"BENEFICIAL EFFECT OF *AZOTOBACTER*, *AZOSPIRILLUM* ON GROWTH AND YIELD OF SPRING ONION (*Allium fistulosum* L.)"

by

Mr. Vivid Babu

(Reg. No. 016/250)

A Thesis submitted to the

MAHATMA PHULE KRISHI VIDYAPEETH,
RAHURI- 413 722, DIST- AHMEDNAGAR,
MAHARASHTRA, INDIA

In partial fulfilment of the requirements for the degree

of

MASTER OF SCIENCE (AGRICULTURE)

in

AGRICULTURAL MICROBIOLOGY

DEPARTMENT OF PLANT PATHOLOGY AND AGRICULTURAL MICROBIOLOGY
COLLEGE OF AGRICULTURE, PUNE

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APPROVED BY

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2018

CANDIDATE'S DECLARATION

I hereby declare that this thesis or part
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The assistance and help received during the course of this investigation have been acknowledged.

Place : Pune

Date : / /2018

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Place : Pune

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LIST OF ABBREVIATION

%	:	Per cent
/	:	Per
@	:	at the rate of
^{0}C	:	Degree Centigrade
BOD		Biological oxygen demand
C. D.	:	Critical Difference
c.f.u	:	Colony forming unit
Cm	:	Centimeter (s)
cv.	:	Cultivar
DAS	:	Days after sowing
DAT		Days after transplanting
E C		Electrical conductivity
e.g.	:	example gratia (for example)
et al.	:	et alli (and others)
etc.	:	et cetera
Fig.	:	Figure
FYM	:	Farmyard manure
G	:	Gram
Ha	:	hectare
i.e.	:	id est (that is)
K	:	Potassium
Kg	:	Kilogram
KSB	:	Potassium solubilising bacteria

Mg	:	milligram
MT	:	Metric tonne
N	:	Nitrogen
No.	:	Number
NS	:	Non significant
P	:	Phosphorus
PGPR	:	Plant Growth Promoting Rhizobacteria
pH	:	Pussance de hydrogen
PP	:	Pertaining page (s)
Ppm	:	Parts per million
PSB	:	Phosphate solubilizing bacteria
PSM	:	Phosphorus solubilizing microorganism
Q		Quintle
S.E.	:	Standard error
spp.	:	Species
TCP	:	Tricalcium phosphate
viz.	:	Videlicent (namely)

ABSTRACT

"BENEFICIAL EFFECT OF *AZOTOBACTER, AZOSPIRILLUM* ON GROWTH AND YIELD OF SPRING ONION (*Allium fistulosum* L.)"

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DIST. AHMEDNAGAR (M. S.), INDIA

2018

Research Guide : Dr. T.K. Narute

Department : Plant Pathology and Agricultural Microbiology

The present investigation entitled "Beneficial effect of *Azotobacter*, *Azospirillum* on growth and yield of spring onion (*Allium fistulosum* L.)" was conducted on glasshouse of All India Co-ordinated Cotton Improvement Project (AICCIP), Department of Plant Pathology and Agril. Microbiology, College of Agriculture, Pune. The objectives of the present research were to isolate effective *Azotobacter* and *Azospirillum* from rhizospheric soil of spring onion from different locations and to compare the efficiency of effective *Azotobacter* and *Azospirillum* isolates on growth and yield as spring onion. In all, there were nine treatments including treatment *Azotobacter* Isolate I (DOGR farm, Rajgurunagar), *Azotobacter* Isolate II (College farm,College of Agriculture , Pune), *Azotobacter* Isolate III (DFR farm, Hadapsar), MPKV *Azotobacter* strain, *Azospirillum* Isolate I (DOGR farm, Rajgurunagar), *Azospirillum* Isolate II (College farm,College of Agriculture , Pune), *Azospirillum* Isolate III (DFR farm, Hadapsar)) MPKV *Azospirillum* strain and uninoculated control replicated three times in the Completely Randomised Design.

The treatment with *Azotobacter* isolate I (DOGR farm, Rajgurunagar) was recorded it's superiority over all the treatments studied for number of leaves (9.23), length of leaves (60.20 cm), fresh weight (24.02 g) and dry weight (6.00 g) of leaves, number of roots per bulb (55.45), weight

of fresh bulb (36.93 g), polar diameter (4.10 cm), equatorial diameter (5.20 cm), total dry matter (15.74 g) and yield (61.50 g). It was superior also for soil *Azotobacter* population (48.33×10^{5} $g^{-1}$of soil) and available soil nitrogen (342.86 $Kgha^{-1}$), available soil phosphorus (28.54 $Kgha^{-1}$), available soil potassium (316.19 $Kgha^{-1}$) and nitrogen uptake (0.71 g) than uninoculated control. The treatment with *Azospirillum* isolate III (DFR farm, Hadapsar) having *Azospirillum* population (33.67 $\times$ 10^{5} $g^{-1}$of soil) was found superior over uninoculated control. There were no significant differences were recorded for pH and E C of soil after harvest of crop. There was increase in available nitrogen, phosphorus and potassium from initial stage to harvesting stage of the crop.

From the present study, the *Azotobacter* and *Azospirillum* isolates were exhibited their positive impact on growth and yield of spring onion and soil properties.

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1. INTRODUCTION

Vegetable crops, especially the leafy ones are important source of vitamins and minerals (Coombs, 1995). They are also excellent source of important nutrients like protein, carbohydrates, fats and oils. They form an essential part of a balanced diet. Vegetables are the excellent source of fibre or roughage which plays an important role in digestion by helping to move food through the digestive system (Foster *et al*., 1985). They may be eaten as side dishes in the raw form or in the cooked form and with meat or fish, in stew, soup and various preparations (Okigbo, 1983).

Spring onion (*Allium fistulosum* L.) belongs to the family *Alliaceae*. It has elongated food storage leaves which are hollow and rounded. It also has a short bulb stem and a fibrous root system which exists at the base of the bulb stem (Tindall, 1986). It was originated in Siberia, and is very popular in the East where it is known as Japanese leek. It has slightly enlarged bulbs, which are very long and are covered with dry membranous, onion-like scales for some distance above ground (Stephens, 2009). The plant requires a well-drained and moist soil. It cannot grow in the shade and it prefers a pH range of 6.5 to 7.5. (Brewster, 1994).

The bulb of spring onion contains an essential oil that is rich in sulphur compounds (Nguyen and Thi Nhu, 1989). It has antibacterial and antiseptic effect. (Duke and Ayensu, 1985). It is used in the treatment of colds and abdominal coldness and fullness (Yeung, 1985). The use of the bulb in the diet impedes internal parasites (Duke and Ayensu, 1985). Externally, the bulb can be made into a poultice to drain pus from sores, boils and abscesses (Chevallier, 1996).

The juice of the plant is used as a moth repellent. The whole plant is said to repel insects and moles (Riotte, 1978).There are number of ways to use spring onions in cooking. They can be chopped and added to sauces, stir fries, and other dishes. They can also be grilled and eaten plain, or roasted and served as a garnish or side vegetable. Some people enjoy eating spring onions raw with a little bit of salt. They can also be used raw in salads.

The entire plant may be pulled and eaten like a green onion or leaf portions may be snipped off and used for flavouring (Stephens, 2009). Nutritional analysis of the leaves showed that they contain 1.4% protein, 0.3% fat, 4.6% carbohydrates, 0.8% ash, some vitamin B1 and moderate levels of vitamin C (Reid, 1977). In India, the spring onion growing area is about 0.5 million hectare and in Maharashtra the area is about less than 0.002% of total leafy vegetable growing areas. The production of spring onion is about 0.5% as compared to total leafy vegetables in Maharashtra.

Biofertilizer are the living microorganisms, which when applied to seeds, plant surfaces or soil, colonizes the rhizosphere or the interior of the plant and promotes growth by increasing the supply or availability of primary nutrients to the host plant. Chemical fertilizers directly increase soil fertility by adding nutrients (Revillas *et al*., 2000 and Vessey, 2003). In agriculture, the use of chemical fertilizers still ensures better yields, but soils and the environment become more polluted and depleted of important nutrients. Biofertilizers may contain symbiotic or non-symbiotic microorganisms that stimulate the growth of plants. Cultivation of plants with biofertilizers can result in higher resistance of plants to diseases and the production of phytohormones and water soluble vitamins.Furthermore, microorganisms can increase plant growth rates (Kumar *et al*., 2001; Behl *et al*., Kumar *et al*., 2008).

Azotobacter genus was discovered by Dutch Microbiologist and Botanist Beijerinck *et al.* in 1901 founder of environmental microbiology. Various species of *Azotobacter* include *Azotobacter chroococcum, A.agilis, A.vinelandii, A. beijerinckii, A.insignis, A.macrocytogenes and A.paspali* (FAO, 1982).Among these species, *Azotobacter chroococcum* is the most prevalent species. It is the first aerobic free-living nitrogen fixer reported by Beijerinck *et al.*, 1901. *Azotobacter* is Gram -ve bacteria, polymorphic in nature. Young cell possess peritrichous flagella as locomotive organ. Old population of bacterial cells includes encapsulated forms and have enhanced resistant to heat, desiccation and adverse conditions. The cyst germinates under favourable conditions to give vegetative cells. *Azotobacter* spp also produce polysaccharides. They are sensitive to low pH, high salts, and temperature above 35^0C. *Azotobacter* spp. is free living bacteria which grow well on a nitrogen free medium. These bacteria utilize atmospheric nitrogen 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 to the crop plants.

Azotobacter spp. are non-symbiotic heterotrophic bacteria capable of fixing an average 20 kg N/ha/per year (Kizilkaya R, 2009). *Azotobacter* is commonly found in rhizosphere and phyllosphere of plants and is very effective for the improvement of soil fertility and crop productivity. It can fix nitrogen directly from the atmosphere that helps plants for better grain production. *Azotobacter* plays an important role to fix nitrogen in the nitrogen cycle.

Besides nitrogen fixation, *Azotobacter* also produces growth hormones *viz;* thiamine, riboflavin, nicotine, indole acetic acid and gibberellins. *Azotobacter* also synthesizes some biologically active substances, including some phytohormones such as auxins, ethylene, cytokinins and gibberellins, there by stimulating plant growth. Seed germination and vigour of the young plants were also observed to be improved due to *Azotobacter* inoculation (Mishustin and Naumova, 1962., Shende *et al.* 1986, Jadhav and Patil, 1985).

Besides *Azotobacter*, *Azospirillum* are the best-characterized genus of plant growth-promoting rhizobacteria (PGPR). They are known to associate with the roots of wheat, tropical grasses, maize, and other cereals (Okon and Hadar 1987 and Oh *et al*. 1999). Members of the genus *Azospirillum* are Gram-negative to Gram-variable, curved-rod shaped, motile, oxidase positive and exhibit acetylene-reduction activity (ARA) under micro-aerophilic conditions.

Azospirillum spp. have been identified mainly as rhizospheric bacteria and its colonization of the rhizosphere has been studied extensively along with reporter gene fusion (Pereg-Gerk *et al*. 2000; Burdman *et al*. 1997; Holguin *et al.* 1999; Steenhoudt and Vanderleyden 2000). The genus *Azospirillum* comprises free-living, nitrogen-fixing bacteria which can colonize, by adhesion, the root surface or the intercellular spaces of the host plant roots. The potential role of the PGPR in association with economically important cereals and other grasses is to promote plant growth by several mechanisms including nitrogen fixation and phytohormone production (Bashan *et al,*2004).

Azospirillum, found in rhizosphere and phyllosphere of plants are effective for the improvement of soil fertility and crop productivity. It can fix nitrogen directly from the atmosphere that help the plants for better production. Several species of *Azospirillum* are able to secrete phytohormones such as auxins, gibberellins, cytokinins, and nitric oxide as signals of plant growth promotion. (Fibach-Paldi *et al*, 2012, M.Kochar and S. Srivastava, 2012).

Biological nitrogen fixation can enhance the long term agricultural production in both economically viable and socially accepted ways by offering an eco-friendly technology for nitrogen replenishment. An application of biofertilizer to seeds improve its germination to a considerable extent and controls plant diseases infection due to antagonistic nature. Now a days, the spring onion is widely accepted and consumed as a leafy vegetable through salad and junk foods. Considering the adverse effect of chemical fertilizers through residues, the present experiment entitled "Beneficial effect of *Azotobacter, Azospirillum* on growth and yield of spring onion (*Allium fistulosu*m L.)" was planned with the following objectives :

1. To isolate effective *Azotobacter* and *Azospirillum* from rhizosphere soil of spring onion from different locations**.**
2. To compare the efficiency of effective *Azotobacter* and *Azospirillum* isolates on spring onion.

2. REVIEW OF LITERATURE

The first Dutch, scientist Beijerinck succeeded for isolation of free living nitrogen fixing bacterium later known *as Azotobacter* in 1901. Similarly *Azospirillum* also comprises free-living, nitrogen-fixing bacteria that are known as plant growth-promoting rhizobacteria (PGPR), which can colonize, by adhesion, the root surface or the intercellular spaces of the host plant roots.

The available literature pertaining to the present study is reviewed here with.

2.1 Characteristics of *Azotobacter* and *Azospirillum* isolates

i. *Azotobacter*

Several research workers *viz.* Beijerinck (1901), Jensen and Petersen (1954) and Johnstone (1974) were reported that the cell of *Azotobacter* spp. are oval, coccoid or rod shaped and produce opaque, circular or irregular, entire, raised or low convex and smooth colonies. The scientists Thompson and Skerman (1979), Apte and Shende (1981) and Tchan and New (1984) reported *Azotobacter* spp. were Gram negative in reaction.

ii. Azospirillum

Dobereiner and Day (1976) observed and stated the vibroid forms of *Azospirillum brasilense.* Tarrand *et al*; (1978) described *Azospirillum* spp. as large Gram negative aerobes, circular, flat or convex having undulate or wrinkled colonies. Krieg and Dobereiner (1986) and Alexander (1977) also reported that *Azospirillum* spp. were Gram negative, slightly curved and straight rod, about 1μm in diameter and 2 to 4 μm length often with pointed ends with varying sizes and shapes.

2.2 Effect of *Azotobacter* and *Azospirillum* inoculation

2.2.1 Effect on seed germination

i. *Azotobacter*

Mahato *et al.* (2009), conducted experiment on effect of *Azotobacter* and nitrogen on seed germination and early seedling growth in tomato and reported maximum number of seedling emergence (90%) with soil + *Azotobacter*. The treatment of soil with nitrogen was found second best and recorded (80%) seedling emergence over the control (60%).

Patil (2015), conducted experiment on lettuce to see the effect of liquid formulation of *Azotobacter* and PSB inoculation on growth and yield and recorded higher seed germination (84.44%) with treatment of liquid *Azotobacter* @ 25ml/kg + Phosphate solubilizing bacteria @ 25ml/kg. Devendra *et al.* (2017), observed effect of *Azotobacter* on growth and yield of onion (*Allium cepa* L.) and recorded maximum onion seed germination in treatment of 166 g/m^2 T5 (85%).

ii. *Azospirillum*

Balasubramani (1988), recorded the effect of *Azospirillum* and nitrogen on growth and yield of okra (*Abelmoschus esculentus* L. Moench) var. Pusa Sawani and found that the seed and soil treatments of *Azospirillum* were superior to five untreated check and *Azospirillum* seed treatment enhanced the germination, plant growth parameters like root length, days to first flowering, first fruit node and dry matter production.

