CHAPTER 6.

EFFECTIVENESS OF FEATHER COMPOST AS GROWTH MEDIUM AND CARRIER MATERIAL FOR BIOFERTILIZERS
Effectiveness of feather compost as growth medium and carrier material for Biofertilizers

Chapter 6

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Highlights

- Feather compost as nutrient for growth and carrier material of biofertilizers like *Rhizobium* sp, *Bacillus megaterium* and *Azotobacter chroococcum*.
- Feather compost as suitable alternative carrier for biofertilizer study.

Abstract

The main objective of this study was to find the efficiency of feather compost as nutrient for growth and carrier material for biofertilizers like *Rhizobium* sp, *Bacillus megaterium* and *Azotobacter chroococcum*. Biofertilizer growth medium was supplemented with feather compost extract and the growth of the bacteria was analyzed by turbidity and viable count methods. The 20% feather compost was the suitable carrier for stability of biofertilizer and improves their longevity. Compare with nodulation and phosphate solubilized efficiency the strain retain its original activity up to the study period. Total viable count of the biofertilizers on the 20% feather compost extract medium were high in *Rhizobium* sp. was about $6.45 \pm 1.19 \times 10^9$ cfu/ml, *Bacillus megaterium* was about $43.33 \pm 3.78 \times 10^8$ cfu/ml and *Azotobacter chroococcum* $7.94 \pm 0.53 \times 10^9$ cfu/ml.
6.1 Introduction

Biofertilizers are commonly called microbial inoculants which are capable of mobilizing important nutritional elements in the soil from non-usable to usable form through biological processes (Chandrasekar, et al., 2005; Selvakumar, 2009). In general, biofertilizers are environment friendly, and play an important role in enhancing crop productivity through nitrogen fixation, phosphate solubilization, plant hormone production, ammonia excretion, siderophore formation and to control various plant diseases (Pankhurst and Lynch, 1995; Pathak et al., 1997; Dadarwal et al., 1997 and Hedge et al., 1999).

The first step in the large scale production of microbial inoculants as biofertilizers required suitable growth medium. Industrially biofertilizers has been produced by fermentations process. The economy of such a process is largely governed by the price of media utilized (Ashok Kumar et al., 2013). In general, microbial growth medium contains expensive components includes mannitol, sucrose or glycerol as the carbon source, yeast extract as a source of nitrogen, growth factors, and mineral salts. For industrial production, it is important to identify inexpensive and easily available sources of nutrients for culture medium. For a laboratory-scale production of biofertilizers like Rhizobium sp., Azotobacter sp. and Phosphobacter sp., the Yeast Mannitol Broth (YMB medium) (Verma et al., 2010). However, its industrial use is limited due to high cost (Gulati, 1979). In view of the growing demand of microbial inoculants it is important to search cheap and readily available substances against these expensive ingredients (Ashok Kumar et al., 2013). Many investigators have used a variety of agricultural and industrial byproducts such as proteolyzed pea husks (Chanda et al., 1987), bagasse (Malik et al., 2001), waste water sludge (Ben Rebah et al., 2007), and sugar waste (Singh et al., 2011) as nutrients for biofertilizer growth.

In addition to that, the production and quality of microbial inoculants in many developing countries is limited by the availability of suitable carriers or technological limitations (Hashem et al., 2010). A carrier must display high water holding capacity, display chemical and physical uniformity, non-toxic to microbial inoculants and must be
environmentally safe. Agricultural by products has been recognized as suitable carrier for biofertilizers. Commonly, fine ground peat (Burton, 1967; 1981), wheat straw (Schiel and Dieguez, 1970), coal and a coal-bentonite mixture (Crawford and Berryhill, 1983), cellulose, filter mud, compost, manures (Muniruzzaman and Khan, 1992), mineral soils, sawdust, sugarcane, vermiculite (Graham-weis et al., 1987) and perlite (Daza et al., 2000) have been used. Little study is available for production of feather compost and their effect on biofertilizer research. Therefore, the present work aimed to evaluate the use of feather bacterial compost as alternative growth medium and carriers for microbial inoculants like Rhizobium sp., Azotobacter chroococcum and Bacillus megaterium.

