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OPTIMIZATION OF ADDITIVE-BASED LIQUID INOCULANT FORMULATIONS FOR IMPROVED SURVIVAL AND STORAGE STABILITY OF *Pseudomonas fluorescens*

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ABSTRACT

The commercial application of liquid bioinoculants is often constrained by reduced microbial viability during prolonged storage. The present investigation was undertaken to develop and optimize additive-based liquid formulations of *Pseudomonas fluorescens* with enhanced shelf life and storage stability under ambient laboratory conditions. Liquid formulations were prepared using King's B broth supplemented with different combinations of polymeric cell protectants [polyvinylpyrrolidone (PVP), polyethylene glycol (PEG), sodium alginate (SA) and glycerol] along with adjuvants [carboxymethyl cellulose (CMC), trehalose and Alphox], while Tween-20 (0.5%) and potassium sorbate (0.5%) were used as common surfactant and preservative, respectively. An untreated broth formulation served as the control. The formulations were inoculated with *P. fluorescens* (10^8 CFU ml^{-1}) and stored at 28 ± 2°C for 360 days. Viable cell populations were determined at regular intervals using the serial dilution and plate count technique. Among the polymeric additives, 2% PVP maintained the highest viable population throughout the storage period, followed by 2% PEG, 0.1% sodium alginate, and 2% glycerol. Among the adjuvants, 0.1% CMC proved superior to trehalose and Alphox in preserving bacterial viability. The interaction of PVP (2%) + CMC (0.1%) exhibited the best performance, recording the highest viable population of 107.90×10^8 CFU ml^{-1} at 30 days and maintaining 9.03×10^8 CFU ml^{-1} after 360 days of storage. Similar trends were observed in PEG + CMC (8.90×10^8 CFU ml^{-1}), SA + CMC (8.87×10^8 CFU ml^{-1}) and glycerol + CMC (8.77×10^8 CFU ml^{-1}) formulations. In contrast, the control formulation showed a rapid decline in viability and completely lost the bacterial population after 120 days of storage. The results demonstrate that polymer-based additives, particularly the combination of PVP and CMC, significantly improve the long-term survival and storage stability of *Pseudomonas fluorescens* in liquid formulations. The optimized formulation offers a promising strategy for the development of stable and effective liquid bioinoculants for sustainable crop production and biological disease management.

Keywords **:** Liquid bioinoculants, *Pseudomonas fluorescens*, Additives, Shelf-life, Storage stability, Cell viability, Biofertilizer formulation.

Introduction

The use of microbial bioinoculants has emerged as an environmentally sustainable alternative to chemical fertilizers and pesticides for improving crop productivity and managing plant diseases. Among the various plant growth-promoting rhizobacteria (PGPR), *Pseudomonas fluorescens* has received considerable attention because of its ability to suppress a wide range of soil-borne pathogens through mechanisms such as antibiotic production, siderophore secretion, induced systemic resistance, competition for nutrients and production of hydrolytic enzymes. In addition to

disease suppression, *P. fluorescens* enhances plant growth by improving nutrient availability and stimulating root development, making it an important component of integrated disease management (IDM) and sustainable agriculture. The successful commercialization of microbial inoculants depends largely on the development of formulations capable of maintaining high populations of viable cells during storage. Conventional carrier-based bioinoculants often suffer from several limitations, including short shelf life, contamination, reduced microbial survival and inconsistent field performance. In contrast, liquid bioinoculants provide several advantages, such as higher microbial populations, ease of application, improved seed adherence, better tolerance to environmental stress and extended storage stability. However, maintaining microbial viability during prolonged storage under ambient conditions remains a major challenge, limiting their large-scale adoption. Various formulation additives have been investigated to enhance the survival and stability of microbial inoculants during storage. Polymeric stabilizers such as polyvinylpyrrolidone (PVP), polyethylene glycol (PEG), sodium alginate, Glycerol and Carboxymethyl cellulose (CMC) have been reported to improve moisture retention, reduce desiccation stress, enhance viscosity and protect microbial cells from adverse environmental conditions. These polymers also facilitate bacterial adhesion to seeds and help maintain high viable cell populations during storage (Mugnier and Jung, 1985; Temprano *et al*., 2002; Bashan, 2004; Deaker *et al*., 2004). Likewise, osmoprotectants such as Trehalose, Glycerol, Glucose and mannitol contribute to microbial survival by stabilizing cellular membranes and proteins under osmotic and desiccation stress. Appropriate combinations of polymers, osmoprotectants, surfactants and preservatives have therefore been shown to substantially improve the shelf life and field performance of liquid bioinoculants (Biradar and Santhosh, 2018). Recent studies have demonstrated that optimized liquid formulations enhance the survival and bio-efficacy of beneficial microorganisms, including *Rhizobium*, *Bacillus*, *Trichoderma*, and *Pseudomonas* spp., thereby improving plant growth and suppressing soil-borne diseases under field conditions (Hannan *et al*., 2012; Hannan *et al*., 2013). Despite these advances, information on the comparative effectiveness of different polymeric cell protectants and their interaction with stabilizing additives such as CMC, Trehalose and Alphox for improving the long-term storage stability of liquid formulations of *Pseudomonas fluorescens* under ambient conditions is still limited. Therefore, the present investigation was undertaken to develop and evaluate additive-based liquid formulations of *Pseudomonas fluorescens* using different combinations of polymeric cell protectants and stabilizing additives. The study aimed to identify suitable formulations capable of maintaining high viable cell populations during prolonged storage, thereby improving the shelf life, storage stability and practical applicability of *P. fluorescens* as an efficient bioinoculant for sustainable crop production.