Barassi *et al*. (2006), studied the influence of *Azospirillum*-inoculated seeds of lettuce (*Lactuca sativa* L., cv.Mantecosa) containing 10^7 bacteria per seed and recorded superior germination rates and vegetative growth compared to non-inoculated plants under NaCl stress. Singh and Singh (2007), studied the role of biofertilizers in vegetable production and reported that the *Azospirillum* @ 400 g/2000 seedling roots with other biofertilizers produce antibiotics for biological control of soil borne diseases and resulting in improved germination and seedling growth.

Mangmang *et al* .(2015), investigated the early seedling growth response of lettuce, tomato and cucumber to *Azospirillum brasilense* inoculated @ 9 CFU/ml by soaking and drenching methods showed that different strains of *Azospirillum brasilense* strongly enhanced root and shoot growth, magnitude of germination value and vigour of lettuce, tomato and cucumber when inoculated by soaking. Mounika *et al.* (2018), studied the influence of biofertilizers and micronutrients on growth, seed yield and quality of coriander (*Coriandrum sativum* l.) cv. Sadhana and result showed that germination was maximum with seed inoculation of *Azospirillum* + PSB + foliar spray of ferrous sulphate @ 0.5%.

iii. *Azotobacter* and *Azospirillum*

Dere (1986), reported the significant increase in seed germination of brinjal plant due to *Azotobacter* and *Azospirillum* inoculation. Musmade and Konde (1987), studied onion crop and recorded highest (96.5%) germination by treatment of combined inoculation of *Azotobacter chroococcum* and *Azospirillum brasiliense* over control.

Das and. Kole (2006), studied the effect of some root associative bacteria on germination of seeds, nitrogenase activity and dry matter production by rice plants and concluded that inoculation of *Azotobacter* (strain AS2) and *Azospirillum* (strain AM3) singly or in combination with the phosphate solubilizing *Bacillus* (strain BP3), sulphur oxidizing *Thiobacillus* (strain BT1) and sulphur mineralizing *Bacillus* (strain BC5), significantly stimulated the germination of rice seeds as compared to uninoculated control .

2.2.2 Effect on plant growth parameters

i. *Azotobacter*

Radhakrishnan and Mallikarjunaiah (1983), reported increase in height and leaf number over control on 30th day due to inoculation with *Azotobacter vinelandii* and *Beijerinckia mobilis* in vegetable crops.

Sharma and Thakur (2001), investigated that the individual treatment of biofertilizers application of *Azotobacter* @ 10^7 cfu/ml on seed resulted significant improvement in the growth of tomato. Bhadoria *et al.* (2005) reported the maximum fresh weight, dry weight and yield in tomato, under the seedling treatment with *Azotobacter* culture over soil inoculation and without inoculation. Kore *et al.* (2006) conducted experiment on garlic and observed that the plant height and bulb fresh and dry weights, increased significantly with combination of 10 MT FYM + 3 kg *Azotobacter* +3kg Phosphate solubilizing bacteria + 75 % RDF ha^{-1} over control. Chattoo *et al.* (2007), conducted an experiment to determine the response of garlic to bio-fertilizer application and reported that bio-fertilizer had a beneficial effect on the growth, yield and quality of garlic. Further they reported that bio-fertilizer mixture of *Azotobacter* + phosphobacteria proved significantly superior than *Azospirillum* + phosphobacteria. Tesfaye *et al.* (2007), studied response of onion (*Allium cepa* L.*)* to combined application of biological and chemical nitrogenous fertilizers. Application of 75 kg N ha-1 along with inoculation of *Azotobacter* strains was found to have significantly increased most of the growth parameters like plant height, root length, bulb fresh and dry weights, bulb diameter and yield.

ii. *Azospirillum*

Kolhe *et al.* (1988), observed that effect of *Azospirillum* alone and in combination with other biofertilizers on the yield of leafy vegetables and perusal of data they indicated that the biofertilizer treatment gave significantly higher yield in all the leafy vegetables as compared to untreated control.

Aly H. El-naggar (2010), studied the effect of biofertilizer, organic compost and mineral fertilizers on the growth, flowering and bulbs production of *Narcissus tazetta* L. and reported that all biofertilizer treatment significantly increased number of leaves/plant, leaf length and width, leaves fresh and dry weight over the untreated control, in which *Azospirillum* treatment showed maximum growth parameters. Sridevi and Ramakrishnan (2010), investigated the effects of combined inoculation of AM (*Arbuscular mycorrhizae*) fungi and *Azospirillum* on the growth and yield of onion (*Allium cepa* l.) and reported that various growth biometrics *viz.*, plant height, leaf length, number of leaf sheaths/plant and total dry matter weight at 30 and 60 days after transplanting in main field was found significantly increased over untreated control.

Ram Vachan and Tripathi (2017), studied the effect of bio-fertilizer with chemical fertilizer on plant growth yield and economics of rabi season onion (*Allium cepa* L.) cv. NHRDF Red 2 and reported that the 100 per cent RDF + *Azospirillum* + phosphate solubilizing bacteria**,** has recorded significantly higher plant height, length of leaves, number of leaves, diameter of bulb, shoot thickness, fresh weight per plant and fresh weight per bulb and shoot weight. Singh *et al.* (2017), studied the role of biofertilizer and chemical fertilizer for sustainable onion (*Allium cepa* L.) production and reported the treatment combination of fertility level F4 (N150P75K100) with inoculation of M4 (*Azospirillum* + VAM) *i.e.*, M4F4 produced maximum bulb yield (467.61q/ha), bulb weight (45.10q/h) and total dry weight of plants (63.92q/ha).

iii. *Azotobacter* and *Azospirillum*

Wange (1995), recorded the response of garlic to combined application of biofertilizers and found that all the treatments significantly improved all the parameters studied over control. Significantly more shoot length and total dry matter weight were recorded in the treatment of *Azotobacter* + *Azospirillum*.

Ghanti and. Sharangi (2009), conducted experiment on onion cv. Sukhsagar and recorded maximum no. of leaves, no. of inflorescence / plot and bulb diameter with the treatment of *Azotobacter+Azospirillum*. Bouthaina *et al.* (2010), observed that the plant height, root and shoot length, fresh and dry weights and leaf area of wheat crop increased significantly with *Azotobacter+Azospirillum* treatments.

Sailaja *et al.* (2014), studied the effect of foliar application of 10% panchagavya solution along with *Azotobacter* +*Azospirillum* @ 10^8 CFU/ml on growth and development of leafy vegetable (*Spinacia oleracea)* and reported that the treated soil help to bring rapid changes in phenotypes of plants and also improves the growth, yield and ultimately improve the productivity of *Spinacia oleracea*. Talwar Dilpreet *et al.* (2016) was concluded the treatments of seedling dipped with the culture of *Azospirillum* and *Azotobacter* @ 10^7 CFU/ml increase the dry mass of onion when utilized along with inorganic fertilizers and significantly improved the nutrient uptake in plants.

2.2.3 Effect on crop yield

i. *Azotobacter*

Lal *et al.* (2002), reported onion yield parameters like bulb equatorial diameter, polar diameter, leaf area per plant and bulb weight were significantly increased by application of FYM+ *Azotobacter*. Rather *et al*. (2003), evaluated the response of onion to microbial inoculation and chemical nitrogen and observed that *Azotobacter* enhanced growth, yield and quality attributes of onion.

Hernando *et al.* (2011), confirmed that the weight, diameter and yield of lettuce and cabbage increased with the *Azotobacter* + Phosphate solubilizing bacteria solution @ 50 mL L^{-1} of water applications.

ii. *Azospirillum*

Ashutosh *et al*. (2007), evaluated onion crop to find out the medium level of nitrogen and treated with *Azospirillum* for obtaining maximum productivity of onion. Results showed that the maximum plant height, number of green leaves, neck thickness, shoot girth, bulb diameter, bulb weight and yield were also higher with treatment of nitrogen 75 kg/ha and treated with *Azospirillum* @ 400 g/2000 seedling roots. Zorita *et al*. (2008) reported the interaction with *Azospirillum* biofertilizer and wheat cultivars and proved significant effect on grain yield and straw yield.

Kaushik *et al*. (2012) revealed that seed inoculation of *Azospirillum* and PSB on wheat @ 10^8 CFU/ml significantly increased the grain and straw yield and net return over control. Gabriela Fasciglione *et al.*(2015), investigated the *Azospirillum* inoculation effects on growth, product quality and storage life of lettuce plants grown under salt stress have reported that *Azospirillum*-inoculated lettuce seeds yield a higher number of transplanted plants with superior traits than non-inoculated controls grown with and without saline stress.

2.3 Effect on microbial population of *Azotobacter* and *Azospirillum* in soil

i. *Azotobacter*

Toukhy and Jana (2000), reported that application of inorganics and biofertilizer significantly increased the microbial activity of rhizosphere of barley and also increased total microbial count.

Josip Colo *et al. (*2014), studied plant growth promoting rhizobacteria in onion production and concluded that the total number of bacteria and the number of *Azotobacter chroococcum* were more in all the inoculated variants than in the control. Sornalatha.T *et al.* (2016), conducted the study on distribution of microbial and diazotrophic *Azotobacter* population in five different rhizosphere soil. The results of enumeration of total microbial population revealed that comparatively the bacterial population found to be higher followed by *Azotobacter* population in various rhizosphere soil.

ii. *Azospirillum*

Yoav Bashan (1986), investigated the enhancement of wheat root colonization and plant development by *Azospirillum brasilense* cd. following temporary depression of rhizosphere microflora. Inoculation of wheat with *Azospirillum brasilense*, combined with the application of four fungal and bacterium-inhibiting substances to which *A. brasilense* is resistant in the soil, decreased the rhizosphere population, while it increased wheat root colonization by *A. brasilense*, even in cases of poor inoculation.

Tever *et al*. (2002) observed the genus *Azospirillum spp.* could colonize the roots and stems of plants without causing disease symptoms to stand out. Bagali *et al.* (2012) and Anand *et al.* (2015), respectively studied onion and carrot crop and recorded maximum population of *Azospirillum* (140 cfu/g soil x10^4) and total bacteria (805 cfu/g soil x10^4) observed in treatment T9 (50 % recommended N through poultry manure + 50 % recommended N through inorganic fertilizers + *Azospirillum* + *P. fluorescens*).

iii. *Azotobacter* and *Azospirillum*

Kanungo *et al*. (1997), examined the cultivars of rice with high N absorbtion efficiency harboured higher population of nitrogen fixing *Azotobacter spp.*, *Azospirillum spp* . and anaerobic bacteria. Borollosy *et al.* (2001), investigated the stimulatory influence on microbial growth, microbial densities and nitrogenise activity in sorghum rhizosphere due to N-biofertilizer application and the effect was more pronounced in case of using rhizobacter in containing *Azotobacter* and *Azospirillum*.

2.4 Effect of *Azotobacter* and *Azospirillum* isolates on application on chemical properties of soil and nitrogen uptake

i. *Azotobacter*

Narula *et al.* (2000), inoculated mutant strain of *Azotobacter chroococcum* and showed greater N, P and K uptake in wheat genotypes and also reported more growth hormone production than soil isolates. Kader *et al.* (2002), concluded that *Azotobacter* inoculation also influenced root growth, total N uptake, and straw yield significantly in wheat.

Kapure and Naik (2004), conducted experiment on chick pea in order to study the effect of biofertilizers i.e. vitromone, bioplin (culture of *Azotobacter*) and phosphobacterin (culture of *Bacillus* spp.) along with culture of *Azotobacter* + phosphorus solubilizing bacteria and observed significant increase in N, P and K in soil.

ii. *Azospirillum*

Yadav *et al*. (2004), studied the use of biofertilizers *Azospirillum* in onion. He recorded that application of *Azospirillum* increased trend of available N, P and K content of soil for all the samplings. Srinivasan *et al.* (2005) and Sangeeth *et al.* (2008), studied beneficial effect of *Azospirillum*, might be due to nitrogen fixation and also due to production of phyto-hormones like IAA, GA and cytokinin, which stimulated the growth and induced changes in root morphology which in turn might influence assimilation of nutrients and better nutrient uptake. Ewulo (2005), reported in maize crop the increase and uniform supply of nutrients at all the stages of plant growth could be due to the combined effect of poultry manure, inorganic fertilizers and *Azospirillum*.

iii. *Azotobacter* and *Azospirillum*

Mahanthesh *et al*. (2009) studied the impact of biofertilizers along with levels of N, P and K on nutrient uptake and yield of onion under irrigated condition in central dry zone of Karnataka and recorded increased bulb yield with increased levels of N, P and K along with *Azotobacter* and *Azospirillum* biofertilizers. Rousk *et al*. 2010 observed that there is a linear relationship between soil bacterial communities and pH value as indicated bacterial population increases in the range of pH=4–8. Mazinani *et al*. (2012) observed and reported the highest population of *Azotobacter* and *Azospirillum* was observed in soil sample with the range of pH=(7–7.8) and it started decreasing above 8 or in acidic pH.

3. MATERIAL AND METHODS

The present investigation "Beneficial effect of *Azotobacter*, *Azospirillum* on growth and yield of spring onion (*Allium fistulosum* L.)" was carried out during 2016-2018 at Department of Plant Pathology and Agricultural Microbiology, College of Agriculture, Pune. The details of material used and methods followed during the period of investigation are described.

3.1. Material :

3.1.1 Pots for glass house experiments:

The plastic pots with 30cm diameter and 30cm height having capacity of 10 kg soil were used for conducting the pot culture experiment.

3.1.2 Disinfectant:

Five per cent aqueous solution of Copper sulphate ($CuSO_4$) was used for disinfecting the plastic pots.

3.1.3 Soil:

3.1.3.1 Collection of Soil samples

The roots and rhizosphere soil samples of onion were collected from various places of Directorate of Onion and Garlic Research (DOGR) farm, Rajgurunagar, College farm, College of Agriculture, Pune and Directorate of Floriculture (DFR) farm, Hadapsar.

3.1.3.2 Soil for pot filling

The well drained, good textured, poyata soil available with All India Co-ordinated Cotton Improvement Project (AICCIP), Department of Plant Pathology and Agril. Microbiology, College of Agriculture, Pune was used for filling pots and conducting the pot culture experiment.

3.1.4 Seeds:

The seed of onion, cv- Phule Samarth was obtained from the Professor, Department of Agronomy, College of Agriculture, Pune.

3.1.5 Microbial Inoculants:

The *Azotobacter* and *Azospirillum* isolates collected from DOGR farm, Rajgurunagar, College farm,College of Agriculture, Pune and DFR farm, Hadapsar were isolated by serial dilution plate technique and isolates were maintained by routine sub-culturing at one month interval on Jensen's and Malate media respectively. MPKV *Azotobacter* and MPKV *Azospirillum* inoculants procured from Bacteriologist, Biological Nitrogen Fixation Scheme, College of Agriculture, Pune .

3.1.6 FYM:

The well decomposed Farm Yard Manure was obtained from Animal Husbandry and Dairy Science Department, College of Agriculture, Pune.

3.1.7 Glasswares:

The necessary branded glassware's *viz.* Test tubes, Petriplates, Conical Flasks, Measuring Cylinders, Beakers, Glass Rods, Pipettes, Funnels, Volumetric Flasks, and Burettes etc. were used.