6.2 Materials and Methods

6.2.1 Microorganisms used

In this study, three different bacterial biofertilizers such as symbiotic nodule forming Rhizobium sp., phosphate solublizing Bacillus megaterium and non-symbiotic nitrogen fixing Azotobacter chroococcum were used. Biofertilizer bacteria were isolated from commercially available biofertilizer packets (Manidharma Biotech Private Limited, Chennai, Tamil Nadu, India), and were further confirmed by morphological and biochemical methods described by Bergey’s Manual of Determinative Bacteriology (9th eds.). Pure culture of the biofertilizer bacteria were maintained on respective agar slop method.

6.2.2 Cultivation and maintainance of biofertilizers

Rhizobium medium (Himedia, M408) was used for the cultivation of Rhizobium species, Azotobacter Agar (Himedia, M372) was used for the cultivation of Azotobacter chroococcum and Pikovskayas Agar (Himedia, M520) was used for isolation and detection of phosphate-solubilizing bacteria, The respective media composition was given below.

Pure isolate of the biofertilizers were grown in their respective liquid medium. 50 ml of the liquid medium was prepared in 100 ml Erlenmeyer flask, after sterilization, biofertilizer bacteria was inoculated in to the medium and incubated at 32°C for 48h, 72h and 96h for Rhizobium species, Bacillus megaterium and Azotobacter chroococcum
respectively. Mass cultures of the biofertilizers were prepared in the same media at the volume of 1.0 lit in 3.0 lit flasks. (Annexure- I).

6.2.3 Growth analysis of biofertilizers in compost extract medium

6.2.3.1 Feather compost extracts preparation

Feather compost was prepared by the standard protocol described in the chapter-5 (section -5.2.2.1). After 40 days of decomposition by using the bacterial isolate \( B. subtilis \) FDS15, the compost was sheaved by using 200μm sized metal sheave to remove the undigested feather waste. Then the feather compost was dried in hot air oven at 45°C for 12 hours. After drying, 50g of feather compost was taken into a 1000 ml Erlenmeyer flask and add 500ml of distilled water. The feather compost aqueous solution was mixed thoroughly for the period of 30 minutes, residues of the compost was filtered by Whatman No1 filter paper and it was used as media supplement.

6.2.3.2 Feather compost extracts media and growth of biofertilizers

Biofertilizer growth medium was prepared with their respective standard growth media replaced with the feather compost extract as described below. For \( Rhizobium \) sp., \( Azotobacter chroococcum \) and \( Bacillus megaterium \) growth, Rhizobium broth (YEMB), Azotobacter broth and Pikovskayas broth were used respectively. (Annexure-I)

a) \( Rhizobium \) sp. growth  (Figure-6.1)
1. Control (YEMB – Yeast Extract Manitol salt Broth)
2. 20% compost extract + 80% YEMB
3. 40% compost extract + 60% YEMB
4. 60% compost extract + 40%YEMB
5. 80% compost extract + 20%YEMB
6. 100% compost extract (alone)

b) Phosphate solubilizer (\( Bacillus megaterium \)) growth (Figure6.3)
1. Control (Pikovskayas broth)
2. 20% compost extract + 80% Pikovskayas broth
3. 40% compost extract + 60% Pikovskayas broth
4. 60% compost extract +40 Pikovskayas broth
5. 80% compost extract +20% Pikovskayas broth
6. 100% compost extract (alone)

c) *Azotobacter chroococcum* growth (Figure-6.5)

1. Control (Azotobacter broth)
2. 20% compost extract + 80% Azotobacter broth
3. 40% compost extract + 60% Azotobacter broth
4. 60% compost extract +40% Azotobacter broth
5. 80% compost extract + 20% Azotobacter broth
6. 100% compost extract (alone)

After the addition of 0.1% (v/v) of bacterial inoculation (0.6 OD value at 595nm) was given to the respective culture flasks, and were incubated at 32°C for the period of 96 h. Bacterial growth was measured after 96h by turbidometric method at 595nm using UV-Vis spectrophotometer (SL159, Elico) and standard plate count method (viable count). All experiments were repeated for three times, and the mean value was accounted for the analysis.