Materials and Methods

Procurement of Microbial Cultures

The bacterial biocontrol agent *Pseudomonas fluorescens* was procured from the Department of Plant Pathology, Dr. Panjabrao Deshmukh Krishi Vidyapeeth (PDKV), Akola, Maharashtra, India. The culture was obtained from the departmental culture collection and maintained under recommended laboratory conditions to preserve its viability and purity until further experimental use.

Preparation of Liquid Formulations of *Pseudomonas fluorescens* Amended with Different Additives

The shelf life and population dynamics of liquid formulations of *Pseudomonas fluorescens* were evaluated under laboratory conditions using King's B broth as the basal medium. Different additives, including carboxymethyl cellulose (CMC, 0.1%), polyvinylpyrrolidone (PVP, 2%), polyethylene glycol (PEG, 2%), sodium alginate (SA, 0.1%), glycerol (2%), Alphox (1%), trehalose (10 mM), and a combination of Tween-20 (0.5%) + potassium sorbate (0.5%), were incorporated separately into the basal medium. A formulation without any additive served as the control (Mugnier and Jung, 1985; Temprano *et al*., 2002).

A volume of 250 ml of King's B broth containing the respective additives was dispensed into conical flasks and sterilized at 121°C for 15 min. After cooling, the media were aseptically inoculated with actively growing cultures of *Pseudomonas fluorescens* to obtain an initial population density of approximately 10^8 CFU ml^{-1}. The inoculated formulations were incubated at 28 ± 2°C for 3–5 days to allow adequate growth of the bioagent. Subsequently, the formulations were stored under laboratory conditions (28 ± 2°C). The viable population of *P. fluorescens* in each formulation was determined at predetermined storage intervals to evaluate the effect of different additives on microbial survival and shelf life. Population density was estimated by serial dilution and plate count technique using King's B agar, and the results were

expressed as colony-forming units (CFU ml^{-1}). The experiment was conducted to identify suitable additive-based liquid formulations capable of maintaining higher viable cell populations during storage.

Table 1 : Treatment Details

Sr. No.	Treatment details
1	Broth + 2% PVP + 0.1 % CMC + 0.5 % Tween-20 + 0.5 % P. Sorbate
2	Broth + 2% PVP + 10mM Trehalose + 0.5 % Tween-20 + 0.5 % P. Sorbate
3	Broth + 2% PVP + 1% ALPHOX + 0.5 % Tween-20 + 0.5 % P. Sorbate
4	Broth + 2% PEG + 0.1 % CMC + 0.5 % Tween-20 + 0.5 % P. Sorbate
5	Broth + 2% PEG + 10mM Trehalose + 0.5 % Tween-20 + 0.5 % P. Sorbate
6	Broth + 2% PEG + 1% ALPHOX + 0.5 % Tween-20 + 0.5 % P. Sorbate
7	Broth + 0.1 % SA + 0.1 % CMC + 0.5 % Tween-20 + 0.5 % P. Sorbate
8	Broth + 0.1 % SA + 10mM Trehalose + 0.5 % Tween-20 + 0.5 % P. Sorbate
9	Broth + 0.1 % SA + 1% ALPHOX + 0.5 % Tween-20 + 0.5 % P. Sorbate
10	Broth + 2 % Glycerol + 0.1 % CMC + 0.5 % Tween-20 + 0.5 % P. Sorbate
11	Broth + 2 % Glycerol + 10mM Trehalose + 0.5 % Tween-20 + 0.5 % P. Sorbate
12	Broth + 2 % Glycerol + 1% ALPHOX + 0.5 % Tween-20 + 0.5 % P. Sorbate
13	Without any additives (Only Broth Liquid Medium)

Shelf-Life Studies of Liquid Formulations of Bioagents

The shelf life of the liquid bioformulations was assessed over a storage period of 360 days under laboratory conditions (28 ± 2°C). Viable microbial populations were determined at 1 h and 24 h after inoculation and subsequently at 30-day intervals until the completion of the storage period. At each sampling interval, formulation bottles were aseptically opened inside a laminar airflow cabinet. An aliquot of 1 ml of the bioformulation was transferred to 9 ml of sterile distilled water to prepare a 10^{-1} dilution, followed by serial ten-fold dilutions using sterile diluent. For bacterial bioformulations, appropriate dilutions (generally up to 10^{-8}) were spread-plated onto the respective selective medium, namely King's B agar for *Pseudomonas fluorescens*. The inoculated plates were incubated at 28 ± 2°C for 24–72 h, depending on the growth characteristics of the test microorganism.

After incubation, the colonies were enumerated, and the viable microbial population was expressed as colony-forming units per millilitre (CFU ml^{-1}) using the standard serial dilution and plate count method described by Aneja (2003). The bioformulations were considered microbiologically stable when viable populations were maintained at or above 1×10^8 CFU ml^{-1} throughout the 360-day storage period.

