International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2019; 7(6): 922-925

Received: 07-09-2019
Accepted: 10-10-2019

Kamlesh Kumar Mohle
Department of Plant Pathology, College of Agriculture, Indira Gandhi Krishi Vishwavidyalaya, Raipur, Chhattisgarh, India

RKS Tiwari
BTCARS, Bilaspur, Chhattisgarh, India

Manisha Tondey
Department of Agricultural Microbiology, PAU, Punjab, India

Kovid Kumar
Department of Plant Pathology, SHIATS, Allahabad, Uttar Pradesh, India

Corresponding Author:
Kamlesh Kumar Mohle
Department of Plant Pathology, College of Agriculture, Indira Gandhi Krishi Vishwavidyalaya, Raipur, Chhattisgarh, India

Shelf life study of *Pseudomonas fluorescens* in talc based carrier and liquid formulations

Kamlesh Kumar Mohle, RKS Tiwari, Manisha Tondey and Kovid Kumar

Abstract
Plant growth promoting rhizobacteria (PGPR) was designed to formulate a suitable carrier from available material and evaluate for shelf life by using locally isolated *P. fluorescens* in soil. Shelf life study of *P. fluorescens* was talc based carrier material and liquid formulations stored at two different temperature regimes *viz*., room and refrigerator. *P. fluorescens* was assessed the initial population and after 6 months of storage. The observations were recorded 48 hours after inoculation by serial dilution technique to obtain 10^{-15} concentrations and different formulations. Shelf life of *P. fluorescens* talc based carrier material compared to liquid formulations.

Keywords: *Pseudomonas fluorescens*, shelf life, formulations, talc and carrier

Introduction
Study was designed to formulate a suitable carrier from locally available cheap material and evaluate for shelf life by using locally isolated plant growth promoting rhizobacteria (PGPR) strains from maize rhizosphere. Significant difference regarding microbial survival was observed between different formulations as well as between different incubation intervals, (Tabassam *et al.*, 2015) [7].

Studies were carried out to evaluate different carriers for shelf life of *Pseudomonas fluorescens* stored at 25±2℃ over a storage period of 6 months. The population dynamics was recorded at monthly intervals. The population of *P. fluorescens* was increased significantly in all carriers *i.e.* Talc, Spent mushroom substrate (SMS) a waste product of mushroom industry, Lignite, Charcoal, Farm yard manure (FYM) and Fly ash up to 60 days storage and there was slow decline in number of viable propagules after 60 days of storage, (Gade *et al.*, 2014) [1].

The shelf life of *P. fluorescens* (PF-4) was recorded in different carrier material stored at two different temperature regimes *viz*., room and refrigerator over a storage period of 10 months. The population dynamics was recorded at monthly intervals. The population of isolated increased significantly in talc, vermicompost, FYM up to 60 days and up to 30 days in king's B broth. The highest mean population of PF-4 was obtained in king B media. Refrigerator temperature supported better population of broth PF- 4 in the carries material, (Keshgond *et al.*, (2012) [3].

The survival of PGPR isolates was investigated by using different carrier materials. The carrier based PGPR consortium with *P. fluorescens* VPS-19 was prepared and the shelf life for each inoculant was studied upto six months of storage. The surviving population in the lignite based consortium was 2.01×10^8 cfu g^{-1} for *P. fluorescens* VPS-19 after six month of storage, (Sangeetha and Stella., 2012) [5].

The culture broths of *pseudomonads* R62, R81 and Pi were successfully used for development of talcum- and vermiculite-based bio-inoculant formulations. In controlled glasshouse experiments, the talcum-based bio-inoculant formulations performed significantly better over vermiculite-based formulations. In field experiments the talcum-based consortium formulation of *pseudomonad* R81 and Pi was most effective, (Sarma *et al*., 2011) [6].

Studies were carried out to evaluate the effect of adding nutrient additives to talc based formulations on the shelf life of bacteria stored under room temperature. Two organisms namely, *Pseudomonas fluorescens* (PDBCAB2) and Bacillus sp. (MTCC6534 chickpea endophyte), a spore forming bacterium, were tested. A gradual increase in *P. fluorescens* population up to 90 days was observed in almost all the treatments that were amended with nutrients, (Rangeshwaran *et al.*, 2010) [4].

