International Journal of Microbiology Research
ISSN: 0975-5276 & E-ISSN: 0975-9174, Volume 11, Issue 6, 2019, pp.-1598-1601.
Available online at https://www.bioinfopublication.org/jouarchive.php?opt=&jouid=BPJ0000234

Research Article

EVALUATION OF SHELF LIFE AND QUALITY OF CARRIER AND LIQUID BASED BIOFERTILIZERS

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Received: June 02, 2019; Revised: June 09, 2019; Accepted: June 12, 2019; Published: June 30, 2019

Abstract- In carrier-based inoculants, peat, wood charcoal and lignite are used as carriers and these inoculants suffer from poor quality, high contamination and unpredictable field performance. Whereas, Liquid biofertilizers of good quality hold great promise in agriculture. It contains special cell protectants or substances that encourage the formation of resting spores or cysts for longer shelf life and protect the cells against seed toxicity after seed application. In the present study, carrier and liquid based Biofertilizers (*Rhizobium* & PSB) were obtained from different firms to evaluate their quality. The shelf life of biofertilizers was estimated using suitable media for viable count. Microbial population of beneficial bacteria in carrier and liquid based Biofertilizers was monitored at monthly intervals. The microbial analysis revealed that there was a decreased in the population (viable count) and contamination of carried based biofertilizers was more when compared to liquid based biofertilizers. The carrier based biofertilizers maintained constant viable count only first three months when compared to liquid it is maintained up to five to six months.

Keywords- *Biofertilizers, Microbial population, Viable count, Quality*

Citation: Shravani K., *et al*., (2019) Evaluation of Shelf Life and Quality of Carrier and Liquid Based Biofertilizers. International Journal of Microbiology Research, ISSN: 0975-5276 & E-ISSN: 0975-9174, Volume 11, Issue 6, pp.-1598-1601.

Introduction

Biofertilizers are not fertilizers. Fertilizers directly increase soil fertility by adding nutrients. Biofertilizers add nutrients through the natural processes of fixing atmospheric nitrogen, solubilizing phosphorus, and stimulating plant growth through the synthesis of growth promoting substances. They can be seen as a technology aligned with principles of sustainable agriculture, as opposed to the increased use of pesticides and fertilizers in recent times [1]. Biofertilizers, one of the important components of the sustainable agriculture are products containing living microorganisms which have the ability to mobilize nutritionally important elements from non- usable to usable form through biological process. These are commercially available as solid products, powder, produced from peat, as granular form, or liquid inoculants using broth medium [2, 3]. In carrier-based inoculants, peat, wood charcoal and lignite are used as carriers and these inoculants suffer from poor quality, high contamination and unpredictable field performance. The population density of microbes in carrier based biofertilizers reduces day by day from the time of production. It is often difficult to uniformly mix peat-based inoculants with seeds. Solid-based inoculants also tend to plug precision air seeders [4]. Because of these difficulties, biofertilizer/bioinoculant producers have been changing to liquid inoculant formulations instead of solid-based inoculants. Liquid biofertilizers of good quality hold great promise in agriculture because of benefits over the conventional carrier based biofertilizers such as longer shelf life, better survival on seed and better nodulation; cost saving on carrier material such as pulverization, neutralization, sterilization, contamination free and convenience of handling, storage and transportation. Moreover, liquid inoculant coats the seeds uniformly and dries when applied through a seed auger. Seeds coated with liquid inoculant flow well when planted by using various types of seeding equipment. Hence, these liquid formulations of biofertilizer application in the field is easily and very simple. In recently they are applied along with inorganic fertilizers in drip irrigation as drip biofertigation [5] to achieve more yields and increase the nutrient efficiency of crop. Because of these results the liquid microbial biofertilizers is the only solution to maintain soil health and soil fertility [6].

Material and methods

Collection of biofertilizers

Carrier and liquid based Biofertilizers (*Rhizobium* & PSB) were collected from different firms and stored at 4°C in refrigerator [Table-1].

Sterilization of glassware and media

Glassware like Petri plates, test tubes, pipettes etc., was sterilized in the hot air oven at 180oC for half an hour before use. Media like yeast extract mannitol agar, nutrient agar, pikovskayas agar, potato dextrose agar, actinomycetes isolation agar was used to grow different bacteria and distilled water was sterilized in an autoclave at 15 lbs psi at (121°C) for 15 min.

