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DEVELOPMENT OF DIFFERENT CARRIER BASED BIOINOCULANT CONSORTIUM AND EVALUATION OF THEIR EFFICACY ON SUNFLOWER

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A B S T R A C T

Inoculation of bioinoculants in crop cultivation plays very important role in sustainable agriculture. It is well known that the carrier based bioinoculants are being very effective that determine the shelf life of the inoculant. Hence the selection of best carrier is very essential for maintaining shelf life of the inoculant during storage and for better field performance. On comparison with the dual inoculant and single inoculant, Microbial Consortium was found to be superior in survival in different carriers analysed. Lignite sustained the highest number of viable cells, followed by Pressmud and vermiculite. A field trail was also conducted to study the efficiency of agriculturally beneficial microbial consortium at graded levels of chemical fertilizers. Agriculturally beneficial microbial consortium was also found to be superior in augmenting the growth and yield of sunflower.

INTRODUCTION

Co-inoculation of plant growth promoting Rhizobacteria is considered to be an innovative approach in plant health management and for the improvement of crop yield and quality (Janisiewicz, 1996; Marimuthu *et al.*, 2002). Indeed, the use of formulated preparations, consisting of a single microbial species or strains as inoculant has often resulted in non-consistent performances in agriculture, and consequently in their low representation in the world wide bio-fertilizer market. One of the reasons of such a failure could be that a single microbial agent is not likely to be active in all soil environments.

A way to overcome this problem is to include different species or strains of beneficial microbes in the same microbial formulation. Application of binary or multiple mixtures would mimic the natural situation more closely and might broaden the spectrum of biocontrol activity (Raupach and Kloepper, 1998). Moreover, they would enhance the efficacy and reliability of health effects on crops (Marimuthu *et al.*, 2002) and would allow the combination of various mechanisms without the need for genetic engineering (Janisiewicz, 1996). The past decades have seen the implementation of these strategies, and a growing interest has focused on the research of new microbial combinations capable of enhanced performances on plant health, with respect to the application of single species. (Roberts *et al.*, 2005). In this context the major role of inoculant formulation is to provide a suitable microenvironment to prevent the rapid decline of introduced bacteria in the soil. Inoculants have to be designed to provide dependable source of beneficial bacteria that survive in the soil and become available to plants. However to be considered as a successful inoculant formulation it has to satisfy the following phenomenon of long shelf life and stability along with the successful bio inoculation effect in the field level experiments. Products lacking this above said characteristics will be unacceptable in the agricultural market (Lethbridge, 1989 and Kennedy, 1997). Hence the present experiment was undertaken to evaluate and to recommend the use of Microbial consortium as inoculant delivery system.

MATERIALS AND METHODS

Bacterial Strains

The Agriculturally beneficial bacterial strains of Nitrogen fixing *Azospirillum lipoferum*, Phosphorus solubilizing *Bacillus megaterium* and Plant growth promoting rhizobacteria *Pseudomonas fluorescens* were isolated from the rhizosphere of sunflower. The isolates were identified and was characterized in the Department of microbiology, Faculty of Agriculture, Annamalai University and used for the further studies.

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Preparation of carrier based inoculants

The selected isolates were multiplied in large quantities in appropriate culture broths by incubating at 28±2ºC in an incubator shaker till they attained log phase with a cell load of $1x10^9$ cfu ml^{-1} and were used for inoculant preparation. Lignite collected from Neyveli Lignite Corporation (NLC), Neyveli and Vermiculite collected from Tamil Nadu Minerals Ltd. Chennai and Pressmud collected from Ambiga Sugars, Pennadam were used as carriers. The individual carrier materials were powdered and the pH was brought to neutral by adding $CaCO_3$ if necessary and sterilized at 15 psi for 1 hour and allowed to cool over night and then mixed with the log phase culture ($1x10^9$ cfu ml^{-1}) of the selected agriculturally beneficial microbial isolates *viz., Azospirillum lipoferum, Bacillus megaterium, Pseudomonas fluorescens* individually in separate quantities of sterile carrier in shallow trays. The moisture content was adjusted to 30-35 per cent. Curing in shallow trays for 24 hr in aseptic rooms and packed in high density opaque polythene bag (300 gauges) at the rate of 200 g bag^{-1} and sealed. Individual inoculant was prepared by mixing equal volumes of each culture broth with sterile carrier and combined inoculant was also prepared by mixing equal volumes of broth with the carrier materials. The populations of individual agriculturally beneficial microorganisms in the inoculant carriers were assessed at monthly intervals upto six months.

Determination of surviving population in the carrier based inoculant by serial dilution and plating technique

A quantity of 10 g of carrier based inoculant was suspended in 100 ml sterile water in 250 ml Erlenmeyer flask. After thorough agitation over a shaker for 10 min, one ml of the supernatant was aseptically transferred to 9 ml sterile water blank in a test tube.

