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A methodology to develop liquid formulation of biofertilizer technology

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Abstract
Microbial based nutrient inputs have emerged as the potential alternative for the productivity, reliability and sustainability of the global food chain. Although, carrier-based biofertilizers are in vogue since long and their use has proved beneficial both for crop yields as well as soil health, but, these are still not popular with the farming community because of varying reasons and mixed results and the main concern has been the viability of the microorganisms. To overcome such problems liquid formulations of biofertilizer have gained popularity because of their storage stability, easy delivery, increased persistence and protection from harmful and damaging environmental factors which seem to be the only alternative for cost-effective sustainable agriculture. In the present study, a new methodology have been designed for development liquid formulation technology where a combination of carrier, emulsifier and thickener was used. Two different types i.e., water-oil based and nutrient broth based formulations were designed and amended with PGPR inoculants. These formulations not only contain the desired microorganisms and their nutrients but also special cell protective agents and additives like emulsifier and thickener that promote for longer shelf life and tolerance to adverse conditions. These formulations were stored at different temperatures (4 °C, 28 °C and 40 °C) for 16 months and analyzed for their physio-chemical and biological properties.

Keywords: Liquid formulations, PGPR, carrier, emulsifier, thickener, protective agents

Introduction
Agriculture has long been associated with the production of essential food crops, particularly in rural India, where it serves as the primary source of livelihood. The Green Revolution significantly increased food grain production, but it often neglected soil productivity and agricultural sustainability. Globalization and technological advancements have brought challenges to agriculture in developing nations like India. Excessive use of chemical fertilizers, while initially beneficial due to their immediate nutrient availability, has led to issues like leaching, water pollution, destruction of beneficial organisms, disease susceptibility in crops, and soil fertility decline, ultimately harming the ecosystem. In this context, microbial-based nutrient inputs have emerged as a promising alternative for enhancing global food production's productivity, reliability, and sustainability. Microorganisms play a vital role in nutrient fixation, solubilization, mobilization, and cycling. Carrier-based biofertilizers have demonstrated superior results over chemical alternatives in improving agriculture productivity and soil health. Despite their proven benefits, carrier-based biofertilizers remain underutilized in the farming community due to various factors, with concerns about the viability of microorganisms being a key issue (Kaminsky *et al*., 2020; Bharti *et al*., 2017) [12, 2]. Liquid biofertilizer technology offers compelling reasons for its adoption, emphasizing the use of agriculturally important microorganisms in liquid formulations. These formulations contain not only the desired microorganisms and their nutrients but also protective substances that enhance shelf life and tolerance to adverse conditions, making them ideal for field application. Different approaches exist for developing these liquid formulations, with the common goal of ensuring storage stability, easy delivery, increased persistence, and protection from environmental factors (Lee *et al*., 2016) [14]. Various agriculturally important microorganisms, such as Azotobacter, PSM, Azospirillum, and potash mobilizing bacteria, are available in liquid formulations (Allouzi *et al*., 2022) [1]. The success of biofertilizers in the field depends on the quality of these bioformulations. Shelf-life of inoculants is crucial, and the choice of an ideal polymer is essential to prevent rapid degradation of microorganisms in the soil. Polymers like sodium alginate, methyl cellulose, trehalose, arabinose, gum arabic, starch, glycerol,

polyethylene glycol (PEG), polyvinyl pyrrolidone, and DMSO have been used as additives or cell protectants to enhance the stability of liquid formulations (Dinesh *et al*., 2020) [6]. To strengthen the shelf life and field efficacy of biofertilizers in India, it is imperative to find a breakthrough in inoculant technology. This study was conducted to explore the impact of different polymers, such as gum arabic, CMC, glycerol, polyvinyl pyrrolidone and DMSO on increasing the survival of liquid PGPR inoculants. This research aims to make biofertilizers more commercially viable and acceptable to farmers.

Methodology

Procurement of cultures

Four PGPR cultures *viz.*, *Bacillus* spp., *Pseudomonas* spp., *Azotobacter* spp. and *Azospirillum* spp. were obtained from stock cultures of Microbiology Department, College of Basic sciences and Humanities, Dr. RPCAU, Pusa, Bihar and used in development of liquid inoculants. These cultures were earlier identified as P, K, Zn and Fe solubilizer.

