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STUDIES ON SUPPLEMENTATION OF *AZOTOBACTER* AND ORGANIC MATTER ON GROWTH AND YIELD IN TOMATO *(LYCOPERSICON ESCULENTUM)*

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ABSTRACT
The pot culture studies were found out to be the effect of *Azotobacter* along with different organic matter supplementation such as *Azotobacter* + PASIC Compost, *Azotobacter* + Farmyard Manure, *Azotobacter* + Pressmud Compost, *Azotobacter* alone on the enhancement of growth and yield in tomato (*Lycopersicon esculentum*). Artificial inoculation of *Pythium aphanidermatum* was done in the inoculated organic matter treatment 15 Days after sowing (DAS) to disease incidence of the pathogen. Among the five treatments, combination of *Azotobacter* + Pressmud Compost was found to be have maximum plant height (43.50 cm), increased dry weight of root (1.840 g/plant) and shoot(5.931 g/plant), increased chlorophyll content (2.79 mg g^{-1}) and increased fruit yield (1001.9 $gplant^{-1}$) followed by the treatments *Azotobacter* + PASIC Compost, AZT + Farmyard Manure and *Azotobacter* alone with minimum plant height , decreased dry weight of root and shoot,decreased chlorophyll content and decreased fruit yield. In case of foliar spray of *Pythium aphanidermatum*, *Azotobacter* + Pressmud Compost were found to be least disease incidence (20.10 mm) followed by more disease incidence with the treatments *Azotobacter* + PASIC Compost (23.10 mm), *Azotobacter* + Farmyard Manure (25.02 mm) and *Azotobacter* alone (28.69 mm) as compared to control.

INTRODUCTION

Tomato (*Lycopersicon esculentum)* is one of the most popular and second largest producer of the vegetable in the world.Tomato occupies large scale cultivation in India with an average production of 4.6 MT per year. The Tomato crop is highly responsible to nitrogen (N) fertilizer application where N availability may be limited and the time of the application is critical (Taber, 2001). The effect of different rates of nitrogen (N) fertilizers with two types of bio-fertilizers and two cultivars on growth and yield of tomato was reported (Najafvand Direkvandi, 2008). *Azotobacter chroococcum* is a coherent group of aerobic, free living diazotrophs able to fix atmospheric nitrogen in nitrogen free or nitrogen poor media with organic compound, as an energy source. Apart from nitrogen fixation, *Azotobacter* produces IAA for plant growth stimulation and siderophore for the suppression of phytopathogen and thus acts as plant growth promoting rhizobacteria. The application of inoculums to the seedling enhanced plant height and stem growth especially from 6 weeks after transplanting and it also increase the fruit yield. The use of *A. chroococcum* inoculum was an effective biological management option in tomato fertilization programme (Taiwo, 2004).The effect of spent wash pressmud on soil chemical properties, growth, yield and quality of seasonal sugarcane was studied (Bhalerae, 2006). The effect of organic manures (Vermicompost, Farmyard manure, neemcake and wood ash), organic amendments and green manures on growth, yield, nutrient uptake and soil chemical properties of Banana cv. Grand Naine has reported (Vanilarasu and Balakrishnamurthy, 2014). Therefore, it is necessities to judicial use of organic matter supplementation at proper time. *Pythium* root rot is one of the most important diseases of tomatoes under field and greenhouse conditions and it kills the newly emerged seedlings. Likewise, many reports suggested improved microbial activity during organic matter supplementation (Bugnall and Jarvis, 2007). To the best of our knowledge, there is no report regarding the interaction effect of *Azotobacter* and organic matter supplementation on Tomato - *Pythium* pathosystem. In the present study, *Azotobacter* was isolated from the rhizosphere soil of tomato plants and characterized the isolates and screened the bacterial isolates. So the interaction effect of *Azotobacter* and organic matter supplementation were studied under pot conditions.

