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Beneficiary Effect of *Azospirillum* Isolates on the Growth and Biochemical Characters of Pearl Millet

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ABSTRACT

Totally 10 *Azospirillum* strains (AC1, AC2, AC3, AC4, AC5, AC6, AC7, AC8, AC9, AC10) were isolated from the soil samples collected from various agricultural regions of Srivilliputtur Taluk, Virudhunagar District, Tamil Nadu, India. The population level of *Azospirillum* was higher in the soil sample collected from Malli area of Srivilliputtur Taluk. The isolated strains were found to be identical with *Azospirillum* sp. by forming typical white, dense and undulating fine pellicle in nitrogen free semi-solid medium. Among ten strains, AC2, AC4, AC5, AC6, AC8, AC9 and AC10 identified as *Azospirillum brasilense* and remaining AC1, AC3 and AC7 identified as *A. lipoferum.* The isolated 10 *Azospirillum* strains were preliminarily screened by noting the change in colour and pH of the nitrogen free malate broth. The selected *Azospirillum* strains (AC2, AC3, AC5, AC6 and AC7) were mass multiplied for bioformulation production. In the nursery experiment, *Azospirillum* inoculation increased the growth and biochemical characters of pearl millet. Among 5 *Azospirillum* strains, the growth response of was higher with AC2 that isolated from Natchiyarpatti. In the case of biochemical characters, the strain AC2 was superior in chlorophyll and glucose content of pearl millet; AC7 (isolated from Athikulam) superior in protein content and NR activity.

INTRODUCTION

PLANT GROWTH PROMOTING RHIZOBACTERIA (PGPR)

PGPRs are soil bacteria that have the ability to colonize roots and to stimulate plant growth through the production of phytohormones. This plant growth promotion activity has been reported for strains belonging to many different genera such as *Azoarcus, Azospirillum, Azotobacter, Arthrobacter, Bacillus, Clostridium, Enterobacter, Glucono acetobacter, Pseudomonas* and *Serratia* (Somers *et al*. 2004). Among them, *Azospirillum, Azotobacter, Azorhizobium, Bacillus* and *Pseudomonas* are widely used as bioinoculants. Rhizobacteria that establish positive interaction with plant roots are called PGPR. Now-a-days, there are renewed interests in the use of rhizobacteria for inoculation of agricultural crops, which are able to colonize plant roots and stimulate plant growth as well as crop yield. The mechanisms by which these rhizobacteria enhance plant growth are multitudinous which include synthesis of growth hormones, increasing the availability and uptake of nutrients, suppression of plant pathogens and production of siderophores (Kloepper *et al*., 1980). PGPR play important role in phytostimulation, phytoremediation and biofertilization. In an ideal system, chemical fertilizer, biofertilizers and organic manures should be given as complementary to each other in a balanced way. The beneficial effect of PGPR is attributed to increase the nitrogen input from biological nitrogen fixation, higher phosphate solubilization, production of plant growth promoting hormones like auxins, gibberellins and cytokinins and reduction of plant diseases and nematode infection (Antoun and Kloepper, 2001; Kamal *et al.*, 2018).

Azospirillum

Azospirillum is a microaerophilic, gram negative and spiral shape bacterium. It is asymbiotic nitrogen fixers able to fix the atmospheric nitrogen make it available to plants. *Azospirillum brasilense*, a free living, PGPR can fix nitrogen under microaerophilic conditions and are frequently associated with the rhizosphere of a large number of agriculturally important crops and cereals (Bashan and Levanony, 1990). *Azospirillum* is benefit to plants by mechanisms related to enhancement of plant growth, increases the mineral uptake, increases the dry matter, improve the water absorption and improve the yield. The carrier based *Azospirillum* inoculant for non-leguminous crops are becoming increasingly popular in India in recent years. *Azospirillum* is a rhizosphere bacterium colonizing the roots of crop plants making use of root exudates and fixes substantial amount of atmospheric nitrogen. They exert beneficial

effects on growth and yield of many economically important crops (Okon and Vanderleyden, 1997). One of the principal mechanisms proposed for *Azospirillum* sp. to explain plant growth promotion of inoculated plants has been related to its ability to produce and metabolize several phytohormones and other plant growth regulation molecules. From a historical perspective, many studies detailing the beneficial effects of inoculation with beneficial rhizobacteria, especially *Azospirillum* sp., have been undertaken and they describe morphological and physiological changes that occur in inoculated plants.

