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Menaka M
Department of Agricultural Microbiology, College of Agriculture. Indira Gandhi Krishi Viswavidhyalaya, Raipur, Chhattisgarh, India

SB Gupta
Department of Agricultural Microbiology, College Of Agriculture. Indira Gandhi Krishi Viswavidhyalaya, Raipur, Chhattisgarh, India

Tapas Chowdhury
Department of Agricultural Microbiology, College of Agriculture. Indira Gandhi Krishi Viswavidhyalaya, Raipur, Chhattisgarh, India

AK Chaubey
Department of Soil Science and Agricultural Chemistry. COA, Banda University of Agriculture and Technology, Banda, Uttar Pradesh, India

Correspondence
Menaka M
Department of Agricultural Microbiology, College of Agriculture. Indira Gandhi Krishi Viswavidhyalaya, Raipur, Chhattisgarh, India

Exploiting Phyllosphere azotobacter and Azospirillum for increasing production of Arjun leaves for silk worm under climatic conditions of Chhattisgarh

Menaka M, SB Gupta, Tapas Chowdhury and AK Chaubey

Abstract
In regions like Chhattisgarh which is exposed to extreme dry and hot climate during summer, the significance of foliar biofertilizers over carrier and liquid based biofertilizers is much higher because of very high use efficiency of biologically fixed amount of nitrogen (BFAN). Foliar *Azotobacter* and *Azospirillum* spraying can increase yields from 10% to 25% similar to that of urea spray. The present investigation was conducted as part of investigation on "Exploiting Phyllosphere *Azotobacter* and *Azospirillum* For Increasing Production Of Leafy Vegetables For Human Being and Green Foliage Feed For Silk Worm Under Climatic Conditions Of Chhattisgarh" at College of Agriculture Raipur and College of Agriculture and Research Station Janjgir Champa, Chhatttisgarh during 2016-17 and 2017-18. It comprises of (i) Survey and collection of Arjun (*Terminalia arjuna*) leaf samples from different locations of Raipur, Durg and Janjgir Champa districts of Chhattisgarh for isolation of foliar *Azotobacter* and *Azospirillum* isolates (ii) Preliminary screening of native foliar *Azotobacter* and *Azospirillum* isolates on the basis of their growth performance and/or nitrogen fixation capacity (iii) Field grown arjun experiments for evaluation of different isolates in order to formulate promising liquid foliar biofertilizers. The data collected and recorded on various characters during the period of investigation were analyzed statistically. Leaf samples were collected from Kulipota, Khisora, Dongari, Baloda, Bachi Hardi, Parsahi I and Parsahi II arjun growing villages of Janjgir Champa district for isolation of foliar *Azotobacter* and *Azospirillum* isolates and after proper characterization, testing and screening, the promising two native foliar *Azotobacter* and *Azospirillum* isolates were selected on the basis of growth and / or atmospheric N fixing capacity and for their further testing under field conditions. Promising isolates Azotobacter A3 and A6 and Azospirillum A15 and 18 were selected. A consistent increase in leaf biomass of field grown arjun from first to second and second to third foliar inoculation at 15 days intervals were observed. Biological nirtrogen fixation increased and also higher dehydrogenase activity was observed.

Keywords: Liquid biofertilizer, Phyllosphere azotobacter, Phylloshere Azospirillum, Phyllosphere biofertilizer

1. Introduction
The above ground parts of the plant have always been very important for the entire ecosystem that dwells on earth. Ruinen [12] coined the term 'phyllosphere' to the above ground part of plants, during her research on Indonesian forest vegetation. Plant parts especially leaves have always been life supporting be it for the plants themselves and some of the organisms around them including human being and economically important insects like silk worms etc. The leaves of Arjun tree constitute the primary food for the Kosi silkworm. The leaves of these plants needs nitrogen, but the usability of the atmospheric Nitrogen by plants is very limited, as compared to Nitrogen trapped in soil by legumes, making them dependent on other means of Nitrogen supply. Although chemical fertilizers made using Habers process do provide significant results in this regards, they have their own limitations. Leaves of plants being exposed to dust and air currents become havens for the establishment of typical flora on their surface aided by cuticle, waxes and appendages which help in the anchorage of microorganisms. As per several scientific reports, the various bacterial genera known to be associated with phyllosphere are *Azotobacter*, *Azospirillum*, *Pseudomonas*, *Micrococcus*, *Xanthomonas*, etc. Among them, *Azotobacter* and *Azospirillum* are the widely used bioinoculants known to benefit a wide variety of crops. Especially they have properties like nitrogen fixation, secretion of growth promoting substances, vitamins, anti-fungal metabolites and phosphate solubilization [12, 13].

