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RESEARCH ARTICLE

EFFECT OF *AZOSPIRILLUM* ON THE GROWTH AND BIOCHEMICAL CHARACTERS OF OKRA (*Abelmoschus esculentus* (L.) Moench.)

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Abstract

Azospirillum is a nitrogen fixing bacterium colonizing the roots of crop plants. It exerts beneficial effects on growth and yield of many economically important crops and extensively used in rice and other cereal crops as biofertilizer. The present study was aimed at to find out the effect of *Azospirillum* on the growth and biochemical characters of Okra (*Abelmoschus esculentus* (L.) Moench.) with respect to different types of carriers. The result found that the isolated *Azospirillum* strains were preliminarily screened under *in vitro* based on the efficiency of colour and pH change in the culture medium. The selected *Azospirillum* was mass multiplied in the laboratory and incorporated into the nursery soil through different carriers. Application of *Azospirillum* bioformulation increased the plant growth and also the dry matter content and it varied with formulations. It also increased the biochemical parameters like total chlorophyll, protein, amino acids, glucose and NR activity. The growth/physiological response were higher in all treatments compared to control. The response was significantly varied between the carriers used for preparation of bioformulation. The application of *Azospirillum* also increased the soil macro and micro nutrients in the bioformulation applied nursery soil. Among various carrier material tested, the composed coir pith is the superior one.

INTRODUCTION

Beneficial microorganisms play a major role in plant growth, development and also help in sustainable agricultural development and protecting the environment. Kloepper and Schroth (1978) described microbial population in the rhizosphere which is beneficial, colonize the roots of plants and shows plant growth promotion activity. Plant rhizospheric region is a unique and versatile region of acute plant microbe interactions for tackling essential macro and micro nutrients from a confined nutrient pool. Multiple types of biological interactions between microorganisms and plants take place in the soil. Numerous microorganisms such as algae, bacteria, protozoa and fungi coexist in the rhizospheric region, but bacteria are the super abundant among them. Plants only prefer those bacteria contributing virtually to their fittingness by releasing sugars, amino acids, organic acids, vitamins, enzymes and organic or inorganic ions through roots exudates producing a very scoop environment where diversity is low.

Plant Growth Promoting Rhizobacteria (PGPR)

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).

Various species of bacteria like *Pseudomonas, Azospirillum, Azotobacter, Klebsiella, Enterobacter, Alcaligenes, Arthrobacter, Burkholderia, Bacillus* and *Serratia* have been reported as PGPR to enhance the plant growth. These PGPR inoculants currently commercialized that seem to promote growth through at least one mechanism; suppression of plant disease (termed Bioprotectants), improved nutrient acquisition (Biofertilizers) or phytohormone production (Biostimulants) (Das *et al* ., 2013).

Biofertilizer

Biofertilizer is a formulation contains living microorganisms which promotes the growth by increasing the supply or availability of primary nutrients to the host plant. They add nutrients through the natural processes of nitrogen fixation, phosphate solubilization and synthesis of growth promoting substances.

Azospirillum

Azospirillum is a PGPR colonizing the rhizosphere of many grasses and cereals all over the world, in tropical as well as in temperate climates. This bacterium is able to fix the atmospheric nitrogen (N_2). It also produces plant growth hormones like IAA, GA and cytokines. *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* inoculants for non-leguminous crops are becoming increasingly popular in India in recent years.

Bioformulation

Various delivery systems such as agar slope based culture, liquid paraffin covered agar cultures, liquid culture in polythene bags and carrier based cultures have been used for the supply of microbial inoculants (Bashan and Holguin, 1997). For large scale production and commercial use in agricultural farms, the carrier based cultures packed in polythene bags with proper sealing, is the common accepted practice in almost all countries. This method has several advantages over others except that the system requires bulk quantities of the product and short shelf life of 3 - 6 months. Longer storage allows proliferation of contaminants and decrease in viability of added microorganisms In India, its use remains restricted due to limited availability. Lignite and wood charcoal were considered as better alternatives to peat (Dadarwal *et al*., 1997).

