



Fluid Bed Dried Microbial Consortium for Enhanced Plant Growth: A Step towards Next Generation Bio Formulation

Pramod Kumar Sahu^{1*}, Lavanya G², Amrita Gupta¹ and BrahmaPrakash GP²

Abstract

A new type of biofertilizer formulation containing microbial consortium was constituted using fluid bed dryer (FBD) with a vision of reducing contamination and enhancing performance of bioinoculants. Three agriculturally beneficial microorganisms viz. *Pseudomonas fluorescens* (plant growth promoter), *Acinetobacter* sp. (phosphate solubilizer) and *Azotobacter chroococcum* (dinitrogen fixer) were used in consortium. Inocula of respective combinations were mixed with carrier material (talc) and dried in FBD. Effectiveness of these inoculants formulations was carried out with finger millet (*Eleusine coracana* Gaertn.) in greenhouse. Enhancement in nitrogen content, phosphorus content and dry weight was reported from triple inoculants consortium followed by dual, single and uninoculated control. Chlorophyll content was highest from dual inoculation of *P. fluorescens* + *Acinetobacter* sp. and *Acinetobacter* sp. + *A. chroococcum*. Plant growth in inoculated treatments was robust when supplied with NPK fertilizers, but effect of inoculation was pronounced in plants not receiving chemical fertilizers. However, performance of plants receiving triple inoculants consortium without nutrients (-NPK) was on par with uninoculated plants with nutrients (+NPK). This can be handy in reducing contamination due to low moisture availability.

Keywords

Fluid bed dryer; Consortium; Bioformulation; Plant growth promotion

Introduction

Inoculation is gaining popularization in farming. Degrading soil quality and enhanced need for food grains had made it imperative to have a judicious blend of organic and chemical fertilizers. In this regard, many researchers and major pesticides companies are moving towards biological products for enhancing agricultural production. Viability of cells is a vital factor for effective bio inoculants [1], thus formulation becomes a crucial step. Many formulations have been tested for its competence like liquid [2], polymer entrapped [3], various solid carrier based bioinoculants, etc [4].

This study focuses on consortium of PGPR and its fluid bed dried (FBD) bioformulation. Synergy in consortia has proven effects on

plant growth promotion [5]. This synergism is now being harnessing for enhancing effects of bio inoculants [6].

Fluid bed dryer (FBD) dries the material in a condition suspended against gravity by upward flowing air stream. Electrical heaters supplement heat for drying the material. This hot air expands the bed of material at its terminal velocity and creating turbulence for drying of product (terminal velocity is the minimum velocity of the air sufficient to keep the given particle floating in the air). This phenomenon is called fluidization and offers more surface area for drying as the entire particle surface comes into contact with heated air. As it produces full agitation of solid particles, it results in faster heat transfer and uniform drying [7]. In pursuit of an appropriate drying technology for bio fertilizer, the idea of fluid bed drying was borrowed from food processing and drug industry. FBD is commonly used in food industries for making instant coffee powder and other drying operations [4].

Dried inoculants formulations have very less moisture content for a contaminant microorganism to grow and multiply in the formulation during storage period. This gives an obvious advantage of purity and protection to the inoculants in formulation. Pre-exposure of microbes to stress may result in better adaptability to natural soil conditions. The loss of viability during storage is relatively low [6,8].

Material and Methods

Preparation of FBD formulation

Fluid bed dried formulation was prepared as described elsewhere [8]. A brief note of the procedure is given here. The cultures were mixed in talc and subjected for fluid bed drying at 38°C for 1-2 hrs till a dry powder is obtained and sealed. These formulations were made in eight treatment combinations- T1 (control), T2 (*Pseudomonas fluorescens*), T3 (*Acinetobacter* sp.), T4 (*Azotobacter chroococcum*), T5 (*P. fluorescens* + *Acinetobacter* sp.), T6 (*P. fluorescens* + *A. chroococcum*), T7 (*Acinetobacter* sp. + *A. chroococcum*), T8 (*P. fluorescens* + *Acinetobacter* sp. + *A. chroococcum*). These treatments were taken with two levels of fertilizers (+NPK and -NPK) and three replications (8 treatments × 2 nutrient levels × 3 replications= 48 experimental units).

