Plant Archives Vol. 18, Special Issue (ICAAAS-2018), 2018 pp. 350-353 *ISSN 0972-5210*

EFFICACY OF LIQUID, GEL AND CARRIER FORMULATION OF *AZOSPIRILLUM* INOCULANTS ON THE GROWTH OF MAIZE

G. Kumareasan, P. Sivasakthivelan* D. Reetha and R. Shanthi

[1]Department of Agricultural Microbiology, Faculty of Agriculture, Annamalai University, Annamalainagar, Tamil Nadu, India-608002

[2]Department of Mathematics, PC. PT. MGR. Govt. Arts and Science College, Puttur, Sirkazhi, Tamil nadu-609108

Abstract

Field experiments were conducted to evaluate the performance of liquid, gel and carrier based formulations of *Azospirillum lipoferum* MAZ - 3 on the dry matter production, *Azospirillum* population, root colonization and chlorophyll content of maize var. Co 1 at graded levels of inorganic nitrogen fertilizer. The liquid formulation of *A. lipoferum* MAZ - 3 inoculation with 75 per cent N level enhanced the dry matter production (259.03 g plant^{-1}), were recorded followed by gel and carrier based formulations of *A. lipoferum* MAZ - 3**.** It was concluded that inoculation of liquid based formulation could augment the growth and yield parameters of maize by fixing higher amount atmospheric nitrogen and secreting higher amount of plant growth promoting substances like Indole acetic acid (IAA) and Gibberellins' when compared to the gel and carrier based formulation in maize crop.

Keywords: *Azospirillum lipoferum*, MAZ - 3, liquid formulation, chlorophyll, Indole acetic acid.

Introduction

Microbial inoculants represent an emerging technology designed to improve the productivity of Agricultural systems in the long run. They can be seen as a technology aligned with principles of sustainable agriculture, as opposed to the increased use of pesticides and fertilizers in recent times. *Azospirillum lipoferum* is one of the potential plant growth promoting rhizobacteria (PGPR).Its positive impacts on plant growth through several mechanisms which include enhancement of root development, production of growth regulators and nitrogen fixation (Garcia *et.al.,* 2001). FAO (1991) reported that most of the international producers of biofertilizers are engaged in the production of carrier-based inoculants. Peat is the most frequently used carrier for rhizobial inoculant industry because it has characteristics such as high water holding capacity and high surface area that support rhizobial growth and survival in large numbers. However, peat is not available in many countries, especially in tropics, and will be depleted in many areas in future (Smith 1992).

The carrier based microbial inoculants produced in India are generally lignite, coal (or) Charcoal based. The major disadvantages associated with these carriers are shorter shelf life, poor quality, high contamination and unpredictable field performance. The cost of solid carrier based inoculant production is high as it is labour and energy intensive process, involving milling, sieving and correcting pH (Somasegaran and Hoben, 1994). Liquid inoculant formulation is one solution to the problems associated with processing of solid carriers. The use of various broth cultures amended with substance that promotes cells survival in the package and after application for seed (or) soil. Additives to liquid inoculant formulations should have a role in protecting *Azospirillum* cells on seed at high temperature and during desiccation. Many kinds of polymers like gum arabic, polyvinyl pyrollidone (PVP), glycerol, trahalose, polyethylene glycol (PEG) and poly vinyl alcohol (PVA) have been used for liquid inoculant production because of their ability to limit heat transfer, their good rheological properties and high water activities (Mugnier and Jung 1985).

Inoculum strategies should include application of carrier materials aimed at providing protective niche together with the provision of nutrient sources. It is opined that the encapsulation method (gel based inoculant) helps to increase the survival rate and easy delivery of bacterial cultures. It also helps in segregating the bacterial cells from adverse environment thereby reducing cell loss. One of the successful, safe and effective methods to introduce bioinoculants in soil is encapsulation of cells in biodegradable gel matrices like sodium alginate solution (2.5 % w/v) with skimmed milk powder (8.0%), starch (25.0%) and humic acid (0.8%) and calcium chloride (0.1 M) (Vassilev *et al*., 2001).

Materials and methods

To evaluate the performance of liquid, gel and carrier (lignite) based *Azospirillum* inoculants, field experiments

Corresponding Author Email: plantdoctorsiva@yahoo.co.in

were laid out with maize var. Co 1. The seeds were surface sterilized and inoculated with the standardized quantity of 15 ml and 20 g kg^{-1} of seed for liquid and carrier based inoculants, whereas, seeds inoculated with of standardized quantity of 4 beads $seed^{-1}$ were sown by dibble the seeds at a depth of 4 cm in soil of gel based *Azospirillum* inoculant. The field experiments were conducted in Randomized Block Design (RBD) with triplicates.