Determination of Shelf Life

The shelf life of the liquid bioagent formulations was assessed by periodically monitoring viable cell populations. Initial viable counts were recorded one hour after inoculation, followed by observations at 24 h and at 30-day intervals thereafter up to 360 days of storage, as described by Dobhal and Hegde (2021). All treatment combinations were stored at room temperature (28 ± 2 °C). At each sampling interval, the containers were aseptically opened under a laminar airflow cabinet to avoid contamination. Viable cell counts were determined using the standard serial dilution and plating technique and microbial survival was expressed as colony-forming units per millilitre (CFU ml^{-1}).

$$\text{Number of cfu/ml} = \frac{\text{Number of colonies} \times \text{dilution factor}}{\text{Volume of culture plate}}$$

Physicochemical Characterization

The physicochemical properties of the liquid formulations were evaluated at each storage interval to assess formulation stability. The pH was measured using a calibrated digital pH meter. Optical density (OD_{600}) was determined with a UV-visible spectrophotometer as an indicator of microbial growth. Viscosity, where applicable, was measured using a viscometer to evaluate changes in rheological properties that could influence handling, storage stability, and field application.

Statistical Analysis

The experiment was conducted using a Factorial Completely Randomized Design (FCRD). All experimental observations were subjected to analysis of variance (ANOVA) using OPSTAT statistical software developed by Prof. O.P. Sheoran, Department of Mathematics and Statistics, CCS Haryana Agricultural University, Hisar, India, and Dr. Vinay Kumar, Central University of Haryana, Mahendergarh. Treatment means were compared at the 5% level of

significance using the appropriate critical difference (CD) test.

Result and Discussion

Effect of Polymer-Based Cell Protectants Combined with Surfactants and Preservatives on the Shelf Life of Liquid *Pseudomonas fluorescens* Bioagent:

The data presented in Table 1 demonstrate that among the four cell-protectant polymer additives evaluated, the formulation amended with 2% polyvinylpyrrolidone (PVP) was the most effective in maintaining the viable population of *Pseudomonas fluorescens* under ambient conditions (28±2°C) for up to 360 days of storage. In the study C_1 (2% PVP) exhibited an initial population of 33.57×10^8 cfu/ml, which increased significantly to 104.29×10^8 cfu/ml by day 30 before gradually declining to 7.56×10^8 cfu/ml at the end of storage. Similarly, C_2 (2% Polyethylene glycol, PEG) showed an initial count of 29.07×10^8 cfu/ml, peaking at 101.08×10^8 cfu/ml on day 30 and slowly decreasing to 6.48×10^8 cfu/ml by day 360, while C_3 (0.1% Sodium alginate, SA) and C_4 (2% Glycerol, GL) followed comparable trends, reaching their highest populations on day 30 (98.72×10^8 and 98.67×10^8 cfu/ml, respectively) and declining to 6.16×10^8 and 6.09×10^8 cfu/ml at the end of storage. These findings indicate that all additives supported initial microbial proliferation, yet a gradual reduction in viable cell counts occurred over prolonged storage. The study revealed that the strategic use of cell protectant polymers, along with 0.5% Tween-20 as a surfactant and 0.5% Potassium sorbate as a preservative, markedly improved the long-term stability of liquid biofertilizer formulations. Among the polymers evaluated, 2% PVP provided the most effective protection, reducing physiological stress and metabolic deterioration and maintaining a higher population of active inoculant cells throughout storage. PEG, Sodium alginate (SA) and Glycerol also enhanced stability, but their effects were comparatively lower than that of PVP, indicating a clear hierarchy in the efficiency of polymers for sustaining microbial viability over time.

The present findings are in agreement with Chattopadhyay (2021), who reported the major constraint of biofertilizers was their limited shelf life despite their crucial role in sustaining agroecosystems. In the context of *Pseudomonas fluorescens*, our results further demonstrate that incorporating stabilizing additives such as 0.1% CMC, 2% PVP and 0.2% hydrogel significantly improves long-term viability compared with conventional charcoal-based inoculants and untreated liquid broth. Such advancements strengthen the reliability and field performance of *Pseudomonas* based formulations. The findings are also consistent with the observations by Biradar and Santhosh (2018), show the polymeric additives markedly enhance the shelf life and bio-efficacy of liquid microbial inoculants. Their optimized formulation of *Pseudomonas fluorescens*, containing polymers such as PVP, PEG, Gum arabic and Sodium alginate with suitable adjuvants, maintained high viable counts (1.76×10^{10} cfu ml^{-1}) up to 180 days and effectively suppressed *Fusarium oxysporum* f. sp. *lycopersici* under greenhouse conditions. Similarly, present findings on *Pseudomonas fluorescens* closely correspond with the results reported by Biradar and Santhosh (2018a), demonstrated the polymeric additives such as PVP, PEG, Gum arabic, Sodium alginate, Xanthan gum and CMC significantly enhanced the shelf life and viability of liquid *Pseudomonas* inoculants. Consistent with their findings, the study demonstrates that incorporating protective polymers and adjuvants, particularly CMC, effectively preserves high cell populations during extended storage while enhancing bio-efficacy against soil-borne pathogens. The sustained viability up to 180 days and improved plant growth under greenhouse conditions corroborate our results, highlighting that well-formulated liquid *Pseudomonas* bioinoculants ensure microbial stability and provide reliable, farmer-friendly solutions for sustainable disease management and enhanced crop productivity. These results are in close agreement with Sivadharshnapriya (2020), demonstrated the Alginate based microencapsulation significantly improved the shelf life and controlled release of *Pseudomonas fluorescens*. The integration of stabilizing additives such as mannitol, PVP, PEG, CMC and Tween-80 enhanced cell retention and encapsulation efficiency, thereby strengthening bio-efficacy against soil-borne pathogens. Similarly, the present investigation confirms that polymeric protectants and adjuvants, particularly CMC and PVP play a crucial role in sustaining high viable populations during prolonged storage.

