crop yield. Therefore, present study was conducted 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. Observations were recorded at different time interval as: 30, 45 and 60, days. 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 observe parameters in treatment T_6 were as: germination (%) 90, plant height 51cm, Leaf area 59cm^2, Branches per plant 8.66 and Leaf per branch 17.33cm. Based on the results, it could be concluded that the strains possess great potential to be developed as biofertilizer to enhance soil fertility and plant growth. However, their performance under field conditions should be assessed before being recommended for commercial applications.

Keywords: *Azotobacter* sp, *Azospirillum* sp, tomato, agriculture, PGPR, crop growth

1. Introduction
Tomato (*Lycopersicon esculentum*) is one of the most popular and second largest producers of the vegetable in the world. Tomato occupies large scale cultivation in India with an average production of 4.6 MT per year. The Tomato crop is highly responsible to nitrogen (N) fertilizer application where N availability may be limited and the time of the application is critical (Taber, 2001). The effect of different rates of nitrogen (N) fertilizers with two types of bio-fertilizers and two cultivars on growth and yield of tomato was reported (Direkvandi, 2008) [19]. *Azotobacter chroococcum* is a coherent group of aerobic, free living diazotrophs able to fix atmospheric nitrogen in nitrogen free or nitrogen poor media with organic compound, as an energy source. Apart from nitrogen fixation, *Azotobacter* produces IAA for plant growth stimulation and siderophore for the suppression of phytopathogen and thus acts as plant growth promoting rhizobacteria. The application of inoculums to the seedling enhanced plant height and stem growth especially from 6 weeks after transplanting and it also increased the fruit yield. The use of *A. chroococcum* inoculum was an effective biological management option in tomato fertilization programme (Taiwo, 2004) [31]. The effect of spent wash press mud on soil chemical properties, growth, yield and quality of seasonal sugarcane was studied (Bhalerae, 2006) [4].
The effect of organic manures (Vermicompost, Farmyard manure, neemcake and wood ash), organic amendments and green manures on growth, yield, nutrient uptake and soil chemical properties of Banana cv. Grand Naine has been reported (Vanilarasu and Balakrishnamurthy, 2014) [34]. Therefore, it is necessities to judicial use of organic matter supplementation at proper time. Pythium root rot is one of the most important diseases of tomatoes under field and greenhouse conditions and it kills the newly emerged seedlings. Likewise, many reports suggested improved microbial activity during organic matter supplementation (Bugnall and Jarvis, 2007) [5]. To the best of our knowledge, there is no report regarding the interaction effect of *Azotobacter* and organic matter supplementation on Tomato - Pythium pathosystem. In the present study, *Azotobacter* was isolated from the rhizosphere soil of tomato plants and characterized the isolates and screened the bacterial isolates. So the interaction effect of *Azotobacter* and organic matter supplementation were studied under pot conditions.
The *Azotobacter* genus belongs to family Azotobacteriaceae comprised of two genera (Tchan, 1984) [32], *Azomonas* (non-cyst forming) with three species and *Azotobacter* (cyst forming)

comprising of 6 species (Tchan *et al*., 1984) [33]. *Azotobacter* spp are Gram negative, free-living, aerobic soil dwelling (Gandora *et al*., 1998) oval or spherical bacteria that form thick-walled cysts (means of asexual reproduction under favorable condition (Salhia, 2013) [28]. The first isolate of the genus was *A. paspali* (Dobereiner and Day, 1975) [9] from the rhizosphere of Paspalum notatum (a tetraploid subtropical grass), is highly host specific. There are around six species in the genus *Azotobacter* (Martyniuk, 2003) some of which are motile by means of peritrichous flagella, others are not. They are typically polymorphic and their size ranges from 2-10µm long and 1-2µm wide (Salhia, 2013) [28]. These bacteria utilize atmospheric nitrogen gas for their cell protein synthesis. This cell protein is then mineralized in soil after the death of *Azotobacter* cells thereby contributing towards the nitrogen availability of the crop plants. *Azotobacter spp* is sensitive to acidic pH, high salts, and temperature (Tchan *et al*., 1984) [33]. *Azotobacter* has beneficial effects on crop growth and yield through biosynthesis of biologically active substances, stimulation of rhizospheric microbes, producing phyopathogenic inhibitors (Chen, 2006; Lenart, 2012). Modification of nutrient uptake and ultimately boosting biological nitrogen fixation. The presence of *Azotobacter* spp in soils has beneficial effects on plants, but the abundance of these bacteria is related to many factors, soil physico-chemical(e.g. organic matter, pH, temperature, soil moisture and microbiological properties (Kizilkaya, 2009). Its abundance varies as per the depth of the soil profile (Vojinoviv, 1961) [35]. Azotobacteria are much more abundant in the rhizosphere of plants than in the surrounding soil and that this abundance depends on the crop species (Sariv and Ragoviv, 1963a). Mishustin and Shilnikora (1961) found *Azotobacter* to be mainly present in root zone of plant growing in poor soil condition. *Azotobacter* populations are quantitatively and qualitatively affected by number of factors soil fertility organic matter content of soil, associative and antagonistication of soil micro oraganism, quality and quantity of root exudates etc (Basavraju *et al*., 1998) [3].

