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Studies on NPK Biofertilizer Consortia and their Influence on Maize (*Zea mays* L.) Seedling Vigour

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A B S T R A C T

Four local efficient biofertilizer strains namely, *Gluconacetobacter* G_1 and *Azospirillum* ACD-15 as nitrogen fixers, *Pseudomonas striata* as phosphorus solubilizing bacterium and a potassium solubilizing bacterium were used in developing NPK biofertilizer consortia. Each NPK consortia consisted of one of the two nitrogen fixing bacteria, the phosphate solubilizing (PSB) and potassium solubilizing (KSB) bacteria in five different formulations namely, lignite, kaolinite, liquid, Na-alginate and bentonite. These formulations were evaluated under *in vitro* on maize germination and seedling vigour. Growth kinetics of each strain was used in developing consortia after their compatibility was confirmed. The population of each of the four strains in the formulations were assessed at ambient conditions over 90 days. The survival of individual strain in the formulations of consortia and their influence on maize seedling vigour were used to identify efficiency of formulations of NPK consortia. Among the five formulations, liquid and Na-alginate formulations supported significantly higher population of biofertilizer strains after 90 DAS. Liquid formulation recorded the highest maize germination (94.25 per cent) and Na-alginate formulation recorded the highest maize seedling vigour (3850.43).

Introduction

Recent improvements in the biofertilizers has shifted the focus of modern agriculture towards environmental friendly sustainable agricultural practices which are known to reduce the dependence on chemical fertilizers and in future it is expected to offer the possibilities to reach our demand for agricultural needs (Desai *et al.*, 2012). Biofertilizers are ready to use products consisting of live microbial inoculants which are capable of fixing atmospheric nitrogen, solubilizing phosphorus, potassium and zinc, bring about organic matter decomposition in the soil.

Plants need macro and micronutrients for their growth and development. Individual application of each of the bioinoculants would be expensive and laborious. Hence, development of consortia with consistent efficiency under field conditions and longer shelf life will pave way for successful commercialization of this technology. Therefore, bioinoculants consisting of biofertilizer strains capable of mobilizing N, P and K nutrients as a single inoculant with enhanced shelf-life will be useful technology in present agriculture.

Among the soil bacteria, diazotrophs such as *Gluconacetobacter* and *Azospirillum* are found beneficial to crops which belong to graminaceous family (Raja et al., 2006). These are most commonly applied for fixing atmospheric nitrogen to enhance its availability and uptake by plants for their growth and development. In order to achieve an increased beneficial effect of these diazotrophs and other nutrient mobilizing microorganisms on growth and yield enhancement (Jain et al., 2012). Microbial consortium is a combination of more than two beneficial microorganisms and is preferred as consortial inoculation performs better than single inoculations (Alagawadi and Gaur, 1998) due to their synergistic effect in consortia.

Earlier studies conducted on the use of microbial consortia in different combinations of inoculants (Jayashree and Jagadeesh, 2017; Vijaykumar and Brahmaprakash, 2018) reported enhanced growth and yield of crops inoculated. However, studies on consortia formulated in different carriers and their effect on maize (*Zea mays* L.) is lacking. Hence, this investigation was conducted to develop two microbial consortia each consisting of a nitrogen fixer (*Gluconacetobacter* or *Azospirillum*), a PSB (*Psuedomonas striata*) and a KSB in different formulations and evaluate their effect on maize under *in vitro*.

