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Evaluate the Shelf Life of Irradiated Carrier Based *Pseudomonas* Biofertilizer Stored At Different Temperatures at Different Intervals

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ABSTRACT

In this study focus on shelf life of different carrier based bioinoculants sterilized by irradiation, for this Pseudomonas cultures collected from different resource labs, then cultures were studied morphologically and biochemically for purity confirmation. The selected Pseudomonas (RGP-1) isolate was multiplied in large quantities in appropriate culture broth by incubating at 28 ± 2°C in an incubator shaker till they attained log phase with a cell load of 1 × 10^{9}cfu ml^{-1}. For biofertilizer production Vermicompost, Vermiculate, Lignite, and Sodium Alginate carriers were selected and carriers sterilized by gamma irradiation at a dose rate of 5.0 kGy for 1h then carriers were used for bioinoculant preparation and stored at different temperatures i.e. 4ºC and 28± 2°C. In first month log$_{10}$ values at 4ºC 9.65 (Vermicompost), 9.76 (Sodium Alginate), 9.64 (Lignite), 9.67 (Vermiculate) and 28± 2°C 9.85 (Vermicompost), 9.92 (Sodium Alginate), 9.80 (Lignite), 9.90 (Vermiculate) showed. Even though bioinoculant populations were higher at first month gradually deceasing upto 8th month, at the end (8th month) of the experiment log$_{10}$ values were at 4ºC 8.40 (Vermicompost), 9.05 (Sodium Alginate), 8.00 (Lignite), 8.70 (Vermiculate) showed which is highest value then minimum 5×10^{7}cfu g^{-1}(7.6 log$_{10}$) viable count of powdered form of carrier based biofertilizer and 28± 2°C was different viable count obtained 7.80 (Lignite) showed upto 6th month, 7.60 (Vermicompost), 8.16 (Sodium Alginate), 8.00 (Vermiculate) showed upto 7th month only.

Key words: *Pseudomonas, Irradiated sterilization, Different temperatures, Sodium alginate.*

INTRODUCTION

Organic farming has emerged as an important priority area globally in view of the growing demand for safe and healthy food and long term sustainability and concerns on environmental pollution associated with indiscriminate use of agrochemicals.

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Though the use of chemical inputs in agriculture is inevitable to meet the growing demand for food in world, there are opportunities in selected crops and niche areas where organic production can be encouraged to tape the domestic export market. Bio-fertilizers are being essential component of organic farming are the preparations containing live or latent cells of efficient strains of nitrogen fixing, phosphate solubilizing or cellulolytic micro-organisms used for application to seed, soil or composting areas with the objective of increasing number of such microorganisms and accelerate those microbial processes which augment the availability of nutrients that can be easily assimilated by plants. Selection of carrier material is very important while preparing biofertilizers. Although, there are no clear cut criteria for the selection of carrier materials but some general characteristics should be present in the material which is going to be used as a carrier for biofertilizer such as it should be cost effective, contain non-toxic compounds and high organic content, easy to process, more than 50% water holding capacity, high buffering capacity, sticky in nature and available in bulk quantity. A variety of materials can be used as carrier but the need of hour is to find out the most suitable carrier which fulfils all the above stated properties.[1]

Carrier based biofertilizers proved to be the best over the agrochemicals and are showing the tremendous effect on the global agricultural productivity, from the last two decades. Rectifying the disadvantages of the carrier based bio fertilizer liquids biofertilizer are developed which would be the alternative for the cost effective sustainable agriculture. Biofertilizer production in India during the period 2013-14, is 51870.67. MT, in Andhra Pradesh is 2137.14 MT.

The knowledge of applied microbial inoculums is long history which passes from generation to generation of farmers. It started with culture of small scale compost production that has evidently proved the ability of biofertilizer. This is recognize when the cultures accelerate the decomposition of organics residues and agricultural by products through various processes and gives healthy harvest of crops[2].

MATERIAL AND METHODS

The present study was carried out in the Department of Agricultural Microbiology & Bioenergy, College of Agriculture, Rajendranagar, PJTSAU, and Hyderabad. Pure cultures of Plant Growth Promoting *Pseudomonas* isolates collected from different laboratories. Screening and characterization of isolates were done with multiple beneficial properties then the efficient PGPR isolate was selected for preparation of carrier based biofertilizers.

COLLECTION OF *Pseudomonas* ISOLATES FROM DIFFERENT SOURCES

Promising bacterial isolates are collected from different laboratories and these isolates were tested for their purity and preservation in Dept. of Agricultural Microbiology & Bioenergy, College of Agriculture, PJTSAU Rajendranagar, Hyderabad.