Dilution process was continued till 10^{-8} dilution was reached. From this dilution, 1.0 ml aliquots were withdrawn and transferred to sterile Petri plates.

Appropriate medium *viz.,* nitrogen free malate medium for *Azospirillum lipoferum*, Pikovaskya's medium for *Bacillus megaterium* and King's B agar medium for *Pseudomonas* fluorescens was prepared and sterilized in an autoclave at 15 psi for 20 min. The medium was cooled and poured in Petri plates and swirled in clockwise and anticlockwise directions for even spreading and allowed for solidification. The plates were incubated in inverted position at room temperature. The developed agriculturally beneficial microbial colonies on the plates were counted and the population was determined and expressed as cfu g^{-1} of carrier material on oven dry basis.

Details of the Experimental Field

The *in vitro* experiments were conducted in the Microbiology Laboratory, Department of Microbiology, Faculty of Agriculture, Annamalai University, Annamalai nagar. The field experiments were conducted in the Experimental Farm, Department of Agronomy, Annamalai University, Annamalai Nagar, Tamilnadu. The Experimental Farm is situated at 11° 24' North latitude and 79° 44' East longitude at an altitude of + 5.79 m above mean sea level.

Plant Height

The height of the plants from each treatment was measured from each treatment was measured on 60^{th} day after sowing. The mean values of the plants from 5 replications were recorded.

Nitrogen Content of the Plant

The plant samples were washed in water, air dried and later dried to a constant weight in an oven at 50°C. Then they were ground, sieved and 100mg of sample was taken for analysis. The total nitrogen content was determined by microkjeldahl method (Bremner, 1960).

Dry Matter Production

Five plants were randomly selected from each treatment and collected, washed and dried in an oven at 80°C till constant weight was observed. The plants were weighed and DMP was expressed in kg ha^{-1} on 60DAS.

Total Number of Seeds per Capitullum

Total number of seeds in the five representative samples was counted and the mean value per plant was recorded. The seeds of the five representative samples were weighed and the mean value $plant^{-1}$ was expressed in g $plant^{-1}$.

Oil Content

The oil content of the seed was estimated using diethyl ether as extractant by soxhelt expressed in percentage.

Protein Content

Crude protein content of seed was calculated by multiplying the nitrogen content of the kernel with 6.25 (Humphries, 1996).

Statistical Analysis

The experimental results were statistically analysed in randomized block design (RBD) and in Duncan's multiple range test (DMRT) as per the procedure described by Gomez and Gomez (1984).

RESULTS AND DISCUSSION

In the present investigation an attempt has been made to compare the role of lignite, Pressmud and vermiculite as carriers in improving the shelf life of bioinoculants as single, dual and consortium inoculation. The population test was carried out in lignite, Pressmud and vermiculite upto 6 months of storage and results are presented in Table 2, 3 and 4 respectively.

The physico- chemical properties of the soil in the experimental site were analysed by the standard procedures and the results are presented in Table 1. Survival of selected agriculturally beneficial microorganisms as bioinoculants was tested with different carrier materials viz., lignite, Pressmud and vermiculite. The results of lignite as carrier material revealed that the consortium of *A. lipoferum* + *B. megaterium* + *P. fluorescens* performed better survival upto 6 months of

Table 1 Soil Characteristics of the Experimental site

S.No	Composition	Values
1.	Course sand (%)	13.02
2.	Fine sand (%)	36.72
3.	Clay (%)	37.12
4.	Textural class	Clay loam
5.	Available Nitrogen Kg ha^{-1} (Calcium hydroxide method)	74.4
6.	Available Phosphorous Kg ha^{-1} (Watanabe and Olsen, 1965)	63.5
7.	Available Potassium Kg ha^{-1} (ammonium acetate extraction method)	76.5
8.	Organic Carbon (%) ''wet'' oxidation by acidified dichromate method	0.32
9.	Organic matter (%)	0.40
10.	Soil reaction (pH)	8.0
11.	Electrical conductivity dsm^{-1}	0.91

Table 2 Survival of selected agriculturally beneficial Microorganisms as bio inoculants in lignite carrier

