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A COMPARITIVE STUDY OF DIFFERENT CARRIERS FOR SHELFLIFE OF *PSEUDOMONAS FLUORESCENS*

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ABSTRACT
Studies were carried out to evaluate different carriers for shelf life of *Pseudomonas fluorescens* stored at 25 ± 2^0C 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 Flyash up to 60 days storage and there was slow decline in number of viable propagules after 60 days of storage.Spent mushroom substrate (78×10^8cfu/g) and fly ash (53.67×10^8cfu/g) maintained viable population count at 90 days of storage.This population was very close to the population recorded in carrier's *viz*., Talc (86.33×10^8cfu /g), Lignite (80×10^8cfu /g) which were usually used in biofertilizer industry. Hence for short storage period, option of SMS and Fly ash can be used for developing commercial formulations of *P. fluorescens*. Talc was found to be the best carrier material that maintained better population of the *P. fluorescens* (18×10^8cfu/g) till the end of storage period.

INTRODUCTION

P. fluorescens encompasses a group of common, Gram negative, rod shaped, nonpathogenic saprophytes that colonize soil, water and plant surface environments. Since they are well adapted in soil, *P. fluorescens* strains are being investigated extensively for use in biocontrol of pathogens in agriculture (Ganeshan and Kumar, 2006). It is known to enhance plant growth and yield and reduce severity of many diseases (Wei *et al.,* 1996) through production of secondary metabolites (Koche *et al*., 2013).In recent years, there has been much success in obtaining effective control of plant pathogens using beneficial biocontrol agents such as strains of *Pseudomonas* species have been used extensively for plant growth promotion and disease control because of their utilization of a large number of organic substrates commonly found in root exudates (Asha *et al*., 2011). But it cannot be used as cell suspension under field conditions as it is done in green house or in research plots with a limited area. Hence, the cell suspensions of *P. fluorescens* should be immobilized in certain carriers and prepared as formulations for easy application, storage, commercialization and field use. Also the carrier should be such that it should support considerable bacterial population for a longer period of time and cost effective.

Presently, talc and lignite powder is being used as carrier material by most of the bioinoculant producing units in India. Often it has also been found that their availability is also made difficult, sometimes not much cost effective. Availability of quality talc and lignite powder is also in doubt because of adulteration by agents and improper mesh size in the pulverizing unit. Several scientists have suggested compost as carrier material for biofertilizers. But the role of good compost in maintaining microbial population has not been studied much.

The existing studies exhibit that the mushroom compost, which is totally waste product and can make hazard if remain as such in court yard. Spent mushroom substrate (SMS) has good physical properties; it includes the water holding capacity, soil pH, soil porosity, salt content i.e electrical conductivity. Addition of SMS will add great amount of macro nutrients (Kim *et al.,* 2011).The biological properties of SMS enhance its marketability as a soil conditioner (Brady and Wiel, 2004).Spent compost is believed to be a source of humus formation and humus is provided to the plants with micronutrients improve the soil aeration, soil water holding capacity and contributes the maintenance of soil structure (Kediri and Mustapha, 2010).By considering all good properties of SMS, it has been tested in the present study to use it as carrier for shelf life of *P. fluorescens* and compared with other carriers like talc, lignite, charcoal, farm yard manure and fly ash.

MATERIALS AND METHODS

Collection and analysis of carrier materials

The carrier materials like talc,lignite, charcoal,farm yard manure (FYM), flyash and spent mushroom substrate (SMS) were collected from different sources and were used in the present study.All the carrier materials were dried in shade, powdered through 60 micron sieve. These materials were

analysed for their physical and chemical characters *viz*., water holding capacity (Emmanuel *et al.,* 2010), bulk density (Emmanuel *et al.,* 2010), pH (Piper, 1966),organic carbon content (Walkley and Black, 1934) and electrical conductivity (Piper**,** 1966).

Morphological studies of *P. fluorescens*

Pure culture of *Pseudomonas fluorescens* isolate was streaked on King's B medium petriplate separately for colony development. The individual colonies were examined for colony colour, and pigmentation. (Buchanon and Gibbson,1974).