In recent years, great attention has been dedicated to study the role of soil microorganisms that play in the dynamics of nitrogen, particularly those able to fix nitrogen from atmosphere [4]. Many investigations have been carried out to find out ways to improve, both in qualitative and quantitative terms, the production of foliage in trees such as *Arjuna terminifolia*. With ecological concerns emerging to the fore, it has become important to avoid chemicals in any form and this has made the use of biofertilizer popular across all types of cultivations. However, the information on the effect of liquid nitrogenous biofertilizers with different levels of nitrogen in different crops is very limited. There were considerable variances in N fixing capacities between isolated *Azotobacter* strains and the soil where it is located [11]. The finding that *Azotobacter* reproduce very well on nitrogen-free nutrient medium, marked the beginning of a new phase in *Azotobacter* research. In soils, *Azotobacter spp*. populations are affected by soil physico-chemical and microbiological properties [2, 9]. As far as physico-chemical soil properties are concerned, numerous studies have focused on the nutrients (i.e. P, K, Ca) and organic matter content and their positive impact on *Azotobacter spp*. populations in soils [3]. The change in microbial population in Chhattisgarh plains have always been a concern. While the spring seems to be favourable for nitrogen fixers such as *Azotobacter* than winters [9] Summer too show a deep decline in these organisms and as a result exhaustion of Nitrogen due to non-existence of nitrogen fixers such as *Azospirillum* [6, 7]. Studies have repeatedly shown that growth of diazotropic bacteria are affected by various physic chemical conditions of the environment such as N and P availability [8], temperature and hydrogen concentration [12]. Govindrajan and Thangaraju [5] observed 50 -70 % success with statistically significant increase in yield in 10 to 30% of trials.

2.1 Materials and Method: Leaf samples of arjun (*Terminalia arjuna*) was collected from Kulipota,Khisora, Dongari, Baloda, Bachi Hardi, Parsahi I and Parsahi II villages of silk producing Janjgir Champa, Chhattisgarh during 2016-17 and 2017-18. The selected isolates were tested with field-grown Arjun in the same region during 2017-2018. The site selected had good irrigation and drainage facilities.

2.2 Isolation, characterization and selection of promising foliar isolates of foliar *Azotobacter* and *Azospirillum:* *Azotobacter* and *Azospirillum* broth with the leaf samples were incubated for 24-72 hours in the shaking incubator at 28^{o}c for optimal growth of the desired bacteria which was then streaked on Jensen's media and nitrogen free bromo thymol blue (Okon's) respectively. Microbial analysis was done by Dilution plating method. Counting of bacterial colonies was done by standard plate count method. Bacterial population density in the broth was calculated on the basis of per ml of leaf sample and bacterial population was calculated on the basis of per gram of leaf/ soil/ ml broth.

2.2 Characterization of the foliar *Azotobacter* and *Azospirillum* isolates: Foliar *Azotobacter* and *Azospirillum* isolates were characterized by Gram staining etc.
On the basis of growth behavior of foliar *Azotobacter* and *Azospirillum* isolates, most effective isolates were selected to use as foliar biofertilizers for testing with field-grown targeted crops.

2.3 Field experiments Field experiments were conducted with arjun (*Terminalia Arjuna*) crop.

2.4 Fertilizer dose: N, P, K: 100, 50, 50 kg/ha (Two split doses).

2.5 Foliar spray schedule: A duration of 15 days was allowed between two consecutive sprays after each foliar inoculation green leaves samples were collected at 15 DAI.

3.0 Results and Discussion

3.1 Morphological characterization of *Azotobacter* and *Azospirillum* isolates: Clear morphological characteristics of *Azotobacter* and *Azospirillum* were identified and asserted using Bergey's Manual of Systematic Bacteriology (Holt *et al*., 1986).