Effect of Adjuvants Combined with Surfactants and Preservatives on the Shelf Life of Liquid *Pseudomonas fluorescens* Bioagent:

The results presented in Table 1 clearly indicate that among the three adjuvants evaluated, 0.1% carboxymethyl cellulose (CMC) was markedly superior in maintaining the long-term viability of *Pseudomonas fluorescens* in liquid formulation at room temperature (28 ± 2°C). The CMC treatment exhibited the highest initial population of 30.83×10^8 cfu/ml, which peaked at 106.75×10^8 cfu/ml by day 30

and retained a relatively high viability of 8.89×10^8 cfu/ml at day 360, demonstrating a superior protective effect compared to the other additives. Trehalose (A_2) and Alphox (A_3) also enhanced cell survival, with initial populations of 31.49×10^8 and 30.33×10^8 cfu/ml, peaking at 99.05×10^8 and 96.27×10^8 cfu/ml on day 30 and declining to 5.98×10^8 and 4.84×10^8 cfu/ml, respectively, by the end of the storage period. When combined with 0.5% Tween-20 and 0.5% Potassium sorbate, 0.1% CMC consistently maintained significantly higher viable counts throughout 360 days, while the other additives showed a gradual and more pronounced decline. The slight decline in *Pseudomonas fluorescens* cell populations treated with 0.1% CMC over the storage period was statistically significant, while the initial population remained stable, indicating that CMC's structural and protective properties effectively minimized cellular stress and mortality during prolonged storage. These findings demonstrate that appropriate adjuvants can substantially enhance the shelf life and stability of liquid biofertilizer formulations. Among the additives tested, 0.1% CMC was the most effective, followed by 10 mM Trehalose and 1% Alphox, ensuring prolonged availability of viable inoculant cells and improving the practical efficiency and reliability of biofertilizer applications.

Similar findings were reported by Souza *et al*. (2022), who demonstrated the biopolymers such as Carboxymethyl cellulose (CMC) and Xanthan gum effectively conserved *Pseudomonas fluorescens* cells during long-term storage while maintaining their antagonistic activity against *Macrophomina* spp., the causal agent of black rot in several crops. Their study, which evaluated cell viability (cfu/ml), pH stability and antifungal performance over 210 days, confirmed that biopolymer-based formulations strengthen microbial survival and sustain biocontrol efficacy. These results align closely with the present study, reinforcing the value of such stabilizing agents for developing reliable, farmer-friendly bioinoculant technologies that support sustainable agriculture and crop protection. Similarly, present findings consistent with those of Dobhal and Hegde (2021), reaffirm the optimized liquid formulations significantly enhance the long-term survival and field reliability of *Pseudomonas fluorescens*. Similar to their report where canola oil–based formulations sustained high viability for twelve months our study demonstrates that the incorporation of effective stabilizing agents, particularly CMC, plays a crucial role in maintaining elevated viable populations during prolonged storage. Similarly, the results confirm that the choice of stabilizing agents particularly CMC significantly improves microbial survival, ensuring the reliable availability of viable inoculant cells. The enhanced shelf life of *Pseudomonas fluorescens* formulations are in close agreement with the results reported by Gade *et al*. (2014), who observed the suitable carrier or stabilizing materials play a crucial role in maintaining high viable populations during storage. Their study showed the carriers such as talc, lignite, spent mushroom substrate and fly ash effectively sustained *P. fluorescens* populations for up to 90 days, with talc performing best over extended storage. Also, similarly result findings, particularly the enhanced survival of *Pseudomonas fluorescens* with Trehalose supplementation, closely align with the results of Kumari and Rani (2024), also demonstrated the Trehalose significantly improves microbial stability in low-cost bran-based liquid bioformulations. In their study, Trehalose (15 mM) markedly sustained the viability and functional traits of *Pseudomonas* and other biofertilizer cultures during long-term storage. Similarly, in the current investigation, Trehalose acted as an effective osmoprotectant, minimizing cellular stress and supporting prolonged survival of *Pseudomonas* in liquid formulations. These consistent observations highlight the vital role of Trehalose in maintaining high quality microbial inoculants.

