Open access Journal International Journal of Emerging Trends in Science and Technology

Effect of Nitrogen Fixing Bacteria *Azotobacter* and *Azospirillum* on the Growth of *Rosa Polyantha*

Author

Inderdeep Kaur

Department of Microbiology and Fermentation Technology,

Sam Higginbottom Institute of Agriculture, Technology and Sciences

Allahabad, India

Email: *inder.sanghera@gmail.com*

ABSTRACT

Nitrogen fixation is an important biological process where atmospheric nitrogen is converted into ammonia, which can easily be assimilated by the plants. In the present study broth containing N-fixing bacteria Azotobacter and Azospirillum were applied on rose plant (Rosa polyantha) and its effect on the growth and yield of rose plant was studied. A pot experiment was conducted to study the effect of two bacterial inoculants Azotobacter, Azospirillum and their combination on growth and yield of rose. The result showed that application of mixed inoculants (Azotobacter + Azospirillum) produced significantly maximum yield with respect to plant height, number of leaves, number of branches per plant, high chlorophyll and protein content, when compared with single inoculation and control. Azospirillum inoculation resulted in higher growth and yield parameters in comparison to Azotobacter inoculation. From this study, it can be concluded that the biofertilizer inoculation of mixed inoculants gives maximum yield of rose plant as compared to single inoculation and control.

Key words: *Biofertilizers, Azotobacter, Azospirillum, Rosa polyantha*

INTRODUCTION

Nitrogen as the most important vital elements of plant in quality and quantity production of cultivation products plays important role. *Azotobacter* and *Azospirillum*as fixing bacteria of nitrogen can freely fix molecular nitrogen and be considered as biological fertilizer (Amiri*et al.*2013). Free-living nitrogen-fixing bacteria *eg Azotobacter chroococcum* and *Azospirillum lipoferum*, were found to have not only the ability to fix nitrogen but also the ability to release phytohormones similar to gibberellic acid and indole acetic acid, which could stimulate plant growth, absorption of nutrients, and photosynthesis (Essam*etal*.2013).

Azotobacter is gram-negative, motile, pleomorphic aerobic bacterium which produces catalase, form thick-walled cysts and may produce large quantities of capsular slime. Members of these genera are mesophillic, which require optimum temperature of about 30°C.*Azotobacter* belongs to family Azotobacteriaceae, aerobic, free living, and heterotrophic in nature. *Azotobacter*s are present in neutral or alkaline soils and *A. chroococcum*is the most commonly occurring species in arable soils. The number of *Azotobacter*

rarely exceeds from 10^4 to 10^5 g^{-1} of soil due to lack of organic matter and presence of antagonistic microorganisms in soil. The bacterium produces anti-fungal antibiotics which inhibits the growth of several pathogenic fungi in the root region thereby preventing seedling mortality to a certain extent .*Azotobacter* have a full range of enzymes needed to perform the nitrogen fixation: ferredoxin, hydrogenase and an important enzyme nitrogenase. (Karunakaran*et al.* 2014). Nitrogen fixation is achieved by the enzyme nitrogenase, which reduces N_2 to ammonia. However, this enzyme is extremely sensitive to oxygen in *Azotobacter* species. High respiration rates and conformational protection of the enzyme are suggested as two factors which make nitrogen fixation possible in an aerobic environment.

Another important Nitrogen fixing bacteria studied is *Azospirillum. Azospirillum* colonized the root regions of crop plants in large numbers and fixes substantial amount of nitrogen and they exerted beneficial effects on plant growth and yield of many crops of economic importance. It is used extensively in rice and other cereal crops as biofertilizers. *Azospirillum* is grown in N-free medium, it behaves as microaerophillic, fix nitrogen and when supplemented with nitrogen it grown as an aerobe.

