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Shelf life of two indigenous isolates of fluorescent pseudmonads as bioagents in different carrier materials

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Shelf life of *Pseudomonas fluorescens* (PF-4) and *Pseudomonas putida* (RFP-13) was recorded in different carrier materials 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 both isolates 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 and RFP-13 was obtained in King's B broth but it showed a rapid decline. Refrigerator temperature supported better population of both Pf-4 and RFP-13 in the carrier materials. Talc was found to be the best carrier material that maintained better population of the bioagents till the end of storage period.

Key words : Fluorescent pseudomonads, *Pseudomonas fluorescens,Pseudomonas putida*, talc, vermicompost, farm yard manure, King's B broth

INTRODUCTION

Pathogens affecting plant health are a major and chronic threat to food production and ecosystem stability worldwide. As agricultural production intensified over the few decades,producers became more and more dependent on agrochemicals as a relatively reliable method of crop protection helping with economic stability of their operations. However, raising ecological problems viz., environmental pollution, development of pesticide resistant strains of plant pathogens and detrimental effects on beneficial organisms caused by excessive use of agrochemicals are the issues of great concern today. In such a scenario, biological control proves to be an effective and non-hazardous strategy to reduce crop damage caused by plant pathogens. In recent years the fluorescent pseudomonads have been extensively used for plant growth promotion and disease control. Several mechanisms have been suggested for disease control by fluorescent pseudomonads involving production of siderophores,hydrogen cyanide (HCN),ammonia, antibiotics, volatile compounds etc.or by competing with pathogens for nutrients or colonization space (Thomashow and Weller, 1996) and also can trigger a plant-mediated resistance mechanism called induced systemic resistance (Pieterse *et al.,* 2001).

Though biocontrol with fluorescent pseudomonads is an acceptable green approach, the availability of commercial bioformulations is very low. Indigenous isolates of fluorescent pseudomonads are well adapted to the particular climatic and edaphic factors of a region. Hence, development of formulations using indigenous isolates of fluorescent pseudomonads with increased shelf life and broad spectrum of action with consistent performance under field conditions could pave the way for commercialization of the technology at a faster rate. Carriers used for formulation of fluorescent *Pseudomonas* spp., must be easy for application, storage, commercialization and field use besides cost effectiveness and easy availability so that the formulation reaches the farmers field in a directly usable form.

Several studies have been made on shelf life of fluorescent pseudomonads using talc (Kloepper and Schroth, 1981; Caesar and Burr, 1991; Vidhyasekaran and Muthamilan, 1995; Amer and

Utkhede,2000, Bora *et al.*; 2004; Rangeshwaran *et al.*, 2010), farm yard manure (Vidhyasekaran and Muthamilan, 1995; Bora and Bora,2008), and vermicompost (Bora and Bora, 2008). In the present study, shelf lives of indigenous isolates of fluorescent pseudomonads of Raichur *viz* ., *Pseudomonas fluorescens* (Pf-4) and *Pseudomonas putida* (RFP-13) have been studied in different carrier materials. The purpose of the study is to generate information on the ability of the carrier materials to support the two bioagents of fluorescent pseudomonads so that farmers can use by storing them in different conditions of room and refrigerator temperature depending on their availability.

MATERIALS AND METHODS

Preparation of the formulations

Talc powder, vermicompost, farm yard manure (FYM) and King's B Broth used as substrates for mass multiplication of *P. fluorescens* and *P. putida,* The carrier materials were first tyndalised, and then air- dried and passed through 350 mesh sieves to obtain fine powders. Mass cultures of *P.fluorescens* and *P. putida* were obtained by adding sterile distilled water to 24 hrs old growth on King's B agar and 15 ml of such bacterial suspensions were aseptically added to two separate 1000 ml of King's B Broth and incubated at 32±2^{0}C for 24 hrs. 500 ml of such bacterial suspensions (10^7 cfu/ml) from King's B Broth cultures were added to 1 kg of carrier material separately. Then the inoculated carrier materials were mixed properly , air dried (except the King's B Broth) and sealed in polythene bags and later stored at two different temperatures *i.e.*,room temperature of 32±2^{0}C and refrigerator temperature of 4^{0}C. Formulation with King's B Broth was kept in conical flasks.

Determination of the population of P. fluorescens and P. putida in different carrier materials

The viable population of *P. fiuorescens* and *P. putida* in the carrier material following different periods of storage was determined at monthly interval by serial dilution technique (Waksman, 1922) and was expressed in terms of cfu/ g (colony forming unit / gram) of carrier material. The enumeration was done at monthly for ten consecutive months.

