



## RESEARCH ARTICLE

# STUDIES ON THE INOCULATION EFFECT OF *Asospirillum* ON GROWTH AND YIELD PARAMETERS OF CHILLI VAR – 1

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### ABSTRACT

Chilli (*Capsicum annum.L*) is one of the most important commercial crops belonging to the family *solanaceae*. Which is grown throughout the tropical and subtropical parts of the world. Agriculture is heavily dependent on the use of chemical fertilizers and pesticides to achieve higher yields. This dependence is associated with problems such as environment pollution, health hazards, destruction of biological communities that otherwise support crop production. The overcome this problem, biofertilizer is the best source to replace the chemical fertilizer. In this study, five locations were selected in cuddalore district, tamil nadu. To isolate the *Azospirillum* and designated as AZS-1 to AZS-5. This *Azospirillum* isolates has capacity to produce IAA and screening for efficiency fixed Nitrogen by utilizing malic acid. The growth and yield parameter such as the highest plant height (78.60 cm), The maximum number of fruits (46.50) per plant, The highest fruit yield (208.26g) per plant. To efficient strains selected and treated with chilli var k-1 among the five treatment the plant growth recorded most excellent in T5 (75% RDF + *Azospirillum sp*).

**Key words:** Chilli, *Azospirillum*, IAA, N-fixation, Growth and yield.

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### INTRODUCTION

Chilli (*Capsicum annum.L*) is one of the most important cash crops belonging to family *solanaceae*. India is the largest producer, consumer and export of chillies in the form of dried whole chillies (Awasthi, and Kumar, 2008). Globally india contributes one fourth to world production of chilli with an area of 8.53 lakh ha and production 8.74 lakh tonnes with a productivity of 1016 kg per ha (Anonymous, 2007). In india chilli is especially grown in Andhra Pradesh, Karnataka, Tamil Nadu, Maharashtra, account for  $\frac{3}{4}$  of the total area besides Madhya Pradesh, West Bengal, Punjab, Bihar and Rajasthan. A large amount of herbicides, pesticides, and fertilizers is applied every year to achieve maximum productivity of chilli and to meet the growing demand, the use of chemical fertilizer in India has increased 170 times in last 50 years ([http://www.fao.org/docrep/article/Agrippa/658\\_en00.htm#topofpage](http://www.fao.org/docrep/article/Agrippa/658_en00.htm#topofpage)) and (Mondal et al., 2003). Among the Nitrogen fixing culture, *Azospirillum* is considered to be an associate symbiotic facultative entophytic diazotrophs group which colonize the surface and interior of roots and this kind of association is considered as the starting point of most ongoing biological Nitrogen fixing programs (Sabalpara et al., 2014).

*Azospirillum* also provides Nitrogen fixation, but also synthesizes growth promoting hormones such as IAA and GA (Dart, 1986). *Azospirillum* spp directly benefits plants improving root and shoot development and increases the yield of crops by mineral uptake and water uptake through roots (Kanchana et al., 2003).

### MATERIALS AND METHODS

#### Details of Location

The survey was conducted at five locations in cuddalore district of Tamil Nadu comprising Annamalai Nagar, keerapalayam, Orathur, Kattumannarkoil, Parathur.

#### Collection of Chilli rhizosphere soil from different location

In each and every location of survey area field which has been under long behind mono culture practice was selected the locations of rhizosphere soil sample were made at different location of Chilli field. The collected soil were brought to laboratory for further analysis.

#### Enumeration of *Azospirillum* population from rhizosphere of Chilli var-k1

The adhered soil of Chilli roots, collected from five Chilli plants of a particular location, were pooled and one gram of

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soil sample was transferred to 100 ml of sterile distilled water in a 250 ml Erlenmeyer flask and incubated on a rotator shaker (100 rpm) for 30 minutes at ambient temperature. The well mixed suspension was then diluted appropriately upto 10-6 dilution was aseptically transferred to sterile petriplates and 10 – 20 ml of selective Nfb medium was added and incubated at 28°C for 48 hrs. Three replications were maintained for each dilution. The colonies were counted by using colony counter. The total number of colonies in the original samples was expressed as cfu g-1 of oven dry soil.