Azotobacter are known to produce physiologically active substances like vitamin B_{12} thiamine, riboflavin, pyredoxin, gibberllins, auxins (IAA), nicotinic acid, folic acid, pantothenic acid and biotin (Mishustin and Shilnikova, 1969). Azotobacter also produces traces of indole acetic acid, folic acid and gibberllin like substances sufficient to cause change in plant physiology.

Azospirillum is a rhizosphere bacterium colonizing the roots of crop plants making use of root exudates and fixes substantial amount of atmospheric nitrogen. At least 15 Azospirillum species have been described, but in terms of physiology and genetics the most studied ones are *A. lipoferum* and *A. brasilense* described by Tarrand *et al*. (1978). The third species *A. amazonense* (Magalhaes *et al*. 1983) [16] was isolated forage grasses planted in Amazonian region. The other species of are *A. halopraeferans* (Reinhold *et al*., 1987) [26], *A. irakense* (Khammas *et al*., 1989) [11], *A. largimobile* (Sly and Stackebrandt, 1999) [30], *A. dobereinerae* (Eckert *et al*., 2001) [10], *A. oryzae* (Xie and Yokota, 2005) [36], *A. melinis* (Peng *et al*., 2006) [23], *A. canadense* (Mehnaz *et al*., 2007a) [18], *A. zeae* (Mehnaz *et al*., 2007b) [18], *A. rugosum* (Young *et al*., 2008) [37], *A. palatum* (Zhou *et al*., 2013) [38], *A. picis* (Lin *et al*., 2009) [15] and *A. thiophilum* (Lavrinenko *et al*., 2010) [14].

Azospirillum is microaerophilic, gram negative and spiral shape bacterium. It is asymbiotic nitrogen fixers able to fix the atmospheric nitrogen make it available to plants. It is beneficial to plants by mechanisms related to enhancement of plant growth, increases the mineral uptake, increases the dry matter, improve the water absorption and improve the yield.

Azospirillum grown in N-free medium behaves as microaerophilic and fixes nitrogen and when supplemented with nitrogen it grows as an aerobe (Day and Dobereiner, 1976) [6]. In culture tubes of semisolid medium with a suitable carbon and energy source, *Azospirillum* develops a growth pellicle just below the surface and fix the nitrogen only under microaerophilic condition, because its nitrogenase is poorly protected from oxygen (Okon *et al*., 1977) [22].

Azospirillum prefers acidic pH for their growth and activity. The optimum pH for the growth of *A. amazonense, A. lipoferum and A. brasilense* strains isolated from a variety of habitats found to be 5.7 - 6.5, 5.7 - 6.8 and 6.0 - 7.3 respectively (Baldani *et al*., 1986) [2]. The optimum temperature for growth of *Azospirillum* was found to be between 32- 40. The growth response of *Azospirillum* strains indicated that D-fructose, D-mannitol, sorbitol, sucrose, tyrosine and tryptophan were poor carbon sources, while α-keto glutarate, L-alanine, L-glutamate, lactate, pyruvate and succinate were good carbon sources (Rai and Gaur, 1982; Del Gallo *et al*., 1984) [25, 7]. *Azospirillum spp*. differed in their utilization of amino acids. *A. lipoferum and A. brasilense* readily utilized many amino acids as the sole source of carbon and nitrogen. Dobereiner and Baldani (1979) [8] found diversity among *Azospirillum* strains with respect to their resistance to various antibiotics. *Azospirillum amazonense* strains were resistant to penicillin but relatively tolerant to chloramphenicol and erythromycin (Magalhaes *et al*., 1983) [16]. The present study was to characterize plant growth promoting activity of Azotobacter and Azospirillum and to evaluate the effect of Azotobacter and Azospirillum on vegetative growth of tomato.

2. Materials and Methods

2.1 Place of the work

The study was conducted at Post Graduate Laboratory, Department of Industrial Microbiology, Sam Higgin bottom University of Agriculture Technology and Sciences, Allahabad.

2.2 Collection of sample

Microbial cultures *Azotobacter sp.* (MCCB 0461), *Azospirillum sp.* (MCCB 0463) were collected from Microbial Culture Collection Bank (MCCB), Department of Industrial Microbiology, Sam Higginbottom University of Agriculture Technology and Sciences, Allahabad.

2.3 Biochemical characterization

Biochemical characterization was done on the basis of following biochemical tests such as Sugar fermentation test; Oxidase test; Catalase test; Urease test; Nitrate reduction test; Indole test; Methyl red test; Voges-Proskauer test.

2.4 Plant Growth Promoting characterization of *Azotobacter* sp. and *Azospirillum* sp.

2.4.1 IAA (Indole acetic acid) production

A modified colorimetric method was used for determination of IAA (Asghar *et al*., 2000).Culture was grown in 50 ml conical flask containing 25 ml King's B (King *et al.,* 1954) with and without L-Tryptophan (0.5%) solution and incubated at 30ºC for 24 hours on a shaker. The culture was then

centrifuged at 4000 rpm for 20 minute. 1ml culture supernatant was put into test tube and mixed with 2ml Salkowski reagent. After 20 – 25 minutes, the test is posistive when the color of supernatant containing IAA turned into red color.

2.4.2 HCN (Hydrogen Cyanide) Production

Screening of bacterial culture for HCN production was done using Castric's method (Castric, 1975). Culture was grown in 10% tryptone soy agar supplemented with glycine (4.4 gl^{-1}). A Whatman filter paper No. 1 soaked in 2% sodium carbonate and 0.5% picric acid solution was placed to the underside of the petridish lids. To avoid the escape of the gas, the plates was sealed with parafilm and incubated at 30°C for 5 days and the production of HCN was determined by the change in color of filter paper from yellow to red-brown.