Soil processing

Soil employed for the experiment was collected from uncultivated field, GKVK, Bangalore. It was red loamy, classified as fine kaolinitic, isohyperthermic, kanhaplustalfs soil. Soil was sieved in 2 mm sieve. 3.8 kg of soil was filled into 4.0 kg capacity pots. Homogenization of soil was done by row and column randomization. Three cycles of wetting and drying were carried out to bring soil into natural condition of compaction. Moisture content of soil was raised to field capacity at the end of each wetting and drying cycle. Field capacity of soil was determined by method proposed by Jackson [9]. Recommended dose of fertilizers for finger millet (*Eleusine coracana* Gaertn.) cv. GPU 28 was provided to +NPK pots and -NPK pots had not been added any chemical fertilizer.

Inoculation of consortium and sowing of seeds

FBD inoculants were applied in the soil at 0.5 cm below the seeds. 4-5 seeds were sown in each of three hills of every pot. Seeds were

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sown at a depth of 1.5 cm. Other agronomic practices were carried out as per the crop requirement. Two plants were maintained per hill after seedlings were stabilized and excess seedlings were thinned out.

Chlorophyll content

Plant height and chlorophyll content was recorded at maximum vegetative growth. Estimation of chlorophyll was done by method suggested by Witham et al. [10]. One gram of leaf sample was crushed in pre-chilled 80 per cent acetone and filtered to extract all the chlorophyll present in the leaf. Final volume was made up to 100 ml with 80 per cent acetone as blank and absorbance was recorded at 663 and 645 nm wavelength.

Dry weight of plant

After harvesting done at 50 per cent flowering, root and shoot dry weight was recorded after drying the samples at 60°C till a constant weight is obtained. Shoots were harvested by separating stem at the collar region from roots. Roots were washed free of soil particles by a slow jet of water.

Nitrogen estimation in plant samples

Amount of nitrogen in root and shoot was estimated by microkjeldahl method as outlined by Jackson [9]. Plant samples (200 mg) were digested with digestion mixture and concentrated sulphuric acid at 400°C till solution become clear. The digested samples were then distilled with 40 per cent sodium hydroxide and ammonia evolved was trapped in boric acid (4 per cent w/v) solution with mixed indicator. After distillation, boric acid solution containing trapped ammonia was titrated against 0.09N sulphuric acid and volume of acid required to neutralize the alkalinity (ammonia) was recorded. Nitrogen content in plant sample was calculated using standard formula.

Phosphorus estimation in plant samples

Phosphorus content of plant sample was determined by ammonium vanadomolybdate method [11]. 100 mg of plant samples were taken and burnt to ash in a muffle furnace. It was then dissolved in distilled water and volume made up to 100 ml. Reagent-A was prepared by mixing 3 solutions-(1) 12g ammonium molybdate

$[(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}\cdot 4\text{H}_2\text{O}]$ dissolved in 250 ml distilled water, warmed for dissolving; (2) 0.2908 g of potassium antimony tartrate dissolved in 100 ml distilled water and (3) 1000 ml, 5 N H_2SO_4 . Reagent-B was prepared by dissolving 1.056 g ascorbic acid into 200 ml of reagent-A. Reagent-B (2.5 ml) was mixed with 10 ml aliquot of sample causing colour to develop and absorbance of this solution was recorded at 840 nm. The phosphorus content was obtained by standard curve developed using KH_2PO_4 as phosphorus source.