3.2. Atmospheric nitrogen fixing capacity of arjun foliar *Azotobacter* and A*zospirillum* isolates: At 7 DAI, the atmospheric nitrogen fixing capacity of the foliar *Azotobacter* and *Azospirillum* isolates was measured and classified as high and low: The top 2 isolates of arjun foliar *Azotobacter* exhibited capacity to fix high amount of atm. nitrogen i.e. Azot A-3 (41.0 mg N/litre broth) and Azot A-6 (40.9 mg N/litre broth). The top 2 isolates of foliar *Azospirillum* exhibited capacity to fix high atm. N i.e. Azos A-18 (70.8 mg N/litre broth) and Azos A-15 (57.4 mg N/litre broth). They were selected for further testing with field-grown Arjun. The data revealed that *Azotobacter* & *Azospirillum* isolates are capable to utilize leaf organic metabolites and can be survived, multiplied and fixed atmospheric N on foliar part of plant for its better use efficiency

3.3 Biomass and N accumulation by field-grown *Arjun (a)*: Consistent increase in leaf biomass of field-grown *Arjun* from first to second and second to third foliar inoculations at 15 days intervals was observed i.e. 8.46 to 13.76, 8.50 to 14.66, 8.35 to 13.95 and 8.69 to 14.51 g average weight of 10 leaves due to first foliar inoculation to third foliar inoculation of different combination of selected foliar *Azotobacter* and *Azospirillum* isolates of *Arjun* over uninoculated control 8.00 to 9.68. Maximum enhancement in leaf biomass was associated with treatment combination of foliar *Azotobacter* A-3 and *Azospirillum*-A 18 isolates isolated from Arjun growing villages Khisora and Buchi Hardi of Janjgir Champa district. The nitrogen content percentage range varied from 3.09 to 3.22%

3.4. Population dynamics of *Azotobacter* and *Azospirillum:* The data clearly indicated significant increase in population density of both *Azotobacter* and *Azospirillum* on inoculated arjun leaf from first foliar spray to third foliar spray compared to uninoculated Arjun plant leaf. Additionally, *Azospirillum* isolates showed more than 10 times population density/ persistency on inoculated leaves over *Azotobacter* isolates.

3.5. Soil enzyme study: Dehydrogenase activity (DHA) of Arjun rhizosphere soil at 15 DAI increased significantly (from 5.66 (control) to 13.98 µg TPF/hour/g soil) over un inoculated control which could be because of indirect influence of three foliar microbial spray (*Azotobacter* and *Azospirillum)* on enhancement intensity of rhizosphere effect.

Table 1: Influence of foliar inoculation on biomass and N accumulation by Arjun leaves

S. No.	Treatment	Average fresh wt. of 10 leaf leave(g)				% N Content (Dry wt. basis)	% Protien (Dry wt. basis)
		After Ist Spray	After IInd Spray	After IIIrd Spray	Mean		
T1	Control	8.00	9.20	9.68	8.96	3.09	19.06
T2	Foliar inoculation by foliar *Azotobacter* - A3 and *Azospirillum-A15*	8.46	10.72	13.76	10.98	3.19	19.94
T3	Foliar inoculation by foliar *Azotobacter* -A3 and *Azospirillum-A 18*	8.50	11.52	14.66	11.56	3.21	20.06
T4	Foliar inoculation by foliar *Azotobacter*-A6 and *Azospirillum*-A15	8.35	11.03	13.95	11.11	3.19	19.94
T5	Foliar inoculation by foliar *Azotobacter*-A6 and *Azospirillum-A18*	8.69	11.44	14.51	11.50	3.22	20.12
	CD (0.05)	NS	1.54	1.74	-	NS	NS

