JOURNAL OF PURE AND APPLIED MICROBIOLOGY, Dec. 2014. Vol. **8**(6), p. 4809-4812

Liquid Formulation of *Azotobacter chroococcum* (ABA-1) for Seed Inoculation

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(Received: 20 August 2014; accepted: 30 October 2014)

Liquid, Carrier and Granular formulations of *Azotobacter chroococcum* (ABA-1) were prepared from Jensen's broth having 9.5x10^{11} cfu/ml . Bacterial survival in formulations as well as on inoculated Pearl millet seeds was studied using Jensen's agar and there *in vivo* efficacy testing on growth parameters of P.millet *cv* GHB-558 in pots. In formulations, *Azotobacter* count was decreased 10 and 100 folds after 3 and 5 months, respectively. Survival of bacteria on seed was reduced from 10^{10} to 10^{5} after 48 hours. Seed germination effect of all formulations on seed agar showed increased root and shoot length with secondary root formation as compared to uninoculated control. In a pot trial, *Azotobacter* formulation containing 2% glycerol + 2% PVP registered highest plant height, shoot and root weight at 60 DAS by seed application in Pearl millet , which was significantly superior over other formulations, recommended dose (80 kg ha^{-1}) of nitrogen and absolute control.

Key words: *Azotobacter*, Liquid formulation, Seed application, Pearl millet.

Azotobacter is an aerobic, free living, heterotrophic N fixer and is used as microbial inoculant since years (Bashan, 1998). The microbial formulation is a crucial aspect for producing inoculants containing an effective bacterial strain and can determine the success or failure of a product. It consists of establishing the microorganisms in a suitable carrier together with additive that aid in the stabilization and protection of the microbial cells during storage and transport at the target site (Xavier *et al*., 2004). Liquid formulations are low in cost, readily available, non perishable, could be grown under normal fermentation conditions, support high cell numbers during storage conditions, have longer shelf life up to 12-24 months and their dosage is 10 times less than carrier formulations. *Azotobacter* inoculation with recommended dose of N and P_2O_5 (80:40) increases the grain yield, plant height, root weight, shoot weight, enzyme activity and provide the nutrients for better growth of plant in pearl millet. Inoculation with the Biofertilizers like *Azotobacter* and *Azospirillum* save half of the recommend dose of Nitrogen and increases the plant growth as compared to control (Vora *et al*., 2008).

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MATERIALS AND METHODS

Development of Liquid, carrier and granular formulations

A. chroococcum (ABA-1) was obtained from stock cultures of Department of Microbiology, AAU, Anand. Inoculation was carried out aseptically in Jensen's broth (250 ml) and incubated

on gyro rotatory shaker at 180 rpm till cell count obtained up to 10^{11}cells/ml. This broth was used as mother culture for all different formulations. From this mother culture, inoculation was carried out in 2 lit sterile Jensen's broth under aseptic condition and incubated on gyro rotatory shaker at 180 rpm till cell count was achieved up to $9.5x10^{11}$cells/ml confirmed by serial dilution technique. This fermented broth (FB) was used for preparation for all formulations. From this FB, 250 ml suspension was withdrawn aseptically in previously sterilized two plastic bottles (250 ml). Sterile glycerol 2% and 2% sterile glycerol+ PVP were added in plastic bottles, respectively. Carrier materials like charcoal, vermiculite, lignite,CMC , talc were passed from 150-212 µ sieves and sterilized in glass containers in autoclave at 15 lbs pressure for 20 minutes. An aliquot of 40 ml of FB and 60 g of sterile carrier was mixed. Similarly, FB was aseptically mixed with sterile 2.5% (w/v) sodium alginate powder and stirred gently for 7 hours. The obtained mixture was added drop wise using Peristaltic pump in 3% $CaCl_2$ solution on rotary shaker to form beads. These beads were washed thrice with D/W and air dried in laboratory on clean filter paper. Initial microbial count from all formulations was carried out following serial dilution technique and they were stored at room temperature for different studies.

Survival study of *A.chroococcum* in different formulations

Serial dilutions ranging from 10^{-5} to 10^{-9} were prepared and 0.5 ml aliquote was taken from each dilution and spreaded on Jensen's agar plate and incubated for 48 hours for CFU using acolyte colony counter.

Survival study of *A.chroococcum* on treated bajra seeds

Survival study was done on bajra seeds (*CV* GHB-558) at 28 ± 1^0C s for both liquid and carrier formulations. Seeds were surface sterilized with 95% alcohol and washed thrice with D/W for 2-3 times following air drying in laboratory. After sterilization, for liquid formulation 0.5 ml FB/100g seeds and for carrier based formulation 2.5 g carrier/ 100g seeds with 5-10 ml D/W was added to maintain moisture on seeds and mixed well by palming. These treated seeds were used for *Azotobacter* population counting upto 48 hrs.