Biochemical studies of *P. fluorescens*

Biochemical tests viz., gelatin liquefaction, citrate utilization, KOH test, catalase activity, siderophore production, and Gram's reaction were carried out for biochemical characterization of *P. fluorescens*.The isolate of *P. fluoresens* were also evaluated for plant growth promoting properties *viz*., IAA production. etc. (Aneja, 2003)

Preparation of carrier based formulations of *Pseudomonas fluorescens*

This formulations were developed as described by Vidhyasekaran and Muthamilan, (1995) by using a mixture of 10g of carboxy methyl cellulose and 1kg of carrier. The carriers were autoclaved for 30 min on each of two consecutive days. *fluorescens* strain was grown on liquid Kings B (King *et al.,* 1954) for 48 h as a shake culture in rotary shaker at 150 rpm. at room temperature ($25 \pm 2°C$) and 400 ml of the bacterial suspension, containing 9×10^8 colony forming units (CFU) per ml was added to 1kg of the talc material and mixed well under sterile conditions. Then packed in polythene bags, sealed and stored at room temperature (25 ± 2ÚC) and with a moisture content of 35%, In each carrier, the population of bacteria was estimated at monthly interval for 6 month by using serial dilution technique.

Counting colonies of *P.fluorescens*

The number of colonies were counted on a Qubec colony counter after the incubation peroid of 48 hrs as colony forming units (cfu) per ml and expressed as cfu per gram of carrier material. The plate count was carried out in triplicates and final value of *cfu* was the average of three readings (Aneja, 2003)

$$\text{Cfu/g of carrier} = \frac{\text{Number of colonies}}{\text{Amount plated} \times \text{dilution}}$$

RESULTS AND DISCUSSION

Morphological and biochemical characterization of *Pseudomonas fluorescens*

P.fluorescens strain was confirmed by performing morphological and biochemical tests. The results are presented in Table 1. Based on the results, *P. fluorescens* was rod shaped, gram-negative and also produced yellow color colonies on Kings B medium and showed green fluorescent color under UV light.It also showed positive for catalase activity, citrate utilization, gelatin liquefication, siderophore and IAA production tests.(Table 1).

According to Todar (2004), more than half of the *Pseudomonas* bacteria produce pyocyanin which is a blue-green pigment, while the nonpathogenic saprophyte *P. fluorescens* produces fluorescent pigment that is soluble and greenish. In this study, all the seven identified gram-negative *Pseudomonas* were found to be green fluorescent on King's B medium under ultraviolet light at 365 nm. The biochemical tests i.e gelatin liquefaction, catalase test, oxidase test, IAA production,siderophore production and hydrogen cyanide production further confirmed the isolates to be *P. fluorescens* as reported by earlier workers (Tiwary *et al.,* 2007; Nathan *et al.,* 2011).

Thus results are also in line with the findings of Ahemad and Khan (2012) who reported that *P. putida* strain PS9 exhibited the plant growth promoting traits like phosphate solubulization, production of siderophor phytoharmone and exo-polysacchrides in substantial amount.

Physico-chemical properties of carrier materials

The physico-chemical properties revealed that the pH values of carrier materials were in the range of 6.9-7.3 and maximum organic carbon content was recorded with farm yard manure (18.7%) followed by lignite, charcoal, spent mushroom substrate and fly ash (7.90%, 5.30%, 0.43%, and 0.36%). Maximum water holding capacity was recorded in talc (189%) followed by spent mushroom substrate, farmyard manure, lignite, fly ash and charcoal (93%, 85%, 65%,63% and 52.6%). (Table 2).

Low bulk density values were recorded in talc (0.3 g/cm^3) followed by spent mushroom substrate, fly ash, FYM, charcoal and lignite (0.35, 0.47,0.65, 0.75 and 0.82 g/cm^3).Low electrical conductivity value was recorded in spent mushroom substrate (0.28 ds/m) followed by FYM, charcoal, Lignite, fly ash and talc (0.30, 0.49, 0.50, 0.65 and 0.67 ds/m).

The physico-chemical characters of carrier materials have got profound influence on the survival of inoculants. The ideal characteristics of an inoculant carrier include more surface area, rich in organic matter, high water holding capacity, neutral pH, easy availability and inexpensiveness.Physico chemical properties of carriers i.e pH of spent mushroom substrate was 6.8 (Polat *et al.*, 2009), organic matter between 40-60% on dry weight basis (Liu *et al.,* 2006). Nigussie and Kissi (2011) recorded the bulk density of charcoal was 1.12 and water holding capacity was 52.77%. The bulk density of fly ash was

Table 1: Morphological and Biochemical characterization of *Pseudomonas fluorescens*

Tests	Character	*P.fluorescens*
Morphological tests	Shape	Rod
	Gram reaction	Negative
	Pigmentation	Yellow
	Fluorescent reaction	Green
Biochemical tests	Citrate utilization	Positive
	Catalase activity	Positive
	Gelatine liquefaction	Positive
Growth promoting	IAA production	Positive
characterization	Siderophore production	Positive

