Research Article

Characterization and Viability Assessment of Freeze Dried Bacterial Liquid Biofertilizer on *Capsicum annum L.*

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ABSTRACT

Low soil fertility is caused by continue crop and using chemical fertilizer. The biofertilizer containing microorganisms able to increase soil fertility. The purpose of this research was to investigate the effect of biofertilizer under freezed condition and exposed to different period of storage and growth parameters of chilli plant grown under field conditions. Nitrogen fixing organisms *(Azotobacter, Azospirillum sp*) and Phosphate solubilizing organisms (*Bacillus, Pseudomonas sp*) were isolated from the soil sample from Edaiyur, Thiruvarur District, Tamil Nadu, South India. Liquid biofertilizer are stored at deep freezer in the time interval 0 to 3 months. Viability assessment was also done by colony forming unit (CFU). The plant namely *Capsicum annum L* was treated with liquid biofertilizer T1**-** Mixture of freeze dried Liquid biofertilizer *(Azospirillum, Azotobacter, Bacillus, Pseudomonas),* T2**-** Freeze dried biofertilizer *Azospirillum,* T3**-** Freeze dried biofertilizer *Azotobacter,* T4- Freeze dried biofertilizer *Bacillus,* T5**-** Freeze dried biofertilizer *Pseudomonas,* C**-** Control. The results was measured that terms of number of leaves, height of plant, shoot length, root length, number of roots and biochemical constituents such as total chlorophyll content and carotenoids was increased at 60 days, after sowing. Among the overall treatments (T1) showed better response for combined inoculations than the other treatments and control. The result showed that viability of bacterium tended to decline during storage of biofertilizer but did not significantly reduce the effect on growth and production of plant. The study suggested that application of biofertilizer improves growth and production and there was no any declined effect of microbial population between 0 and 3 months storage of biofertilizers on plant growth.

Keywords: Liquid biofertilizer, Deep freezer, *Capsicum annum L,* Pot culture.

INTRODUCTION

Biofertilizers are the formulations of living microorganisms which are able to fix atmospheric nitrogen in the available form for plants (Nitrate form) either by living freely in the soil or associated symbiotically with plants. Biofertilizer is one alternative of fertilizer that is able to increase soil fertility and crop production. A biofertilizer is a substance which contains living microorganisms which, when applied to seed, plant surfaces, or soil, colonizes the rhizosphere or the interior of the plant and promotes growth by increasing the supply or availability of primary nutrients to the host plant[1]. The fertilizers are used to improve the fertility of the land using biological wastes, hence the term biofertilizers and biological wastes do not contain any chemicals which are detrimental to the living soil. Biofertilizer may also decrease the pH, which leads to increase availability of trace elements that enhance plant growth[2]. A group of fertilizers contain beneficial rhizobacteria many of which have been called plant growth promoting rhizobacteria [PGPR] that activity colonize the rhizophere, improve the plant growth and increase yield.[11]

Liquid biofertilizer are the microbial preparations containing specific benificial microorganisms which are capable of fixing or solubilizing plant nutrients by their biological activity. Liquid fertilizer formulation is the promising and updated technology which has many advantages over the agrochemicals, left a considerable dispute among the farmer community in terms of several reasons major being the viability of the organisms.[15] One of the benefits from biofertilizer is a contribution from population of microorganism available. Fertilizers are important for success in attaining the challenge of food security at present as well as in future by increasing productivity but high crop yield leads to higher removal of soil nutrients, which have to be replenished for desired productivity in the future[3].

The viability of the microbes in this liquid biofertilizer is two years. They are tolerant to high temperatures 55^{o}C and ultra violet radiations. The count is as high as 10^{9} cfu/ml which is maintained constant up to 2 years[19]. So the application of 1ml of liquid biofertilizer is equivalent to the application of 1kg of 5 months old carrier based biofertilizer (1000) times. Since these are liquid formulations the application in the field is also very simple and easy. Freeze drying has become one of the most important processes for the preservation of biological products[4].