2.4.3 Ammonia production

Bacterial cultures were tested for the production of ammonia in peptone water. Freshly grown culture was inoculated into 10 ml peptone water and incubated at 30°C for 48 hrs. Nessler's reagent (0.5 ml) was added to each tube. Development of brown to yellow colour was a positive test for ammonia production.

2.5 Preparation of Liquid formulation of *Azotobacter* sp and *Azospirillum* sp

For the production of *Azotobacter* and *Azospirillum* bacteria were transferred to liquid broth (100 ml with seed) then broth was transferred to the rotary shaker for 4 days to prepare starter culture. When cell count reached to $10^8 – 10^9$ cells/ml, the broth was used as inoculant.

2.6 Pot Experiment and Statistical Analysis

A pot experiment was carried out to investigate the effect of nitrogen fixing bacteria *Azotobacter spp.* and *Azospirillum spp*. on vegetative growth component of Tomato. The experiment was laid out in Complete Randomized Block Design (CRBD) with three replications. Data regarding growth parameter**,** plant height, number of leaf /branch, number of branches/plant, leaf area was recorded. The observed data were analysed by using Analysis of Variance.

Table 2.1: Treatment details of Biofertiliser under pot experiments

Abbreviation	Treatments
T_1	Uninoculated control
T_2	Azotobacter inoculation
T_3	Azospirillum inoculation
T_4	Azotobacter+Azospirillum in equal proportion (1:1 ratio)
T_5	Recommended dose of nitrogen at 50 % +Azotobacter inoculation+Azospirillum inoculation
T_6	Recommended dose of nitrogen at 75 % + Azotobacter inoculation+Azospirillum inoculation

3. Results and Discussion

3.1 Effect of *Azotobacter* sp., *Azospirillum* sp. and chemical fertilizer on Germination (%) of Tomato

The study was conducted to determine to effect of *Azotobacter sp*, *Azospirillum sp.* and chemical fertilizer on germination percentage (%) of Tomato. In the present study 6 treatments were examined after 7 days of sowing okra crop. T_6 (NPK 75kg/ha+*Azotobacter* sp +*Azospirillum* sp had got highest mean performance and it was followed by T_5 (*Azotobacter sp* +Azospirillum sp+50 kg/ha NPK), T_4 (*Azotobacter sp* +*Azospirillum sp*), T_2 (*Azotobacter sp*), T_3 Azospirillum sp and T_1 (control) (Table 3.1 and Fig 3.1)

Table 3.1: Variataion in Germination (%) of Tomato due to the effect of *Azotobacter* sp, Azospirillum sp and chemical fertilizer

Treatments	Germination (%)
T1-Uninoculated control	60
T2-Azotobacter inoculation	70
T3-Azospirillum inoculation	63
T4—Azotobacter+Azospirillum	77
T5 - Recommended dose of nitrogen at 50 % +Azotobacter inoculation+Azospirillum	83
T6- Recommended dose of nitrogen at 75 % + Azotobacter inoculation+Azospirillum	90
F-Test	S
S.Ed. (±)	
C.D (0.05%)	3.35
F cal	112.3
F tab	1.96

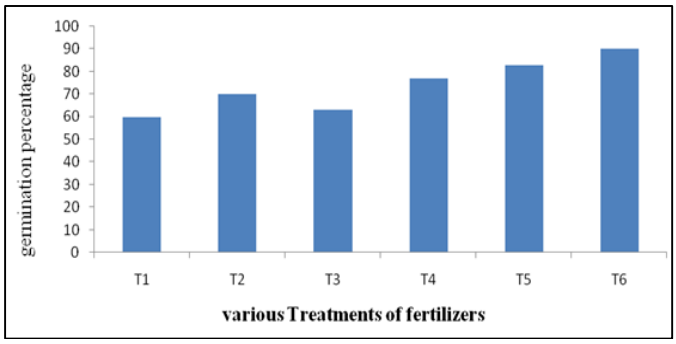

Fig 3.1: Effect of *Azotobacter* sp, *Azospirillum* sp and chemical fertilizer on Germination (%) of Tomato

From the results of the experiments, it is clear that the bio-fertilizer showed better results than the inorganic fertilizer. Since Green Revolution the inorganic fertilizers are used in large amount to increase the yield of the crops. In all the agriculture sectors of India the use of these fertilizers by farmers is increasing day by day to increase the yield and economy. Using inorganic fertilizers farmers can increase the yield of crops but the soil pollution is also increased with this day by day. The use of inorganic fertilizers is increased 6-8 times from the time of green revolution. These fertilizers not only affect the soil but also influence the characteristics and the product of the crop. Fertility of the soil increases due to the continuous use of the fertilizers but it also reduces the crop productivity. The main reason of reduction in crop productivity is due to soil pollution. Soil pollution is caused due to the use of inorganic fertilizers, pesticides, and other chemicals etc (Badoni, 2006) [1]. Martinez *et al*. (1993) [17] reported that soil inoculation with *Azotobacter* increased tomato seed germination by 33-46 %, shortened the period between sowing and transplanting by 5-7 days, increased the yield by 38-60 %. Similar results were also found by Pathak *et al* (2013) who observed maximum percent germination (34.2) in the treatment having FYM + PSB + *Azotobacter* + PGPR; followed by FYM +VAM (29.2). Percent seed germination was slightly better in the treatments having FYM over their respective vermicompost treatments; however, the difference between them was non-significant. During 2008/2009, the maximum germination (51.1) was recorded in FYM +PGPR and FYM + *Azotobacter*; closely followed by FYM+ PSB + *Azotobacter* + PGPR and vermicompost + *Azotobacter* (48.9%). The dual inoculation of

Azotobacter and *G. fasciculatum* had more positive response in peach seedlings as compared to single inoculation or control as reported by Godara *et al*. (1998).

