Available online at www.ijpab.com

DOI: http://dx.doi.org/10.18782/2320-7051.6822

ISSN: 2320 – 7051

Int. J. Pure App. Biosci. **6 (4):** 123-133 (2018)

Research Article

International Journal of Pure & Applied Bioscience

OPEN ACCESS

Role of Polymeric Additives in Formulation, Shelf-life and Bioefficacy of Liquid Inoculant of *Pseudomonas fluorescens*

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Received: 5.07.2018 | Revised: 14.08.2018 | Accepted: 20.08.2018

ABSTRACT

In the present study liquid inoculant formulation of Pseudomonas fluorescens were developed with the use of polymeric additives to study the shelf-life and to evaluate the bioefficacy of Pseudomonas fluorescens. The polymeric additives were used in different combinations and the prepared liquid inoculant formulations of Pseudomonas fluorescens were assessed for their shelf-life at monthly intervals and were evaluated for their bioefficacy against the Fusarium (Fusarium oxysporum f.sp.lycopersici) wilt of Tomato under greenhouse conditions. Polymeric additives used were cell protectants viz., polyvinlypyrrolidone (PVP, 2%), polyethylene glycol (PEG, 1%), gum arabic (0.8%) and sodium alginate (0.1%); adjuvants viz., xanthan gum (0.3%) and carboxymethyl cellulose (CMC, 0.1%); surfactant used was Tween-20 (0.5%) and preservative was potassium sorbate (0.2%). LIF (Liquid inoculant formulation; T5) retained 1.76 X 10^{10} CFU/ml upto 180 days of storage. Even the tomato seedlings treated with the LIF T_5 showed best results in all the growth and yield parameters studied including the disease incidence in these plants. The formulated liquid inoculants were found to have enhanced shelf-life and improved viability.

Key words: *Liquid inoculant formulation, polymeric additives, shelf-life, Pseudomonas fluorescens*

INTRODUCTION

The use of microbial inoculants/ biofertilizers in recent days has gained importance because it is the best solution to solve the environmental issues raised due to the indiscriminate use of chemical fertilizers during the green revolution period. No doubt, the use of chemical fertilizers gave booming results, but they also had their ill effects such as surface/ ground water pollution, freshwater contamination, eutrophication, accumulation of heavy metals in the soil, and soil erosion. In order to have a sustainable production and a lesser impact on the environment, it is wise to go for farming where the usage of chemical fertilizers is reduced. In this context, biofertilizer will serve as the best option.

Cite this article: Praveen Biradar, B.J., Santhosh, G.P., Role of Polymeric Additives in Formulation, Shelf-life and Bioefficacy of Liquid Inoculant of *Pseudomonas fluorescens*, *Int. J. Pure App. Biosci.* **6(4):** 123-133 (2018). doi: http://dx.doi.org/10.18782/2320-7051.6822

Biofertilizer is one of the viable technologies, which is losing its importance among the farmers due to poor shelf-life and inconsistent field performance. Powdered inoculant formulation have been prepared using peat, lignite, talc, charcoal, press mud and saw dust as carriers[8, 11]. According to Fertilizer Control Order (FCO) regulations, minimum number of viable population in a carrier based formulation should be 5.0 X 10^7 CFU/g. But, in general, microorganisms do not survive in these formulations for longer time and moreover population of contaminating microbes build up, as it is not possible to prepare these formulations under total aseptic conditions. In this context, a preparation of liquid formulation appears to be a better alternative than powdered or granular formulations. In liquid formulation it is possible that minimum population density of bacteria (FCO standard is 1 X 10^8 CFU/ml) could be maintained at desired level for longer time and formulation can be prepared under pure culture condition, without contamination.

Unlike solid carrier based biofertilizers, liquid formulations can be developed with sufficient amount of nutrients, cell protectant, adjuvants, surfactants and preservatives responsible for ensuring prolonged shelf-life. The shelf-life of common solid carrier based biofertilizers is less than six months; however, it could be as high as two years for a liquid formulation. Further, solid carrier based biofertilizers are less thermo-tolerant whereas; liquid formulations can tolerate the temperature as high as 55^{o}C. Hence increased shelf-life can be achieved in liquid formulation.

Additives added into liquid inoculant formulations should have a role in protecting cells on seed at high temperature and during desiccation. Many kinds of polymers have been used for inoculant production because of their ability to limit heat transfer, their good rheological properties and high water activities[14]. Polymers that are soluble in liquid inoculant formulations make for convenient batch processing of inoculant and make seed application a simpler process for farmers. Research conducted on liquid inoculants in last few years support higher population density per unit volume for longer time[9, 22]. As the research data on the development of liquid inoculant formulation of *Pseudomonas fluorescens* using the polymeric additives in various suitable combinations that enhance the shelf-life is scanty; the possibility of developing such an inoculant formulation is explored in this investigation. Polymers used in this study were selected based on their properties, such as solubility in water, non-toxicity, and complex chemical nature, which prevents microorganisms in the soil from rapidly degrading the polymeric coating[7].

