Efficacy of phyllosphere microorganisms on growth of bhendi

Mehanathan, T[1*]. M.Deivamani[1], [1]P.Senthilkumar and [2]N.Ramanathan

[1]Horticultural Research Station, Yercaud, Tamil Nadu Agricultural University, Tamil Nadu, India.
[2]Department of Microbiology, Faculty of Agriculture, Annamalai University, Tamil Nadu, India.
*Corresponding author's E-mail: meganathant@ymail.com

Received: January 11, 2016 Accepted: March 3, 2016 Published: June 27, 2016

ABSTRACT

Bhendi (*Abelmoschus esculentus* L. Moench) is an important warm season vegetable crop cultivated in India. It is the world's largest producer of vegetables next to China. Among the vegetables grown extensively, bhendi found an important place due to their significant nutritional value. Phyllosphere microbes often have a direct positive influence in altering plant surface properties, where they may be involved in the fixation of nitrogen, promoting the growth of plants, control of plant pathogens or the degradation of organic pollutants. The present study was undertaken the microbial population and *Azospirillum* population in the phyllosphere region of bhendi. Five *Azospirillum* isolates were isolated from Bhendi Phyllosphere *viz*., *BAZ* 1, *BAZ* 2, *BAZ* 3, *BAZ* 4 and *BAZ* 5 and five strains were characterized as *Azospirillum brasilense*. These strains were screened for their N_2 fixing efficiency and IAA production.

Key words: Bhendi, Phyllosphere, *Azospirillum brasilense*, N_2 fixing efficiency, IAA production.

Bhendi (*Abelmoschus esculentus* L. Moench) is an important warm season vegetable crop cultivated in India. Its adaptability to a wide range of soil and climatic conditions, comparably easy agronomy and feasibility for its round the year cultivation, has made bhendi a popular vegetable. Modern agronomic techniques are yet to be evaluated in the production and productivity of bhendi. The indigenous microbial communities that reside within the phyllosphere are highly diverse and include many different species of bacteria, filamentous fungi, yeasts and algae (Lindow and Brand, 2003). The community composition of phyllosphere microbes are affected by many different factors including plant species, fluctuations in ambient temperature, changes relative humidity, nutrient availability upon the plant surface and direct solar radiation (Lindow and Leveau, 2002). Phyllosphere microbes often have a direct positive influence in altering the plant surface properties, where they may be involved in the fixation of nitrogen, promoting the growth of plants and the control of plant pathogens. Nitrogen is the most important element required for plant growth. Among the biofertilizers, *Azospirillum brasilense* not only aids in the nitrogen fixation but also produces growth promoting substances. *Azospirillum* sp. inoculation is known to increases yield of crop by 5-20 %with saving nitrogen up to 50% of the recommended dose (Dart, 1986). *Azospirillum* sp. gives better returns at very low cost under favourable conditions (Sharma and Bhalla, 1986). Application of *Azospirillum* sp. as seed and seedling treatment significantly increased the seedling vigour in bhendi. *Azospirillum* inoculation to crop resulted in net saving of 25% of recommended doses of nitrogen, improved the soil health and increased productivity. Hence *Azospirillum* is considered as most important microbial liquid Biofertilizer and used in this study.

MATERIALS AND METHODS

The experiment was carried out under the faculty of Agriculture, Annamalai University, at Chidambaram during the period from September 2009 to March 2011. Effect of *Azospirillum brasilense BAZ* 2 foliar inoculation on the plant height, chlorophyll content, fruit length, fresh fruit weight and number of fruits per plant of Bhendi cv. Prabhani kranti was studied in a pot culture

experiment. The following treatments were tested for their study.

T_1- Control (without inoculation of *Azospirillum brasilense*),

T_2- 0.5ml *Azospirillum brasilense* (*BAZ*-2) standard inoculum in 50ml water,

T_3- 1.0 ml *Azospirillum brasilense* (*BAZ*-2) standard inoculum in 50ml water,

T_4 - 1.5ml *Azospirillum brasilense* (*BAZ*-2) standard inoculum in 50ml water

T_5 -2ml *Azospirillum brasilense* (*BAZ*-2) standard inoculum in 50ml water

Observations on plant height, fruit length, fruit weight, No of fruit, chlorophyll content , IAA production and N_2 fixation efficiency were recorded 30, 60 and 90 days after the treatment application. Nitrogen fixation by each *Azospirillum* sp was studied according to the method described by Humphries (1956). All data were statistically analysed and critical differences determined (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