Pearl millet (*Pennisetum glaucum* (L.) is an important grain crop ranking as sixth most important world cereal, (Singh *et al*., 2003), grown, especially in drought-prone semi-arid regions. The crop is cultivated for its grain and as forage. It grows on low fertility soil with annual mean rainfall of 200 mm, compared to maize and sorghum (Ali, 2010). It has low nutrients demand but, could produce appreciable yields with adequate nutrients supply (Maman *et al*., 2000). The present study was aimed at to find out the response pearl millet to *Azospirillum* strains isolated from different regions of Srivilliputtur Taluk, Virudhunagar District, Tamil Nadu.

MATERIALS AND METHODS

Isolation and enumeration of *Azospirillum*

The rhizosphere soil and root samples were collected from various fields of Srivilliputtur Talulk (Natchiyarpatti, Krishnankovil, Sengulam, Athikulam, Malli), Virudhunagar District, Tamilnadu. The soil samples were air dried under shade and used for the isolation and enumeration of *Azospirillum*. For the isolation of *Azospirillum*, the rhizosphere soil samples were serially diluted up to 10^6 dilutions using sterile distilled water. One millimeter of the soil diluent from each dilution was transferred to the tubes containing 10ml of nitrogen free malic acid semi solid medium and kept for incubation for three days at 35±2º C. The presence of *Azospirillum* was indicated by the formation of white characteristic undulating subsurface pellicle with the change of colour of the medium from yellowish green to blue. *Azospirillum* strains were brought to pure culture by streaking the culture on plates containing nitrogen free malic acid medium and by repeated subculture. Pure cultures were maintained in nitrogen free malic acid agar slants.

Enumeration of *Azospirillum* in soil samples were carried out by Most Probable Number (MPN) method. One milliliter of successive dilutions of 10^4,$10^5$and 10^6 were transferred to

test tubes containing nitrogen free malic acid semi solid medium. Five tubes were maintained for each dilution. The tubes were incubated at room temperature for 3 days. The positive tubes were counted and the population was calculated as per MPN tables (Cochran, 1950) and expressed as number of *Azospirillum* per gram dry weight of soil samples.

Identification of *Azospirillum* strains

The isolated *Azospirillum* strains were identified using standard tests as listed in the Bergey's Manual of Determinative Bacteriology (Krieg and Dobereiner, 1984).

Preliminary screening of *Azospirillum* strains

The isolated *Azospirillum* strains were preliminary screened by change in pH and colour of the medium. The *Azospirillum* strains were inoculated in the nitrogen free malic acid broth and incubated for three days. The colour and pH change were observed daily.

Mass multiplication of *Azospirillum*

The selected *Azospirillum* strains were multiplied in the culture flask with nitrogen free malic acid broth. The viable count in the inoculums was checked before mixing the inoculums with carrier material. For mass multiplication of *Azospirillum*, lignite was used as carrier. The lignite was sterilized, sieved and maintained proper water content. The mass cultured *Azospirillum* strains were mixed with the carrier material and used for nursery experiments.