Table 2: Physico-chemical properties of different carrier materials

Carriers	pH	Organic carbon content(%)	Water holding capacity(%)	Bulk density (gcm^{-3})	Electrical conductivity (ds/m)
Talc	7.2	-	189	0.30	0.67
Spent mushroom substrate	7.0	0.43	93	0.35	0.28
Lignite	7.0	7.90	65	0.82	0.50
Charcoal	6.9	5.30	52.6	0.75	0.49
Farm yard manure	7.1	18.7	85	0.65	0.30
Fly ash	6.9	0.36	63	1.2	0.65

Table 3: Viability of *Pseudomonas fluorescens* in different carriers

Treatments	Population of *Pseudomonas fluorescens* ($\times 10^8$cfu/g) of a carrier						
	0 day	30 days	60 days	90 days	120 days	150 days	180 days
Talc	86.00	97.33	115.67	86.33	52.00	31.00	18.00
SMS	83.33	86.33	97.00	78.00	48.33	25.33	12.33
Lignite	84.67	87.67	99.33	80.00	49.33	26.67	14.67
Charcoal	84.00	85.67	95.00	64.00	55.67	23.00	11.00
FYM	84.33	90.33	108.33	81.67	53.00	28.00	15.67
Fly ash	83.00	84.00	88.67	53.67	43.67	21.33	8.00
SEm ±		0.60	0.59	0.62	1.13	1.03	0.45
CD (p = 0.01)		2.59	2.53	2.68	4.90	4.49	1.93

less than 1 g/cm^3 (Kumar *et. al*., 2010).These findings are confirmative with present study.

Survival of *Pseudomonas fluorescens in* different carrier materials at room temperature

The survival of *P. fluorescens* in different carrier materials *viz.,* talc, Spent Mushroom Substrate, lignite,charcoal,farm yard manure and fly ash were estimated under controlled conditions over a period of six months of storage period at room temperature by serial dilution technique on Kings B plate. The results are presented in Table 3.

Initially (at 0 days) all carriers revealed non-significant differences in colony forming units (cfu). The population of *P. fluorescens* was increased up to 60 days of storage in all carrier materials on storage and there was slow decline in number of viable propagule after 60 days of storage. Shelf life studies revealed that among six carrier materials Talc, FYM, Lignite supported maximum mean population of *P.fluorescens* till 60 days of storage and recorded 25.65%, 23.38% and 14.75% addition of bacterial propogules compared to initial population.

Among the different carriers tested, the talc powder supported the maximum population of 18.00×10^8cfu/g at 180 days of storage. It was significantly superior over all treatments.

Talc was followed by FYM of 15.67×10^8cfu/g, Lignite of 14.67×10^8cfu/g ,SMS of 12.33×10^8cfu/g, Charcoal of 11.00×10^8cfu /g and fly ash of 9.00×10^8cfu /g (Table.3)The results clearly indicate that the formulation can be stored for 180 days. Minimum number of cfu was recorded in fly ash at 180 days of storage as compared to other carrier materials. But the population of *P. fluorescens* was well maintained upto 60 days (88.67×10^8cfu /g). However the population decreased drastically at 90 days of storage. As well as SMS was also found to maintain good population of *P.fluorescens* at 60 days (97.00×10^8cfu/g). Kumar *et al*. (2010) reported same results that maximum population of *Azotobacter chrooococcum* in fly ash based formulations. Fly ash formulation can be used upto 60 days as it will be the cheaper carrier source for shelflife of *P. fluorescens*.

The results also indicate that spent mushroom substrate maintained viable population count (78×10^8cfu/g) at 90 days of storage. When it compared with lignite and farm yard manure there was little difference in population level (80×10^8cfu /g ,81×10^8cfu/g).In addition to this SMS had good physical factors includes water holding capacity and electrical conductivity (Kim *et al.,* 2011) and also cheaper than the available carriers for commercial production of *P. fluorescens*

The results of this study are in conformity with the findings of Suryadi *et al*. (2013) who reported that the cell viability of *P. aeruginosa* was decreased ranged from 2.39 to 18.30% among different bioformulations and found talc-A8 based formulation was stable at period of storage showing no viability lost. Rajalaxmi *et al*. (2012) reported talc based formulations of *P. fluorescens* retains mean population of 11×10^7cfu/g at 300 days of storage. Shivkumar *et al*. (2000) studied three carriers for the survival of *P. fluorescens* and reported that talc maintained the highest population level of 18.3×10^7 cfu/g after fourty days of storage and Similar findings were also recorded by Mythukumar (2009), Prathuangwong *et al*. (2013), Chenna *et al*. (2013) in respect of *Pseudomonas fluorescens*. In talc based formulations were found effective for its survival.This is the first time that we tested SMS as a carrier for *P. fluorescens* and found suitable to maintain good population of it for short storage period.

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