	Storage period in months						
	Initial	**1st Month**	**2nd Month**	**3rd Month**	**4th month**	5th Month	6th Month
	Inoculant population (Number of cfu x 10^8 g^{-1} of lignite)						
A. lipoferum	74.22	72.55	63.09	22.66	16.22	5.33	2.33
	(9.87)	(9.86)	(9.80)	(9.35)	(9.21)	(8.72)	(8.36)
B. megaterium	54.22	53.00	45.66	12.22	6.83	3.44	1.88
	(9.73)	(9.72)	(9.65)	(9.08)	(8.83)	(8.53)	(8.27)
P. fluorescens	75.85	72.44	63.09	21.37	15.13	10.00	2.63
	(9.88)	(9.86)	(9.80)	(9.33)	(9.18)	(9.00)	(8.42)
A. lipoferum + B. megaterium	75.85	74.13	63.09	52.48	25.11	18.19	7.24
	(9.88)	(9.87)	(9.80)	(9.72)	(9.40)	(9.26)	(8.86)
A. lipoferum + P. fluorescens	74.13	70.79	60.25	48.97	34.67	17.37	3.80
	(9.87)	(9.85)	(9.78)	(9.69)	(9.54)	(9.24)	(8.56)
B. megaterium + P. fluorescens	63.09	56.23	46.77	22.90	17.37	8.51	6.76
	(9.80)	(9.75)	(9.67)	(9.36)	(9.24)	(8.93)	(8.83)
A. lipoferum + B. megaterium + P. fluorescens	75.85	70.79	60.25	48.97	34.67	27.54	15.84
	(9.88)	(9.85)	(9.78)	(9.69)	(9.54)	(9.44)	(9.20)

Values in parenthesis are log 10 transformed values

Table 3 Survival of selected agriculturally beneficial Microorganisms as bioinoculants in Pressmud carrier

	Storage period in months						
	Initial	**1st Month**	**2nd Month**	3rd Month	4th month	5th Month	6th Month
	Inoculant population (Number of cfu x 10^8 g^{-1} of Pressmud)						
A. lipoferum	75.85	74.13	52.48	33.88	20.89	17.37	1.81
	(9.88)	(9.87)	(9.72)	(9.53)	(9.32)	(9.24)	(8.26)
B. megaterium	56.23	53.70	33.88	25.11	10.47	3.16	1.69
	(9.75)	(9.73)	(9.53)	(9.40)	(9.02)	(8.50)	(8.23)
P. fluorescens	41.68	38.01	26.30	10.00	7.94	4.07	2.18
	(9.62)	(9.58)	(9.42)	(9.00)	(8.90)	(8.61)	(8.34)
A. lipoferum + B. megaterium	75.85	72.44	52.48	16.98	10.00	8.31	2.63
	(9.88)	(9.86)	(9.72)	(9.23)	(9.00)	(8.92)	(8.42)
A. lipoferum + P. fluorescens	75.85	70.79	41.68	34.67	26.30	20.41	2.81
	(9.88)	(9.85)	(9.62)	(9.54)	(9.42)	(9.31)	(8.45)
B. megaterium + P. fluorescens	46.77	39.81	20.89	15.84	7.76	4.16	2.95
	(9.67)	(9.60)	(9.32)	(9.20)	(8.89)	(8.62)	(8.47)
A. lipoferum + B. megaterium + P. fluorescens	75.85	74.13	72.44	60.25	20.41	14.79	8.51
	(9.88)	(9.87)	(9.86)	(9.78)	(9.31)	(9.17)	(8.93)

Values in parenthesis are log 10 transformed values

Table 4 Survival of selected agriculturally beneficial Microorganisms as bioinoculants in Vermiculite carrier

	Storage period in months						
	Initial	**1st Month**	**2nd Month**	**3rd Month**	**4th month**	**5th Month**	**6th Month**
	Inoculant population (Number of cfu x 10^8 g^{-1} of Vermiculite)						
A. lipoferum	75.85	74.13	39.81	18.19	7.41	2.63	1.73
	(9.88)	(9.87)	(9.60)	(9.26)	(8.87)	(8.42)	(8.24)
B. megaterium	67.60	63.09	46.77	10.96	3.98	2.45	1.69
	(9.83)	(9.80)	(9.67)	(9.04)	(8.60)	(8.39)	(8.23)
P. fluorescens	67.60	56.23	13.18	7.94	3.16	2.18	1.81
	(9.83)	(9.75)	(9.12)	(8.90)	(8.50)	(8.34)	(8.26)
A. lipoferum + B. megaterium	75.85	75.85	67.60	25.11	18.19	7.41	2.51
	(9.88)	(9.88)	(9.83)	(9.40)	(9.26)	(8.87)	(8.40)
A. lipoferum + P. fluorescens	72.44	72.44	64.56	22.38	16.21	7.41	2.08
	(9.86)	(9.86)	(9.81)	(9.35)	(9.21)	(8.87)	(8.32)
B. megaterium + P. fluorescens	74.13	74.13	66.06	22.90	17.37	8.31	2.29
	(9.87)	(9.87)	(9.82)	(9.36)	(9.24)	(8.92)	(8.36)
A. lipoferum + B. megaterium + P. fluorescens	75.85	74.13	69.18	26.91	19.05	10.23	5.49
	(9.88)	(9.87)	(9.84)	(9.43)	(9.28)	(9.01)	(8.74)