Biofertilizer have storage limitations. The efforts to keep the organism alive during storage became very important. Biofertilizer in the liquid medium has storage and packaging limitation. The efficiency of biofertilizer depends on the viability of bacteria that may be decreased under exposed in the external factor such as temperature humidity and light. Therefore the biofertilizer should be maintained effectively during storage to avoid the reduction of viability.

Azospirillum sp. and *Azotobacter* sp. is an aerobic, free-living soil microbe which fixes nitrogen from the atmosphere[5]. While *Pseudomonas* sp. and *Bacillus* sp. are bacteria that able to increase the availability of P and K in soil, enhanced N, P and K uptake[6]. *Pseudomonas* sp and *Azospirillum* sp also can produce hormone to enhance plant growth.

Chilli (*Capsicum annum L.*) is one of the most important spice vegetables grown all over the world. Duration 2004-2005 India produced 6.5 to 7 lakh tones and it was declined to 6.25 to 6.35 lakh tones during 2005 to 2006 crop seasons. Red pepper (*Capsicum annum L.*) is a prominent spice of India. Chilli has many culinary and it comprise numerous chemicals including steam volatile oils, fatty oils, capsaicinoids, carotenoids, vitamins, fiber and mineral elements[7]. Many chilli constituents are important for nutritional value, flavour, aroma texture and colour. Chillies are low in sodium and cholesterol free, rich in vitamins A and C, and are a good source of potassium, folic acid and vitamin E[8]. The aim of the present study is to investigate characterization and viability assessment of freeze dried bacterial liquid biofertilizer on *Capsicum annum L.*

MATERIALS AND METHODS

Soil Sample Collection

Soil sample were collected from Edaiyur, Thiruvarur District, Tamil Nadu, South India. *Azospirillum, Azotobacter, Bacillus, Pseudomonas* sp were isolated from soil sample.

Mass Production of Liquid Biofertilizer

The isolated strains were grown in respective broth medium in culture tube. After checking the culture for purity and proper growth, the culture was transferred from culture tube to small conical flask containing sterilized liquid medium as starter culture. Later the starter culture was transferred to a large conical flask on a rotary shaker at 150rpm for 5 days at 28 ± 2 °C.

Freeze Drying -20°C

The bacterial biofertilizer namely *Azospirillum, Azotobacter, Bacillus, Pseudomonas* are freeze dried at Deep freezer RSF170 Remi Instruments private Ltd in Maharashtra. The Instrument are under the maintenance of Centralized Instrumentation Facility (CIF), PG &Research Department of Microbiology, STET Womens college, Sundarakkottai. Freeze dried biofertilizer stored in different storage period such as 0, 1, 2, and 3 months. After storage maximum viability was noticed under 2nd month. Hence second month freeze dried biofertilizer used for this experiment.

Treatment

There were six pots used for the treatment. The pots were maintained in the open shade at the temperature of 27°C-30°C. The crop plant *Capsicum annum L* was treated with liquid biofertilizer. All the pots were watered regularly. T1- Mixture of freeze dried Liquid biofertilizer *(Azospirillum, Azotobacter, Bacillus, Pseudomonas)*, T2- Freeze dried biofertilizer *Azospirillum*, T3- Freeze dried biofertilizer *Azotobacter*, T4- Freeze dried biofertilizer *Bacillus*, T5- Freeze dried biofertilizer *Pseudomonas*, C- Control.

Viability of Bacteria

Viability of bacterial in biofertilizer that used was recorded during storage time. In 2nd month deep freezed liquid biofertilizer are used for pot study due to high microbial count compared to other months.[13]

Morphometric Parameters

Height of the plant (in cm), Number of leaves (per plant), Shoot length (in cm), Root length (in cm), Number of roots (per plant).[16]

Biochemical Constituents

Chlorophyll, Total chlorophyll, Carotenoids were also estimated.[9]

Statistical Analysis

The results obtained in the present investigation were submerged to statistical analysis like mean (x) and standard deviation (SD).[10]

RESULTS AND DISCUSSION

The purpose of this research uses to investigate the effect of liquid biofertilizer exposed to freeze drying of different storage on growth and productivity of *Capsicum annum L.* The results were showed that viability of bacterium tended to decline during storage of biofertilizer but did not significantly reduce the effect on growth and production of plant[12]. The study suggested that application of biofertilizer improve growth and production and there was no significant effect between 0 and 3 months storage of biofertilizers during freeze drying on plant growth.