Nursery experiment

A nursery experiment was conducted to study the nursery performance of different *Azospirillum* strains in pearl millet (*Pennisetum glaucum* (L.) R.Br.). Seeds were pre-soaked for 12 hours in distilled water and were sown in sterilized soil mixture. The soil mixture was prepared by mixing black soil, red soil and sand in the ratio of 1:1:1. The *Azospirillum* bioformulations (AC2, AC3, AC5, AC6 and AC7) were applied 10g each at top soil of the pots. The growth characters such as shoot length, root length, fresh weight and dry weight were analyzed in the *Azospirillum* treated and control plant. Likewise, the biochemical characters such as total chlorophyll, protein content, glucose content and NR activity were estimated.

RESULTS

Population dynamics of *Azospirillum*

In the present study, an attempt was made to isolate the *Azospirillum* strains from the soil sample collected from various field of Srivilliputtur Taluk. Totally ten *Azospirillum* strains (AC1, AC2, AC3, AC4, AC5, AC6, AC7, AC8, AC9 and AC10) were isolated and brought to pure culture. In addition to isolation, the population level of *Azospirillum* was enumerated. The result indicated that the population level of *Azospirillum* was higher in soil sample collected from the Malli ($5.8x10^5$ per gram soil dry weight) followed Athikulam ($0.67x10^5$ per gram soil dry weight). The least population of *Azospirillum* was observed in the soil samples collected from Krishnankovil and Sengulam regions (Table 1).

Table 1: Population level and their code number of *Azospirillum* in different soil

S. No.	Soil sample	Population level (x 10^5/g soil dry wt.)	Code number of the isolated strains	Identified *Azospirillum* strains
1.	Natchiyarpatti	0.55	AC1	*Azospirillum lipoferum*
			AC2	*A. brasilense*
2.	Krishnankovil	0.30	AC3	*A .lipoferum*
			AC4	*A. brasilense*
3.	Sengulam	0.30	AC5	*A. brasilense*
			AC6	*A. brasilense*
4.	Athikulam	0.67	AC7	*A. lipoferum*
			AC8	*A. brasilense*
5.	Malli	5.80	AC9	*A. brasilense*
			AC10	*A. brasilense*

Samples collected from Srivilliputtur Taluk

Identification of *Azospirillum*

The isolated *Azospirillum* strains were identified up to species level based on biochemical tests followed by Bergey's Manual of Determinative Bacteriology. Based on the morphological and pellicle formation, the isolates bacterial strains were identified as *Azospirillum.* The isolated strains were found to be identical with *Azospirillum* by forming

typical white, dense and undulating fine pellicle in nitrogen free semi-solid medium, Gram negative and spiral movement. Among ten isolates, the strains AC1, AC3 and AC7 were found to produce acid from glucose peptone medium and required biotin for their growth characteristics of *A. lipoferum*. The remaining seven isolates, AC2, AC4, AC5, AC6, AC8, AC9 and AC10 were failed to produce acid in glucose peptone medium and do not required biotin for their growth, identified as *A. brasilense* (Table 1).

Preliminary screening of *Azospirillum* strains

To select the best elite strain of *Azospirillum*, the isolated *Azospirillum* strains were preliminarily screened *in vitro*. The *Azospirillum* strains were preliminarily screened by noting the change in colour and pH of the nitrogen free malate broth. The result clearly indicated that the *Azospirillum* strains differed in their ability to change the colour intensity of the medium. The result further indicated that there was a direct correlation between the intensity of bluish colour and the alkalinity of the medium. The strains those changed the colour of the medium and pH drastically were selected for further studies. Further, all the *Azospirillum* strains were able to change the colour of the medium on 3rd day. But some strains started to change the colour even in the first day after inoculation (Table 2). Based on the preliminary screening, the *Azospirillum* strain AC2, AC3, AC5, AC6 and AC7, were selected and used for further studies.