Materials and Methods

The biofertilizer strains used included local efficient strains of *Gluconacetobacter* G_1 and *Azospirillum* ACD-15 as nitrogen fixers, phosphorus solubilizing bacterium *Psuedomonas striata* and local efficient potassium solubilizing bacterium (KSB) collected from the Agricultural Microbiology Laboratory at the Institute of Organic Farming (IOF), University of Agricultural Sciences, Dharwad. All strains were purified by repeated streaking on their selective media *viz*., *Gluconacetobacter* on LGIP agar medium (Sucrose 100 g L^{-1}, K_2HO_4 0.2 g L^{-1}, KH_2PO_4 0.6 g L^{-1}, $MgSO_4.7H_2O$ 0.2 g L^{-1}, $CaCl_2$. $2H_2O$ 0.02 g L^{-1}, $FeSO_4$ 0.02 g L^{-1}, Na_2MoO_4. $2H_2O$ 0.002 g L^{-1}, 5 ml of 0.5 % Bromothymol blue solution in 0.2 N KOH, Agar 18 g L^{-1}), *Azospirillum* on sodium malate agar medium (Malic acid 5 g L^{-1}, KH_2PO_4 0.5 g L^{-1}, $MgSO_4$. $7H_2O$ 0.2 g L^{-1}, $FeSO_4$ 0.1 g L^{-1}, $MnSO_4$ 0.1 g L^{-1}, NaCl 0.02 g L^{-1}, $CaCl_2$ 0.01 g L^{-1}, Na_2MoO_4. $7H_2O$ 0.002 g L^{-1}, 2ml of 0.5 % of Bromothymol blue solution in 0.2 N KOH, Agar 18 g L^{-1}), PSB on Pikovaskaya's medium (Glucose 10 g L^{-1}, $Ca_3(PO_4)_2$ 5 g L^{-1}, KCl 0.5 g L^{-1}, $MgSO_4.7H_2O$ 0.2 g L^{-1}, $FeSO_4$ 0.02 g $L^{-}1$, yeast extract 0.02 g L^{-1}, Agar 18 g L^{-1}) and KSB on Alekshandrov's medium (Glucose 5 g L^{-1}, $FeCl_3$ 0.1 g L^{-1}, $CaCO_3$ 2 g L^{-1}, potassium mineral, mica 2 g L^{-1}, $CaSO_4$ 2 g L^{-1}, $MgSO_4.7H_2O$ 0.005 g L^{-1}, Agar 18 g L^{-1}).

Compatibility test

Before development of consortia, all biofertilizer strains were tested for their compatibility on nutrient agar medium by cross streak method described by Anandraj and Leema (2010).

Growth studies of biofertilizer strains

The growth of four biofertilizer strains were examined in 100 ml broth cultures of respective selective broth media described before. The broths used were amended with cell protectants as described in Table 2 and media without cell protectants as to determine the time taken by each biofertilizer strain to reach its highest population.

Composition of NPK consortia

Two microbial consortia namely, microbial consortium-1 (MC-1) containing *Gluconacetobacter* G_1+PSB+KSB and microbial consortium-2 (MC-2) containing *Azospirillum* ACD-15+PSB+KSB were developed.

Preparation of biofertilizer consortia

Based on the equal populations of microbial strains used, two different microbial consortia consisting of one of the two nitrogen fixers (*Gluconacetobacter* G_1 or *Azospirillum* ACD-15), a PSB (*Psuedomonas striata*) and a KSB were prepared and formulated as lignite, kaolinite, bentonite, Na-alginate and liquid biofertilizer. The microbial strains were grown on respective selective medium and the two consortia were prepared by mixing them in equal proportion (1:1:1) with 10^8 CFU/ml (Table 3) and then, they were mixed with sterilized carrier materials (*viz.,* lignite, kaolinite, bentonite) in the ratio 3 parts of carrier and 1 par of broth and stored in High Density Poly Ethylene (HDPE) bags of 75 gauge and liquid and Na-alginate formulations were stored in High Density Poly Ethylene (HDPE) narrow mouth bottles (Tarsons) for three months under ambient conditions for shelf life studies. By following this method the differences that could have arisen due to variations in their populations and differential growth rate were avoided. To achieve this, the strains were inoculated in a staggered system so as to get their maximum populations coinciding with the time of formulating consortia.

Liquid formulation technology of *Gluconacetobacter* G_1 (Prakash, 2018), *Azospirillum* ACD-15 (Sandesh, 2016), *Psuedomonas striata* (Parvathi and Patil, 2018) and KSB developed at the Institute of Organic Farming (IOF), University of Agricultural Sciences, Dharwad were borrowed and used in this study.

Entrapment of microbial strains within Na-alginate beads was carried out under aseptic conditions as proposed by Saxena (2011). Sodium alginate powder and 0.1 M $CaCl_2$ were prepared and autoclaved separately. The population of each inoculant in consortia was set to 10^8 CFU/ml and to that sterilized Na-alginate powder was added @ 2 g/100 ml separately and thoroughly stirred to obtain a homogenous mixture. Then the mixture was extruded through sterilized burette at steady rate into gently stirred, sterilized 0.1 M $CaCl_2$ solution at room temperature to form uniform sized beads. Thus formed beads were kept in 0.1 M $CaCl_2$ solution at room temperature to obtain regular solid beads and later the $CaCl_2$ solution was drained and the beads were washed twice with sterile distilled water and dried in laminar air flow for 12 hr.