*Azospirillum*belongs to family *Spirilaceae*, heterotrophic and associative in nature, In addition to their nitrogen fixing ability of about 20-40 kg/ha, they also produce growth regulating substances. Although there are many species under this genus like, *A.amazonense, A.halopraeferens, A.brasilense*, but worldwide distribution and benefits of inoculation have been proved mainly with the *A.lipoferum and A.brasilense.*

MATERIALS AND METHODS

Preparation of Jensen broth and Nitrogen free malate broth for plant Inoculation

Jensen's broth was inoculated with pure culture of *Azotobacter* and Nitrogen free malate broth with *Azospirillum*. The broths were then incubated in shaker incubator at 28°C for 7 days. After seven days of incubation, the growth was confirmed by spread plating 0.1µl of broth in their respective media followed by gram staining method for their identification.

Plant inoculation methods

Four seedlings of one month old rose (*Rosa polyantha*) plant were used. The seedlings were measured with respect to plant height, number of leaves and branches were also counted. Seedlings were then planted in four pots containing 2kg of soil and were labelled as Mixed, *Azospirillum*, *Azotobacter*, and Control. The next day 20ml of Jensen's broth was inoculated in *Azotobacter* labelled rose plant, 20ml Nitrogen free malate broth was inoculated in *Azospirillum* labelled plant. Mixed inoculums was prepared by mixing 10ml Jensen's broth with *Azotobacter*and 10ml Nitrogen free malate broth with *Azospirillum* and inoculated into mixed labelled plant. Control labelled was left uninoculated. Every week the plant was measured with respect to plant height, number of leaves, number of branches and number of flowers.

Estimation of Chlorophyll and Protein content of Rose (*Rosa polyantha*) leaves

Chlorophyll estimation

0.5 mg of fresh leaf was ground in a mortar and pestle with 20 ml of 80 per cent acetone. The homogenate was centrifuged at 3000 rpm for 15 min. The supernatant was saved. The pellet was resuspended in 5 ml of 80 per cent acetone each time, until it becomes colourless. All the supernatants were pooled and utilized for

chlorophyll determination. Absorbance was measured at 645 and 663nm in spectrophotometer. The chlorophyll content was determined by using the following formula:

Total Chlorophyll (mg/g fr. wt.) = (0.0202) × (OD645) - (0.00802) × (OD663)

Protein estimation

0.5 mg of plant materials was macerated with a pestle and mortar with 10 ml of 20 per cent trichloroacetic acid. The homogenate was centrifuged for 15 min at 600 rpm. The supernatant was discarded. To the pellet, 5 ml of 0.1 N NaOH was added and centrifuged for 5 min. The supernatant was saved and made up to 10 ml with 0.1 N NaOH. This extract was used for the estimation of protein. From this extract, 1 ml of sample was taken in a 10 ml test tube and 5 ml of reagent C was added. The solution was mixed well and kept in dark for 10 min. Later 0.5 ml folinphenol was added and the mixture was kept in dark for 30 min. The sample was read at 660 nm in the spectronic-20. Blank prepared without protein sample was used for zero setting. Data obtained was plotted in bar graph.

RESULTS AND DISCUSSION

Preparation of *Azotobacter* and *Azospirillum* broth for inoculation in plant as a biofertilizer.

Azotobacter broth was prepared on Jensen's media and *Azospirillum* broth was prepared on Nitrogen free malate media.

Effect of Nitrogen Fixing Bacteria as a biofertilizer on Rose (*Rosa polyantha*) plant

The effect of Biofertilizer treatment on rose plant was significantly higher in single or combined inoculation than control plant. A significant variation in plant height, number of leaves, number of flowers is given in Table 1.

Table 1. Effect of Nitrogen fixing bacteria on morphological parameters of Rose (*Rosa polyantha*).