Efficacy testing of different formulations by seed treatment on growth of Pearl millet

Ten Seeds treated with individual formulations were sown in pots and for T_8 and T_9 seeds were sown without addition of biofertilizer. In T_8 chemical fertilizer in form of urea @ 40 kg N/ ha as basal dose and remaining 40 kg N/ ha as top dressing at 30 DAS was applied. All the pots received 40 kg P_2O_5/ha as basal dose. All agronomic practices were common for all the treatments. At 30 DAS and 60 DAS observations were recorded. Details of treatments:

T_1 : Fermented broth + glycerol 2%
T_2 : Fermented broth + glycerol 2% + PVP 2%
T_3 : Charcoal
T_4 : Vermiculite
T_5 : Lignite + CMC 2%
T_6 : Talc-Powder + CMC 2%
T_7 : Sardar biofertilizer (GSFC Product- Lignite Based)
T_8 : Recommended dose of Chemical fertilizer (80 kg N/ ha)
T_9 : Absolute control (no chemical fertilizer , no biofertilizer)

The data collected on different parameters were subjected to statistical analysis by using completely randomized design (CRD) (Panse and Sukhatme,1978). Data were subjected to analysis of variance and means of samples were compared by Duncan's new multiple range test (DNMRT).(Duncan,1955)

RESULTS AND DISCUSSION

Development of liquid, carrier and granular formulations

For preparation of all formulations, Jensen's broth (mother culture) was made and initial count was taken by serial dilution technique, which was 95×10^{10} CFU/ml. staining was carried out of culture in order to check purity and study Gram reaction, size, shape, and arrangement of *A.chroococcum* (ABA-1). The microscopic examination showed that organisms were small, gram negative, rod shaped to coccoid with occasional cysts. The arrangement of these bacteria was generally in single or pair. This broth was used for preparing liquid, carrier and granular formulations. After preparation of all the

formulations, initial count was carried out from all the products. The results of the investigation showed that T_1 and T_2 treatments contained initial count 90×10^{10} and 94×10^{10} CFU/ml whereas T_3, T_4, T_5, T_6, T_8 and T_7 contained initial count 79×10^{10}, 76×10^{10}, 74×10^{10}, 88×10^{10}, 66×10^{10}, 92×10^{10} (CFU/g), respectively. (Table-1). In present study, all the formulations gave the count up to 10^{10} in initial phase and then decreased by 10 and 100 folds after 3 and 5 months.

Survival study of (ABA-1) on treated bajra seeds

Bajra seeds were treated with all the formulations and the germination effect was studied on solid water agar surface The results showed that among liquid and carrier formulations 2% concentration of various additives like glycerol and PVP to liquid inoculants promoted higher cell density compared to carrier based (charcoal, lignite + CMC 2%, and talc-powder + CMC 2%) formulation. These findings are in agreement with those of Streeter (2007) who also reported that addition of trehalose and yeast extract in the medium increased the *B. japonicum* survival up to 48 hours on soybean seeds. Among all the formulations, liquid formulation with 2% PVP + 2% glycerol gave better germination effect over all formulations and untreated check and after 48 hours of incubation, the germination was started with secondary root formation.

Effect on plant height at 30 DAS and 60 DAS

However, among all formulations T_2: FB + Gly 2% + PVP 2% recorded the highest plant height (83.7 and 95.5 cm) at 30 DAS and 60 DAS respectively (Plate 1), closely followed by T_1 (80.2

(A) (B)

A: liquid formulatiom **B:** control

Plate 1. Response of liquid formulation of Azotobacter on bajra at 60 DAS

Table 1. Seed application efficacy of *A. chroococcum* (ABA-1) formulations on fresh shoot and root weight of pearl millet after 60 DAS

Treatments	Fresh weight		Total N (%)
	Shoot (g)	Root (g)	from leaves
T_1 :FB+glycerol 2%	34.6^{b}	8.7^{a}	1.7^{a}
T_2 :FB+glycerol 2%+pvp 2%	36.9^{a}	9.1^{a}	1.8^{a}
T_3 :Charcoal	28.3^{d}	6.9^{c}	1.6^{b}
T_4 :Vermiculite	27.2^{e}	6.6^{cd}	1.5^{c}
T_5 : Lignite+CMC 2%	26.9^{e}	6.8^{c}	1.4^{d}
T_6 : Talcpowder+CMC 2%	29.7^{c}	7.7^{b}	1.6^{b}
T_7 :Sardar biofertilizer (GSFC product)	26.4^{f}	6.8^{c}	1.4^{e}
T_8 :Chemical fertilizer	32.3^{c}	7.9^{b}	1.6^{b}
T_9 : Absolute control	23.8^{g}	6.0^{d}	1.4^{e}
S.Em±	0.69	0.21	0.005
C.D at 5%	2.06	0.62	0.014
CV %	4.06	4.95	0.54

and 85.3 cm), T_6 (76.7 and 86.5 cm) and T_8 (77 and 83.8 cm) respectively, which were statistically higher over uninoculated control (58.3 and 60.3 cm).

The data pertaining to shoot fresh weight and root fresh weight at 60 DAS due to various treatments of different *A.chroococcum* (ABA-1) formulations are presented in (Table-1). Seed treatment with *liquid* formulation (T_2) recorded maximum fresh shoot weight (36.9 g), and root weight (9.1 g) which was significantly higher over uninoculated control.

Among all formulations liquid based formulation T_2, increased dry shoot weight(8.9 g) and dry root weight (2.5 g) as compared to carrier based formulation T_3 (6.9 g) and (1.3 g), respectively.

The data regarding the total N percentage from leaves at harvest (60 DAS due to inoculation with N fixing *A.chroococcum* formulations reported significant influence which was the highest in T_2 liquid formulation and carrier formulations as compared to control.

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