Original Research Paper

Volume - 11 | Issue - 09 | September - 2021 | PRINT ISSN No. 2249 - 555X | DOI : 10.36106/ijar

Microbiology

# EVALUATION OF THE LIQUID VERSUS SOLID FORMULATION OF *AZOSPIRILLUM* IN PEARL MILLET UNDER STERILE POT CONDITIONS

**Aruna Gupta*** Department of Biotechnology, Institute of Health and Allied Sciences, Muradnagar, Ghaziabad, U.P. *Corresponding Author

**A.K. Jaitly** Department of Plant Science, MJP Rohilkhand University, Bareilly.

**ABSTRACT** In the present investigation, experiments were conducted to study the response of both solid and liquid biofertilizers of Azospirillum brasilense and synthetic fertilizers separately and in combinations in pearl millet under sterile pot conditions. The bacterial inoculants at all levels and in different combinations with chemical fertilizer showed an increase in various growth parameters as compared to the both the controls (i.e.,without biofertilizers and without any chemical fertilizer). The results revealed that the various growth parameters produced were better when used in combination of biofertilizers and chemical fertilizers than when either of them used alone. In this study, our main objective of the experiment was to screen and select the combination of fertilizers which could be proposed best for the growth of pearl millet which can be recommended to be used as the best inoculant and can further be tested in field conditions.

**KEYWORDS :** Azospirillum biofertilizer, chemical fertilizer, plant growth promoting rhizobacteria, pearl millet

## INTRODUCTION

Pearl millet, a C4 plant is the major course crop of the world in the tropical areas and is grown largely for its ability to produce grain under hot dry conditions in unfertile soils of low water-holding capacity. However the response of the crop to applied nutrients is high. In view of escalating input costs and growing concerns on sustainability and soil health, reliance on Integrated Plant Nutrient Supply (IPNS) systems are assuming greater importance in recent days.

Amongst all the major plant nutrients, nitrogen is the most needed one and is specially limiting in Indian soils. Nitrogen is required in large quantities for plants to grow since it is the basic constituent of proteins and nucleic acids. Although many genera and species of nitrogen fixing bacteria have been isolated from the rhizosphere of various cereals, members of *Azospirillum* genera have been widely tested to increase yields of cereals under field conditions.

In the present investigation, the effect of solid and liquid biofertilizer of *Azospirillum* in combinations with synthetic fertilizers was studied to establish their effect of growth yield in pearl millet.

## MATERIAL AND METHODS

### Soil Preparation

Farm soil of IARI, New Delhi having the following characteristics viz., organic Carbon (C) % 0.35, available Phosphorous (P) 39.4 Kg $ha^{-1}$, available Potassium (K) 267 Kg $ha^{-1}$ , Electric Conductivity - 0.14 ds $m^{-1}$, sandy loam sterilized soil and pH of 8.65 was used for pot culture experiments. The soil was further amended with half of the recommended dose of nitrogen (40 Kg N $ha^{-1}$), phosphorous (30 Kg $ha^{-1}$) and potassium (30 Kg $ha^{-1}$) in the form of urea, single super phosphate (SSP) and muriate of potash (MOP) respectively along with solid and liquid culture. SSP and MOP were given as basal dose at the time of sowing. However urea was given in two split doses, half dose i.e., 20 Kg N $ha^{-1}$ was given at the time of sowing and rest half of 20 Kg N $ha^{-1}$ was added after 21 days of sowing. Two set of controls were also kept, one control was kept with complete recommended dose of N, P and K (80:60:60) but without any culture and one set of control was absolute control (i.e., without any chemical fertilizer and culture). In the control treatment also SSP and MOP were given as basal dose at the time of sowing and urea in two split doses, half dose at the time of sowing and half dose was given after 21 days of plant growth.

### Bacterial strain and growth conditions

All the experiments were done with *Azospirillum brasilense* Cd culture procured from Banaras Hindu University, Varanasi. Bacteria were grown in N-free nutrient broth medium and prepared for inoculation at the final concentration of 1 X $10^{-6}$CFU/gm of soil. Plants were grown in disinfected pots containing sterilized soil.

### Preparation of solid carrier based bioinoculant

Carrier based inoculant of *Azospirillum* was prepared by mixing the log phase *Azospirillum brasilense*, Cd culture with carrier (charcoal:soil as 3:1). After mixing, it was kept for curing at room temperature and then packed in high density polythene bags. The presterilized seeds at the time of sowing were coated with carrier-based solid bioinoculant at the final concentration of $10^6$cells/pot.