Growth and maintenance

Different media *viz*., NYSM broth (Nutrient Yeast Extract Salt Medium), King's B broth, Jensen'sbroth, Semi-solid Nitrogen-Free bromothymol malate broth (NFb) were used to revive *Bacillus* spp., *Pseudomonas* spp., *Azotobacter* spp. and *Azospirillum* spp. respectively. The cultures were inoculated in respective sterilized broths and incubated for 24 hrs on a reciprocatory shaker at 28±2 °C.

Compatibility Assessment

We conducted a compatibility assessment for all the collected bacterial cultures, following the method outlined by Raja *et al*. (2006) [19]. On nutrient agar plates, these bacterial cultures were streaked vertically in the centre and then horizontally along the sides of the agar plates. The cross-streaked nutrient agar plates were subsequently incubated at 28 ± 2 °C for a period of 48-72 hours. During incubation, we closely observed the development of inhibition zones around the colonies. Those colonies without inhibition zones were deemed compatible, signifying their non-antagonistic behavior. These compatible strains were selected for the development of the PGPR consortium.

Formation of PGPR Consortium

The PGPR consortium was formed by carefully selecting compatible bacterial strains, demonstrating a lack of antagonistic behavior. To prepare this consortium, 2ml of broth cultures of each of these compatible strains were placed in 250 ml conical flasks, containing 100 ml of Luria Bertani broth. The prepared consortium was kept s subjected to a reciprocating shaker at room temperature for 48 hours to obtain optimal growth. Subsequently, the cell count of individual culture and consortium was enumerated by serial dilution plate method.

Preparation of Formulations

Different liquid formulations were prepared using combination of carriers like vegetable oil, emulsifiers such as Tween 80, and thickening agents including Starch, Gum arabic, and CMC. To enhance stability, different concentrations of protective agents, namely DMSO, PVP, and Glycerol, were introduced into the mix. These experiments were meticulously conducted within 100 ml plastic bottles containing the tailored liquid formulations. Initially, pH of all the formulations were adjusted to 7 by adding drop by drop 1 N NaOH or 1 N HCl. Subsequently, the liquid inoculants underwent sterilization in an autoclave at 121 °C under 15 lbs of pressure. 2.5% v/v late log phase (4 days old) cultures of the PGPR consortia was inoculated into each formulation. The inoculated formulations underwent continuous shaking for four hours daily over the course of one week. After that these formulations were stored under three distinct temperature conditions: 4 °C (in a refrigerator), 28 °C (under room conditions), and 40 °C (in an incubator). These formulations were characterized for shelf life and physio-chemical properties up to 16 months at every two months' time intervals.

Components of liquid formulation

1. Carrier(1%) - Vegetable oil
2. Emulsifier (2%) - Tween 80
3. Thickener (0.5%) - Starch, Gum Arabic and CMC
4. Protective agents - DMSO, Glycerol, PVP

Table 1: Different combinations of protective agents used in formulations

Sl. No.	Protective agents	Concentration (%)
1.	DMSO	1.5% & 3%
2.	Glycerol	1.5% & 3%
3.	PVP	1.5% & 3%
4.	Mixture (DMSO+ Glycerol)	1.5% +1.5%
5.	Mixture (PVP+ Glycerol)	1.5% +1.5%
6.	No Protective agents	-

Methodology developed

(a) Water based formulation

A liquid formulation was prepared through a systematic process (Fig. 1). Initially, 500 mg of a thickening agent was mixed with 95 ml of distilled water, and the blending continued for a duration of 5 minutes. Following this, 2 ml of an emulsifier was introduced into the mixture and blended for an additional 2 minutes. Subsequently, various concentrations of protective agents were incorporated into the blend, followed by an additional 2 minutes of thorough blending. Finally, 1 ml of a carrier was introduced into the mixture, and the blending process was concluded with an additional 1 minute of mixing. The result was a well-prepared liquid formulation. When starch was taken as a thickener, it was first boiled in distilled water till the solution showed transparency. It was then cooled before blending. When solution got cool then blended.