MATERIALS AND METHODS

Isolation and characterization of *Azotobacter*

Rhizosphere soil samples from tomato plants were collected from 10 different locations. Samples of 10 g of the soil were dispersed in 90 ml of sterile water in 250 ml of conical flasks. The supernatant was serially diluted in sterile water with dilution up to 10^{-7} and plated in petridish containing Nitrogen free Waksmann base -77 medium and counted in the colony

counter.The isolates were identified and characterized by using the Gram's staining and colony observation. The isolation and characterization of *Azotobacter* strains from the rhizosphere of sugarcane for their ability to grow on Nitrogen free medium and nitrogenase activity under aerobic or micro aerobic conditions was studied (Tejera, 2004)

Plant growth promoting activities under (*invitro*)

Bio-assay for IAA Production

IAA production was determined *invitro* by the method described by Patten and Glick (1996). The test bacterial culture was inoculated in the Waksmann base - 77 medium with 100 mg/ litre of DL – Tryptophan incubated at 30 + 2ºC. Cultures were centrifuged at 7000 X for 30 minutes. The supernatant was reduced to 50 ml volume by evaporation under vacuum and IAA extracted into ethyl acetate and n-butanol. After extraction, the ethyl acetate fraction and n-butanol was estimated in colorimetric assay (Tien, 1979).

Nitrogen Fixation

Fixation of atmospheric nitrogen by the bacterium was determined by using micro kjeldahl method (Bremner, 1960). 1ml of the *Azotobacter* isolates was transferred to 50ml pyrexmicrokjeldhal flask separately. A quarter teaspoonful of the digestion mixture and 4ml of salicyclic-sulphuric acid mixture were introduced into it and heated till frothing ceased. Completion of the digestion was indicated by the solution turning into bluish green and then cooled by adding 15 ml distilled water.The contents were steam distilled and titrated against 0.1n potassium hydroxide till the appearance of golden yellow colour.

Siderophore Production

Siderophore production was tested using synthetic Waksmann base-77 broth. The growth of *Azotobacter* isolates in iron deficient Waksmann base 77-broth was determined by measuring the absorbancy of 420 nm in Spectronic – 20 colorimeter (Modi, 1985). Siderophore production was extracted and estimated the isolates.The development of wine colour showed the presence of phenolate like siderophores (Reeves, 1983).

***In vitro* assay of antagonistic effect**

The invitro assay was used for *in vitro* test of antagonistic activities of the bacterial isolates (Imran Ali Siddiqui, 2001). The potential bacterial isolates were grown on PDA plates which have been pre-inoculated with *Pythium aphanidermatum*. The plates were incubated at 30 ± 2°C for 72 hours. After the incubation period, antagonistic activities were evaluated by measuring (in mm) the inhibition zone between pathogens and tested bacteria.

Interstrain differences of *Azotobacter* isolates

Interstrain differences of bacterial isolates were determined by using 100 mL volume of Waksmann base-77 medium supplemented with 0.05% (W/V) $(NH_4)_2SO_4$ was dispensed under sterilized condition (Moustafa, 1968).The following carbon sources namely, sucrose, galactose, fructose, mannitol, lactose and xylose were individually filter sterilized at 0.5 % concentration and added to the minimal medium, aseptically.Molar growth yield (Y) of the *Azotobacter* isolates on different carbon sources could be calculated from single

Table 1: Occurrence, designation and community population of *A. chroococcum* from the rhizosphere of tomato grown at different locations of puducherry

Location for the Sample collection	Designation	Log 10 CFU / g of dry soil Total bacterial Population	Population of *A. chroococcum*	Percentage of *A. chroococcum*
PKKVK	AZT-1	7.86	6.92	1.15
Arumbarthapurm	AZT – 2	7.78	6.80	1.05
Arasur	AZT – 3	7.80	6.79	1.11
Thirukanur	AZT – 4	7.32	6.01	0.49
Korkkadu	AZT – 5	7.40	6.12	0.52
Bahour	AZT – 6	7.52	6.26	0.55
Sooramangalam	AZT – 7	7.25	5.86	0.41
Sorapet	AZT-8	7.65	6.52	0.74
Karayamputhur	AZT – 9	7.47	6.26	0.63
T.N.Palayam	AZT-10	6.02	4.56	0.35

