**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, colonize soil, water and plant surface environments. Since negative, rod shaped, nonpathogenic saprophytes that *P. fluorescens* encompases a group of common, Gram

**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 Gibson, 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. fluorescens* 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 ± 2°C) and 400 ml of the bacterial suspension, containing 9 × 10⁹ 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 ± 2°C) 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 period 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 P59 exhibited the plant growth promoting traits like phosphate solubulization, production of siderophor phytohormone and exo-polysaccharides 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 t alc (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 t alc (0.3 g/cm³) followed by spent mushroom substrate, fly ash, FYM, charcoal and lignite (0.35, 0.47, 0.65, 0.75 and 0.82 g/cm³). Low electrical conductivity value was recorded in spent mushroom substrate (0.28 ds/m) followed by FYM, charcoal, Lignite, fly ash and t alc (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>
<thead>
<tr>
<th>Tests</th>
<th>Character</th>
<th><em>P. fluorescens</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Morphological tests</td>
<td>Shape</td>
<td>Rod</td>
</tr>
<tr>
<td></td>
<td>Gram reaction</td>
<td>Negative</td>
</tr>
<tr>
<td></td>
<td>Pigmentation</td>
<td>Yellow</td>
</tr>
<tr>
<td></td>
<td>Fluorescent reaction</td>
<td>Green</td>
</tr>
<tr>
<td>Biochemical tests</td>
<td>Citrate utilization</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td>Catalase activity</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td>Gelatine liquefaction</td>
<td>Positive</td>
</tr>
<tr>
<td>Growth promoting characterisation</td>
<td>IAA production</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td>Siderophore production</td>
<td>Positive</td>
</tr>
</tbody>
</table>

**Table 1:** Morphological and Biochemical characterization of *Pseudomonas fluorescens*
Talc was followed by FYM of 15.67×10⁸ cfu/g of a carrier material at 0 days. It was significantly superior over all treatments. The results clearly indicate that the formulation can be stored for 60 days of storage and recorded 25.65%, 23.38% and 14.75% decrease ranged from 2.39 to 18.30% among different carrier materials at room temperature.

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 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, 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.

Talc was followed by FYM of 15.67×10⁸ cfu/g, Lignite of 14.67×10⁸ cfu/g, Charcoal of 11.00×10⁸ cfu/g and fly ash of 9.00×10⁸ cfu/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 (88.67×10⁸ cfu /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 (97.00×10⁸ cfu/g). Kumar et al. (2010) reported same results that maximum population of Azotobacter chroococcum 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 (78×10³ cfu/g) at 90 days of storage. When it compared with lignite and farm yard manure there was little difference in population level (80×10³ cfu/g,81×10³ cfu/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 11×10⁸ cfu/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 18.3×10⁸ cfu/g after forty 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.

### Table 2: Physico-chemical properties of different carrier materials

<table>
<thead>
<tr>
<th>Carriers</th>
<th>pH</th>
<th>Organic carbon content(%)</th>
<th>Water holding capacity(%)</th>
<th>Bulk density (g/cm³)</th>
<th>Electrical conductivity (ds/m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Talc</td>
<td>7.2</td>
<td>-</td>
<td>189</td>
<td>0.30</td>
<td>0.67</td>
</tr>
<tr>
<td>Spent mushroom substrate</td>
<td>7.0</td>
<td>0.43</td>
<td>93</td>
<td>0.35</td>
<td>0.28</td>
</tr>
<tr>
<td>Lignite</td>
<td>7.0</td>
<td>7.90</td>
<td>65</td>
<td>0.82</td>
<td>0.50</td>
</tr>
<tr>
<td>Charcoal</td>
<td>6.9</td>
<td>5.30</td>
<td>52.6</td>
<td>0.75</td>
<td>0.49</td>
</tr>
<tr>
<td>Farm yard manure</td>
<td>7.1</td>
<td>18.7</td>
<td>85</td>
<td>0.65</td>
<td>0.30</td>
</tr>
<tr>
<td>Fly ash</td>
<td>6.9</td>
<td>0.36</td>
<td>63</td>
<td>1.2</td>
<td>0.65</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Population of Pseudomonas fluorescens (×10⁸ cfu/g) of a carrier</th>
<th>0 day</th>
<th>30 days</th>
<th>60 days</th>
<th>90 days</th>
<th>120 days</th>
<th>150 days</th>
<th>180 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Talc</td>
<td>86.00</td>
<td>97.33</td>
<td>115.67</td>
<td>86.33</td>
<td>52.00</td>
<td>31.00</td>
<td>18.00</td>
<td></td>
</tr>
<tr>
<td>SMS</td>
<td>83.33</td>
<td>86.33</td>
<td>97.00</td>
<td>78.00</td>
<td>48.33</td>
<td>25.33</td>
<td>12.33</td>
<td></td>
</tr>
<tr>
<td>Lignite</td>
<td>84.67</td>
<td>87.67</td>
<td>99.33</td>
<td>80.00</td>
<td>49.33</td>
<td>26.67</td>
<td>14.67</td>
<td></td>
</tr>
<tr>
<td>Charcoal</td>
<td>84.00</td>
<td>85.67</td>
<td>95.00</td>
<td>64.00</td>
<td>55.67</td>
<td>23.00</td>
<td>11.00</td>
<td></td>
</tr>
<tr>
<td>FYM</td>
<td>84.33</td>
<td>90.33</td>
<td>108.33</td>
<td>81.67</td>
<td>53.00</td>
<td>28.00</td>
<td>15.67</td>
<td></td>
</tr>
<tr>
<td>Fly ash</td>
<td>83.00</td>
<td>84.00</td>
<td>88.67</td>
<td>53.67</td>
<td>43.67</td>
<td>21.33</td>
<td>8.00</td>
<td></td>
</tr>
<tr>
<td>SEM ±</td>
<td>0.60</td>
<td>0.59</td>
<td>0.98</td>
<td>0.62</td>
<td>1.13</td>
<td>1.03</td>
<td>0.45</td>
<td></td>
</tr>
<tr>
<td>CD (p = 0.01)</td>
<td>2.59</td>
<td>2.53</td>
<td>0.85</td>
<td>2.68</td>
<td>4.90</td>
<td>4.49</td>
<td>1.93</td>
<td></td>
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</table>

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