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# Azospirillum: Bioformulations, Product Quality and Survivability

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***Abstract:*** ***Bioformulation is the form in which the microbe is applied to the crop field for beneficial effects. Impact of microorganisms is also depends on kind of formulation used. Azospirillum confers several benefits apart from fixing dinitrogen to the host plant like enhanced plant growth by phytohormone production, tolerance from diseases by induced systemic resistance, etc. These effects are achieved by applying them in definite formulation. There are several formulations which can suit the needs of farmers like solid powder based, liquid, polymer entrapped, fluid bed dried formulation, etc. Although traditional formulations are available in different carriers,but continuous improvement in formulation technology is necessary for prompt effects on crop plants. In this review, we will discuss different aspects of Azospirillum bioformulations.***
***Keywords: Azospirillum, bioformulations, carrier, plant growth promotion***

## I. INTRODUCTION

Bioformulation and inoculation has its roots deep into the history. There are several evidences in the traditional farming in India, whose roles were not realized during their performance. Practice of crop rotation and sprinkling soil from previously cultivated farm into new farms brought under the cultivation is ceremoniously on to the new field (Brahmaprakash and Sahu 2012) are one of them. These kinds of rituals today are scientifically proven as inoculation. They are an integral input of organic farming and sustainable agriculture imparting several benefits to crops (Renu et al., 2016a, 2016b; Sahu et al., 2016b; Renu et al., 2017; Meena et al., 2017, Nair et al., 2017). The success of inoculant technology depends on potential of microbial strain and its formulation. Despite this fact that formulation is very crucial for performance of inoculants, there is no formulation which can be used in all inoculants (Brahmaprakash and Sahu, 2012) and the popularity among farmers is low (Sivasakthivelan and Saranraj 2013).

Azospirillum spp. is ubiquitous in different the climatic conditions and soil type in various crops grown. *Azospirillum* spp. fixes atmospheric nitrogen, solubilise phosphorus, enhance mineral and water uptake, phytohormones production, biocontrol, etc. as described elsewhere in this paper. Tarrand et al. (1978) reported *Azospirillum* genes as most promising organisms by its capability of colonizing roots in large numbers and exerting beneficial effects to plants. Thus, a successful bioformulation of *Azospirillum* can have very crucial role in farming community.

### *A. Product quality of Azospirillum inoculants*

All the formulations for *Azospirillum* have to follow the quality of the product as per BIS standards of fertilizer (control) order (amendment, November 2009) as described by Yadav (2009).

Table: Specification of biofertilizers—Azospirillum (Yadav 2009)

| | | |
|---|---|---|
| 1. | Base | Carrier based* in form of moist/dry powder or granules, or liquid based |
| 2. | Viable cell count | CFU minimum $5 \times 10^7$ cell/g of carrier material or $1 \times 10^8$ cell/ml of liquid. |
| 3. | Contamination level | No contamination at $10^{-5}$ dilution |
| 4. | pH | 6.5–7.5 |
| 5. | Particle size in case of carrier based materials | All materials should pass through 0.15–0.212 mm IS sieve |
| 6. | Moisture percent by weight maximum in case of carrier based | 30–40% |
| 7. | Efficiency character | Formation of white pellicle in semisolid nitrogen free bromothimol blue media |

*Type of carrier: The carrier materials such as peat, lignite, peat soil and humus, wood charcoal or similar material favoring growth of organism.

## II. DIFFERENT CARRIERS OF AZOSPIRILLUM AND ITS VIABILITY

Carriers are inert material used for transporting microbes from lab to land. There are various kinds of carriers available for inoculant formulation. Many of them have been used for Azospirillum successfully. Peat is one of the most preferred carriers because it is rich in organic matter and high water holding capacity (Iswaran 1969). Survival of Azospirillum inoculant was tested up to 31 weeks using FYM, soil and charcoal as carriers in different combinations. A mixture of all three was reported highest viable counts of Azospirllum than others (Tilak 1979).

Biochar is also used as a carrier of Azospirillum. Biochar is a kind of charcoal produced by pyrolysis of biomass under limited oxygen availability. Glaser (2007) reported that biochar application with bacterial inoculant enhances plant performance. Saranya et al. (2011) used two different sources of biochar (acacia wood and coconut shell) as a carrier formulation of *Azospirillum lipoferum* (AZ 204) inoculant, and compared with lignite. After 180 days of inoculation among the different carriers, coconut shell based biochar recorded a maximum population of log10.79 CFU $g^{-1}$ of carrier. Coconut shell based biochar was also found enhancing seedling vigour index of green gram (CO 3) higher than other carriers and also increase the survival of *Azospirillum lipoferum* up to 180 days of storage period at a required population as compared to acacia wood based biochar and lignite.