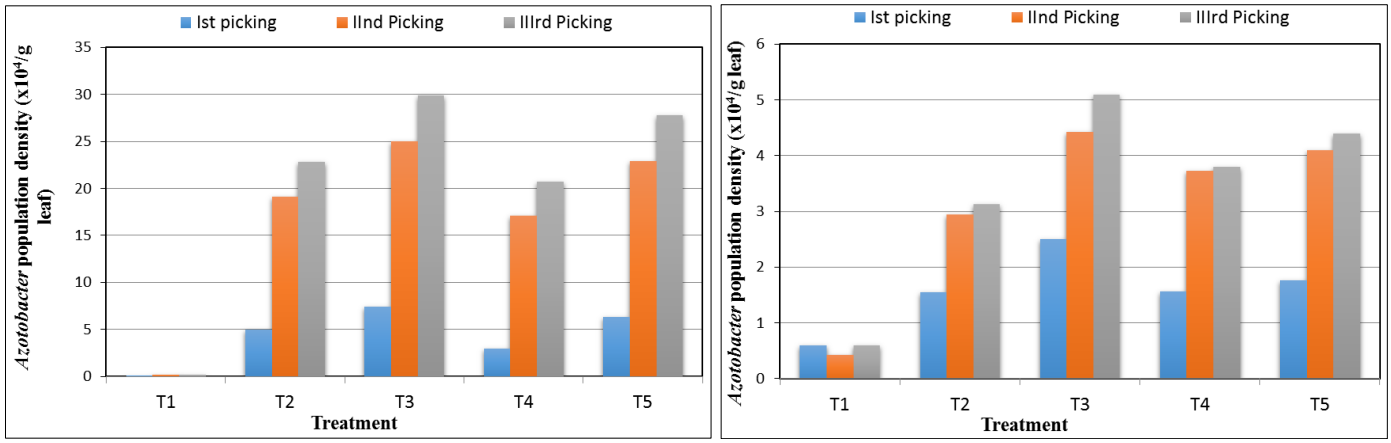

Fig 1: Influence of foliar inoculations on population density of arjun leaf *Azotobacter* and *Azospirillum*

References

1. Bescking JH. Studies on nitrogen-fixing bacteria of the genus *Beijerinckia*. I. Geographical and ecological distribution in soil. Plant Soil. 1961; 14:49-81.
2. Brown ME. Rhizosphere microorganisms-opportunists, bandits or benefactors. In: Soil Microbiology Ed. Walker, N. Halsted Press, New York. 1975, 21-38.
3. Burris RH. Progress in the biochemistry of nitrogen fixation. Proc. Royal Soc. London B. Biological Sci., 1969; 172:339-354.
4. Dobernier J, Baldani JJ, Baldani VLD, Seldin L. Characterization of *Herbaspirillum seropedicae*. Nov. A root association nitrogen fixing bacterium. Int. Syst. J Bacteriol. 1976; 36:86-93.
5. Govindarajan K, Thangaraju M. *Azospirillum* – a potential inoculant for horticultural crops. South Indian Horticulture. 2001; 49:233-235.
6. Gupta SB, Katre RK, Chhonkar PK, Gupta MK, Adil ML. Significant research and development with reference to biofertilizers in Chhattisgarh, Fertilizer News. 2000; 45(11):35-40.
7. Gupta SB, Tedia K, Singh A, Anurag Lakpale N, Pal A, Chhonkar PK. Isolation and identification of stress tolerant bio-inoculants for rainfed cropping system. Soil and Crops. 2002; 12(1):11-16.
8. Kasiviswanathan K, Sitaramaiyengar MN, Krishnaswami S. Effect of feeding leaves grown under different systems of mulberry cultivation on the silkworm cocoon crop. Indian J Seric. 1970; 9:53-58.
9. Lal B, Khanna S. Long term field study shows increased biomass production in tree plants inoculated with *Azospirillum*. Plant and soil. 1996; 184(1):111-116.
10. Menuke L, Georgiu V. *Azotobacter chroococcum* Kak producent auksinopodobnih. V. sb. Bakterijalne udobrenija, Microorganisms. M. Sc. Thesis, Faculty of Science, Tanta Univesity, Egypt, 1964, 166-176.
11. Narula N, Vasudeva M. Terrestrial Environment. Environmental Microbiology, 2006, 23.
12. Ruinen J. Nitrogen fixation in the phylloplane. The Phylloplane, Plant and Soil. 1956; 15(111):81-109.
13. Stotzky G. Activity, ecology, and population dynamics of microorganisms in soil. CRC Crit. Rev. Microbiol. 1972; 2:59-137.
14. Subba Rao NS. Soil microorganism and plant growth. Oxford & IBH Pub. Co, New Delhi, 1999, 252.
15. Subba Rao NS. Prospect of bacterial fertilization in India. Fert. News. 1974; 12:11-12.