MATERIAL AND METHODS

Isolation of *Azospirillum*

Soil and feeder root samples were collected from various crops of Virudhunagar District, Tamil Nadu and air dried under shade and used for the isolation of *Azospirillum*. For the isolation of *Azospirillum*, the rhizosphere soil samples were serially diluted up to 10^6 dilution using sterile distilled water. One milliliter of the soil diluent from each dilution was transferred to the tubes containing 10 ml of nitrogen free malic acid semi solid medium and kept for incubation for three days at 35±2ºC. 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.

Preliminary Screening of *Azospirillum* Strains

Azospirillum strains were preliminarily screened by change in pH and colour of the medium. The isolated *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

The selected elite *Azospirillum* strain was multiplied with nitrogen free malic acid broth and nutrient broth. The broth culture was mixed with different sterilized carrier materials. The viable count in the inoculum was kept as $2x10^9$/ml before mixing with carrier materials. Organic materials like composted coir pith, lignite, organic manure, vermicompost and vermiculite were used for the mass multiplication of *Azospirillum*. The carrier materials were sterilized, sieved and maintained proper water content in the carrier materials. The culture broth was mixed with the carrier materials and used for nursery experiments.

Nursery Experiment

Nursery experiment was conducted to find out the efficacy of selected *Azospirillum* strain with various carrier materials on the growth and biochemical parameters of Okra (*Abelmoschus esculentus* (L.) Moench.). The bioformulations were treated in the pot growing with Okra. The growth parameters such as shoot length, root length and dry weight and biochemical parameters such as Chlorophyll (Wellburn and Lichtenthaler, 1984), Protein (Lowry *et al*. 1951), glucose (Jayaraman 1981), free amino acid (Jayaraman, 1981) and nitrate reductase activity (NRA) by Jaworski (1971) were analyzed in the treated and untreated control plants. In the nursery soil, the total nitrogen was estimated by Kjeldhal method (AOAC, 1996), available phosphorus by Olsen *et al.* (1954) method and microelements were analyzed by Flame Photometer.

Statistical Analysis

The data obtained were subjected to analysis of variance (ANOVA) and the significant means were segregated by critical difference (CD) at various levels of significance.

RESULTS

Preliminary Screening of *Azospirillum* Strains

To select the best strain of *Azospirillum*, the isolates strains were screened *in vitro*. The *Azospirillum* strains were preliminarily screened by noting the change in colour and pH of the nitrogen free malate broth. The results indicated that various strains differed in their ability to change the colour intensity of the medium. The results 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. Among ten strains, the strains AJ 10 was superior in colour change than others. The colour change ability of *Azospirillum* strain reflected in the change of pH of the culture medium. The result showed that the isolated *Azospirillum* strains were differed in their ability to change the pH of the culture medium. Among ten strains, AJ 10 was increased the pH than other strains (Table 1). Based on the colour and pH change, the strain AJ 10 was selected as elite strain and used for further studies.

Mass Multiplication

The elite *Azospirillum* strain (AJ 10) was mass multiplied in the laboratory with different carrier materials and used for nursery experiments.

Standardization of Age of the Culture

Mass multiplication was done in the flask using nitrogen free malic acid medium. The pH of the media was kept around 7.0 and incubation temperature at 30±2^{0}C. The results indicated that the population was on its peak on 3rd day after which it declined.

Quantity of Culture with Respect to Carrier Material

The quantity of culture filtrate which can be mixed with the carrier material varied with their water holding capacity. In the case of lignite 250ml/kg was found optimum, while in the case of vermicompost, organic manure 300 ml/kg and vermiculate and coir pith even with 500ml/kg, did not become pasty (Table 2).

Effect of *Azospirillum* bioformulations on Okra

Growth Attributes

The treatment of bioformulations significantly increased the shoot length of *Abelmoschus esculentus.* The result revealed that the shoot length was higher in the plants treated with *Azospirillum* with lignite as carrier followed coir pith formulation. The effect was least with organic manure bioformulation and control. Results further indicated that the plants grown with coir pith formulation produced taller root. *Azospirillum* inoculation also increased the plant fresh and dry weight .of okra plant. Among different formulations tested, coir pith formulation significantly increased the plant fresh weight followed by vermicompost formulation and least in lignite formulation. Like fresh weight, same trend was observed in plant dry weight also. Plant treated with coir pith formulation significantly increased the plant dry weight than other formulations (Table 3).