Table 2: Screening the *Azotobacter chroococcum* isolates for nitrogen fixation, plant growth promoting and biocontrol characteristics

Place of location	Isolate Number	"N" fixationmg/g	IAA production ng/ml	Siderophore production ($\mu g\ ml^{-1}$)	Zol[a] of *Pythium aphanidermatum*
PKK VK	AZT – 1	16.25	1.582	0.80	15
Arumbarthapuram	AZT – 2	15.52	1.550	0.75	11
Arasur	AZT – 3	15.90	1.475	0.65	12
Thirukanur	AZT – 4	11.26	1.400	0.55	13
Korkkadu	AZT – 5	10.46	1.385	0.58	8
Bahour	AZT – 6	11.15	1.345	0.53	7
Sooramangalam	AZT – 7	13.50	1.285	0.61	5
Sorapet	AZT – 8	15.20	1.450	0.63	9
Karayamputhur	AZT – 9	10.88	1.256	0.56	10
T.N. Palayam	AZT – 10	9.20	1.196	0.50	8

measurement of total growth.

Cultural conditions, Incubation time, pH and Iron Concentration on siderophore production

The static and shake culture conditions (100 rpm), on a rotary shaker were tested. The growth and siderophore production of the *Azotobacter* isolate were monitored at 4 hrs intervals up to 24 hour in Waksmann base-77 broth under shake culture condition (100 rpm).The siderophore production at pH 5.5, 6.0, 6.5, 7.0 and 7.5 levels were tested (Knosp, 1984).Iron at different concentration viz., 0, 50, 100, 150 and 200ìM as $FeCl_3$were tested with Waksmann base-77 broth under shake culture condition (100 rpm).

Pot Culture experiment Setup

Seeds of the tomato were pre-germinated, sown in bed nursery and watered regularly at 2 days intervals. After 15 days, the seedlings were pulled off and transplanted in rectangular cement pots. The bacterial suspension of *Azotobacter* isolates at a concentration of 1 x 10^7 cells along with PASIC Compost, Farm yard Manure and Press mud Compost were applied at a rate of 1.0 ton/acre were kept in the separate cement plot (Dey, 2004). All treatments were replicated thrice. After 45 days the plants collected and measured the plant height (Arts

Table 3: Interstrain differences of *A. chroococcum* on molar growth yield (Y) with different carbon sources

Carbon source	Molar growth yield (g mole $^{-1}$ of carbon source)			
	AZT – 1	AZT -2	AZT – 3	AZT – 8
Glucose	20.00	17.01	10.61	5.69
Sucrose	18.09	11.00	9.50	8.00
Fructose	15.10	14.00	13.15	10.61
Mannitolf	36.10	30.44	26.00	28.00
Lactose	3.75	3.74	3.50	3.00
Xylose	0.00*	0.00*	0.00*	0.00*

*No growth

Table 4: Siderophore production of *Azotobacter chroococcum* ** Isolates under different cultural conditions

Culture condition	Growth * Hydroxamate	Siderophore Production
Static	0.840	5.69
Shake	1.063	6.59

*-Absorbancy at 420nm; **-at 1x10^7 CFU/mL inoculum level.