Treatments	Plant height	Number of branches	No. of leaves	No. of flower
Control	13.02	3.38	36.31	0.54
Azotobacter	16.69	3.23	44.38	0.85
Asospirillium	17.12	3.15	53.69	1.08
Mixed	17.72	4.38	57.08	1.54
F- test	S	S	S	S
S. Ed. (±)	1.117	0.632	6.590	0.487
C. D. (P = 0.05)	2.305	1.304	13.603	1.005

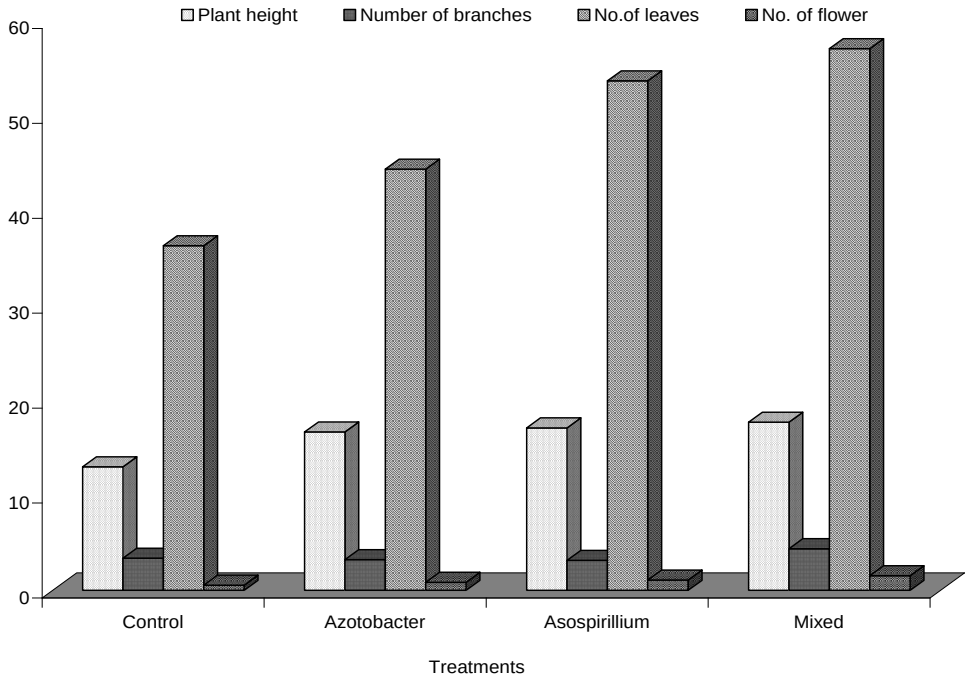

Fig 1:- Morphological parameters of rose (*Rosa polyantha*).

Total Chlorophyll and Protein estimation

Table 2 and 3 shows the protein and chlorophyll content of Rose (*Rosapolyantha*) leaves taken from Control, *Azotobacter*, *Azospirillum* and Mixed plant after three months of planting

Table 2. Total protein estimation

Total protein content	Control	*Azotobacter*	*Azospirillum*	Mixed
	0.21	0.36	0.26	0.39

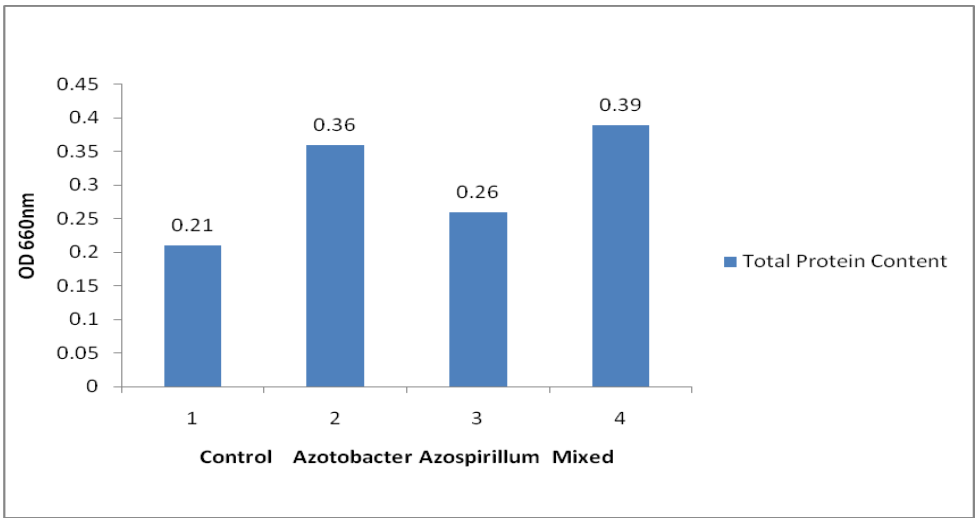

Fig 2 :- Total protein content

Table 3. Total Chlorophyll content

Total chlorophyll content	Control	*Azotobacter*	*Azospirillum*	Mixed
	0.0049	0.0056	0.0065	0.0097

