

## **UGC- Major Research Project**

# **DEVELOPMENT OF NEW FORMULATIONS OF *Azospirillum* BIOINOCULANTS WITH LONGER SHELF LIFE AND THEIR PERFORMANCE ON MAIZE (*Zea mays* L.)**

**Principal Investigator: Dr. D. Reetha, M.Sc.(Ag), Ph.D.**

**Co Principal Investigator: Dr. G. Kumaresan, M.Sc.(Ag), Ph.D.**

**Duration: 3 years (2013-2016)**

### **INTRODUCTION**

The microbial inoculant technology is gaining momentum especially with emphasis on organic farming and sustainable agriculture. Though it offers variety of benefits to sustainable agriculture and farmers, the technology is not being popularized or adapted by the farming community, because of the unpredictable and inconsistent performance of the microbial inoculants. The success of the inoculant technology depends on the two factors *Viz.*, efficiency of the microbial strains and formulation of the inoculant. A lot of research has been formed on the strain improvement and the formulation side is ignored. In order to make a successful inoculation, a suitable formulation is highly inevitable.

A successful formulation consists of viable bacteria in a suitable carrier together with additives to protect the cells during storage, transport and time of application. In addition it should be easy to handle and apply by the farmers. In India, 95% of the microbial inoculant is being produced as carrier based inoculant, which suffers from shorter shelf life, poor quality, high contamination and inconsistent field performance. So it is necessary to develop a alternate new formulation with improved quality and enhanced shelf life to overcome the above said problems and hence the project was undertake with the following objectives.

## OBJECTIVES OF THE PROJECT

- Development and production of three different formulations of *Azospirillum* inoculant viz., liquid formulation, cyst based formulation and immobilized gel based formulation to enhance the shelf life of *Azospirillum*.
- Assessment of the quality parameters of the three different formulations of *Azospirillum* inoculant.
- Standardization of dosage and method of application of three different formulations of *Azospirillum*.
- Evaluation of the comparative field performance of the three different formulations of *Azospirillum* on growth and yield of maize crop under field conditions.

## SUMMARY OF THE FINDINGS

1. Maize rhizosphere soil samples from thirty locations of Cuddalore district were collected and *Azospirillum* isolates were isolated from each sample and identified by morphological and biochemical characteristics. They were designated as MAZ – 1 to MAZ – 30. Among them twenty one were identified as *A. lipoferum* and the remaining nine were *A. brasilense* (Result not shown)
2. Based on the dinitrogen fixing ability, six different isolates, which fixed more than 15 mg of N g<sup>-1</sup> of malate. MAZ-3,6,11,14,25 and 30 were selected for further studies.
3. The selected *Azospirillum* isolates were further screened for their efficiency of nitrogen fixation, phytohormone production, siderophore production, exopolysaccharide production and for their stress tolerance, in comparison with reference strain MTCC 2306. Based on their performance, the isolate MAZ-3 was selected as an efficient *Azospirillum* strain for the development of different formulations.
4. Two different formulations such as liquid and gel based formulations of *A. lipoferum* MAZ – 3 were developed to improve the shelf life.
5. In liquid formulation, the effect of different chemical additives such as PVP, glycerol, gum arabica, trehalose, PEG and PVA at different concentrations on their survival of *A.*

*lipoferum* was studied. The results revealed that trehalose at 10 mM increased the viability of *Azospirillum* compared to other additives.

- 6 To optimize the dosage of liquid formulation of *A. lipoferum* MAZ - 3 for seed inoculation, pot culture experiments were carried out with varied inoculum levels. It was found that the inoculum level 20 ml + 20 ml rice gruel kg<sup>-1</sup> of maize seeds recorded maximum survival of *Azospirillum* on seeds and maximum germination per cent, vigour index, dry matter production and nitrogen content in maize and it was on par with 15 ml inoculum level kg<sup>-1</sup> of seeds. Hence the inoculum level of 15 ml kg<sup>-1</sup> of maize seeds was taken for further study.
- 7 In the development of gel based formulation, the effect of sodium alginate at different concentrations on the immobilization of *Azospirillum* was studied and found that sodium alginate at 2.5 per cent was the optimum level for maximum entrapment of *Azospirillum*
- 8 The influence of different additives such as SMP, starch, HA on the survival of *Azospirillum* in gel based formulation was also assessed. The results showed that, sodium alginate enriched with humic acid at 0.8% supported the viability to a maximum level followed by SMP and starch.
- 9 To optimize the dosage of gel based formulation of *A. lipoferum* MAZ - 3, pot culture experiments were carried out with varied inoculum levels in maize. It was found that gel based formulation at 6 beads seed<sup>-1</sup> recorded maximum population in the rhizosphere soil, it was on par with 4 beads seed<sup>-1</sup>. Hence the inoculum level of 4 beads seed<sup>-1</sup> was taken for further studies.
- 10 The field trial was conducted to evaluate the performance of liquid and gel based formulations of *A. lipoferum* MAZ - 3 in comparison with carrier (lignite) based formulation. The study clearly revealed that inoculation of liquid and gel based formulations with 75% recommended N could augment the growth and yield parameters of maize var. Co 1, on par with the 100% recommended N and thus saving 25% N.

## ACHIEVEMENTS FROM THE PROJECT

- ✓ An efficient strain of *Azospirillum lipoferum* (MAZ-3) was developed for increasing the growth and yield of Maize
- ✓ *Azospirillum lipoferum* (MAZ-3) was developed into three different formulations such as liquid, gel based and cyst based formulations. The quality parameters were assessed and dosage for each was optimized
- ✓ Among the three formulations, the increased shelf life of 12 months was obtained in liquid formulation supplemented with trehalose @ 10 mM/L
- ✓ The inoculation of *Azospirillum lipoferum* (MAZ-3) increased the yield of maize with saving of 25 % recommended dose of N fertilizer