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RESEARCH PAPER

Shelf life study of *Pseudomonas fluorescens* in liquid formulations

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Abstract: Development of liquid formulations has numerous advantages which includes high cell count, prolonged shelf life and improved field efficacy. An experiment was conducted at the Institute of Organic Farming, University of Agricultural Sciences, Dharwad to evaluate different liquid formulations of *Pseudomonas fluorescens* for cell viability up to twelve months of storage at room temperature. The results from shelf life studies indicated that, maximum viability of *Pseudomonas fluorescens* after twelve months of storage at room temperature was found in canola oil based formulation followed by soybean oil based formulation at two per cent concentration. *P. fluorescens* broth and talc based formulation were not competitive in supporting the viability of *Pseudomonas fluorescens*. Thus, canola oil based formulation was found to sustain sufficient viable colonies of *Pseudomonas fluorescens* even after twelve months of storage period, thereby making it the promising one for storage, enhancing the shelf life and its further application in field conditions under organic agriculture.

Key words: Bacteria, Field efficacy, Liquid formulations, *Pseudomonas,* Shelf life

Introduction

The development of microbial bioinoculants for disease management and plant growth promotion has evolved as an alternative to chemical pesticides in last few years, but a broader aspect of their application as formulated product has still remained with experimentation. Even though various bioformulations have shown antagonism to a variety of phytopathogens under labo-ratory conditions, but they have inconsistent field performance which may be attributed to different factors such as poor shelf life, little active material entering the target site, death of antagonist due to desiccation and susceptibility of antagonist to various abiotic stresses. Thus, formulation technologies are being utilized to stabilize the microorganism during manufacturing, storage, delivery, handling and safeguarding the microorganism from adverse environmental conditions, thereby enhancing the organism's efficacy.

Liquid bioformulation are microbial cultures or suspensions amended with compatible substances to improve viability, stickiness, stability, surfactant and dispersal ability. These are referred as aqueous or flowable suspensions and comprises biomass suspensions in water, oils, or sometimes combinations of both (emulsions) (Schisler *et al*., 2004).

Pseudomonas fluorescens (Flugge) Migula comprises a group of common, rod shaped, Gram negative and nonpathogenic saprophytes which inhabit soil, water, and plant surface environments. *Pseudomonas* spp. are the plant growth-promoting rhizobacteria (PGPR), which are vastly used for boosting growth of the plant and disease control (Wei *et al.,* 1996). In order to enhance the efficacy of this bacterial bioagent, it must be prepared as formulations so that it sustains sufficient bacterial population for a longer period of time and ensures its easy application, storage and field use.

Development of liquid formulations has numerous merits such as high cell count, prolonged shelf life, enhanced protection from environmental stresses and improved field efficacy. It increases the contact of organism with the target insect pest or pathogen which leads to enhancement of its activity at the target site. In liquid formulations, the micro-organisms that exists in a dormant cyst state becomes active after application in the field, contributing to an extended shelf life of over a year (Vendan and Thangaraju, 2006). Keeping in view these potential advantages of liquid-based formulations, the present investigation was conducted to assess the shelf life of *Pseudomonas fluorescens* in different liquid formulations.

Material and methods

Preparation of liquid formulation of *Pseudomonas fluorescens*

The *Pseudomonas fluorescens* (IOF) strain (NAIMCC-B-01981) used in this study was obtained from the Institute of Organic Farming, University of Agricultural Sciences, Dharwad.

Pseudomonas fluorescens broth was prepared by inoculating a fresh culture of bacteria in sterilized *Pseudomonas* (Fluorescein) broth, incubated in rotary shaker at 150 rpm for 48 hours at room temperature (25 ± 2 ^{0}C). One ml of the suspension containing the log phase culture (3 x 10^{10} CFU per ml) of *P. fluorescens* was inoculated into the sterilized *Pseudomonas* (Fluorescein) broth containing different oils (soybean oil, groundnut oil and canola oil) and liquid such as glycerol and distilled water at two per cent concentration and one gram of Polyvinylpyrrolidone (PVP), incubated in rotary shaker at 150 rpm for 48 hours at room temperature (25 ± 2 $^\circ$C). For preparation of 2% oil-based formulation (2ml of oil/liquid + 1g of PVP + 97 ml of *P. fluorescens* broth) was used. PVP was added in each liquid formulation to prevent the desiccation and increase the survival of bacterial

cells. This also acts as an emulsifying agent, thus aids in formation of stable emulsion in case of oil based formulations of *P. fluorescens.* The formulations were sealed and further used for shelf life studies and field evaluation.

The oils such as soybean oil, groundnut oil, canola oil and market sample were procured from the market.

