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ORIGINAL RESEARCH ARTICLE

Studies on Improve Survivability and Shelf Life of Carrier Using Liquid Inoculation of *Pseudomonas striata*

l.Mugilan[1], P. Gayathri[1], EK.Elumalai[2], R.Elango[*1]

[1]*Department of Microbiology, Annamalai university,Chidambram- 608 002(T.N.),India.*
[2]*P.G. and Research Department of Zoology, Voorhees College,Vellore - 632001(T.N.) India.*

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ABSTRACT

Biofertilizers are also known as microbial inoculants or bioinoculants. It is a product of selected, beneficial and live microorganisms which helps to improve plant growth and productivity, mainly through supply of plant nutrients. Death of the organisms in the inoculated seed is one of the important factors contributing the failure of inoculation response in field condition. This present study was conducted to improve the shelf life and survivability of inoculant in liquid form. *Pseudomonas straita* was used as an inoculant which having phosphate solubilizing capacity Three different treatment like vermiculite, lignite and liquid inoculants were used for survival of Pseudomonas straita. . Four different broths were used such as GPS (Glucose peptone broth), PVB (Pikovasky broth), NB (Nutrient broth) and PSB (Peptone sodiumchloride broth) to prepare liquid bioinoculants. Upon that liquid inoculant shows high phosphate solubilizing efficiency than other two treatments and also luquid inoculant show more survivability of Pseudomonas straita than other two treatments. Based on the results, liquid inoculant is considered as best bioinoculants in growth of paddy than control and other two treatments.

Key words: Biofertilizer, bioinoculants, *Pseudomonas straita*

INTRODUCTION

Biofertilizers play an important role for supplementing the essential plant nutrients for sustainable agriculture, economy and eco-friendly environment. The term biofertilizers generally are defined as preparation containing live or latent cells of efficient strains of N-fixing, P-solubilizing or cellulolytic microorganisms used for application to seed or soil [1]. It is also known as microbial inoculants or bio inoculants. Bio-fertilizers manufactured in India presently are carrier based; in general, it is suffer from short shelf life, poor quality, high contamination and unpredictable field performance. Death of the organisms in the inoculated seed is one of the important factors contributing the failure of inoculation response in field condition. Much research is done in India on *Rhizobium* strain selection and inoculation response. However research conducted on the inoculants production and formulation technologies is limited. A break through is needed in the inoculants technology to improve the shelf life and field efficacy of bio-fertilizers in India to make them commercially viable and acceptable to farmers.

One of the main problem in inoculants technology is the survival of micro-organisms during storage and several parameters such as culture medium, physiological state of the microorganisms when harvested [2] the process of dehydrates, rate of drying the temperature storage and water activity (Aw) of the inoculums have an influence on their shelf life. So, studies to increase the shelf life of inoculants or finding alternate formations for carrier based inoculants are important. In the present study experiments have been conducted to improve the shelf life and quality parameters of microbial inoculants of *Pseudomonas striata* by the addition of suitable additives and also developing new formulation such as liquid inoculants.

MATERIALS AND METHODS

Isolation of phosphate solubilizing bacteria *Pseudomonas striata*

The phosphorus solubilizing bacteria *P.striata* were isolated from the soil sample following the techniques of Dhingra and Sinclair [3]. Ten gram of rhizosphere soil sample transferred to 250ml conical flask containing 100ml sterile water blank to get a dilution of 10^{-1}. From these dilutions

***Corresponding Author:** R.Elango, **Email:** relango2000@yahoo.co.in

were prepared up to a dilution of 10^{-5} by transferring 10 ml of 10^{-1} to a conical flask containing 90 ml of sterile water. One ml of 10^{-5} dilution was transferred to petriplates. The plates were poured with apatite medium and plates with three replications were incubated for two weeks. After the incubation period, the plates were examined for colonies having a circular halo or zone around the colonies were counted on the second week.

Characterization of bacterial isolates

Isolated bacterial cultures were characterized based on morphological tests [4], biochemical tests [5,6].