3.2 Effect of *Azotobacter* sp., *Azospirillum* sp. and chemical fertilizer on plant height (cm) of tomato

The plant height was the highest in T6 treatment and it was followed by T5; while it was lowest in T1.

Table 3.2: Variation in plant height (cm) of Tomato due to the application of *Azotobacter sp, Azospirilum sp* and chemical fertilizer

	Treatments	Plant height(cm)		
		30 DAS	45 DAS	60 DAS
T_1	Uninoculated control	15.333	20.333	23.666
T_2	Azotobacter inoculation	20.000	25.666	34.000
T_3	Azospirillum inoculation	19.000	24.333	31.000
T_4	Azotobacter+Azospirillum	24.333	31.333	37.000
T_5	Recommended dose of nitrogen at50%+Azotobacterinoculation+Azospirillum inoculation	25.666	34.333	38.666
T_6	Recommended dose of nitrogen at 75 % + Azotobacter inoculation+Azospirillum inoculation	27.666	38.333	51.000
	F_{cal}	37.786	58.889	34.145
	F_{tab}	6.151	5.028	1.076
	F_{test}	s	s	s
	C d-(0.05%)	2.338	2.716	4.796
	S.Ed			

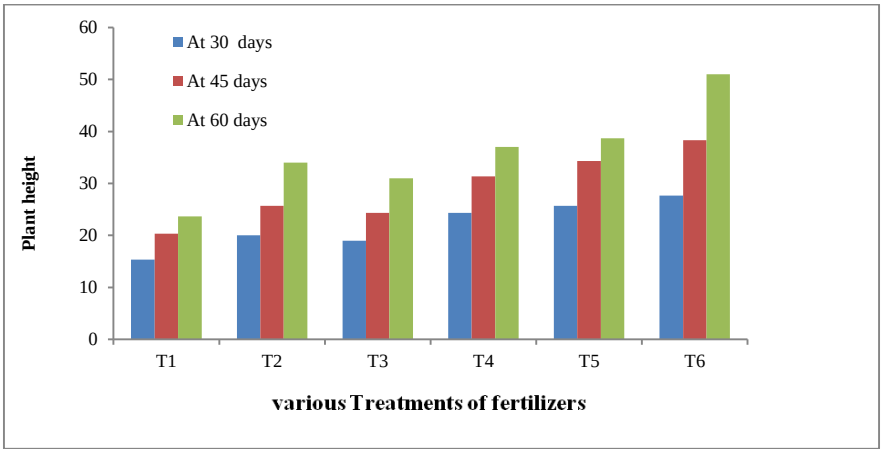

Fig 3.2: Effect of Biofertilizer and chemical fertilizer on plant height (cm) of Tomato

Similar findings were observed by Sahu *et al*., (2014) [27]. The treatments included different biofertilizers (*Azospirillum*, *Azotobacter* and PSB) with inorganic fertilizers (N, P, K). The results showed that application of PSB along with *Azotobacter* and full dose of nitrogen, potash and half dose of phosphorus results significantly vigorous growth and also increased plant height of okra. Sharma *et al.* (2014) [29] found out the effect of biofertilizer application methods and inorganic fertilizers on the growth, seed application with three biofertilizers, *Azospirillum*, *Azotobacter* and Phosphorus solubilizing bacteria. Similar results were also reported by Kandil *et al*. (2011) who studied the effects of inoculation with *Azotobacter* sp. and *Azospirillum* sp. on wheat and observed higher plant height in inoculated wheat plants. Similarly Kumar *et al* (2013) [13] also reported same findings and recorded higher plant height. Significantly higher plant height was recorded with the application of 80 kg N ha^{-1} + inoculation with *Azotobacter* + *Azospirillum*.

3.3 Effect of *Azotobacter sp, Azospirillum sp* and chemical fertilizer on Leaf area (cm^2) of Tomato

The plant height was the highest in T6 treatment and it was followed by T5; While it was lowest in T1. (Table 3.3; Fig 3.3)

Table 3.3: Variation in Leaf area (cm^2) of tomato due to the effect of *Azotobacter sp, Azospirilum sp* and chemical fertilization.