MATERIAL AND METHODS

King's B broth (peptone: 20g/l; glycerol: 15ml/l; K_2HPO_4: 1.5 g/l; $MgSO_4.7H_2O$: 1.5 g/l) was used to culture *Pseudomonas fluorescens*. Sterilized King's B broth was inoculated with the *Pseudomonas fluorescens* and incubated at 28±2°C on a reciprocatory shaker for 24 hrs. One ml of day old culture of *Pseudomonas fluorescens* was used to inoculate the media prepared using polymeric additives and incubated in BOD incubator at 28±2 °C. Different liquid inoculant formulations were prepared by amending the King's B broth using polymeric additives in varying combination and optimum concentrations. Different group of polymeric additives used were cell protectants *viz.*, Polyvinlypyrrolidone (PVP, 2%), polyethylene glycol (PEG, 1%), gum arabic (0.8%) and sodium alginate (0.1%). Adjuvants used were xanthan gum (0.3%) and carboxymethyl cellulose (CMC, 0.1%), tween-20 (0.5%) as a surfactant and potassium sorbate (0.2%) as a preservative.

A total of 11 formulations were prepared for this study. Treatments (T_4-T_{11}) were prepared by using the cell protectants, adjuvants, surfactant and a preservative in various combinations. One formulation was prepared by amending King's B broth (T_2) with tween-20 and potassium sorbate. Only King's B broth was maintained as treatment T_1. A talc based formulation (T_3) was prepared

by employing finely powdered talc whose pH was adjusted to 6.5-7.0 by using calcium carbonate ($CaCO_3$).

Shelf life studies of liquid inoculants

Liquid inoculants 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 their shelf-life at monthly interval upto 180 days of storage. using Standard Plate Count (SPC).Values obtained on shelf-life studies are mean of three replications ± standard deviation and were statistically analysed using Duncan's multiple range test ($p<0.05$).

Bioefficacy of liquid inoculant formulation.

Bio-efficacy of liquid inoculants was studied by pot culture assay with tomato as the test crop. The medium red soil was mixed with sand at 1: 1 ratio. The sand-soil mixture was sterilised at 121 °C, 15 lb pressure for an hour. The mixture was mixed thoroughly and filled in the earthen pots of 30 cms diameter at the rate of 5 kg per pot. The required quantity of vermicompost (100 g/pot) was weighed separately for each pot and incorporated into the soil. Twenty five days old tomato seedlings were dipped in Liquid formulation of *Pseudomonas fluorescens* (100 ml dissolved in 10 L of water) and talc inoculant formulation (1 kg dissolved in 10 L of water) for thirty minutes. Inoculated seedlings were transplanted at the rate of 4 seedlings/pot. Seven days after transplanting, seedlings were thinned to retain 2 plants/pot. All the parameters were studied at 30, 60 Days after transplanting and at harvest.

RESULTS AND DISCUSSION

The survivability of *Pseudomonas fluorescens* in different LIF is explained in Table 1. Among the LIF, T_5 supported higher survival of *Pseudomonas fluorescens* upto 180 DAS (Days after storage) as compared to other LIF. The higher survival of *Pseudomonas fluorescens* in liquid inoculant T_5 could be due to presence of cell protectant polyvinylpyrrolidone (PVP) (2%) in combination with xanthan gum (0.3%), tween-20 (0.5%) and potassium sorbate (0.2%). The next best to treatment T_5 in sustaining more number of population upto 180 DAS was T_4. It was prepared by using PVP as a cell protectant, along with CMC as an adjuvant, tween-20 as surfactant and potassium sorbate as a preservative.

Polyvinylpyrrolidone is a synthetic polymer of vinyl groups with pyrrole ring. It is a high molecular weight compound (40000), it is a water soluble compound with stabilization and adhesive properties, with high water holding capacity that appears to slow down the drying rate of media, thus maintaining the moisture level in the media[7]. Polyvinylpyrrolidone also has a capacity to bind bacterial toxins that were constantly released into the media, when bacterial cells were in stationary phase. Maintenance of macromolecular structure may improve biological integrity, thus leading to improved survival.

Along with PVP, in treatment T_5, xanthan gum is used as adjuvant, which has also helped the survival of *Pseudomonas fluorescens.* Xanthan gum helps in stabilizing the liquid inoculants, by preventing cells from separating. Due to its thicky consistency, the relative drying of media was very less as compared to other adjuvant carboxymethyl cellulose (CMC). Tween-20 was used as surfactant, which will lower the surface tension while, potassium sorbate helps in preserving the bacterial cell density for a longer period of storage. Adjuvant CMC added in LIF T_4 increases the gel viscosity, and this rheological feature helped to maintain the viability of cells for longer period of time.