Equipment and apparatus used

Hot air oven and autoclaves were used for sterilization of heat stable and media, respectively. BOD incubators were used for incubating cultures at different temperatures. Cultures were stored and maintained in a refrigerator at 4ºC. The pH was measured by using digital pH meter. Cyclomixer was used for homogenization during serial dilution. Plate mixer was used for spread plate technique. Quebech digital colony counter was used for counting the viable population of microorganisms. Compound electron microscope was used to observe the cell morphology of bacterial cultures.

Table-1 Details of different bioinoculants used in the present study

SN	Microbial inoculant	Type of formulations	Source / Production centre
1	*Rhizobium*	Liquid	K.N Biosciences (India). Pvt. Ltd.
		Carrier	K.N Biosciences (India). Pvt. Ltd. Agricultural Research Institute, PJTSAU, Rajendranagar. Agri Biotech Foundation, Rajendranagar, PJTSAU, Hyderabad.
2	PSB	Liquid	K.N Biosciences (India). Pvt. Ltd.
		Carrier	K.N Biosciences (India). Pvt. Ltd. Agricultural Research Institute, PJTSAU, Rajendranagar. Agri Biotech Foundation, Rajendranagar, PJTSAU, Hyderabad

Table-2 Evaluation of shelf life of carrier based biofertilizers collected from different production centers

Months	Carrier based biofertilizers (× 10^7 log no. of cfu g^{-1})					
	ARI		ABT		K.N. Biosciences. (India) Pvt. ltd.	
	Rhizobium	PSB	*Rhizobium*	PSB	*Rhizobium*	PSB
Mfg.date	Jul-16	Jul-16	Jul-16	Jul-16	Jul-16	Jul-16
Exp.date	Nov-16	Nov-16	Dec-16	Dec-16	Dec-16	Dec-16
Date of collection	06-Jul-16	06-Jul-16	04-Jul-16	04-Jul-16	10-Jul-16	10-Jul-16
July	6.84	6.67	7.34	6.79	7.4	7.34
August	6.79	6.63	6.26	5.73	7.15	6.23
September	5.72	5.54	5.18	5.69	6.43	6.08
October	4.61	4.45	5.08	4.51	6.28	5.2
November	4.58	4.34	4.15	4.26	4.08	4
December	3.36	3.34	3	4.08	4	3

Table-3 Evaluation of shelf life of liquid biofertilizers collected from K.N. Biosciences, India Pvt. Ltd

Months	Liquid (× 10^8 log no. of cfu ml^{-1})	
	Rhizobium	PSB
Mfg.date	Jul-16	Jul-16
Exp.date	Feb-16	Feb-16
Date of collection	10-Jul-16	10-Jul-16
July	8.38	8.15
August	7.28	7.11
September	7.08	6.4
October	6.32	6.18
November	6.26	5.15
December	6.08	4.28
January	5.23	4.2

Table-4 Evaluation of quality of (Initial) different formulations of biofertilizers collected from different production

Production centers	Type of biofertilizer Carrier	pH	Consistency	Moisture content (%)	Level of contaminants (log no. of cells cfu g^{-1} or ml^{-1})
ARI, Rajendranagar	*Rhizobium*	6.8	Clumps	35	0
ARI, Rajendranagar	PSB	6.7	Clumps	25	0
ABT	*Rhizobium*	6.5	Clumps	45	0
ABT	PSB	6.8	Clumps	35	0
K.N Biosciences	*Rhizobium*	6.8	Powdery	35	0
K.N Biosciences	PSB	7	Powdery	45	0
Liquid biofertilizers					
K.N. Biosciences	*Rhizobium*	7	Thick liquid	-	0
K.N. Biosciences	PSB	7	Thick liquid	-	0

Viable cell count

By using standard serial dilution plate count method, the collected microbial inoculants of *Rhizobium* and phosphate solubilizing bacteria were analyzed for checking the viable population [7] and plating on selective media. Triplicated plates incubated at 28 ± 2°C in the incubator. The microbial colonies appeared after the stipulated time of incubation was counted as colony forming units per gram (cfu g^{-1}) fresh weight of the sample. For analysis of *Rhizobium* the 1.0 ml sample was taken and different dilutions was prepared and 10^{-6} to 10^{-8} dilutions were taken and plated on YEMA plates. Whereas, for phosphate solubilizing bacteria 10^{-5} to 10^{-7} dilutions were used to enumerate the bacteria.