Shelf life of Azospirillum bioinoculant was tested with different organic amendments as carrier material like sawdust, straw powder, paddy wood, charcoal, poultry manure, farmyard manure and lignite. Observations of Stella and Sivasakthivelan (2009) revealed the sawdust sustaining high population (log9.80 CFU g-1 of carrier)

## III. FORMULATIONS OF AZOSPIRILLUM

Biofertilizers are the preparations containing live or latent microorganisms, beneficial for plants; and a bioformulation is physical form of carrier on which the inoculum is being supplied to plant or soil. Like different carriers, there are different formulations available of varying qualities, like solid (Warren et al. 2009), liquid (Deaker et al. 2004; Dayamani 2010; Valineni and Brahmaprakash 2011), polymer entrapped (Fages 1992), fluid bed dried (Brahmaprakash and Sahu 2012; Sahu 2012; Sahu et al. 2013); infected root inoculum (Hung and Sylvia 1988), etc.

### *A. Solid formulation*

It is traditional formulation of Azospirillum. Survival of Azospirillum in solid carriers like vermiculite, peat, corn cobs, powdered peanut shell, and polyacrylamide gel were studied. These studies reported that vermiculite supported $10^7$ cells of Azospirillum $g^{-1}$ of carrier even after seven months (Sparrow and Han 1981).

Several waste products can be used as carrier in solid formulations. A number of locally available material like lignite, pressmud, charcoal, soil, peat and coffee waste and found to be better than other in enhancing survival of Azospirillum up to 200 days. In pressmud formulation of Azospirillum the rate of decline of population was least (Kumar Rao et al. 1982).

### *B. Liquid inoculants*

Liquid formulations are aqueous, oil, or polymer-based products. It is a formulation containing desired microorganisms, nutrients, cell protectant and additives that promote cell survival in storage and after application to seed or soil (Brahmaprakash and Sahu 2012; Sahu and Brahmaprakash, 2016). Liquid inoculants are known to maintain a good population density of inoculum which is an important parameter to measure the quality of biofertilizer. Additives and osmoprotectants in liquid biofertilizer protect the cells upon application from toxicity, desiccation and osmotic shock (Vithal Navi 2004).

The liquid inoculants of *Rhizobium* sp., *Azotobacter* sp., *Azospirillum* sp. and PSB found maintaining population up to the level of $10^8$ cells per ml (Sridhar et al. 2004; Dayamani 2010; Valineni and Brahmaprakash 2011). Since the liquid biofertilizer have sufficiently higher cell count, each seed receives more than thousands of cells in seed inoculation.

Dayamani (2010) has studied the effects of nature and concentration of additives on the performance of liquid inoculum. Inoculants of Azotobacter sp., Azospirillum sp., Acinetobacter sp., *Bacillus* sp. and *Pseudomonas* sp. were tested with different osmolytes in different concentration to optimize it for liquid inoculant preparation. It was clear from this study that each organism responds variably to different osmolytes and its concentration. PVP K-15 at 2% concentration was found optimum for *Pseudomonas* sp. and Bacillus sp. PEG 4000 at 2% concentration found best for Acinetobacter sp., glycerol at 2% level for Azotobacter sp., PVP and PEG both at 1% and 2% levels for Azospirillum sp.

## IV. POLYMER ENTRAPPED FORMULATION

Continuous improvements in inoculant technology resulted in polymer entrapment as a method of inoculant formulation. In polymer entrapment, the polymer and subjected to chemical solidification after mixing with mass multiplied cells. Solidification forms uniform beads entrapping live cells inside. These beads are fermented for further growth in nutrient broth and then dried. These beads are degraded after application to soil by microorganisms and release the entrapped cells into soil. These polymers are proven potential bacterial carriers (Jung et al. 1982).

The dry beads give an interesting and excellent survival rates over a long period. Experiment started on 1983 with two plant growth promoting bacteria (Azospirillum brasilense Cd and Pseudomonas fluorescens 313) immobilized in two types of alginate-bead inoculant (with and without skim-milk supplement), dried and stored at ambient temperature. Beads are recovered after 14 years in 1996 and found that the population in each type of bead had decreased, yet significant numbers $10^5$-$10^6$ CFU $g^{-1}$ beads survived (Bashan and Gonzalez 1999). They found that morphology as well as plant growth promotion activity was similar to their 1983 cultures.

A successful inoculation of alginate beads of Azospirillum brasilense and Pseudomonas fluorescens were done on wheat plants under field conditions (Fages 1992). Fages (1992) also reported that survival of Azospirillum in different carriers such as peat, vermiculite, alginates and liquid formulation and reported that dried microgranulated alginate formulation was performing better. Experiments on Azospirillum inoculant formulations have been proposed on different biopolymer like alginate, xanthan gum and pero-dextrin which were shown to be good carriers as protect the inocula against stress conditions (Somasegaran 1985).

