Assessment of Shelf Life and Quality of Biofertilizers using Tricalcium Phosphate as an Anticaking Agent and Aluminium Silicate as the Inert Carrier

R. Matura, V. Bahuguna, M. Bhandari, I. Thapa, S. Jain

ABSTRACT
Background: Bio-fertilizers are the substances which contain living microorganisms, when applied to soil, seeds and plant root these fertilizers increases soil fertility and promote growth of the plant. Biofertilizers help plants to utilize important mineral resources, phosphorous and nitrogen. Microorganisms like Rhizobacteria, fungi and algae which provide nutrient to the soil and which are produced commercially are known as biofertilizers. The microorganisms which present in biofertilizers are Rhizobium species, Pseudomonas species and Azospirillum species etc. These biofertilizers have potential to replace conventional chemical fertilizers. The quality of biofertilizers is utmost important as they have to be used by farmers and should work well when applied to the soil. It should not form clumps after preparation. In this study, anticaking property provided by tricalcium phosphate (TCP) to individual biofertilizer containing Pseudomonas, Rhizobium and Azospirillum respectively (each separately) was studied.

Methods: In our study, we have used serial dilution and direct count method (CFU) for checking viability of live microorganism for 15, 30 and 90 days duration in respective biofertilizers in our laboratory. Different percentage viz 5%, 10%, 15% and 20% of tricalcium phosphate (TCP) was used in addition to aluminium silicate as an inert carrier.

Conclusion: Our study has validated that all percentage (5%, 10%, 15% and 20%) of tricalcium phosphate (TCP) is reducing clump formation as compared to control with no TCP added. On the basis of plate count method (CFU result) 10% TCP is found to be optimum to be used as an anticaking agent for biofertilizer containing Pseudomonas, Rhizobium and Azospirillum respectively.

Key words: Anticaking agent, Biofertilizers, CFU, Microorganism, TCP.

INTRODUCTION
Plants for their survival and normal functioning require different minerals from soil. This can be achieved by normal physiological process such as osmosis. There are certain microorganisms which are present in soil and help in accelerating nutrient availability to plants. These soil microorganisms when used as an inoculum and when provided to unfertile soil increase the fertility of the soil. Thus they are named as biofertilizers. Biofertilizers are generally carrier based microbial preparations which contain beneficial microorganisms in a viable state for certain period of time and which when applied to seed or soil application enhances plant growth through nutrient uptake and also elevates growth hormone production (Brahmaprakash et al 2012). There are different definitions available in literature for biofertilizers. According to the definition proposed by Vessey (Vessey 2003), biofertilizers are substances which contain living microorganisms which, when applied to seed, plant surfaces, or soil, colonize the rhizosphere or the interior of the plant and promote growth by increasing the supply or availability of primary nutrients to the host plant. Seed inoculation of pigeonpea (Cajanus cajan) + mungbean (Phaseolus radiatus) with Rhizobium and phosphorous solubilizing bacteria (PSB) recorded significantly higher nutrient uptake (N and P2O5) (Singh et al 2013). Thus Rhizobium based biofertilizer along with Phosphate solubilizing bacteria (PSB) are important for plant growth.

Biofertilizers help in making the soil environment rich in all kinds of micro- and macro-nutrients via number of processes such as nitrogen fixation, mineralization as phosphate and potassium solubilization, release of plant growth regulating substances, production of antibiotics and biodegradation of organic matter in the soil (Sinha et al., 2014; Sivakumar et al., 2013) which provide efficient nutrient uptake and help in improving plant tolerance towards drought and moisture stress (Abdelraouf et al 2013). Sometimes combine treatment of urea and Rhizobium resulted in maximum plant growth as seen in cluster bean
In India Rhizobium was the first microbial inoculant, which was introduced as biofertilizer in the beginning of the seventies with the introduction of soybean into the country. Azospirillum and Azotobacter were added to the list in mid-nineties. Phosphate solubilising biofertilizer (PSB) was introduced in late nineties (Yadav et al. 2014). It is studied in Rhizobium based liquid biofertilizer that on addition of different polymeric additives; polyvinyl pyrrolidone, gum arabic and glycerol it support growth and promote survival of liquid inoculants (Rhizobium sp. strain MB1503) during the storage. (Sherawat et al. 2017).