3.1.8 Equipment's and other appliances:

The laboratory equipment *viz.* Autoclave, Hot air oven, Biological Oxygen Demand (BOD) incubator, Laminar air flow cabinet, Refrigerator, Weighing balance, Micro-kjeldhal's digestion unit, Photo spectrometer, Flame photometer, from mother department and Soil Science and Agril. Chemistry department of College of Agriculture Pune were used whenever necessary.

3.1.9 Culture media:

Jensen's medium was used for *Azotobacter* and Malate media for *Azospirillum* during research work as specified in Appendix.

3.1.10 Miscellaneous material

Brown paper bags, test tube racks, micropipette, labels, meter scale, polyethene bags, spirit lamp, plastic trays, inoculation needle cotton, scalpel, sterilized water, distilled water, from mother department were used whenever necessary.

3.2 Methods :

3.2.1 Sterilization:

All the media, solutions, cotton wool, etc. unless mentioned otherwise were sterilized in autoclave at 15 lbs. pressure (121.5^{0}c) for 15 minutes by following standard procedure (kopeloff and Beerman,Society of American Bacteriologist, 1957).

3.2.2 Aseptic precautions

The normal aseptic techniques for bacteriological work were followed throughout the study.

3.2.3 Isolation of *Azotobacter* and *Azospirillum*

The *Azotobacter* and *Azospirillum* population from collected soil sample were enumerated on Jensen's medium and Malate medium, respectively by serial dilution pour plate technique.

3.2.4 Purification

The isolated colony from the plates of *Azotobacter* and *Azospirillum* were picked and streaked on plates of Jensen's and Malate media respectively. The culture of MPKV *Azotobacter* and MPKV *Azospirillum* were procured from Bacteriologist, Biological Nitrogen Fixation Scheme, College of Agriculture, Pune .However, the *Azotobacter* and *Azospirillum* isolates collected from DOGR farm, Rajgurunagar, College farm, College of Agriculture, Pune and DFR farm, Hadapsar were isolated by serial dilution plate technique.These plates were kept in BOD and incubated for 72hr at 28±2^{0}C temperature and each plate was observed. *Azotobacter* and *Azospirillum* colonies were transferred into broth of Jensen and Malate media and kept in BOD with shaker for 72 hrs. Then the cultures were serially diluted up to 10^{-5} for purification by pour plate method.

These plates were labelled properly and incubated at 28±2^0C temperature for 72 hr and colonies were counted in order to study the colony characters. Pure colony was transferred to slants of respective media, incubated for 72 hrs and preserved.

Sub culturing was done time to time to get the pure culture till the end of the experiment. The total *Azotobacter* and *Azospirillum* population in one gram soil was calculated by following formula.

$$\text{No. of bacteria per gram of soil} = \frac{\text{Av. Plate colony count x Dilution factor}}{\text{Inoculation volume}}$$

3.2.5 Morphological tests

3.2.5.1 Gram's staining

A thin bacterial smear from fresh culture was made on a clean slide, fixed by heat fix method and Gram stained as described by Kopeloff and Beerman (SAB, 1957) and observed through microscope under oil immersion lens having 1000 x magnification.

3.2.5.2 Colony characteristics

Plates of Jensen's agar and Malate media were streaked with respective culture and incubated for a week and well separated colonies were examined daily in order to study the various colony characters which include form, elevation and margin.

3.2.6 Methods used for soil Analysis:

Soil sample collected from different locations were analysed to study chemical and biological properties of soil.

Table: 3.1 Analysis of soil samples

Sr. No	**Parameters**	**Method**	**Reference**
A	**Chemical properties**		
1	Available Nitrogen (Kg/ha)	Alkaline permagnate method	Subbiah and Asija, (1956)
2	Available Phosphorus (Kg/ha)	0.5 M NaHCO3 Olsen's method	Olsen *et al*.(1965)
3	Available Potassium (Kg/ha)	Ammonium acetate extraction method	Knudsen *et al.* (1982)
4	ElectricalConductivity (ds/m)	Electrical Conductivity meter	Jackson. (1971).
5	pH	pH meter	Jackson. (1971).

B	**Biological properties**		
	Total *Azotobacter* Count	Serial dilution and pour plate technique	Subba Rao (1999)
	Total *Azospirillum* Count	Serial dilution and pour plate technique	Subba Rao (1999)

3.2.7 Methods used for plant analysis

Collected plant sample were analyzed for chemical properties i.e. nitrogen per cent and nitrogen uptake by plant by using Kjeldhal's method.

Table: 3.2 Analysis of plant samples

Sr. No.	**Parameter**	**Method**	**Reference**
1	Total Nitrogen (%)	Kjeldhal's method	Parkinson and Allen,(1975)

3.3 Experimental details

3.3.1 Experimental site

Pot culture experiment was laid out in the glasshouse of All India Co-ordinated Cotton Improvement Project (AICCIP), Department of Plant Pathology and Agril. Microbiology College of Agriculture, Pune and *In vitro* studies were carried out in laboratory of Department of Plant Pathology and Agril. Microbiology, College of Agriculture, Pune.

3.3.2 Pot Culture Study

3.3.2.1 Treatment details

Design of Experiment : Completely Randomized Design (CRD)

Test Crop : Spring onion

Variety : Phule Samarth

Number of Treatments : 09

Number of Repetitions : 03

Treatment

T_1 : *Azotobacter* Isolate I (DOGR farm, Rajgurunagar)

T_2 : *Azotobacter* Isolate II (College farm,College of Agriculture , Pune)

T_3 : *Azotobacter* Isolate III (DFR farm, Hadapsar)

T_4 : MPKV *Azotobacter* Strain

T_5 : *Azospirillum* Isolate I (DOGR farm, Rajgurunagar)

T_6 : *Azospirillum* Isolate II (College farm,College of Agriculture , Pune)

T_7 : *Azospirillum* Isolate III (DFR farm, Hadapsar))

T_8 : MPKV *Azospirillum* Strain

T_9 : Uninoculated Control.

3.3.2.2 Seed inoculation

For inoculation of liquid *Azotobacter* (18×10^{-5}/ ml) and *Azospirillum*(16×10^{5}/ ml), the seeds of Spring onion were dipped in respective liquid suspension @ 10 ml/ 50g of seeds for 1 hour separately.

3.3.2.3 Pot filling / Transplanting

The plastic pots used for pot culture experiment were surface sterilized by using $CuSO_4$ @ 5% solution and filled with soil and FYM in ratio of 2:1 @ 10 kg/pot and same were marked and labelled according to treatments.

3.3.2.4 Fertilizer application

The fertilizer recommended dose of 60:60:30 kg/ha (N: P: K) was applied through straight fertilizer form i.e. Urea, Single Super Phosphate and Murate of Potash, respectively. Half dose of Nitrogen was applied at the time of sowing and remaining half dose of N was given 30 days after sowing. It was uniformly applied to all treatments on weight basis of soil in the pots.

3.3.2.5 Irrigation and thinning

Immediately after seed sowing soil were irrigated lightly and thereafter every alternate days interval. Thinning was done after 15 days of sowing keeping healthy growing seedlings for further observations.Normal cultural practises i.e. weeding, irrigation etc. were carried out. Five seedlings were maintained per pot.

3.3.2.6 Foliar application of biofertilizer

The foliar application of liquid *Azotobacter* and *Azospirillum* at the rate of 25 ml per litre of water was given at 20 day after transplanting (DAT).

3.3.2.7 Plant protection measures

As a part of preventive measures against pests and diseases, the due care was taken and spraying of Deltametrin and Metasystox were adopted as and when required.

3.3.2.8 Harvesting

Harvesting of spring onion plant was done at 70 days after transplanting (DAT).

3.3.3 Observations recorded

Following observations were recorded as mean of three replications during crop growth period.

3.3.3.1 Seed germination

The seed germination count was recorded by using rolled towel paper technique. The rolled towel paper soaked with sterile water,100 seeds treated with respective treatments were placed uniformly in the rolled towel paper for seed germination. Each treatment was replicated thrice. The periodic observation were recorded reqularly and final seed germination count was recorded after 10 days.

$$\text{Germination Percentage} = \frac{\text{Total number of seeds germinated per bed}}{\text{Total number of seeds sown}} \times 100$$

After germination count five plants/pot were maintained for further studies.

3.3.3.2 Number of leaves

Numbers of leaves per plant were recorded at 30 days interval by taking leaf count per spring onion plant from 30 to 70 days after transplanting.

3.3.3.3 Length of leaves

The length of leaves was recorded in centimetre by using meter scale at 30 days intervals from 30 to 70 days after transplanting.

3.3.3.4 Fresh and Dry weight of leaves

At harvest, weight of leaf recorded immediately after the uprooting the plant sample. The leaves were detached from shoot and weigh on weighing balance and recorded leaf weight. Dry weight of leaves was recorded by air drying of leaves and it was wrapped in brown paper bags followed by oven dried at 70± 2^0C till constant weight is obtained.

3.3.3.5 Number of roots per bulb

The number of roots per bulb was recorded after uprooting the plant sample.

3.3.3.6 Bulb weight and Girth/ Diameter of bulb

The weight and the girth (diameter) of bulbs were recorded after uprooting the plant sample. The weights of bulbs were taken on weighing balance and the girth (diameter) of bulbs was measured by using measuring scale.

3.3.3.7 Total dry matter weight of plant

Plants uprooted at maturity stage with gentle wash to roots to remove adhering soil on root surface, air dried and the shoots, bulbs and roots were wrapped in brown paper bags separately and oven dried at 70± 20^0C till constant weight is obtained and recorded as a dry weight of shoot and root which ultimately gives the total dry matter weight of plant.

3.3.3.8 Yield of Spring onion per plant

Fresh spring onion plants were harvested and treatment wise weight was recorded immediately as yield.

3.3.4 Chemical analysis

3.3.4.1 Soil analysis

Soil samples were collected from each plastic pot from the rhizosphere of plants at the time of harvest. Initial soil samples were taken before the sowing of seeds. The collected soil samples were air dried, crushed in wooden mortar and pestle and sieved through 2 mm sieve. The soil samples were analysed for their chemical properties by using standard analytical methods. Available nitrogen was estimated by alkaline permanganate method (Subbiah and Asija, 1956), Available phosphorus by Olsen's method (Olsen *et al.* 1965) and available potassium by Ammonium acetate extraction method (Knudsen *et al.* 1982). EC and pH by Electrical Conductivity meter and pH meter respectively (Jackson. 1971).

3.3.4.2 Plant analysis

The plant samples were collected at harvest stage. The samples were dried in air and wrapped in brown paper bags. The samples were then dried in hot air oven at temperature of 70 ± 2^0C. The oven dried samples (shoot and root) were ground to fine powder form and used for analysis by adopting the standard laboratory method.

Collected spring onion plant samples were analyzed for total nitrogen percentage by Kjeldhal's method (Parkinson and Allen, 1975). The data on percent nitrogen content in plant were converted into N uptake values.

3.4 Statistical analysis

The data obtained in different observations were computed statistically as per Completely Randomized Design (CRD) by using the standard statistical methods as described by Panse and Sukhatme (1967) for its statistical significance. The data were presented in tabular form with suitable graphical illustrations and figures at appropriate places.

4. RESULTS AND DISCUSSION

A pot culture experiment was conducted to study the combined effect of *Azotobacter* and *Azospirillum* isolates on spring onion. The variety of onion used for growing was Phule Samrath. The results obtained from the experiment are presented in this chapter.

4.1 Study of different characteristics of *Azotobacter* and *Azospirillum* isolates

4.1.1 Morphological tests

Observations of morphological characters like shape of the cells and Gram staining reaction by efficient *Azotobacter* and *Azospirillum* isolates are presented in Table: 4.1.

Table: 4.1 Morphological characters of *Azotobacter* and *Azospirillum* Isolates

Tr. No.	Isolates	Gram staining Reaction	Shape of the cells
T_1	*Azotobacter* isolate I	Gram -ve	Cocci
T_2	*Azotobacter* isolate II	Gram -ve	Cocci
T_3	*Azotobacter* isolate III	Gram -ve	Oval
T_4	MPKV *Azotobacter* strain	Gram -ve	Cocci
T_5	*Azospirillum* isolate I	Gram -ve	Vibroid
T_6	*Azospirillum* isolate II	Gram -ve	Vibroid
T_7	*Azospirillum* isolate III	Gram -ve	Vibroid
T_8	MPKV *Azospirillum* strain	Gram -ve	Vibroid

-ve = negative

Among the isolates *Azotobacter* isolate I, *Azotobacter* isolate II and MPKV *Azotobacter* strain showed coccoid shape of cell while *Azotobacter* isolate III showed oval shape. The *Azospirillum* isolate I, *Azospirillum* isolate II, *Azospirillum* isolate III and MPKV *Azospirillum* strain showed vibroid shape. All the eight efficient isolates were found Gram negative. Similar types of morphological characters of *Azotobacter* and *Azospirillum* were observed and recorded by Beijerinck (1901), Jensen and Petersen (1954), Dobereiner and Day (1976), Alexander (1977), Thomson and Skerman (1979), Apte and Shende (1981) and Tarrand *et al*; (1978). Thus the observations in respect of morphological characters are in agreement with earlier research workers.

4.1.2 Colony characters

The isolates under study exhibited the following trend of colony characters are presented in Table: 4.2. The *Azotobacter* isolate I, *Azotobacter* isolate II and MPKV *Azotobacter* strain were observed to be circular form, Raised elevation and entire margin while *Azotobacter* isolate III showed irregular form, convex elevation and curled margin. In case of *Azospirillum*, *Azospirillum* isolate I, *Azospirillum* isolate II, and *Azospirillum* isolate III were showed circular form, flat elevation and wrinkled margin while MPKV *Azospirillum* strain showed circular form, convex elevation and undulate margin. These observations are in confirmity with those recorded by

Table: 4.2 Colony characters of *Azotobacter* and *Azospirillum* isolates under study

Tr. No.	Isolates	Colony characters		
		Form	**Elevation**	**Margin**
T_1	*Azotobacter* isolate I	Circular	Raised	Entire
T_2	*Azotobacter* isolate II	Circular	Raised	Entire
T_3	*Azotobacter* isolate III	Irregular	Convex	Curled
T_4	MPKV *Azotobacter* strain	Circular	Raised	Entire
T_5	*Azospirillum* isolate I	Circular	Flat	Wrinkled
T_6	*Azospirillum* isolate II	Circular	Flat	Wrinkled
T_7	*Azospirillum* isolate III	Circular	Convex	Undulate
T_8	MPKV *Azospirillum* strain	Circular	Flat	Wrinkled

Johnstone (1974), Tchan and New (1984) and Krieg and Dobereiner (1986) showing colony characters: circular form, Raised elevation and entire margin in *Azotobacter* and circular form, flat elevation and wrinkled margin in *Azospirillum* strains.

4.2 Effect of *Azotobacter* and *Azospirillum* isolates on growth of spring onion.

4.2.1 Effect on seed germination

The seed germination percentage was recorded as mean of three replications. There were 100 seeds kept for seed germination count per treatment and observations were recorded at 10 days after sowing.

Table: 4.3 Effect of *Azotobacter* and *Azospirillum* isolates treatment on seed germination of spring onion .