b) Nutrient broth based liquid formulation

Three types of nutrient broth based liquid formulations were prepared, denoted as NB-100%, NB-75%, and NB-50%. The formulations were crafted by blending nutrient broth with varying concentrations of distilled water along with 250 ml of thickener. This mixture underwent thorough shaking for a duration of 5 minutes. Subsequently, 1% emulsifier was introduced into the blend, and the mixture was shaken for an additional 3 minutes. Following this step, different protective agents were added, and the blend underwent further shaking for 2 minutes (Fig. 2).

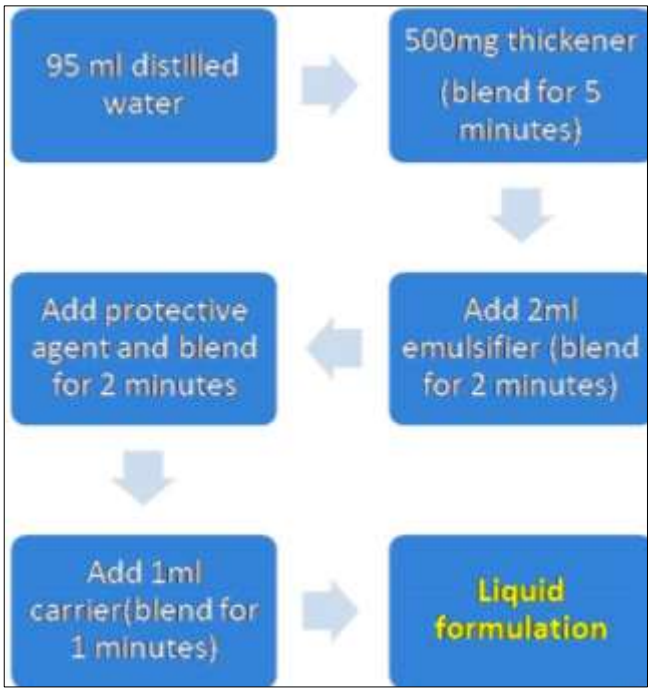

Fig 1: Flow diagram of preparation of water based formulation

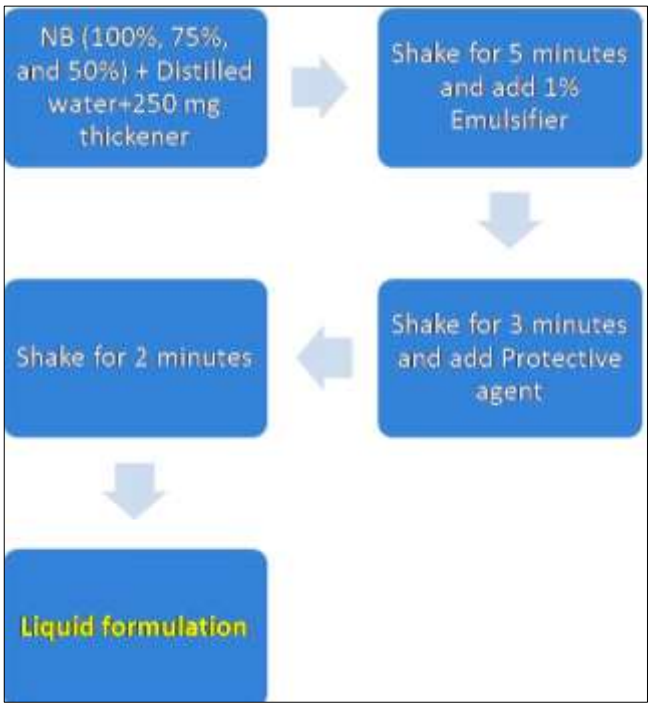

Fig 2: Flow chart of preparation of nutrient broth based liquid formulation

Characterization of formulations

Suspensibility (Width of suspension)