Viability of Bacteria

The result of viability test for bacteria during storage 0, 1, 2 and 3 months of storage are presented in (Table 1). Viability of bacterium is declined slightly after freeze drying mechanism, then it was stable until 2 month storage. After 2 months, the viability of each bacterium contained in biofertilizers was declined.

Height of the Plant

Among the overall treatments combined inoculation of four liquid biofertilizer treatments such as mixture of freeze dried liquid biofertilizers of (*Azospirillum, Azotobacter, Bacillus, Pseudomonas)* (T1) in 30th day was showed better response in height of plants (25.9±1.03cm) followed by other treatments and control. In 30th day liquid biofertilizer alone *Azospirillum* (T2) was exhibited better response in height of plants (22.7±0.51cm) followed by *Azotobacter, Bacillus, Pseudomonas* and control.

In 45th day combined inoculation of four liquid biofertilizer inoculated treatments such as mixture of freeze dried liquid biofertilizers of (*Azospirillum, Azotobacter, Bacillus, Pseudomonas*) (T1) was showed maximum response in height of the plants (35.5±1.52cm) followed by treatments and control. In 45th day of liquid biofertilizer *Azotobacter* (T3) was showed better response in height of the plants (19.3±0.97cm) followed by other treatments and control.

Combined inoculation of four liquid biofertilizer treatments such as, mixture of freeze dried liquid biofertilizers of (*Azospirillum, Azotobacter, Bacillus, Pseudomonas)* (T1) in 60th day the yield concepts such as height of the plant (44.21±0.91cm) was noted in (Table-2) followed by other treatments and control (Table-5). In 60th day liquid biofertilizer of *Bacillus* (T4) was agreed with above said response in height of plants (27.4±1.82cm) followed by other treatments and control (Table-2).

Number of Leaves

Overall treatments, combined inoculation of four liquid biofertilizers such as mixture of freeze dried liquid biofertilizers of (*Azospirillum, Azotobacter, Bacillus, Pseudomonas)* (T1) in 30th day was showed maximum response in leaves of plants (16.3±1.69) followed by other treatments and control. In 30th day liquid biofertilizer alone *Azospirillum* (T2) was showed better response in leaves of plants (16±0.46) followed by *Azotobacter, Bacillus, Pseudomonas* and control.

Similarly in 45th day combined inoculation of four liquid biofertilizers inoculated treatments such as mixture of freeze dried liquid biofertilizers (*Azospirillum, Azotobacter, Bacillus, Pseudomonas)* (T1) was showed maximum response in leaves of plants (30±0.96) followed by treatments and control. In 45th day liquid biofertilizers *Azotobacter* (T3) was showed better response in leaves of plants (28±0.73) followed by other treatments and control.

The same was also obtained combined inoculation of four liquid biofertilizers treatments such as mixture of freeze dried liquid biofertilizers of (*Azospirillum, Azotobacter, Bacillus, Pseudomonas)* (T1) in 60th day was showed better response in leaves of plants (39±1.8) followed by other treatments and control (Table-2). In 60th day liquid biofertilizer alone *Pseudomonas* (T5) was exhibited better response in leaves of plants (33±1.3) followed by *Azospirillum, Azotobacter, Bacillus* and control (Table-2).

Shoot Length

Among the overall treatments, combined inoculation of four liquid biofertilizers such as mixture of freeze dried liquid biofertilizers of (*Azospirillum, Azotobacter, Bacillus, Pseudomonas)* (T1) in 30th day was showed maximum response in shoot length of plants (15.1±0.87cm) followed by other treatments and control. In 30th day liquid biofertilizer alone *Azospirillum* (T2) was showed better response in leaves of plants (14.8±0.92cm) followed by *Azotobacter, Bacillus, Pseudomonas* and control.