Results and Discussion

Shelf life of carrier based biofertilizers (× 10^7 log no. of cells cfu g^{-1})

Rhizobium-carrier based biofertilizer was collected on July 6, 2016 from Agricultural Research Institution (ARI), Rajendranagar (Mfg date - July 1, 2016). The initial population of *Rhizobium* estimated on yeast extract Mannitol agar (YEMA) with congo red medium was 6.84 log no. of cells. The viability of microorganisms was evaluated on monthly intervals upto December, 2016 (6 months). The microbial analysis revealed that there was a decline in the population of *Rhizobium* from 5.72 to 3.36 log no. of cells noticed from September, 2016 to December, 2016. Level of contaminants observed in during the last 3 months were 7.00, 7.08 and 7.18 log no. of cells in October, November and December respectively. The biofertilizer retained desired population only in the first three months period, but later level their contamination was observed [Table-2] and [Fig-1]. *Rhizobium*-CBBF was collected on July 4, 2016 from Agri Biotech Foundation (ABT), Rajendranagar, (Mfg date-July 1, 2016). The initial population of *Rhizobium* on YEMA with congo red medium was 7.34 log no. of cells. The viability of microorganisms was evaluated on monthly intervals upto December, 2016 (6 months). The microbial analysis revealed that there was a decline in the population of *Rhizobium* 5.08 and 3.00 log no. of cells was noticed with increase in storage time *i.e.*, October and December. Level of contaminants were observed 7.50, 7.80 log no. of cells during last two months *i.e.*, November and December respectively. The quality was good and the microbial population was retained up to October (4 months). *Rhizobium* - carrier based biofertilizer was collected on July 10, 2016 from K.N. Biosciences (Pvt.) ltd. (Mfg date - July 5, 2016). The initial population of *Rhizobium* was 7.40 log no. of cells on YEMA with congo red medium. The viability of microorganisms was evaluated on monthly intervals upto December, 2016 (6 months).

The microbial analysis revealed that there was a decline in the population of *Rhizobium* in the month of November and December recorded 4.00 and 4.08 log no. of cells respectively [Table-2] and [Fig-1]. Level of contaminants were observed 4.00 and 5.08 log no. of cells during the last two months *i.e.*, November and December respectively. The quality was good and prescribed population was found even within one month. After evaluating the shelf life of *Rhzobium* inoculants which were obtained from different production centers and the results displayed in [Table-2] revealed that the viable count of *Rhizobium* spp. strain decreased with increase in storage time. Phospahate solubilizing bacteria (PSB) - CBBF was collected on July 6, 2016 from ARI, Rajendranagar (Mfg date - July 1, 2016). The initial population of PSB estimated on pikovskaya's agar was 6.67 log no. of cells. The viability of microorganisms was evaluated on monthly intervals upto December, 2016 (6 months) [Table-2] and [Fig-1]. The microbial analysis revealed that there was a decline in the population of PSB 4.45 to 3.34 log no. of cells noticed from October, 2016 to December, 2016 respectively. The biofertilizer retained desired population in the initial three months and then considerable contamination took place (7.08, 7.26, 8.20) log no. of cells during the last three months *i.e.*, October, November, December respectively [Table-2] and [Fig-1]. PSB – CBBF was collected on July 4, 2016 from ABT, Rajendranagar, (Mfg date-July 1, 2016). The initial population of PSB enumerated on pikovskaya's agar was 6.79 log no. of cells [Table-2] and [Fig-1]. The viability of microorganisms was evaluated on monthly intervals upto December, 2016 (6 months). The microbial analysis revealed that there was a decline in the population of PSB from November and December were 4.26 and 4.08 log no. of cells respectively. The biofertilizer retained desired population in the first four months without contamination. PSB – CBBF was collected on July 10, 2016 from K.N. Biosciences (Pvt.) ltd. (Mfg date-July 5, 2016). The initial population of PSB found on pikovskaya's agar was 7.34 log no. of cells. The viability of microorganisms was evaluated on monthly intervals upto December, 2016 (6 months). The microbial analysis revealed that there was a decreased in the population of PSB noticed from 4.00 to 3.00 log no. of cells with increase in storage time *i.e.*, November and December, 2016 respectively. Very low contamination of microbial population was observed during the last two months *i.e.*, November and December 5.08, 5.20 log no. of cells respectively. The quality was good as prescribed population was found [Table-2] and [Fig-1]. The efficiency of biofertilizers heavily depends on carrier material because carrier acts as delivery vehicle to transfer live microorganisms from an agar slant of laboratory to plant rhizoshere. Presently, talc and lignite powder were used as carrier material by most of the bioinoculant producing units. These inoculants suffer with major drawback of short shelf life resulting in inconsistent performance under field conditions. The cost of production of carrier-based inoculants was also high, being energy and labour intensive process as discussed [8]. Soil carriers have the disadvantage of poor spread on seeds when coated on large amounts of seeds [9].