To assess the dispersion of the emulsion in water, we measured its suspensibility, specifically focusing on the width of the suspension. This assessment involved taking 10 ml of the formulation and transferring it into a 15 ml graded test tube. These test tubes were then placed in incubation overnight at varying temperatures, including 4 °C, 28 °C, and 40 °C. The following day, we meticulously measured the width of the settled suspension at the bottom of each test tube for all formulations. This evaluation was conducted at regular intervals over a 16-month period.

pH Measurements

Under sterile conditions, 10 ml of each formulation was meticulously collected, and their pH values were recorded using a pH meter. Initially, we adjusted the pH of all formulations to a neutral level by adding either 1 N NaOH or 1 N HCl. Subsequently, we maintained routine pH readings, monitoring them at monthly intervals throughout the 16-month storage period.

Shelf Life Assessment (CFU Enumeration)

The assessment of the formulation's shelf life involved determining the cell population through Colony Forming Unit (CFU) enumeration. We employed the serial dilution plate method on nutrient agar to perform this assessment. In this process, we started by collecting 10 ml of the formulation, ensuring thorough mixing. This collected mixture was then placed into a sterilized centrifuge tube, followed by centrifugation at 5000 rpm for a duration of 10 minutes. After centrifugation, the supernatant was carefully discarded, and the pellet was retained. The pellet was subsequently cultivated in nutrient broth for a period of 72 hours. After this incubation period, we extracted 1 ml of the broth using a micropipette (1000 µl) and diluted it into 9 ml of distilled water. This mixture underwent vigorous shaking on a vortex for 2 minutes, and further dilution was performed to achieve a final dilution of 10^8. From this dilution, 0.1 ml was evenly spread onto sterilized nutrient agar plates by gently rotating them in both clockwise and anti-clockwise directions to ensure the uniform distribution of the suspension on the medium. These plates were then incubated for 48 hours in an incubator, following which the colonies were counted (Fig. 3).

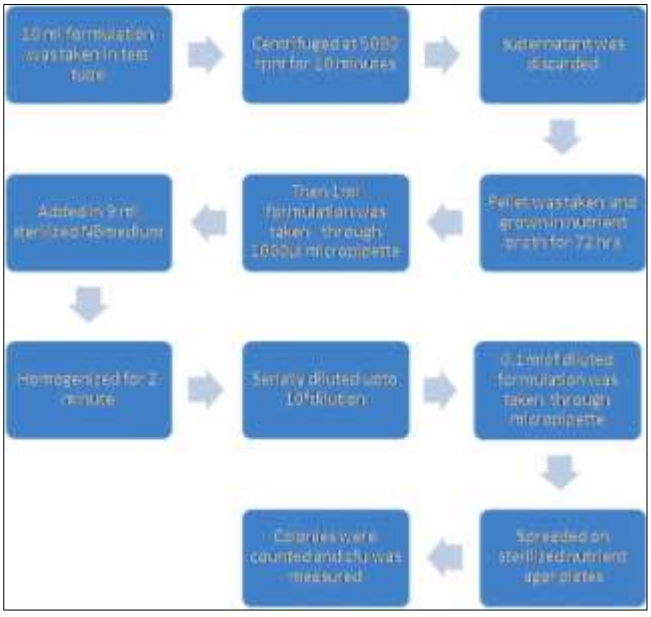

Fig 3: Flow diagram of measurement of cell population

Revival of culture from formulation

Ten ml formulation containing culture was taken in a pre-sterilized centrifuge tube and centrifuged at 5000 X g for 10 minutes. The cell pellet was washed with sterilized distilled water, centrifuged and finally re-suspended in 10 ml sterilized NB medium. This re-suspended cell pellet was used as inoculum to inoculate 100 ml NB medium taken in 250 ml conical Erlenmeyer flasks. The flasks were incubated in a culture room at 28 ± 2 °C under incubator for 48 hours and

analyzed for their PGPR characteristics. This activity was done at every month interval up to 16 months (Fig. 4).

