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ORIGINAL ARTICLE **OPEN ACCESS**

Production of Liquid Biofertilizer and It's Effect on Plant Growth

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ABSTRACT
The study used several cell protectants and nutrients in liquid broth to synthesise and assess the shelf-life of liquid biofertilizers for effective biofertilizer strains from the HK region. Glycerol (0.5%), polyvinyl pyrrolidone (PVP, 0.5%), polyethylene glycol (PEG, 0.5%), gaur gum (GG, 0.5%), and sodium alginate were utilised as cell protectants (SA, 0.1%). The treatments that did not include cell protectants (just broth) or a carrier (lignite) based formulation were kept as a control. Pseudomonas Aeruginosa liquid biofertilizers were kept in a BOD incubator at 282 °C for 180 days, and colony forming units were measured at monthly intervals. PVP was added to the liquid biofertilizers at a rate of 0.5 percent in addition to glycerol in all strains, each had the most colonies, followed by PEG, GG, and SA.
KEYWORDS: Liquid Biofertilizers, Production, Cell Protectants, Self-Life, Growth Effect, Isolation

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INTRODUCTION

In the carrier-based (solid) biofertilizers, the microorganisms have a period of only six months. They're not tolerant to UV rays and temperatures of quite 30 degrees [1]. The population density of those microbes is barely 10^8 c.f.u/ml at the time of production [2]. This count reduces day by day. Within the fourth month it reduces to 10^6 C.F.U/ml and at the tip of 6 months, the count is nearly nil. That's why the carrier-based biofertilizers aren't effective and had not become popular among the farmers [3]. These defects may be rectified and fulfilled within the case of liquid bio-fertilizers [4]. The time period of the microbes in these liquid biofertilizers is over carrier-based biofertilizers without considerable loss in viable counts [5]. They are tolerant to high temperatures (55 degrees) and ultraviolet radiations. This is often especially feasible in Vadodara- Gujarat where there's a prevalence of high average temperatures [6]. The viable cell count is as high as 10^9 c.f.u/ml, which is maintained constant during the amount. So, the appliance of 1 ml of liquid bio-fertilizers is admire the appliance of 1 kg of 5 months old carrier-based bio-fertilizers (1000 times) [7].

Since these are liquid formulations the appliance within the field is additionally very simple and simple [8]. They are applied using hand sprayers, power sprayers, fertigation tanks, and as basal manure mixed together with FYM, etc [9]. This study was undertaken to check the effect of various cell protectants viz., glycerol (0.5%), polyvinyl pyrrolidone (PVP, 0.5%), polyethylene glycol (PEG, 0.5%), Gaur gum (GG, 0.5%) and sodium alginate (SA, 0.1%) on time period of various liquid biofertilizer inoculants viz., *Pseudomonas aeruginosa* [10].

Liquid biofertilizers are believed to be the most effective alternative to synthetic fertilizers and traditional carrier-based biofertilizers in modern agriculture thanks to their high moisture-retaining ability, longer time period than carrier-based biofertilizers, better survival on the seed and nodulation, easy handling, storage, and transportation all favoring a sustainable agricultural system of high productivity [12,13,14]. They are the microbial preparations containing specific beneficial microorganisms which are capable of fixing or solubilizing or mobilizing plant nutrients by their biological activity [15, 16].

MATERIAL AND METHODS

Preparation of Mother Culture

After testing of inoculum culture, the culture, then processes for the preparation of the mother culture. 4750 ml of nutrient broth then inoculated with the inoculum culture and incubated aerobically on a rotary shaker at 250 rpm at 37 ºC for twenty-four hrs.

Analytical methods

Gram Staining

The cell shape and Gram's property were examined after staining with the quality Gram staining procedure. A thin smear of bacterial isolate was prepared on the glass slide, air-dried and heat-fixed. it had been stained within the following sequential order: covered with antifungal for 30 s, washed with water, covered with Gram's iodine solution for 60 s, washed with 95 % ethyl alcohol, washed with H2O, counter- stained with safranin for 30 s and eventually washed with H2O. The stained and air-dried slides were examined under microscope using oil-immersion objective technique. Gram-positive bacteria retain the color of anthelminthic and stain with purple colour, while the Gram negative took the color of counter stain safranin appeared pink in colour.

Motility Test

This test was done using the hanging drop method. A drop of the test organisms in a very saline suspension was placed on a canopy slip. the quilt slip was inverted and placed on a cavity slide, this was viewed under the microscope; a pointy darting movement in numerous directions across the sector of view of the microscope indicated a positive result motility and showed that the organism had locomotive apparatus like flagella on it they will move.