Materials and Methods

Mother culture of *Pseudomonas fluorescens* was obtained from State Biocontrol laboratory, Sesal farm Chorbhhatti BTC CARS Bilaspur, C.G. cultures was preserved in room temperature and refrigerated condition.

Preparation of Talc based formulation of *Pseudomonas fluorescens*

Nutrient agar medium was used for making talc based formulation of *P. fluorescens*. Sterilized nutrient agar medium was inoculated with Petri dishes of *P. fluorescens* and incubated at 29 ± 2 ℃ inside the BOD Incubator for two days. Culture media was homogenized in the form of green biomass and mix with sterilized talc in the ratio of 1: 100 (one part of bio mass in 100 parts of talc) and thus 10% (W/V) talc based formulation of were obtained. The products were packed in polythene bags and kept under room temperature conditions as well as under refrigerated conditions for the purpose of storage.

Preparation of liquid formulation of *Pseudomonas fluorescens*

Nutrient broth medium was used for making liquid formulation of *P. fluorescens*. Sterilized broth was inoculated with culture discs of *Pseudomonas fluorescens* and incubated at 29 ± 2 ℃ inside the BOD Incubator for two days. Culture broth was homogenized in the form of green biomass and mix with sterilized water in the ratio of 1: 100 (one part of bio mass in 100 parts of sterilized water) and thus 10% (V/V) liquid based formulations were obtained. Prepared product was packed and sealed in sterilized bottles and kept under room temperature conditions as well as under refrigerated conditions for the purpose of storage.

Shelf life of *P. fluorescens* in powder and liquid formulations was studied by taking samples of stored products after 06 months of storage. Twenty ml of nutrient agar medium and nutrient broth medium was poured in plates. The initial population of *P. fluorescens* was assessed by serial dilution technique. One gram of sample was taken from each samples and serially diluted to obtain 10^{-15} concentrations. One ml was poured in separate sterilized Petri plates and spreaded uniformly with the help of spreader and plates were incubated in BOD incubator at 29 ± 2 ºC for two days. Each sample was replicated thrice. The observations were recorded 48 hours after inoculation. The data were statistically analysed with the help of completely randomized design.

Result and Discussions

Shelf life study of *Pseudomonas fluorescens* in talc based formulations.

Data presented in table 1. showed that the viability in different concentration and dilution at 10^{-15} talc based formulation of *P. fluorescens* under storage in room temperature. The maximum CFU (19.66×10^{-15} $CFUg^{-1}$) of six month old *P. fluorescens* was recorded on 20% talc based formulation followed by 15% talc based formulation (17.00×10^{-15} $CFUg^{-1}$), 10% talc based formulation (15.33×10^{-15} $CFUg^{-1}$), 5% talc based formulation (13.00×10^{-15} $CFUg^{-1}$) and minimum CFU of (10.66×10^{-15} $CFUg^{-1}$) was observed at 1% talc based formulation.

The viability in different concentration and dilution at 10^{-15} talc based formulation of *P. fluorescens*. The maximum CFU (35.00×10^{-15} $CFUg^{-1}$) of fresh *P. fluorescens* was recorded on 20% talc based formulation followed by 15% talc based formulation (33.33×10^{-15} $CFUg^{-1}$), 10% talc based formulation (30.66×10^{-15} $CFUg^{-1}$), 5% talc based formulation (24.00×10^{-15} $CFUg^{-1}$) and minimum CFU of (21.00×10^{-15} $CFUg^{-1}$) was observed at 1% talc based formulation.

Data presented in table 2. Showed that the viability in different concentration and dilution at 10^{-15} talc based formulation of *P. fluorescens* under storage in refrigeration. The maximum CFU (22.66×10^{-15} $CFUg^{-1}$) of six month old *P. fluorescens* was recorded on 20% talc based formulation followed by 15% talc based formulation (20.33×10^{-15} $CFUg^{-1}$), 10% talc based formulation (18.00×10^{-15} $CFUg^{-1}$), 5% talc based formulation (16.33×10^{-15} $CFUg^{-1}$) and minimum CFU of (12.00×10^{-15} $CFUg^{-1}$) was observed at 1% talc based formulation.