Treatment T_7 (King's B broth + 1% PEG + 0.3% Xanthan gum + 0.5% Tween 20 + 0.2% Potassium sorbate) and T_6 (King's B broth + 1% PEG + 0.1% CMC + 0.5% Tween 20 + 0.2% Potassium sorbate) were found to be best after T_4 in retaining viable population by recording 1.51×10^{10} and 1.42×10^{10} CFU/ml at 180 DAS respectively. This was possible because both these LIF were prepared using Polyethylene glycol (PEG) as cell

protectant. Polyethylene glycol is small molecular weight (3000), water soluble compound with adhesive property[13, 20]. Polyethylene glycol has adhesive property and has a sticky consistency, which will enhance cell adherence to seed, and its viscous nature will slow the drying process of the inoculant.

In treatment T_7, xanthan gum was used as adjuvant along with cell protectant PEG, which would help in fighting against stress condition. This was in agreement with the work of Somasegaran (1985). The great value of porosity and capacity of CMC to absorb the hydrophobic and hydrophilic liquids may have been useful in enhancing the shelf life of liquid inoculants. Further, the effects of tween-20 and potassium sorbate might have given added benefits as discussed earlier.

Treatment T_9 (King's B broth + 0.8% Gum arabic + 0.3% Xanthan gum + 0.5% Tween 20 + 0.2% Potassium sorbate) and T_8 (King's B broth + 0.8% Gum arabic + 0.1% CMC + 0.5% Tween 20 + 0.2% Potassium sorbate) maintained 1.35 X 10^{10} and 1.29 X 10^{10} CFU/ml upto 180 DAS respectively. This was possible because both these treatments were prepared using gum arabic as cell protectant. Gum arabic, is a biopolymer, with large molecular weight with adhesive, emulsification and stabilization properties which limits heat transfer and has high water activity[10, 14, 25]. The properties of xanthan gum in T_9, the property of CMC in T_8 and; the properties of tween-20 and potassium sorbate in both the treatments has already been discussed above and it holds good with respect to these treatments also.

Treatment T_{11} (King's B broth + 0.1% Sodium alginate + 0.3% Xanthan gum + 0.5% Tween 20 + 0.2% Potassium sorbate) and T_{10} (King's B broth + 0.1% Sodium alginate + 0.1% CMC + 0.5% Tween 20 + 0.2% Potassium sorbate) maintained 1.22 X 10^{10} and 1.21 X 10^{10} CFU/ml upto 180 DAS. Sodium alginate is a large molecular weight non-toxic compound with adhesive property, limits heat transfer, has high water activity; and these properties are useful in supporting long term survival of inoculant[3, 4, 14]. The positive effect of xanthan gum in T_{11}, the property of CMC in T_{10} and; tween-20 and potassium sorbate in both the treatments has already been discussed earlier.

Treatment T_3 could maintain 1.17 X 10^{10} CFU/ml upto 180 DAS, because this treatment was prepared by amending King's B broth with tween-20 as surfactant and potassium sorbate as preservative. The added chemicals might have enhanced the survival when compared to using only King's B broth (T_1).

King's B broth (T_1) and talc based formulation (T_2) were poor in supporting the survival of *Pseudomonas fluorescens*. This could be due to depletion of nutrients from the media, loss of moisture content and desiccation stress during the storage period. The other possible reason for poor survival of *Pseudomonas fluorescens* in these formulations could be due to absence of cell protectants, resulting in the failure of bacteria to protect them against desiccation. The death of bacteria due to desiccation could be attributed to the changes in membrane permeabilities and quantities of water retained at a known relative pressure[6]. The outcome of the work of Vidyashekaran and Muthamilan[24] suggested that the talc based formulation could not sustain higher population of *Pseudomonas fluorescens* more than 4 months of storage.

The experiment on formulation and shelf life studies of LIF of *Pseudomonas fluorescens*, revealed that the cell protectants, adjuvants, surfactant and preservative added into the media while preparation increased the shelf life of biofertilizer inoculant. Treatment T_5 (King's B broth + 2% PVP + 0.3% Xanthan gum + 0.5% Tween 20 + 0.2% Potassium sorbate) was found to be superior to all the other LIF even after 180 days of storage. The next best LIF after T_5 was T_4 (King's B broth + 2% PVP + 0.1% CMC + 0.5% Tween 20 + 0.2% Potassium sorbate). These treatments were followed by T_7 and T_6 which were formulated using PEG; T_9 and T_8 formulated using gum arabic; T_{11} and T_{10} formulated using sodium alginate. Least population density was observed in treatments T_1 (only King's B broth) followed by T_2 (talc based

formulation) and T_3 (King's B broth + 0.5% Tween 20 + 0.2% Potassium sorbate). All the different LIF of *Pseudomonas fluorescens* increased the plant height, number of branches, shoot dry weight, root dry weight, nitrogen concentration, phosphorous concentration and number of tomato fruits.

