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Effect of Fluid Bed Dried Formulation in Comparison with Lignite Formulation of Microbial Consortium on Finger Millet (*Eleusine coracana* Gaertn.)

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A formulation was developed using fluid bed dryer in a suitable carrier with agriculturally beneficial microorganisms; *Acinetobacter* sp., *Azotobacter chroococcum* and *Bacillus subtilis* in single, dual and triple combinations by using talc and skim milk powder as carrier material. Survival of these inoculant microorganisms was determined at different intervals till 365 days of storage. There was a maximum survival in triple inoculants with no contamination during the storage period when compared to lignite formulation. Green house experiment was conducted to study the effect of this inoculant formulation on Finger millet (*Eleusine coracana* Gaertn.). The results indicated that plant growth parameters, nitrogen and phosphorus uptake were maximum in plants inoculated with triple inoculants followed by dual and single inoculants as compared to uninoculated control. Results were more pronounced in plants receiving nutrients (NPK).

Key words: Fluid bed dryer, *Acinetobacter* sp., *Azotobacter chroococcum* and *Bacillus subtilis, Eleusine coracana* Gaertn., skim milk powder and talc.

The use of microorganisms with an aim of improving nutrient availability for plants is an important practice and necessary for agriculture. During the past couple of decades, the use of plant growth promoting rhizobacteria (PGPR) for sustainable agriculture has increased tremendously in various parts of the world. Significant increases in growth and yield of agronomically important crops in response to inoculation with PGPR have been repeatedly reported (Gray and Smith, 2005; Figueiredo *et al*., 2008).

There are several PGPR inoculants currently commercialized that seem to promote growth through at least one mechanism: suppression of plant disease (bioprotectants), improved nutrients acquisition (biofertilizers), or phytohormone production (biostimulants). Biofertlilizers are available for increasing crop nutrient uptake of nitrogen from nitrogen-fixing bacteria associated with roots (Bashan and Holguin, 1997), iron uptake from siderophore-producing bacteria (Scher and Baker, 1982), sulfur uptake from sulfur-oxidizing bacteria (Stamford *et al*., 2008), and phosphorus uptake from phosphate-mineral solubilizing bacteria (Chabot *et al*., 1996).

Biofertlilizers with suitable carrier can cater different needs of growing plants, act as a consortium along with other microorganisms in the rhizosphere. There is a necessity of a suitable carrier material for successful field application of any biofertilizers as these carrier based preparation of microbial inoculants generally have shorter shelf life, poor quality, high contamination, which affects inoculation response under field conditions.

To overcome this, an experiment was conducted to develop dry formulation using Fluid bed dryer (FBD) with different carrier materials which has been successfully employed almost in

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all chemical, food and other process industries to dry materials. These dry and granular formulations are convenient as they allow control of placement and application rate (Stephens and Rask, 2000). The effect of fluid bed dried formulation was studied on Finger millet (*Eleusine coracana* Gaertn.). Since understanding the interaction between consortium of microbial inoculants and plant systems will pave the way to harness enhanced benefits from microbial inoculants for improving plant growth and yield (Raja *et al*., 2006).

MATERIALS AND METHODS

Plant growth promoting rhizobacteria

Acinetobacter sp., *as phosphorus (P) solubiliser, Bacillus subtilis* as biocontrol agent and *Azotobacter chroococcum* as nitrogen (N) fixer were used in this experiment. These cultures were procured from the Department of Agricultural Microbiology, University of A gricultural Sciences, Bangalore, India.

Carrier materials

Skim milk powder and Talc in equal quantities, Sugar (1 %) and Gelatin as sticking agent (2 %) were used for formulation preparation in a fluid bed dryer.

Fluid bed dryer (FBD)

FBD is one where material is maintained suspended against gravity in an upward flowing air stream. The air is heated by means of electrical heaters. This hot air expands the bed of material at a certain velocity and creating turbulence in the product (fluidization) and offers more surface area for drying. Fluidization produce full agitation of solid particles, it results in heat transfer and uniform drying.