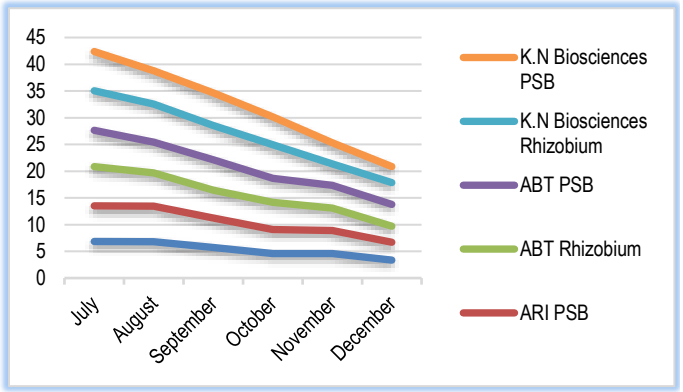

Fig-1 Evaluation of shelf life of *Rhizobium* and PSB carrier based biofertilizers collected from different production centers

Shelf life of liquid based biofertilizers (×10⁸ log no. of cfu ml⁻¹)

Rhizobium - liquid based biofertilizer was collected on July 10, 2016 from K.N. Biosciences (Pvt.) ltd. (Mfg date-July 5, 2016). The initial population of *Rhizobium* on YEMA with congo red medium was 8.38 log no. of cells. The viability of microorganisms was evaluated on monthly intervals upto January, 2017. The microbial analysis revealed that there was a decreased in the population of *Rhizobium* from 6.08 to 5.23 log no. of cells noticed in December, 2016 and January, 2017 respectively. After six months from its manufactured *i.e.*, January month microbial count was reduced to 5.23 log no. of cells. The biofertilizer retained desired population till six months and there was no contamination [Table-3] and [Fig-2]. PSB - LBBF was collected on July 10, 2016 from K.N. Biosciences (Pvt.) ltd. (Mfg date-July 5, 2016). The initial population of PSB found on pikovskaya's agar was 8.15 log no. of cells. The viability of microorganisms was evaluated on monthly intervals upto January, 2017. The microbial analysis revealed that there was a decreased in the population of PSB noticed in the month of December and January recorded as 4.28 and 4.20 log no. of cells respectively [Table-3] and [Fig-2]. After six months of its manufacturing, the microbial count has reduced to 4.20 log no. of cells. The liquid biofertilizers maintained the constant population and less contamination compared to carrier biofertilizers up to six months. According to biofertilizer control order specifications, the viable count of liquid based biofertilizers must be 1 x 108 cfu ml^{-1}. In the present study revealed that K.N. Biosciences ltd. produced liquid based biofertilizers supported and maintained viable count up to six months and without any contamination. Liquid formulations available today sustained with high viable microbial counts for about one year. It is possible to make *Rhizobium* survive in a liquid medium for more than six months with the help of cell protectants such as trehalose, poly vinyl pyrrolidine, etc. Cell protectants used in liquid formulation enhance cell tolerance to desiccation, osmotic pressure, temperature stress and stabilize both enzymes and cell membranes, which are helpful in ensuring longer shelf life and better adaptability to survive in the harsh conditions in the soil environment [10]. The current study also agrees with the above-mentioned studies and its proved that liquid biofertilizers have longer shelf life.

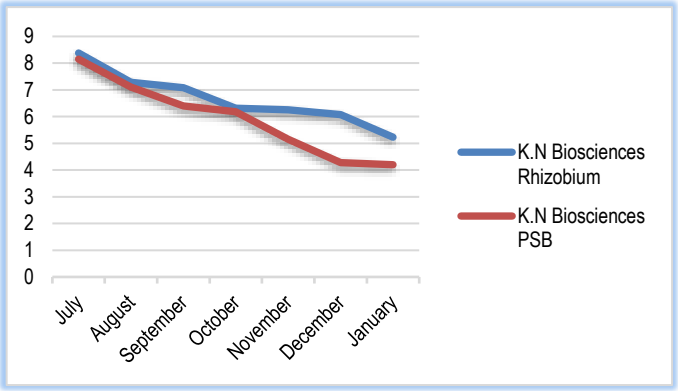

Fig-2 Evaluation of shelf life of *Rhizobium* and PSB liquid based biofertilizers collected from K.N.Biosciences (India) Pvt. Ltd.