Biochemical Attributes

In the nursery experiment, *Azospirillum* inoculation with *Abelmoschus esculentus* increased the biochemical parameters such total chlorophyll, protein, amino acids, glucose and NR activity. The effect was varied with types of formulations. The result revealed that the total chlorophyll content was higher in plants treated with coir pith bioformulation followed by vermicompost bioformulation. But there was not much variation in total chlorophyll content with bioformulations with organic manure, lignite and vermiculite. Estimation of protein content in leaves of *A. esculentus* indicated that protein content was significantly higher in coir pith formulation and least in vermiculite. There was not much variation in protein content in plants treated with formulations with organic manure, lignite and vermiculite. But effect was far better than control plants. The glucose content was higher in plants treated with coir pith formulation and least in vermiculite formulation. Application of *Azospirillum* with different types of carriers significantly increased the free amino acid in the leaves of *A. esculentus* in all treated plants. Among them, the effect was higher in coir pith formulation over other formulations and control. NR activity was estimated in leaves of treated and control plants. The NR activity was maximum in plants treated with *Azospirillum* as coir pith formulation (Table 4).

Effect of *Azospirillum* on the soil nutrient content

Azospirillum treatment increased the soil nutrient content like macro and micro nutrients. The result revealed that macronutrients such as total nitrogen (N) and available phosphorus (P) and micronutrients such as Fe, Mn, Zn and Cu were higher in irrespective of the carrier material. Among the treatments, the effect was the higher in the coir pith bioformulation. The soil total N as well as available P were higher in coir pith formulation followed by formulation with vermicompost. Likewise, the micronutrients were also higher in all bioformulation treated soil compared to control. Among them, coir pith and vermicompost formulations were superior in micronutrient response (Table 5).

Table 1: Preliminary screening of *Azospirillum* strains

S. No.	*Azospirillum* Strains	Colour Change			pH
		1st day	2nd day	3rd day	
1.	AJ1	Green	Light Blue	Dark Blue	9.1
2.	AJ 2	Green	Light Blue	Dark Blue	9.0
3.	AJ 3	Green	Light Blue	Dark Blue	9.2
4.	AJ 4	Green	Light Blue	Dark Blue	9.3
5.	AJ 5	Green	Light Blue	Dark Blue	9.0
6.	AJ 6	Green	Light Blue	Dark Blue	9.1
7.	AJ 7	Green	Light Blue	Dark Blue	9.1
8.	AJ 8	Green	Light Blue	Dark Blue	9.3
9.	AJ 9	Green	Light Blue	Dark Blue	9.2
10.	AJ 10	Light Blue	Dark Blue	Dark Blue	9.9
11.	Control	Green	Green	Green	7.0

Table 2: Quantity of culture filtrate require for preparation of inoculum with different carrier materials

Carrier material	Quantity of culture filtrate (ml/kg)	Nature of formulation
Coirpith	50	Powdery
	100	Powdery
	200	Powdery
	250	Powdery
	300	Powdery
	400	Powdery
	500	Powdery
	550	Pasty
Vermicompost	50	Powdery
	100	Powdery
	200	Powdery
	250	Pasty
Organic manure	50	Powdery
	100	Powdery
	200	Powdery
	250	Pasty
Lignite	50	Powdery
	100	Powdery
	200	Powdery
	250	Pasty
Vermiculite	50	Powdery

100	Powdery
200	Powdery
250	Powdery
300	Powdery
400	Powdery
500	Powdery
550	Pasty

Table 3: Effect of *Azospirillum* formulations on the growth of *Abelmoschus esculentus*

S. No.	Treatments	Shoot length (cm)	Root length (cm)	Fresh Weight (g)	Dry Weight (g)
1.	Control	8.9[e]	18.8[f]	3.9[f]	1.6[f]
2.	Coir pith formulation	16.5[ab]	28.9[a]	10.2[a]	5.9[a]
3.	Vermicompost formulation	15.9[b]	27.2[b]	8.5[b]	4.5[b]
4.	Organic manure formulation	10.12[d]	20.9[e]	6.8[c]	2.4[d]
5.	Lignite formulation	16.9[a]	25.4[d]	8.0[b]	3.5[c]
6.	Vermiculate formulation	12.7[c]	26.7[c]	6.9[c]	2.8[e]
	CD P= 0.05%	0.5449	0.215	0.1102	0.475