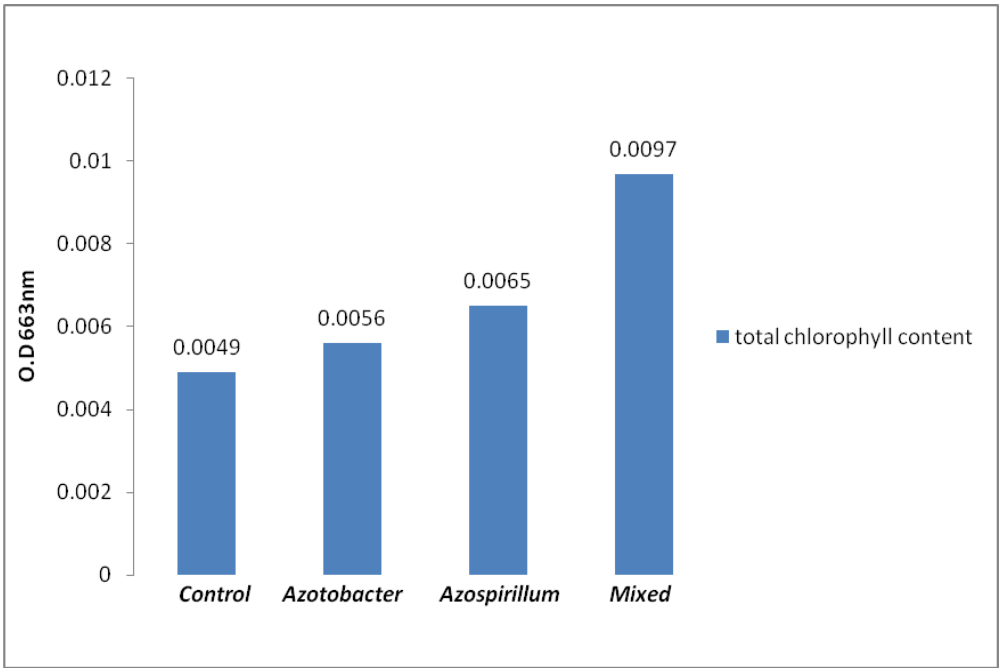

Fig 3:- Total Chlorophyll content

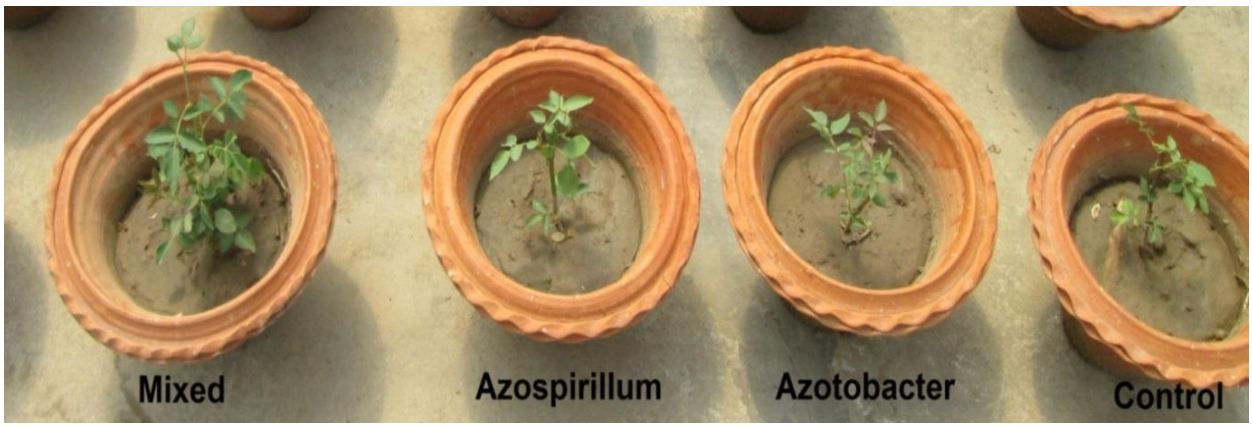

Fig. 4:-Rose (*Rosa polyantha*) plant after one month

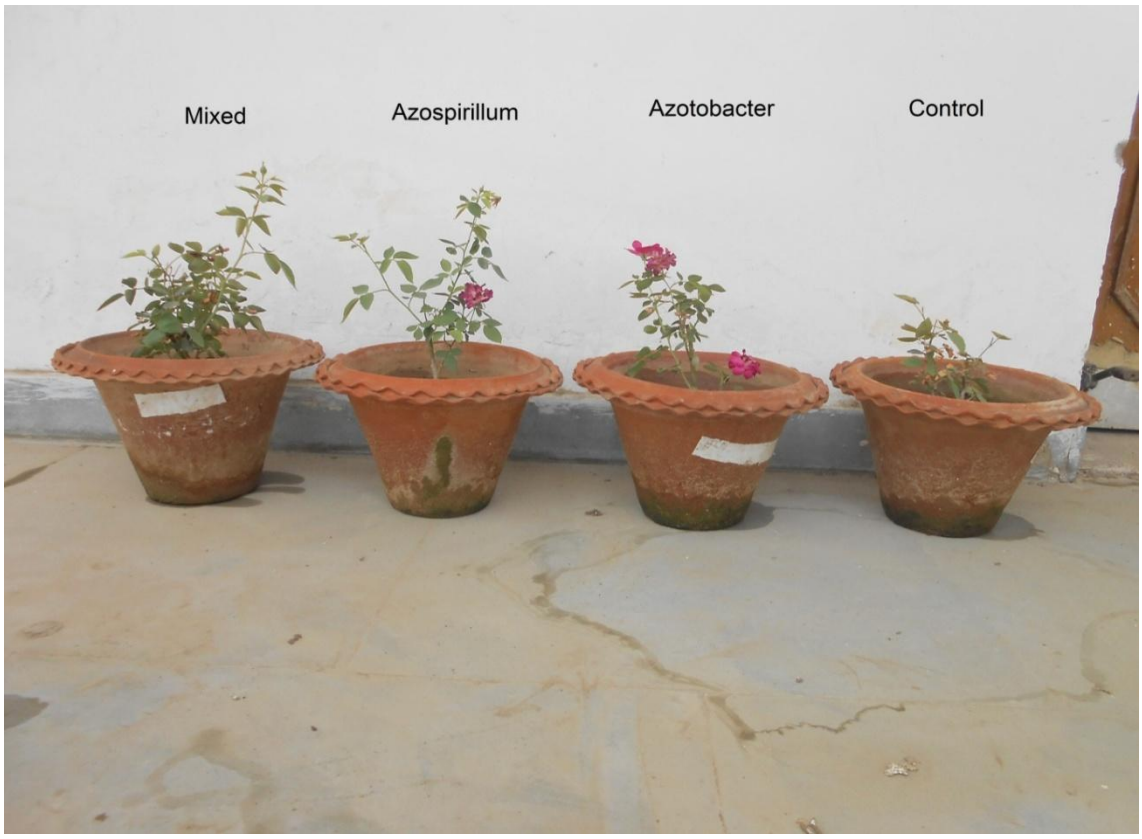

Fig. 5:-Rose (*Rosa polyantha*) plant after three month

The identified colonies of *Azotobacter* and *Azospirillum* were inoculated in broth medium i.e Jensen's broth for *Azotobacter* and Nitrogen free malate broth for *Azospirillum* and incubated in shaker incubator for one week at 28°C. The growth was confirmed by spread plating the inoculated broth of *Azotobacter* and *Azospirillum* in their respective media mentioned above. After confirmation of growth, the broth was inoculated in plants.