The viability in different concentration and dilution at 10^{-15} talc based formulation of *P. fluorescens*. The maximum CFU (41.00×10^{-15} $CFUg^{-1}$) of fresh *P. fluorescens* was recorded on 20% talc based formulation followed by 15% talc based formulation (37.00×10^{-15} $CFUg^{-1}$), 10% talc based formulation (33.66×10^{-15} $CFUg^{-1}$), 5% talc based formulation (27.66×10^{-15} $CFUg^{-1}$) and minimum CFU of (25.33×10^{-15} $CFUg^{-1}$) was observed at 1% talc based formulation.

Table 1: Total colony forming units of *Pseudomonas fluorescens* on fresh talc based formulation and kept for 6 months under storage in room temperature

Talc based formulations at dilution ends 10^{-15}	*P. fluorescens* fresh ($CFUg^{-1}$) Observation of 48 hours	*P. fluorescens* six months old ($CFUg^{-1}$) Observation of 48 hours
1% talc formulation	21.00	10.66
5% talc formulation	24.00	13.00
10% talc formulation	30.66	15.33
15% talc formulation	33.33	17.00
20% talc formulation	35.00	19.66
S E m (±)	1.56	1.00
CD 5%	4.99	3.19

Table 2: Total colony forming units of *Pseudomonas fluorescens* on fresh talc based formulation and kept for 6 months under storage in refrigeration

Talc based formulations at dilution ends10^{-15}	*P. fluorescens* fresh ($CFUg^{-1}$) 48 hours	*P. fluorescens* six months old ($CFUg^{-1}$) 48 hours
1% talc formulation	25.33	12.00
5% talc formulation	27.66	16.33
10% talc formulation	33.66	18.00
15% talc formulation	37.00	20.33
20% talc formulation	41.00	22.66
S E m (±)	0.68	0.96
CD 5%	2.18	3.08

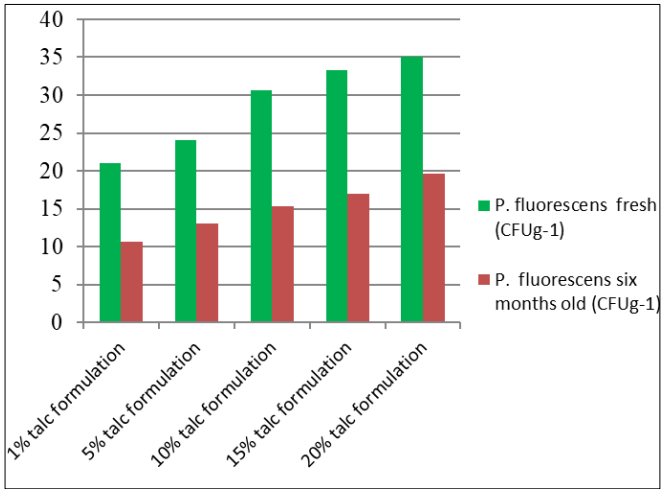

Fig 1: Total colony forming units of *Pseudomonas fluorescens* on fresh talc based formulation and kept for 6 months under storage in room temperature

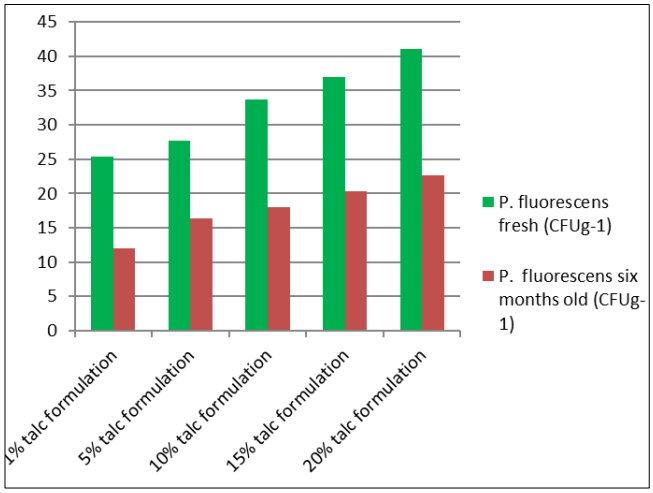

Fig 2: Total colony forming units of *Pseudomonas fluorescens* on fresh talc based formulation and kept for 6 months under storage in refrigeration

Shelf life study of *Pseudomonas fluorescens* in liquid formulations

Data presented in table 3. Showed that the viability in different concentration and dilution at 10^{-15} liquid formulation of *P. fluorescens* under storage in room temperature. The maximum CFU (29.33×10^{-15} $CFUg^{-1}$) of six month old *P. fluorescens* was recorded on 20% liquid formulation followed by 15% liquid formulation (25.00×10^{-15} $CFUg^{-1}$), 10% liquid based formulation (21.00×10^{-15} $CFUg^{-1}$), 5% liquid formulation (16.00×10^{-15} $CFUg^{-1}$) and minimum CFU of (12.33×10^{-15} $CFUg^{-1}$) was observed at 1% liquid formulation.