In vitro study on maize

Clean, bold and healthy maize (Super 900M Gold) seeds were surface sterilized with alcohol (70 %) for one minute, followed by sodium hypochlorite (2 % active chlorine) for three minutes. These treated seeds were then washed six times with the sterile distilled water and were allowed to dry under a laminar air flow chamber then treated with the developed formulations of microbial consortia. The surface sterilized, dried seeds

were treated with carrier based NPK consortia (lignite, kaolinite and bentonite) @ 20 g kg^{-1} of seeds and liquid consortia @ 4 ml kg^{-1} and Na-alginate consortia dissolved in small quantity of sterile distilled water @ 4 g kg^{-1} of seeds. Recommended dose of lignite based *Azospirillum* ACD-15 and PSB @ 20 g kg^{-1} was used as a check. The germination test was conducted by following the procedure given by ISTA (1999) using between paper method.

Shelf life evaluation

The shelf life of individual biofertilizer strains in each formulation was assessed at 30 days interval for 90 days after incubation by Direct Plate Count Technique (DPCT) for all the four strains and Most Probable Number Estimation (MPNE) method for *Gluconacetobacter* G_1 and *Azospirillum* ACD-15 correspondingly on semi-solid LGIP and sodium malate medium.

The selective media used were LGIP for *Gluconacetobacter*, sodium malate medium for *Azospirillum*, Pikovaskaya's medium for PSB and Alekshandrov's medium for KSB.

Statistical analysis

The data obtained from *in vitro* studies were subjected to statistical analysis using Factorial Completely Randomized Design (FCRD) and data on shelf life of formulations of biofertilizer consortia were subjected to statistical analysis using Completely Randomized Design (CRD). Interpretation of the data was carried out in accordance with Panse and Sukhatme (1985).

The level of significance used in the 'F' and 't' test was $P<0.01$. The mean values between treatments were compared using the least significance differences (L. S. D). The treatment means were compared by applying Duncan's Multiple Range Test (DMRT).

Results and Discussion

Compatibility of biofertilizer strains

An *in vitro* experiment was conducted to test the compatibility of the biofertilizer strains used in this study. The four local efficient microbial strains *viz*., *Gluconacetobacter* G_1, *Azospirillum* ACD-15, *Psuedomonas striata* (PSB) and a KSB were found compatible with each other as evident from a cross streak assay on a common growth medium (Nutrient agar). This ensured no variation in formulations developed using the four biofertilizer strains was due to competition among them. Secondly, physical mixing of the strains did not have any deterrent effect on the performance of each other in the final consortia. The results also demonstrated the significance of conducting compatibility test among constituent biofertilizer strains following a simple procedure. If carefully followed, this test can reveal compatibility or competition arising due to physical competition and antibiosis as revealed on nutrient agar plates and is prerequisite for developing consortia.

Shelf life of formulations

Before mixing for developing formulations, the populations (CFU/ml) of strains *viz.*, *Gluconacetobacter* G_1, *Azospirillum* ACD-15, PSB and KSB on their respective selective medium were 7.43×10^{10}, 38.6×10^{8}, 34×10^{9} and 23×10^{8} respectively. Among them KSB had the least population. As consortia were developed based on equal population of each biofertilizer strain volume of each culture with different population required was calculated. One ml of KSB culture containing 23×10^{8} CFU/ml was equivalent in population to 0.03 ml of *Gluconacetobacter* G_1 containing 7.43×10^{10} CFU/ml, 0.06 ml of PSB containing 34×10^{8} CFU/ml and 0.59 ml of *Azospirillum* ACD-15 containing 38.60×10^{8} CFU/ml.