Plant height (Table 2) was highest in the treatment T_5 recording 43.85, 66.40 and 78.18 cm at 30, 60 DAT and at harvest respectively while, T_{12} (uninoculated control) recorded lowest plant height with 21.25, 39.21 and 48.77 cm at 30, 60 DAT and at harvest respectively. The next best after T_5 was T_4 with 40.88, 61.81 and 73.08 cm at 30, 60 DAT and at harvest respectively followed by T_7 with 40.50, 60.10 and 71.55 cm at 30, 60 DAT and at harvest respectively. Plants treated with talc based formulation resulted in 21.75, 42.75 and 53.36 at 30, 60 DAT and at harvest respectively followed by the plants treated with only King's B broth with 21.66, 40.06 and 50.29 cm of plant height at 30, 60 DAT and at harvest respectively.

Number of branches (Table 2) was significant in plants inoculated with LIF of *Pseudomonas fluorescens* against uninoculated control. Highest number of branches was recorded in T_5 showing 9.04, 12.64 and 13.30 branches per plant at 30, 60 DAT and at harvest respectively. Lowest branches per plant were observed in T_{12} with 2.69, 3.48 and 4.57 branches per plant at 30, 60 DAT and at harvest respectively. Treatment T_4 showed best response after treatment T_5 with 8.08, 11.66 and 12.19 branches per plant at 30, 60 DAT and at harvest respectively. Treatment T_7 treated plants recorded 7.16, 11.31 and 11.66 branches per plant at 30, 60 DAT and at harvest respectively. The plants treated with T_2 (talc based formulation) recorded 3.06, 5.77 and 6.36 branches per plant at 30, 60 DAT and at harvest respectively and was non-significant with the plants treated with T_1 (only King's B broth) with 2.92, 4.62 and 5.94 branches per plant at 30, 60 DAT and at harvest respectively.

Highest nitrogen content (Table 3) was observed in treatment T_5 followed by T_4 and T_7 and the lowest was recorded in T_{12}. Nitrogen concentration in T_5 was 1.37, 1.53 and 1.28% at 30, 60 DAT and at harvest respectively. Nitrogen concentration in T_4 and T_7 was found to be 1.34, 1.50 and 1.26% at 30, 60 DAT and at harvest respectively and; 1.32, 1.49 and 1.25% at 30, 60 DAT and at harvest respectively. Plants treated with talc based formulation and with only King's B broth did not differ much in their effects and recorded 1.17, 1.33 and 1.11% at 30, 60 DAT and at harvest respectively and; 1.16, 1.32 and 1.08% at 30, 60 DAT and at harvest respectively.

Similar trends were recorded for phosphorous content (Table 3). Highest content was recorded in T_5 with 0.443, 0.539 and 0.471% at 30, 60 DAT and at harvest respectively and the lowest was recorded in T_{12} with 0.230, 0.329 and 0.295% at 30, 60 DAT and at harvest respectively. The next best treatments to T_5 was T_4 and T_7 which recorded 0.412, 0.480 and 0.434% at 30, 60 DAT and at harvest respectively and; 0.392, 0.462 and 0.426% at 30, 60 DAT and at harvest respectively. Plants treated with talc based formulation and only King's B broth recorded lower phosphorous concentration with 0.282, 0.357 and 0.324% at 30, 60 DAT and at harvest respectively and; 0.273, 0.347 and 0.309% at 30, 60 DAT and at harvest respectively.

Highest shoot dry weight (Table 4) was observed in T_5 while, lowest was observed in T_{12} (uninoculated control). Shoot dry weight in T_5 was 19.11, 28.37 and 39.15 gram per plant at 30, 60 DAT and at harvest respectively. Shoot dry weight was lowest in T_{12} (uninoculated control) with 9.03, 14.47 and 18.31 gram per plant at 30, 60 DAT and at harvest respectively. Treatments T_4 and T_7 were the best treatments next to treatment T_5 by recording 17.44, 26.22 and 37.18 gram per plant at 30, 60 DAT and at harvest respectively and; 15.27, 28.37 and 39.15 gram per plant at 30, 60 DAT and at harvest respectively. Plants treated with talc based formulation and only King's B broth were non-significant with each other by recording 9.33, 17.12 and 22.25 gram per plant at 30, 60 DAT and at harvest respectively and; 9.06,

16.69 and 20.69 gram per plant at 30, 60 DAT and at harvest respectively.

Similar trends were observed for root dry weight (Table 4). Highest root dry weight was observed in T_5 with 5.07, 6.28 and 6.82 gram per plant at 30, 60 DAT and at harvest respectively. The lowest was in T_{12} which recorded 2.80, 3.78 and 4.90 gram per plant at 30, 60 DAT and at harvest respectively. The next best treatments were T_4 with 4.61, 5.82 and 6.77 gram per plant at 30, 60 DAT and at harvest respectively followed by T_7 with 4.30, 5.59 and 6.61 gram per plant at 30, 60 DAT and at harvest respectively. Plants treated with talc based formulation and only broth recorded lower root dry weight. Plants treated with talc based formulation recorded 3.20, 4.37 and 5.44 gram per plant at 30, 60 DAT and at harvest respectively and plants treated with only King's B broth recorded 3.06, 4.21 and 5.24 gram per plant at 30, 60 DAT and at harvest respectively.