Effect on dry matter production

Five plants from each treatment were randomly selected for recording growth parameters plant height and dry matter production periodically at 30 and 60 days after sowing (DAS) and at harvest.

Enumeration of *Azospirillum* population (MPN method) and Root colonization

The MPN counts of *Azospirillum* were calculated on the basis of positive tubes using the table provided by Cochran (1950). A random sample of root pieces of 5 cm in length from each treatment was used to determine the number of cells colonized on the ectorhizosphere of maize roots. Root pieces in glass tubes containing 5 ml of sterile phosphate buffer (0.2 M pH - 7.0) and 2.5 g of 3 mm diameter glass beads were shaken thoroughly for 1 min. The phosphate buffer containing bacterial cells were serially diluted and plated on Nfb agar medium supplemented with yeast extract for counting the number of cells on the rhizoplane or ectorhizosphere.

Results

Field experiments were conducted to evaluate the performance of liquid, gel and carrier based formulations of *A. lipoferum* MAZ - 3 on the dry matter production, *Azospirillum* population, root colonization and chlorophyll content of maize var. Co 1 at graded levels of inorganic nitrogen fertilizer and parameters were recorded on 30 DAS, 60 DAS and at harvest.

Effect on dry matter production

The effect of *A. lipoferum* formulations (liquid, gel and lignite) on the plant height and dry matter production of maize Co1 were studied. It was found that, the liquid formulation was better performance on dry matter production followed by gel formulation and carrier formulation. The maximum dry matter production of 260.34 g $plant^{-1}$ were obtained from the treatment T_3 (LFA + 100% N) at the time of harvest respectively. The treatment was found to be statistically on par with T_6 (LFA + with 75% N) which recorded 259.03 g $plant^{-1}$. The corresponding values of 257.63 and 256.43 g $plant^{-1}$ (gel formulation) and 252.75 and 250.93 g $plant^{-1}$ (carrier formulation) were recorded. Poor performance was recorded in the control treatment with 186.23 g $plant^{-1}$. It was observed that the treatment T_6 (LFA + 75%N) significantly increase the dry matter production over T_2which receives 100% N without inoculation *A. lipoferum*.

Effect on rhizosphere population and root colonization of *Azospirillum*

The results on total population and root colonization of *Azospirillum* spp. in maize rhizosphere soil and root as influenced by liquid, gel and carrier based formulations of *Azospirillum* are presented in Table 2.

The maximum *Azospirillum* population of 96.67 $x10^6$ CFU g^{-1} of soil was recorded in LFA + 75% N (T_6) followed by GFA +75% N (T_7) as 71.00 × 10^6 CFU g^{-1} of soil. The results revealed that gel based formulation resulted lower population at all the levels of N at 30 DAS when compared to carrier formulation of *Azospirillum* and the trend was changed on 60 DAS onwards. The maximum root colonization of *Azospirillum* (98 × 10^6 CFU g^{-1} of root) was sustained in LFA + 75% N (T_6) followed by T_7 (GFA + 75% N) as 81.00 × 10^6 CFU g^{-1} of root. Whereas, the control (T_1) recorded minimum root colonization of 0.16 $x10^6$ followed by the treatment received 100% N (T_2) as 0.20 × 10^6 CFU g^{-1} of root. In all the treatment combinations, the population and root colonization of *Azospirillum* spp. were increased up to 60 DAS and thereafter a declining trend was observed. The results indicated that LFA was favouring the population than gel and carrier base formulations. Among the levels of inorganic N fertilizer, 75% supported higher population of *Azospirillum* in all the formulations.

Discussion

The effect of different formulations *viz.,* liquid, gel and carrier based *A. lipoferum* MAZ - 3 with graded levels of recommended dose of N fertilizer on various growth parameters were studied under field conditions. The liquid formulation of *A. lipoferum* MAZ - 3 inoculation with 75 per cent N level enhanced the plant height and biomass followed by gel and carrier based formulations of *A. lipoferum* MAZ - 3. The effect of *Azospirillum* inoculation in augmenting the growth parameters of maize has been studied by many authors (Verma, 2011 and Braccini *et al.,* 2012).

Increased cell elongation and multiplication due to enhanced nutrient uptake by plants following inoculation of *Azospirillum* + nitrogen fertilizer probably caused the increase in plant height. The plant growth promoting substances *viz*., IAA and GA_3 secreted by *Azospirillum* might play an important role in root elongation and shoot growth (Gutierrez-Manero *et al.,* 2001). In general, *Azospirillum* inoculation enhanced proliferation of root system which in turn accelerated minerals uptake and consequently increased the biomass content (Ding *et al.,* 2005).