Tr. No.	Treatment details	Mean Germination %
T_1	*Azotobacter* isolate I	86.44
T_2	*Azotobacter* isolate II	83.00
T_3	*Azotobacter* isolate III	85.88
T_4	MPKV *Azotobacter* strain	85.22
T_5	*Azospirillum* isolate I	84.88
T_6	*Azospirillum* isolate II	83.77
T_7	*Azospirillum* isolate III	86.11
T_8	MPKV *Azospirillum* strain	84.44
T_9	Uninoculated control.	73.33
	SE ±	0.25
	C.D. (0.05)	0.75

The results in respect of germination of spring onion seeds as influenced by *Azotobacter* and *Azospirillum* isolates treatments were presented in Table: 4.3 and graphically shown in Fig: 4.1. Inoculation with liquid *Azotobacter* and *Azospirillum* isolates showed differential increase in germination percentage over uninoculated control and ranged from 73.33 to 86.44 per cent. It was revealed that the application of *Azotobacter* and *Azospirillum* isolates through seed treatment showed significant effect on seeds germination ability over uninoculated control. Significantly higher germination was recorded in seeds treated with *Azotobacter* isolate I (86.44) which was at par with *Azospirillum* isolate III (86.11) and *Azotobacter* isolate III, followed by MPKV *Azotobacter* strain(85.22%), *Azospirillum* isolate I (84.88%), MPKV *Azospirillum* strain(84.88%), *Azospirillum* isolate II (83.77) and treatment *Azotobacter* isolate II (83.00). The least germination percentage was recorded in uninoculated control (73.33). Similar results were recorded by Musmade and Konde (1987), who studied on onion crop and recorded highest (96.5%) germination by treatment of combined inoculation of *Azotobacter chroococcum* and *Azospirillum brasiliense* over control. Barassi *et al.* (2006), also studied the influence of *Azospirillum*-inoculated seeds of lettuce (*Lactuca sativa* L., cv.*Mantecosa*) and recorded superior germination rates compared to non-inoculated plants. Devendra *et al.* (2017), observed the effect of *Azotobacter* on growth and yield of onion (*Allium cepa* L.) and maximum onion seed germination in treatment with *Azotobacter* (85%) over control.

4.2.2 Effect on number of leaves per plant

It was revealed that, inoculation with *Azotobacter* and *Azospirillum* isolates significantly increased the number of leaves per plant over the uninoculated control. The observations recorded at periodic interval on average number of leaves per plant influenced by application of different *Azotobacter* and *Azospirillum* isolate treatments were presented in Table: 4.2 and graphically shown in Fig: 4.2 and Plate 4.1, 4.2 & 4.3.

i. 30 Days after transplanting

The number of leaves per plant started increasing at 30 days after transplanting ranged from 2.30 to 3.50. The results revealed that application of *Azotobacter* and *Azospirillum* isolates were found statistically significant in increase the number of leaves per plant at 30 days after sowing. Significantly higher number of leaves (3.50) per plant was observed in *Azotobacter* isolate I which is superior over all the treatments, followed by *Azospirillum* isolate III (3.20), *Azotobacter* isolate III and MPKV *Azotobacter* strain (3.13), *Azospirillum* isolate I (3.07), MPKV *Azospirillum* strain (3.00) *Azospirillum*) isolate II (2.98) and *Azotobacter* isolate II (2.92). The least number (2.30) of leaves were recorded in the uninoculated control.

ii. 50 Days after transplanting

The results revealed that application of *Azotobacter* and *Azospirillum* isolates were found statistically significant in increase the number of leaves per plant at 50 days after transplanting. Significantly higher number of leaves (7.23) per plant was observed in *Azotobacter* isolate I which is superior to other treatments, followed by *Azospirillum* isolate III (6.75), *Azotobacter* isolate III (6.15), MPKV *Azotobacter* strain (6.08), *Azospirillum* isolate I (6.00) and MPKV *Azospirillum* strain (5.75), *Azospirillum* isolate II (5.50) and *Azotobacter* isolate II (5.33). The least number of leaves were recorded in the uninoculated control (4.35).

Table: 4.2 Effect of *Azotobacter* and *Azospirillum* isolate on the number of leaves per plant of spring onion at periodic interval

Tr. No.	Treatment details	Average number of leaves/plant		
		30 DAT	50 DAT	70 DAT
T_1	*Azotobacter* isolate I	3.50	7.23	9.23
T_2	*Azotobacter* isolate II	2.92	5.33	7.50
T_3	*Azotobacter* isolate III	3.13	6.15	8.67
T_4	MPKV *Azotobacter* strain	3.13	6.08	8.33
T_5	*Azospirillum* isolate I	3.07	6.00	8.25
T_6	*Azospirillum* isolate II	2.98	5.50	7.67
T_7	*Azospirillum* isolate III	3.20	6.75	9.05
T_8	MPKV *Azospirillum* strain	3.00	5.75	7.83
T_9	Uninoculated control.	2.30	4.35	6.28
	SE ±	0.07	0.10	0.09
	C.D. (0.05)	0.21	0.30	0.27

DAT = days after transplanting

iii. 70 Days after transplanting

The similar trend of the results were also noticed in respect of number of leaves after 70 DAT and increased in number of leaves ranged from 6.28 to 9.23. The significantly maximum number of leaves was recorded with the application of treatment with *Azotobacter* isolate I (9.23) which is superior to other treatments, followed by *Azospirillum* isolate III (9.05), *Azotobacter* isolate III (8.67), MPKV *Azotobacter* strain (8.33), *Azospirillum* isolate I (8.25) and MPKV *Azospirillum* strain (7.83), *Azospirillum* isolate II (7.67) and *Azotobacter* isolate II (7.50). The least number (6.28) of leaves were recorded in uninoculated control.It was revealed that inoculation with *Azotobacter* and *Azospirillum* isolates significantly increased the number of leaves per plant over the uninoculated control. The results obtained were statistically significant at 30 DAT, 50 DAT and subsequently at 70 DAT due to application of treatment with *Azotobacter* and *Azospirillum* isolates over uninoculated control. The results are in confirmity with the observation recorded by Radhakrishnan and Mallikarjunaiah (1983), reported that an increase in height and leaf number over control on 30th day due to inoculation with *Azotobacter vinelandii* and *Beijerinckia mobilis* in vegetable crops. Kolhe *et al.* (1988), observed that effect of *Azospirillum* alone and in combination with other biofertilizers increased the number of leaves, fresh weight and yield of leafy vegetables. Ghanti and. Sharangi (2009), conducted experiment on onion cv. Sukhsagar and recorded maximum no. of leaves, no. of inflorescence / plot and bulb diameter with the treatment of *Azotobacter+Azospirillum*.

4.2.3 Effect on length of leaves

The length of leaves was also recorded at periodic interval after transplanting with the help of metric scale. It was observed that the *Azotobacter* and *Azospirillum* isolates showed significantly increase in the length of leaves at different growth stages of spring onion over the uninoculated control. The results were presented in Table: 4.5 and graphically shown in Fig: 4.3.

i. 30 Days after transplanting

The result presented in Table: 4.5 and graphically shown in Fig: 4.3 on length of leaves at 30 days after transplanting was revealed that the *Azotobacter* and *Azospirillum* isolates recorded significantly increase length of leaves ranging from 15.38 to 20.13 cm. Significantly maximum length of leaves was recorded in treatment of *Azotobacter* isolate I (20.13 cm) which was superior over rest of the treatments and uninoculated control, followed by remaining treatments of *Azospirillum* isolate III (19.32 cm), *Azotobacter* isolate III (19.00 cm), MPKV *Azotobacter* strain (18.92 cm), *Azospirillum* isolate I (18.85 cm), MPKV *Azospirillum* strain (18.62 cm) *Azospirillum* isolate II (18.25 cm) and *Azotobacter* isolate II (17.62 cm). The lowest length of leaves was observed in uninoculated control (15.38 cm).

ii. 50 Days after transplanting

It was observed that the length of leaves increased per plant at 50 days after transplanting was statistically significant and ranged from 31.45 cm to 40.20 cm. Significantly maximum length of leaves was recorded with application of *Azotobacter* isolate I (40.20 cm) followed by *Azospirillum* isolate III (39.70 cm), *Azotobacter* isolate III (39.33 cm), MPKV *Azotobacter* strain (39.25 cm), *Azospirillum* isolate I (39.15cm), MPKV *Azospirillum* strain (38.67 cm) *Azospirillum* isolate II (38.08 cm) and *Azotobacter* isolate II (38.00 cm). The least length of leaves was observed in uninoculated control (31.45 cm).

iii. 70 Days after transplanting

The similar trend of the results were also noticed in respect of length of leaves after 70 DAT and increase in length of leaves were ranged from 45.64 cm to 60.20 cm. The significantly maximum length of leaves was recorded with the application of *Azotobacter* isolate I (60.20 cm) and it was superior over all other treatments, followed by *Azospirillum* isolate III (59.32 cm), *Azotobacter* isolate III (59.00 cm), MPKV *Azotobacter* strain (58.42 cm), *Azospirillum* isolate I (58.33 cm), MPKV *Azospirillum* strain (58.25 cm), *Azospirillum* isolate II (57.33 cm) and *Azotobacter* isolate II (56.67 cm).The minimum length of leaves was observed in uninoculated control (45.64 cm).

Table: 4.5 Effect of *Azotobacter* and *Azospirillum* isolates on the length of leaves in cm plant^{-1} of spring onion at periodic interval

Tr. No.	Treatment details	Average length of leaves in cm		
		30 DAT	50 DAT	70 DAT
T_1	*Azotobacter* isolate I	20.13	40.20	60.20
T_2	*Azotobacter* isolate II	17.67	38.00	56.67
T_3	*Azotobacter* isolate III	19.00	39.33	59.00
T_4	MPKV *Azotobacter* strain	18.92	39.25	58.42
T_5	*Azospirillum* isolate I	18.85	39.17	58.33
T_6	*Azospirillum* isolate II	18.25	38.08	57.33
T_7	*Azospirillum* isolate III	19.32	39.70	59.32
T_8	MPKV *Azospirillum* strain	18.62	38.67	58.25
T_9	Uninoculated control.	15.38	31.45	45.64
	SE ±	0.15	0.12	0.17
	C.D. (0.05)	0.44	0.37	0.51

DAT = Days after transplanting

Finally it was noticed that the *Azotobacter* and *Azospirillum* isolates showed more length of leaves as compared to uninoculated control. Similar findings were reported by Sharma and Thakur (2001), studied on tomato crop and investigated that the individual treatment of biofertilizer application of *Azotobacter* resulted significant improvement in the growth parameters like length of leaves, leaf area index, fresh and dry weight and yield in tomato. Bouthaina *et al.* (2010), observed that the plant height, root and shoot length, fresh and dry weights and leaf area of wheat crop increased significantly with *Azotobacter+Azospirillum* treatments. Sridevi and Ramakrishnan (2010), investigated the effects of combined inoculation of AM (*Arbuscular mycorrhizae*) fungi and *Azospirillum* on the growth and yield of onion (*Allium cepa* l.) and reported that various growth biometrics *viz.*, plant height, leaf length, number of leaf sheaths/plant at 30 and 60 days after transplanting in main field was found significantly increased over untreated control.

4.2.4 Effect on fresh weight of leaves

The fresh weight of leaves was recorded immediately after harvesting. It was observed that the application with *Azotobacter* and *Azospirillum* isolates significantly increased the fresh weight of leaves in spring onion over the uninoculated control. The results are presented in Table: 4.6 and graphically shown in Fig: 4.4 and plate 4.4.

i. After harvest (70 days after transplanting)

There was significant increase in fresh weight of leaves in spring onion ranged from 15.91 g to 24.02 g. Significantly higher fresh weight leaves recorded with the application of *Azotobacter* isolate I (24.02 g), followed by *Azospirillum* isolate III (23.06 g), *Azotobacter* isolate III (22.81 g), MPKV *Azotobacter* strain (22.77 g), *Azospirillum* isolate I (22.47 g), MPKV *Azospirillum* strain (22.35 g), *Azospirillum* isolate II (21.93 g) and *Azotobacter* isolate II (21.60 g). The least fresh weight of leaves was recorded in uninoculated control (15.91g). Similar observations were also reported by Bhadoria *et al.* (2005) under the seedling treatment with *Azotobacter* culture in tomato. Aly H. El-naggar (2010), studied the effect of biofertilizer, organic compost and mineral fertilizers on the growth, flowering and bulbs production of *Narcissus tazetta* L., reported that all biofertilizer treatment significantly increased number of leaves/plant, leaf length and width, leaves fresh and dry weight over the untreated control, in which *Azospirillum* treatment showed maximum growth parameters.

Table: 4.6 Effect of *Azotobacter* and *Azospirillum* isolates on the fresh weight and dry weight of leaves g plant^{-1} of spring onion at harvest.

Tr. No.	Treatment details	Fresh weight of leaves (g/plant)	Dry weight of leaves (g/plant)
T_1	*Azotobacter* isolate I	24.02	6.00
T_2	*Azotobacter* isolate II	21.60	5.53
T_3	*Azotobacter* isolate III	22.81	5.70
T_4	MPKV *Azotobacter* strain	22.77	5.67
T_5	*Azospirillum* isolate I	22.47	5.63
T_6	*Azospirillum* isolate II	21.93	5.59
T_7	*Azospirillum* isolate III	23.06	5.82
T_8	MPKV *Azospirillum* strain	22.35	5.62
T_9	Uninoculated control.	15.91	2.92
	SE ±	0.12	0.04
	C.D. (0.05)	0.35	0.12

4.2.5 Effect on dry weight of leaves

The dry weight of leaves were recorded after harvest and drying (70 days after transplanting) as influenced by application of *Azotobacter* and *Azospirillum* isolates were presented in Table: 4.6 and graphically shown in Fig: 4.5. It revealed that application of *Azotobacter* and *Azospirillum* isolates significantly increased the dry weight of leaves over uninoculated control.

i. After harvest (70 Days after transplanting)

The dry weight of leaves was increased in the range of 2.92 g to 6.00 g due to the application of of *Azotobacter* and *Azospirillum* isolates. The significantly maximum dry weight of leaves were noticed 70 DAT in *Azotobacter* isolate I (6.00 g), followed by the *Azospirillum* isolate III (5.82 g), *Azotobacter* isolate III (5.70 g), MPKV *Azotobacter* strain (5.67 g), *Azospirillum* isolate I (5.63 g), MPKV *Azospirillum* strain (5.62 g), *Azospirillum* isolate II (5.59 g) and *Azotobacter* isolate II (5.53 g). The minimum dry weight of leaves was recorded in uninoculated control (2.92g). Tesfaye et al. (2007), studied the response of onion (*Allium cepa* L.) to combined application of biological and chemical nitrogenous fertilizers and observed that application of 75 Kg N ha^{-1} along with inoculation of *Azotobacter* strains was significantly increased most of the growth parameters like plant height, root length, bulb fresh and dry weights, bulb diameter and yield. Bouthaina *et al*. (2010), observed that the plant height, root and shoot length, fresh and dry weight and leaf area of wheat crop increased significantly with *Azotobacter+Azospirillum* treatments.

4.2.6 Effect on number of roots per bulb

The number of roots per bulb were counted and presented in Table: 4.7. It revealed that application of *Azotobacter* and *Azospirillum* isolates showed signific increase in number of roots over uninoculated control.

i. After harvest (70 days after transplanting)

The significant increase was noted in number of roots per bulb in spring onion due to application of the *Azotobacter* and *Azospirillum* isolates over uninoculated control and ranged from 31.50 to 55.45. Significantly higher number of roots was recorded with the application of *Azotobacter* isolate I (55.45) which was at par with *Azospirillum* isolate III (52.53), followed by *Azotobacter* isolate III (50.08), MPKV *Azotobacter* strain (49.33), *Azospirillum* isolate I (47.75), MPKV *Azospirillum* strain (46.90), *Azospirillum* isolate II (46.03) and *Azotobacter* isolate II (43.33). The minimum number of roots (31.50) per bulb was observed in uninoculated control. There was no past reference found on this parameter.