**6.2.3.3 Standard plate count method**

After the growth of biofertilizer in the feather compost extract medium, the viable count of the bacteria was analyzed by serial dilution method. 1.0 ml of the culture was serially diluted upto $10^{-11}$ in 9.0 ml sterile saline blanks. Then, 0.1ml of the diluted bacterial suspension was added to a sterile solid biofertilizer growth medium (spread plate method) in Petri plates and it was incubated at 30°C for 48 h for both *Rhizobium* sp. and *Bacillus megaterium*, and 96 h for *Azotobacter chroococcum*.

**6.2.3.4 Growth cure of biofertilizers in carrier extract medium**

After finding the ideal concentration of compost extract for biofertilizer growth medium, growth pattern of the biofertilizers were analyzed by standard growth curve experiments (viable count method).
For the growth curve analysis, standard plate count method was used, 0.1% of log phase bacterial suspension was given as inoculum for 100 ml fresh biofertilizer growth medium and the compost extract medium. The culture flasks were kept dark and incubated at 30°C for 48 hrs for \textit{Rhizobium} sp., 60 hrs for \textit{B. megaterium} and 90hrs \textit{A. chroococcum}. Every 3 hours interval sample was (0.5 ml) recovered from the respective culture flask and they were serially diluted up to \(10^{-11}\) dilutions. 0.1 ml of the diluted bacterial culture was spread on their respective solid growth medium, and incubated at 30°C for 48 hrs for \textit{Rhizobium}, 72 hrs for \textit{B. megaterium} and 96 hrs for \textit{A. chroococcum}. Total number of viable colonies on the respective media was counted with colony counter and plot the growth curve.

### 6.2.4 Carrier stability of Biofertilizers

#### 6.2.4.1 Feather compost carrier preparation

Chicken feather was collected, washed with tap water and detergent. After making them fat content of the feather was removed by washing with chloroform and then the solvent was evaporated, washed three to four times with distilled water and air-dried. The cow dung was collected from local area and sun dried. Then combination of feather and cow dung was taken in the ratio of 1:4 for studies. Then, it was mixed uniformly in a plastic bin. The preparation was then inoculated with 10% \textit{Bacillus subtilis} FDS15 culture (\(10^8\)cfu/ml) and separated control also maintained for evaluation. The plastic bin was covered with polythene bag use to avoid contamination and the moisture content was maintained with sterilized water at 35 to 40%. The feathers were kept 40 days at room temperature for degradation (Figure- 6.12). After 40 days, the feather compost was sieved with 0.5 mm mesh screen and dried in a hot air oven at 60°C for two days. Then the compost was treated with CaCO\(_3\) to adjust its pH to 7. The carriers were analyzed for the total N, organic carbon and organic matter (described in the chapter – 4). Maximum water holding capacity, pH, total soluble salts, total P and total K were determined according to the standard protocol described by Jackson (1973). The materials were autoclaved at 121°C at a pressure of 15 lbs for 20 minutes.
6.2.4.2 Preparation of lignite carrier (Control)

The lignite fly ash was collected from Thermal power station, Neyveli, was used as control carrier. Carrier material collected were sun dried, powdered and sieved through 500μ sieve (Figure- 6.12). The carrier was adjusted to pH. 7 and the physical and chemical properties were analyzed (previously described in chapter - 4). Then the carrier material was sterilized at 15lb psi for 20 minutes.

6.2.4.3 Starter culture preparation

*Rhizobium* sp.

A loopfull of *Rhizobium* sp. pure culture was transferred into 250ml Erlenmeyer flask containing 100ml of Rhizobium broth (Himedia) and incubated at 30°C on 130 rpm rotary shaker for 3 days.