Number of tomato fruits per plant (Table 4) was found to be highest in plants treated with treatment T_5 with 24.33 fruits per plant, followed by T_4 and T_7 with 21.66 and 19.66 fruits per plant respectively. Lowest fruits were recorded in plants treated with T_{12} (11.00 fruits per plant). There was a non-significant result found when tomato was treated with talc based formulation and with only King's B broth by recording 13.30 and 13.00 fruits per plant respectively.

To evaluate the bioefficacy of LIF of *Pseudomonas fluorescens*, Tomato seedlings were treated with different formulations of *Pseudomonas fluorescens* after shelf-life studies of 180 days. In greenhouse studies, the increased plant growth and yield parameters were observed in T_5 followed by T_4 and T_7. These treatments under *in vitro* LIF condition could sustain good number of viable population upto 180 days of storage when compared to all other LIF. Henceforth, the results may be due to higher population density of these LIF during the time of inoculation to Tomato seedlings. As these LIF could retain higher population in the shelf-life studies, so was their positive effect on the Tomato crop. There was an increased plant height, number of branches, shoot and root dry weight, nitrogen and phosphorous concentration in the tomato treated with the LIF of *Pseudomonas fluorescens*, because of its plant growth promoting rhizobacteria (PGPR) mechanisms. Generally, plant growth promoting rhizobacteria facilitate the plant growth directly by either assisting in resource acquisition (nitrogen, phosphorus and essential minerals) or modulating plant hormone levels, or indirectly by decreasing the inhibitory effects of various pathogens on plant growth and development in the forms of biocontrol agents.

Plant growth promoting rhizobacteria stimulate plant growth through mobilizing nutrients in soils, producing numerous plant growth regulators and protecting plants from phytopathogen by controlling or inhibiting them[1, 2, 5, 16].

Pseudomonas fluorescens is able to produce the plant growth hormone, i.e. indole acetic acid, which acts to stimulate root growth and provide it with more branching, increase in its height and large surface area. It is considered that indole secretion, by PGPRs, is a vital mechanism to clarify plant promotion[23].

Phosphate solubilization is the common feature of *Pseudomonas fluorescens*. It will solubilise inorganic phosphate, making soil phosphorous available to the plants. Therefore, the unavailable forms of phosphorous can be partially dissolved and enhance its availability to the plant.

Per cent disease incidence.

The LIF of *Pseudomonas fluorescens* had shown greater inhibitory activity for per cent disease incidence (Table 4) in Tomato. In greenhouse experiment, out of 12 treatments T_5, T_4 and T_7 have shown good control over the *Fusarium* wilt (*F. oxysporum* f.sp. *lycopersici*) of Tomato, while T_{12} (uninoculated control) showed highest per cent disease incidence. Treatment T_5 controlled per cent disease incidence to a maximum level because it sustained higher number of population upto 180 days when studied *in vitro* followed by T_4 and T_7.

The per cent disease incidence in T_{12} (uninoculated control) was 66.00% and the lowest percent disease incidence of 10.23% was observed in T_5, followed by T_4 (11.63%) and T_7 (11.90%). Per cent disease incidence in plants treated with talc based formulation and with only King's B broth was 13.18 and 13.20% respectively.

Production of Siderophore, HCN and antibiotics like DAPG and pylouretin by *Pseudomonas fluorescens* were correlated with its efficacy in the management of plant diseases[15, 17]. Jagadeesh *et al.*[12] also reported the role of fluorescent siderophores in the biological control of bacterial wilt of tomato. Selvakumar *et al.*[18]studied the effects of *Pseudomonas fluorescens* and *Bacillus subtilis* on pathogen development and plant growth under pot culture conditions. Both organisms reduced the incidence of *Fusarium* wilt in Tomato significantly, by 79.69-75.24% which indicated that these organisms induced systemic resistance to *Fusarium* wilt in Tomato.

Table 1: Survival of *Pseudomonas fluorescens* in different liquid inoculant formulations

