Available online at www.ijpab.com

DOI: http://dx.doi.org/10.18782/2320-7051.2442

ISSN: 2320 – 7051

Int. J. Pure App. Biosci. **5 (1):** 395-399 (2017)

Research Article

International Journal of Pure & Applied Bioscience

OPEN ACCESS

Influence of Agriculturally Beneficial Microbial Formulations in Survival on Seeds of Cowpea and Finger Millet

Lavanya*, G. and Brahmaprakash, G. P.

Department of Agricultural Microbiology, GKVK, Bengaluru

*Corresponding Author E-mail: lavanya.agri@gmail.com

Received: 11.01.2017 | Revised: 20.01.2017 | Accepted: 23.01.2017

ABSTRACT

A study on survival of fluid bed dried and lignite formulations of agriculturally beneficial microorganisms on seeds of cowpea and finger millet was undertaken to know the efficacy of bio formulations where these biological formulations applied to seeds helps to deliver the agents to the spermosphere and the plants obtain a conducive environment. Seed treatment has the potential to deliver microbiological agents in the right amount, at right time and at right place. The seeds treated with fluid bed dried and lignite formulations at single, dual and triple combinations were tested for the survival of microbial inoculants at five different intervals of seed treatment (0 4, 8, 16 and 24 hrs) for both cowpea and finger millet by drop plate method. The higher survival of inoculants was observed in triple inoculants formulation followed by dual and single inoculants in fluid bed dried formulations when compared to lignite based formulation.

Key words: *Fluid Bed Dried Formulation, Lignite Formulation, Cowpea, Finger Millet, Microbial Survival on Seed*

INTRODUCTION

Application of plant growth promoting rhizobacteria is done through several means based on survival nature and mode of action of the pathogen. The methods used most commonly are through seed treatment, root dip, soil application, foliar spray, etc. An ideal formulation is expected to facilitate the delivery of the living biological agents in its active state, at the right place and at the right time. Biological formulations applied to seeds greatly help to deliver the agents to the spermosphere of plants where in extremely conducive environment prevail. Seed treatment has the potential to deliver microbiological agents in the right amount, at right time and at right place[4]. Soil application requires large volumes of inoculant for adequate distribution so inoculation of seed is a more economical way of introducing inoculant microorganisms to the rhizosphere. These inoculant microorganisms in rhizosphere exerts beneficial effects on plant growth[2] as it act like phytostimulators, regulates plant growth, production of antibiotics and also by providing nutrients like N, P, Zn, etc.

Cite this article: Lavanya, G. and Brahmaprakash, G.P., Influence of Agriculturally Beneficial Microbial Formulations in Survival on Seeds of Cowpea and Finger Millet, *Int. J. Pure App. Biosci.* **5(1):** 395-399 (2017). doi: http://dx.doi.org/10.18782/2320-7051.2442

In addition, few of the crop beneficial microbes have unique tolerance to harsh environments including drought stress[1], salt stress[8], high temperature and also in contaminated environments[3] this shows the ability of these PGPR for contributing in higher production of crops in such areas. The application of microbial inoculants to seeds is likely to increase with increasing public awareness of the potential environmental and health hazards of agrochemicals. Hence, a study was conducted to know the survival of fluid bed dried formulations on seeds of cowpea and finger millet crop.

MATERIAL AND METHODS

A laboratory study was conducted in the Department of Agricultural Microbiology, UAS, GKVK, Bengaluru. The fluid bed dried and lignite formulations of 6 different agriculturally beneficial microorganisms were used. Preparation of inoculants formulation was done according to the procedure of Lavanya *et al*.[6]. Seven different treatments of two consortia in single, dual and triple combinations using milk powder+ Talc+ one per cent sugar+ two per cent gelatin + centrifuged cells were prepared using the microorganisms; *Pseudomonas fluorescens*, *Bacillus megaterium*, *Rhizobium* sp., considering their role in plant growth promotion, P-solubilisation and nitrogen (N) fixing capability respectively for cowpea. Another microbial consortium of *Acinetobacter* sp., *Azotobacter chroococcum* and *Bacillus subtilis* with their role in P solubilization, N- fixation and biocontrol activity respectively was prepared for finger millet crop using Fluid bed dryer. Lignite based formulations were prepared by using these microorganisms at the ratio of 1:4 (broth culture: lignite).