Evaluation of quality (Initial) of carrier based and liquid based biofertilizers

pH

The pH of the *Rhizobium* and PSB carrier based biofertilizer collected from different production centers ranged from 6.5 to 7.0. The pH of the *Rhizobium* and PSB collected from ARI, Rajendranagar were 6.8 and 6.7 respectively. Similarly, the pH of the *Rhizobium* and PSB collected from ABT, Rajendranagar were 6.5 and 6.8 respectively. The pH of the *Rhizobium* and PSB collected from K.N. Biosciences (Pvt.) ltd. were 6.8 and 7.0 respectively. Whereas, the pH of the *Rhizobium* and PSB of liquid based biofertilizer collected from K.N Biosciences was 7.0 pH [Table-4]. There was more fluctuation in case of pH in carrier based biofertilizer than in liquid based biofertilizers.

Moisture content (%)

Moisture content of carrier based biofertilizers must be 30-40 %. Biofertilizers brought from ARI, Rajendranagar had moisture content of 25-35 %. Whereas, ABT Rajendranagar had moisture content of about 25-45 %. Biofertilizers brought from K.N. Biosciences (Pvt.) ltd. had moisture content of about 35-45 % [Table-4]. The moisture content of biofertilizers gradually decreased with the time period.

Consistency

Consistency of carrier based biofertilizers must be powdery and flowable for easy application in fields, but the biofertilizers obtained from different firms for the present study was not having proper consistency. Carrier based biofertilizers brought from ABT and ARI, Rajendranagar were having small clumps. Carrier based biofertilizers brought from K.N. Biosciences (Pvt.) ltd. were powdery and free flowing in nature. Consistency of liquid based biofertilizers brought from K.N Biosciences were thick liquid in nature [Table-4]. In liquid based biofertilizers viable count was constant for initial four months period followed by decreased in viable count was observed in fifth and sixth months but liquid biofertilizers maintained the desirable population up to six months. Hence, quality parameters of liquid based biofertilizers were proved good when compared to carrier biofertilizers. In carrier biofertilizers, the quality was too low, moisture content was high and count was decreased more with increase in storage time.

Conclusion

Liquid bio-fertilizes is considered the best way for replacing the traditional carrier based biofertilizers in modern agriculture. Because, the quality standards of liquid based biofertilizers are good and stable for six months. In carriers-based biofertilizers, the quality is very low and the viable count has decreased by every month interval. Hence, these liquid biofertilizers we can use in drip tank as drip bio fertigation to which helps in achieving increased crop yields, soil health and sustainable global food production.

Application of research: After evaluating the different formulations of biofertilizers based on the quality the best performed carrier and liquid biofertilizers applied in Greengram crop as different methods *i.e.*, Seed treatment, Soil application and Drip fertigation. Among all methods the liquid biofertilizers applied with drip fertigation along with inorganic fertilizers the treatment showed highest yields when compared to different methods of application. Hence, the liquid biofertilizers having long shelf life and without any clogging effect in drip like carrier based biofertilizers to increased more yield and maintain the sustainable agriculture, Soil fertility.

Research Category: Soil fertility and Biofertilizers

Abbreviations:

RDF: Recommended dose of NPK fertilizers
LBBF: Liquid based biofertilizers
CBBF: Carrier based biofertilizers
LCBF: Liquid culture based biofertilizers
PSB : Phosphate solubilizing bacteria
ARI: Agricultural Research Institute
ABT: Agri Biotech Foundation
PSB: Phosphate solubilizing bacteria

Acknowledgement / Funding: Authors are thankful to Department of Agricultural Microbiology & Bioenergy, College of Agriculture, Professor Jayashankar Telangana State Agricultural University, Rajendranagar, Hyderabad, 500030, Telangana

***Research Guide or Chairperson of research: Dr S. Triveni**
University: Professor Jayashankar Telangana State Agricultural University, Rajendranagar, Hyderabad, 500030
Research project name or number: MSc Thesis, Evaluation of carrier based and liquid based biofertilizer and their application methods in Greengram

Author Contributions: All authors equally contributed

Author statement: All authors read, reviewed, agreed and approved the final manuscript. Note-All authors agreed that- Written informed consent was obtained from all participants prior to publish / enrolment

Study area / Sample Collection: Department of Agricultural Microbiology & Bioenergy, College of Agriculture, Rajendranagar, Hyderabad, 500030

Cultivar / Variety name: Greengram

Conflict of Interest: None declared

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.
Ethical Committee Approval Number: Nil

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