Formulations	Population density (X 10^{10}CFU/ml or g)						
	Storage (days)						
	0	30	60	90	120	150	180
T_1	2.43^{a}(±0.246)	1.45^{i}(±0.050)	1.18^{i}(±0.028)	1.08^{i}(±0.028)	0.05^{j}(±0.004)	0.00^{j} (±0.00)	0.00^{j}(±0.00)
T_2	0.19^{e}(±0.001)	0.18^{j}(±0.002)	0.17^{j}(±0.002)	0.16^{j}(±0.002)	0.15^{i}(±0.002)	0.13^{i}(±0.001)	0.11^{i}(±0.002)
T_3	2.14^{d} (±0.005)	1.87^{h}(±0.020)	1.77^{h}(±0.025)	1.67^{h}(±0.020)	1.57^{h}(±0.02)	1.37^{h}(±0.020)	1.17^{h} (±0.020)
T_4	2.36^{abc} (±0.076)	2.28^{b}(±0.030)	2.17^{b}(±0.025)	2.07^{b}(±0.025)	1.97^{b}(±0.025)	1.77^{b}(±0.025)	1.57^{b} (±0.025)
T_5	2.45^{a} (±0.050)	2.40^{a}(±0.010)	2.30^{a}(±0.010)	2.20^{a}(±0.01)	2.09^{a}(±0.005)	1.89^{a}(±0.005)	1.76^{a} (±0.055)
T_6	2.37^{ab} (±0.020)	2.12^{d}(±0.025)	2.02^{d}(±0.025)	1.92^{d}(±0.025)	1.82^{d}(±0.025)	1.62^{d}(±0.025)	1.42^{d} (±0.025)
T_7	2.42^{ab} (±0.025)	2.21^{c}(±0.028)	2.11^{c}(±0.011)	2.01^{c}(±0.028)	1.91^{c}(±0.028)	1.71^{c} (±0.028)	1.51^{c} (±0.028)
T_8	2.23^{cd} (±0.032)	2.04^{e}(±0.037)	1.95^{e}(±0.026)	1.85^{e}(±0.030)	1.75^{e}(±0.03)	1.55^{e}(±0.030)	1.29^{f} (±0.011)
T_9	2.30^{bc}(±0.020)	2.05^{e}(±0.030)	1.97^{e}(±0.020)	1.86^{e}(±0.036)	1.76^{e}(±0.036)	1.56^{e}(±0.036)	1.35^{e} (±0.030)
T_{10}	2.16^{d} (±0.002)	1.92^{g} (±0.025)	1.82^{g} (±0.025)	1.72^{g} (±0.025)	1.62^{g} (±0.025)	1.42^{g} (±0.025)	1.21^{gh} (±0.028)
T_{11}	2.16^{d}(±0.015)	1.99^{f}(±0.011)	1.89^{f}(±0.030)	1.79^{f}(±0.011)	1.69^{f}(±0.011)	1.49^{f}(±0.011)	1.22^{g} (±0.025)

Note: T_1 = King's B broth; T_2 = talc based formulation; T_3 = King's B + 0.5% Tween 20 (surfactant) + 0.2% Potassium sorbate (preservative); T_4 = King's B + 2% PVP + 0.1% CMC + 0.5% Tween 20 + 0.2% Potassium sorbate; T_5 = King's B +2% PVP + 0.3% Xanthan gum + 0.5% Tween 20 + 0.2% Potassium sorbate; T_6 = King's B + 1% PEG + 0.1% CMC + 0.5% Tween 20 + 0.2% Potassium sorbate; T_7 = King's B + 1% PEG + 0.3% Xanthan gum + 0.5% Tween 20 + 0.2% Potassium sorbate; T_8 = King's B + 0.8% Gum arabic + 0.1% CMC + 0.5% Tween 20 + 0.2% Potassium sorbate; T_9 = King's B + 0.8% Gum arabic + 0.3% Xanthan gum + 0.5% Tween 20 + 0.2% Potassium sorbate; T_{10} = King's B + 0.1% Sodium alginate + 0.1% CMC + 0.5% Tween 20 + 0.2% Potassium sorbate; T_{11} = King's B + 0.1% Sodium alginate + 0.3% Xanthan gum + 0.5% Tween 20 + 0.2% Potassium sorbate.

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.

Table 2: Effect of LIF of *Pseudomonas fluorescens* on plant height and branches of Tomato

Treatments	Plant height (cm)			Number of branches per plant		
	30 DAT	60 DAT	At harvest	30 DAT	60 DAT	At harvest
T_1	21.66^{g} (±0.66)	40.06^{fg} (±2.84)	50.29^{h} (±1.09)	2.92^{h} (±0.17)	4.62^{i} (±0.56)	5.94^{g} (±0.05)
T_2	21.75^{g} (±2.08)	42.75^{ef} (±2.01)	53.36^{g} (±1.82)	3.06^{h} (±0.11)	5.77^{h} (±0.59)	6.36^{g} (±0.53)
T_3	25.96^{f} (±2.52)	44.12^{e} (±1.25)	53.85^{fg} (±1.23)	3.31^{h} (±0.82)	6.94^{g} (±0.21)	7.52^{f} (±0.46)
T_4	40.88^{ab} (±1.72)	61.81^{b} (±2.62)	73.08^{b} (±1.50)	8.08^{b} (±0.24)	11.66^{b} (±0.51)	12.19^{b} (±0.31)
T_5	43.85^{a} (±2.35)	66.40^{a} (±1.72)	78.18^{a} (±1.50)	9.04^{a} (±0.15)	12.64^{a} (±0.55)	13.30^{a} (±0.62)
T_6	37.91^{bc} (±1.94)	55.35^{c} (±1.92)	69.21^{c} (±0.80)	6.59^{cd} (±0.38)	10.79^{c} (±0.30)	10.93^{c} (±0.13)
T_7	40.50^{ab} (±1.88)	60.10^{b} (±1.77)	$71.55b^{c}$ (±1.17)	7.16^{c} (±0.57)	11.31^{bc} (±0.54)	11.66^{b} (±0.38)
T_8	36.33^{cd} (±2.67)	48.64^{d} (±1.72)	63.56^{de} (±2.50)	5.38^{ef} (±0.44)	9.15^{de} (±0.48)	9.71^{d} (±0.51)
T_9	37.75^{bc} (±2.24)	50.80^{d} (±1.85)	65.01^{d} (±1.60)	5.93^{de} (±0.05)	9.76^{d} (±0.30)	10.50^{c} (±0.50)
T_{10}	30.88^{e} (±2.17)	44.92^{e} (±1.08)	56.41^{f} (±2.80)	4.05^{g} (±0.18)	8.16^{f} (±0.75)	8.64^{e} (±0.48)
T_{11}	32.75^{de} (±3.27)	45.16^{e} (±2.00)	61.83^{e} (±0.38)	4.99^{f} (±0.79)	8.65^{ef} (±0.49)	9.20^{de} (±0.24)
T_{12}	21.25^{g} (±1.39)	39.21^{g} (±1.37)	48.77^{h} (±1.41)	2.69^{h} (±0.19)	3.48^{j} (±0.49)	4.57^{h} (±0.35)