RESULTS AND DISCUSSION

The mean population of *P. fluorescens* in talc, vermicompost and FYM increased significantly for first 60 days of storage and later showed a declining trend.The population of *P. fluorescens* was significantly high in King's B Broth (270.33 x 10^7 - 275.00 x 10^7 cfu ml^{-1}) but was for only a short period *i.e.,* 30 days after which it declined very rapidly and lasted for 120 days and 150 days at room and refrigerator temperatures respectively. The highest mean population at both room and refrigerator temperatures over the entire period of storage was found in talc (11.00 x 10^7 - 13.00 x 10^7 cfu g^{-1}) followed by vermicompost (5.33 x 10^7 - 6.67 x 10^7 cfu g^{-1}). Though FYM recorded a good mean population count (146.00 x 10^7 cfu g^{-1}) till 60 days but it also showed a rapid decline thereafter (Table1).

The mean population of *P. putida* in talc, vermicompost, FYM increased significantly for first 60 days of storage and later showed a declining trend. King's B Broth maintained the highest mean population of *P. putida* at 30 days of storage (263.00 x 10^7 - 266.00 x 10^7 cfu ml^{-1}) but later there was a rapid decline and recovery of colonies only up to 150 and 180 days at room and refrigerator temperatures respectively. The highest mean population at both room and refrigerator temperatures was found over the entire period of storage of 300 days in talc (9.33 x 10^7 - 11.00 x 10^7 cfu g^{-1}) followed by vermicompost (6.00 x 10^7 - 9.00 x 10^7 cfu g^{-1}). FYM recorded a high mean population count (148.00 x 10^7 cfu g^{-1}) till 60 days but it also showed a rapid decline therefter. The refrigerator temperator temperature (4^{0}C) helped in better maintenance of population of both *P. fluorescens* and *P.putida* in all the carrier materials (Table 2).

The results indicated that for both *P.fluorescens* and *P. putida,*talc was the best since it showed uniform trend in maintaining the populations over the entire period of storage as compared to other substrates with 12 x 10^7 cfu g^{-1} and 13 x 10^7 cfu g^{-1} respectively.

However, in the present investigation, King's B Broth recorded the highest mean populations of *P. fluorescens* and *P. putida* with 272.67x10^7 cfu ml^{-1} and 264.50 x 10^7 cfu ml^{-1} respectively at 30th day of storage but was for only a short period and there-

Table 1 : Effect of different carrier materials on the shelf life of *P. fluorescens* (Pf-4) [1X10⁷ cfu/g].

Storage Days	Tale			Vermicompost			Farmyard manure (FYM)			King's B Broth		
	Room T (32+2°C)	Refri T (4°C)	Mean	Room T (32+2°C)	Refri T (4°C)	Mean	Room T (32+2°C)	Refri T (4°C)	Mean	Room T (32+2°C)	Refri T (4°C)	Mean
30	110.67	115.33	113.00	111.67	115.67	113.67	132.67	142.00	137.33	270.33	275.00	272.67
60	127.33	132.33	129.83	130.33	136.67	133.50	140.00	152.00	146.00	91.00	94.00	92.50
90	94.67	100.33	97.50	102.33	112.33	107.33	79.00	82.67	80.84	39.33	51.00	45.17
120	84.00	92.00	88.00	68.33	71.67	70.00	52.67	61.67	57.17	4.33	16.33	10.33
150	50.33	54.33	52.33	58.67	60.67	59.67	42.33	43.67	43.00	0.00	4.67	2.33
180	33.67	38.33	36.00	23.67	25.33	24.50	27.67	28.67	28.17	0.00	0.00	0.00
210	21.67	25.00	23.33	16.00	21.67	18.83	18.67	21.33	20.00	0.00	0.00	0.00
240	16.00	19.00	17.50	12.33	14.67	13.50	9.00	11.33	10.17	0.00	0.00	0.00
270	13.00	18.67	15.84	8.00	10.33	9.17	7.00	9.00	8.00	0.00	0.00	0.00
300	11.00	13.00	12.00	5.33	6.67	6.00	3.00	5.00	4.00	0.00	0.00	0.00
Mean	56.23	60.83	58.53	53.67	57.57	55.62	51.20	55.73	53.47	40.50	44.10	42.30

Room T :Room temperature (32±2°C); Refri T : Refrigerator temperature (4°C)

Comparing of means	S.Em±	CD at 1%
Carrier materials (A)	0.39	1.44
Temperature (B)	0.28	1.02
Storage Days (C)	0.62	2.28
A x B	0.55	2.04
A x C	1.24	4.56
B x C	0.87	3.23
A x B x C	1.75	6.45

Table 2 : Effect of different carrier materials on the shelf life of *P. putida* (RFP-13) [1X10⁷ cfu/g].