Biofertilizer once made for commercial production purposes should pass the entire quality mandate. Quality of biofertilizer is one of the most important factors which has to be maintained, before it reaches to the farmers, thus proper quality control is required. It has to be properly assessed in laboratory itself where it is initially prepared that shelf life of biofertilizer should be for long time. According to forum for nuclear cooperation in Asia (FCNA) quality is meaning the number of selected microorganism in the active form per gram or milliliter biofertilizer. (Biofertilizer Manual by FNCA Biofertilizer, 2006).

It is necessary that biofertilizer produced should be of high standard quality and should pass all the quality mandates. It becomes utmost important to evaluate the produced inoculum from commercial units comparable with some reference values so it can be ensured that protocols are strictly followed as recommended by recognized laboratories. This is most crucial as several handling errors may occur during product generation at industrial level thus resulting in poor product quality. This may lead to quite dissatisfaction to both producers and users. Thus, specific protocols for checking the quality of microbial biofertilizers should be there for proper monitoring of commercial biofertilizers. Biofertilizers should have prolonged shelf life. The Biofertilizer should not form clumps when mixed with the soil. It should be free flowing so that it could mix well with the soil. To overcome this problem anticaking agents are routinely added to biofertilizers during their initial production. In recent years concern has been developed to find alternative to the mineral and chemical fertilizers for increased yield of crops. Since chemical fertilizers imposes health and some environmental consequences. There is need to replace these chemical fertilizers with other available alternatives. It is the crop nutrient uptake and crop yields that are the principal factors that determine optimum fertilization practices (Ju et al. 2011). Microbial inoculant is used as biofertilizers in recent past in agriculture sector which represents an attractive environmentally friendly alternative (Suyal et al. 2016). This new approach to agriculture farming is often referred to as sustainable agriculture.

**MATERIALS AND METHODS**

**Formulation of liquid biofertilizer inoculants**

The strains used for liquid biofertilizer formulation were *Azospirillum, Pseudomonas and Rhizobium*. Malic acid, dipotassiumhydrogen phosphate (K₂HPO₄), Ferrous sulphate (FeSO₄), Calcium chloride (CaCl₂), Manganese sulphate (MnSO₄), Sodium molybdate (Na₂MoO₄), Sodium chloride (NaCl) and Magnesium sulphate (MgSO₄) were used to culture *Azospirillum*. Glycerol, dipotassiumhydrogen phosphate (K₂HPO₄), Sodium chloride (NaCl) and Magnesium sulphate (MgSO₄) were used to culture *Rhizobium*. Glycerol, peptone, dipotassiumhydrogen phosphate (K₂HPO₄) and Magnesium sulphate (MgSO₄) were used to culture *Pseudomonas* respectively. The sterilized broths were inoculated with 2% pre-inoculum of the respective strains; pH maintained 6.8 for *Azospirillum* and *Rhizobium* and 7.0 for *Pseudomonas*. After incubation period microscopic observation is done to check for bacterial growth, contaminations and pH of the culture is also checked. Now Colony forming unit (CFU) of the overnight cultured broth is estimated using plate count method. Further fresh sterilized prepared media were inoculated with 1.0 ml respective overnight grown mother culture and incubated in BOD incubator at 28°C for 40 hours and 20 hours respectively.

Mixing of the given cultivated respective strain is done in inert carrier aluminium silicate and tricalcium phosphate is used as anticaking agent in different proportion viz 5%, 10%, 15% and 20% of the inert carrier. Shelf life of the formulation is checked using serial dilution and plate count method. This work is carried out in Biotechnology Laboratory in School of Applied and Life Sciences, Utranchal University Dehradun under Institutional funded project. The project was started in November 2019.