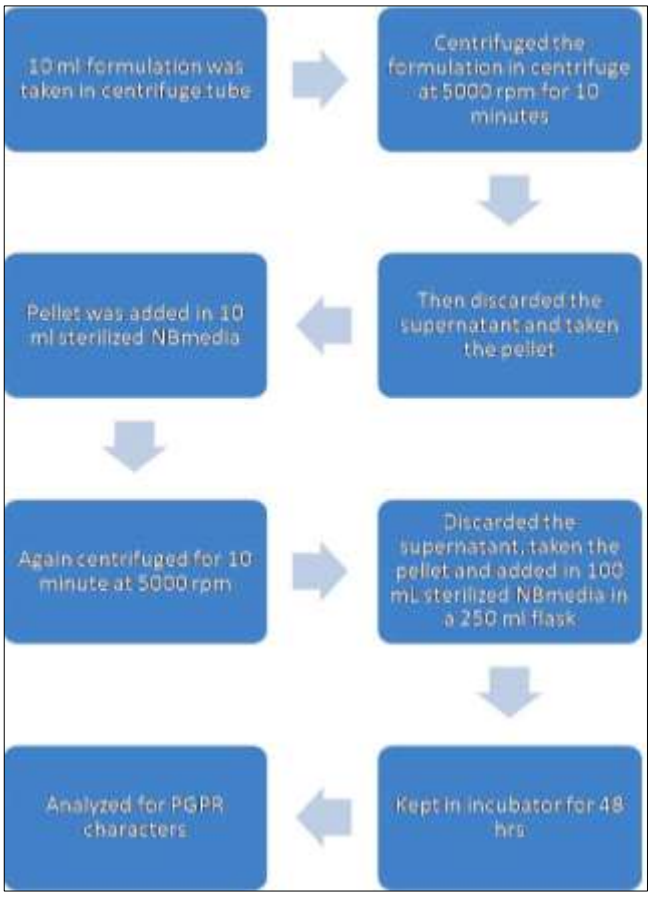

Fig 4: Flow diagram of revival of culture from formulation

Result

Compatibility test of collected bacterial cultures

In the cross streak assay on nutrient agar plates, all the six bacterial cultures didn't show inhibition zones around the colonies *i.e.* they didn't show antagonistic behavior with each other. Hence they were compatible, therefore all the six bacterial cultures were used to form PGPR consortium.

Suspensibility (Width of suspension) of liquid formulations

It was observed that the width of suspension was variable in the range of 0 to 3.2 cm across all the formulations and at all storage temperatures. In all types of formulations, the width of suspension was increased with increase in storage period up to 4 months, afterwards it was decreased with further storage and became stable in the final months of storage. It was observed that all the formulations showed least value of width of suspension in final storage periods. At 40 °C decrease in suspensibility was minimum when compared to 4 °C and 28 °C temperatures. It might be because during initial months the components were not utilized by microbes results in increased with of suspension and later it was decreased due to utilization by microbes. This meant that when formulations were applied to seeds, the bacterial cells in the formulations would stay in the upper layers and obtain enough light and oxygen to grow and multiply.

pH of liquid formulations

By using pH meter, pH of both the nutrient broth based and water based formulations stored at different temperatures (4 °C, 28 °C and 40 °C) were analysed at different intervals of storage. The pH of the formulations was observed to be variable and dependent on the type of the formulation. It was observed that pH of most of the formulations gradually decreased with increasing days of storage. The pH of the formulations stored at temperature 4 °C was more stable followed by 28 °C and then 40 °C. The pH at was observed in range of 6.20 to 7.18 across the formulations during 16 months of storage. Also pH was stable in formulations prepared with all three types of thickeners followed by two thickeners and single thickener. Least pH was observed in formulation without any thickener. It was found that unlike the nutrient broth based formulations, in water based formulations, pH was decreased during initial months of storage period then increased and became stable in later months.