### Preparation of liquid carrier based bioinoculant

The liquid based bioinoculant was prepared by inoculating log phase culture of *Azospirillum brasilense*, Cd culture in N-free malate medium broth with 50 µM trehalose. The pre-sterilized seeds were treated directly at $10^6$ cells/pot with the liquid bioinoculant for 15-20 minutes before sowing.

### Preparation of wells for sowing of seeds

Holes up to 5 cm depth were made in each pot and two seeds were sown in each hole. Care was taken to maintain normal soil moisture at the time of sowing. Plants were watered with sterile water at regular intervals. Plants were grown in the growth chamber maintained at controlled conditions; day and night temperatures at $35^0$C and $25^0$C respectively; humidity at 60-70 %; light intensity was maintained at 700 lux and photoperiod of 14 hrs.

### Harvesting

Plants were harvested after 60 days of plant growth.

### Details of the experiment

| S.No. | Treatment |
|---|---|
| 1 | Absolute control (No chemical fertilizer and bioinoculant) |
| 2 | Positive control (full dose of N, P, K i.e., 80:60:60 with no culture |
| 3 | Control with ½ dose of N, P, K i.e., 40:30:30 but with no culture |
| 4 | Carrier bioinoculant + ½ dose of N, P, K |
| 5 | Liquid bioinoculant + ½ dose of N, P, K |

No. of treatments - 5
No. of replications - 6
No. of plants /pot - 2
Pearl millet variety - Pusa 444

## RESULTS

The plant growth parameters such as plant height, fresh and dry shoot and root weights, N-content in straw were found to be better in plants given treatment with liquid bioinoculant plus half the recommended chemical fertilizer dose as compared to solid carrier based bioinoculant treatment. The population of Azospirillum in the rhizospheric soil of pearl millet was found to be more in liquid bioinoculant followed by solid bioinoculant. However the control plants with full doses of N, P, K; half dose of N, P, K and absolute control with no N, P, K and biofertilizer, the population was found to be almost negligible.

The bacterial count in liquid bioinoculant, solid bioinoculant, treatment with half dose of N, P, K, positive control with full dose of N, P, K and control treatment with no N, P, K and no culture recorded 410,

330, 68, 61 and 45 cells per gram of soil weight respectively.

**Table 1. Inoculation effect of liquid-bioinoculant, solid-bioinoculant and controls in pearl millet, var. Pusa 444 under sterile pot conditions after 60 days of plant growth (Average of 6 replications).**

| S. No. | Treatment | Plant height (cm) | Shoot fresh wt. (gm/plant) | Shoot dry wt. (gm/plant) | Root fresh wt. (gm/plant) | Root dry wt. (gm/plant) | Plant dry matter nitrogen (%) | Bacterial count (cells/gm soil) |
|---|---|---|---|---|---|---|---|---|
| 1. | Full dose of N,P,K | 107 | 23.98 | 2.82 | 1.34 | 0.50 | 0.78 | 45 |
| 2 | Half dose of N,P,K | 93 | 19.50 | 2.17 | 1.02 | 0.34 | 0.69 | 68 |
| 3 | Absolute control | 87 | 17.15 | 1.54 | 0.61 | 0.26 | 0.57 | 61 |
| 4 | Solid culture + half N,P,K | 100 | 20.90 | 2.47 | 1.32 | 0.48 | 0.72 | 330 |
| 5 | Liquid culture + half N,P,K | 106 | 22.80 | 2.69 | 1.35 | 0.51 | 0.74 | 410 |
| CD at 5% | | 12.00 | 2.023 | 0.279 | 0.106 | 0.075 | 0.029 | |

**Plate 1 Growth effect of liquiz and solid based bioinoculants of Azospirillum brasilense, Cd culture on growth of pearl millet.**

**(From left to right – Control with ½ dose of N, P, K; solid bioinoculant + ½ dose of N, P, K; control with full dose of N, P, K; liquid bioinoculant + ½ N, P, K; absolute control with any dose of N, P, K and bioinoculant).**

## CONCLUSION

The efficiency of the liquid formulation developed was tested in pearl millet under sterile pot conditions. Results showed that the growth parameters such as plant height, fresh and dry weight of root, fresh and dry weight of shoot and the nitrogen content of the straw when inoculated with solid and liquid bioinoculants were better as compared to the control. However the various parameters studied were not significant when compared between the plants inoculated with the solid-carrier based bioinoculant and liquid bioinoculant but morphologically the growth of root and root hairs was found to be better in liquid bioinoculant treated plants which in turn helps the plants in better absorption of water and nutrients thereby resulting in better growth of plants. Better colonization or cell number of *Azospirillum* in the rhizospheric soil of the liquid bioinoculant also indicates suitability of liquid biofertilizers over solid-carrier based bioinoculant. This might be due to the fact that the cell protectants added in the liquid formulation protect the cells from dessication under deleterious conditions. Thus we can conclude that the liquid bioinoculant with half the recommended chemical fertilizer dose performs better as compared to solid carrier based combinations of bioinoculant under controlled conditions.