IDENTIFICATION OF BACTERIAL ISOLATES PURITY CHECKING.

Morphological and Biochemical Characterization

The isolated bacteria were studied for their morphological like gram reaction, pigmentation, cultural characteristics and biochemical characteristics like Indole production, methyl red, voges-praskaure's test, citrate utilization test, oxidase, catalase and sugar fermentation tests.

Screening for plant growth promoting properties

Screening will be carried out for different plant growth promoting properties such as mineral solublization like Phosphorus Solubilisation[3], Zinc Solubilization[4], Potassium releasing[5], Plant growth promoting substances such as IAA production[6], biocontrol activity such as HCN production[7] and Siderophore production[8] and antifungal activity with soil born plant pathogens all ten isolates were checked for their purity and then

studied for the colony morphology and pigmentation. The cell shape and gram reaction were also recorded as per the standard procedures given by[9]

Gram's staining.

A loopful of inoculum from young culture was taken, mixed with water, and placed in the center of the slide. The suspension was spread out on slide using the tip of inoculation needle to make a thin suspension. The smear was dried in air and fixed through mild heating by passing the slide 3 to 4 times over the flame. The smear was then flooded with Crystal violet solution for 1 min and washed gently with flow of tap water. Then the slide was flooded with Iodine solution. After incubation at room temperature for 1 min, Iodine solution was drained out followed by washing with 95% decolorizer. After that, it was washed with water within 15 to 30 sec and blot carefully. The smear was incubated with Saffranin solution for 1 min. The slide was washed gently in flow of tap water and dried in air. The slide was examined under microscope at 100X power with oil immersion and data was recorded.

Cultural Characterization

Morphological characteristics of the colony of each isolate were examined on specialized medium. Cultural characterization of isolates observed by different characteristics of colonies such as shape, size, elevation, surface, margin, colour, odour, pigmentation etc were recorded.

Growing of culture

King's medium B Base

Kings Medium B Base is recommended for non-selective isolation, cultivation and pigment production of *Pseudomonas* species

Composition

Ingredients	**Gms / Litre**
Proteose peptone	20.000
Dipotassium hydrogen phosphate	1.500
Magnesium sulphate. Heptahydrate	1.500
Agar	20.000
Final pH (at 25°C)	7.2±0.2

Directions

Suspend 42.23 grams of dehydrated medium in 1000 ml distilled water containing 15 ml of glycerol. Heat to boiling to dissolve the medium completely. Mix well. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Aseptically pour into sterile Petri plates.

Collection of Carrier Materials

Sodium alginate collected from Department of Agricultural Microbiology and Bioenergy College of Agriculture Rajendranagar, Hyderabad. Vermiculate collected from Navaratna Crop Science Pvt Ltd, Cherlapally, Hyderabad. Lignite collected from Department of Agricultural Microbiology and Bioenergy College of Agriculture Rajendranagar, Hyderabad. Vermicompost collected from NIRD Rajendranagar, Hyderabad.

Physico-chemical properties of carriers

For the preparation of bioformulation, the collected different carriers such as lignite, vermicompost, sodium alginate and vermiculite were tested for their moisture content and pH at initial and end of the experiment.

Irradiation Sterilization

Lignite, vermicompost, vermiculate, and sodium alginate was sterilized by gamma irradiation at a dose rate of 5.0 kGy for 1h. (Gamma irradiation was carried out at the Irradiation unit PJTSAU, Hyderabad).

Preparation of Biofertilizers

Suspend 12.8 grams in 1000 ml distilled water. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Mix well and dispense into sterile test tubes. The selected isolate was multiplied in large quantities in culture broth by incubating at 28±2°C in an incubator shaker till they attained log phase with a cell load of 1×10^{9}cfu ml^{-1} and were used for inoculant preparation. The individual carrier materials were powdered and the pH was brought to neutral by adding $CaCO_3$ then sterilized by gamma irradiation at a dose rate of 5.0 kGy for 1h after then mixed with the log phase culture (1×10^{9}cfu ml^{-1}) of the selected plant growth promoting bacterial isolate viz.,

Pseudomonas (RGP-1) in separate quantities of sterile carrier in shallow trays. The optimum moisture content was adjusted to (30-40%) prior to preparation, followed by curing in shallow trays for 24 hours in aseptic rooms and then packed in high density opaque polythene bag (12g) at the rate of 100g bag^{-1} and sealed. Individual inoculant was prepared by mixing with lignite (1:3v/w), vermicompost (1:2v/w), vermiculite (1:2v/w) volumes of each culture broth with sterile carrier materials. The populations of individual Plant Growth Promoting Rhizobacteria in the inoculant carriers were assessed at monthly intervals upto 8 months at different storage temperature at 4ºC 28 ± 2°C.