Shelf life (CFU) of liquid formulations

The formulations incorporating protective polymers demonstrated significantly enhanced viability and metabolic activities of PGPR compared to those lacking protective polymers. In particular, all formulations amended with protective polymers and stored in the refrigerator exhibited notably higher viable cell counts and other metabolic activities of PGPR when compared to formulations without these protective agents. Throughout the 16-month incubation period, across various storage temperatures, all formulations maintained a cell population ranging from 2.3 x 10^7 to 6.2 x 10^7 cells ml^{-1}. This was in contrast to the initial cell population of 3.5 x 10^9 cells ml^{-1} added to each formulation. Notably, combinations of protectants yielded superior results compared to individual protectant usage, irrespective of the storage temperature. Formulations containing DMSO, particularly in combination with glycerol as protective polymers, displayed extended shelf life and improved metabolic activities at 4 °C. In contrast, at 28 °C and 40 °C, formulations containing PVP and combinations of PVP with glycerol exhibited better shelf life and metabolic activities. Over the initial three months, an increase in cell count was observed, with NB-100% formulations showing the highest increase, followed by NB-75% and NB-50%. However, after 16 months of storage at all temperatures, NB-75% formulations displayed the highest cell count, followed by NB-100% and NB-50% formulations. During the initial months of storage (up to 3 months), formulations stored at 4 °C exhibited lower cell counts compared to those stored at 28 °C and 40 °C. Greater fluctuations in cell count were observed in formulations stored at 40 °C in comparison to those stored at 28 °C and 4 °C. In all storage temperatures, cell counts initially increased and then declined from the 3rd month onward, stabilizing later on. Notably, the decline in cell count was more pronounced in formulations stored at 40 °C, followed by 28 °C and 4 °C, with the lowest cell count observed in the formulations stored at 40 °C after 16 months of storage. A distinct trend was observed in water-based formulations, where cell counts decreased during the initial storage period, then increased in the later months, ultimately stabilizing. This was in contrast to nutrient broth-based formulations, which exhibited a continuous increase in cell count across the 16-month storage period.

Fig 5: Liquid formulations

Fig 6: Growth of revived consortium from formulation stored at 40 °C, 280 °C and 400 °C on nutrient agar plates respectively

Discussion

The conducted assessments demonstrated the overall stability of the formulations at all tested temperatures, both in terms of physical characteristics and cell viability. Notably, the higher survival rate observed under freezing conditions (4 °C) can be attributed to the fact that lower temperatures restrict or minimize microbial growth and reduce nutrient consumption during storage. This allows the organisms to remain at an optimal concentration for an extended period, while limiting cell death in the inoculums at lower temperatures. In contrast, storage at room temperature (around 28 °C) may lead to increased microbial growth, nutrient depletion, and the accumulation of potentially hazardous components (Tittabutret *et al*., 2007) [26]. Studies conducted by Jaiswal *et al*. (2022) [11] also found that formulations stored at 4 °C exhibited extended shelf life and stable pH compared to those stored at higher temperatures like 28 °C and 40 °C. Similarly, Patil *et al.* (2012) reported that the viable cell count of certain bacterial strains was lower at 25 °C compared to 4 °C. This aligns with our study's findings, suggesting that higher temperatures (28 °C and 40 °C) are suitable for short-term storage, while 4 °C offers extended shelf life for long-term storage.

Regarding the number of viable bacteria in nutrient broth-based formulations, a significant increase was observed during the first three months, followed by a subsequent decline and stabilization. This trend is likely due to the ready availability of nutrients in the nutrient broth and various thickeners, which initially support bacterium growth (Dinesh *et al*., 2020) [6]. These components promote the growth of the PGPR consortium, resulting in an extended bacterial growth phase. However, the subsequent decrease in cell count can be attributed to nutrient depletion as the bacteria grew and the available nutrients became insufficient to meet their requirements. This phenomenon is consistent with findings from studies conducted by Santhosh (2015) [20] and Kumaresan and Reetha (2011) [13], where the cell count decreased during prolonged storage due to nutrient depletion and cell autolysis, possibly influenced by competition and the production of toxic compounds by bacteria. In contrast to nutrient-based formulations, water-based formulations showed an initial decrease in cell count during the early months, likely because an immediate nutrient source was not readily available. However, in later months, the cell count increased as microbes began to access nutrients by degrading them. Over the 16-month storage period, more fluctuations in cell count were observed in nutrient broth-based formulations compared to water-based formulations, potentially due to the higher availability of nutrients in the nutrient broth. After the 16-month storage period, the readings indicated that the cell count was higher in nutrient broth-based formulations compared to water-based formulations, in line with similar findings by Phua & Khairuddin (2010) [18] and other researchers who observed longer shelf life in media-based liquid formulations. In the case of NB-100% formulations, they initially promoted more microbial growth compared to NB-75% and NB-50%. However, after 16 months, the cell count was higher in NB-75% followed by NB-100% and NB-50%. This change in trend may be attributed to the initial availability of nutrients that promoted growth, but in the later months, the release of toxic compounds by microbes and nutrient deficiencies led to microbial death in NB-100% formulations. In contrast, NB-75% formulations consistently promoted microbial growth due to the optimal availability of nutrients, while growth in NB-50% formulations was lower due to limited nutrient availability (Buntić *et al*., 2019; Surendra and Baby, 2016; Vendan and Thangaraju, 2006) [3, 24, 27].