Shelf life studies of *Pseudomonas fluorescens* in different formulations

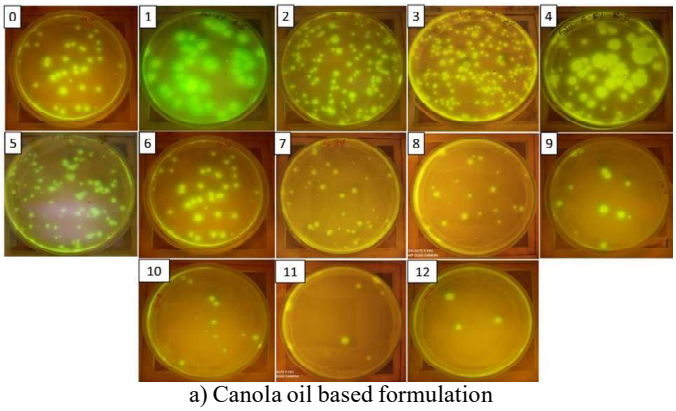
The studies on shelf life of different liquid formulations, one talc based and one commercially available liquid formulation were conducted starting from the first day when the formulation was prepared with a regular interval of thirty days till 12 months. The microbial analysis for number of viable *Pseudomonas fluorescens* bacteria was analyzed at monthly intervals by serial dilution with plate count method (Cappucinno and Sherman, 2001).

One ml aliquot from each liquid formulation and the checks was drawn and serially diluted in 9 ml distilled water till 10^8 dilutions. From 10^8 dilutions, one ml aliquot was transferred on sterile petriplate. These plates were poured with *Pseudomonas* agar (Fluorescein) medium and incubated at 28 °C and next day colonies were counted from each treatment. Observations on initial colony forming units (cfu) and colony forming units (cfu) after every month were recorded.

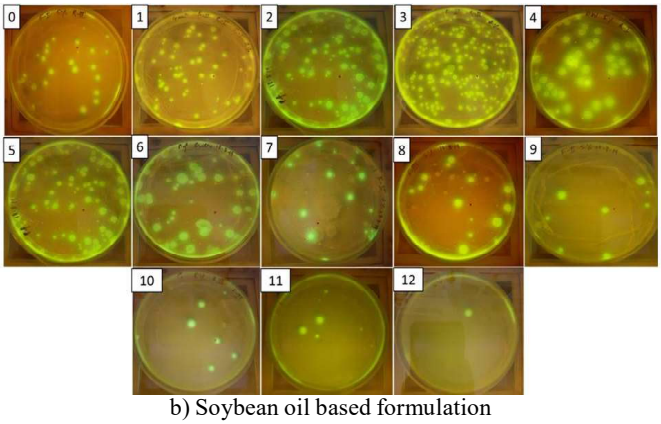
Results and discussion

Studies on shelf life were conducted at monthly intervals to assess the survivability of *Pseudomonas fluorescens* in different liquid formulations in order to identify the suitable liquid carrier for formulating *P. fluorescens.* The results of shelf life study are presented in Table 1 and Plate 1.

The results of the shelf life study revealed that the initial colony forming units was observed maximum in canola oil-based formulation (25 x 10^8 CFU ml^{-1}) which was significantly superior to all other treatments followed by glycerol based formulation (22.33 x10^8 CFU ml^{-1}), soybean oil based formulation (21.33 x 10^8 CFU ml^{-1}) and groundnut oil-based formulation (20.00 x 10^8 CFU ml^{-1}). The minimum population was recorded in distilled water (19.33 x 10^8 CFU ml^{-1}) and talc powder (18.33 x 10^8 CFU ml^{-1}). Whereas, in case of *P. fluorescens* broth and market sample, the initial population was (24.00 x 10^8 CFU ml^{-1} and 20.67 x 10^8 CFU ml^{-1}) respectively. Canola oil-based formulation followed by soybean oil-based formulation supported higher survival of *Pseudomonas fluorescens* upto 360 days of storage in comparison to other liquid formulations. This could be due to the reason that, the oil engulfs the water around the organism,



a) Canola oil based formulation



b) Soybean oil based formulation

Plate1. Viable colonies of *Pseudomonas fluorescens* in oil based formulations at monthly interval

Table 1. Shelf life of *Pseudomonas fluorescens* at different days of interval in various liquid formulations at 2% concentration