Table.1 Time taken by biofertilizer strains to reach maximum population

Biofertilizer strains	Time (hr)	Maximum population (media without cell protectants)	Maximum population (media with cell protectants-for Liquid formulation)
***Gluconacetobacter* G_1**	96	7.43×10^{10}	12.56×10^{10}
***Azospirillum* ACD-15**	56	38.6×10^{8}	125.6×10^{9}
***Pseudomonas striata* (PSB)**	48	34×10^{9}	57×10^{9}
KSB	36	23×10^{8}	32×10^{8}

Table .2 Additives, adjuvant, surfactants, antioxidant and their concentrations used in developing liquid formulations of microbial consortia

Amendments		Concentrations of amendments			
		G_1	ACD-15	PSB	KSB
Additives	Polyethylene Glycol (PEG) (%)	0.5	0.5	0.5	0.5
	Trehalose (mM)	5	5	-	-
	Glycerol (mM)	5	5	5	5
Adjuvant	Gum Arabica (%)	0.15	0.15	0.15	0.15
Surfactant	Polysorbate-20 (ppm)		250	250	250
Antioxidant	Ascorbic acid	0.02	-	-	-

Table.3 Proportion of the three microbial inoculants in formulation of two different microbial consortia in 1:1:1 ratio

Sl. No.	Microbial consortia	Different formulations developed	Volume of microbial inoculants used in developing microbial consortia (ml)			Final volume of microbial consortia (ml)
			G1	PSB	KSB	
1	MC-1	Lignite Kaolinite Bentonite Na-alginate	1.83	4.24	60.61	66.68
		Liquid	4.63	10.37	185.18	200.18
2	MC-2	Lignite Kaolinite Bentonite Na-alginate	24.09	2.81	40.16	67.06
		Liquid	4.63	10.37	185.18	200.18

Table.4 Population of *Gluconacetobacter* strain, G_1 in different formulations of microbial consortium-1 at different interval under ambient condition

Formulations	Population G_1 (x 10^8 CFU/ml) at regular intervals			
	Days after incubation (DAI)			
	0	30	60	90
Lignite	9.700[c] (50.167)	9.072[b] (12.000)	8.100[a] (1.333)	7.20[a] (0.167)
Kaolinite	9.729[c] (53.833)	9.033[b] (11.167)	8.059[a] (1.167)	7.318[a] (0.217)
Liquid	10.049[a] (112.000)	9.438[a] (27.500)	8.159[a] (1.500)	7.812[a] (0.650)
Na-alginate	9.868[b] (74.000)	9.704[a] (52.667)	8.100[a] (1.333)	7.693[a] (0.500)
Bentonite	9.673[c] (47.333)	8.933[b] (8.833)	8.100[a] (1.333)	7.301[a] (0.233)
S.E.m (±)	0.022	0.069	0.091	0.101
L.S.D (p≤0.01)	0.101	0.308	NS	0.452

Note: The values in parenthesis are real values next to the $\log_{10}$ transformed values and statistical analysis was done to $\log_{10}$ transformed values.
Means followed by the same superscript within factors (A and B) and their interaction (A × B) do not vary significantly at P<0.01 by DMRT.

Table.5 Population of PSB in different formulations of microbial consortium-1 at different interval under ambient condition

Formulations	Population PSB (x 10^8 CFU/ml) at regular intervals			
	Days after incubation (DAI)			
	0	30	60	90
Lignite	9.602[b] (40.000)	8.735[a] (5.500)	8.059[b] (1.500)	7.059[a] (0.117)
Kaolinite	9.618[b] (41.500)	8.522[a] (3.333)	8.259[c] (1.667)	7.259[a] (0.200)
Liquid	9.828[a] (68.667)	8.583[a] (3.833)	8.577[a] (2.167)	7.577[a] (0.417)
Na-alginate	9.699[ab] (50.333)	8.573[a] (4.500)	8.360[ab] (1.667)	7.360[a] (0.233)
Bentonite	9.651[b] (44.833)	8.059[b] (1.167)	8.259[ab] (1.500)	7.259[a] (0.200)
S. Em (±)	0.033	0.098	0.115	0.115
L.S.D (p≤0.01)	0.147	0.438	NS	NS

Note: The values in parenthesis are real values next to the $\log_{10}$ transformed values and statistical analysis was done to $\log_{10}$ transformed values.
Means followed by the same superscript within factors (A and B) and their interaction (A × B) do not vary significantly at P<0.01 by DMRT.