Abr.	Treatments	Leaf area (cm2)		
		30 DAS	45 DAS	60 DAS
T_1	Uninoculated control	34.66	37	40.33
T_2	Azotobacter inoculation	38.66	41.33	43.3
T_3	Azospirillum inoculation	36.66	40.66	43.33
T_4	Azotobacter+Azospirillum	43	44.66	52.66
T_5	Recommended dose of nitrogen at50%+Azotobacterinoculation+Azospirillum inoculation	46.66	51.33	54.66
T_6	Recommended dose of nitrogen at 75 % + Azotobacter inoculation+Azospirillum inoculation	48.66	54.33	59
	F_{cal}	74.237	35.491	45.232
	F_{tab}	1.320	8.708	2.247
	F_{test}	s	s	S
	C d-(0.05%)	2.011	3.450	3.450

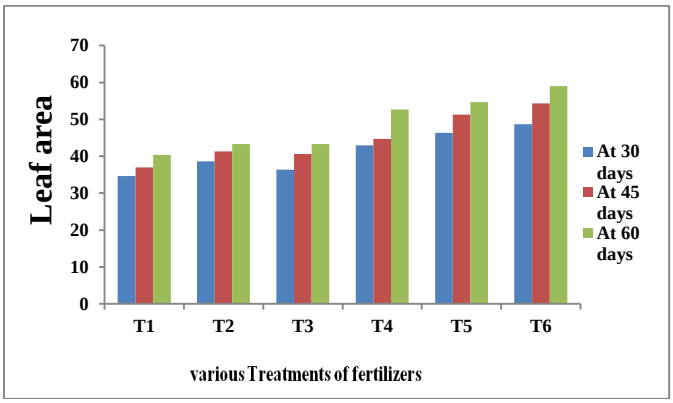

Fig 3.3: Effect of *Azotobacter sp, Azospirillum sp* and chemical fertilizer on Leaf area (cm^2) of Tomato

Similar results were observed by Kumar *et al* (2013) [13] in which all the growth attributes like plant height, leaf area and number of leaf per branch were significantly influenced by the fertility levels, FYM and Azotobacter. Leaf area (181.8 cm2) increased significantly with corresponding increase in fertility levels up to 100 % NPK, but these values were remained at par to the treatment of 50 % NPK. Alone treatment did not improve growth attributes significantly. Combined application of 100 % NPK with Azotobacter resulted an increase in leaf area (187.3 cm^2) The highest values of all growth attributes were observed due to an integrated application of 100%NPK and FYM + Azotobacter followed by 50% NPK + FYM + Azotobacter and 100% NPK+FYM. Similar results were found by who reported that the strawberry plant attained 74.95 cm^2 leaf area with the application of 25% nitrogen through FYM augmented with *Azotobacter* which was at par with the plant with cent percent nitrogen in the form of Urea in combination with *Azotobacter*. Similar findings were observed by Nagoni *et al.* (2017) [20] in which Leaf area (cm^2) was significantly influenced by the integrated application of nutrients. The highest leaf area was recorded in T1 57.5 % RDF + *Azotobacter* + Phosphorus Solubilizing Bacterium [PSB] + VAM.

3.4 Effect of *Azotobacter sp, Azospirillum sp* and chemical fertilizer on branches per plant of tomato.

Table 3.4: Variataion in Branches per plant of Tomato due to the Effect of *Azotobacter sp*, Azospirillum sp and chemical fertilizer

	Treatments	Branches per plant		
		30DAS	45 DAS	60 DAS
T_1	Uninoculated control	1.66	2.66	3.33
T_2	Azotobacter inoculation	3.66	4.0	5.33
T_3	Azospirillum inoculation	4.33	5.66	7.0
T_4	Azotobacter+Azospirillum	5.33	6.0	7.33
T_5	Recommended dose of nitrogen at50%+Azotobacterinoculation+Azospirillum inoculation	6.0	6.66	8.0
T_6	Recommended dose of nitrogen at 75 % + Azotobacter inoculation+Azospirillum inoculation	6.33	7.33	8.66
	F_{cal}	20.300	41.050	29.625
	F_{tab}	1.771	3.872	2.348
	F_{test}	s	S	s
	C d-(0.05%)	1.180	0.836	1.104

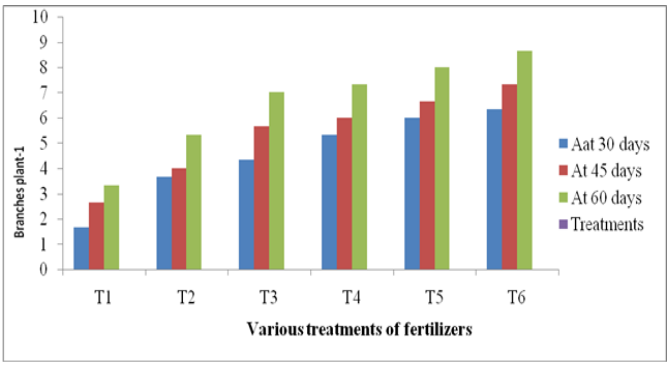

Fig 3.4: Effect of *Azotobacter sp*, Azospirillum sp and chemical fertilizer on Branches per plant of tomato