Enumeration of bacteria and fungi on bhendi leaves

The average phyllosphere bacterial population was observed 5, 00,000 g^{-1} of bhendi fresh leaves (Table. 1) and the population of fungi in bhendi fresh leaves in phyllosphere region 90,000 g^{-1} was observed. Among the five isolates, the highest population count in *Azospirillum* was done by MPN method. Blum (2007) reported occurrence of a natural microbial population of 100 to 1,000 CFU of cultural phyllosphere bacteria per g (dry weight) of leaf as similar to present study. Also Bashan (1989) reported that the total numbers of cultured bacteria in the phyllosphere and in the rhizosphere region plants growing under dry ambient temperature conditions were enumerated by the plate count method on nutrient agar supplemented with cycloheximide after 72 h of incubation at 28 ± 2°C. Production of Indole acetic acid (IAA) by *Azospirillum* isolates has been reported by many workers Okon *et al*. (1988); Tapia – Hernandez *et al*. (1990) Zimmer *et al*. (1991); Bar and Okon, (1993b); Baca *et al*. (1994). In the present study, IAA production of all the five isolates under *in vitro* condition was evaluated. The highest amount of IAA (6.15) was produced by *BAZ* 2 followed by *BAZ* 5 (5.28) in 10 days (Table 2). On the occurrence of free-living nitrogen fixing bacteria in the phyllosphere and their possible role as providers of organic nitrogen to the plant has triggered the research on phyllosphere nitrogen fixation. Many phyllosphere bacteria also produced variety of plant growth promoting substances on leaf surface (Hegazi, 1979). Similar to the present study, the nitrogen fixing efficiency of the *Azospirillum brasilence* strains was estimated and the highest amount of N_2 (18.80 mg of N_2 g^{-1} malate) was fixed by *BAZ* 2 followed by *BAZ* 5 (18.40 mg of N_2 g^{-1} malate) (Table 3).

Table 1. Enumeration of bacteria and fungi in bhendi leaf

Organism	Cfu per gm of fresh leaf (g)
Bacteria	5,00,000
Fungi	90,000

Table 2. Screening of *Azospirillum* isolates for N_2 fixing efficiency (*In vitro*)

Azospirillum isolates	mg N_2 fixed g^{-1} of malate
BAZ 1	10.61
BAZ 2	18.80
BAZ 3	13.62
BAZ 4	08.10
BAZ 5	18.40

Table 3. Screening of *Azospirillum* isolates for IAA production (*In vitro*)

Azospirillum isolates	Quantity of IAA production (µg ml^{-1}) (10 Days)
BAZ 1	2.16
BAZ 2	6.15
BAZ 3	3.90
BAZ 4	1.65
BAZ 5	5.28

Moreover, plant height, chlorophyll contend, fruit length, fresh fruit weight and number of fruit per plant were increased by *Azospirillum* in fixing atmospheric nitrogen and producing growth promoting substances like IAA compared to untreated control. The inoculated plants recorded significantly higher plant height than un-inoculated.

The highest plant height was recorded in T_5 with (37.4cm, 76.2cm and 99.7cm) followed by T_4 (36.7cm, 74.3cm and 96.3 cm), T_3 (94.1 cm), T_2 (92.7 cm) and lowest in T_1-(Control) with (85.3 cm) on 30th, 60th and 90th day respectively (Table 4). The highest fruit length was recorded in T_5 (7.9cm, 10.2cm, 12.7cm and 12.7 cm), followed by T_4 (7.5cm, 8.9cm, 10.4cm and 10.4cm) on 45th, 60th, 75th and 90th day (Table 5). The highest fruit fresh weight was recorded in T_5 (16.8g, 18.1g, 18.3g and 22.7 g), followed by T_4 (14.3g, 16.7g, 20.3g and 21.9 g) on 45th, 60th, 75th and 90th day respectively (Table 6). Also the highest fruit number was recorded in T_5 (7.9, 14.8, 21.6 and 22.9 no. of fruit per plant), followed by T_4 (7.4, 13.6, 20.2 and 22.6 no of fruit per plant) on 45th, 60th, 75th and 90th days (Fig 1). The chlorophyll content of leaf extract was higher in T_5 (4.9 µg) followed by T_4 (4.8 µg) on 60th day (Fig 2). Similar to present study was supported by various workers Anajali Deshmukh *et al*. (1999); Amirtalingam. (1988); Parvatham *et al*. (1989); Bashan and Hranony, (1990) and Naver *et al*. (1985). The positive effect of foliar inoculation of *Bacillus* sp in increasing the fruit length, fruit yield of bhendi either alone or in combination with *Azospirillum lipoferum* (*AZ* 204) was reported by earlier workers (Deka *et al*., 1992).