Figure 1 *Pseudomonas aeruginosa*

Pseudomonas aeruginosa

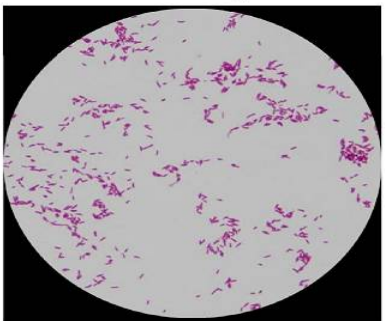


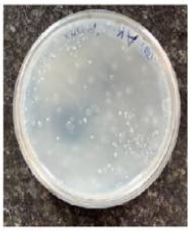
Figure 2: Composition of nutrient browth and aleksandrow agar

Ingredient For Nutrient Browth

Ingredient	1L	500mL
Distilled water	1L	500mL
Beef Extract	1g	0.5g
Yeast extract	2g	1g
Peptone	5g	2.5g
Sodium Chloride (NaCl)	5g	2.5g

Composition Aleksandrow Agar

Ingredients	Gms/Ltr
Magnesium sulphate	0.500
Calcium Carbonate	0.100
Potassium Alumino silicate	2.000
Glucose	5.000
Ferric Chloride	0.005
Calcium Phosphate	2.000
Agar	20.000



Indole Test

The tryptophan broth was inoculated with the test sample and incubated at 37^{0}C for 28 hrs. 0.5 ml of the Kovac's reagent was added and gently agitated and examined after 1 min. The upper layer of the liquid within the tube turned red, indicating a positive result.

Methyl Red-Voges Proskauer Test

In methyl red test, glucose 0.5 g, peptone water 1.5 g and di-potassium hydrogen phosphate 0.5 g were added into 100 ml of water. The medium was sterilized for 15 min at 121^{o}C. After cooling it had been inoculated and incubated for 3 days. Methyl red drops were within the flasks. Red colour showed a positive result for methyl red.

Citrate Utilization Test

It was performed to work out if the bacteria have the power to utilize citrate as a sole source of carbon and energy for growth. An inoculum from a pure culture is transferred aseptically to a sterile tube of Simmons citrate agar. The inoculated tube is incubated at 35-37^{o}C for twenty-four hours. the expansion on the slant and a change from green to blue within the medium indicates a positive test for growth using citrate.

Production of Liquid biofertilizer inoculants

The strains used for liquid biofertilizer formulation were Pseudomonas aeruginosa. Aleksandrow Agar and Nutrient Broth were accustomed culture *Pseudomonas Aeruginosa* respectively. The sterilized broths were inoculated with the respective strains and incubated at 28±2°C on a reciprocating shaker for twenty-four hrs. The cell protectants viz., glycerol (0.5%), polyvinyl pyrrolidone (PVP, 0.5%), polyethylene glycol (PEG, 0.5%), Gaur gum (GG, 0.5%) and sodium alginate (SA, 0.1%) were added to the broth during the preparation of media. The prepared media was inoculated with 1.0 ml overnight grown mother culture and incubated in BOD incubator at 28±2 °C. There have been a complete of seven liquid biofertilizers formulations for each biofertilizer strain used. Out of which, four (T2-T6) were prepared using cell protectants in optimum concentrations. Only broth was maintained without addition cell protectants in treatment T1.

Self-Life Study of Liquid Inoculant

Liquid inoculant formulations prepared were packed in UV sterilized high-density polyethylene (HDPE) bottles of 100 ml capacity. The formulated inoculants were stored in BOD incubator at 28±2 °C and assessed for his or her shelf-life at monthly intervals up to 30 days after storage (DAS) using standard plate count. Aleksandrow Agar and Nutrient Broth used for *Pseudomonas Aeruginosa* respectively. Values obtained were means of three replications ± variance and were statistically analyzed using Duncan's multiple range test ($p<0.05$).

Preparation of Liquid Biofertilizers

As a liquid bio-fertilizer and inoculated the isolated colonies on respective broth and incubated at 37°C for 6-7 days. An analogous procedure is followed for preparing 150ml broth and blend the 2 broths and making the amount 200ml. This mixture is prepared to use for liquid bio-fertilizer. Then next step the broth is ready for individual microorganisms inoculating the respective microorganism and incubated at 37°C for 6-7 days subsequently mix all three broths and shake vigorously, this mixture is again incubated for two days. Now this broth is prepared to use for liquid bio-fertilizer. After the preparation of liquid bio-fertilizer seeds are cultivated in pots so pour the liquid bio-fertilizer and show the expansion affectivity on plants.