*Bacillus megaterium* (Phosphobacter)

A loop full of *B. megaterium* pure culture was transferred into 250ml Erlenmeyer flask containing 100ml of Pikovskaya broth and incubated at 30°C on 120 rpm rotary shaker for 4 days.

*Azotobacter chroococcum*

A loop full of *A. chroococcum* pure culture was transferred into 250ml Erlenmeyer flask containing 100ml of Azotobacter broth and incubated at 30°C on 120 rpm rotary shaker for 4 days.

6.2.4.4 Mass culture production of *Rhizobium* sp.

A loop-full of *Rhizobium* sp. pure culture was transferred into 250ml Erlenmeyer flask containing 100 ml of Yeast extract mannitol broth supplemented with 20% feather compost extract and incubated at 30°C on 120 rpm rotary shaker for 72 hours. After incubation, 10ml of the inoculums was transferred to 1000ml of respective broth and kept in shaking incubator for mass multiplication. The concentration of viable cells in the growing culture reached to $10^8 - 10^9$ cells/ml was used as inoculants.
6.2.4.5 Mass culture production of *B. megaterium* and packaging

A loop-full of *B. megaterium* pure culture was transferred into 250ml Erlenmeyer flask containing 100ml of Pikovskaya's broth supplemented with 60% feather compost extract and incubated at 30°C on 120 rpm rotary shaker for 72 hours. After incubation, 10ml of the inoculums was transferred to 1000ml of respective broth and kept in shaking incubator for mass multiplication. The concentration of viable cells in the growing culture reached to $10^8$-$10^9$ cells/ml was used as inoculants.

6.2.4.6 Mass culture production of *A. chroococcum*

A loop-full *A. chroococcum* of pure culture was transferred into 250ml Erlenmeyer flask containing 100ml of Azotobacter broth supplemented with 20% feather compost extract and incubated at 30°C on 120 rpm rotary shaker for 72 hours. After incubation, 10ml of the inoculums was transferred to 1000ml of respective broth and kept in shaking incubator for mass multiplication. The concentration of viable cells in the growing culture reached to $10^8$-$10^9$ cells/ml was used as inoculants (Sudhakar et al., 2000).

6.2.4.7 Production of sterile carrier-based inoculant (packaging)

The production requires a completely sterile carrier in sterile package. The simplest way is to mix the sterile carrier with bacterial liquid culture. The carrier material was packed in 12cm x 15cm autoclavable polythene covers (100g) and sealed using an electric sealer. It was sterilized at 15lps for 20 minutes. Each pre-sterilized carrier bag is injected aseptically with culture by means of a syringe fitted with a sterile needle. The area of puncture must be disinfected with ethanol. Quantity of broth should be adequate to wet 40% of the carrier by carrier weight. If the carrier material contains nutrients available for the incorporating bacteria to grow, injection into the carrier package of starter culture of the bacterial cells together with sterile water for the sufficient moisture adjustment will be sufficient. The puncture hole is then immediately sealed with preprinted self-sticking label. The bags are then kneaded by hand or by shaker until the liquid inoculum has been uniformly absorbed in to the carrier. The final moisture of inoculant should be 45-50%. After the injection, the carrier package should be placed in temperature-controlled area for appropriate period (60 days). Every 15 days interval
viability and efficiency of carrier based biofertilizer was analyzed. All the experiments were performed in triplicates.

