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EFFECT OF DIFFERENT FORMULATIONS OF *AZOTOBACTER* BIOINOCULANT ON THE GROWTH AND YIELD OF MAIZE

G. Kumaeasan, B. Karthikeyan and R. Shanthi*

Depart. of Agricultural Microbiology, Faculty of Agriculture, Annamalai Univ., Annamalainagar-608002 (T.N.)India

*Deprtment of Mathematics, PC. PT. MGR. Govt. Arts and Science College, Puttur, Sirkazhi-609108 (T.N.) India

Abstract

The present study was conducted to evaluate the performance of liquid, gel and carrier based formulations of *Azotobacter chroococcum* MAC- 4 on the growth and yield of maize var. Co 1 at graded levels of inorganic nitrogen fertilizer under field conditions. The liquid formulation of *A. chroococcum* MAC- 4 inoculation with 75 per cent N level enhanced the plant height (178.33 cm), dry matter production (259.03 g plant^{-1}), yield components like no. of grains cob^{-1}(483.67), grain weight plant^{-1}(146.45 g), cob weight plant^{-1}(276.45 g), 100 seeds weight^{-1}(28.49 g), grain yield (6.50 t ha^{-1}) and stalk yield of maize (10.53 t ha^{-1})were recorded followed by gel and carrier based formulations of *A. chroococcum* MAC- 4**.** It was concluded that inoculation of liquid based formulation could augment the growth and yield parameters of tomato 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 tomato crop.

Key words **:** *Azotobacter chroococcum* MAC- 4, liquid formulation, Gibberellins', Indole acetic acid, Maize.

Introduction

Bioinoculants are used as preparations that contain one or more beneficial microbial strains or species in an easy-to-use economical way. Microbial inoculants form an integral part of integrated plant nutrient supply system (IPNS), as they are cost effective and renewable source of plant nutrients to supplement the chemical fertilizers for sustainable agriculture. *A. chroococcum* is one of the potential plant growth promoting rhizobacteria (PGPR). The genus *Azotobacter* includes 6 species, with *A. chroococcum* most commonly inhabiting in various soils all over the world (Mahato *et al*., 2009). Besides nitrogen fixation, *Azotobacter* also produces thiamin, riboflavin, indole acetic acid and gibberellins. When *Azotobacter* is applied to seeds, *seed* germination is improved to a considerable extent, so also it controls plant diseases due to above substances produced by *Azotobacter*. The exact mode of action by which *Azotobacteria* enhances plant growth is not yet fully understood. Three possible mechanisms have been proposed: N2 fixation; delivering combined nitrogen to the plant; the production of phytohormone-like substances that alter plant growth and morphology and bacterial nitrate reduction, which increases nitrogen accumulation in inoculated plants (Mrkovacki and Milic, 2001).

***Author for correspondence :** E-mail : microsen1974@gmail.com

Despite good potentiality of microbial inoculants, the actual utilization is very low at about 2% of its potential. Meager adoption among countryman is ascribed mostly to their unpredictable response, low quality in terms of total viable counts at the time of use, short shelf life and temperature sensitiveness (Yadav and Chandra, 2014). The possible scope of contamination is very huge in the case of carrier based bio-inoculants as massive sterilization does not provide the desired outcomes (Bhavya *et al*., 2017) Liquid inoculants formulations could be a possible solution to the aforementioned tribulations as it contains cell protectants/additives for promotion of lengthy shelf life and tolerance to unpropitious conditions of the desired microorganisms in addition to their growth nutrients (Hegde, 2008). The contamination can be managed by means of proper sterilization techniques and maintenance of rigorous hygiene conditions by appropriate quality control measures in the case of liquid based biofertilizer (Bhavya *et al*., 2017). Depending upon the ability to heat transfer, high water activities and rheological properties of different polymers like polyethylene glycol (Temprano *et al*., 2002), polyvinyl alcohol (Deaker *et al*., 2004), gum Arabic (Mugnier and Jung, 1985), polyvinyl pyrrolidone (Singleton, 2002) and sodium alginate (Bashan *et al*., 2004) have been used for inoculants production.

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 find the efficacy of liquid, gel and carrier (lignite) based *Azotobacter* inoculants, field experiments 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 *Azotobacter* inoculant. The field experiments were conducted in Randomized Block Design (RBD) with triplicates.

Effect on plant height and 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 *Azotobacter* population in rhizosphere soil

To estimate the number of *Azotobacter* population were calculated on the basis of serial 10 fold dilution technique, using the pour plate methods and Waksman's base 77 medium and replicate of 10 g soil samples and an appropriate dilution as described by Johnson and Curl, (1972).

Yield parameters

The yield components *viz*; grain number per cob, number of grains per row, length and girth of cob, filled to unfilled grain, grain weight, cob weight, hundred Seed weight, grain yield and stalk yield were recorded at the time of harvest.