Table.6 Population of KSB in different formulations of microbial consortium-1 at different interval under ambient condition

Formulations	Population KSB (x 10^8 CFU/ml) at regular intervals			
	Days after incubation (DAI)			
	0	30	60	90
Lignite	9.561[b] (36.833)	8.218[c] (1.667)	8.000[a] (1.000)	7.133[a] (0.150)
Kaolinite	9.618[b] (41.500)	8.788[a] (6.167)	8.159[a] (1.500)	7.059[a] (0.117)
Liquid	9.705[a] (50.667)	8.774[a] (6.000)	8.100[a] (1.333)	7.300[a] (0.200)
Na-alginate	9.777[a] (59.833)	8.558[b] (3.667)	8.059[a] (1.167)	7.218[a] (0.183)
Bentonite	9.607[b] (40.500)	8.799[a] (6.333)	8.100[a] (1.333)	7.100[a] (0.133)
S. Em (±)	0.022	0.040	0.079	0.101
L.S.D (p≤0.01)	0.098	0.180	NS	NS

Note: The values in parenthesis are real values next to the $\log_{10}$ transformed values and statistical analysis was done to $\log_{10}$ transformed values.
Means followed by the same superscript within factors (A and B) and their interaction (A × B) do not vary significantly at P<0.01 by DMRT.

Table.7 Population of *Azospirillum* strain, ACD-15 in different formulations of microbial consortium-2 at different interval under ambient condition

Formulations	Population ACD-15 (x 10^8 CFU/ml) at regular intervals			
	Days after incubation (DAI)			
	0	30	60	90
Lignite	9.657[b] (45.500)	9.327[b] (21.333)	8.059[a] (1.167)	7.541[a] (0.400)
Kaolinite	9.695[b] (49.667)	9.415[b] (27.000)	8.000[a] (1.000)	7.259[a] (0.200)
Liquid	10.038[a] (109.500)	9.356[b] (22.833)	8.259[a] (2.000)	7.618[a] (0.467)
Na-alginate	9.935[a] (86.167)	9.630[a] (42.667)	8.208[a] (1.633)	7.700[a] (0.517)
Bentonite	9.669[b] (47.000)	9.191[b] (16.000)	8.100[a] (1.333)	7.100[a] (0.133)
S. Em (±)	0.026	0.055	0.084	0.132
L.S.D (p≤0.01)	0.114	0.246	NS	NS

Note: The values in parenthesis are real values next to the $\log_{10}$ transformed values and statistical analysis was done to $\log_{10}$ transformed values.
Means followed by the same superscript within factors (A and B) and their interaction (A × B) do not vary significantly at P<0.01 by DMRT.

Table.8 Population of PSB in different formulations of microbial consortium-2 at different interval under ambient condition

Formulations	Population PSB (x 10^8 CFU/ml) at regular intervals			
	Days after incubation (DAI)			
	0	30	60	90
Lignite	9.607^{b} (40.500)	8.597^{bc} (4.167)	8.201^{a} (1.333)	7.201^{a} (0.167)
Kaolinite	9.645^{b} (44.500)	8.685^{ab} (5.000)	8.191^{a} (1.000)	7.191^{a} (0.167)
Liquid	9.798^{a} (63.000)	8.678^{ab} (4.8330)	8.259^{a} (1.500)	7.259^{a} (0.183)
Na-alginate	9.751^{ab} (56.500)	9.107^{a} (13.000)	8.259^{a} (2.667)	7.259^{a} (0.200)
Bentonite	9.640^{b} (44.167)	8.201^{c} (1.667)	8.159^{a} (1.333)	7.159^{a} (0.150)
S. Em (±)	0.031	0.080	0.102^{a}	0.102
L.S.D (p≤0.01)	0.138	0.361	NS	NS

Note: The values in parenthesis are real values next to the $\log_{10}$ transformed values and statistical analysis was done to $\log_{10}$ transformed values.
Means followed by the same superscript within factors (A and B) and their interaction (A × B) do not vary significantly at P<0.01 by DMRT.