Interaction Effects of Additives Combinations on the Shelf Life of Liquid *Pseudomonas fluorescens* Bioagent at Room Temperature

The survival and population dynamics of *Pseudomonas fluorescens* in liquid formulations were significantly influenced by the interaction between different additive combinations and storage duration (Table 1(a); Fig. 1; Plate 1). At the initial stage, bacterial densities ranged from 26.97×10^8 cfu/ml in the control to 34.87×10^8 cfu/ml in the T_2 treatment (PVP + Trehalose) indicating that the incorporation of polymer-based additives immediately enhanced microbial proliferation. Within the first 24 hours, all treatments exhibited a sharp increase in viable counts, with the highest population recorded in T_1 (PVP + CMC, 96.23×10^8 cfu/ml) closely followed by T_4 (PEG + CMC, 95.67×10^8 cfu/ml) and T_7 (SA + CMC, 95.50×10^8 cfu/ml) demonstrating the synergistic effect of these additives in promoting early bacterial growth. During extended storage a gradual decline in population density was observed across all formulations, reflecting the typical physiological and metabolic stress experienced by microbial cells over time. However, at 30 days after storage (DAS), formulations containing PVP + CMC (107.90×10^8 cfu/ml), PEG + CMC (107.43×10^8 cfu/ml), SA + CMC (106.20×10^8 cfu/ml) and PVP + Trehalose

(106.00 × 10^8 cfu/ml) maintained relatively high cell counts, indicating that these carrier combinations effectively supported cell survival under ambient conditions. Beyond 120 DAS a steady reduction in viable counts occurred, yet the formulations containing PVP in combination with CMC consistently sustained higher populations compared to Alphox based carriers, emphasizing their superior protective role. At 150 days after storage (DAS), appreciable viable cell counts were maintained in treatments T_1 (PVP + CMC; 62.00 × 10^8 cfu/ml), T_4 (PEG + CMC; 62.40 × 10^8 cfu/ml) and T_7 (SA + CMC; 61.93 × 10^8 cfu/ml), whereas the untreated control (T_{13}) completely lost cellular viability after 90 DAS, highlighting the vulnerability of unprotected microbial inoculants to prolonged storage conditions. By the conclusion of 360 DAS, formulations containing PVP + CMC (9.03 × 10^8 cfu/ml), PEG + CMC (8.90 × 10^8 cfu/ml), SA + CMC (8.87 × 10^8 cfu/ml) and GL + CMC (8.77 × 10^8 cfu/ml) continued to sustain significantly higher populations of viable cells compared to Alphox-based treatments (T_3, T_6, T_9, T_{12}), which recorded markedly lower counts of 4.40- 5.20 × 10^8 cfu/ml. respectively. These results clearly indicate that polymer-assisted formulations, particularly PVP + CMC, PEG + CMC and SA + CMC, are highly effective in maintaining the stability and functional viability of *P. fluorescens* over extended periods, thereby ensuring a reliable supply of active inoculants for agricultural use. In contrast, Alphox based treatments demonstrated comparatively limited efficacy and the complete loss of viability in the untreated control underscores the critical need for scientifically optimized formulation strategies to safeguard microbial inoculants. The findings emphasize that the use of appropriate polymeric protectants preserves microbial populations.

The findings of the present investigation on *Pseudomonas fluorescens* are in strong agreement with earlier reports, particularly those of Biradar and Santhosh (2018), emphasized the pivotal role of polymeric additives in enhancing the shelf life and functional stability of liquid microbial inoculants. Their work demonstrated the incorporation of cell-protectant polymers such as PVP, PEG, Gum arabic and Sodium alginate combined with adjuvants like Xanthan gum and CMC, along with Tween-20 as a surfactant and Potassium sorbate as a preservative, substantially improved the survival of *P. fluorescens* during storage. The optimized formulation (T_5) in their study retained a remarkably high viable count of 1.76 × 10^{10} cfu ml^{-1} up to 180 days. Comparable trends were recorded in the present study, wherein formulations containing CMC, PVP and similar stabilizing polymers significantly enhanced the long-term survival and metabolic integrity of *Pseudomonas fluorescens*. The protective matrix formed by these polymers minimized cellular desiccation stress, mitigated oxidative damage and supported the maintenance of high viable cell populations throughout the storage period. Furthermore, the current results corroborate the extended shelf life and enhanced bio-efficacy reported in a subsequent study by Biradar and Santhosh (2018a), which also highlighted the positive influence of PVP, PEG, Gum arabic, Sodium alginate, Xanthan gum and CMC on the survival dynamics of *Pseudomonas* formulations. In line with their observations, the present investigation confirms that CMC-based combinations were particularly effective in sustaining microbial viability during prolonged storage. The outcomes of the present study align the observations reported by Sivadharshnapriya (2020), demonstrated the effectiveness of polymer-enriched alginate formulations in enhancing the survival and bio-efficacy of *Pseudomonas fluorescens*. Their work showed that enriching King's B broth with adjuvants such as mannitol, PVP/PEG, CMC and Tween-80 significantly improved cell viability with treatments T_1 and T_3 sustaining the highest populations over nine months. These enriched formulations produced high-quality alginate beads with greater structural stability, reduced swelling, higher water retention and prolonged release of the bioagent attributes that directly contributed to extended shelf life and improved pathogen suppression. Similarly, the present investigation confirms that incorporation of polymers such as PVP, PEG, and CMC markedly enhances the long-term survival and functional performance of *Pseudomonas fluorescens*. In agreement with the findings of Sivadharshnapriya, polymer-fortified formulations maintained higher viable counts and exhibited superior antagonistic activity against major soil-borne pathogens. Also, Dobhal and Hegde (2021), demonstrated the liquid formulations offer clear advantages in maintaining high cell counts, extended shelf life and enhanced field efficacy of *Pseudomonas fluorescens*. Their year-long storage experiment showed that oil-based formulations, particularly those using canola oil, supported the highest viability after twelve months, followed by soybean oil at 2% concentration. These parallel results reinforce that scientifically optimized liquid formulations provide superior microbial stability and are well suited for reliable, long-term application in sustainable and organic agriculture. The present findings corroborate those of Chattopadhyay (2021), who identified limited shelf life as a major constraint in biofertilizer technology and demonstrated that stabilizing additives