Note: T_{12}: Uninoculated control

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.

Table 3: Effect of LIF of *Pseudomonas fluorescens* on nitrogen and phosphorous content of Tomato

Treatments	Nitrogen content (%)			Phosphorous content (%)		
	30 DAT	60 DAT	At harvest	30 DAT	60 DAT	At harvest
T_1	1.16^{g} (±0.013)	1.32^{fg} (±0.025)	1.08^{e} (±0.015)	0.273^{h} (±0.010)	0.347^{f} (±0.015)	0.309^{hi} (±0.010)
T_2	1.17^{fg} (±0.017)	1.33^{ef} (±0.020)	1.11^{de} (±0.024)	0.282^{h} (±0.001)	0.357^{ef} (±0.014)	0.324^{gh} (±0.012)
T_3	1.20^{f} (±0.020)	1.36^{ef} (±0.024)	1.11^{de} (±0.015)	0.297^{g} (±0.007)	0.380^{de} (±0.015)	0.335^{fg} (±0.004)
T_4	1.34^{ab} (±0.016)	1.50^{ab} (±0.026)	1.26^{a} (±0.016)	0.412^{b} (±0.007)	0.480^{b} (±0.014)	0.434^{b} (±0.009)
T_5	1.37^{a} (±0.020)	1.53^{a} (±0.045)	1.28^{a} (±0.015)	0.443^{a} (±0.007)	0.539^{a} (±0.012)	0.471^{a} (±0.015)
T_6	1.28^{c} (±0.012)	1.47^{bc} (±0.025)	1.22^{b} (±0.017)	0.388^{c} (±0.008)	0.457^{b} (±0.020)	0.411^{c} (±0.010)
T_7	1.32^{b} (±0.026)	1.49^{ab} (±0.019)	1.25^{ab} (±0.014)	0.392^{c} (±0.015)	0.462^{b} (±0.009)	0.426^{bc} (±0.020)
T_8	1.26^{cd} (±0.015)	1.43^{c} (±0.025)	1.18^{c} (±0.027)	0.348^{de} (±0.010)	0.413^{c} (±0.012)	0.364^{de} (±0.005)
T_9	1.27^{c} (±0.007)	1.46^{bc} (±0.026)	1.21^{bc} (±0.022)	0.350^{d} (±0.008)	0.424^{c} (±0.021)	0.380^{d} (±0.009)
T_{10}	1.20^{ef} (±0.020)	1.37^{de} (±0.020)	1.14^{d} (±0.020)	0.307^{f} (±0.007)	0.399^{cd} (±0.012)	0.347^{ef} (±0.007)
T_{11}	1.23^{de} (±0.011)	1.42^{cd} (±0.065)	1.18^{c} (±0.016)	0.339^{e} (±0.01)	0.401^{cd} (±0.010)	0.352^{ef} (±0.014)
T_{12}	1.12^{h} (±0.025)	1.27^{g} (±0.020)	1.03^{f} (±0.014)	0.230^{i} (±0.012)	0.329^{f} (±0.012)	0.295^{i} (±0.018)

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.