Table 4: Effect of *Azospirillum* formulations on biochemical characters of *Abelmoschus esculentus*

S.No	Treatment	Total Chl (mg/g LFW)	Protein (mg/g LFW)	Glucose (mg/g LFW)	Free Amino acid (mg/g LFW)	NRA (m.mole NO_2 formed/hr/g of LFW)
1.	Control	0.44[e]	3.08[d]	0.24[e]	1.47[d]	0.593[f]
2.	Coirpith formulation	1.02[a]	7.78[a]	1.44[a]	5.71[a]	1.013[a]
3.	Vermicompost formulation	0.91[b]	7.47[a]	1.20[b]	3.33[b]	0.919[b]
4.	Organicmanure formulation	0.86[c]	5.95[b]	0.96[c]	2.52[c]	0.651[e]
5.	Lignite formulation	0.87[d]	5.95[b]	0.72[d]	2.27[c]	0.680[d]
6.	Vermiculate formulation	0.81[d]	5.34[c]	0.68[d]	3.43[b]	0.702[c]
CD P=0.05%		0.039	0.034	0.034	0.189	0.031

Table 5: Effect of *Azospirillum* formulations on the soil nutrient content

S. No.	Treatments	Macro Nutrients		Micro Nutrients			
		N (%)	P (ppm)	Fe (ppm)	Mn (ppm)	Zn (ppm)	Cu (ppm)
1.	Control	0.14 [d]	8.9 [d]	0.6 [d]	4.7 [f]	0.7 [d]	0.4 [e]
2.	Coir pith formulation	0.29 [a]	13.7 [a]	1.5 [a]	9.9 [a]	1.4 [a]	0.9 [a]
3.	Vermicompost formulation	0.20 [b]	13.7 [a]	1.3 [a]	8.5 [b]	1.0 [b]	0.8 [b]
4.	Organic manure formulation	0.25 [b]	11.1 [c]	1.1 [b]	5.8 [e]	0.8 [c]	0.5 [c]
5.	Lignite formulation	0.19 [c]	13.7 [a]	0.8 [c]	6.5 [c]	0.8 [c]	0.5 [d]

6.	Vermiculate formulation	0.25 [b]	12.4 [b]	0.9 [c]	6.3 [d]	0.8 [c]	0.6 [c]
CD P=0.05%		0.064	0.70	0.071	0.57	0.083	0.066

DISCUSSION

Bacterial inoculant is one major group which includes *Azospirillum*, *Rhizobium*, phosphate solubilizing bacteria, plant growth-promoting rhizobacteria and so on. Biofertilizers are usually prepared as carrier-based inoculants containing effective microorganisms. Incorporation of microorganisms in carrier material enables easy-handling, long-term storage and high effectiveness of biofertilizers. Basically, the carrier-based inoculant of these bacteria can be prepared by a common procedure. In the present study, totally 10 *Azospirillum* strains were isolated from rhizosphere soil different crop plants of Virudhunagar District. The isolated strains were preliminarily screened under *in vitro*. *Azospirillum* strains differed in their ability in changing the colour intensity of the medium. There was a direct correlation between the intensity of bluish colour and the alkalinity of the medium. Based on the preliminary studies, the strain AJ 10 was selected and used for further studies.

The nature of carrier material, shelf life and inoculum potential are important in the quality of bioinoculants. 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 such as *Azospirillum, Pseudomonas* and *Bacillus* sp. In the case of slow growing organisms, it took about 6-7 days to reach such counts (Dadarwal *et al*., 1997). 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).

The quality of bioformulations was also determined by their shelf life. The shelf life of *Azospirillum* was higher in composted coir pith followed by vermicompost (Rajannan *et al*., 1996; Ramalingam and Ranganathan, 2001).

There was a significant difference was observed in growth and biochemical parameters in *Abelmoschus esculentus* with reference to treatment of *Azospirillum* with different carrier materials. The response was varied with respect to carrier materials. Among them, the coir pith formulation had superior effect in all the growth response. The effect was mainly due the incorporation of *Azospirillum* strain and addition to this the nature of carrier material also have the beneficiary effect in the response. So, the nature of formulation by means of nature of carrier also determines the positive efficacy of biofertilizers. The success of application of biofertilizers is mainly based on the delivery system or nature of carrier material. The carrier material determines the shelf life and survival of bacterial strain in the soil. The survival is very important in the biofertilizer application because the applied strain should have the optimum population the rhizosphere region of applied crop plants.