Preparation of inoculant formulation

Inoculant formulation was prepared using sterilized talc (25 g) and skim milk powder (25 g). Centrifuged cells (10000 rpm at 4°C for 10 minutes) of *Acinetobacter* sp., *Bacillus subtilis* and *Azotobacter chroococcum* were collected in sterile distilled water and mixed with the carrier material in the ratio of 1:2. One per cent sugar and two per cent gelatin was also added. The single, dual and triple combinations of all three rhizobacteria with carrier and sticking agent was subjected to drying in a fluid bed dryer at 40 °C for a period of 45 minutes. The dried formulations were sealed in a sterile polythene cover for further study.

Survival of single, dual and triple inoculant formulation of fluid bed dried formulation was studied in comparison to lignite formulation. The viability of inoculants in formulation was ascertained by standard plate count technique at monthly intervals till 365 days of storage period.

Effect of fluid bed dried single, dual and triple inoculant formulation on finger millet (*Eleusine coracana* Gaertn.) was assessed under green house conditions. Seeds of finger millet (GPU- 48) were procured from AICRP on millets, ZARS, UAS, GKVK, Bengaluru. Finger millet seeds were sown in pots with eight different treatments and three replications of single, dual and triple combinations of fluid bed dried and lignite formulations. Two level of nutrients (+ NPK and - NPK) for fluid bed dried and lignite formulation were imposed. At maximum vegetative stage (45 days after sowing) plant growth parameters, total nitrogen (Microkjeldahl method, Jackson, 1973) and total phosphorus (Ascorbic acid method, Murphy and Riley, 1962) in finger millet was determined.

Treatment details

The eight different treatments of single, dual and triple inoculants combinations considered in the experiment are given below.

1. Control
2. *Acinetobacter* sp.
3. *Azotobacter chroococcum*
4. *Bacillus subtilis*
5. *Acinetobacter* sp.+ *Azotobacter chroococcum*
6. *Acinetobacter* sp.+ *Bacillus subtilis*
7. *Azotobacter chroococcum*+ *Bacillus subtilis*
8. *Acinetobacter* sp.+ *Azotobacter chroococcum*+ *Bacillus subtilis*

Statistical analysis

The survival study was statistically analyzed by complete randomized design (CRD) and pot study was analyzed by using two way factorial complete randomized design (CRD) and means were separated by critical difference value (Little and Hills, 1978).

RESULTS AND DISCUSSION

Survival study of fluid bed dried and lignite formulation

Among single, dual and triple inoculant formulation of fluid bed dried and lignite

Table 1. Survival of microorganisms in consortium (FBD) prepared using fluid bed dryer