Statistical analysis

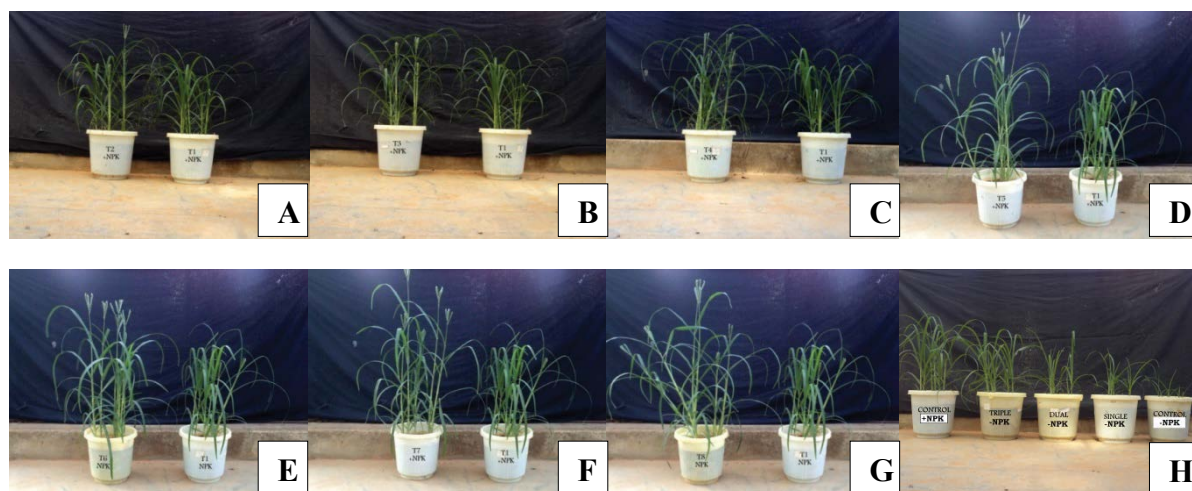
Statistical analysis of the data from green house investigation was done by using complete randomized design (CRD) and means were separated by critical difference value [12]. Data was interpreted based on main treatment effect.

Results and Discussion

Plant growth in inoculated treatments was robust when supplied with NPK fertilizers, but effect of inoculation was pronounced in plants not receiving chemical fertilizers (Figure 1). However, performance of plants receiving triple inoculants consortium without nutrients (-NPK) was on par with uninoculated plants with nutrients (+NPK).

Nitrogen content

Nitrogen content of the roots (mg/plant) found highest in plant receiving triple inoculation (22.93 mg/plant). Dual inoculation of *P. fluorescens* + *Acinetobacter* sp. (T5) and *Acinetobacter* sp. + *A. chroococcum* (T7) were also on par with triple inoculation (Table 1). Least (7.41 mg/plant) was recorded from uninoculated control (Table 1). Nitrogen content of shoot (mg/plant) was significantly higher in plants receiving triple microorganism consortium (63.37 mg/plant). Dual inoculation of *P. fluorescens* + *A. chroococcum* (T6) and *Acinetobacter* sp. + *A. chroococcum* (T7) were also on par with triple inoculation (Table 1). Least (14.59 mg/plant) was observed from uninoculated control (Table 1). Enhance in nitrogen uptake may be a result of nitrogen fixation by *A. chroococcum* [13], its ability to produce growth hormones [14] and synergistic effects with *P.*



A=T2, T1; B=T3, T1; C=T4, T1; D=T5, T1; E=T6, T1; F=T7, T1; G=T8, T1; H=T1(+NPK) and triple, dual, single, control (-NPK) Treatments: T1= Control; T2= *Pseudomonas fluorescens* (Pf); T3= *Acinetobacter* sp. (Aci); T4= *Azotobacter chroococcum* (Azo); T5= Pf + Aci.; T6= Pf + Azo; T7= Aci + Azo; T8= Pf + Aci + Azo

Figure 1: Effect of fluid bed dried inoculant formulations in finger millet (*Eleusine coracana* Gaertn.)

fluorescens and *Acinetobacter* sp. which might be a reason of improved nitrogen uptake and plant growth [15].

Phosphorus content

Phosphorus content of the roots (mg/plant) found highest in plants receiving triple inoculation (1.63 mg/plant) and least (0.84 mg/plant) from uninoculated control (Table 2). Triple and a dual inoculants formulations containing *P. fluorescens* + *A. chroococcum* recorded highest shoot phosphorus content (3.28 mg/plant) and least from uninoculated control (1.68 mg/plant). Shoot phosphorus of finger millet was observed higher in the treatments receiving *Acinetobacter* sp. (T3, T5, T7 and T8) as a constituent (Table 2). *Acinetobacter* sp. enhanced the phosphorus availability to the plants in individual and consortium [16]. This effect of *Acinetobacter* sp. in finger millet may be due to its phosphorus solubilisation and plant growth promotion by producing growth hormones like gibberellins which help the plant in nutrient uptake and building up its biomass as discussed by Kang et al. [17]. The synergistic effects with other constituents of consortium were also visible.