Table 2: Preliminary screening of *Azospirillum* strains by colour and pH change of the medium

S. No.	*Azospirillum* Strains	Colour Change			pH of the medium
		1st day	2nd day	3rd day	
1.	AC1	Blue	Dark Blue	Dark Blue	8.0
2.	AC2	Blue	Dark Blue	Dark Blue	8.8
3.	AC3	Blue	Dark Blue	Dark Blue	8.4
4.	AC4	Light Blue	Blue	Dark Blue	8.1
5.	AC5	Light Blue	Blue	Dark Blue	8.5
6.	AC6	Light Blue	Blue	Dark Blue	8.5
7.	AC7	Green	Light Blue	Dark Blue	8.6
8.	AC8	Light Blue	Blue	Dark Blue	8.2
9.	AC9	Light Blue	Blue	Dark Blue	8.3
10.	AC10	Light Blue	Blue	Dark Blue	8.0
11.	Control	Green	Green	Green	7.0

Mass multiplication and preparation of bioformulations

The selected *Azospirillum* strains (AC2, AC3, AC5, AC6 and AC7) were mass multiplied in the laboratory with lignite as carrier material. The mass multiplication of *Azospirillum* strains was done in the flask with nitrogen free malic acid broth. The pH of the media was kept around 7.0 and incubation temperature at 28 ± 2^{0}C. The mass multiplied *Azospirillum* strains were mixed with lignite as carrier material. Further, the quantity of culture broth needed for mass multiplication was also standardized. The result revealed that the optimum quantity for lignite found to be 250ml/kg. The mass multiplied *Azospirillum* bioinoculants were used for the nursery experiment.

Effect of *Azospirillum* on the growth characters

Effect of *Azospirillum* on the growth characters were studied from the control and treated seedlings of pearl millet. The growth characters such as rate of shoot length, root length, fresh weight and dry weight were analyzed. The treatment of *Azospirillum* significantly increased the shoot length of *Pennisetum glaucum*. The result revealed that the shoot length was higher in the plants treated with AC2 strain followed AC7 strain. The effect was least with AC6 strain. The application of *Azospirillum* was significantly increased the root length of pearl millet seedlings. The result indicated that the plants grown with AC2 and AC6 strains produced taller root than other strains. *Azospirillum* inoculation also increased the plant fresh weight of pearl millet. Among different strains tested, the *Azospirillum* strain, AC2 significantly increased the plant fresh weight followed by AC7 strain. The effect was least in AC3 strain. Like fresh weight, similar trend was observed in the plant dry weight also. The plants treated with AC2 strain significantly increased the plant dry weight than other *Azospirillum* strains (Table 3).

Table 3: Growth response of *Azospirillum* strains in pearl millet

S. No.	Treatments	Shoot length (cm)	Root length (cm)	Fresh Weight (g)	Dry Weight (g)
1.	Control	14.0 ±0.44 (100)	5.0 ±0.17 (100)	0.70 ±0.06 (100)	0.31 ±0.05 (100)
2.	AC 2	20.0 ±0.57 (143)	6.1 ±0.12 (122)	0.93 ±0.03 (133)	0.42 ±0.07 (135)
3.	AC 3	17.0 ±0.26 (121)	6.0 ±0.30 (120)	0.75 ±0.04 (107)	0.33 ±0.03 (106)
4.	AC 5	18.5 ±0.72 (132)	5.5 ±0.43 (110)	0.87 ±0.05 (124)	0.38 ±0.04 (122)
5.	AC 6	16.5 ±0.29 (117)	6.1 ±0.27 (122)	0.77 ±0.06 (110)	0.35 ±0.02 (113)
6.	AC 7	19.5 ±0.49 (139)	5.5 ±0.14 (110)	0.90 ±0.03 (129)	0.40 ±0.03 (129)