Similar results were found by Nagoni *et al.* (2017) [20] they revealed that applicationof 75 per cent RDF along with biofertilizer increase the number of branches over control at harvesting stage which might be attributed to stimulatory effect of biofertilizers especially *Azotobacter* and phosphate solubilizing bacteria for the development of photosynthetic structures like size of the chloroplast and the number of grana mm^{-2}. Similar results were found by Kumaran *et al.* (1998) evaluated the effect of organic fertilizers on growth, yield and quality of tomato and the results revealed that application of FYM, *Azospirillum* and other biofertilizers combined with recommended dose of inorganic fertilizers showed superior performance in respect of growth and fruit yield of tomato. The most pronounced expression of yield contributing characters such as plant height, number of branches per plant, mean fruit weight and number of fruits per plant was obtained with inorganic fertilizers in combination with Azospirillum and other bio fertilizers. Similar results were found by Naseeruddin *et al*. (2016) [21] reported maximum number of branches/plant (8.23) was recorded under treatment T8 (NPK 100% + Azotobacter) followed by (8.15 cm) T10 (NPK 50% + Vermicompost), whereas minimum of branches/plant (4.65) was recorded under treatment T12 (control). Number of branches were Increased Due to an integrated application of 75%NPK with *Azotobacter sp and Azospirillum sp*. Similar results were also reported by Kumar *et al*. (2013) [13] in which combined application of 100 % NPK and 10 t FYM ha-1 resulted an increase in plant height (76.0 cm), leaf area (187.3 cm^2) and number of branches (6.8) significantly over an individual application of either 50 % NPK or 10 t FYM ha^{-1}. The highest values of all growth attributes were observed due to an integrated application of 100 % NPK and FYM + Azotobacter followed by 50% NPK + FYM + Azotobacter and 100% NPK + FYM.

3.5 Effect of *Azotobacter sp, Azospirillum sp* and chemical fertilizer on Leaf per Branch of Tomato.

The leaves per plant was the highest in T_6 treatment and it was followed by $T_{5;}$ while it was lowest in T_1treatment (Table 3.5 and Fig 3.5).

Table 3.5: Effect of *Azotobacter sp, Azospirillum sp* and chemical fertilizer on Leaf per Branch of Tomato.

Abr.	Treatments	Leaf Branch-1		
		30 DAS	45 DAS	60 DAS
T_1	Uninoculated control	4.33	5.0	7.33
T_2	Azotobacter inoculation	5.33	7.66	9.0
T_3	Azospirillum inoculation	8.0	9.0	11.66
T_4	Azotobacter+Azospirillum	9.0	10.0	13.33
T_5	Recommended dose of nitrogen at50%+Azotobacterinoculation+Azospirillum inoculation	9.33	10.86	14.66
T_6	Recommended dose of nitrogen at 75 % + Azotobacter inoculation+Azospirillum inoculation	10.33	11.33	17.33
	F_{cal}	8.991	13.871	66.699
	F_{tab}	0.009	0.001	2.469
	F_{test}	s	s	S
	C d-(0.05%)	2.442	1.926	1.398

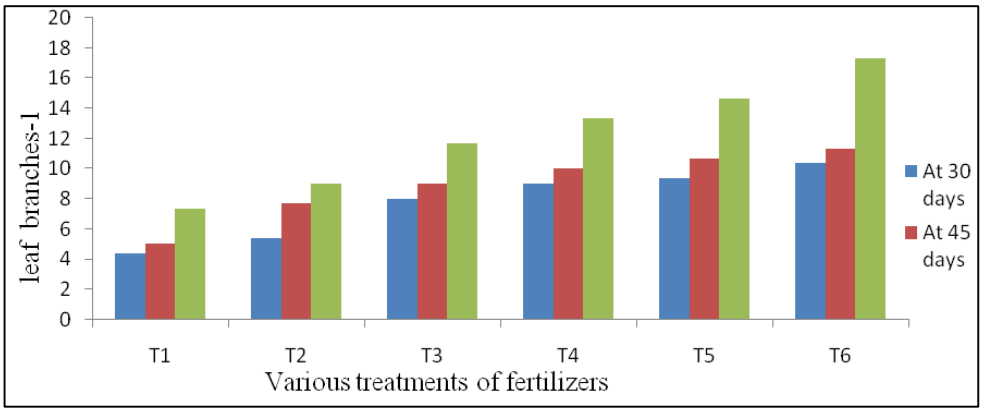

Fig 3.5: Effect of *Azotobacter sp, Azospirillum sp* and chemical fertilizer on Leaf per Branch of Tomato.

Similar results were observed by Kumar *et al.*(2013) [13] in which they evaluated combined application of 100 % NPK and 10 t FYM ha^{-1} resulted an increase in number of leaf per branches (16.5) were significantly higher due to 100 % NPK over control, but these values were remained at par to the treatment of 50 % NPK. Alone treatment of *Azotobacter* did not improve growth attributes significantly. Combined application of 100 % NPK and 10 t FYM ha^{-1} resulted an increase in number of leaves per branch significantly over an individual application of either 50 % NPK or 10t FYM ha^{-1}. The highest values of all growth attributes were observed due to an integrated application of 100 % NPK and FYM + *Azotobacter* followed by 50% NPK + FYM + *Azotobacter* and 100% NPK + FYM. Similar results were found by Naseeruddin *et al.* (2016) [21] evaluated the Leaf per branches (18.59) were found maximum under treatment T8 (NPK 100% + Azotobacter) followed by (17.58) T11 (NPK 50% + Azotobacter), while minimum (9.65) was found under treatment T12 (control).

Plate 3.1: Seedlings of tomato before transplanting

Plate 3.2: Tomato growth under pot experiment

4. Summary and Conclusion

The study was conducted to charscterize the plant growth promoting rhizobacteria and to evaluate their potential on the vegetative growth of tomato. A pot experiment was conducted on Tomato (*Lycopersicon esculentum*) with the objective to know the effect of biofertilizers *(Azotobacter sp*. And *Azospirillum* sp) with the different doses of NPK on growth of Tomato. The treatment comprised of varying treatments from T_0 to T_6. The experiment was conducted in Randomized Block Design with three replications. The NPK full dose was 120:60:40 NPK/ha.