Table: 4.7 Effect of *Azotobacter* and *Azospirillum* isolates on the number of roots bulb^{-1} of spring onion

Tr. No.	Treatment details	Number of roots per bulb
T_1	*Azotobacter* isolate I	55.45
T_2	*Azotobacter* isolate II	43.33
T_3	*Azotobacter* isolate III	50.08
T_4	MPKV *Azotobacter* strain	49.33
T_5	*Azospirillum* isolate I	47.75
T_6	*Azospirillum* isolate II	46.08
T_7	*Azospirillum* isolate III	52.53
T_8	MPKV *Azospirillum* strain	46.90
T_9	Uninoculated control.	31.50
	SE ±	0.37
	C.D. (0.05)	1.10

4.2.7 Effect on weight of fresh bulbs

The weight of bulbs was recorded immediately after harvest (70 days after transplanting) as influenced by application of *Azotobacter* and *Azospirillum* isolates were presented in Table: 4.8 and graphically shown in Fig: 4.7. The results obtained due to application of *Azotobacter* and *Azospirillum* isolates significantly increased the fresh weight of bulbs over uninoculated control.

i. After harvest (70 days after transplanting)

Data on weight of bulb per plant was revealed that application of *Azotobacter* and *Azospirillum* isolates improved the weight of bulbs significantly over uninoculated control. The significantly highest weight of bulb was observed due to application of *Azotobacter* isolate I (36.93 g),which was at par with *Azospirillum* isolate III (36.70 g), followed by *Azotobacter* isolate III (36.24 g), MPKV *Azotobacter* strain (36.15 g), *Azospirillum* isolate I (36.08 g), MPKV *Azospirillum* strain (35.88 g), *Azospirillum* isolate II (35.72 g) and *Azotobacter* isolate II (34.95 g). The least fresh weight of bulb was recorded in uninoculated control (25.31 g). Similar result were noticed by Kore *et al*. (2006) in garlic and reported that the plant height and bulb fresh and dry weights were increased significantly with combination of 10 MT FYM + 3 kg *Azotobacter* +3kg phosphate solubilizing bacteria + 75 % RDF ha^{-1} over control.

Table: 4.8 Effect of *Azotobacter* and *Azospirillum* isolates on the weight of bulb g plant^{-1} and diameter of bulb cm plant^{-1} of spring onion

Tr. No.	Treatment details	weight of bulb (g/plant)	Polar Diameter (cm)	Equatorial Diameter (cm)
T_1	*Azotobacter* isolate I	36.93	4.10	5.20
T_2	*Azotobacter* isolate II	34.95	3.69	4.57
T_3	*Azotobacter* isolate III	36.24	3.93	4.99
T_4	MPKV *Azotobacter* strain	36.15	3.90	4.95
T_5	*Azospirillum* isolate I	36.08	3.88	4.80
T_6	*Azospirillum* isolate II	35.72	3.74	4.76
T_7	*Azospirillum* isolate III	36.70	3.98	5.10
T_8	MPKV *Azospirillum* strain	35.88	3.79	4.78
T_9	Uninoculated control.	25.31	2.57	3.24
	SE ±	0.14	0.02	0.07
	C.D. (0.05)	0.42	0.07	0.21

Ashutosh Singh *et al*. (2007), evaluated onion crop to find out the medium level of nitrogen and treated with *Azospirillum* for obtaining maximum productivity of onion. Result showed that the maximum plant height, number of green leaves, neck thickness, shoot girth, bulb diameter, bulb fresh weight and yield were also higher with treatment of nitrogen 75 kg/ha and treated with *Azospirillum* @ 400 g/2000 seedling roots.

4.2.8 Effect on diameter of bulbs

The polar and equatorial diameter of bulb per plant was calculated and presented in Table: 4.8. It was revealed that application of *Azotobacter* and *Azospirillum* isolates improve the polar and equatorial diameter of bulb significantly over uninoculated control.

i. After harvest (70 days after transplanting)

The result presented in Table: 4.8 and graphically shown in Fig: 4.8 on polar diameter of bulb revealed that the *Azotobacter* and *Azospirillum* isolates significantly increase polar diameter of bulb ranging from 2.57 cm to 4.10 cm. Significantly maximum polar diameter of bulb was recorded with the application of *Azotobacter* isolate I (4.10 cm) which was superior over rest of treatments and uninoculated control. Followed by remaining treatments of *Azospirillum* isolate III (3.98 cm), *Azotobacter* isolate III (3.93 cm), MPKV *Azotobacter* strain (3.90 cm), *Azospirillum* isolate I (3.88 cm), MPKV *Azospirillum* strain (3.79 cm) *Azospirillum* isolate II (3.74 cm) and *Azotobacter* isolate II (3.69 cm). The lowest polar diameter (2.57 cm) of bulb was observed in uninoculated control.

ii. After harvest (70 days after transplanting)

The data presented in Table: 4.8 and graphically shown in Fig: 4.9 on equatorial diameter of bulb revealed that the *Azotobacter* and *Azospirillum* isolates significantly increase equatorial diameter of bulb ranging from 3.24 cm to 5.20 cm. The maximum equatorial diameter of bulb was recorded with the application of *Azotobacter* isolate I (5.20 cm) which was superior over rest of treatments and uninoculated control, followed by remaining treatments of *Azospirillum* isolate III (5.10 cm), *Azotobacter* isolate III (4.99 cm), MPKV *Azotobacter* strain (4.95 cm), *Azospirillum* isolate I (4.80 cm), MPKV *Azospirillum* strain (4.78 cm) *Azospirillum* isolate II (4.76 cm) and *Azotobacter* isolate II (4.57 cm). The lowest equatorial diameter of bulb was observed in uninoculated control (3.24 cm). Similar result were also recorded by Lal *et al.* (2002), in respect of bulb equatorial diameter, polar diameter, leaf area per plant and bulb weight which were significantly increased by application of FYM+ *Azotobacter*. Ghanti and. Sharangi (2009), studied onion cv. Sukhsagar and recorded maximum no. of leaves, no. of inflorescence / plot and bulb diameter with treatment of *Azotobacter+Azospirillum*. Hernando *et al.* (2011), confirmed that the weight, diameter and yield of lettuce and cabbage favoured with the *Azotobacter* + Phosphate solubilizing bacteria applications.

4.2.9 Effect on dry matter weight of whole plant

The results found that with respect to dry matter weight i.e. whole plant dry matter weight include shoot , bulb and root dry matter weight of spring onion at harvesting stage were influenced by *Azotobacter* and *Azospirillum* isolates were presented in Table: 4.9 and graphically shown in Fig: 4.10 which were statistically significant. The observation on dry matter weight was recorded after the oven drying of plant sample at 70±2 c0 for 24 hours. The dry matter weight of spring onion was increased in the range of 7.05 g to 15.74 g due to the application of *Azotobacter* and *Azospirillum* isolates. The significantly maximum dry matter weight of spring onion were noticed in *Azotobacter* isolate I (15.74 g), followed by the *Azospirillum* isolate III (15.03 g), *Azotobacter* isolate III (14.60 g), MPKV *Azotobacter* strain (13.93 g), *Azospirillum* isolate I (13.03 g), MPKV *Azospirillum* strain (12.55 g), *Azospirillum* isolate II (11.98 g) and *Azotobacter* isolate II (11.03 g). The minimum dry matter weight (7.05 g) of spring onion was recorded in uninoculated control. Wange (1995), recorded the response of garlic to combined application of biofertilizers and found that all treatments significantly improved all the parameters over control. Significantly more shoot length and total dry matter weight were recorded in treatment of *Azotobacter* + *Azospirillum*. Talwar Dilpreet *et al.* (2016) concluded the treatments of seedlings with the culture of *Azospirillum and Azotobacter* increase the dry mass of onion when utilized along with inorganic fertilizers and significantly improve the nutrient uptake in plants

Table: 4.9 Effect of *Azotobacter* and *Azospirillum* isolates on the dry matter weight g plant^{-1} of spring onion

Tr. No.	Treatment details	Dry matter weight(g/plant)
T_1	*Azotobacter* isolate I	15.74
T_2	*Azotobacter* isolate II	11.03
T_3	*Azotobacter* isolate III	14.60
T_4	MPKV *Azotobacter* strain	13.93
T_5	*Azospirillum* isolate I	13.03
T_6	*Azospirillum* isolate II	11.98
T_7	*Azospirillum* isolate III	15.03
T_8	MPKV *Azospirillum* strain	12.55
T_9	Uninoculated control.	7.05
	SE ±	0.21
	C.D. (0.05)	0.62

4.2.10 Effect on yield of spring onion

The data on yield of spring onion as influenced by application of bioinoculants were recorded and presented in Table: 4.10 and graphically shown in Fig: 4.11 and plate 4.5. There was significant increase in yield of spring onion due to application of *Azotobacter* and *Azospirillum* isolates and it was varied between 45.43 g plant^{-1} to 61.50 g plant^{-1}.

i. After harvest (70 days after transplanting)

Data on yield of spring onion revealed that application of *Azotobacter* and *Azospirillum* isolates improved the yield of spring onion significantly over uninoculated control. Significantly highest yield of spring onion was observed due to application of *Azotobacter* isolate I (61.50 g), followed by *Azospirillum* isolate III (60.38 g), *Azotobacter* isolate III (59.41 g), MPKV *Azotobacter* strain (58.99 g), *Azospirillum* isolate I (58.16 g), MPKV *Azospirillum* strain (58.04 g), *Azospirillum* isolate II (57.22 g) and *Azotobacter* isolate II (56.38 g). The minimum yield of spring onion was recorded uninoculated control (45.43 g). These observations are in confirmity with those recorded by Rather *et al.* (2003), evaluated the response of onion to microbial inoculation and chemical nitrogen and observed that *Azotobacter* enhanced growth, yield and quality attributes of onion.

Table: 4.10 Effect of *Azotobacter* and *Azospirillum* isolates on yield of spring onion

Tr. No.	Treatment details	Yield of spring onion (g / plant)
T_1	*Azotobacter* isolate I	61.50
T_2	*Azotobacter* isolate II	56.38
T_3	*Azotobacter* isolate III	59.41
T_4	MPKV *Azotobacter* strain	58.99
T_5	*Azospirillum* isolate I	58.16
T_6	*Azospirillum* isolate II	57.22
T_7	*Azospirillum* isolate III	60.38
T_8	MPKV *Azospirillum* strain	58.04
T_9	Uninoculated control.	45.43
	SE ±	0.35
	C.D. (0.05)	1.04

Zorita *et al*. (2008) reported the interaction with *Azospirillum* biofertilizer and wheat cultivars and proved significant effect on grain yield and straw yield. Sailaja *et al.* (2014), studied the effect of foliar application of panchagavya along with *Azotobacter* +*Azospirillum* on growth and development of leafy vegetable (*Spinacia oleracea*) and reported that the treated soil help to bring rapid changes in phenotypes of plants and also improved the growth, yield and ultimately improve the productivity of *Spinacia oleracea*.

4.3 Effect of *Azotobacter* and *Azospirillum* isolates on biological properties of soil during crop growth period

4.3.1 Microbial population of *Azotobacter* in soil

Soil *Azotobacter* population as influenced by application of biofertilization were recorded after the harvest of spring onion. The soil *Azotobacter* population was observed by using serial dilution and pour plate technique on Jensen medium. The results regarding the *Azotobacter* population in soil was statistically significant and it was presented in Table: 4.11 and graphically shown in Fig: 4.12. The initial *Azotobacter* population in soil before sowing was 18

$\times 10^5$ $g^{-1}$of soil. The increasing trend of *Azotobacter* population in soil due to application with bioinoculants was observed after the harvest of crop.

i. 70 Days after transplanting

Application of bioinoculants to spring onion increased the *Azotobacter* population over uninoculated control from 22.67 to 48.33 $\times 10^5$ $g^{-1}$of soil. Significantly highest population (48.33$\times 10^5$ $g^{-1}$of soil) was recorded in *Azotobacter* isolate I over all other treatments, followed by *Azotobacter* isolate III (43.33$\times 10^5$ $g^{-1}$of soil), MPKV *Azotobacter* strain(40.33$\times 10^5$ $g^{-1}$of soil), *Azotobacter* isolate II (34.33$\times 10^5$ $g^{-1}$of soil), *Azospirillum* isolate III (32.67$\times 10^5$ $g^{-1}$of soil), *Azospirillum* isolate I (31.33$\times 10^5$ $g^{-1}$of soil), MPKV *Azospirillum* strain (30.67$\times 10^5$ $g^{-1}$of soil) and *Azospirillum* isolate II (29.67$\times 10^5$ $g^{-1}$of soil). The lowest *Azotobacter* population was observed in uninoculated control (22.67$\times 10^5$ $g^{-1}$of soil). Josip Colo *et al.* (2014), studied plant growth promoting rhizobacteria in onion production and concluded that the total number of bacteria and the number of *Azotobacter chroococcum* were more in all the inoculated variants than in the control. Sornalatha. *et al.* (2016), studied distribution of microbial and diazotrophic *Azotobacter* population in five different rhizosphere soil. The results of enumeration of total microbial population revealed that comparatively the bacterial population found to be higher followed by *Azotobacter* population in various rhizosphere soil.

4.3.2 Microbial population of *Azospirillum* in soil

The soil *Azospirillum* population was observed by using serial dilution and pour plate technique on Malate medium. The results regarding the *Azospirillum* population in soil was statistically significant and it was presented in Table: 4.11 and graphically shown in Fig: 4.13.

i. 70 Days after transplanting

Inoculation with liquid bioinoculants formulations increased the *Azospirillum* population in pot soil from 20.67 to 43.33 $\times 10^5 g^{-1}$of soil. Significantly maximum *Azospirillum* population (43.33$\times 10^5 g^{-1}$of soil) was recorded in *Azospirillum* isolate III, followed by *Azospirillum* isolate I(41.33$\times 10^5 g^{-1}$of soil), MPKV *Azospirillum* strain (39.33$\times 10^5$ $g^{-1}$of soil), *Azospirillum* isolate II (37.67$\times 10^5$ $g^{-1}$of soil), *Azotobacter* isolate I (33.33$\times 10^5$ $g^{-1}$of soil), *Azotobacter* isolate III (31.33$\times 10^5$ $g^{-1}$of soil), MPKV *Azotobacter* strain (30.67$\times 10^5$ $g^{-1}$of soil) and *Azotobacter* isolate II (28.67$\times 10^5$ $g^{-1}$of soil). The minimum *Azospirillum* population was observed in uninoculated control (20.67$\times 10^5$ $g^{-1}$of soil). Tever *et al.* in 2008 observed the genus *Azospirillum* spp. could colonize the roots and stems of plants without causing disease symptoms to stand out. Bagali *et al.* (2012) and Anand *et al.* (2015), respectively studied onion and carrot crop and recorded maximum population of *Azospirillum* (140 cfu/g soil x10^4) and total bacteria (805 cfu/g soil x10^4) is observed in treatment T_9 (50 % recommended N through poultry manure + 50 % recommended N through inorganic fertilizers + *Azospirillum* + *P. fluorescens*).