Sl no	Treatments	Population density ($\log_{10}$ CFU) Duration of storage (days)											
		0	3	7	15	30	60	90	120	150	180	240	365
1	*Azotobacter chroococcum.*	7.66^{abc}	7.70^{bcd}	7.68^{bc}	7.54^{abc}	7.49^{bc}	7.46^{cd}	6.90^{d}	6.70^{f}	6.40^{d}	6.48^{d}	5.70^{d}	5.53^{a}
2	*Acinetobacter* sp.	7.63^{abc}	7.62^{d}	7.60^{c}	7.54^{abc}	7.58^{bc}	7.34^{d}	7.08^{d}	7.04^{e}	7.00^{bc}	6.70^{cd}	6.40^{bc}	5.82^{a}
3	*Bacillus subtilis*	7.9^{abc}	7.92^{abc}	7.94^{ab}	7.91^{abc}	7.84^{a}	7.68^{abc}	7.60^{abc}	7.46^{abc}	7.15^{ab}	6.70^{cd}	6.00^{cd}	6.22^{a}
4	*Azotobacter chroococcum+ Acinetobacter* sp.	7.59^{bc}	7.66^{cd}	7.59^{c}	7.64^{abc}	7.64^{ab}	7.52^{bcd}	7.45^{bc}	7.08^{e}	6.70^{cd}	7.08^{b}	6.85^{ab}	5.52^{a}
	Azotobacter chroococcum Acinetobacter sp.	7.66^{abc}	7.69^{cd}	7.70^{bc}	7.67^{abc}	7.68^{ab}	7.52^{bcd}	7.40^{bc}	7.23^{cde}	7.34^{ab}	7.23^{ab}	7.00^{a}	5.52^{a}
5	*Azotobacter chroococcum + Bacillus subtilis*	7.52^{c}	7.54^{d}	7.55^{c}	7.49^{c}	7.39^{c}	7.49^{cd}	7.38^{c}	7.32^{bcd}	7.34^{ab}	7.15^{ab}	6.95^{a}	5.52^{a}
	Azotobacter chroococcum Bacillus subtilis	8.04^{a}	8.06^{a}	8.03^{a}	7.89^{abc}	7.73^{ab}	7.59^{abcd}	7.72^{a}	7.69^{a}	7.45^{a}	7.20^{ab}	7.18^{a}	6.64^{a}
6	*Acinetobacter* sp.+ *Bacillus subtilis* .	7.54^{c}	7.54^{d}	7.59^{c}	7.59^{abc}	7.51^{bc}	7.41^{cd}	7.49^{abc}	7.15^{de}	7.18^{ab}	7.00^{bc}	6.85^{ab}	5.52^{a}
	Acinetobacter sp *Bacillus subtilis*	7.99^{ab}	7.96^{ab}	7.97^{a}	7.95^{ab}	7.87^{a}	7.81^{a}	7.63^{ab}	7.47^{ab}	7.32^{ab}	7.11^{ab}	6.90^{ab}	6.87^{a}
7	*Acinetobacter* sp.+ *Azotobacter chroococcum*	7.65^{abc}	7.65^{d}	7.62^{c}	7.51^{bc}	7.49^{bc}	7.43^{cd}	7.40^{bc}	7.47^{ab}	7.23^{ab}	7.30^{ab}	7.00^{a}	6.37^{a}
	+ *Bacillus subtilis Azotobacter chroococcum* .	7.62^{abc}	7.62^{d}	7.61^{c}	7.60^{abc}	7.70^{ab}	7.36^{d}	7.43^{bc}	7.47^{ab}	7.38^{ab}	7.40^{a}	7.00^{a}	5.52^{a}
	Acinetobacter sp *Bacillus subtilis*	8.04^{a}	7.96^{ab}	7.97^{a}	7.97^{a}	7.85^{a}	7.78^{ab}	7.61^{abc}	7.63^{a}	7.32^{ab}	7.40^{a}	6.95^{a}	6.64^{a}
	LSD at 5 %	0.42	0.27	0.27	0.45	0.24	0.29	0.23	0.23	0.42	0.30	0.53	1.79

NOTE: Means with same superscript within similar days of storage donot differ significantly at P=0.05

Table 2. Survival of microorganisms in consortium (lignite) prepared using lignite

Sl no	Treatments	Population density ($\log_{10}$ CFU) Duration of storage (days)											
		0	3	7	15	30	60	90	120	150	180	240	365
1	*Azotobacter chroococcum*	7.56cd	7.76bc	7.82ab	7.60bc	7.61a	7.59a	6.90g	6.60g	6.30a	6.30c	6.00a	5.52a
2	*Acinetobacte r* sp.	7.52cd	7.67c	7.60b	7.54bc	7.67a	7.68a	6.78g	6.90f	6.78a	6.60abc	6.18a	5.83a
3	*Bacillus subtilis*	7.95ab	8.02ab	7.99a	7.61bc	7.86a	7.66a	7.46abc	6.90f	6.90a	6.48bc	6.32a	6.11a
4	*Acinetobacter+ Azotobacter chroococcum*	7.56cd	7.66c	7.63b	7.75abc	7.77a	7.69a	7.30def	7.08def	6.30a	6.30c	5.7a	5.52a
	Azotobacter chroococcum Acinetobacter	7.60cd	7.75bc	7.70ab	7.67abc	7.68a	7.61a	7.20f	7.11cde	6.90a	6.95abc	6.30a	6.00a
5	*Azotobacter chroococcum + Bacillus subtilis*	7.55cd	7.56c	7.56b	7.49c	7.72a	7.61a	7.26ef	7.26abcd	7.15a	7.11ab	6.5a	5.82a
	Azotobacter chroococcum Bacillus subtilis	8.08a	8.05a	8.03a	7.70abc	7.64a	7.63a	7.41abcd	7.41a	6.90a	7.11ab	6.70a	6.12a
6	*Acinetobacter sp.+ Bacillus subtilis*	7.48d	7.66c	7.59b	7.59bc	7.68a	7.41a	7.49ab	7.04ef	7.11a	7.00ab	6.70a	6.11a
	Acinetobacter sp. Bacillus subtilis	7.84abc	8.08a	8.02a	7.84ab	7.81a	7.54a	7.52a	7.30ab	7.23a	7.11ab	6.65a	6.30a
7	*Acinetobacter sp.+ Azotobacter chroococcum*	7.63bcd	7.70c	7.62b	7.68abc	7.61a	7.61a	7.40abcd	7.32ab	7.15a	7.11ab	6.85a	6.10a
	+ Bacillus subtilis Azotobacter chroococcum	7.58cd	7.62c	7.61b	7.70abc	7.70a	7.81a	7.38bcde	7.18bcde	7.18a	6.98ab	6.48a	5.70a
	Acinetobacter sp. Bacillus subtilis	8.05a	8.12a	8.02a	8.01a	7.71a	7.64a	7.34cde	7.28abc	7.23a	7.15a	6.81a	6.16a
	LSD at 5 %	0.34	0.28	0.36	0.34	0.29	0.40	0.14	0.18	1.26	0.67	2.05	1.08