significantly enhance long-term microbial viability and functional efficiency. Consistently the results show that polymers such as CMC, PVP and hydrogels markedly improved survival, metabolic activity and plant growth promoting traits of liquid formulations compared to conventional charcoal-based inoculants. Likewise, the observations align with Karunya and Reetha (2014), reported the superior storage stability of saline-tolerant PGPR strains *Azospirillum brasilense* PA-17, *Bacillus subtilis* PB-15 and *Pseudomonas fluorescens* PP-15 in liquid formulations amended with PVP, Trehalose and Glycerol. PVP based formulations maintained the highest viable populations over six months. These findings are in support of Dayamani and Brahmaprakash (2014), who showed the suitable osmolytes markedly enhance the survival of liquid PGPR inoculants. Optimized levels of PVP, PEG and Glycerol significantly improved viability of *Azotobacter*, *Azospirillum*, *Acinetobacter*, *Bacillus* and *Pseudomonas* with maximum populations at 2% Glycerol, 1% PEG-400, 2% PEG-4000 and 2% PVP K-15. Similarly, the present study confirms osmolyte-based polymer stabilization as an effective strategy to enhance viable counts, shelf life and performance of liquid inoculants.

Conclusion

The present study demonstrated that polymeric cell protectants and stabilizing additives significantly improved the shelf life and storage stability of liquid formulations of Pseudomonas fluorescens. Among the additives tested, 2% PVP and 0.1% CMC were the most effective in maintaining bacterial viability. The combination of 2% PVP + 0.1% CMC, along with 0.5% Tween-20 and 0.5% potassium sorbate, retained the highest viable population (9.03×10^8 CFU ml^{-1}) after 360 days of storage, whereas the untreated control lost viability completely. These findings indicate that the optimized liquid formulation can effectively enhance microbial survival during long-term storage and represents a promising alternative to conventional carrier-based inoculants for sustainable agriculture and integrated disease management. Further validation under greenhouse and field conditions is required to confirm its bio-efficacy.

Table 1: Viable cell count of liquid based bioagent (*Pseudomonas fluorescens)* amended with additives during storage period of one year at room temperature.

Treatments Details	Population density (×10^8 cfu/ml)													
	Storage Days													
	Initial count*	24 hrs.*	30*	60*	90*	120*	150*	180*	210*	240*	270*	300*	330*	360*
C1- (PVP)	33.57	94.46	104.29	90.38	74.21	68.71	59.43	52.37	40.27	30.36	24.52	20.52	13.69	7.56
C2- (PEG)	29.07	94.13	101.08	88.98	69.77	65.47	55.94	49.58	37.59	28.33	21.52	18.34	12.03	6.48
C3- (SA)	29.24	92.79	98.72	88.29	70.81	63.97	54.34	48.71	34.10	26.46	21.20	16.96	11.13	6.16
C4- (GL)	31.64	93.18	98.67	87.91	72.22	63.58	53.57	48.38	31.26	26.13	21.04	17.26	11.02	6.09
SE(m) ±	**1.38**	**0.26**	**0.32**	**0.15**	**0.22**	**0.22**	**0.22**	**0.24**	**0.24**	**0.12**	**0.20**	**0.16**	**0.14**	**0.15**
CD at 1%	**NS**	**1.02**	**1.25**	**0.59**	**0.86**	**0.88**	**0.87**	**0.97**	**0.94**	**0.49**	**0.79**	**0.64**	**0.57**	**0.59**
A1- (CMC)	30.83	95.64	106.75	91.69	76.53	70.73	61.92	55.36	40.18	32.33	26.35	21.66	14.68	8.89
A2- (TREAHALOSE)	31.49	93.81	99.05	88.87	69.68	64.99	54.04	48.69	35.14	26.98	20.81	17.67	11.39	5.98
A3- (ALPHOX)	30.33	91.47	96.27	86.11	69.05	60.58	51.51	45.23	32.09	24.15	19.06	15.48	9.83	4.84
SE(m) ±	**1.20**	**0.22**	**0.27**	**0.13**	**0.19**	**0.19**	**0.19**	**0.21**	**0.21**	**0.11**	**0.17**	**0.14**	**0.12**	**0.13**
CD at 1%	**NS**	**0.88**	**1.08**	**0.51**	**0.74**	**0.76**	**0.75**	**0.84**	**0.81**	**0.42**	**0.69**	**0.55**	**0.49**	**0.51**

**Note- ***Means of three replications

Factor 1:- C_1= PVP 2**%** (Polyvinyl Pyrrolidone), **C_2**= PEG 2% (Polyethylene glycol), **C_3**= SA 0.1% (Sodium alginate), **C_4**= GL 2% (Glycerol)

Factor 2:- A_1= CMC 0.1% **(**Carboxy methyl cellulose**)**, **A_2**= Trehalose 10mM, **A_3**= Alphox 1%

Common use (Preservatives and Surfactant/emulsifiers):- Potassium sorbate (0.5%) and Tween-20-(0.5%)