The effect of *Azotobacter*and *Azospirillum* on rose (*Rosa polyantha*) plant was observed on the basis of following observation

1- The maximum height, number of leaves, number of flowers, chlorophyll and protein content was observed on plant inoculated with mixed culture of *Azotobacter* and*Azospirillum.*
2- Comparing the effect on plants inoculated with *Azospirillum*and *Azotobacter* the maximum overall increasing effect was observed in *Azospirillum* inoculated plant.
3- Control showed least overall increase as compared to bacterial inoculated plants.

These Nitrogen fixing bacteria can be called as biofertilizers. Biofertilizers are ecofriendly and environmentally safe. Utilization of biofertilizer increased all growth and yield promoting traits. The application of free living Nitrogen fixing *Azotobacter* and *Azospirillum* as biofertilizers is known to result in increased productivity of a variety of crops. As in this study, the effect of biofertilizer on rose (*Rosa polyantha*) was enhanced when compared to uninoculated control. Plant height, number of leaves, number of branches, number of flowers , the parameters used to assess performance for plant showed that co-inoculution of biofertilizersi. e*Azotobacter* mixed with *Azospirillum* show significant effect compared to single inoculation and control because these bacteria help in increasing plant productivity by way of increased biological

nitrogen fixation, increased absorption, stimulation of plant growth through hormonal action or antibiosis, or by decomposition of organic matter. Since rose (*Rosa polyantha*) being an ornamental plant, profitable in market, for maximum flower yield soil should be inoculated with mixed inoculums of *Azotobacter* and *Azospirillum.*

ACKNOWLEDGEMENTS

The author is thankful to Head, Dept. of Microbiology and Fermentation Technology, Sam Higginbottom Institute of Agriculture, Technology and Sciences, Allahabad for providing necessary facilities to conduct the experiment.

REFERENCES

1. **Akbari, Ghalavand.P., A.,Sanavy.M and Alikhani.M.A.2007**. The effect of Biofertilizers, nitrogen fertilizer and farmyard manure on grain yield and seed quality of sunflower (*Helianthusannus*L.). *Journal of Agricultural Technology* .Vol. 7(1): 173-184.
2. **Akhani., Darzi.M.T., HadiandM.S. 2012**. Effects Of Biofertilizer And Plant Density On Yield Components And Seed Yield Of Coriander (*CoriandrumSativum*).*International Journal of Agriculture and Crop Sciences.*Vol 4: 1205-1211.
3. **Aazadi .M.S., Siyadat .S.A., Syahbidi .M and Younesi .E.2014**.To study effect of Nitrogen, *Azotobacterspp* and *Azospirillum spp*. on phonological and morphological traits of Durum Wheat cultivars in Dehloran region, Iran. *CercetariAgronomice in Moldova.*Vol XLVII: 157.
4. **Alireza., and Farahani.H.A.2013**. Influence of biofertilizer on essential oil, harvest index and productivity effort of black cumin (*Nigella sativa*L.).*International Journal for Biotechnology and Molecular Biology Research*. Vol.4: 24-27.
5. **Amiri .A and Rafrie.M.2013.**Effect of soil inoculation with *Azospirillum* and *Azotobacter*bacteria in nitrogen use efficiency and agronomic characteristics of Corn. *Annals of Biological Research*. Vol 4(2): 77-79.
6. **Cassan. F,Perrig .D, Sgroy, V., Masciarelli , O., Penna , C. and Luna , V. 2009**. *Azospirillumbrasilense*Az 39 and *Bradyrhizobiumjaponicum* EI09, inoculated singly or in combination, promote seed germinaton and early seedling growth in corn (*Zea mays L.*) and soybean (*Glycine max L.*). *European Journal of soil Biology*. Vol.45: 28-35.
7. **Chairman .K., Amuthan .M., Ramesh .S., Vasanthi .K and Ranjit .S.A.,2013**. Isolation and identification of biofertilizing microorganisms from soil samples and determination of growth conditions in chilly and cluster beans. *Medicinal plant Research*. Vol.3.No 6 44-51.
8. **Chaudhari., Jadav. R.G. and Masu.M.M. 2009**. Effect of biofertilizers and their combinations with nitrogen fertilizer on growth, yield and quality of rose (*Rosa damascene L.*).*Academic Journal of Plant Sciences.* Vol. 4: 373-376.
9. **Dhevendharan .K.,Preetha .G and Bodethala .N.V.2013**.Studies on nitrogen fixing bacteria and their application on the growth of seedling of *Ocimum sanctum*. *Pharmacognosy Journal*. Vol 5: 60-65.
10. **Essam and Lattief.A.E.2013**. Impact of integrated use of bio and mineral nitrogen fertilizers on productivity and profitability of wheat (*Triticumaestivum L.*) under upper Egypt conditions .*International Journal of Agronomy and Agricultural Research (IJAAR).* Vol. 3: 67-73.