b. Preparation of Alginate based inoculant

Pseudomonas (RGP-1) was grown in respective medium to get a population of $lxl0^9$cfu ml^{-1} Sodium alginate beaded inoculant was prepared as per the methods described by[10]. Two gram of sodium alginate was added to 100 ml of culture broth of PGPR and mixed for 30 minutes in a magnetic stirrer. The mixture was added drop wise through a 10 ml syringe into 100 ml sterile 0.1N $CaCl_2$ to obtain uniform Alginate beads. One gram of material contained 16 to 17 beads, each bead approximately weighing 60mg. The beads were washed twice in sterile distilled water and incubated for seven days in a psychrotherm (model environ shaker) incubator at 28±2°C to allow PGPR to multiply inside the beads. The beads were again washed in sterile distilled water and air dried in Laminar air flow chamber under aseptic condition. The Alginate beads were then stored in polythene bags at room temperature (28±2ºC) and refrigerator (4ºC) upto 8 months.

Treatments

T 1: $S_1C_1O_1$ (Irradiated Vermicompost with *Pseudomonas spp*)

T 2: $S_1C_2O_1$ (Irradiated Sodium alginate with *Pseudomonas spp)*

T3: $S_1C_3O_1$ (Irradiated Lignite with *Pseudomonas spp).*

T4: $S_1C_4O_1$ (Irradiated Vermiculite with *Pseudomonas spp),*

Determination of viable bacterial population in the carrier based inoculants by serial dilution and plating technique. Influence of storage temperature on the survival of the inoculants as consortium in different carrier materials .The carrier based microbial inoculants prepared with different carrier material was kept in different temperature levels *viz.*, Room temperature (28±2ºC) and Refrigerator (4°C). The surviving populations of PGPB at different temperatures were determined and population was enumerated by dilution plate technique at different intervals i.e monthly upto 8 months.

RESULTS AND DISCUSSION

First month results revealed that $\log_{10}$ values were 9.65, 9.85 (vermicompost), 9.76, 9.92 (sodium alginate), 9.64, 9.80, (lignite), 9.67, 9.90 (vermiculite) showed at 4^0C, 28 ± 2^0C storage temperature respectively. When compared among carrier materials sodium alginate having highest population and lignite having least population at both temperatures.

Eight month results revealed that $\log_{10}$ values were 8.40, 6.80 (vermicompost), 9.05, 7.35 (sodium alginate), 8.00, 6.50, (lignite), 8.70, 7.00 (vermiculite) showed at 4^0C, 28 ± 2^0C storage temperature respectively.

From 1st month to 8th month *Pseudomonas* population gradually decreased at both temperatures (table no.1. and 2.) As per specification of biofertilizers, carrier should be minimum 5×10^7cfu g^{-1}($\log_{10}$ 7.6) viable count of powdered form of carrier based biofertilizer. So that the above results revealed that at 4^0C, lignite carrier based *Pseudomonas* bioinoculants supported and maintained optimum viable count $\log_{10}$ 8.00 more than 5×10^7cfu g^{-1}($\log_{10}$ 7.6) viable count of powdered form of carrier based biofertilizer upto 8th month. but showed the viable count least among different carrier materilas, whereas sodium alginate, vermiculite and vermicompost based carrier bioinoculants supported and maintained optimum viable count $\log_{10}$ 9.05, 8.70, 8.40, respectively upto 8th month fig no.1. Which is more than the

minimum 5×10^7cfu g^{-1}(log_{10} 7.6) viable count of powdered form of carrier based biofertilizer.

At 28 ± 2^0C storage temperature lignite carrier based *Pseudomonas* inoculants supported and maintained optimum viable count log_{10} 7.80 upto 6th month only, but sodium alginate, vermiculite and vemicompost based carrier bioinoculants supported and maintained optimum viable count log_{10} 8.16, 8.00 and 7.60 respectively more than 5×10^7cfu g^{-1} (log_{10} 7.6) upto 7th month and in 8th month showed viable count log_{10} 6.80 (vermicompost), 7.35 (sodium alginate), 6.50 (lignite), 7.00 (vermiculite) fig no 2. When comparing all carrier based bio inoculants shelf life sodium alginate *Pseudomonas* carrier based biofertilizer showed highest viable count upto 8th moths at both storage temperatures *viz.,* log_{10} 7.35 Room temperature (28±2ºC) and log_{10} 9.05 Refrigerator (4°C).