Table 1 (a): Interaction effect on viable cell count of liquid based bioagent (*Pseudomonas fluorescens)* amended with additives during storage period of one year at room temperature

Treatments Details		Population density (×10^8 cfu/ml)													
		Storage Days													
		Initial count*	24 hrs.*	30*	60*	90*	120*	150*	180*	210*	240*	270*	300*	330*	360*
T_1	C1A1- (PVP+CMC)	34.57	96.23	107.90	92.20	77.30	71.53	62.00	56.00	41.80	35.73	26.83	22.33	15.13	9.03
T_2	C1A2-(PVP+TREAHALOSE)	34.87	95.43	106.00	91.30	76.27	70.00	60.97	54.37	40.23	29.17	25.83	21.37	14.30	8.60
T_3	C1A3- (PVP+ ALPHOX)	31.27	91.70	98.97	87.63	69.07	64.60	55.33	46.73	36.53	26.47	20.90	17.87	11.63	5.03
T_4	C2A1- (PEG+CMC)	30.03	95.67	107.43	91.77	76.40	70.87	62.40	55.70	40.90	33.00	26.37	21.47	14.70	8.90
T_5	C2A2- (PEG+TREAHALOSE)	29.83	93.50	99.30	89.10	66.20	64.67	53.37	46.73	37.40	27.83	19.60	18.23	11.33	5.33
T_6	C2A3- (PEG+ ALPHOX)	27.33	93.23	96.50	86.07	66.70	60.87	52.07	46.30	33.37	24.50	18.60	15.33	10.07	5.20

T_7	C3A1- (SA+CMC)	28.97	95.50	106.20	91.47	76.20	70.37	61.93	55.13	40.53	31.83	26.23	21.40	14.53	8.87
T_8	C3A2- (SA+TREAHALOSE)	28.33	92.57	95.70	88.10	69.17	63.43	51.87	48.47	34.27	24.73	19.07	15.73	10.20	5.20
T_9	C3A3- (SA+ ALPHOX)	30.43	90.30	94.27	85.30	67.07	58.10	49.23	42.53	29.33	23.47	18.30	13.73	8.67	4.40
T_{10}	C4A1- (GL+CMC)	29.73	95.17	105.47	91.33	76.20	70.13	61.33	54.60	40.53	29.40	25.97	21.43	14.37	8.77
T_{11}	C4A2- (GL+TREAHALOSE)	32.93	93.73	95.20	86.97	67.10	61.87	49.97	45.20	29.70	23.43	18.73	15.33	9.73	4.77
T_{12}	C4A3- (GL+ ALPHOX)	32.27	90.63	95.33	85.43	73.37	58.73	49.40	45.33	29.40	21.73	18.43	15.00	8.97	4.73
T_{13}	CONTROL	26.97	74.33	88.20	37.50	1.70	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
SE(m)		**2.39**	**0.45**	**0.55**	**0.26**	**0.38**	**0.39**	**0.38**	**0.42**	**0.41**	**0.21**	**0.35**	**0.28**	**0.25**	**0.26**
Factor (A X B) CD at 1%		**NS**	**1.77**	**2.17**	**1.03**	**1.49**	**1.53**	**1.50**	**1.67**	**1.62**	**0.84**	**1.37**	**1.11**	**0.99**	**1.02**

*Values are means of three replications.

Note- **$T_{1=}$** YEM-Broth + 2% PVP + 0.1 % CMC + 0.5 % Tween-20 + 0.5 % P. Sorbate, **$T_{2=}$** YEM -Broth + 2% PVP + 10mM Trehalose + 0.5 % Tween-20 + 0.5 % P. Sorbate, **$T_{3=}$** YEM - Broth + 2% PVP + 1% ALPHOX + 0.5 % Tween-20 + 0.5 % P. Sorbate, **$T_{4=}$** YEM -- Broth + 2% PEG + 0.1 % CMC + 0.5 % Tween-20 + 0.5 % P. Sorbate, **$T_{5=}$** YEM - Broth + 2% PEG + 10mM Trehalose + 0.5 % Tween-20 + 0.5 % P. Sorbate, **$T_{6=}$** YEM - Broth + 2% PEG + 1% ALPHOX + 0.5 % Tween-20 + 0.5 % P. Sorbate, **$T_{7=}$** YEM - Broth + 0.1 % SA + 0.1 % CMC + 0.5 % Tween-20 + 0.5 % P. Sorbate, **$T_{8=}$** YEM - Broth + 0.1 % SA + 10mM Trehalose + 0.5 % Tween-20 + 0.5 % P. Sorbate, **$T_{9=}$** YEM - Broth + 0.1 % SA + 1% ALPHOX + 0.5 % Tween-20 + 0.5 % P. Sorbate, **$T_{10=}$** YEM - Broth + 2 % Glycerol + 0.1 % CMC + 0.5 % Tween-20 + 0.5 % P. Sorbate, **T_{11}=** YEM - Broth + 2 % Glycerol + 10mM Trehalose + 0.5 % Tween-20 + 0.5 % P. Sorbate, **T_{12}=** YEM - Broth + 2 % Glycerol + 1% ALPHOX + 0.5 % Tween-20 + 0.5 % P. Sorbate, **T_{13}**= YEM -Broth + Without additives