Azospirillum inoculation enhanced shoots and root growth with increase in nitrogen assimilation and were attributed to growth substances produced by the associated bacteria (Tian *et al*., 1979). Positive effects of *Azospirillum* inoculation were demonstrated in various root parameters including increased in number and length of root, root 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.*, 1982), 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, 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 plant growth hormones such as auxins, gibberellins, cytokinins *etc*. Several investigators (Vlassak and Reynders (1978) found that azospirilla may produce plant growth substances, mainly indole acetic acid, indole lactic acid, gibbrellin and cytokinins. They 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.

Application of *Azospirillum* also increased the biochemical parameters of okra plants. The effect was varied with the nature of carrier materials. Application of biofertilizers resulted in a significant increase in chlorophyll content of barley leaves. The significantly higher chlorophyll content of barley leaves observed in the pots with organic manures could be due to differences in nitrogen content of the organics manures and its uptake. The greater chlorophyll values in leaves on plots treated with organic manure are of importance because photosynthetic activity and crop yield may increase with increased chlorophyll content of leaves (Ramesh *et al*., 2009).

Application of biofertilizer increased the quality parameters might be due to the higher protein content Kachot *et al*. (2001) in groundnut. *Azospirillum* physiologically influenced the activity of number of enzymes which lead to increased cell metabolism and enzymatic activity which in turn change the biochemical composition in *Amaranthus* (Ramanathan and Subbaiah, 1982). In *Capsicum,* microorganisms might have helped in faster decomposition of organic manures there by increasing the availability of nutrients, specially protein synthesis further it was suggested that increase in fruit weight might have accelerated the mobility of photosynthates from source to the sink which was influenced by the growth hormones (Sivakumar *et al*., 1999). *Azospirillum* application significantly increased the biochemical parameters like the Chlorophyll a, and b, carotenoid, protein and abscorbic acid (Singh *et al.,* 2014). Ferreira *et al.* (1987) reported that wheat plants inoculated with *Azospirillum* showed greater activity of the nitrate reductase enzyme. Inoculation with nitrogen fixing bacteria always increased leaf NRA suggesting a greater supply of NO_3 to the plants over uninoculated control. The increased NO_3 uptake may relate to increased root development in response to production of hormones (Tien *et al.,* 1979; Tilak and Subba Rao, 1987).

Azospirillum inoculation increased the soil nutrient status especially in macro and micronutrients. This is mainly due to N fixation by *Azospirillum* strains as biofertilizer. In all the *Azospirillum* treatments, the soil total N was increased over control. This increase in the soil nutrient level was responsible for plant growth and development. *Azospirillum* inoculation increased the total nitrogen content and yield of cereal and forage grasses (Nur *et al.,* 1980; Kundu, 1988). *Azospirillum* inoculated plants showed increase in the level of total nitrogen in different parts of the plant and the inoculated soil (Saxena *et al.,* 1990).

The application of different organic manures and biofertilizers along with lower dose of fertilizers plays a significant role in enhancing the soil fertility in terms of macronutrients, secondary nutrients and microbial population. The biofertilizers like *A. brasilense* and *A. awamori* helped in enriching the soil with the major nutrients like N and P which are mainly essential for luxuriant growth of mulberry crop. Like macronutrients, micronutrients also significantly increased in the bioformulation treated nursery soil. The increased availability of nitrogen, phosphorus, potassium, exchangeable calcium, exchangeable magnesium and available sulphur were noticed in the soil treated with biofertilizer and organic compost (Shashidhar *et al.,* 2009). These macro and micronutrients are responsible for the betterment of growth and biochemical parameters of Okra.

CONCLUSION

Based on the *in vitro* studies, *Azospirillum* strains were screened for their efficiency and selected the best one. The selected strain of *Azospirillum* was mass multiplied and used for nursery experiments. *Azospirillum* strain was incorporated into the nursery soil through different carriers. The inoculation of *Azospirillum* bioformulations increased the growth and biochemical attributes on Okra. The response was varied with carrier materials used in bioformulations. Further, *Azospirillum* incorporation increased the soil nutrient status. The response was higher in plants treated with *Azospirillum* as coir pith formulation.