Effect of *Azospirillum* on the biochemical characters

In the nursery experiment, *Azospirillum* inoculation increased the biochemical parameters of *Pennisetum glaucum* such as total chlorophyll, protein, glucose and NR activity. The results clearly indicated that the effect was higher than the control plants and the effect was varied with *Azospirillum* strains. The application of *Azospirillum* strains increased the total chlorophyll content over the control plants. The total chlorophyll content was significantly higher in plants treated with AC2 strain. The estimation of protein content in leaves of pearl millet indicated that the protein content was significantly higher in plants treated with AC7 strain and least in AC6 strain. The results revealed that there was a marked difference observed in the glucose content among the treatments. Among them, glucose content was higher in plants treated with AC2 strain. The least glucose content was noticed with AC6 strain. The *in vitro* NR activity was estimated in leaves of treated and control plants. The results indicated that NR activity was higher in plants treated with *Azospirillum* (AC7 strain) over the control plants (Table 4).

Table 4: Biochemical response of *Azospirillum* strains in pearl millet

S. No.	Treatment	Total Chlorophyll (mg/g LFW)	Protein (mg/g LFW)	Glucose (mg/g LFW)	NRA (mM NO_2 formed/hour/g LFW)
1.	Control	1.37 ±0.05 (100)	3.42 ±0.31 (100)	7.5 ±0.31 (100)	1.067 ±0.28 (100)
2.	AC 2	1.91 ±0.04 (139)	5.03 ±0.55 (147)	13.38 ±0.20 (178)	1.973 ±0.19 (185)
3.	AC 3	1.72 ±0.07 (126)	5.49 ±0.33 (161)	13.24 ±0.55 (177)	1.680 ±0.26 (157)
4.	AC 5	1.63 ±0.06 (119)	4.71 ±0.52 (138)	10.82 ±0.49 (144)	1.723 ±0.13 (161)
5.	AC 6	1.88 ±0.08 (137)	4.41 ±0.47 (129)	9.02 ±0.48 (120)	1.663 ±0.21 (156)

DISCUSSION

Isolation and population dynamics of *Azospirillum*

The pellicle formation in the nitrogen free semi solid malic acid medium is characteristic of *Azospirillum*. Semisolid malate medium has been recommended to isolation and detection of nitrogen fixing spirilla in soil and rhizosphere. Azospirilla were isolated from a wide variety of plants including many grasses and cereals from all over the world, in tropical, temperate and cold climates, from desert plants, from water-flooded rice paddies and from salt affected soils (Reinhold *et al.*, 1987). *Azospirillum* strain was isolated from the rhizosphere soil of paddy field. These isolated bacterial strains were confirmed by its morphological and physical characteristics on the growth on nitrogen free medium. The cultural characteristics are used for the confirmation. The isolated strains were formed pellicles in nitrogen free semisolid medium that is the characteristic feature of *Azospirillum*. These isolated strains

were confirmed by observation of gram negative staining (Sagadevan *et al.*, 2014). The associations of the *Azospirillum* sp. were initially reported to be restricted to the Gramineae (Dobereiner and Day, 1976). The association of the organisms with roots of non-Gramineae plants was also reported by Lakhmikumari *et al.*, (1976). Seasonal variation in MPN counts, which showed a similar pattern of decrease or increase with the variable climatic conditions, confirm that all types of microorganisms were influenced by the temperature fluctuations in a similar fashion in tea soils (Bezbaruah, 1999). Plantation crops like arecanut, cashew, cocoa, rubber, cardamom and sapota grown in acid soils colonized *Azospirillum* in their root system (Hu *et al*., 2006). Yadav and Singh (1991) opined that the large variation in the population of *Azospirillum* in different soils was due to the difference in organic carbon content and types of crops (Gaind, 1987).