Storage Days	Tale			Vermicompost			Farmyard manure (FYM)			King's B Broth		
	Room T (32+2°C)	Refri T (4°C)	Mean	Room T (32+2°C)	Refri T (4°C)	Mean	Room T (32+2°C)	Refri T (4°C)	Mean	Room T (32+2°C)	Refri T (4°C)	Mean
30	118.33	122.00	120.17	117.33	122.33	119.83	135.33	139.33	137.33	263.00	266.00	264.50
60	130.33	136.00	133.17	127.67	132.00	129.83	146.00	150.00	148.00	100.33	105.33	102.83
90	96.33	101.00	98.67	90.00	93.33	91.67	66.67	70.00	77.34	48.33	51.00	49.67
120	85.33	91.67	88.50	66.33	69.00	67.67	56.00	58.33	57.17	11.33	12.67	12.00
150	54.00	57.33	55.67	43.00	47.33	45.17	44.00	46.33	45.17	3.00	5.00	4.00
180	36.33	38.67	37.50	32.67	35.00	33.84	22.00	24.00	33.84	0.00	2.33	1.17
210	24.33	27.00	25.67	20.33	23.67	22.00	16.00	19.00	22.67	0.00	0.00	0.00
240	18.00	21.33	19.67	14.00	15.67	14.84	10.33	12.67	18.00	0.00	0.00	0.00
270	12.00	16.33	14.17	10.00	12.00	11.00	8.00	10.33	9.17	0.00	0.00	0.00
300	9.33	11.00	10.17	6.00	9.00	7.50	3.00	5.00	7.50	0.00	0.00	0.00
Mean	58.43	62.23	60.33	52.73	55.93	54.33	46.23	49.00	55.62	42.60	44.23	43.42

Room T :Room temperature (32±2°C); Refri T : Refrigerator temperature (4°C)

Comparing of means	S.Em±	CD at 1%
Carrier materials (A)	0.37	1.35
Temperature (B)	0.26	0.95
Storage Days (C)	0.52	1.90
A x B	0.58	2.13
A x C	1.16	4.28
B x C	0.82	3.01
A x B x C	1.63	6.02

after it declined very rapidly but they lasted for 120 and 150 days at room and refrigerator temperature.The present results are similar to those obtained by Devika Rani *et al.* (2006) who reported highest population of *Pseudomonas fluorescens* in liquid broth and also concluded that the formulations can be stored at room temperature up to 60 days without losing the viability and strength of bioagent.

Though King's B Broth was found to support a very high population and growth at a very faster rate, the decline in the population was also very fast comparatively which might be due to the exhaustion of nutrients and accumulation of larger amounts of antibiotic compounds in liquid media produced by the isolate itself leading to the death of cells. Madigan and Martinko (2006) reported that bacteria proliferating in normal culture media will reach an abrupt stationary phase upon exhaustion of nutrients. Cell proliferation is halted completely and some cell death occurs which supports our result.

Vermicompost and FYM could encourage better population of both *P. fluorescens* and *P. putida* initially because of their physical properties *viz*., organic matter and moisture capacity but could maintain till the end of storage period of 300 days. In addition, their quality parameters differ with locations as per the availability of raw materials, their composition like organic carbon, method of preparation and their storage conditions. These reasons influence their limited use as carrier materials on commercial basis for marketability.

Talc is a natural mineral, chemically referred to as magnesium silicate [$Mg_3Si_4O_{10}(OH)_2$] available in powder form. It has a high surface area, very low moisture equilibrium, relative hydro-phobicity, chemical inertness, reduced moisture absorption and prevents the formation of hydrate bridges that enable longer storage periods of the bioagent. Owing to inert nature and easy availability as raw material from soapstone industries, it is used as a carrier for formulation (Nakkeeran *et al.*,2004). Also, use of talc is much easier, and the quality parameters too do not vary to greater extent across different locations. In addition, it is possible to maintain the same population for a definite period which makes the product easily marketable due to better reproducibility.

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