Chlorophyll content

Highest chlorophyll-a content was recorded highest in plants of two treatments receiving dual inoculation of *P. fluorescens*+ *Acinetobacter* sp. (T5, 1.88 mg/g leaf) and *Acinetobacter* sp.+ *A. chroococcum* (T7, 1.87 mg/g leaf); and least (T1, 0.88 mg/g leaf) from uninoculated control plants. Similar trend was observed for chlorophyll-b content (Table 3). The effect of nitrogen fixation by *A. chroococcum* and PGPR activity of *P. fluorescens* and *Acinetobacter* sp. might be one of the reasons in improving overall plant growth and chlorophyll content. These results are similar to those obtained by Kang [17] and Shehata [18].

Plant height

Plants receiving triple inoculation recorded maximum plant height (66.31 cm) and also from other treatments contained *Acinetobacter* sp. as a constituent. Least (45.03 cm) was reported from uninoculated control (Graph 1). Combined effect of phosphorus solubilisation and plant growth promotion by *Acinetobacter* sp. and other inoculants might be one of the reasons for improvement in plant height [16].

Dry weight of plants

Highest root and shoot dry weight was observed in plants receiving triple inoculation (0.61 and 1.13 g/plant respectively) and least (0.35 and 0.61 g/plant respectively) from uninoculated control (Table 4). Total dry weight of plant was highest in triple inoculation in -NPK plants (Graph 2). This could be due to the plant bacterial interaction in rhizosphere which provide nitrogen (*A. chroococcum*), solubilize phosphorus (*Acinetobacter* sp.) and plant growth promotion by all three microbes. Various direct and indirect effects by these microorganisms like production of auxins, cytokinins, gibberellins, anti-pathogenic compounds, secondary metabolites and siderophores for plant growth promotion and suppression of growth of harmful microorganisms [19,20] might be one of the reasons. Consortium of microorganisms that interacts synergistically by providing nutrients and stimulating each other through physical and biochemical activities have beneficial effects on plant growth and dry weight. These results are in agreement with findings of Rather [5] and Abbasi et al. [21].

Conclusion

The efforts made in this investigation with forethought of microbial consortium in a novel inoculant formulation brought about remarkable outcomes. *P. fluorescens* and *A. chroococcum* with a rather new agriculturally important microorganism (AIM) *Acinetobacter* sp.

Table 1: Nitrogen content of root and shoot (mg/plant) as influenced by inoculation of fluid bed dried inoculant formulations in finger millet (*Eleusine coracana* Gaertn.).

Treatments	Root nitrogen content			Shoot nitrogen content		
	+NPK	-NPK	Main effect of treatment	+NPK	-NPK	Main effect of treatment
T1 (C)	13.92 ^b	0.90 ^c	7.41 ^c	27.73 ^d	1.45 ^d	14.59 ^d
T2 (Pf)	25.48 ^a	4.73 ^{bc}	15.10 ^b	60.54 ^c	4.76 ^{cd}	32.65 ^c
T3 (Aci)	27.65 ^a	3.16 ^{bc}	15.40 ^b	68.21 ^{bc}	5.73 ^{bcd}	36.97 ^c
T4 (Azo)	32.29 ^a	3.96 ^{bc}	18.13 ^{ab}	65.71 ^c	4.25 ^d	34.98 ^c
T5 (Pf+ Aci)	28.44 ^a	7.55 ^b	18.00 ^{ab}	75.36 ^{abc}	14.74 ^{bc}	45.05 ^{bc}
T6 (Pf+ Azo)	26.08 ^a	3.80 ^{bc}	14.94 ^b	103.67 ^a	10.33 ^{bcd}	57.00 ^{ab}
T7 (Aci+ Azo)	28.73 ^a	7.24 ^b	17.99 ^{ab}	101.48 ^a	14.97 ^b	58.22 ^{ab}
T8 (Pf+Aci+ Azo)	33.40 ^a	12.47 ^a	22.93 ^a	96.71 ^{ab}	30.03 ^a	63.37 ^a
LSD at 5%	10.88	4.46	5.65	30.23	10.16	15.33

Note- Means with same superscript within a column are on par at P=0.05 and data is interpreted on the basis of main treatment effect

Table 2: Phosphorus content of root and shoot (mg/plant) as influenced by inoculation of fluid bed dried inoculant formulations in finger millet (*Eleusine coracana* Gaertn.).