#### Isolation of *Azospirillum* from rhizosphere of chilli

Ten g of air dried Chilli rhizosphere soil sample was transferred to 100 ml of sterile distilled water in a 250 ml Erlenmeyer flask and incubated on a rotary shaker (100 rpm) for 30 minutes at ambient temperature. The well mixed suspension was diluted appropriately upto 10-6 dilution. One ml of the suspension from 10-5 and 10-6 dilution was aseptically transferred to sterile petriplates and 15 – 20 ml of selective Nfb medium was added to each petriplates mixed well with agar by rotating at clockwise and anticlockwise and allowed to set. Then, the plates were incubated at 28±2°C for 48 hrs. After the incubation period, the colonies were developed and transferred to Nfb agar slants and maintained at 4°C for further study.

#### Purification of *Azospirillum* isolates and designation of *Azospirillum* isolates

All the five *Azospirillum* isolates were purified by streak plate method using Nfb medium frequently. The *Azospirillum* isolates, obtained from the rhizosphere of chilli grown at five different location of Cuddalore district, were designated as AZS and numbered randomly.

#### Characterization of *Azospirillum* isolates

All the five *Azospirillum* isolates, obtained from rhizosphere of chilli grown at Cuddalore district were characterized based on LOPAT test.

#### Quantitative estimation of Indole Acetic Acid in *Azospirillum* isolates

100 ml of N- free malate broth and Kings B broth containing 0.005 ml tryptophan was sterilized in 250 ml conical flask. Each isolate of *Azospirillum* was grown over night in nutrient broth was inoculated to the above each culture medium and incubated at 37°C for 7 days in dark. The culture were centrifuged at 6000 rpm at refrigerated centrifuge at 4°C and the supernatant was collected in a conical flask and used for estimation of IAA. The quantity of IAA in the culture filtrate was determined and expressed as mg/100 ml of the medium.

#### Screening of *Azospirillum* isolates for Nitrogen fixation efficiency

The N- free semisolid malate medium supplied with L- glutamic acid at 100 mg /l was used in this study. To a 250 ml conical flask 100 ml of the above solution was dispensed and autoclaved 15 lps mts. The *Azospirillum* isolates were grown separately for 24 hrs in Nfb broth and inoculated at 2 ml / 100 ml of the medium, duplicate sample were kept for each isolates the flasks were incubated at 37°C for seven days. After seven

days of incubation the Nitrogen fixed invitro was calculated and expressed in mg N fixed/g of malate supplied.

#### Effect of *Azospirillum* sp. On the growth and yield of Chilli

The pot culture experiment was conducted to study the effect of *Azospirillum* sp. On the growth and yield of Chilli. The study was conducted at department of microbiology, Annamalai University, Annamalai Nagar. The soil used in the pot culture experiment was clay loamy in nature. The experiment was arranged in Randomized Block Design (RBD) with three replications. For sowing in inoculated pots, Chilli seeds were soaked with trehalose at 15 Mm, polyvinyl pyrrolidone (PVP) at 2% and glycerol for 30 min in different formulations (20 ml /kg of seeds), (spacing, 15 cm ×10 cm: 3 seedlings / hill and 12 seedling / plot). Gap filling was done after 10 DAS. The crop was given hand weeding on 30 DAS and well protected against pests and diseases. A water level of 5 cm depth was maintained through the crop period. Five representative samples of plant in each pot were peg marked for periodical observation.

#### Treatment schedule

Design – RBD, Replication – 3

T1 – Control, T2 – 100% RDF

T3 – 25% RDF + *Azospirillum* sp.,

T4 – 50% RDF + *Azospirillum* sp.

T5 – 100% RDF + *Azospirillum* sp.

(The growth and yield parameters will be analysed after harvesting)

For each treatment, three replications were chosen for measuring and recording the biometric observation of plants and were recorded at periotic intervals vis., 20<sup>th</sup>, 40<sup>th</sup> and 60<sup>th</sup> days after sowing (DAS).

## RESULT AND DISCUSSION

The occurrence of *Azospirillum* population in the rhizosphere of Chilli var-k1 grown at five selected locations were designated as AZS-1 to AZS-5 respectively and numbered randomly. The total bacterial population, *Azospirillum* population and percentage of *Azospirillum* population were estimated and above selected Chilli var-k1 and result are presented in the (Table – 1) and (Table – 2).

s.no	Location	<i>Azospirillum</i> Designation
1	Annamalai Nagar	AZS-1
2	Keerapalayam	AZS-2
3	Orathur	AZS-3
4	Kattumannarkoil	AZS-4
5	Parathur	AZS-5

The location, namely kattumannarkoil recorded maximum of 5.0 percent as community population of *Azospirillum*, from parathur recorded least population of 0.23 percent as community population in the rhizosphere. All other locations recorded the community population of *Azospirillum*. The occurrence and activities of *Azospirillum* in the rhizosphere have already reported by many researchers (Usharani *et al.*, 2014) and (Sivasakthivelan and Saranraj, 2013). Five strains of *Azospirillum* were isolates from various area from cuddalore district as described in “Materials and Methods”. They were designated as “AZS” series and numbered randomly.