Different observations were collected out of 6 treatments. Maximum seed germination was observed in T_6 (90%). The maximum plant height was observed in T_6 at 30 days, 45 days, 60 days, was as: 27,38 and 51cm, respectively. The max Leaf area was found in T_6 at, 30 days, 45days and 60 days was as: 48,54, 59cm, respectively. The maximum number of Branches per plant was observed in T6 at 30 days, 45 days and 60 days was as: 6.33, 7.33, 8.66 respectively Leaf branch-1 also maximum in T6.The leaf per branch inT6 at 30 days,45 days and 60 days was as: 10.33,11.33,17.33.

From the above results it can be concluded that full dose of NPK with *Azotobacter sp* and *Azotobacter spp* was the most effective treatment in Tomato cultivation. Therefore, Integrated use of inorganic, organic and bio-fertilizer had a significant and positive influence on maximum growth.

5. Recommendation

Application of liquid *Azotobacter and Azospirillum* biofertilizers with reduced recommended dose of nitrogen fertilizers is capable of improving soil health and fertility as well as to achieve more productivity. Gradually it can reduce the use of chemical fertilizers and maintain the natural habitat of the soil.

6. References

1. Badoni Anoop. Effect of inorganic fertilizers and organic manure on seed germination and seedling growth of *Solanum melongena* L. under environmental condition. M. Sc. Thesis, Department of Seed Science and Technology, H. N. B. Garhwal University, Srinagar, 2006.
2. Baldani JI, Baldani VLD, Seldin L, Dobereiner J. Characterizatio of Herbaspirillum seropedicae gen. nov. sp. nov., a root associated N2–fixing bacterium. Int. Syst. Bacteriol. 1986; 36:86-93.
3. Basavraju O, Rao ARM, Shankrappa TH. Effect of Azotobacter inoculation and Nitrogen levels on growth and yield of radish (*Radish Sativus* L.) In: Proceedings of Microbial Technology for Sustainable Productivity and development, (Ed., Rajak D.C.), Jabalpur, Biotechnology of Microbes and Sustainable Utilisation, 2002, 155-160.
4. Bhalerae VP, Jadhav MB, Bhoe PG. Effect of spentwash pressmud compost on soil properties, yield and quality of seasonal sugarcane. Ind Sugar. 2006; 56:9.
5. Bugnall P, Jarvis M. Influence of bacterial sources of indole acetic acid and root elongation of sugar beet. Phytopathology. 2007; 76:386-389.
6. Day JM, Dobereiner J. Physiological aspects of nitrogen fixation by a Spirillum from Digitaria roots. Soil Biol. Biochem. 1976; 8:45-50.
7. Del Gallo M, Martinez-Drets G, Goebel EM, Burris RH, Krieg NR. Carbohydrate metabolism in Azospirllum spp. In: Advances in Nitrogen Fixation Research. (eds.), 1984.
8. Dobereiner J, Baldani VLD. Selective infection of maize roots by streptomycin resistant Azospirillum lipoferum and other bacteria. Can. J Microbiol. 1979; 25:1264-1269.
9. Dobereiner J, Day JM. Nitrogen fixation in rhizosphere of grasses. In: Nitrogen Fixation by Free-Living Microorganisms. (Eds.) Stewart, W.D.P. Cambridge: Cambridge University Press, 1975, 39-56.
10. Eckert B, Weber OB, Kirchhof G, Halbritter A, Stoffels M, Hartmann A. Azospirillum doebereinerae sp. nov., a nitrogen fixing bacterium associated with the C4-grass Miscanthus. Int. J Syst. Evol. Microbiol. 2001; 51:17-26.
11. Khammas KM, Ageron E, Grimont PAD, Kaiser P. Azospirillum irakense sp. Nov., a nitrogen fixing bacterium associated with rice roots and rhizosphere soils. Res. Microbiol. 1989; 140:679-693.
12. Kumar A, Singh R, Giri DD, Singh PK, Pandey KD. Effect of *Azotobacter chroococcum* inoculation on growth and curcumin content of turmeric (*Curcuma longa* L.), International Journal Current Microbiology Applied Sciences. 2014; 3(9):275-283.
13. Kumar T, Kumar JS Arun, Dawson Joy. Effect of different levels of nitrogen and biofertilizers on growth and yield of barley (*Hordeum vulgare* L.), Adv. Res. J Crop Improv. 2013; 4 (1):59-61.
14. Lavrinenko K, Chernousova E, Gridneva E, Dubinina G, Akimov V, Kuever J, *et al*. Azospirillum thiophilum sp. nov., diazotrophic bacterium isolated from a sulfide spring. Int. J Syst. Evol. Microbiol. 2010; 60:2832-2837.
15. Lin SY, Young CC, Hupfer H, Siering C, Arun AB, Chen WM, *et al*. Azospirillum picis sp. nov., isolated from discarded tar. Int. J Syst. Evol. Microbiol. 2009; 59:761-765.
16. Magalhaes FM, Baldani JI, Souto SM, Kuykendall JR, Dobereiner J. New acid tolerant Azospirillum species. Anal. di Acadeni Brasilian de Cience. 1983; 55:417-430.
17. Martinez R, Dibut B, Gonzalez R. Stimulation of tomato development and yield by inoculation of red ferrallitic with Azotobacter chroococcum, 1993.
18. Mehnaz S, Weselowski B, Lazarovits G. *Azospirillum* zeae sp. nov., adiazotrophic bacterium isolated from rhizosphere soil of Zea mays. Int. J Syst. Evol. Microbiology. 2007; 57:2805-2809.
19. Najafvand Direkvandi S, Alemzadeh Ansari N. Effect of different levels of Nitrogen fertilizer with two types of bio-fertilizers on growth and yield of two cultivars of tomato (*Lycopersicon esculentum* Mill). Asian J Plant Sciences. 2008; 7(8):757-761.
20. Nagoni Siddaling, Kempegowda K, Hadora R. Effect of Integrated Nutrient Management on Growthand Yield of Tomato (*Solanum lycopersicum* L.) International journal of plant and soil science. 2017; 16(2):1-7.
21. Naseeruddin Shah KH, Singh V, Rana DK. Role of integrated nutreients management on growth, yield and quality of tomato under garhwal hills. 2016; 5(4):339-341. ISSN: 2250-2823.
22. Okon Y, Albrecht SL, Burris RH. Methods for growing Spirillum lipoferum an for counting it in pure culture and in association with plants. Appl. Environ. Microbiol. 1977; 33:85-88.
23. Peng G, Wang H, Zhang G, Hou W, Liu Y, Wang ET, *et al*. Azospirillum melinis sp. nov., a group of diazotrophs isolated from tropical molasses grass. Int. J Syst. Evol. Microbiol. 2006; 56:1263-1271.
24. Rai AK, Basu AK. Pre-sowing seed bio-priming in okra: response for seed production. International Quirterly Journal of Life Science. 2014; 9(2):643-647.
25. Rai SN, Gaur AC. Nitrogen fixation by Azospirillum spp. and effect of Azospirillum lipoferum on the yield and N uptake of wheat crop. Plant Soil. 1982; 69:233-238.
26. Reinhold B, Hurek T, Fendrik I, Pot B, Gillis M, Kersters K, *et al*. *Azospirillum halofraeferens* sp. nov., a nitrogen¬ fixing organism associated with roots of kallar grass (*Leptochola fusca*) (L.) Kunth.). Int. J Syst. Bacteriol. 1987; 37:43-51.
27. Sahu AK, Kumar S. Effect of biofertilizers and inorganic fertilizers on vegetative growth and yield of okra, [*Abelmoschu sesculentus* (L.) Moench], International Journal of Agricultural Sciences. 2014; 10(2):558-561.
28. Salhia B. The Effect of *Azotobacter chrococcum*as Nitrogen biofertilizer on the growth and yield of *Cucumis sativus.* The Islamic University Gaza, Deanery of Higher Education Faculty of Science, Master of Biological Sciences, Botany, 2013.
29. Sharma I, Samnotra RK, Kumar V. Influence of biofertilizer application methods and inorganic fertilizers