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REFERENCES

1. Antoun, H. and Kloepper, J.W. (2001): Plant growth promoting rhizobacteria (PGPR). In Encyclopedia of Genetics. Academic Press, New York. Edited by Brenner S, Miller JH, 1477–1480.
2. AOAC, (1996): In: Official methods of analysis, 15th edition. (eds.) S. Williams and D.C. Wahington. pp. 105-107.
3. Bashan, Y. (1986): Migration of the rhizosphere bacteria *Azospirillum brasilense* and *Pseudomonas fluorescence* towards wheat roots in the soil. J. Gen. Microbiol,. 132: 3407-3414.
4. Bashan, Y. and Holguin, G.(1997): *Azospirillum* - Plant relationships: Environmental and Physiological Advances (1990-1996). Can. J. Microbiol., 43: 103-121.
5. Dadarwal, K.R., Sindhu, S. S. and Suneja, S. (1997): Plant growth promoting rhizobacteria and their role in crop productivity. In: *Biotechnological Approaches in Soil Microorganisms for Sustainable Crop Production.* (ed.) K.R. Dadarwal, Scientific Publishers, Jodhpur. pp. 149-192.
6. Das, A.J., Kumar, M. and Kumar, R. (2013): Plant Growth Promoting Rhizobacteria (PGPR): An alternative of chemical fertilizer for sustainable, environment friendly agriculture. Res. J. Agriculture and Forestry Sci., 1(4): 21-23.