Table 4. Effect of *Azospirillum brasilense* (BAZ -2) inoculation on the plant height of bhendi

Treatments	Plant height (cm)			
	Days			
	30	60	90	Mean
T_1	28.6	65.3	85.3	59.7
T_2	32.7	69.9	92.7	65.1
T_3	35.4	71.4	94.1	66.9
T_4	36.7	74.3	96.3	69.1
T_5	37.4	76.2	99.7	71.1
Mean	34.16	71.4	93.6	

	SED	CD (P=0.05)
Treatments	0.44486	0.88681
Days	0.44486	0.88681
Treatments x Days	0.99474	1.98298

Table 5. Effect of *Azospirillum brasilense* (BAZ -2) inoculation on fruit length

S.No	Treatments	Fruit length (in cm)				
		Days				
		45	60	75	90	Mean
1	T_1	5.7	6.1	6.8	6.4	6.3
2	T_2	6.5	6.9	7.8	7.3	7.1
3	T_3	6.8	8.4	9.1	8.9	8.3
4	T_4	7.5	8.9	10.4	9.7	9.1
5	T_5	7.9	10.2	12.7	11.6	10.6
Mean		6.9	8.1	9.4	8.8	

	SED	CD (P=0.05)
Treatments	0.05719	0.11451
Days	0.05115	0.10242
Treatments x Days	0.11437	0.22903

Table 6. Effect of *Azospirillum brasilense* (BAZ -2) inoculation on fruit fresh weight (g fruit^{-1})

Treatments	Fruit fresh weight(g fruit^{-1})				Mean
	Days				
	45	60	75	90	
T_1	7.5	10.2	13.7	12.8	11.05
T_2	11.2	13.5	16.1	14.5	13.83
T_3	10.7	15.2	19.4	16.8	15.53
T_4	14.3	16.7	21.9	18.3	17.80
T_5	16.8	18.1	22.7	20.3	19.48
Mean	12.1	14.74	18.76	16.54	

	SED	CD (P=0.05)
Treatments	0.11188	0.22404
Days	0.10007	0.20039
Treatments x Days	0.22377	0.44809

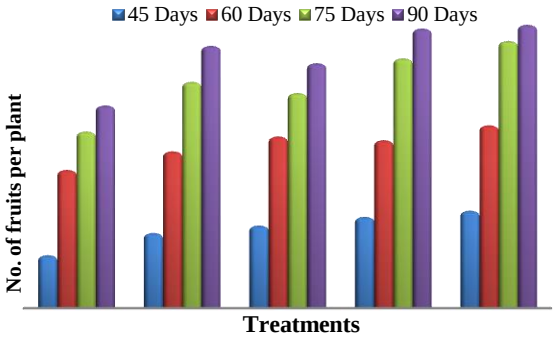

Fig 1. Effect of *Azospirillum brasilense* (BAZ -2) inoculation on the number of fruits per Bhendi

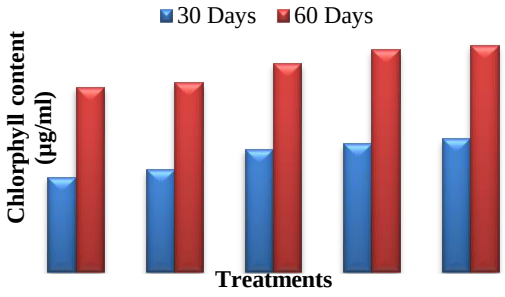

Fig 2. Effect of *Azospirillum brasilense* (BAZ -2) inoculation on Chlorophyll content of bhendi leaf

CONCLUSION

Indole Acetic Acid and nitrogen fixing efficency was improved in bhendi crop treated with *Azospirillum brasilense* (BAZ-2) and the chlorophyll content, plant height, fruit weight and yield also improved. The foliar application of 2 ml of *Azospirillum brasilense* (BAZ-2) the selected organism is effective alternate biocontrol agent in controlling pest and diseases in bhendi crop and there was no health hazard in using them.