Treatments	Root phosphorus content			Shoot phosphorus content		
	+NPK	-NPK	Main effect of treatment	+NPK	-NPK	Main effect of treatment
T1 (C)	1.51 ^c	0.17 ^c	0.84 ^b	3.13 ^c	0.24 ^d	1.68 ^c
T2 (Pf)	2.47 ^{ab}	0.51 ^{abc}	1.49 ^a	4.17 ^{bc}	0.53 ^{cd}	2.35 ^{bc}
T3 (Aci)	2.48 ^a	0.38 ^{bc}	1.43 ^a	4.57 ^{bc}	0.55 ^{cd}	2.56 ^{ab}
T4 (Azo)	2.50 ^a	0.34 ^{bc}	1.42 ^a	4.11 ^{bc}	0.48 ^{cd}	2.30 ^{bc}
T5 (Pf+ Aci)	2.09 ^{ab}	0.68 ^{ab}	1.38 ^{ab}	4.54 ^{bc}	1.08 ^b	2.81 ^{ab}
T6 (Pf+ Azo)	1.56 ^b	0.31 ^c	0.93 ^b	6.01 ^a	0.75 ^{bc}	3.38 ^a
T7 (Aci+ Azo)	1.69 ^{ab}	0.67 ^{ab}	1.18 ^{ab}	5.33 ^{ab}	1.11 ^b	3.22 ^a
T8 (Pf+Aci+ Azo)	2.41 ^{ab}	0.84 ^a	1.63 ^a	4.75 ^{bc}	1.81 ^a	3.28 ^a
LSD at 5%	0.91	0.36	0.47	1.69	0.44	0.84

Note- Means with same superscript within a column are on par at P=0.05 and data is interpreted on the basis of main treatment effect

Table 3: Chlorophyll-a and chlorophyll-b content (mg/g leaf) as influenced by inoculation of fluid bed dried inoculant formulations in finger millet (*Eleusine coracana* Gaertn.).

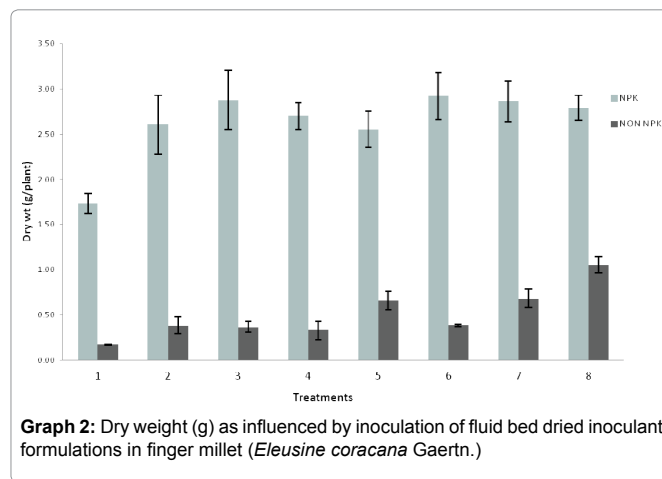
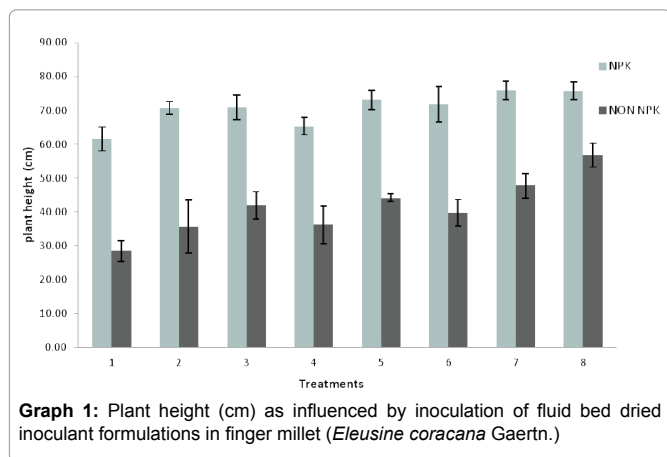
Treatments	Chlorophyll-a content (mg/g leaf)			Chlorophyll-b content (mg/g leaf)		
	+NPK	-NPK	Main effect of treatment	+NPK	-NPK	Main effect of treatment
T1 (C)	1.12 ^f	0.65 ^a	0.88 ^f	0.53 ^d	0.31 ^e	0.42 ^f
T2 (Pf)	1.20 ^f	0.91 ^f	1.06 ^e	0.70 ^b	0.38 ^e	0.54 ^{de}
T3 (Aci)	1.57 ^e	1.40 ^{be}	1.48 ^d	0.59 ^c	0.40 ^d	0.49 ^e
T4 (Azo)	1.98 ^b	1.49 ^b	1.73 ^b	0.83 ^a	0.53 ^{bc}	0.68 ^b
T5 (Pf+ Aci)	2.02 ^{ab}	1.74 ^a	1.88 ^a	0.85 ^a	0.69 ^a	0.77 ^a
T6 (Pf+ Azo)	1.87 ^c	1.39 ^{be}	1.63 ^c	0.68 ^b	0.56 ^b	0.62 ^c
T7 (Aci+ Azo)	2.09 ^a	1.66 ^a	1.87 ^a	0.86 ^a	0.62 ^{ab}	0.74 ^a
T8 (Pf+Aci+ Azo)	1.70 ^d	1.34 ^e	1.52 ^d	0.66 ^{bc}	0.48 ^{cd}	0.57 ^{cd}
LSD at 5%	0.09	0.14	0.08	0.08	0.10	0.06