## ACKNOWLEDGEMENT

The financial assistance from Department of Science and Technology, New Delhi through the Young Women Scientist Fellowship Scheme and the infrastructure for the research facility provided by Division of Microbiology, is gratefully acknowledged.

## REFERENCES

1. Abrantes, G.T.V., Day, J.M. and Dobereiner, J. (1995). Methods for the study of nitrogenase activity in field grown grasses. Bull. Inter. Inf. Biol., 21: 1-7.
2. Agarwala, R. and Tilak, K.V.B.R. (1989). Azospirillum brasilense and Rhizobium sp. (cowpea misc.) inoculation : Effect on yield and nitrogen uptake of various minor millets. Indian J. Microbiol. 29: 229-232.
3. Albrecht, S.L., Okon, Y., Lonnquist, J. and Burris, R. H. (1981). Nitrogen fixation in corn-Azospirillum associations in a temperate climate. Crop. Sci. 21: 301-306.
4. Baldani, V.L.D. and Dobereiner, J. (1980). Host-plant specificity in the infection of cereals with Azospirillum Spp. Soil Biol. Biochem., 12: 433- 439.
5. Barber, L.E., Russel, S.A. and Evans, H.J. (1979). Inoculation of millet with Azospirillum. Plant and Soil, 52: 49-57.
6. Bashan, Y. and Holguin, G. (1997). Azospirillum plant relationship: environmental and physiological advances. Can J. Microbiol., 43: 102-121.
7. Bashan, Y. and Levanomy, H. (1990). Current status of Azospirillum inoculation technology : Azospirillum as a challenge for agriculture. Can J. Microbiol., 36: 591-608.
8. Chaudhary, K., Yadav, K.S., Tauro, P. (1992). Nitrogen fixation by Azospirillum brasilense-temperature effect. Indian J. Microbiol., 32: 41-44.
9. Del Gallo, M. and Fendrik, I. (1994). The rhizosphere and Azospirillum. In Azospirillum-Plant Association (ed). Edited by Y. Okon. CRC Press, Boca Raton, Fla pp. 57-75.
10. Eskew, D.L., Focht, D.D. and I.P. Ting. (1977). Nitrogen fixation, denitrification and pleomorphic growth in a highly pigmented Spirillum lipoferum. Appl. Environ. Microbiol. 34 : 582-585.
11. Fages, J. (1992). An industrial view of Azospirillum inoculants formulation and application technology. Symbiosis, 13: 15-26.
12. Hegazi, N.A., Khawas, H. and M. Monib. (1981). Inoculation of wheat with Azospirillum under Egyptian conditions, pp. 493.
13. Hegde, S.V. (2002). Liquid Biofertilizers in Indian Agriculture. In current perspectives in nitrogen fixation. Australian Academy Science, Canberra. Biofert. News Lett., 12 : 17-22.
14. Kennedy, I.R. and Tchan, Y.T. (1992). Biological nitrogen fixation in non-leguminous field crops : Recent advances. Plant Soil, 141: 92-118.
15. Mikhailouskaya, N. and Bogdevitch, I. (2009). Effect of biofertilizers on yield and quality of long-fibred flax and cereal grains. Agronomy Research 7(Special issue I): 412-418.
16. Reynders, L. and Vlassak, K. (1982). Use of Azospirillum brasilense as biofertillzer in intensive wheat cropping. Plant Soil, 66 : 217-223.
17. Sudhakar, C. and C. Sudha Rani. (2008). Effect of inclusion of biofertilizers as part of INM on yield and economics of Safflower (Carthamus tinctorius, L). 7 th International Safflower Conference, Australia.
18. Tilak, K.V.B.R. and Saxena, A.K. (2000). Azospirillum – its impact on crop production. In : Recent Advances in Biofertilizer Technology (Eds. Yadav, Sibgh and Raychoudhary), pp. 177-189.