Preparation of liquid inoculants

The Pikovskaya's broth inoculated with *P. striata* in 250ml conical flask. It was allowed to multiply by incubating at 30±2^{0}C in a psychrothem. (Model environ Shaker 3597-1L BGM) incubator cum shaker at 100rpm for 72 hours. The broth containing approximately 25x10^{11}cfu/ml was used as a starter culture for the production of liquid bioinoculants.

Mixing of sticking and spreading agents

The broth was mixed with sticking and spreading agents viz., Glycerol, Tween 20 were added at the rate of 1.5ml, 2 ml, 2.5 ml / 100ml. They were packed in 100ml lot in opaque low density grade polypropylene bags of thickness 75 micron as per the procedure followed by Somasegaran [7].

Analysis of liquid inoculants

The liquid inoculants of *P. striata* were analyzed for their survival at for night intervals over a period of three months of storage. The estimation of population was done by serial dilution and plating method.

Serial dilution was prepared by transfer of 1 ml of inoculums into 9 ml sterile water blanks to get 10^{-1} dilution. Similarly the dilutions were made serially upto 10^{-9} from 10^{-1} dilution, one ml was pipette out into sterile glass petriplates and sterile apatite medium were poured on it. The plates were rotated clockwise and anti clockwise direction for uniform spread of the dilution mixture, then the plates were incubated at 28±2^{0}C for 24hrs and the population was estimated.

Effect of inoculation of different formulations of microbial inoculants on the growth parameters of paddy (var. Adt-43)

The inoculation of different formulations of *P. striata* on germination, vigour index of paddy var. ADT43 was determined by roll towel method.

Inoculation of different formulation

The carrier based inoculants @ 20g/kg, Alginate beads @ 10g/kg and liquid inoculants @ 5ml/kg of seeds were mixed with 20ml of rice gruel and made into a uniform slurry. The seeds were thoroughly mixed with the slurry; shade dried for 30min. and was sown.

Determination of growth parameters

Vigour index

The treated paddy seeds were placed in the pre-soaked germination paper. The seeds were held in position by placing another pre-soaked germination. These germination papers along with seeds were then rolled from left to right and the roll was secured by putting two rubber bands on either end of the roll. Germination per cent and seedling length were recorded at 15th day of sowing. Five normal seedlings from each treatment were taken at random and used for the seedling measurement. The vigour index was computed by multiplying germination per cent and seedling length and expressed as a whole number.

Statistical analysis

The data were subjected to a statistical analysis by following the procedure suggested by Nageswara Rao [8].

RESULTS

Authentication of *P.striata*

The microbial inoculants *P.striata* used in the present study were isolated from rhizosphere soils of paddy. The above strains were authenticated by performing morphological and biochemical tests. Based on the results *P.striata* was rod in shape, showed Gram negative reaction and actively motile. It also showed positive catalase activity, Gelatin liquiefication and not able to hydrolyse starch.

Physico-chemical properties of carrier materials

The physico-chemical properties such as organic matters, total nitrogen, Bulk density, and water holding capacity were estimated for the carrier materials like vermiculite and lignite were used in this study and the results are presented in (**Table 1**). The organic matter content was maximum in lignite (75.46%). Vermiculite recorded minimum organic matter content of (1.07%). The nitrogen content was maximum in lignite (0.31%) and vermiculite recorded the least nitrogen content of (0.01%).Bulk density of 0.75g/cm^3 was recorded lignite and vermiculite. Lignite was observed to show the maximum water holding capacity (198.9%), whereas vermiculite recorded (152.4%).

Table 1: Physico-chemical properties of different carrier materials

S.No	Carrier	Organic matter content (%)	Total Nitrogen (%)	Bulk density (gcm3)	Water holding (%)
1	Lignite	75.46	0.31	0.75	198.9
2	Vermiculite	1.07	0.01	0.75	152.4

Survival of *P.striata* in different formulation under sterile condition

The survival of *P.striata* in different formulations viz., lignite, vermiculite and liquid inoculant were estimated under sterile condition over a period of three months of storage period at room temperature. The result is presented in (**Table 2**).The results of this study showed that, the required population of *P.striata* was maintained in all the carriers tested up to three months of storage period, without reduction. Among the different carriers tested the liquid inoculants supported the maximum population of 28.3 x 10^9 cfu ml^{-1} 2 months of storage followed by lignite and vermiculite.