Results

Field experiments were conducted to evaluate the performance of liquid, gel and carrier based formulations of *A. chroococcum* MAC-4 on the growth and yield characters 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 plant height and dry matter production

The effect of *A. chroococcum* 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 plant height and dry matter production followed by gel formulation and carrier formulation. The maximum plant height and dry matter production of 179.32 cm and 260.34 g $plant^{-1}$ were obtained from the treatment T_6 (LFA + 75% N) at the time of harvest respectively. The treatment was found to be statistically on par with T_3 (LFA + with 100% N) which recorded 178.31 cm and 259.03 g $plant^{-1}$ respectively.

The corresponding values of 176.14 and 174.79 cm and 257.63 and 256.43 g $plant^{-1}$ (gel formulation) and 173.01 and 172.02 cm and 252.75 and 250.93 g $plant^{-1}$ (carrier formulation) were recorded. Poor performance was recorded in the control treatment with 102.31 cm and 184.43 g $plant^{-1}$. It was observed that the treatment T_6 (LFA + 75%N) significantly increase the plant height and dry matter production over T_2 which receives 100% N without inoculation *A. chroococcum*.

Effect on rhizosphere population of *Azotobacter*

In the present study, results revealed that the total

Table 1 : Effect of inoculation of liquid and gel based formulations of *A. chroococcum* MAC- 4 with graded levels of nitrogen on plant height and dry matter production in maize

Treatments*	Plant height (cm)			Dry matter production (g $plant^{-1}$)		
	30 DAS	60 DAS	Harvest	30DAS	60 DAS	Harvest
T_1 - Control	34.00	63.15	102.31	19.14	64.34	184.43
T_2 - 100 % N	75.26	121.67	173.43	34.06	105.13	255.04
T_3 - LFA + 100% N	77.47	127.94	178.31	36.04	109.83	259.03
T_4 - GFA + 100% N	75.14	126.62	176.14	34.20	105.54	254.53
T_5 - CFA + 100% N	73.34	122.23	172.14	33.40	104.33	252.75
T_6 - LFA + 75% N	78.00	127.61	179.32	37.40	111.33	260.34
T_7 - GFA + 75% N	74.23	124.71	173.79	34.63	106.73	256.43
T_8 - CFA + 75% N	73.83	123.33	172.02	32.86	103.14	250.93
T_9 - LFA + 50%N	69.23	121.33	167.56	29.67	90.70	231.88
T_{10} - GFA + 50% N	68.01	118.63	165.89	28.75	86.43	226.67
T_{11} - CFA + 50% N	67.35	115.15	161.46	26.13	79.78	221.33
SEd	0.392	0.523	0.590	0.323	0.901	0.637
CD(p=0.05)	0.780	1.023	1.181	0.670	2.011	1.311

LFA-Liquid formulation of *Azotobacter,* GFA-Gel formulation of *Azotobacter*
CFA-Carrier formulation of *Azotobacter*

Table 2 : Effect of inoculation of liquid and gel based formulations of *A. chroococcum* MAC-4 with graded levels of nitrogen on the survival of *Azotobacter* in the maize rhizosphere soil.

Treatments	*Azotobacter* population × 10^6 CFU g^{-1} of soil)		
	30 DAS	60 DAS	Harvest
T_1 - Control	0.45 (5.65)	0.73 (5.86)	0.60 (5.78)
T_2 - 100 % N	0.50 (5.69)	0.96 (5.98)	0.71 (5.85)
T_3 - LFA + 100% N	08.53 (6.93)	37.54 (7.56)	7.32 (6.86)
T_4 - GFA + 100% N	5.67 (6.75)	34.26 (7.54)	6.68 (6.82)
T_5 - CFA + 100% N	7.06 (6.85)	28.15 (7.45)	4.23 (6.63)
T_6 - LFA + 75% N	25.64 (7.41)	98.65 (7.99)	27.14 (7.43)
T_7 - GFA + 75% N	13.63 (7.13)	71.11 (7.85)	18.62 (7.27)
T_8 - CFA + 75% N	13.50 (7.13)	51.32 (7.71)	8.32 (6.92)
T_9 - LFA + 50%N	13.24 (7.12)	65.06 (7.81)	16.05 (7.21)
T_{10} - GFA + 50% N	9.38 (6.97)	48.65 (7.69)	10.26 (7.01)
T_{11} - CFA + 50% N	9.14 (6.96)	35.00 (7.54)	6.11 (6.79)
SEd	0.056	0.016	0.042
CD(p=0.05)	0.115	0.037	0.088

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

population of *Azotobacter* sp. in maize rhizosphere soil as influenced by liquid, gel and carrier based formulations of *Azotobacter* are presented in Table 2.