6.2.4.7 Viability of biofertilizers on carrier inoculation (Hardy et al. 1973)

The survival of Rhizobium sp., B.megaterium and A.chroococcum were determined after the inoculum was subjected to different carriers at room temperature. The ten grams of each sample was taken for estimating viable cells at the starting day (0 day), 15th, 30th, 45th and 60th day after carrier inoculation (storage) using dilution plating method on Yeast extract manitol agar, Pikovskaya’s agar and Azotobacter agar medium respectively. Serial dilution was prepared by transfer of 1g each of inoculum into 9 ml sterile water blanks to get 10⁻¹ dilution. Similarly the dilution were made serially upto 10⁻⁹ from 10⁻¹ dilution. One ml was pipette out into sterile glass Petri plates. The sterilized Yeast extract manitol agar, Pikovskaya's agar and Azotobacter agar medium for Rhizobium sp., B.megaterium and A.chroococcum was added. The plates were rotated clockwise and anticlockwise direction for uniform spread of the dilution mixture and the plates were incubated at 30ºC for 3, 3, 5 days respectively. The number of apparent Rhizobium sp., B.megaterium and A.chroococcum colonies after incubation were counted using Lapiz digital colony counter and calculated into viable cells. The plate count was carried out in triplicates and final value of cfu was the average of three readings (Aneja, 2003).

6.2.4.8 Analysis of physio chemical properties

a. Estimation of pH

(The pH were analysis in 0, 15, 30, 45, 60 days intervals)

Twenty gram of carrier inoculant of Rhizobium sp. B.megaterium and A.chroococcum from each treatment was weighed and 80ml of distilled water was added. The sample was then vortexed for 5 minutes to get uniform mixing of carrier with the distilled water. The vortexed sample was allowed to settle for 30 min and then pH of each treatment was determined using digital pH meter.
b. Moisture content
(The moisture content 0, 15, 30, 45, 60 days intervals)

The moisture content was also expressed based on wet weight, which gives the percentage of original wet weight sample containing water (Bressollier et al. 1999). Initial weight of the compost was measured and final weight of the compost after drying is also determined, thereby water loss can be calculated, which indicates the original moisture content of the compost. 10g of carrier-inoculant material from each treatment at different storage days was taken. Then the sample were oven dried at 70°C till constant weight was arrived and the moisture content of the inoculant carrier was expressed in percentage.

6.2.4.9 Physical and chemical properties of the carrier materials

The carriers were analyzed for organic carbon and organic matter total Nitrogen total Phosphorus and total Potassium were determined according to standard methods (Jackson, 1973) described in chapter -5.

6.2.5 Assessment of nodulation efficiency of Rhizobium sp.

Seed treatment method

The seeds of groundnut were surface sterilized with 80% ethanol, 0.1% mercuric chloride and washed the seeds with sterile distilled water for 3 to 4 times. The seeds were mixed with carrier based plant growth promoting Rhizobium sp, (with $1 \times 10^8$ CFU/ml$^{-1}$) and shade dried for 30 min. After shade drying, the seeds were sown in experimental pots with unsterilized soil. Six seeds were sown per pot and thinned to three plants/pot. After 10 weeks of plant growth, plant roots were harvested to assess nodulation and nodule numbers per plants were recorded. Nodulation efficiency, equated to nodule numbers as well as symbiotic efficiency of carrier-based Rhizobium sp. during storage period was determined through evaluation.

6.2.6 Assessment of phosphate solubilizing activity

Pure culture of phosphate solubilizing bacteria was isolated from the carrier inoculants during different storage period and it was spot inoculated at the centre of sterile Pikovskaya’s agar plates (minimum 10 bacterial isolates from each time point was
taken for analysis). The inoculated plates were incubated at 30±1°C for 7 days (Pikovskaya, 1948). Both Bacillus megaterium and Azotobacter chroococcum were used for phosphate solubilization study. Size of the bacterial colony and the zone of solubilization were measured with calibrated Himedia scale.

The solubilizing efficiency of the microorganism was calculated using the following formula:

$$\text{Solubilizing efficiency (\% S.E)} = \frac{Z - C}{C} \times 100$$

$Z =$ Solubilization zone (mm)
$C =$ Colony diameter (mm)

6.3 Results

The main objective of this study was to evaluate the efficiency of feather compost as nutrients for growth and carrier material of biofertilizers like Rhizobium sp, Bacillus megaterium and Azotobacter chroococcum. The feather compost extract was prepared and it was supplemented with respective biofertilizers growth medium. Growth in the biofertilizer in the feather compost extract supplemented media was analyzed by the turbidity method and viable count method. Further, carrier efficiency of the compost was studied by, evaluation for survival of the biofertilizer, and moisture and pH of the carrier. The study was conducted for a period of 15 days interval for 60 days.