Role of different additives on shelf life

Liquid biofertilizer formulations are a crucial component of modern agricultural practices, designed not only to contain beneficial microorganisms and essential nutrients but also to incorporate specialized cell protectants and polymeric additives that extend their shelf life and enhance their resilience under adverse conditions. These formulations often employ various types of polymers known for their ability to limit heat transfer, maintain high water activity, and exhibit favorable rheological properties (Jaiswal *et al*., 2022 Hindersah *et al*., 2020; Hegde, 2008) [11, 10]. Commonly utilized polymers include sodium alginate, polyvinyl pyrrolidone (PVP), polyvinyl alcohol (PVA), gum arabic, and polyethylene glycol (PEG), which serve as both thickeners and adhesives. These liquid inoculants comprise several essential components, including carriers (such as nutrient broth and water-oil mixtures), emulsifiers (e.g., Tween-80), thickeners (e.g., carboxymethyl cellulose and gum arabic), and protective agents (including polyvinyl pyrrolidone, glycerol, and dimethyl sulfoxide or DMSO). The selection of substrates for these liquid biofertilizers aims to provide microorganisms with complex sources of nutrition, resulting in slow nutrient digestion and a sustained presence in the broth suspension. It has been observed that the highest cell

count is achieved in formulations containing all types of thickeners, followed by those with two types of thickeners and, lastly, formulations with a single type of thickener. These thickeners are beneficial due to their high nutrient content, water retention capacity, and aeration properties, which collectively play a protective role in promoting bacterial growth (Jaiswal *et al.*, 2022; Rocha *et al*., 2011; Khavazi *et al*., 2007) [11]. In addition to thickeners, emulsifiers like Tween-80 are employed in liquid inoculants, primarily for their non-ionic surfactant properties. These properties contribute to the stability and relative non-toxicity of Tween-80, making it a suitable emulsifier for various domestic, pharmaceutical, and scientific applications (Daniel *et al.*, 2013) [4]. CMC already proved to support high bacterial populations. The present findings are similar to the results obtained by Daniel *et al*. (2013) [4] who demonstrated that liquid biofertilizers with CMC @ 0.1 percent had excellent cell retention property. He *et al*. (2015) also found that the formulations modified with sodium carboxymethyl cellulose, corn flour, sodium alginate, bentonite and urea had a larger number of live bacteria (1×10^8 CFU/ml after six months at 25±3 °C) than that of control. Gum arabic, known for its adhesive, emulsifying, and stabilizing properties, plays a vital role in limiting heat transfer, maintaining high water activity, and protecting cells from desiccation and drying. This ability to prevent complete dehydration and ensure the survival of essential microbial components makes gum arabic an important adhesive agent in liquid inoculants (Hindersah *et al*., 2020) [10].