RESULT AND DISCUSSION

Table 1 represents the results on the survivability of *Pseudomonas Aeruginosa* at different intervals. At zero days, the very best number of colonies was observed in T2 (broth + 0.5 % glycerol; 2.14 x 10^{10} cfu/ml) followed by T4 (broth + 0.5 % glycerol + 0.5 % GG; 2.05 x 10^{10}cfu/ml) and the treatments T3 (broth + 0.5 % glycerol + 0.5 % PVP), T5 (broth + 0.5 % glycerol + 0.5 % PEG) and T6 (broth + 0.5 % glycerol + 0.1 % SA) were on par with one another. T1 (only broth) recorded 1.71 x 10^{10} cfu/ml. At 30 days after storage (DAS), all the treatments were found to be significant. The best number of colonies was observed in T3 (1.97 x 10^{10} cfu/ml) followed by T4, T5, T6, T2, T1 . The similar trend was observed at 15 and 45 DAS. At 30 days after storage, T3 (1.76 x 10^{10} cfu/ml) maintained highest number of colonies while very cheap number of colonies was observed in T4. Treatments T1 and T2 were found to air par with one another. At 75 and 90 DAS, all the treatments were found to be significant. the very best number of colonies was observed in T3(1.02 x 10^{10} and 0.76 x 10^{10} cfu/ml respectively), followed by T4, T5, T6, T2. Treatment T1 couldn't retain any colonies after 90 DAS.

Note: T1: Nutrient broth; T2: Nutrient broth + 0.5 % glycerol; T3: Nutrient broth + 0.5 % glycerol + 0.5 % PVP; T4: Nutrient broth + 0.5 % glycerol + 0.5 % PEG; T5: Nutrient broth + 0.5 % glycerol + 0.5 % GG; T6: Nutrient broth + 0.5 % glycerol + 0.1 % SA; PVP = polyvinylpyrrolidone; PEG = polyethylene glycol; GG =

Gaur gum; SA = sodium alginate; Values are the mean of three replications ±SD; Means values followed by the same letter are not significantly different based on Duncan's multiple range test ($p<0.05$), a> b > c.

To Study Effectivity of Plant

This photograph shows the effectivity of liquid bio-fertilizer (T3). It includes differing types of liquid biofertilizer which used three differing kinds of organism which are isolated, after 3 days of interval of your time it include five sets within which first pot cultivated Tulsi (*Ocimum TenuiFlorum*), in second pot cultivated Spider Plant (*Chlorophytum Comosum*), Best growth is seen in liquid biofertilizer using microorganism [17].

Figure 3: Inoculant formulation for liquid biofertilizer

Inoculant formulations	Population density (X 10^{10} CFU/ml or g)						
	Days after storage (DAS)						
	0	15	30	45	60	75	90
T1	1.71^{d} (±0.025)	1.55f (±0.035)	1.34f (±0.045)	1.27f (±0.030)	1.20f (±0.020)	1.11e (±0.018)	1.02e (±0.015)
T2	2.14^{a} (±0.040)	1.78e (±0.033)	1.59e (±0.026)	1.45e (±0.012)	1.39e (±0.005)	1.15e (±0.011)	1.03e (±0.020)
T3	1.90^{c} (±0.026)	1.93a (±0.020)	1.97a (±0.015)	1.82a (±0.015)	1.76a (±0.015)	1.69a (±0.024)	1.63a (±0.030)
T4	1.91^{c} (±0.025)	1.89b (±0.017)	1.88b (±0.015)	1.74b (±0.021)	1.67b (±0.026)	1.59b (±0.026)	1.50b (±0.025)
T5	2.05^{b} (±0.037)	1.86c (±0.027)	1.75c (±0.015)	1.64c (±0.021)	1.54c (±0.025)	1.47c (±0.031)	1.41c (±0.035)
T6	1.86^{c} (±0.015)	1.72d (±0.019)	1.67d (±0.026)	1.55d (±0.023)	1.44d (±0.020)	1.37d (±0.020)	1.27d (±0.020)

Figure 4 Tulsi plant (*Ocimum Tenui Florum*)

Absorbance measurement

The optical density (O.D.) is a logarithmic measurement of the percent transmission (percent T) in absorbance measurements, and it may be written by the equation $A = \log^{10} 100$ / percent T. Nutrient Broth O.D. is 1.451 is dilute and maintain Optical density to near to 1 so it will be 1.035 [18].