The viability in different concentration and dilution at 10^{-15} liquid formulation of *P. fluorescens.* The maximum CFU (40.33×10^{-15} $CFUg^{-1}$) of fresh *P. fluorescens* was recorded on 20% liquid formulation followed by 15% liquid formulation (36.00×10^{-15} $CFUg^{-1}$), 10% liquid formulation (32.66×10^{-15} $CFUg^{-1}$), 5% liquid formulation (25.33×10^{-15} $CFUg^{-1}$) and minimum CFU of (22.00×10^{-15} $CFUg^{-1}$) was observed at 1% liquid formulation.

Data presented in table 4. Showed that the viability in different concentration and dilution at 10^{-15} liquid formulation of *P. fluorescens* under storage in refrigeration. The maximum CFU (33.33×10^{-15} $CFUg^{-1}$) of six month old *P. fluorescens* was recorded on 20% liquid formulation followed by 15% liquid formulation (29.00×10^{-15} $CFUg^{-1}$), 10% liquid based formulation (24.33×10^{-15} $CFUg^{-1}$), 5% liquid formulation (19.00×10^{-15} $CFUg^{-1}$) and minimum CFU of (15.33×10^{-15} $CFUg^{-1}$) was observed at 1% liquid formulation.

The viability in different concentration and dilution at 10^{-15} liquid formulation of *P. fluorescens.* The maximum CFU (45.33×10^{-15} $CFUg^{-1}$) of fresh *Pseudomonas fluorescens* was recorded on 20% liquid formulation followed by 15% liquid formulation (40.33×10^{-15} $CFUg^{-1}$), 10% liquid formulation (36.33×10^{-15} $CFUg^{-1}$), 5% liquid formulation (30.00×10^{-15} $CFUg^{-1}$) and minimum CFU of (26.66×10^{-15} $CFUg^{-1}$) was observed at 1% liquid formulation.

Reported that the effect of Vermicompost in maintaining the shelf life of bio-inoculant such as *Azospirillum lipoferum*, *Bacillus megaterium* and *Pseudomonas fluorescens* was studied up to 12 months from the date of preparation of inoculant by comparing with lignite carrier comparatively, Vermicompost based bioinoculants showed longer shelf life than lignite based bioinoculants. Among Vermicompost based bioinoculants *B. megaterium* showed maximum population of 7.60 x 10^{8} cfu/g of dry wt on 360th day followed by *Pseudomonas* 10^{8} cfu/g of dry wt respectively (Gandhi and Saravanakumar. 2009) [2].

It may be concluded from present findings that the maximum population of *P. fluorescens* fresh formulation and the minimum population of *P. fluorescens* on six month old formulation.

Table 3: Total colony forming units of *Pseudomonas fluorescens* on fresh liquid formulation and kept for 6 months under storage in room temperature

Liquid based formulations at dilution ends	*P. fluorescens* fresh ($CFUg^{-1}$)	*P. fluorescens* six months old ($CFUg^{-1}$)
10^{-15}	Observation of 48 hours	Observation of 48 hours
1% liquid formulation	22.00	12.33
5% liquid formulation	25.33	16.00
10% liquid formulation	32.66	21.00
15% liquid formulation	36.00	25.66
20% liquid formulation	40.33	29.33
S E m (±)	1.09	1.26
CD 5%	3.49	4.03

Table 4: Total colony forming units of *Pseudomonas fluorescens* on fresh liquid formulation and kept for 6 months under storage in refrigeration