Survival of single, dual and triple inoculant formulations in cowpea and finger millet seeds

Cowpea and finger millet seeds were surface sterilized with 70 per cent alcohol for one minute and with sodium hypochlorite (1%) for three minutes. Seeds were washed six times with sterile water. Surface sterilized seeds were then treated with fluid bed dried inoculants formulation and lignite inoculant formulation. Surface sterilized seeds were pre-coated with gum-arabica sticker solution (4 %) and treated with FBD and lignite inoculant at the rate of 20.0 g / kg seed of cowpea and 75 g/ kg of seeds of finger millet seeds (Package of Practice).

Immediately after seed treatment, 10 seeds were placed in a conical flask containing 10 ml diluent (0.85% NaCl and 0.01% Tween 80) and was mixed thoroughly for one minute. Viable counts per ml of diluent were determined by following drop-plate method[11]. The microbial population at different hours of seed treatment was recorded. Viable counts per seed were calculated by dividing viable counts per ml of diluent by the number of inoculant treated seeds placed in diluent. Similarly, viable counts per seed were determined at 4, 8, 16 and 24 hours interval after seed treatment.

The survival study of microbial inoculants was analyzed by Complete Randomized Block Design (CRD)[7].

RESULTS AND DISCUSSION

Survival of viable cells of inoculant microorganisms on seeds at the time of seed germination is important for plant growth promotion, nitrogen fixation, phosphorus solubilization and biocontrol activity. Seed inoculation of N-fixers and P-solubilizers is one of the widely accepted and most commonly used and easiest means of inoculation. Hence, this study was undertaken to determine the influence of fluid bed dried and lignite based inoculant formulations on survival of cowpea and finger millet seeds.

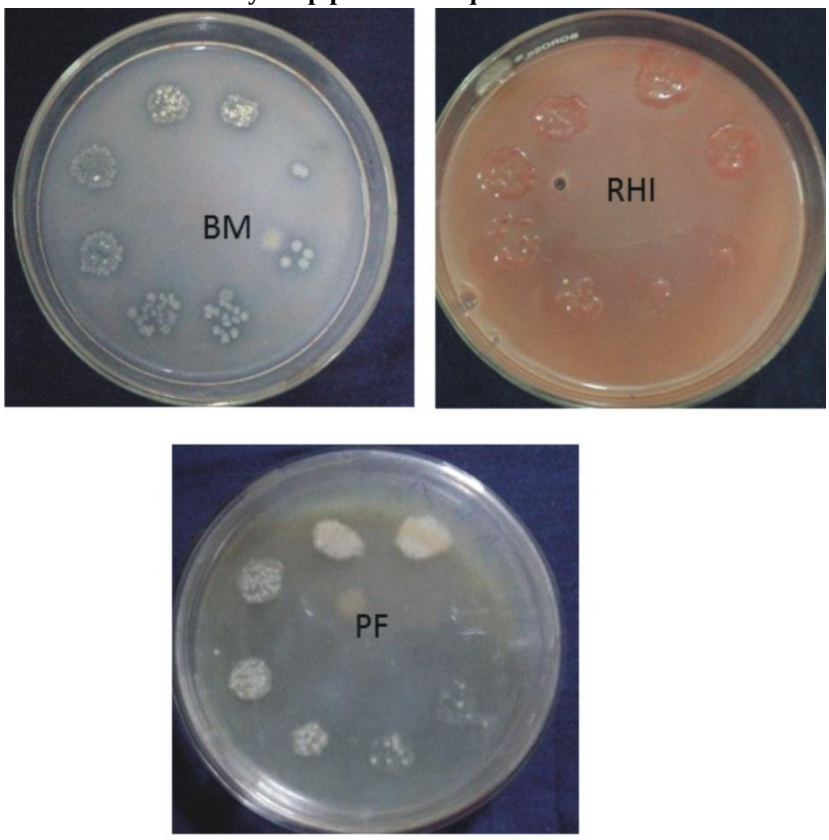
Survival of inoculants in cowpea and finger millet crop