Table 2 : Survival of *Pseudomonas striata* in different formulation

S.No	Treatment	Population of *P.striata* in 1 x 10^9 cfu.			
		15	**30**	**45**	**60**
1	Vermiculite	4.0	5.21	7.15	9.0
2	Lignite	4.42	6.18	7.41	10.0
3	Liquid	15.33	19.0	20.41	28.3

Population dynamics of *P.striata* in different media composition

The population dynamics of *P.striata* in different media composition are presented in (**Table 3**). The results of the experiment revealed that maximum population of *P.striata* was found in Pikovskaya's medium 28.35 x 10^9 cfu ml^{-1} at 2 months of storage followed by nutrient broth 24.33 x 10^9 cfu ml^{-1}. It was also observed that the population of *P.striata* in peptone sodium chloride broth and Glucose peptone broth was found to be on par.

Table 3 : Population dynamics of *Pseudomonas* in different media compostition

S.No	Media	Population in *Pseudomonas* inoculants 1x10^{10} cfu ml^{-1}			
		15 days	**30 days**	**45 days**	**60 days**
1	Pikovskaya's Media	15.33	19.0	20.41	28.35
2	Nutrient media	15	17.93	19.8	24.33
3	Peptone sodium chloride media	12.87	14.33	19.61	20
4	Glucose peptone media	9.31	10.15	11.72	13.82

Survival of microbial inoculants *P.striata* in different formulation with different additives

A study was conducted to improve the shelf life and survival of *P.striata* inoculants in different formulation by adding certain additives such as Glycerol and Tween 20 at 1.5%, 2% and 2.5% levels. The survival of *P.striata* was estimated at fortnight intervals over a storage period of three months and the results are presented in (**Table 4**). The results of the experiment revealed that all the additives tested were able to increase the surviving population of *P.striata*. Among the different additives, Glycerol 2.5% and Tween 20, 2.5% level was found to record a maximum population of 20.11 x 10^9 on the 3rd month of storage followed by 2% of Glycerol and 2% of Tween 20. Whereas uninoculated additives (control) recorded only 12 x 10^9 populations.

Table 4: Survival of *P.striata* in different formulations with different additives

S.No	Additives Glycerol Tween 20 (%)	Population in *P. striata* in 1x10^{10} cfu ml^{-1}			
		15 days	**30 days**	**45 days**	**60 days**
1	1.5ml	11.76	13.12	15.52	17.72
2	2ml	12.32	14.32	15.36	18.00
3	2.5 ml	14.32	18.0	18.52	20.11
4	Nil	6.18	7.41	9.0	12

Phosphate solubilizing efficiency of *P.striata*

An *in vitro* experiment was conducted to evaluate the phosphate solubilizing efficiency of *P.striata* in different formulation such as carrier based (lignite and vermiculite) and liquid formulation on the production of area of clear zone were studied and result are given in (**Table 5**).

Table 5: Phosphate solubilizing efficiency on *Pseudomonas striata*

S.No	Media	Size of clear zone	
		5 days	**10 days**
1	Lignite	4 mm	7mm
2	Vermiculite	2mm	5mm
3	Liquid inoculant	7mm	11.5mm

Survival of *P.striata* in liquid formulation

The population of *P.striata* was found to be supported at a higher level in liquid formulation

throughout the storage period. The maximum surviving population of *P.striata* was 25 x 10^{12} cfu ml^{-1} on the 2 month of storage results are given in (**Table 6**).

Table 6: Survival of *P. striata* in liquid formulation

S.No	Interval	Population of *P. striata* in cfu ml^{-1}
1	Initial	1.62 x 10^4
2	15 days	14.58 x 10^9
3	30 days	17.44 x 10^9
4	45 days	19.20 x 10^9
5	60 days	25.10 x 10^9

***In vitro* evaluation of different formulation of *P.striata* on seed germination of paddy.**

The results of the study revealed that, in general the inoculation of *P.striata* through different formulation could augment the growth parameters of paddy, compared to uninoculated control. Among the different formulation tested, liquid formulation showed the maximum inoculation effect followed by lignite and vermiculite carrier based material.