In all the treatment combinations, the population of *Azotobacter* 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 *Azotobacter* in all the formulations.

Effect on yield and yield components

Inoculation of maize with liquid, gel and carrier based formulations of *A. chroococcum* MAC-4 were significantly increased number of grains per cob and grains per row grain and cob weight, 100 seeds weight, grain yield and stalk yield over uninoculated control (T_1) and 100 per cent N (T_2). It was evidenced that the yield and yield components were recorded higher in liquid formulation followed by gel and carrier based formulations of *A. chroococcum* (Table 3). The higher values of 483.67 grains cob^{-1}, 33.67 grains row^{-} of 146.45 g grain weight $plant^{-1}$, 276.45 g cob weight $plant^{-1}$, 28.49 g 100 seeds $weight^{-1}$, 6.50 t ha^{-1} grain yield and 10.53 t ha^{-1} stalk yield of maize were recorded in LFA with 75% N (T_3) followed by LFA with 100% N (T_6) with respective values as 482.33, 31.33, 145.36 g, 276.41 g, 27.62 g 6.45 t ha^{-1} and 10.50 t ha^{-1}. The treatment T3 (LFA with 100% N) is on par with T_6 (LFA with 75% N).

Discussion

The effect of different formulations *viz.,* liquid, gel and carrier *A. chroococcum* MAC- 4 with graded levels of recommended dose of N fertilizer on various growth parameters were studied under field conditions. The liquid formulation of *A. chroococcum* MAC- 4 inoculation with 75 per cent N level enhanced the plant height and biomass followed by gel and carrier based formulations of *A. chroococcum* MAC-4. The effect of *Azotobacter* inoculation in augmenting the growth parameters of maize has been studied by many authors (Braccini *et al.,* 2012).

Increased cell elongation and multiplication due to enhanced nutrient uptake by plants following inoculation of *Azotobacter* + nitrogen fertilizer probably caused the increase in plant height. The plant growth promoting substances *viz*., IAA and GA_3 secreted by *Azotobacter* might play an important role in root elongation and shoot growth (Gutierrez-Manero *et al.,* 2001). In general, *Azotobacter* 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 *Azotobacter* inoculation. In this study also the total N content of maize was increased due to the inoculation of *A. chroococcum* MAC-4 and the higher content was obtained with liquid formulation. Since plants inoculated with *Azotobacter* 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 *Azotobacter* inoculation.

In this study, liquid formulation at 75 per cent N level

Table 3 : Effect of inoculation of liquid, gel based AND carrier formulations of *A. chroococcum* MAC-4 with graded levels of nitrogen on yield attributes and yield of maize.

Treatments	Number of grains cob^{-1}	Number of grains row^{-1}	Grain per plant (g)	Cob weight per plant (g)	100 seeds Weight (g)	Grain yield (t/ha)	Stalk yield (t/ha)
T_1 - Control	219.66	19.00	101.33	173.36	20.18	3.78	6.97
T_2 - 100 % N	466.33	29.66	137.24	265.56	25.10	6.01	9.81
T_3 - LFA + 100% N	482.33	31.33	145.36	276.41	27.62	6.45	10.50
T_4 - GFA + 100% N	474.00	29.33	142.74	270.06	26.00	6.21	10.34
T_5 - CFA + 100% N	471.67	28.33	139.13	264.16	25.53	6.10	9.86
T_6 - LFA + 75% N	483.67	33.67	146.45	276.45	28.49	6.50	10.53
T_7 - GFA + 75% N	476.33	28.66	141.26	267.96	25.66	6.10	10.23
T_8 - CFA + 75% N	472.00	27.67	138.65	263.33	25.00	5.95	9.69
T_9 - LFA + 50%N	376.33	25.00	134.31	254.86	25.53	5.46	9.21
T_{10} - GFA + 50% N	346.67	23.66	132.23	250.06	24.91	5.10	9.01
T_{11} - CFA + 50% N	345.33	20.33	126.06	238.33	24.00	4.79	8.37
SEd	1.445	0.322	0.844	1.464	0.451	0.082	0.039
CD(p=0.05)	2.904	0.681	1.675	2.968	0.936	0.164	0.083

supported higher survival of *A. chroococcum* MAC-4 in the rhizosphere followed by gel and carrier based formulations of *A. chroococcum* MAC-4. 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. chroococcum* MAC-4 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. chroococcum* MAC-4 might have contributed to the growth, N uptake and chlorophyll content of crops as discussed earlier in this chapter.

Conclusion

It was concluded that *Azotobacter* inoculation of liquid and gel based formulation with 75 per cent recommended N could augment the growth and yield parameters 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 and carrier formulation regarding crop productivity of irrigated maize.

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