Positive increase in maize and sorghum biomass was observed in green house and field experiments by Dobbelaere *et al.* (2001) in gel and liquid formulations. Due to the prolonged survival in the rhizosphere region by the liquid and gel formulations might be the reason for the better performance than the carrier based *Azospirillum* inoculation. In this study also the total N content of maize was increased due to the inoculation of *A. lipoferum* MAZ - 3 and the higher content was obtained with liquid formulation. Since plants inoculated with *Azospirillum* had maximum N content, it is reasonable to think that the inoculation might have enhanced

'N' uptake by the plants due to increased availability of N in the rhizosphere by the activity of the inoculated bacteria. The present result is in agreement with Freitas and Stanford (2002) wherein they found increased total nitrogen content in maize due to *Azospirillum* inoculation.

In this study, liquid formulation at 75 per cent N level supported higher survival of *A. lipoferum* MAZ - 3 in the rhizosphere followed by gel and carrier based formulations of *A. lipoferum* MAZ - 3. It may be due to the fact that liquid formulations are amended with cell protectants which enhance the cell tolerance to desiccation, osmotic and temperature stress. These amendments might induce the cells to synthesize metabolites that protect against stress (Gomez Zavaglia *et al.,* 2003).Gel based formulation support higher *A. lipoferum* MAZ - 3 next to liquid formulation; it might be due to fact that encapsulated beads provide microenvironment in the soil and protect the cells from a biotic stresses and biotic stresses. The main goals of encapsulation of PGPB is to protect them from harsh soil environment, reduce microbial competition and release them gradually to facilitate colonization of plant roots (Bashan *et al.,* 2002).Thus, liquid and gel based formulations sustained prolonged survival in the rhizosphere. The higher survival of liquid followed by gel based formulations of *A. lipoferum* MAZ - 3 might have contributed to the growth, N uptake and chlorophyll content of crops as discussed earlier in this chapter.

It was concluded that inoculation of liquid and gel based formulation with 75 per cent recommended N could augment the dry matter production, *Azospirillum* population, root colonization and chlorophyll content of maize when compared to the maize crop grown in 100 percent recommended N without any further bioinoculation and thus a saving of 25 per cent recommended N level could be possible in maize crop. Moreover, the liquid formulation showed better performance than gel formulation regarding crop productivity of irrigated maize.

References

Bashan, Y., Jaun-Pablo Mernandez, L.A. Leyva and M. Bacilio (2002). Alginate microbeads as inoculant carriers for plant growth-promoting bacteria. *Biol. Fertil Soils.,* **35:** 359-368.

Braccini, A.L., L.G.M. Dan, G.G. Piccinin, L.P. Albrecht, M.C. Barbosa and A.H.T. Ortiz (2012). Seed inoculation with *Azospirillum brasilense*, associated with the use of bioregulators in maize. *Rev. Caatinga,* **25 (2)**: 58–64.

Cochran, W. G. (1950). Estimation of bacterial densities by means of the "Most probable number" *Biometrics,* **6**: 105-116.

Day, J. M. and J. Dobereiner (1976). Physiological aspects of nitrogen fixation by a *Spirillum* from *Digitaria* roots. *Soil Biol. Biochem.,* **8**: 45-50.

Ding, Y., J. Wang, Y. Liu and S. Chem (2005). Isolation and identification of nitrogen - fixing bacilli from plant rhizospheres in Beijing region. *J. Appl. Microbiol.,* **99:** 1271-1281.

Dobbelaere, S., A. Croonenborghs, A. Thys, D. Ptacek, J. Vanderleyden, P. Dutto, C. Labendera – Gonzalez and J. Caballero- Mellado (2001). Response of Agronomically important crops to inoculation with *Azospirillum*. *Aust. J. Plant physiol.,* **28**: 871-879.

FAO (1991). Report of Expert consultation on Legume Inoculant production and Quality control. FAO, Rome., pp. 148.

Freitas, A.D.S. and N.P. Stanford (2002). Associative nitrogen fixation and growth of maize in a Brazilian rainforest soil as affected by *Azospirillum* and organic materials. *Trop. Grassl.,* **36**: 77-82.

Garcia De Salamone, I. E., R. K. Hynes and L.M. Nelson (2001). Cytokinin production by plant growth promoting rhizobacteria and selected mutants. *Can. J. of Microbiol.,* **47**: 404-411.

Gomez Zavaglia, A., E. Tymezyszyn, G. De Antoni and E. Anibal disalva (2003). Action of trehalose on the preservation of *Lactobacillus delbrueckii* sp. *bulgaricus* by heat and osmotic dehydration. *J. Appl. Microbiol.,* **95**: 1315-1320.

Gutierrez - Manero, F.J., B. Ramos - Solano, A. Probanza, J. Mehouachi, F.R. Tadeo and M. Talon (2001). The plant growth promoting rhizobacteria *Bacillus pumilus* and *Bacillus licheniformis* produce high amounts of physiologically active gibberellius. *Physiol. Plant.,* **111**: 206-211.