Identification of *Azospirillum* strains

In the present study, among ten *Azospirillum* strains, 3 strains were identified as *Azospirillum lipoferum* and 7 strains were as *A. brasilense.* On the basis of colony morphology, gram staining, and carbon/nitrogen utilization pattern the isolated strains from maize (*Zea mays* L.) were identified as members of genus *Azospirillum* (Ilyas *et al*., 2012). Azospirilla were gram negative, cured rods of variable size exhibits spirillar movement and contain PHB as reserve food material (Okon and Itzigsohn, 1992) and gram negative, motile, curved rod of variable size, ranging from 0.5 – 1µm in length, exhibits spirillar movement and polymorphism, containing poly-hydroxybutyrate (PHB) granules and fat droplets. More detailed studies on a large number of isolates from various parts of the world led them to the description of a new genus named *Azospirillum* and they distinguished two species namely, *A. brasilense* and *A. lipoferum* based on the physiological and morphological differences. The two species were also subsequently distinguished based on the DNA homology experiments. Later, four additional *Azospirillum* species were described, *A. amazonense* (Falk *et al.*, 1985; Magalhaes *et al.*, 1983), isolated from many grasses in the Amozonian area of Brazil, the salt tolerant species *A. halopraeferans*, associated exclusively with roots of kallar grass *A. irakense* (Khammas *et al.*, 1989) and *A. dodereinerae* (Eckert *et al*., 2001). Ten *Azospirillum* strains were isolated from paddy field rhizosphere soil of Thanjavur district. The isolates strains were characterized and identified up to species level. The *Azospirillum* strains were identified by morphological, physiological and biochemical characters. All the 10 *Azospirillum* strains were identified based on morphological and biochemical characters such as shape,

temperature, pH and biochemical character such as IMViC, catalase, citrate, starch hydrolysis and urease test (Usha and Kanimozhi, 2011).

Mass multiplication of *Azospirillum*

The nature of carrier material, shelf life and inoculum potential are important in the quality of bioinoculants. The mass production technology for biofertilizers involves careful selection of the microbial strains, a low cost growth medium and a suitable carrier material for a long shelf life. Quality of bioinoculants is one of the most important factors deciding their performance. A good carrier material is one which can keep up the viability of microbes for a longer period by providing organic food base to the organisms and retaining the moisture content. The selected *Azospirillum* strain was mass multiplied with different types of carrier materials. The quality of bioinoculants was determined by several factors such as nature of carrier materials, age and quantity of culture filtrate *etc*. A viable count ranged from 10^9 to 10^{10} ml^{-1} is preferred for the preparations of bioformulation and this population was attained within 3 - 5 days in the case of fast growing organisms like *Azospirillum*. In the case of slow growing organisms, it took about 6-7 days to reach such counts. Most of the laboratories the practice is in using logarithmic or late logarithmic phase culture with fermentation period of 30 - 72 h or 10 - 15 day old culture (Sadasivan and Neyra, 1985). Sivasakthivelan and Saranraj (2013) reviewed that the biofertilizers manufactured in India are mostly carrier based, using lignite or charcoal as carriers.

Effect of *Azospirillum* strains on the growth characters

Azospirillum inoculation enhanced shoots and root growth with increase in nitrogen assimilation and were attributed to growth substances produced by the associated bacteria (Kapulnik *et al.*, 1982). Positive effects of *Azospirillum* inoculation were demonstrated in various root parameters including increased length of root and dry weight (Hadas and Okon, 1987), increased in the number, density and appearance of root hairs increased in root surface area (Bashan, 1986) and stimulation of root exudation (Heulin *et al.*, 1987), increased root hair development, root branching and root surface area (Fallik *et al.*, 1988). *Azospirillum* inoculation on wheat, sorghum and panicum, significantly increased the total shoot and root weight, total N content, plant and leaf length (Kapulnik *et al.*, 1981) and in winter wheat (*Triticum aestivum*) inoculated with *Azospirillum brasilense* showed significantly increase in

the number of fertile tillers, shoot and root dry weight and root to shoot ratio (Warembourg *et al.*, 1987).