**Table 2. Occurrence and community population of *Azospirillum* from the rhizosphere of Chilli grown at Cuddalore district**

S.No	Location for Sample collection	Total bacterial population	<i>Azospirillum</i> Population	Percentage of <i>Azospirillum</i> population
1	Annamalai Nagar	17.5	1	0.588
2	Keerapalayam	09.8	2	2.22
3	Orathur	16.8	3	0.75
4	Kattumannarkoil	10.2	5	5.0
5	Parathur	13.3	3	0.23

**Table 3. Characterization of *Azospirillum* isolate from the rhizosphere soil in chilli var-k1**

Characters studied	AZS-1	AZS-2	AZS-3	AZS-4	AZS-5
Gram stain reaction	-	-	-	-	-
Motility	+	+	+	+	+
Catalase activity	+	+	+	+	+
Starch hydrolysis	-	-	-	-	-
Gelatin liquefaction	+	+	+	+	+
Citrate utilization	-	-	-	-	-
Biotin requirement	+	+	+	+	+
Culture identification	<i>Azospirillum lipoferum</i>				

**Table 4. Screening the *Azospirillum* isolates for plants growth promoting characteristics and nitrogen fixation efficiency**

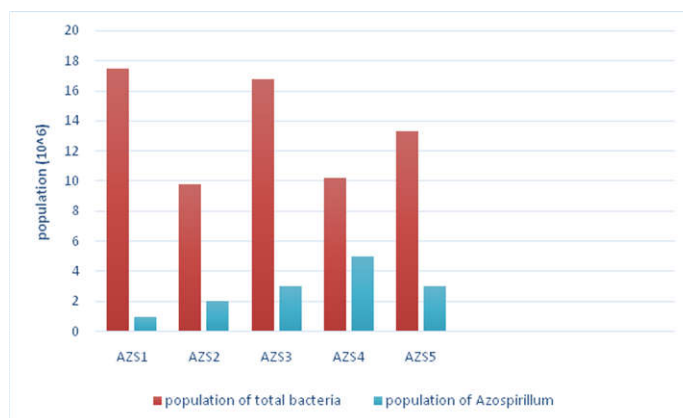
Isolates	IAA production (mg/ml)	N-fixed mg/g malate
AZS-1	18.30	05.64
AZS-2	29.90	17.80
AZS-3	24.82	10.40
AZS-4	04.70	13.62
AZS-5	13.26	16.30

**Table 5. Effect of *Azospirillum* sp. on the growth and yield parameters of Chilli**

Treatment	Plant height (cm)	Number of fruits per plant	Fruit yield per plant (gram)
T1- control	68.50	30.38	176.42
T2- 100% RDF	76.80	37.20	190.82
T3- 25% RDF+ <i>Azospirillum</i> sp.	72.80	32.15	180.25
T4-50% RDF+ <i>Azospirillum</i> sp.	73.11	40.20	195.26
T5-75% RDF+ <i>Azospirillum</i> sp.	78.60	46.50	208.26