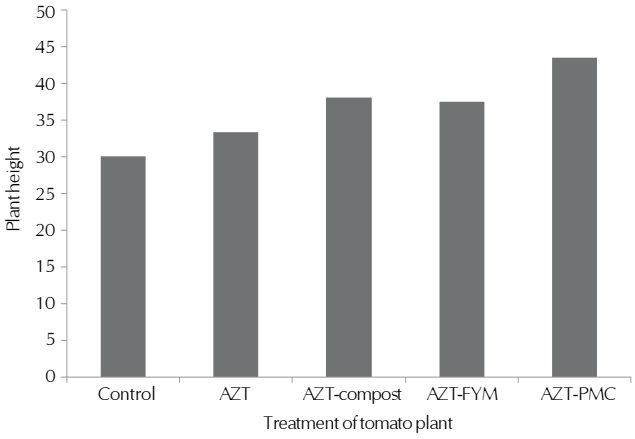

Figure1: Effect of PGPR application on growth enhancement of plant height of tomato

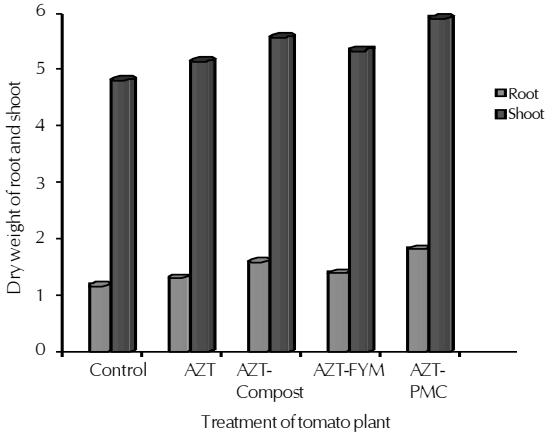

Figure 2: Effect of PGPR application on growth enhancement of dry weight of tomato

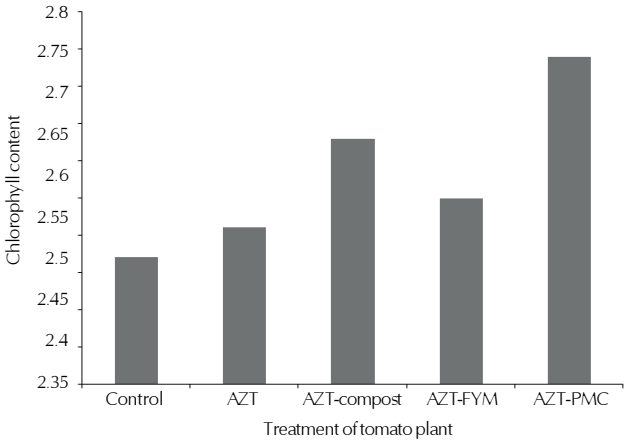

Figure 3: Effect of PGPR applications on growth enhancement of chlorophyll content of Tomato

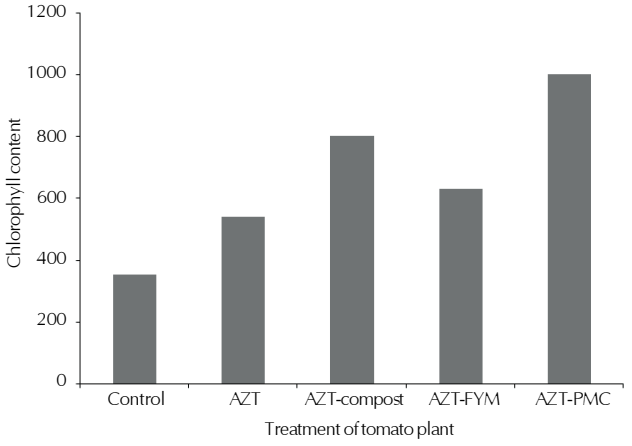

Figure 4: Effect of PGPR applications on growth enhancement of fruit yield of tomato

Table 5: Effect ofculture pH and various concentration on growth and sideropho reproduction of *Azotobacter chroococcum* ** (AZT-1)

pH level	Growth	Siderophore production(μg/ml) Hydroxamate	Iron Concentration (ppm)	Growth	Siderophore Production (μgml^{1}) Hydroxamate
5.5	-	-	-	-	-
6.5	0.868	4.40	0	0.890	6.0
7.0	0.975	6.20	50	0.900	4.1
7.5	0.831	7.10	100	0.990	1.8

*OD at 420 nm; ** at 1x10^{7} CFU/mL inoculum level.