NOTE: Means with same superscript within similar days of storage donot differ significantly at P=0.05

formulation, the survival of triple inoculants was found better in triple inoculant formulations when compared to single and dual inoculants. Viability of triple inoculants was observed till 365 days of storage period. In beginning, triple inoculants recorded $\log_{10}$ 7.65, $\log_{10}$ 7.62 and $\log_{10}$ 8.04 cells/ g which reached $\log_{10}$ 6.37, $\log_{10}$ 5.52 and $\log_{10}$ 6.64 cells/ g at 365 days of storage period in *Azotobacter chroococcum, Acinetobacter* sp. and *Bacillus subtilis* respectively in fluid bed dried formulation (Table 1) where as lignite formulation recorded $\log_{10}$ 6.10, $\log_{10}$ 5.70 and $\log_{10}$ 6.16 cells/g at the end of 365 days in *Azotobacter chroococcum, Acinetobacter* sp. and *Bacillus subtilis* respectively (Table 2).

Maximum viable cells with no contamination were noticed in fluid bed dried formulation than lignite formulation. The use of skim milk powder might have supported survival of viable cells for a longer period in fluid bed dried formulation. Archana (2011) has reported that the use of skim milk powder has supported maximum population beyond 8 months of storage period. Sahu (2012) reported that the number of cells in talc based fluid bed dried formulation of triple inoculants survive better. These results shows that dry formulation are preferred over wet formulation where fluid bed dried formulation results in better formulation with no contamination which can be given priority. Similar outcome was reported by Hegde and Brahmaprakash (1992) where dried inoculant formulation prepared using sodium alginate and perlite for soil application, cells survived beyond 180 days.

Effectiveness of fluid bed dried formulation on Finger millet (Eleusine coracana Gaertn.)

Total Nitrogen content

Total nitrogen content was found better in plants inoculated with fluid bed dried formulation than lignite formulation. Maximum total nitrogen content of 462.70 mg/ plant in triple inoculation was influenced by plants inoculated with fluid bed dried formulation. Plants inoculated with lignite based formulation influenced higher nitrogen content in triple inoculation (339.87 mg/ plant) (Fig 1). Plants which were not under the influence of nutrients (without NPK) supported higher total nitrogen content in triple inoculation (108.66 and 99.60 mg/ plant) and least in control plants (29.36 and 27.02 mg/ plant) in fluid bed dried and lignite formulations respectively (Fig 1a).

This might be due to the inoculation of *Azotobacter chroococcum* a nitrogen fixer along with PGPR organisms which play an active role in N uptake. Inoculation of finger millet cultivar Indaf-7 with diazotrophs cultures increased yield and nitrogen uptake over control in a green house study (Thanuja and Ambika, 2010) also the application of PGPR microorganisms with N fertilizer increased the fertilizer N efficiency by increasing N content and N uptake in plants (Kumar and Chandra, 2008).