The details of designation of the isolates their rise of collection are presents in (Table – 1). The isolates of *Azospirillum* were characterized by bio-chemical tests and designated of *Azospirillum* AZS-1 to AZS-5 (Table – 3). All the above five *Azospirillum* isolates were screened for Nitrogen fixing capacity and plant growth promoting characteristics namely production of IAA taken from showed positive results. The maximum IAA production was recorded by the isolate AZS-2 (29.90 mg / ml) followed by AZS-3 (24.82 mg /ml). The minimum production of IAA was found in AZS-4 (04.70 mg /ml) isolates. The ability of Nitrogen fixation among the isolates of *Azospirillum* AZS-2 fixed higher amount of Nitrogen (17.80 mg / g of malate) followed by AZS-5 (13.62 mg / g of malate) in the medium AZS-1, fixed least Nitrogen (05.64 mg / g of malate) present in (Table – 4). Chilli (*Capsicum annum.L*) is the most widely grown in one of the major vegetable crops in India. Nitrogen is one of the most important nutrients for the growth and yield of several crop. Nitrogen is the most limiting nutrient for vegetable crops production. Also, it is unessential component of structural amino acid, amides, nucleotides, nucleoproteins and is essential to cell division, expansion and nonstructural components of plant cells. Ammonium and urea in soils are the two main sources of available N for plant growth. Although, other nitrogen sources such as fertilizer applications have noticeable effects on growth of Chilli plants. The effect of *Azospirillum* sp. on the growth and yield of Chilli was investigated and the results were furnished in (Table – 5). Maximum growth and yield parameters were observed in the treatment T5 - 75% RDF + *Azospirillum* sp. followed by the treatment T2 - 100% RDF. The treatment T5 was on par with the treatment T2.

Minimum growth and yield parameters were recorded in the control treatment T1. Many *Azospirillum* strains produce plant hormones both in liquid culture and natural situation. The major hormones produced in Indole -3 acetic acid (IAA) (Fallik *et al.*, 1989). The phytohormone synthesized by *Azospirillum* influenced the root hair development, respiration rate, metabolism and root proliferation which in turn resulted in better mineral uptake of the inoculated plant (Okon, 1985).

**Fig. 1. Relative occurrence of *Azospirillum* in chilli rhizosphere soil**

Characterized the production of two aromatic amino acid and amino transferases and production of IAA in *Azospirillum* strains. Experiments were also made to quantify IAA production by different wild strains of *Azospirillum* spp. They reported that during the late stationary phase. IAA production was significantly increased in the presence of tryptophan. They

also observed that the two aromatic amino acid, amino transferases were constitutively produced and they were not repressed by tyrosine plus phenylalanine (Barbar *et al.*, 1987).

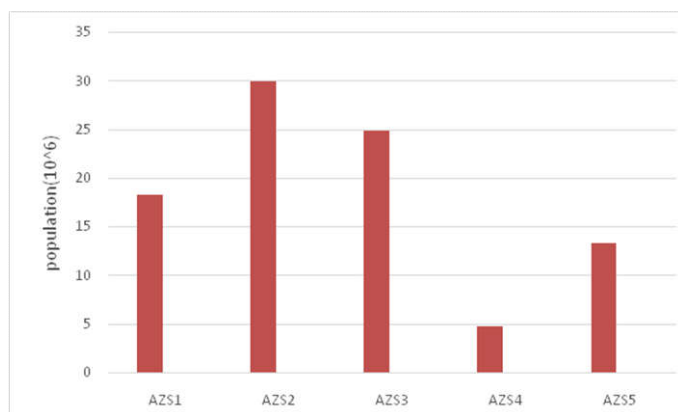


Fig. 2. IAA production of *Azospirillum* isolates

*A. lipoferum* and *A. brasilense* showed Nitrogen fixation in the range of 7.54 to 25.53 mg of Nitrogen per gram of malic acid after seven days at 28° under static condition (Tamilvendian and Purushothaman, 1996). Plant growth and yield attributes increased significantly when the 15 rhizospheric isolates were applied to a local Chilli cultivar 'Suryamukhi' in pots. On the basis of their performance in the pot experiment, three rhizobacteria (C2, C25 and C32) were selected for further study in field. Remarkable increase in growth characteristics such as total number of fruits, fruit-weight and yield was recorded in plants with combined inoculation under field conditions. The result clearly demonstrate the rhizocompetence and plant growth enhancing efficacy of these strains (Moumita Datta *et al.*, 2011). Okon (1985) and wani (1990) evaluated the worldwide success of *Azospirillum* and conducted that positive effects on yield were obtained in approximately 65% of all field experiments about 70 to 75% of all pot experiments in cotton and several vegetables resulted in a yield increased (Bashan and Holguin, 1995).

## Conclusion

In this present study, *Azospirillum* enhanced the growth of Chilli var-k1 by improve the growth of plant and yield by N fixation also release of various growth promoting agents which might have lead to better yield. This study recorded 75% of Recommended Dose of Fertilizer and *Azospirillum* combination (T5) recorded maximum plant height (78.60cm), no of fruits (46.50) per plant and fruit yield (208.26 g) per plant.

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