## V. FLUID BED DRIED BIOFORMULATION

Fluid bed dryer (FBD) is a dryer in which material is maintained suspended against gravity in an upward flowing air stream creating a fluidized condition. This have been tried in several plant growth promoting rhizobacteria including Azotobacter chroococcum, Pseudomonas fluorescens, Acinetobacter sp., Bacillus megaterium, etc. (Sahu et al., 2013; Lavanya et al., 2015a, 2015b; Lavanya et al., 2016; Sahu et al., 2016a). It can be a potential bioformulation for Azospirillum as the decline in number of cells is very limited (Sahu et al. 2013; Sahu et al., 2016a), absolutely no contamination builds up (Brahmaprakash and Sahu 2012; Sahu and Brahmaprakash, 2016) and several ingredients can be mixed and dried (Srivastava and Mishra 2010). With all these features, the FBD can be a better candidate for use in Azospirillum inoculant industry.

## VI. CONCLUSION

The performance of inoculant microbes is very much dependent on nature of formulation it is applied. Therefore, beneficial impacts on plant can be enhanced by providing a balanced bioformulation which can support higher cell counts of *Azospirillum* spp. and provide better yield benefits to plants. It is also necessary for efficient transportation, product performance, contamination level, longevity of inoculants, etc. Thus, Advancement in formulation technology with higher standards is need of the hour for enhancing crop productivity by microbial inoculants.

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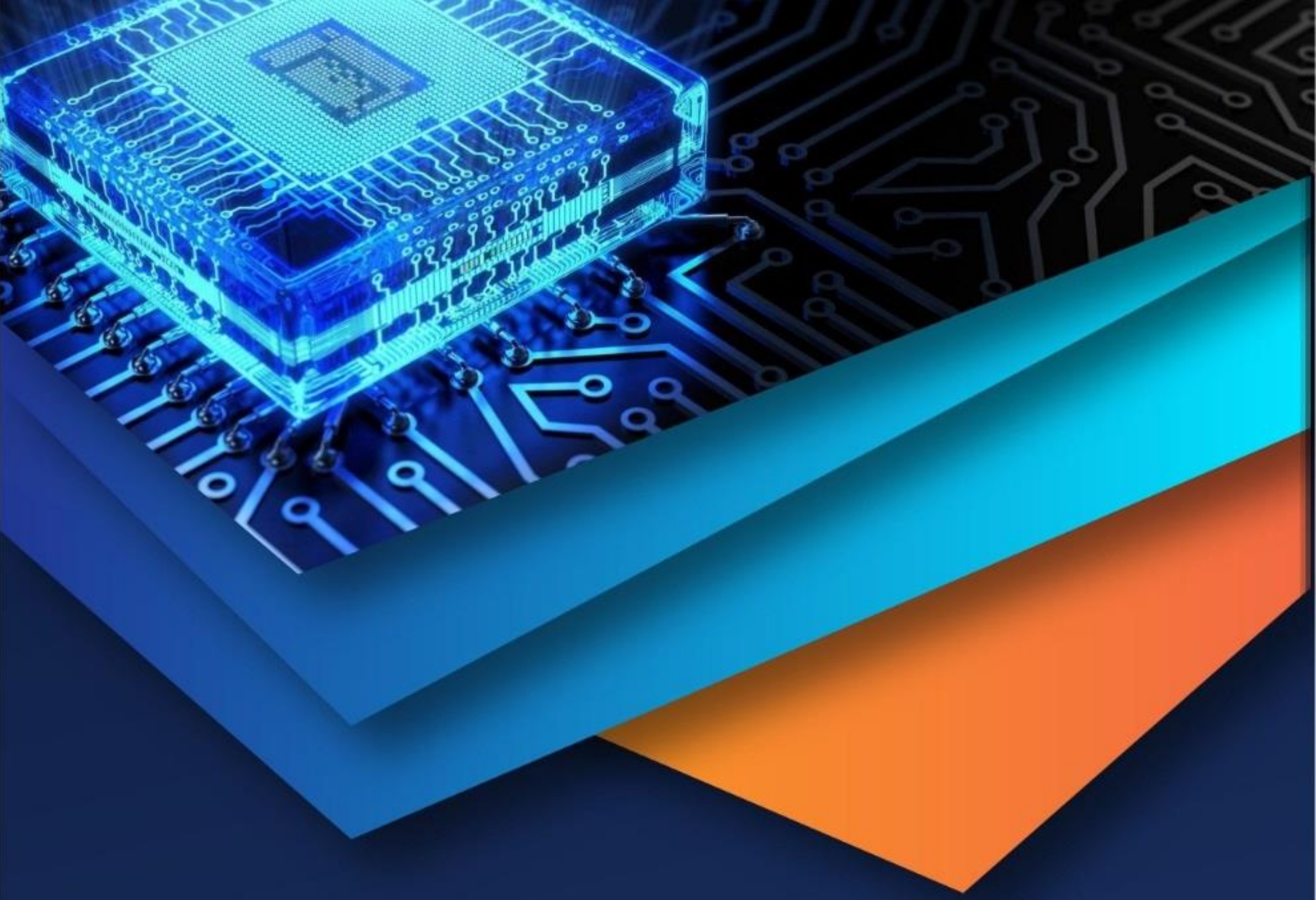


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