Table 6: Studies on the combined effect of *Azotobacter chroococcum* siderophore production application on growth parameters of tomato var. PKM -1 during *Pythium aphanidermatum*

S. No	Treatment	Plant Height	Dry Weight (g plant – 1)		Chlorophyll (mg g^{-1})	Fruit yield (g Plant^{-1})	Disease incidence
			Root	Shoot			
1.	Control	30.00	1.180	4.838	2.52	353.02	80.30
2.	*Azotobacter* alone	33.40	1.320	5.168	2.56	540.50	28.69
3.	*Azotobacter* + PASIC Compost	38.00	1.600	5.594	2.68	803.50	23.10
4.	*Azotobacter*+ FYM	37.50	1.410	5.364	2.60	630.60	25.02
5.	*Azotobacter*+ Pressmud Compost	43.50	1.840	5.931	2.79	1001.9	20.10

and Marks, 1971), dry weight on root and shoot (Girdthai, 2008),total chlorophyll content of tomato leaves (Jiwan, 1990) and fruit yield (Zehnder, 2000).

Statistical Analysis

The experiment results were statistically analyzed in Randomized Block Design (RBD) and in Duncan's Multiple Range Test (DMRT) as per the procedure described by Gomez and Gomez (1984).

RESULTS AND DISCUSSION

Azotobacter population in the rhizosphere of tomato from ten selected locations were designated as 'AZT' series and numbered randomly. The location, viz, PKKVK recorded maximum of (1.15%) as community population of *Azotobacter* (Table 1). Kumar and Narula, 2004 reported that the occurrence of *Azotobacter chroococcum* in the rhizosphere of many crops.The plant growth promoting activities were tested (Table – 2) and the isolates showed positive results for nitrogen fixation, IAA Production and siderophore production. Out of the ten PGPR isolates, AZT-1 recorded maximum nitrogen fixation (16.25 mg/g), IAA production (1.582ng/ml), siderophore production (0.80 ìg ml^{-1})and zone of *Pythium aphanidermatum*(15 mm) followed by AZT- 3, AZT-2 and AZT-8(Table 2). IAA production by *A. chroococcum* strains have been reported by many authors (Lippmann, 2000 and Farah ahmad, 2005). Production of growth hormone such as IAA by PGPRs has also been reported by (Dilfuza, 2008). The siderophore mediated disease suppression of *A. chroococcum* against phytopathogens has been reported (Verma, 2001). The use of PGPR isolates as inoculants biofertilizers might be beneficial for cauliflower cultivation as they enhanced the growth of cauliflower by inducing IAA production and phosphorus solubilization (Kushwaha, 2013).The growth behaviour and molar growth yield (Y) with different carbon sources of four *A. chroococcum* isolates was studied (Table 3). A very high molar growth yield, namely 36.10 g mol^{-1} of mannitol was recorded with the isolate AZT-1 followed by AZT-2, AZT-8 and AZT- 3 respectively. No growth was recorded in xylose. Mannitol was utilized as carbon source preferentially by *A. chroococcum* isolates followed by sucrose (Page, 1985).