In 45th day combined inoculation of four liquid biofertilizer inoculated treatments such as mixture of freeze dried liquid biofertilizers of (*Azospirillum, Azotobacter, Bacillus, Pseudomonas*) (T1) was showed maximum response in shoot length of plants (23.5±1.02cm) followed by treatments and control. In 45th day of liquid biofertilizer *Azotobacter* (T3) was showed better response in shoot length of plants (17±0.88cm) followed by other treatments and control.

Similar observations were made, combined inoculation of four liquid biofertilizers treatments such as mixture of freeze dried liquid biofertilizers of (*Azospirillum, Azotobacter Bacillus, Pseudomonas)* (T1) in 60th day was showed better response in shoot length of plants (32.5±1.93cm) followed by other treatments and control (Table-2). In 60th day liquid biofertilizer alone *Bacillus* (T4) was exhibited better response in shoot length plants (29.3±0.96cm) followed by *Azospirillum, Azotobacter, Pseudomonas* (Table-2).

Root Length

Effect of combined inoculation of four liquid biofertilizers such as mixture of freeze dried liquid biofertilizers of (*Azospirillum, Azotobacter, Bacillus, Pseudomonas)* (T1) in 30th day was showed maximum response in root length of plants (5.2±0.16cm) followed by other treatments and control. In the 30th day liquid biofertilizer *Azotobacter* (T3) was showed better response in root length of plants (3.5±0.21cm) followed by other and control (Table-2).

In 45th day combined inoculation of four liquid biofertilizer inoculated treatments such as mixture of freeze dried liquid biofertilizers of (*Azospirillum, Azotobacter, Bacillus, Pseudomonas)* (T1) was showed maximum response in root length of plants (8.9±0.18cm) followed by treatments and control. In 45th day of liquid biofertilizer *Bacillus* (T4) was showed better response in root length of plants (7.5±0.92cm) followed by other treatments and control.

The comparative account of overall treatments combined inoculation of four liquid biofertilizers treatments such as mixture of freeze dried liquid biofertilizers of (*Azospirillum, Azotobacter, Bacillus, Pseudomonas)* (T1) in 60th day was showed better response in root length of plants (11.5±0.96cm) followed by other treatments and control (Table-2). In 60th day liquid biofertilizer alone *Pseudomonas* (T5) was exhibited better response in root length plants (8±0.47cm) followed by *Azospirillum, Azotobacter, Bacillus* (Table-2).

Number of Roots

Overall treatments, combined inoculation of four liquid biofertilizers such as mixture of freeze dried liquid biofertilizers of (*Azospirillum, Azotobacter, Bacillus, Pseudomonas)* (T1) in 30th day was showed maximum

response in number of roots plants (17±0.99) followed by other treatments and control. In 30th day liquid biofertilizer alone *Azospirillum* (T2) was showed better response in number of roots of plants (13.5±0.72) followed by *Azotobacter, Bacillus, Pseudomonas* and control.

In 45th day combined inoculation of four liquid biofertilizer inoculated treatments such as mixture of freeze dried liquid biofertilizers of (*Azospirillum, Azotobacter, Bacillus, Pseudomonas)* (T1) was showed maximum response in number of roots of plants (19±1.02) followed by treatments and control. In 45th day of liquid biofertilizer *Bacillus* (T4) was showed better response in root length of plants (15±0.85) followed by other treatments and control.

Better results were noticed in combined inoculation of four liquid biofertilizers treatments such as mixture of freeze dried liquid biofertilizers of (*Azospirillum, Azotobacter, Bacillus, Pseudomonas)* (T1) in 60th day was showed better response in number of roots plants (25±1.6) followed by other treatments and control (Table-2).

In 60th day liquid biofertilizer alone *Pseudomonas* (T5) was exhibited better response in number of roots plants (19±0.67) followed by *Azospirillum, Azotobacter, Bacillus* (Table-2).