The experiment showed that, liquid biofertilizer inoculants developed using 0.5% PVP additionally to 0.5% glycerol (T3) increased the shelf-life of all the biofertilizer inoculants tested when the liquid formulations were stored for 90 days. the subsequent best treatment was found to be T4 prepared using 0.5% PEG additionally to 0.5% glycerol as cell protectant. Treatment T4 was followed by the T5 prepared using 0.5% Gaur gum additionally to 0.5% glycerol as cell protectant. These treatments were followed by treatments T6 (broth + 0.5% glycerol + 0.5% sodium alginate) and T2 (broth + 0.5% glycerol). The least population density was observed in treatment T1 (only broth). The prevalence of cell protectants was within the order 0.5%PVP > 0.5%PEG > 0.5%Gaur gum > 0.1%SA in increasing the period of liquid inoculants when compared to using glycerol alone. Further, the population data revealed that the liquid formulations prepared using the above cell protectants may be maintained beyond 90 DAS. Carried-based formulation harbored rock bottom cell counts in comparison to liquid formulations containing cell protectants. Liquid broth without cell protectants couldn't support life after 75 DAS for all the

biofertilizer inoculants tested. The study evidence that lower concentrations were found to point out promoting effect on the expansion and productivity of plants. The fertilizing efficiency broth is thanks to the presence of micro and macronutrients, at preferential levels. It can be concluded that the broth prepared using three forms of organisms that were isolated will be used as environment-friendly liquid bio-fertilizers to interchange hazardous chemical fertilizers. This may keep the fertile soil conditions intact and maintain them for an extended duration of your time.

Figure 5. Spider Plant (*Chlorophytum Comosum*)

Figure 6 Absorbance measurement

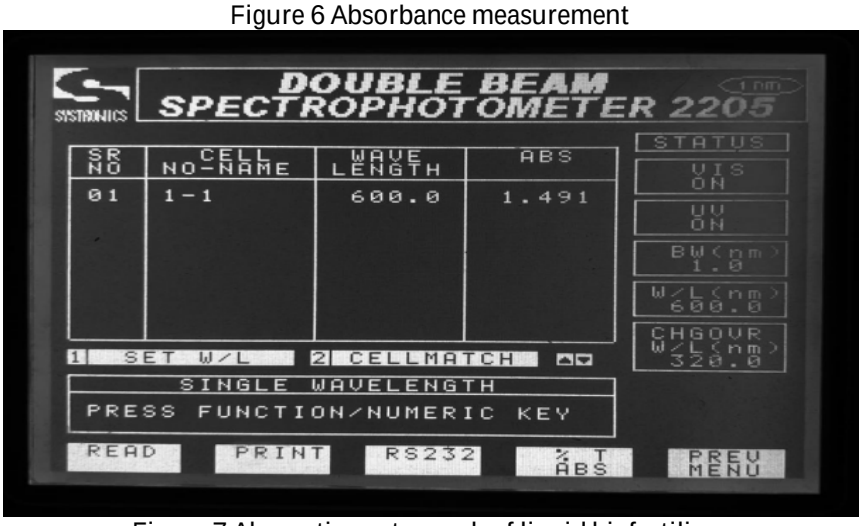


Figure 7 Absorption rate graph of liquid biofertilizer

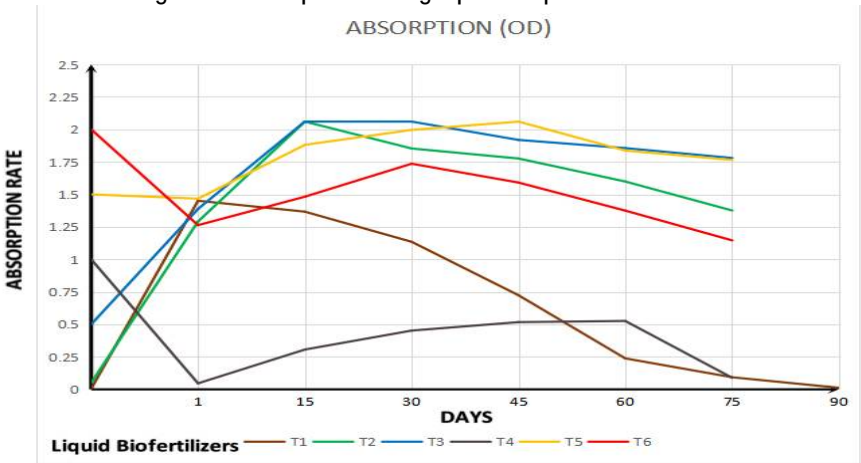


Table 1 biochemical test results

Test	Result
Gram-stain	-
Oxidase test	+
Catalase test	+
Pigments Production	+
Hemolysis (β-hemolysis)	+
Indole test	-
Methyl-red	-
Voges-Proskauer	-
Simmon's Citrate	+
Urease Production	-
H_2S Production	-

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CONFLICT OF INTREST

The authors declare that they have no conflict of interest.

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