The siderophore production (Hydroxamate type) of AZT -1 isolatewas studied at different cultural conditions, different p^{H} level and different iron concentration (Table 4 and 5). It was observed that the growth of *A. chroococcum* was recorded more at shake culture 6.59, maximum siderophore production at 7.5 p^{H} (7.10ìg/ml) and increased siderophore (Hydroxamate) production (1.8ìg/ml^{-1}) was observed in medium containing 100 ppm of iron respectively. The role of iron depletion and siderophore production of *A. chroococcum* has been reported (Fekete, 1989). Siderophore are also known to act as growth factors and as phytopathogenic suppressive agents. The effects of *Azotobacter* inoculation of the growth, yield and quality of tomato in clay loam soil has been reported (Rahman, 2005). In the present study, treatment 5 containing AZT + PMC found to be have maximum plant height, increased dry weight of root and shoot, chlorophyll content, fruit yield and least incidence of *P. aphanidermatum* followed by AZT + PASIC Compost, AZT + FYM and AZT alone as compared to other treatments (Table 6). The increased total 'N" content of groundnut due to the inoculation of *Azotobacter* has been reported (Barber, 1976). The influence of cashew leaves in pressmud mixture in combination with gramwaste, urea and lignocellulolytic fungi on the growth and reproduction of *Eudrilus eugenia* has been reported (Raja and Ramalingam, 2007). Finally, it can be concluded that combination of *Azotobacter* and Pressmud compost as the best treatment for the growth and yield of tomato.

REFERENCES

Arts and Marks 1971. Effect of *Azotobacter* on the growth of groundnut plant height. *Indian J. Experimental Biology*. **38:** 373-378.

Barber, S. and Pate, J. S. 1976. Nitrogen fixation by *Azotobacter* maximum at low level nitrogen fertilizer application. *Annual Review*.

2: 87-97.

Bhalerae, V. P., Jadhav, M. B. and Bhoe, P. G. 2006. Effect of spentwash pressmud compost on soil properties, yield and quality of seasonal sugarcane. *Ind Sugar.* **56:** 9.

Bremner, J. M. 1960. Determination of nitrogen by Micro kajeldhal method. *J. Agricultural Science*. **55:** 11-33.

Bugnall, P. and Jarvis, M. 2007. Influence of bacterial sources of indole acetic acid and root elongation of sugar beet. *Phytopathology*. **76:** 386-389.

Dey, R., Pal, K. K., Bhatt, D. M. and Chauhan, S. M. 2004. Growth promotion and yield enhancement of peanut by application of PGPR. *Microbial Res.* **159(4):** 371-394.

Dilfuza, E. 2008. Plant growth promoting properties of Rhizobacteria isolates from wheat and peas grown in loamy sand soil. *Turk. J. Biol.* **32:** 9-15.

Farah Ahmad, Iqbal Ahmad and MohdSaghir, K. 2005. Indole Acetic Acid production by the indigenous isolates of *Azotobacter* and fluorescent *Pseudomonas* in the presence and absence of tryptophan. *Turk. J. Biol*. **29:** 29-34.

Fekete, F. A., Lanzi, R. A. and Beaulieu, J. B. 1989. Isolation and Preliminary characterization of Hydroxamic Acids formed by Nitrogen fixing *Azotobacterc hroococcum* B-8. *App. Env. Microbio*. **55(2):** 298-305.

Girdthai, T., Jogloy, S., Kesmala, T., Vorasoot, N. and Akkasaeng, C. 2008. Relationship between root characteristics of peanuts in hydroponics and pot studies. *Crop Science Society of America*. **9:** 529.

Gomez, K. A. and Gomez, A. A. 1984. Statistical procedure for agricultural research with emphasis on rice. International Rice Research Institute. *Los Bancos, Phillipines*. p.294.

Imran Ali Siddiqui 2001. Effect of microbial antagonists on invitro growth of *P. aphanidermatum*. *J. Biological Science.* **1(4):** 224 -226.

Jiwan, P. Palta. 1990. Leaf Chlorophyll Content. Department of Horticulture University of Wisconsin Madison, USA. *Remote Sensing Reviews*. **5(1):** 207-213.

Knosp, O., Von Tigerstron, M. and Page, W. 1984. Siderophore mediated uptake of iron by *A. vinelandii. J. Bacteriology*. **159:** 341-347.

Kumar, V. and Narula, B. 2004. Solubilizing of inorganic phosphate and growth emergence of wheat as affected by *A. chroococcum* mutants. *Biology and Fertility of soil.* **28:** 301-305.