Values in parenthesis are log 10 transformed values

Table 5 Effect of selected agriculturally beneficial Microorganisms as bioinoculants in different carriers on the plant growth and yield of Sunflower

S.No	Treatments		Plant Height (cm)	'N' uptake	Dry Matter Production (Kg ha^{-1})	Seed. Yield (Kg ha^{-1})	Oil content (%)	Protein Content (%)
1.	T_0	Control	80.54	124.60	1791.24	956.50	36.12	9.14
2.	T_1	75% N, P & K	110.26	168.60	2362.50	1230.80	37.92	11.62
3.	T_2	50% N, P & K	96.50	130.50	1964.26	998.50	37.42	10.06
4.	T_3	Single inoculant in Lignite as carrier	108.74	154.20	2114.24	1138.40	38.44	10.32
5.	T_4	Dual inoculant in Lignite as carrier	115.24	174.60	2442.52	1342.60	38.92	11.87
6.	T_5	Consortium in Lignite as carrier	124.24	190.60	2628.24	1472.20	39.94	12.17
7.	T_6	Single inoculant in Pressmud as carrier	105.54	154.20	2204.42	1120.80	38.14	10.24
8.	T_7	Dual inoculant in Pressmud as carrier	112.24	168.40	2304.52	1204.60	38.68	11.74
9.	T_8	Consortium in Pressmud as carrier	122.24	188.40	2624.42	1460.40	39.42	12.08
10.	T_9	Single inoculant in Vermiculite as carrier	102.84	152.60	2110.10	1116.50	38.10	10.18
11.	T_{10}	Dual inoculant in Vermiculite as carrier	109.74	162.40	2250.54	1174.60	38.24	10.42
12.	T_{11}	Consortium in Vermiculite as carrier	120.56	186.40	2614.42	1364.50	39.12	11.96
		SEd	0.98	1.56	1.42	5.62	0.42	0.12
		CD (p=0.05)	1.99	2.24	3.82	10.50	0.89	0.25

storage (9.20 log cfu x 10^8 g $^{-1}$ of lignite) which was followed by dual inoculation and the least survival was noticed in single inoculation.

Bioinoculants survival was also evaluated in Pressmud as carrier. The maximum survival of (8.93 log cfu x 10^8 g $^{-1}$ of Pressmud) was noticed in consortium followed by dual and single inoculation. Similarly vermiculite also recorded the maximum survival in consortium (8.74 log cfu x 10^8 g $^{-1}$ of Vermiculite) followed by dual and single inoculation. Among the different carriers tested it was found that the survival of microbial consortium of *A. lipoferum* + *B. megaterium* + *P. fluorescens* showed better results of survival in lignite followed by Pressmud and vermiculite as carriers.

Effects of selected agriculturally beneficial microorganisms as bioinoculants in different carriers on the growth, yield, and quality parameter of sunflower were presented in Table 5. The maximum plant height (124.24 cm) was recorded in microbial consortium A. *lipoferum* + *B. megaterium* + *P. fluorescens* of lignite as carrier material, Which was closely followed by (122.24 cm) in microbial consortium with Pressmud as carrier and (120.56 cm) in microbial consortium with vermiculite as carrier. The least height was noticed in control (80.54 cm). Goel *et al.,* (1999) reported that the inoculation with certain PGPR may enhance crop productivity either by making the other nutrients available or protecting plants from pathogenic microorganisms.

The similar trend was followed in "N" uptake the maximum uptake was recorded in consortium of bioinoculant in lignite (190.60) as carrier followed by Pressmud (188.40) and vermiculite (186.40). Highest amount of Dry matter production was recorded in microbial consortium in lignite as carrier (2628.24 Kg ha^{-1}) followed by dual inoculation and single inoculation. Maximum seed yield was obtained from microbial consortium of lignite carrier (1472.20 Kg ha^{-1}) which was followed by microbial consortium of Pressmud (1460.40 Kg ha^{-1}) and vermiculite (1364.50 Kg ha^{-1}) as carrier. The least was recorded in control (956.50 Kg ha^{-1})

Chandrasekhar *et al*., (2005) reported that both morphological and yield parameters showed a better results through the combination of bioinoculants and chemical fertilizer than using either method alone. The highest protein content (39.94%) and oil content (12.17%) was noticed in microbial consortium of lignite carrier. which was followed by dual inoculation in lignite, Pressmud, and vermiculite respectively.

CONCLUSION

The results of the present study clearly indicate that the lignite is the best suited carrier material for enhanced shelf life when compared to pressed and vermiculite and it also states that inoculation of sunflower with bioinoculant consortium were highly beneficial than the dual or single inoculation which increases the yield besides reducing the cost if inorganic fertilizer.

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