Table.9 Population of KSB in different formulations of microbial consortium-2 at different interval under ambient condition

Formulations	Population KSB (x 10^8 CFU/ml) at regular intervals			
	Days after incubation (DAI)			
	0	30	60	90
Lignite	9.570^{a} (37.167)	8.869^{a} (7.500)	8.059^{a} (1.167)	7.201^{a} (0.167)
Kaolinite	9.576^{a} (37.667)	8.760^{ab} (5.833)	8.000^{a} (1.000)	7.259^{a} (0.200)
Liquid	9.684^{a} (48.500)	8.651^{b} (4.500)	8.100^{a} (1.333)	7.401^{a} (0.267)
Na-alginate	9.720^{a} (53.333)	8.724^{ab} (5.333)	8.000^{a} (1.000)	7.324^{a} (0.217)
Bentonite	9.609^{a} (41.000)	8.883^{a} (7.667)	8.000^{a} (1.000)	7.059^{a} (0.117)
S. Em (±)	0.034	0.040	0.052	0.098
L.S.D (p≤0.01)	NS	NS	NS	NS

Note: The values in parenthesis are real values next to the $\log_{10}$ transformed values and statistical analysis was done to $\log_{10}$ transformed values.
Means followed by the same superscript within factors (A and B) and their interaction (A × B) do not vary significantly at P<0.01 by DMRT.

Table.10 Most Probable Number Estimation of *Gluconacetobacter* strain, G_1 in microbial consortium-1 under ambient condition

Formulations	Most probable number at × 10^9 at regular intervals			
	Days after incubation (DAI)			
	0	30	60	90
Lignite	9.142 (1.440)	8.950 (0.907)	8.407 (0.310)	8.011 (0.120)
Kaolinite	9.159 (1.467)	9.150 (1.433)	8.246 (0.187)	8.111 (0.130)
Liquid	9.104 (1.300)	9.079 (1.240)	8.665 (0.700)	8.277 (0.203)
Na-alginate	9.244 (2.000)	9.167 (1.500)	8.872 (0.933)	8.442 (0.320)
Bentonite	9.030 (1.133)	9.093 (1.333)	8.174 (0.150)	7.950 (0.091)
S. Em (±)	0.100	0.080	0.187	0.123
L.S.D (p≤0.01)	NS	NS	NS	NS

Note: The values in parenthesis are real values next to the $\log_{10}$ transformed values and statistical analysis was done to $\log_{10}$ transformed values.
Means followed by the same superscript within factors (A and B) and their interaction (A × B) do not vary significantly at P<0.01 by DMRT.

Table.11 Most Probable Number Estimation of *Azospirillum* strain, ACD-15 in microbial consortium-2 under ambient condition

Formulations	Most probable number at × 10^9 at regular intervals			
	Days after incubation (DAI)			
	0	30	60	90
Lignite	9.507 (3.467)	9.001 (1.113)	8.993 (1.080)	8.312 (0.223)
Kaolinite	9.564 (6.533)	9.159 (1.467)	8.967 (1.020)	8.412 (0.260)
Liquid	9.437 (3.833)	9.049 (1.157)	9.093 (1.447)	8.599 (0.673)
Na-alginate	9.177 (1.667)	9.116 (1.407)	9.044 (1.147)	8.484 (0.437)
Bentonite	9.407 (3.000)	8.913 (0.853)	8.904 (0.957)	7.873 (0.078)
S. Em (±)	0.226	0.103	0.152	0.199
L. S. D (p<0.01)	NS	NS	NS	NS

Note: The values in parenthesis are real values next to the $\log_{10}$ transformed values and statistical analysis was done to $\log_{10}$ transformed values.
Means followed by the same superscript within factors (A and B) and their interaction (A × B) do not vary significantly at P<0.01 by DMRT

Table.12 Seed germination percentage and seedling vigour index of maize as influenced by inoculation with formulations of microbial consortia

Formulations	Seed germination (%)								Seedling vigour index							
	T_1	T_2	T_3	T_4	T_5	T_6	T_7	Mean (Consortia) A	T_1	T_2	T_3	T_4	T_5	T_6	T_7	Mean (Consortia) A
MC1	89.50^{e}	91.50^{bcd}	92.25^{b}	95.00^{a}	90.25^{de}	91.75^{b}	89.25^{c}	91.7^{a}	2552.33^{f}	2585.68^{f}	3235.47^{d}	4102.33^{b}	2647.89^{f}	2514.36^{c}	2135.00^{d}	3024.74^{b}
MC2	90.50^{cde}	90.00^{de}	96.25^{a}	93.00^{b}	91.00^{cde}	91.75^{b}	89.25^{c}	92.15^{a}	2613.83^{f}	2686.17^{ef}	4266.75^{a}	3598.54^{c}	2790.71^{e}	2514.36^{c}	2135.00^{d}	3191.20^{a}
Mean (Formulations) B	90.00^{b}	90.75^{b}	94.25^{a}	94.00^{a}	90.63^{b}	91.75^{b}	89.25^{c}		2583.08^{b}	2635.92^{b}	3751.11^{a}	3850.43^{a}	2719.30^{b}	2514.36^{c}	2135.00^{d}	
Comparison of	**S. Em (±)**				**L. S. D (p<0.01)**				**S. Em (±)**				**L. S. D (p<0.01)**			
A (Consortia)	0.49				NS				36.40				141.57			
B (Formulation)	0.78				3.03				57.56				223.84			
Interaction (A×B)	1.10				NS				81.40				316.55			