Table 4: Effect of LIF of *Pseudomonas fluorescens* on shoot dry weight, root dry weight, fruit yield and disease incidence of Tomato

Treatments	Shoot dry weight (g/plant)			Root dry weight (g/plant)			Fruits per plant	Per cent disease incidence
	30 DAT	60 DAT	At harvest	30 DAT	60 DAT	At harvest	At harvest	At harvest
T_1	9.06^{i} (±0.20)	16.69^{i} (±0.11)	20.69^{i} (±0.51)	3.06^{fg} (±0.30)	4.21^{fg} (±0.20)	5.24^{de} (±0.20)	13.00^{fg} (±1.00)	13.20^{b} (21.31)
T_2	9.33^{i} (±0.28)	17.12^{hi} (±0.51)	22.25^{hi} (±0.50)	3.20^{efg} (±0.29)	4.37^{ef} (±0.26)	5.44^{cde} (±0.43)	13.30^{fg} (±2.00)	13.18^{b} (21.29)
T_3	10.05^{h} (±0.58)	17.60^{h} (±0.53)	23.83^{h} (±1.55)	3.40^{efg} (±0.29)	4.42^{ef} (±0.31)	5.46^{cde} (±0.13)	13.66^{f} (±1.15)	13.13^{bc} (21.25)
T_4	17.44^{b} (±0.19)	26.22^{b} (±0.29)	37.18^{b} (±0.23)	4.61^{ab} (±1.09)	5.82^{ab} (±0.07)	6.77^{a} (±0.24)	21.66^{b} (±1.15)	11.63^{e} (19.94)
T_5	19.11^{a} (±0.12)	28.37^{a} (±0.26)	39.15^{a} (±0.40)	5.07^{a} (±0.03)	6.28^{a} (±0.35)	6.82^{a} (±0.58)	24.33^{a} (±1.52)	10.23^{f} (18.65)
T_6	14.38^{d} (±0.04)	22.23^{d} (±0.40)	34.16^{cd} (±0.55)	4.13^{bcd} (±0.36)	5.31^{bc} (±0.18)	6.31^{ab} (±0.26)	18.33^{cd} (±1.52)	12.13^{cde} (20.38)
T_7	15.27^{c} (±0.01)	23.30^{c} (±0.50)	35.5^{c} (±0.50)	4.30^{bc} (±0.04)	5.59^{b} (±0.1)	6.61^{a} (±0.1)	19.66^{bc} (±1.52)	11.90^{de} (20.17)
T_8	12.44^{f} (±0.41)	20.36^{f} (±0.64)	31.34^{e} (±0.54)	3.67^{cdef} (±0.02)	4.79^{cde} (±0.4)	5.79^{bcd} (±0.42)	16.66^{de} (±2.08)	12.60^{bcde} (20.79)
T_9	13.43^{e} (±0.47)	21.50^{e} (±0.52)	33.36^{d} (±1.42)	3.78^{cde} (±0.5)	5.01^{cd} (±0.45)	6.00^{bc} (±0.49)	17.66^{cd} (±2.08)	12.37^{bcde} (20.59)
T_{10}	10.55^{h} (±0.29)	18.78^{g} (±0.25)	26.81^{g} (±0.29)	3.47^{defg} (±0.46)	4.49^{def} (±0.28)	5.45^{cde} (±0.21)	14.33^{ef} (±1.52)	13.12^{bc} (21.24)
T_{11}	11.49^{g} (±0.49)	19.42^{g} (±0.32)	28.94^{f} (±0.09)	3.62^{cdef} (±0.05)	4.60^{def} (±0.32)	5.62^{cd} (±0.12)	16.33^{de} (±0.57)	12.86^{bcd} (21.02)
T_{12}	9.03^{i} (±0.05)	14.47^{j} (±0.39)	18.31^{j} (±2.19)	2.80^{g} (±0.35)	3.78^{g} (±0.52)	4.90^{e} (±0.48)	11.00^{g} (±1.00)	60.00^{a} (50.78)

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.

CONCLUSION

Polymeric additives such as cell protectants, adjuvants, surfactant and preservative used in the study of development of LIF have a pronounced effect on the viability of *Pseudomonas fluorescens* during storage period. Among the cell protectants used, PVP showed the best results in shelf-life studies. The next best cell protectant in retaining higher colony counts after PVP was PEG followed by gum arabic and sodium alginate. Xanthan gum as an adjuvant yielded best results in combination with cell protectants when compared to CMC in shelf-life studies. Surfactant tween-20 and preservative potassium sorbate had considerable effect in maintaining good population density in different LIF. King's B broth could maintain the population density upto 90 days of storage, after that there was a gradual decrease in colony counts and at the end of 180 days there were no colonies observed. The population density in talc based formulation was much lower when compared to all other LIF of *Pseudomonas fluorescens.* The results of bioefficacy studies followed same trend as that of shelf-life studies. Tomato seedlings inoculated with the LIF T_5 (King's B broth + 2% PVP + 0.3% Xanthan gum + 0.5% Tween-20 + 0.2% Potassium sorbate), showed the best result in all growth and yield parameters. Treatment T_5 was found best in controlling wilt disease incidence in greenhouse studies.

Acknowledgement

PGPR, *Pseudomonas fluorescens* culture was obtained from culture collection of Department of Agricultural Microbiology and the pathogen, *Fusarium oxysporum* f.sp. *lycopersici* was obtained from culture collection of Department of Plant Pathology, College of Agriculture, Bheemarayanagudi,

University of Agricultural Sciences, Raichur. The authors are greatly indebted for providing the culture.

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