Further, application of *Azospirillum* increased growth characters due to N-fixation and plant growth hormones such as auxins, gibberellins, cytokinins *etc*. Several investigators (Fayez and Vlassak, 1984) found that azospirilla may produce plant growth substances, mainly indole acetic acid, indole lactic acid, gibbrellin and cytokinin - like substances. The results indicated that inoculation with azospirilla or pure hormone substances induce the proliferation of lateral roots and root hairs which increase nutrient absorbing surfaces and many more root tips available for infection. *Azospirillum*, one among these strains is well known for its ability to enhance plant growth and yield under a variety of environmental conditions (Blaha and Schrank, 2003) its growth promotion activity under field conditions is not consistent (Ogut *et al*., 2005). Field inoculation with *Azospirillum* spp. has been evaluated worldwide in different crops, demonstrating that these bacteria are capable to improve yields of important crops in different soils and climatic regions (Okon and Labandera- Gonzalez, 1994). The stimulatory effect exerted by *Azospirillum* has been attributed to several mechanisms including secretion of phytohormones (e.g. auxins and gibberellins), biological nitrogen fixation, and enhancement of mineral uptake by plants (Bashan *et al*., 2004).

Effect of *Azospirillum* strains on the biochemical characters

Application of biofertilizers resulted in a significant increase in chlorophyll content of barley leaves. The chlorophyll content can potentially provide an estimate of the N status of crops. The greater chlorophyll values due to the photosynthetic activity and crop yield may increase with increased chlorophyll content of leaves (Lal and Srivastava Ramesh, 2010). Biofertilizers had a beneficial effect on growth attributes of *Echinochloa frumentacea*. Biochemical parameters showed a good response to the bacterial inoculants. The beneficial effects of bacterial inoculation on increased chlorophyll content might have been due to the supply of higher amount nitrogen to the growing tissue and organs supplied by N_2 fixing *Azospirillum*. Demonstrated the effect of *Azospirillum* on various growth and yield characters in Okra where the treatment with *Azospirillum* resulted in significant increase in total chlorophyll content (Rukumani, 1990; Bartolini *et al.*, 2017).

Under nursery, the response of *Azospirillum* was varied with respect to various types of carrier materials. Carrier materials are determining the quality of bioinoculants as well as

nursery and field performance. The most appropriate carrier material was the peat, which was commonly used in USA, Australia and other European countries. In India due to poor quality of available peat and also due to its availability in limited areas, its' use remain restricted. After peat, lignite and wood charcoal were considered to be the better alternatives. Lignite had acidic pH and required around 10% (W/W) addition of calcium carbonate to bring pH near neutral. Wood charcoal on the other hand, had pH around 7.5-8.0 with water holding capacity equivalent to peat and therefore serves as better carrier. Lignite and wood charcoal were in common use in India by research institutions and by private produces (Dadarwal *et al*., 1997). Quality of the bioinoculants is one of the most important factors deciding their field performance. The growth and survival of *Azospirillum lipoferum* in peat, coir pith and peat plus coir pith mixture. Peat supported the maximum proliferation of *Azospirillum* than other carriers and pressmud supported higher survival of *Azospirillum lipoferum* than peat and lignite (Suresh Kumar, 1996). Data emphases the potential of *Azospirillum* is a biofertilizer, reducing costs and improving agricultural sustainability. In conclusion, soil inoculation with *Azospirillum* enhanced plant growth and protein content (El Sayed *et al.*, 2015; Bartolini *et al.*, 2017).

CONCLUSION

The present study revealed that the population level of *Azospirillum* was varied with reference to nature of host plants. This may due to the host specificity of *Azospirillum* strains. All selected *Azospirillum* strains performed well in the nursery experiments but response was varied between the strains. *Azospirillum* strains performed well than control plants in both morphological and biochemical characters of pearl millet. The nursery experiment proved that the N-fixer, *Azospirillum* is best suit for sustainable growth and development of pearl millet. The differences in the crop response of *Azospirillum* strains may be due the nature of agroclimate and host plants. Therefore, these two factors should be considered during the selection of *Azospirillum* strains during the production biofertilizer.

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