Total phosphorus content

Plants under the influence of NPK showed enhanced total phosphorus content when compared to the plants which were not receiving NPK. Treatment of plants with Fluid bed dried formulations were found to be better than lignite formulations. Triple inoculant treatment enhanced higher total phosphorus content of 57.63 mg/ plant and 53.92 mg/ plant followed by dual inoculation of *Acinetobacter* sp. and *Bacillus subtilis* (51.46 mg/ plant and 46.43 mg/ plant) and lower content was present in uninoculated control (20.30 mg/ plant and 19.81 mg/ plant) in plants inoculated with fluid bed dried and lignite based formulation respectively (Fig 2). Plants not receiving NPK in

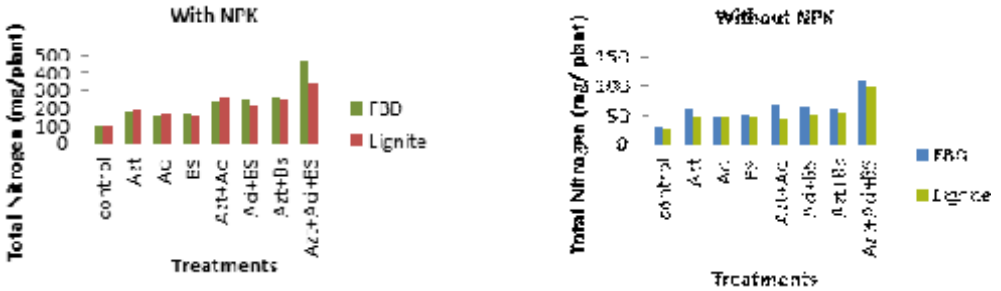

Fig. 1 & 1a. Total nitrogen content (mg/ plant) in finger millet (*Eleusine coracana* Gaertn.) on inoculation of fluid bed dried and lignite formulations.

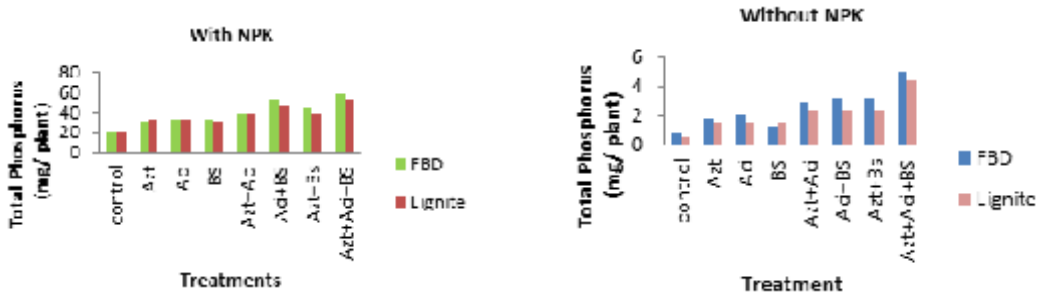

Fig. 2 & 2a. Total phosphorus content (mg/ plant) in finger millet (*Eleusine coracana* Gaertn.) on inoculation of fluid bed dried and lignite formulations

fluid bed dried formulation recorded highest of 4.97 mg/ plant of total phosphorus content and lowest of 0.78 mg/ plant in control plants. Similarly plants inoculated with lignite based formulation enhanced higher phosphorus content of 4.34 mg/ plant in triple inoculant formulation and least of 0.53 mg/ plant in uninoculated control plants (Fig 2a).

Acinetobacter sp. and *Bacillus subtilis* inoculation which play a role in P solubilisation showed a higher uptake of phosphorus content in finger millet plant. Earlier reports say that *Bacillus subtilis* as bioinoculant can be successfully used for P solubilization and for maintaining soil health (Swain *et al*., 2012). The production of plant growth promoting harmones by PGPR organisms and P solubilisation by P solubilisers play a vital role in higher uptake of nutrients. These findings were similar with reports of Fan *et al.* (2011).

These findings indicated that Fluid bed drying process is found to be successful in preservation of microbial population for a very long period of time which could meet the Bureau of Indian Standards. It was also possible to deliver more than one inoculant microorganisms in a single system (carrier) with enhanced plant growth in finger millet (*Eleusine coracana* Gaertn.) crop. Hence fluid bed dryer can be a promising tool for biofertilizer formulation which is easier for handling and application with low levels of contamination.

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