In fluid bed dried formulation, it was observed that the cowpea seeds treated with single, dual and triple inoculant formulation containing *Pseudomonas fluorescens*, *Bacillus megaterium* and *Rhizobium* sp., showed maximum cell numbers at 24 hours of seed treatment (Table 1). There was a gradual

increase in cell numbers from 0 to 24 hours of incubation. Triple inoculant formulation of *Pseudomonas fluorescens, Bacillus megaterium* and *Rhizobium* sp. supported a maximum of $\log_{10}$ 6.50, $\log_{10}$ 6.50 and $\log_{10}$ 6.53 cells/ seed at 24 hours of incubation period (Table 1 and Plate 1) where as the lignite formulation recorded a maximum of $\log_{10}$ 6.50, $\log_{10}$ 6.47 and $\log_{10}$ 6.51 cells/ seed respectively at 24 hours of seed treatment (Table 1 and Plate 1). Additives such as gum arabica and xanthan gum are used to prolong the survival of microbial agents applied to seeds. Similar findings were reported where the survival of inoculants microorganism (*Rhizobium* sp.) on seeds may be enhanced by addition of sticky additives to the carriers such as gum arabica, methocel, mixture of sucrose and sodium glutamate[10] and also the Talc - glucose, Talc - yeast and Cellulose – clay based powder formulations results in highest levels of viability[9].

Similarly the triple inoculant fluid bed dried formulation of *Azotobacter chroococcum, Acinetobacter* sp. and *Bacillus subtilis* in finger millet seeds recorded $\log_{10}$ 6.10 cells/ seed, $\log_{10}$ 6.04 cells/ seed, $\log_{10}$ 6.18 cells/ seed at 0 hours and reached its maximum at 24 hours which recorded $\log_{10}$ 6.46 cells/ seed, $\log_{10}$ 6.48 cells/ seed and $\log_{10}$ 6.51 cells/ seed respectively when compared to dual and single inoculants formulation (Table 2). The lignite formulation of triple inoculants treatment showed higher survival of inoculants on finger millet seeds which reached its maximum of $\log_{10}$ 6.45, $\log_{10}$ 6.47 and $\log_{10}$ 6.48 cells/ seed respectively at 24 hours interval of seed treatment (Table 2). The higher survival of microbial population in triple inoculants was observed in fluid bed dried followed by lignite formulations. The results were on par with the study who reported that the seed treatment of formulation prepared using *Bacillus subtilis* showed higher viability up to one year which was recorded in talc based and lignite based powder formulation[5].

Plate 1: Survival of inoculant microorganisms on cowpea seeds treated with fluid bed dried formulation by drop plate technique method



BM: ***Bacillus megaterium*** **RHI:** ***Rhizobium sp. ;*** **PF:** ***Pseudomonas fluorescens***

Table 1: Survival of microbial inoculants on seeds of cowpea (*Vigna unguiculata* L.) in fluid bed dried and lignite based formulation at different intervals

Sl No	Treatments		Population density (log $_{10}$ CFU/ seed of cowpea)									
			FBD					Lignite				
			Duration (Hours)									
			0	4	8	16	24	0	4	8	16	24
1	*Pseudomonas fluorescens*		5.52^{f}	6.26^{bc}	6.36^{bc}	6.38^{cde}	6.46^{bc}	5.48^{d}	6.29^{ab}	6.43^{a}	6.37^{ab}	6.44^{ab}
2	*Bacillus megaterium.*		6.10^{ab}	6.11^{d}	6.21^{e}	6.34^{e}	6.47^{abc}	6.15^{ab}	6.15^{c}	6.16^{bc}	6.33^{b}	6.46^{ab}
3	*Rhizobium* sp.		5.64^{ef}	5.90^{e}	6.03^{f}	6.36^{de}	6.41^{c}	5.57^{d}	5.97^{d}	6.08^{cd}	6.36^{ab}	6.47^{ab}
4	*Pseudomonas fluorescens + Bacillus megaterium*	*Pseudomonas fluorescens*	6.17^{ab}	6.25^{bcd}	6.33^{cd}	6.44^{bc}	6.48^{ab}	6.18^{a}	6.18^{bc}	6.40^{a}	6.40^{ab}	6.47^{ab}
		Bacillus megaterium	6.03^{bc}	6.14^{cd}	6.18^{e}	6.47^{ab}	6.51^{ab}	6.12^{ab}	6.20^{bc}	6.23^{b}	6.44^{a}	6.50^{a}
5	*Pseudomonas fluorescens + Rhizobium* sp.	*Pseudomonas fluorescens*	6.08^{ab}	6.37^{b}	6.46^{a}	6.43^{bcd}	6.51^{ab}	6.22^{a}	6.39^{a}	6.49^{a}	6.40^{ab}	6.48^{ab}
		Rhizobium sp.	5.76^{de}	6.15^{cd}	6.20^{e}	6.42^{bcd}	6.41^{c}	5.89^{c}	6.23^{bc}	6.20^{b}	6.39^{ab}	6.39^{b}
6	*Bacillus megaterium.+ Rhizobium* sp	*Bacillus megaterium*	5.89^{cd}	6.15^{cd}	6.18^{e}	6.44^{bc}	6.50^{ab}	5.95^{bc}	6.15^{c}	6.18^{bc}	6.40^{ab}	6.50^{a}
		Rhizobium sp	5.78^{de}	6.15^{cd}	6.08^{f}	6.43^{bcd}	6.49^{ab}	5.86^{c}	5.97^{d}	6.00^{d}	6.43^{a}	6.49^{a}
7	*Pseudomonas fluorescens. + Bacillus megaterium.+ Rhizobium* sp.	*Pseudomonas fluorescens*	6.17^{ab}	6.88^{a}	6.41^{abc}	6.44^{bc}	6.50^{ab}	6.22^{a}	6.23^{bc}	6.45^{a}	6.37^{ab}	6.50^{a}
		Bacillus megaterium	6.10^{ab}	6.32^{b}	6.25^{de}	6.37^{cde}	6.50^{ab}	6.18^{a}	6.14^{c}	6.12^{bc}	6.45^{a}	6.47^{ab}
		Rhizobium sp.	6.20^{a}	6.37^{b}	6.44^{ab}	6.52^{a}	6.53^{a}	6.18^{a}	6.28^{ab}	6.39^{a}	6.45^{a}	6.51^{a}
LSD at 5%			**0.14**	**0.15**	**0.09**	**0.73**	**0.07**	**0.22**	**0.13**	**0.12**	**0.1**	**0.1**