The maximum germination percentage of 97% was observed with liquid inoculation of *P.striata* and the maximum of 70.00% was observed in uninoculated control. Among the two carrier based inoculation the lignite based inoculants recorded 87% of germination followed by vermiculite (83%) alone.The maximum root length of 8.5 cm was observed with liquid inoculation and the minimum of 4 cm was the two carrier based inoculation lignite recorded 7 cm of root length followed by vermiculite (5.5cm).Liquid formulation was recorded maximum shoot length (22.5cm) followed by lignite and vermiculite. The minimum shoot length was recorded with uninoculated control (10.5cm). The maximum vigour index of 3007 was recorded with liquid inoculation and minimum of 1015 was observed in uninoculated control (**Table 7**).

Table 7: Effect of inoculation of *P. striata* inoculants in different formulation on growth of paddy

S.No	Treatments	Germination (%)	Root length (cm/plant)	Shoot length (cm/plant)	Vigour index
1	Uninoculated control	70.00	4.0	10.5	1015
2	Vermiculite	83	5.5	13.85	1606.05
3	Lignite	87	7	15.89	1991.43
4	Liquid inoculant	97	8.5	22.5	3007

DISCUSSION

In the present day situation intensive agriculture needs the use of integrated nutrient management systems involving organic and inorganic sources of plant nutrients to sustain the yield of crop plants. Biofertilizers form an integral part of IPNS and organic farming, which constitutes the present as well as the future mandate of agriculture.

Authentication of *P.striata*

P.striata used in the study were isolated from rhizosphere soil of paddy were subjected to cultural and biochemical test. The results revealed that the biochemical characteristic and morphological characteristics of *P.striata* are in line with the descriptions of Rosa et al[9].

Physico-chemical properties of carrier material

The carrier material used for inoculants production should have the characters like high organic matter content, water holding capacity and natural pH for better survival of organisms. The physico-chemical properties revealed that the bulk density was maximum in lignite followed by vermiculite. Maximum water holding capacity was observed in lignite followed by vermiculite. Similar results were also observed by Tilak and Subba Rao[10].

Survival of *P. striata* in carrier and liquid based inoculants

Due to the scarcity of good quality carrier material peat which is widely used as a carrier material at present and also its problem in transport an alternative to peat has to be identified. In this study two different carrier materials viz., lignite and vermiculite were tested for their suitability to support higher survival of *P.striata*. Among them, lignite supported higher population followed by vermiculite. Kandasamy and Prasad [11] also recommended the use of lignite as a carrier due to high carbon content, in contrast to vermiculite which contents very low organic matter and N content.

Population dynamics of *P.striata* in different media composition

In present study, to improve the survival of *P.striata* in different media such as pikovskaya's, nutrient broth, Glucose peptone broth and peptone sodium chloride broth. The surviving populations of P.striata were found to be increased in Pikovskaya's broth was noticed. Many works have been carried out in this aspect. The increase in survival of microorganisms by the addition of different amendments like soyameal [12].

Survival of microbial inoculants *P.striata* in different formulation with different additives.

The present study, the possibility of using different formulation and different additives were used in liquid inoculants of *P.striata*. The results

revealed that the higher survivals of inoculants were recorded in liquid formulation followed up to a storage period of 3 month. Better survival of *P.striata* in Glycerol and Tween 20 for longer period was observed and it could be improved by the addition of skimmed milk and controlled dehydration.

***In vitro* evaluation of different formulation of *P.striata* on seed germination of paddy**

In the present study, the effect of inoculation of *P.striata* in different formulation such as carrier based inoculants and liquid formulation on growth of paddy was studied. The result revealed that three different formulations enhance the growth of paddy compared to uninoculated control. Among the three formulations, liquid formulation was found to enhance higher growth of paddy compared to other formulation. The increased effect of liquid formulation may be due to higher population of *P.striata*. Based on the result of the study, the vermiculture can be used as an alternate carrier material to prepare the microbial inoculants. The new formulation of liquid inoculants was found to be better in supporting the population of inoculants than the carrier based inoculants. The effect of inoculants of liquid formulation of *P.striata* was found to be better in increasing the growth parameters such as seed germination, seedling high and vigour index.

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