on growth, seed yield and economics cost of okra [*Abelmoschus esculents* (L.) Moench] under sub-tropical irrigated area of Jammu. International Journal of Agricultural Sciences. 2014; (10):322-328.
30. Sly LI, Stackebrandt E. Description of Skermanella parooensis gen. nov., sp. nov. to accommodate Conglomeromonas largomobilis subsp. parooensis following the transfer of *Conglomeromonas largomobilis* subsp. largomobilis to the genus Azospirillum. Int. J Syst. Bacteriol. 1999; 49:541-544.
31. Taiwo LB. Growth response of tomato (*Lycopersicon esculentum* Mill) inoculated with *Azotobacter chroococcum* in an alfisol. J Agri. Research. 2004; 5(1):13-18.
32. Tchan YT. Azotobacteriaceae. In: Bergey's mannual of systemic bacteriology. Williams and Wilkins, Baltimore, London. (ed. Krieg J and Holt G). 1984; 1:219-225.
33. Tchan YT, New PT, Genus I. *Azotobacter beijerinck*. In: Bergey's Manual of Systematic Bacteriology, Vol. I, (Eds.) Krieg, J. and Holt, G.), Williams and Wilkins, Baltimore, London. 1984, pp. 219-225.
34. Vanilarasu K, Balakrishnamurthy G. Influences of organic manures and amendments in soil physiochemical properties and their impact on growth, yield and nutrient uptake of banana. The Bioscan. 2014; 9(2):525-529.
35. Vojinoviv Z. Microbiological properties of main types soil in Serbia for nitrogen cycling. Journal for Scientific Agricultural Research, 1961.
36. Xie CH, Yokota A. *Azospirillum oryzae* sp. nov., a nitrogen-fixing bacterium isolated from the roots of the rice plant Oryza sativa. Int. J Syst. Evol. Microbiol. 2005; 55:1435-1438.
37. Young CC, Hupfer H, Siering C, Ho MJ, Arun AB, Lai WA, *et al*. Azospirillum rugosum sp.noy isolated from oil-contaminated soil. Syst. Evol. Microbiol. 2008; 58:959-963.
38. Zhou S, Han L, Wang Y, Yang G, Zhuang L, Hu P. *Azospirillum humicireducens* sp. nov., a nitrogen-fixing bacterium isolated from a microbial fuel cell. Int. J Syst. Evol. Microbiol. 2013; 63:2618-2624.