Note: **MC_1**: Microbial consortia-1, **MC_2**: Microbial consortia-2, **T_1**: Lignite formulation, **T_2**: Kaolinite formulation, **T_3**: Liquid formulation, **T_4**: Na-alginate formulation, **T_5**: Bentonite formulation, **T_6**: Dual inoculation of ACD-15+PSB, **T_7**: Uninoculated control and **NS**: Non-significant.

Means followed by the same superscript within factors (A and B) and their interaction (A × B) do not vary significantly at P<0.01 by DMRT.

Table.13 Shoot and root length of maize seedling as influenced by inoculation with formulations of microbial consortia

Formulations	Shoot length (cm)								Root length (cm)							
	T_1	T_2	T_3	T_4	T_5	T_6	T_7	**Mean (Consortia) A**	T_1	T_2	T_3	T_4	T_5	T_6	T_7	**Mean (Consortia) A**
MC1	13.04^{et}	12.24^{t}	16.32^{c}	20.59^{a}	13.70^{e}	12.77^{c}	11.22^{d}	15.18^{a}	15.46^{g}	16.01^{tg}	18.76^{d}	22.60^{b}	15.64^{tg}	14.62^{c}	12.70^{d}	17.69^{b}
MC2	12.53^{t}	12.51^{t}	20.97^{a}	18.79^{b}	14.71^{d}	12.77^{c}	11.22^{d}	15.90^{a}	16.31^{f}	17.35^{e}	23.38^{a}	19.93^{c}	15.94^{g}	14.62^{c}	12.70^{d}	18.58^{a}
Mean (Formulations) B	12.78^{c}	12.37^{c}	18.65^{a}	19.69^{a}	14.21^{b}	12.77^{c}	11.22^{d}		15.88^{b}	16.68^{b}	21.07^{a}	21.26^{a}	15.79^{b}	14.62^{c}	12.70^{d}	
Comparison of	**S. Em (±)**				**L. S. D (p<0.01)**				**S. Em (±)**				**L. S. D (p<0.01)**			
A (Consortia)	0.24				NS				0.19				0.75			
B (Formulation)	0.38				1.49				0.31				1.19			
Interaction (A×B)	0.54				2.11				0.43				1.68			

Note: **MC_1**: Microbial consortia-1, **MC_2**: Microbial consortia-2, **T_1**: Lignite formulation, **T_2**: Kaolinite formulation, **T_3**: Liquid formulation, **T_4**: Na-alginate formulation, **T_5**: Bentonite formulation, **T_6**: Dual inoculation of ACD-15+PSB, **T_7**: Uninoculated control and **NS**: Non-significant.

Means followed by the same superscript within factors (A and B) and their interaction (A × B) do not vary significantly at P < 0.01 by DMRT

The population (CFU/ml) of strain *Gluconacetobacter* G_1, *Azospirillum* ACD-15, PSB and KSB on their respective selective medium amended with protectants (for liquid formulation) were 12.56×10^{10}, 125.6×10^{8}, 57×10^{9} and 32×10^{8} CFU/ml respectively. Among them KSB had the least population. One ml inoculum of KSB containing 32×10^{8} CFU/ml was equivalent in population to 0.03 ml of *Gluconacetobacter* G_1 containing 12.56×10^{10}, 0.05 ml of PSB containing 57×10^{8} and 0.44 ml of *Azospirillum* ACD-15 containing 73.72×10^{8} CFU/ml.