But survival rate of *Pseudomonas* cells was more upto 8th month at 4^0C compared to 28 ± 2^0C. This results revealed that 4^0C storage temperature is best suitable for storage of carrier based inoculants because of low level of moisture content in the carrier inoculants stored at 28 ± 2^0C temperature compared to 4^0C.

Table 1: *Pseudomonas* Population in different carrier based bioinoculants at 28±2ºC upto 8th months

MONTH	VERMI COMPOST	SODIUM ALGINATE	LIGNITE	VERMICULITE
	(28±2ºC)	(28±2ºC)	(28±2ºC)	(28±2ºC)
1	9.85	9.92	9.80	9.90
2	9.77	9.84	9.74	9.80
3	9.66	9.75	9.63	9.69
4	9.34	9.54	9.27	9.33
5	8.60	9.18	8.45	8.78
6	8.20	8.70	7.80	8.42
7	7.60	8.16	7.40	8.00
8	6.80	7.35	6.50	7.00

Table 2:

MONTH	VERMI COMPOST	SODIUM ALGINAT	LIGNITE	VERMICULITE
	4 ºC	4 ºC	4 ºC	4 ºC
1	9.65	9.76	9.64	9.67
2	9.64	9.73	9.60	9.70
3	9.54	9.69	9.50	9.65
4	9.48	9.62	9.40	9.60
5	9.31	9.51	9.18	9.40
6	9.12	9.33	8.80	9.26
7	8.90	9.20	8.62	9.00
8	8.40	9.05	8.00	8.70

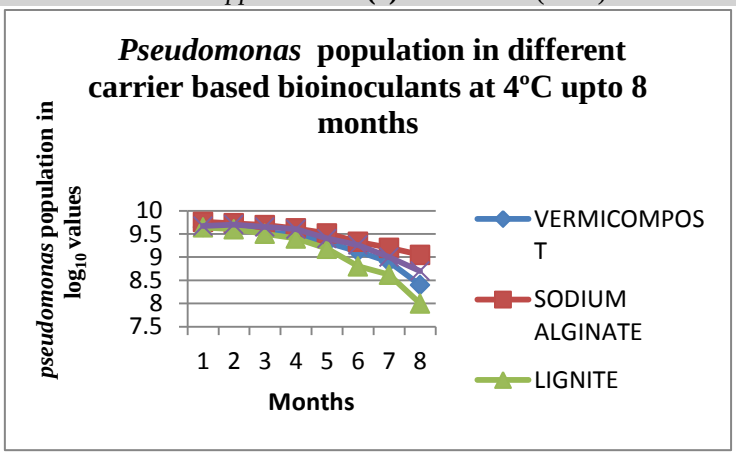

Fig. 1:

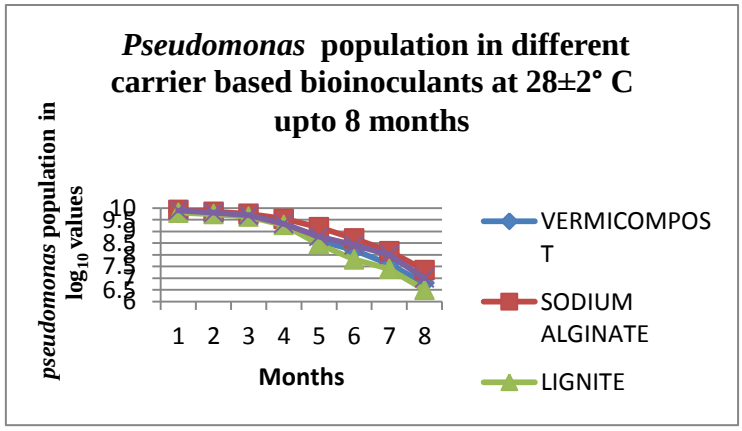

Fig. 2:

CONCLUSION

For the increasing the shelf life of *Pseudomonas* carrier based biofertilizers should be store at 4°C temperature then Room temperature (28±2ºC), and also for best carrier based biofertilizer production sodium alginate and vermiculite carriers were showed highest population with long time storage capacity at both temperatures *viz.*, Room temperature (28±2ºC) and Refrigerator (4°C).

ABBREVIATIONS

RGP; Red Gram *Pseudomonas*, IAA; Indole Acetic Acid, PGPR; Plant Growth Promoting Rhizobacteria, HCN; Hydrogen Cyanide, PJTSAU; Professor Jayashanker Telangana State Agricultural University, NIRD; National Institute of Rural Development.

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