Formulation	Colony forming units (x 10^8) per ml of formulation at different days of interval												
	Initial	30	60	90	120	150	180	210	240	270	300	330	360
Soybean oil-based formulation	21.33	44.00	67.40	80.93	64.10	46.24	36.54	24.33	16.67	10.33	5.67	3.00	1.33
Groundnut oil-based formulation	20.00	28.33	52.70	74.33	32.67	25.03	19.00	16.17	11.00	7.67	3.33	1.00	0.67
Canola oil-based formulation	25.00	51.67	72.33	86.00	64.67	54.33	38.67	27.00	22.67	18.33	10.00	5.67	3.33
Glycerol-based formulation	22.33	42.67	64.00	82.33	60.67	45.00	36.67	22.67	17.33	12.00	5.33	2.67	1.00
Distilled water	19.33	34.67	50.33	28.67	12.67	6.33	2.67	1.33	0.67	0.00	0.00	0.00	0.00
Pseudomonas fluorescens broth	24.00	40.33	32.67	22.33	15.33	7.67	2.00	0.00	0.00	0.00	0.00	0.00	0.00
Talc based formulation	18.33	26.97	31.67	25.10	19.07	10.33	2.33	0.00	0.00	0.00	0.00	0.00	0.00
Market Sample (Liquid formulation of *P. fluorescens*)	20.67	28.33	48.00	34.67	23.73	20.37	11.00	4.33	2.00	1.00	0.00	0.00	0.00
Mean	21.38	37.12	52.39	54.30	36.61	26.91	18.61	11.98	8.79	6.17	3.04	1.54	0.79
					S.Em. ±						CD @ 1%		
Formulation (F)					0.19						0.53		
Interval (I)					0.24						0.68		
F x I					0.69						1.92		

thereby, slowing the evaporation of water; which is advantageous for organisms that are susceptible to desiccation, thus preventing antagonist *Pseudomonas fluorescens* from dessication and thereby increasing the shelf life (Jean *et al*., 2006).

The results are in accordance with Mujtaba, (2011) who reported that canola oil + glycerol based formulation showed maximum viability after six months and viable conidia of *Trichoderma* were found upto twelve months of storage. Archana *et al.* (2015) opined that the maximum population of *Pseudomonas fluorescens* was recorded in rice bran oil followed by soybean oil after 175 days. Reddy *et al.* (2017) calculated the colony forming units of *T. harzianum* on 56th day of observation during the shelf life study and reported that paraffin oil (20 x 10^7CFU ml^{-1}) and soybean oil (2.1 x10^7 CFUml^{-1}) gave the better performance of spore viability. Nadare *et al.* (2018) also studied the effectiveness of various liquid carriers like soybean oil, mustard oil, sunflower oil, paraffin oil and talc powder on the viability of *Trichoderma viride* and reported that the colony forming unit of *Trichoderma viride* were maximum in paraffin oil which was followed by soybean oil. Jayasudha *et al.* (2018) reported that the pongamia oil retain the highest per cent survival of the different bacterial bioagents including *Brevibacillus borstelensis*, *Bacillus subtilis*, *Brevibacillus* sp. and *Lysinibacillus xylanilyticus* followed by ground nut oil, sunflower oil, distilled water, nutrient broth even after 3 months of storage.

The rate of decline in population was relatively slow in canola oil, soybean oil, groundnut oil and glycerol based formulations. After six months of incubation period, the highest population density was recorded in canola oil-based formulation (38.67 x 10^8 CFU ml^{-1}) succeeded by glycerol based formulation (36.67 x 10^8 CFU ml^{-1}) and soybean oil-based formulation (36.54 x 10^8 CFU ml^{-1}). The minimum population was observed in *P. fluorescens* broth (2.00 x 10^8 CFU ml^{-1}) followed by talc based formulation (2.33 x 10^8 CFU g^{-1})and distilled water (2.67 x 10^8 CFU ml^{-1}) after six months of storage. This may be due to loss of nutrients from the media, loss of moisture content and desiccation during the storage period. Another possible explanation for the low survival of *Pseudomonas fluorescens* in these formulations may be the lack of cell protectants, leading to the inability of bacteria to safeguard them from desiccation. Vidyashekaran and Muthamilan (1995) opined that the talc based formulation could not retain sufficient population of *Pseudomonas fluorescens* more than 4 months of storage.

Glycerol based formulation ranked next to canola oil and soybean oil-based formulation as it also maintained shelf life up to twelve months of storage (1.00 x 10^8 CFU ml^{-1}) mainly due to the reason that it holds a higher quantity of water and guard cells from the consequence of desiccation by reducing the rate of drying. These results were in consonance with Manikandan *et al.* (2010) who claimed that the introduction of glycerol and trehalose in nutrient broth has amplified the shelf life of liquid formulation of *P. fluorescens* (Pf1) for the storage period of 6 months.

Conclusion

Canola oil based liquid formulation encouraged th e shelf life of *P. fluorescens*.

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