In the present study, various protective agents, including Polyvinyl Pyrrolidone (PVP), glycerol, and dimethyl sulfoxide (DMSO), were incorporated into all liquid inoculants. These agents were crucial for enhancing shelf life and metabolic activities.Notably, formulations containing DMSO, as well as combinations of DMSO and glycerol as protective polymers, exhibited extended shelf life and metabolic robustness, particularly at a lower storage temperature of 4 °C. This observation aligns with the findings of who emphasized the superior cryoprotective capabilities of DMSO compared to glycerol and PVP for preserving microorganisms under frozen storage conditions. PVP, a water-soluble compound with colloidal stabilization and adhesive properties, played a pivotal role in these liquid inoculants. Its high water-holding capacity effectively slowed down the drying rate of the medium, ensuring the maintenance of adequate moisture levels for microbial metabolism. Additionally, PVP exhibited an ability to bind bacterial toxins, which was advantageous in reducing protein precipitation or cell coagulation, thereby enhancing biological integrity and overall survival. Bacteria did not utilize PVP as an energy source, ensuring its effectiveness as a protective agent (Jaiswal *et al.*, 2022; Buntić *et al*., 2019; Singleton *et al.*, 2002; Deaker *et al*., 2004) [11, 3, 22, 5]. The combination of PVP and glycerol, with their substantial water-binding capacity, maintained a conducive environment around the microbial cells for continued metabolic activity and prevented rapid desiccation of the inoculant post-application. The adhesive nature of PVP further facilitated its adherence to seeds, enhancing its effectiveness. Furthermore, PVP exhibited colloidal stabilization properties that protected bacteria within colloidal systems (Gopi *et al*., 2019) [8]. Studies by Sridhar *et al*. (2004) [23] supported the role of glycerol in liquid formulations, particularly in maintaining the viability of cells during extended storage periods. Glycerol, along with PVP and glucose, was found to support higher populations of live bacteria and endospores for up to six months of storage. Girisha *et al*. (2006) [7] also reported that liquid inoculant formulations with PVP as an osmoprotectant exhibited superior shelf life compared to those without PVP. The combination of PVP and glycerol, as well as DMSO and glycerol, showed enhanced shelf life and metabolic activities under various storage conditions, validating their effectiveness (Santhosh., 2015; Daniel *et al*., 2013; Gopal and Baby., 2016) [20, 24, 4]. In the realm of liquid biofertilizers, the inclusion of glycerol and DMSO is noteworthy. These protectants operate through colligative mechanisms, lowering the freezing point of water and biological fluids (Gopi *et al*., 2019; Mugilan *et al.*, 2011) [8, 17, 22]. They also intracellularly bind water, preventing excessive dehydration, reducing salt toxicity, and inhibiting the formation of large ice crystals within cells. Glycerol's high water-binding capacity contributes to its protective role, safeguarding cells from the adverse effects of desiccation by slowing down the drying process. Jaiswal *et al*. (2022) [11] reported the efficacy of formulations containing DMSO and glycerol, emphasizing their capacity to enhance shelf life and metabolic activities under different temperature conditions. Furthermore, horticultural oil was introduced in this study and seamlessly assimilated into the formulations without affecting bacterial growth. Lee *et al*. (2016) [14] discovered that horticultural oil could enhance the viability of specific microbial strains, such as *Rhodopseudomonas palustris* strain PS3, while the exopolysaccharides produced by these cells helped reduce stress during storage.

Conclusion

Liquid formulation represents an emerging and distinctive technology in India. It offers unique production methods and characteristics. Liquid biofertilizers play a pivotal role in prolonging the survival of microorganisms by providing them with an optimal medium throughout the entire crop cycle. Liquid formulations provide manufacturers with the flexibility to incorporate ample nutrients, cell protectants, and inducers responsible for cell, spore, or cyst formation. This comprehensive approach ensures an extended shelf-life for these formulations. In the present study, a methodology was meticulously developed, leveraging various carriers, emulsifiers, thickeners, and protective agents. These formulations exhibited remarkable longevity, maintaining pH stability for up to 16 months of storage. Therefore, liquid biofertilizers emerge as a compelling alternative to conventional carrier-based biofertilizers in contemporary agricultural research. They hold the potential to contribute significantly to enhanced crop yields, soil health restoration, and the pursuit of sustainable global food production. In essence, they serve as preparations designed to preserve microorganisms and deliver them to target regions, thus enhancing their biological activity. These consortia of microorganisms are provided with a suitable medium to sustain their viability over a defined period, ultimately augmenting the biological activity at the intended site.

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