Table: 4.11 Soil *Azotobacter* and *Azospirillum* population as influenced by application of biofertilizer (c.f.u. × 10^{5} g^{-1} of soil)

Tr. No.	Treatment details	*Azotobacter* (c.f.u. × 10^{5} g^{-1} of soil)	*Azospirillum* (c.f.u. × 10^{5} g^{-1} of soil)
T_1	*Azotobacter* isolate I	48.33	33.67
T_2	*Azotobacter* isolate II	34.33	28.67
T_3	*Azotobacter* isolate III	43.33	31.33
T_4	MPKV *Azotobacter* strain	40.33	30.67
T_5	*Azospirillum* isolate I	29.67	37.67
T_6	*Azospirillum* isolate II	31.33	41.33
T_7	*Azospirillum* isolate III	32.67	43.33
T_8	MPKV *Azospirillum* strain	30.67	39.33
T_9	Uninoculated control.	22.67	20.67
	SE ±	0.61	0.64
	C.D. (0.05)	1.81	1.90

Note :-

Initial *Azotobacter* population in soil (before transplanting) was 18 × 10^{5} $g^{-1}$of soil.

Initial *Azospirillum* population in soil (before transplanting) was 16 × 10^{5} $g^{-1}$of soil.

4.4 Effect of liquid *Azotobacter* and *Azospirillum* application on chemical properties of soil

4.4.1 pH of soil at harvest

The data on pH of soil at harvest stage presented in Table: 4.12 and graphically shown in Fig: 4.14.The initial pH of soil before sowing was 8.1. The observations on pH of soil in spring onion were found non significant after harvest of crop. However, the average pH per treatment ranged from 7.80 to 7.97.

The relatively higher pH (7.97) was observed in uninoculated control, followed by *Azotobacter* isolate I (7.90), *Azospirillum* isolate II, MPKV *Azospirillum* strain, *Azospirillum* isolate III and *Azospirillum* isolate I (7.87). Further the pH of *Azotobacter* isolate II and *Azotobacter* isolate III (7.83).The lowest pH of soil was observed in MPKV *Azotobacter* strain (7.80). Similar result was pointed out by Rousk *et al*. (2010), observed that there is a linear relationship between soil bacterial communities and pH value as indicated bacterial population increases in the range of pH=(4–8). Mazinani *et al*. (2012), reported that the highest population of *Azotobacter* and *Azospirillum* was observed in soil samples with the range of pH= (7–7.8) and it started decreasing above 8 or in acidic pH.

4.4.2 Electrical conductivity (EC) of soil at harvest

The results on EC of soil at harvest stage are statistically non significant over uninoculated control, which were presented in Table: 4.12 and graphically shown in Fig: 4.15.The initial EC of soil before sowing was 0.24. However, the average EC per treatment ranged from 0.21 to 0.23. The relatively higher EC (0.23) was observed in uninoculated control, *Azotobacter* isolate II, *Azotobacter* isolate III and *Azotobacter* isolate I, followed by *Azospirillum* isolate II, *Azospirillum* isolate I, MPKV *Azotobacter* strain and MPKV *Azospirillum* strain (0.22). The lowest EC (0.21) of soil was observed in *Azospirillum* isolate III. There was no past literature found on this trait.

Table: 4.12 Soil pH and Electrical Conductivity (E.C) influenced by application of *Azotobacter* and *Azospirillum* isolates

Tr. No.	Treatment details	Soil pH	Electrical Conductivity (EC)
T_1	*Azotobacter* isolate I	7.93	0.23
T_2	*Azotobacter* isolate II	7.83	0.23
T_3	*Azotobacter* isolate III	7.77	0.23
T_4	MPKV *Azotobacter* strain	7.80	0.22
T_5	*Azospirillum* isolate I	7.87	0.22
T_6	*Azospirillum* isolate II	7.83	0.22
T_7	*Azospirillum* isolate III	7.90	0.21
T_8	MPKV *Azospirillum* strain	7.87	0.22
T_9	Uninoculated control.	7.97	0.23
	Initial pH and E.C in soil	8.30	0.24
	SE ±	0.04	0.004
	C.D. (0.05)	(N.S)	(N.S)

4.4.3 Available Nitrogen content in soil at harvest (Kg/ha)

The data on available nitrogen content in soil at harvest stage presented in Table: 4.13 and graphically shown in Fig: 4.16. The initial nitrogen content in soil before sowing was 175.62 Kg/ ha. It was revealed that available nitrogen content of soil was increased due to application of *Azotobacter* and *Azospirillum* isolates significantly over the uninoculated control in all treatments.

The available nitrogen content varied between 204.86 to 342.86 Kg ha^{-1}. Significantly highest available nitrogen content (342.86 Kg ha^{-1}) was observed in application of *Azotobacter* isolate I which was superior over rest of treatments and uninoculated control, followed by *Azospirillum* isolate III (338.69 Kg ha^{-1}), *Azotobacter* isolate III (330.32 Kg ha^{-1}), MPKV *Azotobacter* strain (326.14 Kg ha^{-1}), *Azospirillum* isolate I (321.96 Kg ha^{-1}), MPKV *Azospirillum* strain (317.78 Kg ha^{-1}), *Azospirillum* II isolate (309.42 Kg ha^{-1}) and *Azotobacter* isolate II (305.24 Kg ha^{-1}). The lowest available nitrogen (204.89 Kg ha^{-1}) content was recorded in uninoculated control.

4.4.4 Available Phosphorus content in soil at harvest (Kg/ha

The results revealed that an application of *Azotobacter* and *Azospirillum* isolates increased the available phosphorus in soil as compared to uninoculated control. The initial phosphorus content in soil before sowing was 14.14 Kg ha^{-1}.

Table: 4.13. Effect of *Azotobacter* and *Azospirillum* isolates on available N, P and K (Kg ha^{-1}) content in soil at harvest

Tr. No.	Treatment details	Available in Kg ha^{-1}		
		N	P	K
T_1	*Azotobacter* isolate I	342.86	28.54	316.69
T_2	*Azotobacter* isolate II	305.24	24.07	278.55
T_3	*Azotobacter* isolate III	330.32	27.21	297.25
T_4	MPKV *Azotobacter* strain	326.14	25.88	292.39
T_5	*Azospirillum* isolate I	321.96	25.63	283.04
T_6	*Azospirillum* isolate II	309.42	24.40	279.30
T_7	*Azospirillum* isolate III	338.69	27.90	310.53
T_8	MPKV *Azospirillum* strain	317.78	24.91	280.79
T_9	Uninoculated control.	204.89	17.90	222.09
	Initial value of soil N,P,K status	175.62	14.14	209.76
	SE ±	6.54	0.49	2.85
	C.D. (0.05)	19.42	1.46	8.47

The available phosphorus was ranged between 17.90 to 28.54 Kg ha^{-1} and it was presented in Table: 4.13 and graphically shown in Fig: 4.17. Significantly highest available phosphorus content (28.54 Kg ha^{-1}) was observed in *Azotobacter* isolate I which was superior over rest of all treatments and uninoculated control, followed by *Azospirillum* isolate III (27.90 Kg ha^{-1}), *Azotobacter* isolate III (27.21 Kg ha^{-1}), MPKV *Azotobacter* strain (25.88 Kg ha^{-1}), *Azospirillum* isolate I (25.63 Kg/ ha), MPKV *Azospirillum* strain (24.91 Kg ha^{-1}), *Azospirillum* isolate II (24.40 Kg ha^{-1}) and *Azotobacter* isolate II (24.07 Kg ha^{-1}). The lowest available phosphorus content was recorded in uninoculated control (17.90 Kg ha^{-1}).

4.4.5 Available Potassium content in soil at harvest (Kg/ha)

The data on potassium content of soil at harvesting stage as influenced by bioinoculation of *Azotobacter* and *Azospirillum* isolates was presented in Table: 4.13 and graphically shown in Fig: 4.18. It was observed that potassium content of soil before sowing was 209.76 Kg ha^{-1}.Significantly highest available potassium content (316.69 Kg ha^{-1}) was observed in *Azotobacter* isolate I , followed by *Azospirillum* isolate III (310.53 Kg ha^{-1}), *Azotobacter* isolate III (297.25 Kg ha^{-1}), MPKV *Azotobacter* strain (292.39 Kg ha^{-1}), *Azospirillum* isolate I (283.04 Kg ha^{1}),MPKV *Azospirillum* strain (280.79 Kg ha^{-1}), *Azospirillum* isolate II (279.30 Kg ha^{-1}) and *Azotobacter* isolate II (278.55 Kg ha^{-1}).

The lowest available potassium content was recorded in uninoculated control (280.79 Kg/ ha). These observations are in conformity with those recorded by Kapure and Naik (2004), who conducted experiment on chick pea in order to study the effect of biofertilizers i.e. vitromone, bioplin (culture of *Azotobacter*) and phosphobacterin (culture of *Bacillus* spp.) along with pure culture of *Azotobacter* + phosphate solubilizing bacteria and observed significant increase in N, P and K content in soil. Yadav *et al.* (2004), studied the use of biofertilizers *Azospirillum* in onion. He recorded that application of *Azospirillum* increased trend of available N, P and K content of soil for all the samplings.

4.5 Effect of *Azotobacter* and *Azospirillum* isolates on nitrogen uptake by spring onion

The uptake of nitrogen as influenced by application of *Azotobacter* and *Azospirillum* isolates were studied and calculated by considering concentration of nutrients and dry matter production of spring onion plant. It was observed from data given in Table: 4.14 and graphically shown in Fig: 4.19. The nitrogen uptake in spring onion was significantly increased over uninoculated control and ranged from 0.20g to 0.70g. Significantly highest nitrogen uptake was observed with application of *Azotobacter* isolate I (0.71 g), followed by the *Azospirillum*, isolate III (0.67 g), *Azotobacter* isolate III (0.63 g), MPKV *Azotobacter* strain (0.58 g), *Azospirillum* isolate I (0.50 g), MPKV *Azospirillum* strain (0.49g), *Azospirillum* isolate II (0.45 g) and *Azotobacter* isolate II (0.40 g). The minimum uptake (0.21 g) of nitrogen was recorded in uninoculated control

Table: 14. Effect of liquid *Azotobacter* and *Azospirillum* isolates on the nitrogen uptake (g plant^{-1}) in spring onion.

Sr. No.	**Treatment details**	**N Concentration (%)**	**N uptake (g plant^{-1})**
T_1	*Azotobacter* isolate I	4.62	0.71
T_2	*Azotobacter* isolate II	3.55	0.40
T_3	*Azotobacter* isolate III	4.20	0.63
T_4	MPKV *Azotobacter* strain	4.15	0.58
T_5	*Azospirillum* isolate I	3.81	0.50
T_6	*Azospirillum* isolate II	3.64	0.45
T_7	*Azospirillum* isolate III	4.34	0.67
T_8	MPKV *Azospirillumr* strain	3.73	0.49
T_9	Uninoculated control.	2.71	0.21
	SE ±		0.008
	C.D. (0.05)		0.026

The results reported in present investigation are on the same line as reported by Kader *et al.* (2002) and Mahanthesh *et al*. (2009), reported that usefulness of biofertilizers along with chemical fertilizers uptake, growth, yield and soil properties.

5. SUMMARY AND CONCLUSION

The present experiment was undertaken to study the "Beneficial effect of *Azotobacter*, *Azospirillum* on growth and yield of spring onion (*Allium fistulosum* L.)" The spring onion variety used for this experiment was Phule samrath. The pot culture experiment was conducted at the Department of Plant Pathology and Agricultural Microbiology, College of Agriculture, Pune-05. The experiment laid out in completely randomized design was conducted using spring onion as a test crop under glasshouse condition and results of the experiment are summarized below.

5.1 Effect of *Azotobacter* and *Azospirillum* isolates on growth of spring onion

5.1.1 Effect on seed germination

Inoculation with liquid *Azotobacter* and *Azospirillum* isolates showed differential increase in germination percentage of spring onion. The seed treatment with *Azotobacter* Isolate I (86.44) showed significantly highest germination over uninoculated control (73.33).

5.1.2 Effect on number of leaves per plant

Application of *Azotobacter* and *Azospirillum* isolates increased the number of leaves per plant at different intervals during crop growth stages. It was maximum at 70 days after transplanting (DAT) and ranged from 6.28 to 9.23. The significantly higher number of leaves was recorded in treatment with *Azotobacter* Isolate I (9.23) followed by rest of the treatments and least (6.28) was recorded in uninoculated control.

5.1.3 Effect on length of leaves

The length of leaves per plant increased due to application of *Azotobacter* and *Azospirillum* isolates and observed maximum length of leaves at 70 DAT ranging from 45.64 cm to 60.20 cm. The significantly maximum length of leaves was recorded with the application of treatment with *Azotobacter* isolate I (60.20 cm) and it was superior over all other treatments. The lowest length of leaves was observed in uninoculated control (45.64 cm).

5.1.4 Effect on fresh weight of leaves

It was observed that the inoculation with *Azotobacter* and *Azospirillum* isolates significantly increased the fresh weight of leaves in spring onion over the uninoculated control. The results obtained were statistically significant and higher at 70DAT ranging from 15.91 g to 24.02 g. The significantly maximum fresh weight of leaves were noticed in treatment with *Azotobacter* isolate I (24.02 g) and minimum was found in uninoculated control (15.91 g).

5.1.5 Effect on dry weight of leaves

The dry weight of leaves was recorded and found maximum at 70 DAT ranging from 2.92 g to 6.00 g. The significantly maximum dry weight of leaves were recorded in treatment with *Azotobacter* isolate I (6.00 g) all other treatments and the lowest dry weight of leaves (2.92 g) was noticed in uninoculated control.

5.1.6 Effect on number of roots per bulb

It was concluded that application of *Azotobacter* and *Azospirillum* isolates improve number of roots per bulb significantly over uninoculated control and ranged from 31.50 to 55.45. The maximum number of roots was recorded with the application of *Azotobacter* isolate I (55.45) which was superior over rest of treatments and uninoculated control. The minimum number of roots was recorded in uninoculated control (31.50).

5.1.7 Effect on weight of fresh bulbs

The result obtained due to application of *Azotobacter* and *Azospirillum* isolates significantly increased the fresh weight of bulbs over uninoculated control after harvest (70 DAT). The significantly highest weight of bulb was observed due to application of *Azotobacter* isolate I (36.93 g) which was at par with *Azospirillum* isolate III (36.70 g) and minimum fresh weight (25.31 g) of bulbs was noticed in uninoculated control.

5.1.8 Effect on diameter of bulbs

It was revealed that application of *Azotobacter* and *Azospirillum* isolates improve the polar and equatorial diameter of bulb significantly over uninoculated control. The maximum polar diameter (4.10 cm) of bulb was recorded with the application of *Azotobacter* isolate I which was superior over rest of treatments and uninoculated control respectively. Significantly maximum equatorial diameter of the bulb was recorded in Azotobacter isolate I (5.20 cm) which was at par with Azotobacter isolate III (4.99 cm) treatment. The lowest polar diameter (2.57 cm) and equatorial diameter (3.24 cm) was observed in uninoculated control.

5.1.9 Effect on total dry matter weight of whole plant

The results found that with respect to dry matter weight i.e. whole plant dry matter weight include shoot , bulb and root dry matter weight of spring onion at harvesting stage were significantly influenced by *Azotobacter* and *Azospirillum* isolates. Significantly maximum dry matter weight of spring onion were noticed in *Azotobacter* isolate I (15.74 g) to all other treatments. The lowest dry matter weight of spring onion (7.05 g) was noticed in uninoculated control.