Hence our study was clearly highlighted that combined inoculation of freeze dried liquid biofertilizer (*Azospirillum, Azotobacter, Bacillus, Pseudomonas)* (T1) could enhance the morphological parameters such as height of the plant, number of leaves, shoot length, root length, number of roots[17], viability of bacteria and biochemical constituents such as chlorophyll, carotenoids, compared to individual inoculation and control (Table-3). This could be the collective effect of liquid biofertilizer and also reduce the use of chemical fertilizer.[18]

CONCLUSION

In this present study, the nitrogen fixing bacterium *(Azospirillum, Azotobacter*) and phosphate solubilizing organisms *(Bacillus, Pseudomonas*) isolated from soil sample. Liquid biofertilizer are kept under Deep freezer and storage at 0, 1, 2, 3 months. Viability was properly attained in only 2nd month storage due to that 2nd month Deep freezed liquid biofertilizer are used for pot culture study. The seedling of *Capsicum annum L* transplanted in 6 pots of equal size, which were noted as Treatment (T1) Mixture of Freeze dried liquid biofertilizers (*Azospirillum, Azotobacter, Bacillus, Pseudomonas*), (T2) Freeze dried biofertilizer *Azospirillum* (T3) Freeze dried biofertilizer *Azotobacter* (T4) Freeze dried biofertilizer *Bacillus* (T5) Freeze dried biofertilizer *Pseudomonas* (T6) Control. The seedlings of pot treated with freeze dried biofertilizers. The uninoculated pots were denoted as control. Freeze dried biofertilizers was applied on plants at 15 days intervals.

Then morphological parameters such as height, leaves number, shoot length and root length, number of roots, Chlorophyll content, carotenoids, viability of cells after storage analysed at different intervals (30th, 45th, 60th) respectively. Compare to all pots the mixture of freeze dried liquid biofertilizers (T1) in 60th day was showed better response. Prevent the environmental pollution from extensive application of chemical fertilizers, the liquid biofertilizers could be recommended farmers to insure the public health and a sustainable agriculture.[14]

Table 1: Viability of bacteria used as biofertilizer

S. No	Technique	Species	Storage Time			
1			0 month	1 month	2 months	3 months
	Freeze drying	*Azospirillum*	1.9×10^7	4.6×10^6	9.8×10^5	6.2×10^4
		Azotobacter	5.1×10^6	1.53×10^6	1.28×10^5	1.13×10^5
		Bacillus	4.1×10^7	1.2×10^7	3.17×10^6	2.88×10^6
		Pseudomonas	2×10^7	3×10^8	2.66×10^5	1.74×10^5

Table 2: Impact of liquid biofertilizers on different morphological parameters in *Capsicum annum L.* (60th day)

Treatments	Height of the plant (cm)	Number of leaves /Plant	Shoot length (cm)	Root length (cm)	Number of roots / plant
T1	44.2 ± 1.96	39 ± 1.8	23.5 ± 1.93	11.5 ± 0.96	25 ± 1.67
T2	43.1 ± 1.87	37 ± 1.72	19.2 ± 1.80	10.3 ± 0.87	23 ± 1.02
T3	42.5 ± 1.56	35 ± 1.52	30.2 ± 1.31	10 ± 0.67	22 ± 0.99
T4	40.5 ± 1.44	34 ± 1.34	29.3 ± 0.96	9 ± 0.53	20 ± 0.82
T5	40 ± 1.40	33 ± 1.30	27.3 ± 0.85	8 ± 0.47	19 ± 0.67
C	38 ± 01.28	29 ± 0.89	25 ± 0.51	6 ± 0.38	15 ± 0.38

Table 3: Biochemical constituents in *Capsicum annum L.* (60th day)

Treatments	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)	Total chlorophyll (mg/g)	Carotenoids (mg/g)
T1	0.644 ± 0.66	0.599 ± 0.77	0.679 ± 0.089	0.997 ± 0.98
T2	0.497 ± 0.035	0.425 ± 0.66	0.496 ± 0.093	0.667 ± 0.96
T3	0.521 ± 0.40	0.494 ± 0.49	0.521 ± 0.423	0.515 ± 0.64
T4	0.494 ± 0.30	0.415 ± 0.53	0.498 ± 0.097	0.412 ± 0.76
T5	0.493 ± 0.53	0.399 ± 0.96	0.495 ± 0.12	0.315 ± 0.56
C	0.321 ± 0.09	0.311 ± 0.76	0.412 ± 0.56	0.201 ± 0.46

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