11. **Farhan and Shokheri. 2008**. Effect of Organic &Biofertilizer on the growth of Patcholi plant (*Pogostemoncablin*). *Faculty of Chemical and Natural Resources Engineering University Malaysia Pahang.*
12. **Jolly ,S.N., Shanta, N.A. and Khan, Z.U.M. 2010**. Quantification of heterophobic bacteria and *Azospirillum* from Rhizosphere of Taro (*Colocasiaesculenta L. Schott*.) and the nitrogen fixing potential of isolated *Azospirillum*. *International Journal of Botany*.Vol.6(2) : 117-121.
13. **Kadasamy .D.,Ganesan.P and Bodethala.N.V.H ,2013.** Studies on Nitrogen fixing bacteria and their application on growth of seedlings of *Oscimum sanctum*. *Pharmacognosy Journal* .Vol. 5: 60-65.
14. **Karunakaran .A.R., Dhanasekaran.s.,Hemalatha . K., Monika .R.,Shanmugapriya .P and Sornalatha .T. 2014.** Isolation and Mass production of Biofertilizer(*Azotobacter* and *Phosphobacteria*). *International Journal of Latest Research in Science and Technology*. Vol 3:79-81.
15. **Khan .I., Masood .A and Ahmad.A.2010**. Effect of Nitrogen Fixing bacteria on plant growth and yield of Brassica juncea. *Journal of Phytology*. Vol 2(9) :25-27.
16. **Maurya .B.R., Kumar .A.,Raghuwanshi .R and Singh .V., 2012**. Diversity of *Azotobacter* and *Azospirillum* in Rhizosphereof Different Crop Rotations in Eastern Uttar Pradesh of India. *Research Journal of Microbiology* .Vol.7: 123-130.
17. **Nepolean .P.,Jayanthi .R., Vidhya P.R., Balamurugan .A., Kuberan .T., Beulah .T., Premkumar and R.,2012.** Role of biofertilizers in increasing tea productivity. *Asian Pacific Journal of Tropical Biomedicine*.Vol.4:S1443-S1445.
18. **Panchal ,Parekh.N.S., Parmar.A.B and Pate.H.C.2010**. Effect of Biofertilizers and nitrogenous fertilizer on growth, flowering and yieldof annual white chrysanthemum (*Chrysanthemum coronariumL.*) under middle Gujarat agroclimatic conditions. *The Asian Journal of Horticulture*.Vol-5:22-25.
19. **Ramakrishnan .K and Selvakumar .G.2012**. Effect of Biofertilizers on enhancement of growth and yield on Tomato (*LycopersicumesculentumMill*.). *International Journal of Research in Botany*. Vol 4: 20-23.
20. **Selvakumar, Reetha.Sand Thamizhiniyan.P. 2012**. Response of Biofertilizers on Growth, Yield Attributes and Associated Protein Profiling Changes of Blackgram (*Vignamungo*L. Hepper).*World Applied Sciences Journal.Vol.*16: 1368-1374.
21. **Shivananda .T and Dang. R,.2008.**Influence of bio-fertilizers on the availability of nutrients (N, P and K) in soil in relation to growth and yield of *Stevia rebaudiana*grown in South India. *International Journal of Applied Research in Natural Products* .Vol. 1: 20-24.