NOTE: Means with same superscript within similar hours of storage do not differ significantly at P=0.05with DMRT, FBD: fluid bed dryer

Table 2: Survival of inoculant microorganisms in fluid bed dried and lignite based formulation on finger millet (*Eleusine coracana* Gaertn.) seeds at different intervals

Sl no	Treatments		Population density (log $_{10}$ CFU/ seed of Finger millet)									
			FBD					Lignite				
			Duration (Hours)									
			0	4	8	16	24	0	4	8	16	24
1	*Azotobacter chroococcum*		5.43^{c}	6.14^{bc}	6.27^{cde}	6.28^{cd}	6.36^{de}	5.43^{c}	6.21^{ab}	6.29^{bcd}	6.28^{e}	6.35^{cd}
2	*Acinetobacter* sp.		6.03^{abc}	6.09^{c}	6.17^{e}	6.18^{d}	6.30^{e}	6.03^{ab}	6.12^{b}	6.15^{e}	6.14^{d}	6.29^{d}
3	*Bacillus subtilis*		5.52^{bc}	6.31^{a}	6.34^{bcd}	6.30^{bcd}	6.44^{abcd}	5.43^{c}	6.29^{a}	6.34^{ab}	6.30^{bc}	6.44^{ab}
4	*Azotobacter chroococcum + Acinetobacter* sp.	*Azotobacter chroococcum*	6.22^{a}	6.23^{abc}	6.34^{bcd}	6.43^{ab}	6.39^{cde}	6.07^{a}	6.20^{ab}	6.32^{abc}	6.30^{bc}	6.44^{ab}
		Acinetobacter sp.	6.04^{abc}	6.22^{abc}	6.43^{ab}	6.43^{ab}	6.47^{abc}	6.01^{ab}	6.20^{ab}	6.33^{abc}	6.42^{a}	6.47^{ab}
5	*Acinetobacter* sp *+ Bacillus subtilis*	*Acinetobacter* sp.	6.18^{ab}	6.22^{abc}	6.32^{bcd}	6.43^{ab}	6.47^{abc}	6.18^{a}	6.20^{ab}	6.38^{ab}	6.39^{abc}	6.48^{a}
		Bacillus subtilis	5.90^{abc}	6.27^{ab}	6.29^{cde}	6.41^{abc}	6.40^{bcd}	5.85^{b}	6.27^{ab}	6.29^{bcd}	6.40^{ab}	6.39^{bc}
6	*Azotobacter chroococcum + Bacillus subtilis*	*Azotobacter chroococcum*	5.86^{abc}	6.23^{abc}	6.23^{de}	6.36^{abc}	6.48^{abc}	5.83^{b}	6.19^{ab}	6.18^{de}	6.35^{abc}	6.48^{a}
		Bacillus subtilis	5.86^{abc}	6.15^{bc}	6.40^{abc}	6.41^{abc}	6.49^{ab}	5.86^{b}	6.11^{b}	6.43^{a}	6.37^{abc}	6.47^{ab}
7	*Azotobacter chroococcum+ Acinetobacter* sp..+ *Bacillus subtilis*	*Azotobacter chroococcum*	6.10^{abc}	6.15^{bc}	6.26^{de}	6.41^{abc}	6.46^{abc}	6.09^{a}	6.11^{b}	6.26^{bcde}	6.41^{ab}	6.45^{ab}
		Acinetobacter sp.	6.04^{abc}	6.25^{ab}	6.24^{de}	6.41^{abc}	6.48^{abc}	6.029^{ab}	6.22^{ab}	6.20^{cde}	6.40^{ab}	6.47^{ab}
		Bacillus subtilis	6.18^{ab}	6.32^{a}	6.50^{a}	6.48^{a}	6.51^{a}	6.17^{a}	6.29^{a}	6.43^{a}	6.45^{a}	6.48^{a}
LSD at 5 %			**0.67**	**0.15**	**0.14**	**0.14**	**0.10**	**0.013**	**0.17**	**0.13**	**0.11**	**0.09**