5.1.10 Effect on yield of lettuce

There was significant increase in yield of spring onion due to application *Azotobacter* and *Azospirillum* isolates and it was varied between 45.53 g plant^{-1} to 61.50 g plant^{-1}. Significantly the highest yield of spring onion was recorded with application of treatment with *Azotobacter* isolate I (61.50 g) and minimum yield of spring onion (45.43 g) was found in uninoculated control.

5.2 Effect of *Azotobacter* and *Azospirillum* isolates on biological properties of soil during crop growth period

There was an increase in *Azotobacter* and *Azospirillum* population in soil during crop growth stages due to application of *Azotobacter* and *Azospirillum* isolates on spring onion**.**

5.2.1 Microbial population of *Azotobacter* in soil

The *Azotobacter* culture increased the *Azotobacter* population in soil. The *Azotobacter* population ranged from 22.67 × 10^{5} g^{-1} to 48.33 × 10^{5} $g^{-1}$of soil. Significantly highest population (48.33 × 10^{5} $g^{-1}$of soil) was recorded in seed treatment with *Azotobacter* isolate I over all other treatments, followed by treatment with liquid *Azotobacter* isolate III (43.33 × 10^{5} $g^{-1}$of soil). The lowest *Azotobacter* population was observed in uninoculated control (22.67 × 10^{5} $g^{-1}$of soil). Initial *Azotobacter* population in soil (before transplanting) was 18 × 10^{5} $g^{-1}$of soil.

5.2.2 Microbial population of *Azospirillum* in soil

The *Azospirillum* population in soil due to inoculation with liquid bioinoculants was observed at 70 DAT and ranging from 20.67 × 10^{5} g^{-1} to 33.67 × 10^{5} $g^{-1}$of soil. Significantly the highest population (33.67 × 10^{5} $g^{-1}$of soil) was recorded due to application of treatment with *Azospirillum* isolate III and the lowest *Azospirillum* population (20.67 × 10^{5} $g^{-1}$of soil) was recorded in uninoculated control. Initial *Azospirillum* population in soil (before transplanting) was 16 × 10^{5} $g^{-1}$of soil.

5.3 Effect of liquid *Azotobacter* and *Azospirillum* application on chemical properties of soil

5.3.1 pH of soil at harvest

The observations on pH of soil in spring onion were found non significant after harvest of crop. However, the average pH per treatment ranged from 7.80 to 7.97. The relatively higher pH (7.97) was observed in uninoculated control and lowest pH of soil was observed in MPKV *Azotobacter* strain (7.80). Initial pH in soil (before transplanting) was 8.30.

5.3.2 EC of soil at harvest

The results on EC of the soil at harvest stage showed statistically non significant over uninoculated controI. The initial EC of soil before sowing was 0.24. However, the average EC per treatment ranged from 0.21 to 0.23. The relatively higher EC (0.23) was observed in uninoculated control and the lowest EC (0.21) of soil was observed in *Azospirillum* isolate III.

5.3.3 Available nitrogen content in soil at harvest (Kg/ha)

Available nitrogen content in soil recorded significant increase from initial level up to harvesting stage. The application of liquid bioinoculants contributes to available nitrogen content by increasing the population of nitrogen fixing microorganisms. The available nitrogen content of the soil at harvest stage varied between 204.89 to 342.86. Kg ha^{-1}. Significantly highest available nitrogen content (342.86 Kg ha^{-1}) was observed in treatment with *Azotobacter* isolate I followed by treatment with *Azospirillum* isolate III (338.69 Kg ha^{-1}) and treatment with *Azotobacter* isolate III (330.32 Kg ha^{-1}) these three treatments was at par with each other and lowest in uninoculated control (204.89 Kg ha^{-1}).

5.3.4 Available phosphorus content in soil at harvest (Kg/ha)

Available phosphorus content in soil was higher at harvest stage and it was ranged from 17.90 to 28.54 Kg ha^{-1}. The highest available phosphorus content (28.54 Kg ha^{-1}) was observed in treatment with *Azotobacter* isolate I and lowest available phosphorus content was recorded in uninoculated control (17.90 Kg ha^{-1}).

5.3.5 Available potassium content in soil at harvest (kg/ha)

Available potassium content in soil recorded significant increase from initial level up to harvesting stage. The available potassium content of the soil at harvest stage varied between 204.89 to 342.86. Kg ha^{-1}. Significantly highest available potassium content (316.69 Kg ha^{-1}) was observed in treatment with *Azotobacter* isolate I which was at par with treatment with *Azospirillum* isolate III (310.53 Kg ha^{-1}) and lowest in uninoculated control (222.09 Kg ha^{-1}).

5.4 Effect of *Azotobacter* and *Azospirillum* isolates on nitrogen uptake by spring onion

Application of liquid bioinoculants significantly increased nitrogen uptake by spring onion crop over uninoculated control and ranged from 0.21 g to 0.71 g. Significantly highest nitrogen uptake was observed with the application of treatment with liquid *Azotobacter* isolate I (0.71 g) which was superior to all other treatments. The lowest nitrogen uptake was recorded with uninoculated control (0.21 g).

Conclusion

Application of *Azotobacter* and *Azospirillum* isolates increased growth parameters and yield of spring onion crop significantly over uninoculated control. The significantly increased total *Azotobacter* and *Azospirillum* count in plant rhizosphere which further increased the available nitrogen and phosphorus content of soil. The uptake of nitrogen by spring onion crop was also increased due to inoculation with *Azotobacter* and *Azospirillum* bioinoculants.

The treatment with *Azotobacter* isolate I (DOGR farm, Rajgurunagar) was recorded it’s superiority over all the treatments studied for number of leaves (9.23), length of leaves (60.20 cm), fresh weight (24.02 g) and dry weight (6.00 g) of leaves, number of roots per bulb (55.45), weight of fresh bulb (36.93 g), polar diameter (4.10 cm), equatorial diameter (5.20 cm), total dry matter (15.74 g) and yield (61.50 g). It was superior also for soil *Azotobacter* population (48.33×10^{5} $g^{-1}$of soil) and available soil nitrogen (342.86 $Kgha^{-1}$), available soil phosphorus (28.54 $Kgha^{-1}$), available soil potassium (316.19 $Kgha^{-1}$) and nitrogen uptake (0.71 g) than uninoculated control.

The treatment with *Azospirillum* isolate III (DFR farm, Hadapsar) was found superior for soil *Azospirillum* population (33.67×10^{5} $g^{-1}$of soil) over uninoculated control. There were no significant differences recorded for pH and EC of soil after harvest of crop. Increase in available nitrogen, phosphorus and potassium from initial stage to harvesting stage of the crop. From the present study, the *Azotobacter* and *Azospirillum* isolates were exhibited their positive impact on growth and yield of spring onion and soil properties.

7. LITERATURE CITED

Alexander M. (1977), Introduction to Soil Microbiology. 2nd Edn., John Wiley and Sons Inc., New York

Aly H. El-naggar (2010). Effect of biofertilizer, organic compost and mineral fertilizers on the growth, flowering and bulbs production of *Narcissus tazetta, l. J.Agr ic. & Env.Sci*.Alex.Univ., Egypt Vol.9 (1) 2010.

Anand, G. P., A. R. M. Sayeed, and F.Mohammed (2015). Integrated nutrient management in carrot (*Daucus carrot* L.) under North Eastern transitional track of Karnataka. Res. *J. Agril. Sci*., 6 (3) : 571-572.

Apte R and Shende S.T (1981), studies on morphological, biochemical and physiological characteristics of *Azotobacter* spp.Zb I. Bakt. II Abt, 136: 548- 554.

Bagali, A. N., H. B. Patil, P. Chimmad, P. L. Patil and R. V Patil, (2012). Effect of inorganics and organics on growth and yield of onion (*Allium cepa* L.). Karnataka *J. Agric. Sci*., 25 (1) : 112-115.

Balasubramani P (1988). Effect of *Azospirillum* and nitrogen on growth and yield of bhindi (*Abelmoschus esculentus* L. Moench) var. Pusa Sawani. S. *Indian Hort*. 36(4) : 216-18.

Barassi, C.A., G. Ayrault, C.M. Creus, R.J. Sueldo, and M.T. Sobrero. (2006). Seed inoculation with *Azospirillum* mitigates Nacl effects on lettuce. *Sci. Hort*. 109:8–14.

Bashan Y., G.Holguin, and L. E. de-Bashan (2004). "*Azospirillum*-plantrelationships: physiological, molecular, agricultural, and environmentaladvances (1997–2003)," *Canadian Journal of Microbiology*,vol. 50, no. 8, pp. 521–577.

Behl, R.K. and N. Narula, (2003). Establishment of phosphate solubilizing strains of *Azotobacter chroococcum* in the rhizosphere and their effect on wheat cultivers under green house conditions. *Microbiological research.* 156: 87-94.

Beijerinck M W (1901). Ueber Oligonitophile Mikroben, Zentralblattfiir Bakteriologie, Parasitenkunde, Infektionskrankheiten and Hygiene, Abteilung II 7: 561-582.

Bhadoria S. K. S., Y. C. Dwivedi, and S. S. Kushwah (2005). Effect of *Azotobacter* inoculation with nitrogen level on quality character of tomato. *Veg. Sci*. 32 (1) : 94-95.

Borollosy M.A., M. A. Gawade, and A. L. Shahawy (2001).Evaluation of some nitrogenious commercial biofertilizers on growth of some non-legumes.*Annals Agric. Sci*. Moshtohor. 39 (4) : 2183-2196.

Bouthaina A. E. G., Rhawhia A. M., E. R.Tomader, E. S.Mona Morsy (2010). Effect of some soil microorganisms on soil properties and wheat production under north Sinai conditions. *J. of Appl. Sci. Res*. 4(5): 559-579.

Brewster, J.L. (1994). Onions and other Vegetable Alliums. Horticulture Research International, Wellesbourne, CAB International. pp 236.

Burdman S, J Kigel, Y Okon (1997). Effects of *Azospirillum brasilense* on nodulation and growth of commonbean (*Phaseolus vulgaris* L.). Soil Biology Biochemistry **29,** 923–929.

Chattoo M A, N Ahmed, S Faheema, S Narayan, SHK Khan and Hussain (2007). Response of garlic (*Allium sativum* L.) to bio-fertilizer application.*Asian J. of Horti*. 2 (2) :249-252.

Chevallier, A. (1996). The Encyclopedia of Medicinal Plants Dorling Kindersley. London.

Coombs, B. (1995). Horticulture Australia, Morescope Publishing Co., Hawthorn East, Vic. Pp 1-7.

Das A. C. and S. C. Kole (2006). Effect of some root associative bacteria on germination of seeds, nitrogenase activity and dry matter production by rice plants. *Journal of crop and weed* 2(2) : 47-51.

Dere, K. B. (1986). Effect of *Azotobacter chroococcum* and *Azospirillum brasilense* inoculation under graded levels of nitrogen on growth and yield of brinjal (*Slolanum melongena*) cv. Manjari gota. M.Sc. (Agri.). Thesis submitted to MPKV, Rahuri (M.S.), India.

Devendra Kumar Kurrey, Mahendra Kumar Lahre and Gaurav S Pagire (2017). Studies on effect of *Azotobacter* on growth and yield of onion (*Allium cepa* L.) *Journal of Pharmacognosy and Phytochemistry* (2018); 7(1): 1171-1175.

Dobereiner J and J M Day (1976). Associative symbiosis in tropical grasses: characterization of microorganisms and nitrogen fixing sites. In: Newton WE, Nyman CJ (eds) Proceedings of the First International Symposium on Nitrogen Fixation. Pullman: Washington State University Press, pp. 518–536.

Duke, J. A. and E. S. Ayensu, (1985). Medical Plants of China Reference Publications, Inc.

Ewulo, B. S., (2005). Effect of poultry manure and cattle manure on sandy clay loam soil. *J. Anim. Vet. Sci.*, 4: 839 – 841.

Fibach-Paldi S, S Burdman & Y Okon (2012) . Key physiological properties contributing to rhizosphere adaptation and plant growth promotion abilities of *Azospirillum* brasilense . FEMS Microbiol Lett 326:99–108.

Food and Agriculture Organization (1982). Application of Nitrogen-Fixing Systems in Soil Improvement andManagement. Food and Agriculture Organization of the United Nations.FAO Soils Bulletin 49, Rome.

Foster, J.A., M.J. Hogan, B.M. Herring, and W. Gieseking, (1985). Creative Living, Basic Concepts in Home Economics. Third Edition. Pp 274-275.

Gabriela Fasciglione, M.Elda Casanovas, Victoria Quillehauquy, K. Alejandra Yommi, María, G. Goni, I.Sara, Rour, Carlos, A. Barassi (2015). *Azospirillum* inoculation effects on growth, product quality and storage life of lettuce plants grown under salt stress. Scientia Horticulturae 195, 154–162.

Ghanti S. and A.B. Sharangi (2009). Effect of bio-fertilizers on growth, yield and quality of onion cv. Sukhsagar. *Journal of Crop and Weed*, 5(1):120-123.

Hernando C., L.Tulio, P. Edwin, and P. Ruth, (2011). The effect of three liquid biofertilizers in the production of lettuce (*Lactuca sativa* L.)and cabbage (*Brassica oleracea* L. var *capitata*). Agronomia Colombiana 29 (3) : 415-421.

Holguin G., C.L Patten , and B.R.Glick, (1999). Genetics and molecular biology of *Azospirillum*. Biol. Fertil. Soils, 29: 10–23.

Jackson, M.L. (1971). Soil chemical analysis. Prentice Hall of India Pvt Ltd., New Delhi.

Jadhav S.W. and P.L. Patil, (1985). Effect of *Azotobacter* on growth and yield of paddy. *Indian J. Microbiol*. 21 (3): 212-213.

Jensen V and E J Petersen (1954). Studies on the occurrence of *Azotobacter* in Danish forest soils. Asskr. K. Vet. Landbkojsk. 94-108

Johnstone D B (1974), GenusI.*Azotobacter* Beijerinck 1938. In Bergey's Manual of Determinative Bacteriology. 8 ed. Willliams and Wilkins, Baltimore Publ. pp. 254- 256.

Josip Colo, Timea Hajnal-Jafari , Simonida Durić , Dragana Stamenov and Saud Hamidović (2014). Plant growth promotion rhizobacteria in onion production. Polish *Journal of Microbiology* 2014, Vol. 63, No 1, 83–88.

Kader M. A., M. H. Mian, and M. S. Hoque, (2002). Effects of *Azotobacter* inoculant on the yield and nitrogen uptake by wheat.Online *J. Biol. Sci*., 2: 259-261.

Kanungo P.K., D. Panda, T.K. Adhya, Ramakrishnan and Rajaramamohan Rao, (1997). Nitrogenase activity and nitrogen fixing bacteria associated of rice cultivars with varying n absorption efficiency .*J. Sci. Food and Agri*.73 (4): 487 – 488.

Kapure R. M. and R. M. Naik, (2004). Effect of biofertilizer on N, P content of leaves, available P from soil, leghaemoglobin and chlorophyll content in chickpea. *J. Soils and Crops*. 14 (1) : 22-25.