Note- Means with same superscript within a column are on par at P=0.05 and data is interpreted on the basis of main treatment effect

Table 4: Root and shoot dry weight (g/plant) as influenced by inoculation of fluid bed dried inoculant formulations in finger millet (*Eleusine coracana* Gaertn.).

Treatments	Root dry weight			Shoot dry weight		
	+NPK	-NPK	Main effect of treatment	+NPK	-NPK	Main effect of treatment
T1 (C)	0.62 ^b	0.07 ^c	0.35 ^c	1.12 ^b	0.10 ^d	0.61 ^c
T2 (Pf)	0.96 ^a	0.20 ^{bc}	0.58 ^a	1.64 ^{ab}	0.19 ^{cd}	0.92 ^{bc}
T3 (Aci)	1.05 ^a	0.16 ^{bc}	0.60 ^a	1.83 ^a	0.21 ^{cd}	1.02 ^{ab}
T4 (Azo)	1.01 ^a	0.14 ^c	0.58 ^a	1.69 ^{ab}	0.19 ^{cd}	0.94 ^b
T5 (Pf+ Aci)	0.83 ^a	0.26 ^{ab}	0.55 ^{ab}	1.72 ^{ab}	0.40 ^b	1.06 ^{ab}
T6 (Pf+ Azo)	0.62 ^b	0.12 ^c	0.37 ^b	2.30 ^a	0.26 ^{bc}	1.28 ^a
T7 (Aci+ Azo)	0.70 ^b	0.27 ^{ab}	0.48 ^{ab}	2.17 ^a	0.42 ^b	1.29 ^a
T8 (Pf+Aci+ Azo)	0.87 ^a	0.35 ^a	0.61 ^a	1.92 ^a	0.70 ^a	1.31 ^a
LSD at 5%	0.36	0.15	0.19	0.66	0.16	0.33

Note- Means with same superscript within a column are on par at P=0.05 and data is interpreted on the basis of main treatment effect



in a consortium resulted in a superior inoculant formulation for crop plants combining proficiency of these microorganisms for inclusive plant growth and development. At the same time, on the other hand the fluid bed dryer formulation sustained more number of viable cells indispensable for performance of any microorganism in a formulation. FBD formulation not only gives stability in the performance but also reduce contaminants to a permissible level. This finding opens a contemporary vista of practice of biofertilizers in a demanding world which would otherwise face obscurity in present scenario.

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