Among the five formulations, relatively better shelf life of biofertilizer strains in consortium-1 (Table 4, 5 and 6) was recorded in liquid formulation where it recorded significantly higher population of 6.5×10^{7} CFU/ml of *Gluconacetobacter* G_1, PSB 4.2×10^{7} CFU/ml of PSB and 2.0×10^{7} CFU/ml KSB after 90 days after incubation (DAI). Whereas, the population of the biofertilizer strains in consortium-2 (Table 7, 8 and 9) were found significantly higher in Na-alginate formulation with the population of 5.2×10^{7} CFU/ml *Azospirillum* ACD-15, 2.0×10^{7} CFU/ml of PSB and 2.6×10^{7} CFU/ml of KSB after 90 DAI. In the present investigation, it was noticed that, the initial set population of all biofertilizer strains as mentioned earlier was equal and population marginally declined in all the formulations after 90 DAI.

The cell protectants in liquid formulation and encapsulation of biofertilizer strains in Na-alginate formulation played a crucial role in reducing the process of cell desiccation due to their high water holding capacity and thereby maintained water around the cells as compared to other formulations. The main advantages of Na-alginate encapsulation are their slow release of biofertilizer strains in soil, biodegradable and non-toxic in nature besides having long shelf life.

Viable population of microbial strains (*Gluconacetobacter* G_1 and *Azospirillum* ACD-15) as estimated by MPNE method are presented in table 10 and 11. It was recorded that there were no significant differences in the population of microbial strains in all the five formulations of two microbial consortia at all stages of incubation. However, the population of *Gluconacetobacter* G_1 in terms of most probable number recorded was higher in Na-alginate formulation upto 90 DAI (1.5×10^{9} CFU/ml at 30 DAI, 9.3×10^{8} CFU/ml at 60 DAI and 3.2×10^{8} CFU/ml at 90 DAI) and *Azospirillum* ACD-15 in kaolinite formulation at 30 DAI (1.4×10^{9} CFU/ml) and in liquid formulation at 60 (1.4×10^{9} CFU/ml) and 90 DAI (6.7×10^{8} CFU/ml). Therefore, among the five formulations of microbial consortia, liquid formulation of microbial consortium-1 and Na-alginate formulation of microbial consortium-2 supported higher population of each strain until 90 days of storage under ambient conditions. Among the two consortia, the population of PSB and KSB were higher in microbial consortium-1 compared to microbial consortium-2 (Table 5, 7, 8 and 9). However, the population of strains G_1 and ACD-15 estimated using Dilution Plate Count Technique (DPCT) were found to be lower than the population values recorded by MPNE method (Table 10 and 11). Amalraj *et al.*, (2016) had reported that liquid and Na-alginate formulation of microbial consortia containing a nitrogen fixer, a PSB and PGPR were found higher for to 60 days of storage at room temperature and these formulations were also found effective.

In vitro study on maize

Developed formulations were also tested for their effectiveness on maize under *in vitro* after 90 DAI of shelf life studies. Maize seeds inoculated with NPK consortia in liquid formulation resulted in significantly the highest seed germination (94.25 %) whereas

Na-alginate formulation resulted in significantly higher shoot length (19.69 cm), root length (21.26 cm) and seedling vigour index (3850.43) irrespective of the type of N_2 fixer used as compared to dual inoculation and uninoculated control. These results were in accordance with the earlier studies with *Azospirillum* and *Phosphobacterium* (Mariappan et al., 2014) and phosphorus and potassium solubilizing bacteria on maize (Abou and Abdel, 2012). The improved germination and seedling vigour observed in all the treatments over uninoculated control could be attributed to the release of growth promoting substances besides their ability to mobilize three major (N, P and K) nutrients by microbial strains in NPK consortia. It is seldom difficult to enhance plant growth by the application of single biofertilizer strain as it can provide only single nutrient element which can be overcome by the application of NPK consortia which provide all the three major nutrients by multiple microbial mechanisms and also other benefits from different biofertilizer strains in consortia on plant growth. From this *in vitro* study on maize seedling vigour and also shelf life studies, liquid and Na-alginate formulations of microbial consortia were found promising.

In this study, biofertilizer consortia with Na-alginate and liquid formulations proved effective with respect to population stability of individual strain and their effectiveness on maize seedling vigour irrespective of microbial consortium used. This suggested the importance of carrier material in effectiveness of a microbial consortium as the constituent biofertilizer strains in each consortium remained the same but the carrier material used were different. The findings are useful for developing biofertilizer consortia consisting of a nitrogen fixer, a P-solubilizing bacterium and K-solubilizing bacterium formulated in liquid and Na-alginate entrapment.

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