Kaushik, M.K., N.R. Bishnoi, and H.K Sumeriya (2012). Productivity and economics of wheat as influenced by inorganic and organic sources of nutrients. Annals of Plant and Soil Research 14 (1): 61- 64.

Kizilkaya R (2009). Nitrogen fixation capacity of *Azotobacter* spp. Strains isolated from soils in different ecosystems and relationship between them and the microbiological properties of soils. *J Environ Biol* 30(1): 73-82.

Knudsen D., G. A. Peterson, and P. F. Pratt, (1982).Lithium, Sodium and Potassium in methods of soil anylysis part-2. Chemical and Microbilogical properties.Page A. L. (11) edition Amer. Soc. Agron.Ind., Soil Sci. Soc. of Amer. Inc. Madison. Wisconsion U.S.A. 225-245

Kochar M. and S. Srivastava (2012). "Surface colonization by *Azospirillum brasilense* SM in the indole-3-acetic acid dependent growth improvement of sorghum," *Journal of Basic Microbiology*, vol. 52, no. 2, pp. 123–131.

Kolhe D. S, V. A Khaire, J.D Patil and M. D Nayakwadi (1988). A note on the effect of *Azotobacter* and *Azospirillum* alone and in combination on the yield of leafy vegetables. Haryana *J. Hort. Sci*. 17(3-4): 280-81.

Kopeloff and Beerman (1957). Manual of microbiology methods, Society of American Bacteriologist,McGraw –Hill Book Co.,p. 16-17.

Kore M S, R.Z Shembekar, N.K Chopde, O.D Kuchanwar, S.S Pillewan and S.B Godse (2006). Nutrient management in garlic (*Allium sativum* L.). *J. of Soils and Crops,* 16 (2):465-468.

Krieg, N. R. and J. Döbereiner, (1986). Genus *Azospirillum,* Bergey's Manual of Systematic Bacteriology, Willliams and Wilkins, Baltimore, vol. 1, pp. 94–104.

Kumar G P, S.K Yadav, P.R Thawale, S.K Singh, A.A Juwarkar (2008).Growth of *Jatropha curcas* on heavy metal contaminated soil amended with industrial wastes and *Azotobacter*-A greenhouse study. Bioresour. Technol. 99: 2078-2082.

Kumar V, R.K Behl, N Narula (2001). Establishment of phosphate solubilizing strains of *Azotobacter chroococcum* in the rhizosphere and their effect on wheat cultivars under greenhouse conditions. Microbiol. Res. 156: 87-93.

Lal S, A C Yadav, J.L Mangal, Singh Avtar and V K. Batra (2002). Effects of FYM and irrigation levels on growth and yield of onion cv. Hisar-2. Haryana *J. Hort. Sci*. 31 **(**3-4):256-258.

Mahanthesh M, M Harshavardhan and M Raviprasad (2009). Impact of Bio-fertilizers with levels of NPK on nutrient uptake and yield of onion (*Allium cepa* L.) cv. Bellary Red in Kharif season under irrigated situation in central dry zone of Karnataka. Mysore *J. Agric. Sci*., 43(4) : 650-655.

Mahato, Anoop Badoni and J. S. Chauhan (2009). Effect of *Azotobacter* and Nitrogen on Seed Germination and Early Seedling Growth in Tomato. H. N. B. Garhwal University, Srinagar, Researcher, 1(4).

Mangmang J.S., R. Deaker, G. Rogers (2015) . Early seedling growth response of lettuce, tomato and cucumber to *Azospirillum brasilense* inoculated by soaking and drenching. *Hort. Sci*. (Prague) Vol. 42, 2015 (1): 37–46.

Mazinani1 Z, M,Aminafshar, Ahmad, M Chamani (2012). Effect of *Azotobacter* and *Azospirillum* population on physico–chemical characteristics of some soil samples in Iran. *Annals of Biological Research*.; 3:3120-3125. ISSN 0976- 1233.

Mishustin E.N. and A.N.Naumova, (1962). Bacterial fertilizers, their effectiveness and mode of action. Microbiologiya, 31: 543-555.

Mounika Y., G. Thanuja Sivaram, P. Syam Sundar Reddy and M. Ramaiah (2018). Influence of biofertilizers and micronutrients on growth, seed yield and quality of coriander (*coriandrum sativum* l.) cv. sadhana. int.*j.curr.microbiol.app.sci 7*(1): 2099-2107.

Musmade B.B and B.K Konde. (1987). Effect of *Azotobacterization* on growth and yield of onion (*Allium cepa L.)* under field conditions. Department of Plant Pathology and Agril.Microbiology MPKV. Rahuri-413722.

Narula, N., V. Kumar, R.K. Behl,, A Deubel, A Gransee, and W Merbach,. (2000). Effect of P solubilizing *Azotobacter chroococcum* on N, P and K uptake in P responsive wheat genotypes grown under greenhouse conditions. *J. Plant Nutrition and soil sci*. 163: 393- 398.

Nguyen, V.D. and D Thi Nhu,. (1989). Medicinal Plants in Vietnam. World Health Organization.

Oh K H, CS Seong, S W Lee, O S Kwon, Y S Park (1999). Isolation of psychrotrophic *Azospirillum* sp., and characterization of its extracellular protease. FEMS Microbiology Letters 174, 173–178.

Okigbo, B.N. (1983). Fruit and Vegetable Production and Extension Services in Africa. Act. Hortic. 84: 23.

Okon Y. Hadar (1987). Microbial inoculants as crop-yield enhancers. CRC Critical Review Biotechnology **6,** 61–85.

Olsen S. R, C. V. Cole, F. S. Watanbe,. and L. A. Dean (1965). Estimation of available phosphorus in soil by extraction with $NaHCO_3$. Cir. U. S. Dept. Agric. Pp.939.

Panse V. G. and P. V. Sukhatme,. (1967). Statistical methods for agricultural workers .ICAR publication New Delhi.

Parkinson J.A. and S.E. Allen, (1975).A wet oxidation procedure suitable for determiniation of nitrogen and mineral nutrients in biological material.Commun. *Soil. Sci. Plant Annial*. 6 (1): 1-11.

Patil Dipak Ashok (2015). Effect of liquid formulation of *Azotobacter* and PSB inoculation on growth and yield of lettuce. Thesis submitted to the Mahatma Phule Krishi Vidyapeeth**,** Rahuri.

Pereg-Gerk L, K Gilchrist, I.R Kennedy (2000). Mutants with enhanced nitrogenase activity in hydroponic *Azospirillum brasilense*-wheat associations. Applied Environmental Microbiology 66, 2175–2184.

Pereyra, M.A., C.A. Zalazar, and C.A. Barassi, (2006). Root phospholipids in *Azospirillum*-inoculated wheat seedlings exposed to water stress. *Plant Physiol Biochem* 44, 873-879.

Radhakrishanann, D. and R. R. Mallikarjuniah (1983). Effect of bacterial inoculants on growth of palak and amaranthus. VIth Southern Reg. Conf. On Microbial Inoculants, held at U.A.S Banglore on Sep. 22-23 (Abst.) pp. 16.

Ram Vachan and S M Tripathi (2017).Studied on the effect of bio-fertilizer with chemical fertilizer on plant growth, Yield and economics of Rabi season onion (*Allium cepa* L.) cv. NHRDF Red 2. Journal of Pharmacognosy and Phytochemistry; 6(5): 1496-1499.

Ranjan J.K., N Ahmed, B. Das and Pragya Hare Krishna (2010). Effect of biofertilizers in combination with reduced dose of fertilizers on growth and yield of garlic at high altitude of north-west Himalayas. *Indian J.of Horti.*, 67:263-267.

Rather S A, M Ahmed and M A Chattoo (2003). Response of onion to microbial inoculation and chemical nitrogen. Haryana *J. Hort. Sci*. 32(3-4): 270-71.

Reid, B. E. (1977). Famine Food of the Chiu-Huang Pen-ts‟ao. Taipei. Southern Materials Centre.

Revillas J J, B Rodelas, C Pozo, MV Martínez-Toledo, González-LópezJ (2000). Production of B-group vitamins by two *Azotobacter* strainswith phenolic compounds as sole carbon source under diazotrophic and adiazotrophic conditions. *J. Appl. Microbiol*. 89: 486-493.

Riotte, L. (1978). Companion Planting for Successful Gardening. Garden Way, Vermont, USA.

Rousk J, E Baath, P C Brookes and, J G Caporaso.(2010), Soil bacterial and fungal communities across a pH gradient in an arable soil; 4:1340-51

Sailaja V., N. Naga Ragini, Kanderi Dileep Kumar, B. Rajasekhr Reddy and S.V Satyanarayana (2014). Effect of foliar application of panchagavya on growth and development of leafy vegetable (*Spinacia oleracea).International Journal of Agricultural and Food Science*; 4(4): 119-122.

Sangeeth, K. P., R. Susheelabhai and V.Srinivasan, (2008). Evaluation of indigenous *Azospirillum* isolates for growth promotion in black pepper (*Piper nigrum* L.) rooted cuttings. *J. Spices Arom. Crops*, 11: 128-33.

Sharma, S. K. and K. S. Thakur, (2001). Effect of *Azotobacter* and nitrogen on plant growth and fruit yield of tomato. Veg. Sci. 28 (2): 146-148.

Shende S.T., G.B.Rudraksha, , R.Apte, and R.S Raut,.(1986). *Azotobacter* inoculation: Nitrogen economy and response of sorghum (CSH-1)cereal nitrogen fixation. In:proc. of working group meetings, held during 9-12 oct.,1984 at ICRISAT Center, Patancheru,A.P.,India.

Singh .V.K., Amrita Kumari, V.K. Chaudhary and S. Shree (2017). Role of Biofertilizer and Chemical Fertilizer for Sustainable Onion (*Allium cepa* L.) Production. *Int.J.Curr.Microbiol.App.Sci 6*(9): 2034-2040.

Singh, D. R.;Ashutosh Singh (2007). Effect of nitrogen and *Azospirillum* inoculation on growth and yield of onion (*Allium cepa* L.).*Haryana Journal of Horticultural Sciences* 2007 Vol.36 No.1/2 pp.153-155 ref.10.

Sornalatha.T , P.U Mahalingam, V. Jansi. (2016).Study on distribution of microbial and diazotrophic *Azotobacter* population in five different rhizosphere soil. *International Journal of Environmental & Agriculture Research* , [Vol-2, Issue-7, ISSN:[2454-1850].

Sridevi S. and K. Ramakrishnan (2010). Effects of combined inoculation of AM (Arbuscular mycorrhizae) fungi and *Azospirillum* on the growth and yield of onion(*Allium cepa* l.) *Journal of Phytology* 2(1): 88-90.

Srinivasan, V., S. Hamza and A. K. Sadanandan, (2005). Evaluation of composted coir pith with chemical and bio-fertilizers on nutrient availability, yield and quality of black pepper (*Piper nigrum* L.). *J. Spices Arom. Crops*, 14: 15-20.

Steenhoudt O, J Vanderleyden (2000) *Azospirillum*, a free living nitrogen-fixing bacteria closely associated with grasses: genetic, biochemical and ecological aspects. FEMS Microbiology Review 24, 487–506.

Stephens, J.M. (2009). A document (HS635) of the Horticultural Sciences Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida.

Subba Rao N. S. (1999). Soil Microbial. 5th edition, Oxford and IBH Publ.Co. Pvt. Ltd. Chapter: 3 :63.

Subbiah, B. V. and G. L. Asija, (1956). A rapid procedure for the estimation of available nitrogen in soil. Curr. Sci. 25 : 258-260.

Talwar Dilpreet, Kulbir and S.S Walia (2016). Influence of biofertilizers on Microbial Count and Nutrient uptake of kharif onion (*Allium cepa* L.). *International Journal of Agriculture Sciences*,Volume 8, Issue 51, pp.-2295-2298.

Tarrand J J, NR Krieg and J Dobereiner (1978). A taxonomic study of the *Spirillum lipoferum* group with descriptions of a new genus ,*Azospirillum* gen nov and two species *A. lipoferum* (Beijerinki), comb. nov. and *A. brasilense* sp. nov. *Can J Microbiol* 24:967–980.

Tchan Y.T and P B New (1974), Genus *Azotobacter* Beijerinck (1901). In Bergey’s Manual of Systematic Bacteriology. Willliams and Wilkins, Baltimore Publ. vol.1. pp. 220- 229.

Terver, I.W. and J.P. Hollis, (2002). Bacteria in the storage organs of Healthy tissue. *Phyto. Patothol.J*.38:960-967.

Tesfaye Balemi, Netra Pal, Anil Kumar Saxena (2007). Studies on response of onion (*Allium cepa* L.) to combined application of biological and chemical nitrogenous fertilizers. Acta agriculturae Slovenica, 89 - 1,str. 107 – 114.

Thompson J P and V B D Skerman (1979), *Azotobacteriaceae*. The taxonomy and ecology of aerobic nitrogen fixing bacteria. Academic Press. London. pp. 138-145.

Tindall, H.D. (1986). Vegetable in the Tropics, Macmillan Press Ltd, UK. pp: 130–139.

Toukhy B. and J.C.Jana, (2000). Integrated nutrient management for better growth, yield and quality of green chilli (*Capsicum annum*) in teria region of West Bengal. Indian J.Fert.3(1):111-118.

Vessey J.K (2003). Plant growth promoting rhizobacteria as biofertilizers. Plant Soil, 255: 571-586.

Wange S S (1995). Effect of biofertilizers and different levels of major nutrients on growth and yield of garlic (*Allium sativum* Linn.). *J. Soils and Crops*., 5(4): 101-103.

Yadav B D, R B Khandelwal and Y K Sharma (2004). Use of biofertilizers (*Azospirillum*) in onion. Haryana *J. Hort. Sci*. 33(3-4): 281-83.

Yeung, H. (1985). Handbook of Chinese Herbs and Formulas. Institute of Chinese Medicine, Los Angeles.

Yoav Bashan (1986). Enhancement of wheat root colonization and plant development by *Azospirillum brasilense* cd. following temporary depression of rhizosphere microflora. Applied and environmental microbiology, may, Vol. 51, No. 5 p. 1067-1071.

Zorita, M.D. and M.V.F. Canigia, (2008). Field performance of a liquid formulation of *Azospirillum brasilense* on dryland wheat productivity .*Eur.J.Soil Biol*., 30(9): 1-10.

APPENDIX

Composition of Media

1) Jenson's media

Ingredients

i) Sucrose	20 g
ii) Dipotassium phosphate	1 g
iii) Magnesium sulphate	0.5 g
iv) Sodium chloride	0.5 g
v) Ferrous sulphate	0.1 g
vi) Sodium molybdate	0.005 g
vii) Calcium carbonate	2 g
viii) Agar	20. g
ix) Distilled water	1000 ml

2) Malate media

i) Malic acid	5 g
ii) Dipotassium hydrogen orthophosphate	0.5 g
iii) Potassium dihydrogen phosphate	0.4 g
iv) manganese sulphate	0.01 g
v) Magnesium sulphate	0.1 g
vi) Iron sulphate	0.05 g
vii) Sodium chloride	0.02 g
viii) Sodium molybdate	0.002 g
ix)Calcium chloride	0.01 g
x) Bromothymol blue	2 ml
xi) Potassium hydroxide	4 g
xi) Agar	15.0 g
xiii) Distilled water	1000 ml

VITA

Mr. VIVID BABU

MASTER OF SCIENCE (AGRICULTURE)

IN

AGRICULTURAL MICROBIOLOGY

2018

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