Note: Means with same superscript within same intervals do not differ significantly at P=0.05 with DMRT, FBD: fluid bed dryer

CONCLUSION

Survival of inoculant formulations on seeds of cowpea (*Vigna unguiculata* L.) and finger millet (*Eleusine coracana* Gaertn.) crop were determined at different intervals of incubation where the consortia of microorganisms resulted in higher multiplication and survival of cells followed by dual and single inoculants formulations. The increase in microbial population has shown that seed treatment is one best among the different types of application which has helped to deliver microbial inoculants in a better way.

Acknowledgement

Funding received from Department of Biotechnology (DBT) for development of FBD inoculants and consortium of agriculturally beneficial microorganisms and Dept. of Agricultural Microbiology, UAS, GKVK, Bangaluru is gratefully acknowledged.

REFERENCES

1. Arzanesh, M. H., Alikhani H. A, Khavazi, K., Rahimian, H. A., and Miransari, M., Wheat (*Triticum aestivum* L.) growth enhancement by *Azospirillum* sp. under drought stress. *World Journal of Microbiology and Biotechnology* **27:** 197–205 (2011).
2. Bakker, P. A. H. M., Raaijmakers, J. M., Bloemberg, G. V., Hofte, M., Lemanceau, P., and Cooke, M., New perspectives and approaches in plant growth-promoting rhizobacteria research. *European Journal of Plant Pathology* **119:** 241–242 (2007).
3. Burd, G. I., Dixon, D. G., and Glick, B. R.., Plant growth-promoting bacteria that decrease heavy metal toxicity in plants. *Canadian Journal of Microbiology* **46:** 237–245 (2000).
4. Chandra, K., and Greep, S., Liquid based bio-fertilizers. *Journal of Eco-Friendly Agriculture* **5(1):** 1-7 (2010).
5. Jayaraj, J., Radhakrishnan, N.V., Kannan, R., Sakthivel. K., Suganya, D., Venkatesan, S., and Velazhahan, R., Development of new formulations of *Bacillus subtilis* for management of tomato damping-off caused by *Pythium aphanidermatum*. *Biocontrol Sci. Technol*., **15:** 55-65 (2005).
6. Lavanya, G., Sahu, P. K., Manikanta, D. S, and Brahmaprakash, G. P., Effect of fluid bed dried formulation in comparision with lignite formulation of microbial consortium on Finger millet (*Eleucine coracana* Gaertn.). *Journal of pure and applied Microbiology*.,**9:** 193-199 (2015).
7. Little, T. M., and Hills, J. F., Agricultural experimentation. John Wiley and sons, New York, USA (1978).
8. Mayak, S., Tirosh, T., and Glick, B. R., Plant growth-promoting bacteria confer resistance in tomato plants to salt stress. *Plant Physiology and Biochemistry* **42:** 565–572 (2004).
9. Omer, M. A., Bioformulations of *Bacillus* Spores for using as Biofertilizer. *Life Science Journal* **7:** 124-131 (2010).
10. Salema, M. P., Parker, C. A., and Kidby, D. K., Death of rhizobia on inoculated seed. *Soil Biology and Biochemistry* **14:** 13-14 (1982).
11. Somasegaran, P., and Hoben, H. J., Methods in legume *Rhizobium* Technology. Department of Agronomy and Soil Science, University of Hawaii. pp 367 (1985).