REFERENCES

Amirathalingam, S. (1988). Studies on the effect of *Azospirillum*, nitrogen and NAA growth and yield of chilli. South Indian Horticulture, 36:218.

Anajali Deshmukh, D.B.Matte and Bharthi Bhaisare. (1999). Fly ash as a source of plant nutrients and soil conditions. *Jornal soils and Crops*, **9(2)**: 276 – 279.

Baca, B.E., L. Soto - Urzua, Y.G. Xochihua and A. Cuervo – Garcia. (1994). Characterization of two aromatic amino acid aminotransferases and production of Indole acetic acid in *Azospirillum* strains. *Soil Biol. Biochem.*, **26**: 57- 63.

Bar, T. and Y. Okon. (1993) b. Induction of indole -3-acid synthesis and possible toxicity of tryptophan in *Azospirillum brasilense* Sp7. *Sym. Rehovot.*, **13**:191 – 198.

Bashan, Y. (1989). Nonspecific responses in plant growth, yield, and root colonization of noncereal crop plants to inoculation with *Azospirillum brasilense*. *Cd. Can. J. Bot.*, **67**:1317-1324.

Bashan, Y. and Hranony. (1990). Effect of bacterial inoculant on rice. Can. J. Microbiol., 36: 591.

Blum, B.J., (2007). Concepts and Strategies for a successful product Development: the industry development concept. In: Cost Action 850 – Conference Schloss Salzau, Germany.

Dart, P.J. (1986). Nitrogen associated with non-legumes in agriculture. *Plant and Soil*, **90**: 303-304.

Deka , B.C., G.C. Bora and A. shadeque. (1992). Effect of *Azospirillum* on growth and yield of chilli (Capsicum annuum L.) cultivar pusa Jwala. *Haryana J. Hort .,Sci.,* **38**: 41 – 46.

Gomez, K.A.Gomez. (1984). Statistical procedure for Agricultural research. John Wiley and Sons, New York, Pp.140-156.

Hegazi,N.M. Eid, R.S. farag and M.Monih. (1979). A symbiotic nitrogen fixation in rhizosphere of sugarcane planted under semi arid condition of Egypt. *Rev. Ecol. Biol. Soil,* **16** : 23 – 37.

Humphries, E.C. (1956). Mineral components and analysis: Modern methods of plant analysis. Springer-Verlag, Berlin. pp. 468-502.

Lindow SE, Brand MT. (2003). Microbiology of the phyllosphere. *Appl. Environ. Microbiol*., **69**: 1875-1883.

Lindow SE, Leveau JH. (2002). Phyllosphere Microbiology. *Curr. Opin. Biotechnol.,* **31**: 238-243.

Naver, G., A. Kronenberg and H. Bothe . (1985). Denitrification and nitrogen fixation by *Azospirillum. Arch Microbiol.,* **141**: 364 – 370.

Okon, Y.,Y .Kapulnik and S. Sarig. (1988). Field inoculation studies with Azospirillum in Israel. In: Biological Nitrogen Fixation: Recent Developments (ed.) Subba Rao, N.S. oxford and I.B.H. Publishing Company, New Delhi.pp.175-195.

Parvatham, A., K .P. vijayan and A. nazar. (1989). Effect of *Azospirillum* on growth and nutrient uptake of Pusa Sawani bhendi. *South Indian Horti.,* **37(4)** : 227 – 229.

Sharma, N.K. and P.L.Bhalla. (1986). Influence of integrated nutrient management on growth field and economics of okra (*Abelmoschus esculentus* L.). *Veg.Sc.,* **22(1)**:1-4

Tapia- Hernander, A., Mascarua and J. Caballeso. (1990). Production of bacteriocins and siderophore – like activity by *Azospirillum brasilense*. *Microbiol.,* **64**: 73 – 83.

Zimmer, W.C. H.Elmerich, H.Hennecke and D.D.S. Verma. (1991). Regulation of the synthesis of indole-3-acetic acid in *Azospirillum*. Adv. Mole Gene. *Plant Microbe Int.,* **11**: 465-468.