**Mixing of Bio-fertilizer with its carrier (Aluminium silicate) and anticaking agent (tri calcium phosphate).**

Each and every bio-fertilizer sample has to be mixed with an appropriate carrier in which the microbial activity remains stable for some period. After production of the liquid broth culture of each biofertilizer (*Azospirillum, Pseudomonas and Rhizobium*), the bioinoculant is mixed with its carrier i.e. aluminium silicate aseptically and packed. The ratio for mixing is fixed so that we can have particular moisture content which is required for microbial growth. Sometimes anticaking agent is also used to prevent clump formation. Generally clumps get formed due to high moisture content. In the present study tri calcium phosphate (TCP) is tested in different proportion (i.e. 5%, 10%, 15% and 20% of inert carrier Aluminium silicate) for *Azospirillum, Pseudomonas and Rhizobium* to be used as anticaking agent.

**For estimation of shelf life of particular bio-fertilizer**

For every bio-fertilizer shelf life is to be determined which gives an idea of using that particular sample before its activity gets demolished. The self-life of common carrier based biofertilzer is around six months. (Brar et al. 2012). In the present studies powder formulation shelf life of three independent biofertilizer formulation containing viz *Azospirillum, Pseudomonas and Rhizobium* is taken at...
RESULTS AND DISCUSSION

The shelf life of *Rhizobium* based bioinoculant with tricalcium phosphate (TCP) as an anticaking agent

After mixing anticaking agent (TCP) to respective bioinoculant it is seen that TCP is reducing clump formation in generally all the percentage (viz 5%, 10%, 15% and 20%) used to the inert carrier. On day one i.e. the very first day of mixing TCP to bioinoculant highest no of colonies were seen in 15% (2 x 10^8 cfu/gm) followed by 20% (2 x 10^8 cfu/gm each) of TCP. After 90 days on the basis of CFU result highest no of colonies were seen in 10% TCP (9 x 10^8 cfu/gm) followed by 5% TCP (4 x 10^7 cfu/gm) (Table 1).

The shelf life of *Pseudomonas* based bioinoculant with tricalcium phosphate (TCP) as an anticaking agent

After mixing anticaking agent (TCP) to respective bioinoculant it is seen that TCP is reducing clump formation in generally all the percentage (viz 5%, 10%, 15% and 20%) used to the inert carrier. At very first day of mixing TCP to bioinoculant highest no of colonies were observed in 10%, 15% and 20% (1 x 10^9 cfu/gm each) followed by 5% (9 x 10^8 cfu/gm). After 90 days based on the CFU result highest no of colonies were seen in 10% TCP (2 x 10^9 cfu/gm) followed by 5% TCP (1 x 10^7 cfu/gm) (Table 2). From table data it is found that when percentage of TCP is increased CFU count also increases (in 10% TCP) and then decreases afterwards this may be due to 10% TCP concentration is favoring the growth of the *Pseudomonas* as compared to other percentage of TCP.

The shelf life of *Azospirillum* based bioinoculant with tricalcium phosphate TCP as an anticaking agent

After mixing anticaking agent (TCP) to respective bioinoculant it is seen that TCP is reducing clump formation in generally all the percentage (viz 5%, 10%, 15% and 20%) used to the inert carrier. On the very first day of mixing TCP to bioinoculant highest no of colonies was observed in 15% (9 x 10^7 cfu/gm) followed by 20% (2 x 10^8 cfu/gm). After 90 days based on CFU result highest no of colonies were seen in 10% TCP (6 x 10^7 cfu/gm) followed by 5% and 15% TCP (2 x 10^7 cfu/gm each) (Table 3).

| % of TCP | After mixing | After 15 days | After 30 days | After 90 days |
|---------|-------------|---------------|---------------|---------------|
| 5%      | 1 x 10^7    | 1 x 10^7      | 1 x 10^7      | 4 x 10^7      |
| 10%     | 2 x 10^8    | 2 x 10^8      | 2 x 10^8      | 9 x 10^8      |
| 15%     | 3 x 10^8    | 1 x 10^9      | 2 x 10^9      | 1 x 10^9      |
| 20%     | 2 x 10^9    | 8 x 10^9      | 2 x 10^9      | 1 x 10^9      |

**Table 1:** CFU count of powder formulation at different proportion and different time interval for *Rhizobium* based biofertilizer.