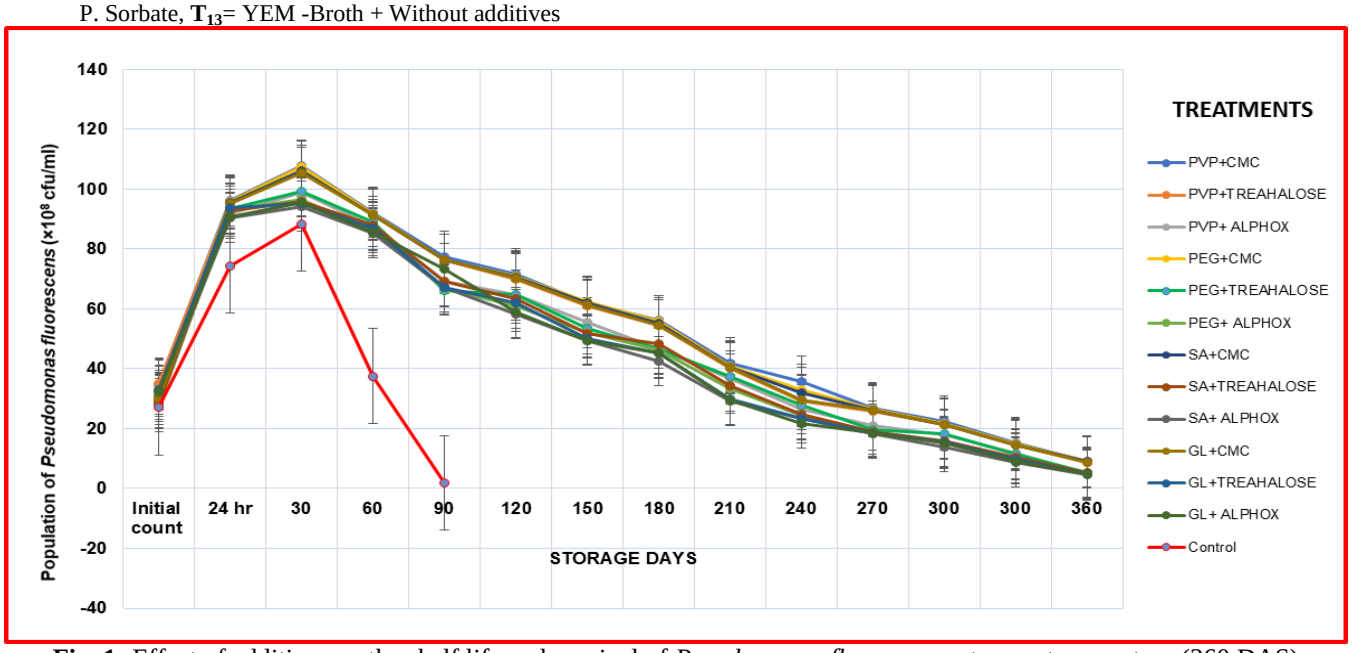

Fig. 1: Effect of additives on the shelf life and survival of *Pseudomonas fluorescens* at room temperature (360 DAS)

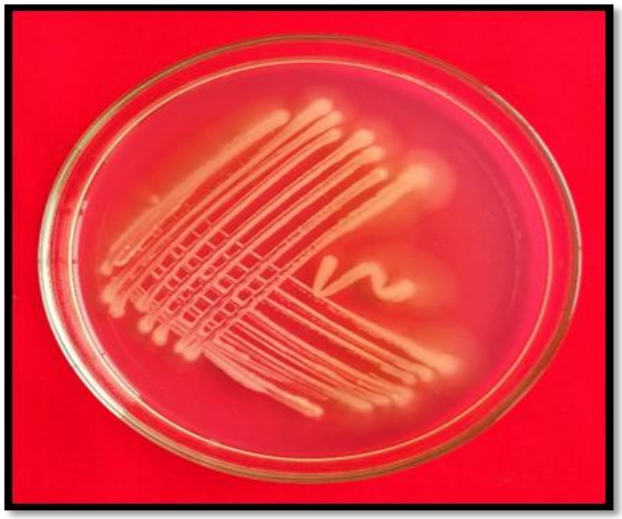

Plate 1 : Pure culture of *Bacillus megaterium* var. *Phosphaticum*

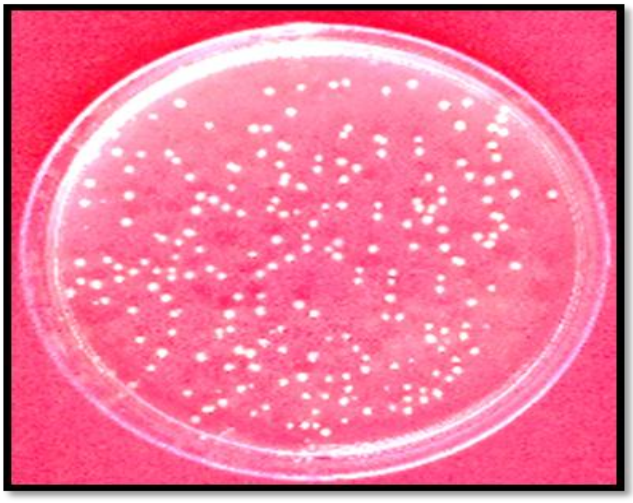

Plate 2 : Colonies of *Pseudomonas fluorescens* on King's 'B' agar Medium

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