Liquid based formulations at dilution ends	*P. fluorescens* fresh ($CFUg^{-1}$)	*P. fluorescens* six months old ($CFUg^{-1}$)
10^{-15}	Observation of 48 hours	Observation of 48 hours
1% liquid formulation	26.66	15.33
5% liquid formulation	30.00	19.00
10% liquid formulation	36.33	24.33
15% liquid formulation	40.33	29.00
20% liquid formulation	45.33	33.33
S E m (±)	1.04	0.73
CD 5%	3.33	2.33

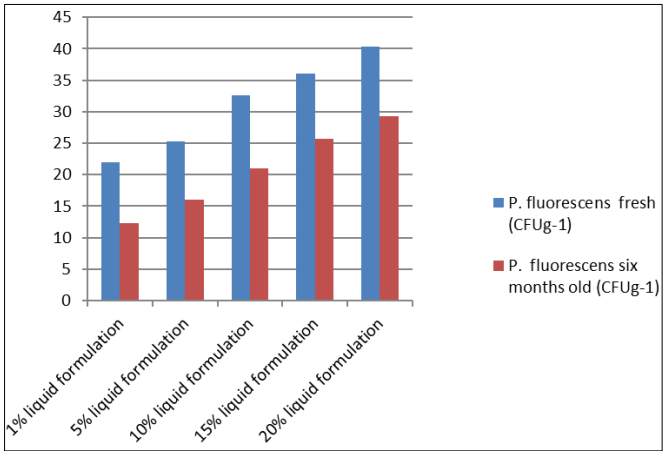

Fig 3: Total colony forming units of *Pseudomonas fluorescens* on fresh liquid formulation and kept for 6 months under storage in room temperature

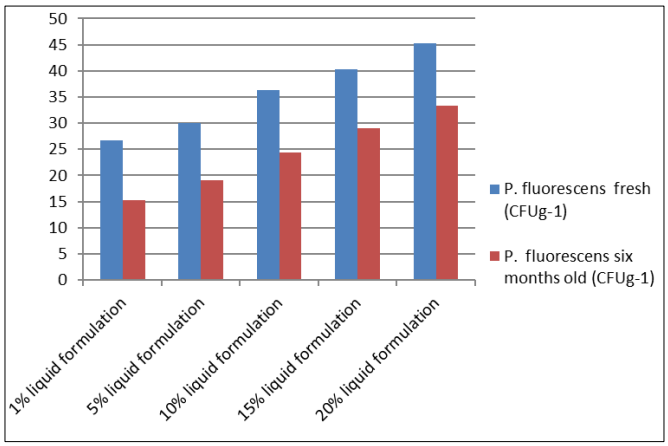

Fig 4: Total colony forming units of *Pseudomonas fluorescens* on fresh liquid formulation and kept for 6 months under storage in refrigeration

References

1. Gade RM, Chaithanya BH, Khurade KC. A comparative study of different carriers for shelf life of Pseudomonas fluorescens. The Bio. 2014; 9(1):287-290.
2. Gandhi A, Saravanakumar K. Studies on shelf life of *Azospirillum* lipoferum, *Bacillus megaterium* and *Pseudomonas fluorescens* in Vermicompost carrier. J Phytol. 2009; 1(2):100-107.
3. Keshgond R, Naik MK, Amaresh YS, Sunkad G, Patil MG, Hosamani AC. Shelf life of two indigenous isolates of fluorescent pseudmonads as bioagents in different carrier materials. J Mycopathol. Res. 2012; 50(1):85-88.
4. Rangeshwaran R, Vajid NV, Ramanujam B, Sriram S, Bhaskaran TV, Kumar S. Additives in powder based formulation for enhanced shelf life of *Pseudomonas fluorescens* and *Bacillus* sp. J Biol. Cont. 2010; 24(20):158-163.
5. Sangeetha D, Stella D. Survival of plant growth promoting bacterial inoculants in different carrier materials. Int. J Pharmacol. Biol. Arch. 2012; 3(1):170-178.
6. Sarma MVRK, Kumar V, Saharan K, Srivastava R, Sharma AK, Prakash A *et al*. Application of inorganic carrier-based formulations of *fluorescent pseudomonads* and Piriformospora indica on tomato plants and evaluation of their efficacy. J Appl. Microb. 2011; 111:456-466.
7. Tabassam T, Sultan T, Akhtar ME, Mahmood-ul-Hassan M, Ali A. Suitability of different formulated carriers for sustaining microbial shelf life. Pak. J Agri. Res. 2015; 28(2):143-151.