**Conclusion**

The present studies showed that liquid biofertilizer inoculants when mixed with anticaking agent tricalcium phosphate and aluminium silicate as an inert carrier the clump formation has been reduced in all the proportions used. Nevertheless, shelf life of bioinoculant is also important as it states stability of the biofertilizers. It was seen in our studies that 10% TCP as an anticaking agent reduces clump formation in the tested bioinoculants and also it has shown good growth as estimated from CFU even after 90 days in all the bioinoculant...
Assessment of Shelf Life and Quality of Biofertilizers using Tricalcium Phosphate as an Anticaking Agent and Aluminium...

(\textit{Azospirillum, Pseudomonas and Rhizobium}) tested. Thus 10% tricalcium phosphate can be used as an anticaking agent in the stated biofertilizers for maximum efficacy.

REFERENCES

Abdelraouf, R.E., El-Habbasha, S.F., Hozayn, M., Hoballah, E. (2013). Water stress mitigation on growth, yield and quality traits of wheat (\textit{Triticum aestivum} L.) using biofertilizer inoculation. Journal of Applied Sciences Research. 9(3): 2135-2145.

Biofertilizer Manual by FNCA Biofertilizer, (2006). Japan Atomic Industrial Forum (JAIF).

Brahmaprakash, G.P. and Sahu, P.K. (2012). Biofertilizers for sustainability. Journal of the Indian Institute of Science. 92(1): 37-62.

Brar S.K., Sarma, S.J. and Chaabouni, E. (2012). Shelf life of biofertilizers: An accord between formulations and genetics. Journal of Fertilizers and Pesticides. doi:10.4172/2155-6202.1000e109.

Gul, A., Salam, A., Afridi, M.S., Bangash, N.K., Ali, F., Ali, M.Y., Khan, S. and Mubeen, R. (2019). Effect of urea, biofertilizers and their interaction on the growth, yield and yield attributes of \textit{Cyamopsis tetragonoloba}. Indian Journal of Agricultural Research. (53): 423-428.

Ju, X. and Christie, P. (2011). Calculation of theoretical nitrogen rate for simple nitrogen recommendations in intensive cropping systems: A case study on the North China Plain. Field Crops Res. 124: 450-458, doi:10.1016/j.fcr. 2011.08.002.

Sehrawat, A., Yadav, A., Anand, R.C., Kukreja, K. and Suneja, S. (2017). Enhancement of shelf life of liquid biofertilizer containing \textit{Rhizobium} sp. infecting mungbean (\textit{Vigna radiata} L.). Legume Research-An International Journal. 40: 684-690.

Singh, R., Malik, J.K., Thenua, O.V.S. and Jat, H.S. (2013). Effect of phosphorus and bio-fertilizer on productivity, nutrient uptake and economics of pigeonpea (\textit{Cajanus cajan}) + mungbean (\textit{Phaseolus radiatus}) intercropping system. Legume Research-An International Journal. 36: 41-48.

Sinha, R.K., Valani, D. and Chauhan, K. (2014) "Embarking on a second green revolution for sustainable agriculture by vermiculture biotechnology using earthworms: reviving the dreams of Sir Charles Darwin". International Journal of Agriculture and Biology. 1: 50-64.

Sivakumar, T., Ravikumar, M. and Prakash, M., Thamizhmani, R. (2013). Comparative effect on bacterial biofertilizers on growth and yield of green gram (\textit{Phaseolus radiatus} L.) and cow pea (\textit{Vigna siensis Edhl.}). International Journal of Current Research and Academic Review. 1(2) 20-28.

Suyal, D.C., Soni, R., Santosh Sai and Goel, R. (2016) Microbial Inoculants as Biofertilizer Microbial Inoculants in Sustainable Agricultural Productivity. 10.1007/978-81-322-2647-5_18 pp 311-318.

Yadav, A.K. and Chandra, K. (2014). Mass Production and Quality Control of Microbial Inoculants. Proc Indian Natn. Sci. Acad. 80(2): 483-489.

Vessey, J.K. (2003). Plant growth promoting rhizobacteria as biofertilizers. Plant Soil. 255: 571-586.