7. Fallik, E., Okon, Y., Epstein, E., Goldeman, A. and Fisher, M. (1988): Growth response of maize roots to *Azospirillum* inoculation: effect of soil organic matter content, number of rhizosphere bacteria and timing of inoculation. Soil Biol. Biochem., 20: 45-49.
8. Ferreira, M.C.B., Fernandes, M.S. and Dobereiner, J. (1987): Role of *Azospirillum brasilense* nitrate reductase in nitrate assimilation by wheat plants. Biol. Fertil. Soil, 4: 47-53
9. Hadas, R. and Okon Y. (1987). Effect of *A. brasilense* on root morphology and respiration in tomato seedlings. Biol. Fertil. Soil, 5:241-247
10. Heulin, T., R. Bally and J. Balandreau. (1982): Isolation of a very efficient nitrogen fixing bacterium from the rhizosphere of rice. In: *Azospirillum Il. Genetics, Physiology, Ecology*. (ed.) W. Klingmuller, Brikhauser, Verlag,Basal, pp.92-99.
11. Jaworski, E.G. (1971): Nitrate Reductase assay intact plant tissues. Biochem. Biophy. Res. Commun., 43: 1274-1279.
12. Jayaraman, J. (1981): Laboratory manual in Biochemistry, Willey-eastern Company limited. Madras. pp. 1-65.
13. Kachot, N. A., Malavia, D. D., Solanki, R. M. and Sagarka, A., (2001): Integrated nutrient management in rainy-season groundnut (*Arachis hypogaea)*. Indian J. Agron., 46: 516-522.
14. Kapulnik, Y., S.Sarig, I. Nur. Y. Okon, J. Kigel, and Y. Henis. (1981): Yield increases in summer cereal crops of Israel in field inoculated with *Azospirillum*. Exp. Agric., 17: 171 – 178.
15. Kloepper, J.W. and Schroth, M.N. (1978): Plant growth-promoting rhizobacteria on radishes: Proceedings of the 4th International Conf. on Plant Pathogenic Bacteria, Station de Pathologie Vegetable et Phytobacteriologie, INRA, Angers, France, pp. 879-882.
16. Kloepper, J.W., Leong, J., Teintze, M. and Schorth, M. N. (1980): Enhanced plant growth by siderophores produced by plant growth promoting rhizobacteria. Nature, 286:885-886.
17. Kundu, B.S. (1988): Effect of dual inoculation with *Azospirillum* and *Rhizobium* on nodulation and nitrogen fixation in pigeon pea. Indian J. Microbiol., 28: 233.
18. Lowry, O.H., RosenBurg, J.N., Farr, A.L. and Randall, R.J. (1951): Protein measurement with Folin-phenol reagent. J. Biol. Che., 193: 262-275.
19. Nur, I., Y. Okon, and Y. Henis. (1980): An increase in nitrogen content of Setaria italica and *Zea mays* inoculated with *Azospirillum*. Can. J. Microbiol., 26: 481 – 485.
20. Olsen, S.R., Cole, C. V., Watanabe, F.S. and Dean, L. (1954): Estimation of available phosphorus in soils by extraction with sodium bicarbonate U.S. D.A.Circular, U.S. Govt. Printing Office, Washington D.C. pp.939.
21. Rajannan, G., Sara Parwin Banu, K., Helkiah, J. and Ramaswamy, P.P. (1996): Pressmud as carrier material for biofertilizers. *Paper presented in National Seminar on Biofertilizer Production - Problems and Constraints.* Department of Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore. pp. 24-25.
22. Ramalingam, R. and Ranganathan, L.S. (2001): Vermicomposting of pressmud boosts nutrient quality of compost. Ecol. Env. Cons., 7 (3): 297-299.
23. Ramanathan, S. and Subbaiah, K. (1982): Studies on the effect of plant density and graded levels of nitrogen in yield and yield components of chillies (*Capsicum annuum* L.). South Indian Horticulture, 29: 178-181
24. Ramesh, P., Panwar, N.R., Singh, A.B. and Ramana, S. (2009): Production potential, nutrient uptake, soil fertility and economic of soybean (*Glycine max*)-based cropping systems under organic, chemical and integrated nutrient management practices. Indian J. Agronomy, 54 (3): 278-283.
25. Sadasivan, L. and Neyra, C.A. (1985): Flocculation in *Azospirillum brasilense* and *Azospirillum lipoferum* exopolysaccharides and cyst formation. J. Bacteriol., 163: 716-723.
26. Saxena, B., Desai, A. and Modi, V. V. (1990): External adsorption of *Azospirillum lipoferum* strain D-2 plant roots and its effect on plant growth. Curr. Sci., 59 (8): 25.
27. Shashidhar, K.R., Narayanswamy, T.K., Bhaskar, R.N., Jagdesh, B.R., Mahesh, M., Krishna, K.S. (2009): Influence of organic nutrients on soil health and mulberry (*Morus indica* L.) production. J. Biol. Sci., 1(1):94-100.
28. Singh,C. K., John, S.A. and Jaiswal, D. (2014): Effect of organics on growth, yield and biochemical parameters of chilli (*Capsicum annum* L.). J. Agri. Veteri. Sci., 7: 27-32.
29. Sivakumar, J., Muralidhar, A.P and Kumargouda, V. (1999): Fertilizer and irrigation use efficiency as influenced by furrow and ferti-drip irrigation in *Capsicum*-maizesunflower cropping sequence. Proceedings of Natl. Seminar on Problems and Prospects of Micro irrigation - A Critical Apprasil, pp.74-78.

30. Tien, T.M., Gaskins, M.H. and Hubbel, D.H. (1979): Plant growth substances produced by *Azospirillum brasilense* and their effect on the growth of pearl millet *(Pennisetum americanum* L.). Appl. Environ. Microbiol., 37: 1016-1024.
31. Tilak, K.V. B. R. and Subba Rao, N. S. (1987): Association of *Azospirillum brasilense* with pearl millet (*Pennisetum americanum* (L.) Leeke). Biol. Ferti. Soil, 4: 97-102.
32. Vlassak, K. and Reyners, L. (1978): Conversions of tryptophan to auxins by *Azospirillum* spp. In: *Proceedings of the Steenbock – Kettering International Symposium on Nitrogen Fixation*, held in Madison, WI, June 1978. University of Wisconsin Press, Madison, WI.pp. 44.
33. Warembourg, F.R., Dressen, R., Vlassak, K., and Lafont, F. 1987. Peculiar effect of *Azospirillum* inoculation on growth and nitrogen balance of winter wheat (*Triticum aestivum*). *Biol. Fertil. Soils* 4: 55-59.
34. Wellburn. A.R. and Lichtenthaler, H. 1984. Formulae and Program to determine total chlorophyll *a* and *b* and total Carotenoids of different solvents, In Advances In Photosynthesis Research (Sybesmaed) MartinusNijihoff / Dr. W. Junk. The Hague. 9-12.