Mugnier, J. and G. Jung (1985). Survival of bacteria and fungi in relation to water activity and the solvent properties of water in biopolymers gels. *Appl. Environ.Microbiol.* **50:** 108-114.

Smith, R.S. (1992). Legume inoculant formulation and application. *Can. J. of Microbiol.,* **38**: 485-492.

Somasegaran, P. and H.J. Hoben (1994). Handbook for Rhizobia-Methods in legume-*Rhizobium* technology. Springer-Verlag, New York, USA. p. 450.

Vassilev, N., M. Vassileva, M. Fenice and F. Federici (2001). Immobilized cell technology applied in solubilization of insoluble inorganic (rock) phosphates and plant acquisition. *Bioresour. Technol*. **79:** 263 271.

Verma, N.K. (2011). Integrated nutrient management in winter maize (*Zea mays* L.) Sown at different dates, *J. Plant Breeding and Crop Sci*., **3(8):** 161-167.

Table 1: Effect of inoculation of liquid and gel based formulations of *A. lipoferum* MAZ - 3 with graded levels of nitrogen on dry matter production in maize

Treatments	Dry matter production (g plant $^{-1}$)		
	30 DAS	60 DAS	Harvest
T_1 - Control	20.12	65.34	186.23
T_2 - 100 % N	32.06	102.13	250.00
T_3 - LFA + 100% N	36.70	110.88	260.34
T_4 - GFA + 100% N	35.20	107.76	257.63
T_5 - CFA + 100% N	33.43	104.33	252.75
T_6 - LFA + 75% N	36.04	109.83	259.03
T_7 - GFA + 75% N	34.63	106.73	256.43
T_8 - CFA + 75% N	32.86	103.14	250.93
T_9 - LFA + 50%N	29.67	90.70	231.88
T_{10} - GFA + 50% N	28.75	86.43	226.67
T_{11} - CFA + 50% N	26.13	79.78	221.33
SEd	0.348	1.001	0.647
CD(p=0.05)	0.700	2.011	1.321

Table 2: Effect of inoculation of liquid and gel based formulations of *A. lipoferum* MAZ - 3 with graded levels of nitrogen on the survival and root colonization of *Azospirillum* in the maize rhizosphere soil and roots

Treatments	*Azospirillum* population (× 10^6 CFU g $^{-1}$ of soil)			Root colonization of *Azospirillum* (× 10^6 CFU g $^{-1}$ of root)		
	30 DAS	30 DAS	60 DAS	Harvest	60 DAS	Harvest
T_1 - Control	0.49 (5.67)	0.16 (5.20)	0. 53 (5.70)	0.34 (5.53)	0.83 (5.92)	0.62 (5.78)
T_2 - 100 % N	0.53 (5.72)	0.20 (5.30)	0.86 (5.93)	0.65 (5.81)	0.96 (5.98)	0.76 (5.88)
T_3 - LFA + 100% N	08.63 (6.94)	25.32 (7.40)	44.54 (7.65)	14.23 (7.15)	38.54 (7.58)	8.32 (6.92)
T_4 - GFA + 100% N	5.67 (6.7 5)	20.63 (7.31)	37.65 (7.58)	10.14 (7.00)	34.26 (7.54)	6.68 (6.82)
T_5 - CFA + 100% N	7.06 (6.85)	21.06 (7.32)	32.34 (7.51)	7.56 (6.88)	28.15 (7.45)	4.23 (6.63)
T_6 - LFA + 75% N	22.64 (7.35)	46.65 (7.67)	98.06 (7.99)	31.38 (7.50)	96.65 (7.99)	25.14 (7.40)
T_7 - GFA + 75% N	13.63 (7.13)	29.63 (7.47)	81.08 (7.91)	26.08 (7.41)	71.11 (7.85)	18.62 (7.27)
T_8 - CFA + 75% N	14.56 (7.17)	31.06 (7.49)	53.14 (7.72)	13.62 (7.14)	54.32 (7.74)	9.32 (6.97)
T_9 - LFA + 50%N	12.24 (7.09)	29.06 (7.46)	63.63 (7.80)	18.06 (7.26)	63.06 (7.80)	14.58 (7.17)
T_{10} - GFA + 50% N	9.38 (6.97)	23.32 (7.37)	58.38 (7.77)	14.65 (7.17)	48.65 (7.69)	10.26 (7.01)
T_{11} - CFA + 50% N	10.62 (7.03)	25.64 (7.41)	47.36 (7.68)	9.36 (6.97)	36.23 (7.56)	7.11 (6.85)
SEd	0.059	0.034	0.037	0.039	0.019	0.044
CD(p=0.05)	0.118	0.069	0.074	0.079	0.039	0.089

Values in parenthesis are $\log_{10}$ transformed values