Kushwaha, A., Baily, S. B., Maxton, A. and Ram, G. D. 2013. Isolation and characterization of PGPR associated with cauliflower roots and its effect on plant growth. *The Bioscan.* **8(1):** 95-99.

Lippmann, B., Bela, S., Sandhu, S. S., Kumari, P., Sheoran, R. K. 2000. Analogue resistant mutants of *A. chroococcum* depressed for nitrogenase activity and early ammonia excretion having potential as inoculants for cereal crops. *Indian J. Experimental Biology*. **38:** 373-378.

Modi, M., Shah, K. S. and Modi , V. V. 1985. Isolation and characterization of catechol like siderophore from cowpea *Rhizobium* RA-1. *Arch. Microbiology*. **141:** 156-158.

Moustafa, H. H. and Colins, E. B. 1968. Molar growth yields of certain lactic acid bacteria as influenced by autolysis. *J. Bacteriology*. **96(1):** 17-25.

Najafvand Direkvandi, S. and Alemzadeh Ansari, N. 2008. Effect of different levels of Nitrogen fertilizer with two types of bio-fertilizers on growth and yield of two cultivars of tomato (*Lycopersicon esculentum Mill)Asian J. Plant Sciences*. **7(8):** 757-761.

Page, W. J. and Grand, G. A. 1985. Partial repression of siderophore mediated iron transport in *Azotobacter vinelandii* grown with mineral iron *Can. J. Microbiol*. **34:** 675-679.

Patten, C. and Glick, B. R. 1996. Bacterial biosynthesis of indole-3-acetic acid. *Can. J. Microbiol*. **42:** 207-220.

Rahman 2005. Effect of *Azotobacter* inoculation and organic matter supplementation on the growth, yield and quality of tomato. *Indian Jour. Microbiology*. pp. 1112-1113.

Raja, R. P. and Ramalingam, R. 2007. Utilization of cashew leaf litter and sugar mill wastepressmud for the culture of a compost earthworm *Eudrilus eugeniae* (Kinberg). *The Bioscan.* **2(1):** 37-40.

Reeves, M., Pine, L., Neilands, J. B. and Ballows, A. 1983. Absence of siderophore activity in *Legionella sp*. Grown in iron deficient media. *J. Bacterilogy*. **154:** 324-329.

Taber 2001. Effect of different levels of nitrogen fertilizer with two types of bio-fertilizers on growth and yield of two cultivars of tomato (*Lycopersicon esculentum* Mill). *Asian J. Plant Sciences*. **7(8):** 757-761.

Taiwo, L. B. 2004. Growth response of tomato (*Lycopersicon esculentum* Mill) inoculated with *Azotobacter chroococcum* in an alfisol*, J. Agri. Research*. **5(1):** 13-18.

Tejera, N., Llunch, C., Martinez-Toledo, M. V. 2004. Isolation and characterization of *Azotobacter* and *Azospirillum* strains from the sugarcane rhizosphere. *Plant and soil*. **270:** 223-232.

Tien, T. M., Gaskins and Hubbel, M. H. 1979. The plant growth substances produced by *Azotobacter* and their effect on the growth of groundnut. *Applied Environ Microbiology*. **37:** 1016-1024.

Vanilarasu, K. and Balakrishnamurthy, G. 2014. Influences of organic manures and amendments in soil physiochemical properties and their impact on growth, yield and nutrient uptake of banana. *The Bioscan.* **9(2):** 525-529.

Verma , S., Kumar, V., Narula, N., and Merbach, Co. 2001. Studies on *invitro* production at antimicrobial substances by isolates/ mutants. *J. Plant Diseases and Protection* .**108:** 152-158.

Zehnder Geoffrey, W., Murphy John, F., Sikora Edward, J. and Kloepper Joseph, W. 2000. Application of rhizobacteria for induced resistance. *European J. Plant Pathology*. **107:** 39 -50.