6.3.1 Isolation and identification of bio-fertilizers strains

The results of morphological and bio-chemical characteristics of isolated strains of bio-fertilizers are summarized in Table- 6.1.
### Table- 6.1: Morphological and biochemical characterization strain

<table>
<thead>
<tr>
<th>Test</th>
<th><em>Rhizobium sp</em></th>
<th><em>Bacillus sp</em></th>
<th><em>Azotobacter sp</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Morphology</td>
<td>rod</td>
<td>rod</td>
<td>rod</td>
</tr>
<tr>
<td>Gram staining</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Endo spore</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Indole</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Methyl red</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>V P test</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Citrate utilization</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Oxidase</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Catalase</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Urease</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>NO₃</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Starch</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Sucrose</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Mannitol</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Xylose</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Dextrose</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Lactose</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

+ Positive, - Negative.

### 6.3.2 Growth of biofertilizers in compost extract medium

#### 6.3.2.1 Growth of *Rhizobium sp* in compost extract medium

The growth of *Rhizobium* sp. in, YEM broth replaced with 20%, 40%, 60%, 80% and 100% feather compost extract were prepared and was inoculated with log phase *Rhizobium* sp. and the medium were incubated at 30°C for 48 h. The result of *Rhizobium* sp. population was determined for YEM agar plate by colony forming unit method (Figure- 6.2). The growth turbidity measurement was presented in (Table 6.2). The feather compost extract medium for 20% was high population growth (1.9067 ±0.0251), compared to others supplementation and control medium. Similarly viable cell count also high in medium replaced with 20% compost extract. It was about 6.45 ± 1.19 x10⁹ cfu/ml after 48 hours of growth followed by 40% extract replacement 9.54 ± 2.1 x 10⁸ cfu/ml (Figure-6.2). The one way ANOVA analysis revealed that supplementation of feather...
compost extract significantly influence (P< 0.01) on the growth of *Rhizobium* sp. (Figure-6.2).

**Figure-6.1:** Growth of *Rhizobium* sp. in different growth media. C = Control (YEMB – Yeast Extract Manitol Salt Broth); 20% compost extract + 80% YEMB; 40% compost extract + 60% YEMB; 60% compost extract + YEMB; 80% compost extract + YEMB; 100% compost extract (alone).

**Table- 6.2:** Growth of *Rhizobium* sp. in Yeast Extract Manitol Salt Broth supplemented with different concentration (%) of feather compost extract.

<table>
<thead>
<tr>
<th>Analysis</th>
<th>Different media</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>Growth*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.7833 ±0.0351</td>
</tr>
<tr>
<td>Final pH</td>
<td>7.597 ±0.020</td>
</tr>
</tbody>
</table>

Note: Growth of *Rhizobium* sp. in different growth media. C = Control (YEMB – Yeast Extract Manitol Salt Broth); 20% compost extract + 80% YEMB; 40% compost extract + 60% YEMB; 60% compost extract + YEMB; 80% compost extract + YEMB; 100% compost extract (alone). Values are mean with S.D. of three repeated experiments. One-way ANOVA analysis revealed that percentage supplementation of feather compost extract significantly influence (P< 0.01) the growth of *Rhizobium* sp.
Figure -6.2: Growth of *Rhizobium* sp. in different growth media. C = Control (YEMB – Yeast Extract Manitol Salt Broth); 20% compost extract + 80% YEMB; 40% compost extract + 60% YEMB; 60% compost extract + YEMB; 80% compost extract + YEMB; 100% compost extract (alone). Values are mean with S.D. of three repeated experiments. One-way ANOVA analysis revealed that percentage supplementation of feather compost extract significantly influence the growth of *Rhizobium* sp. except same * superscript.

### 6.3.2.2 Growth of *Bacillus megaterium* in compost extract medium

The growth of *Bacillus megaterium* in Pikovskaya’s broth replaced with 20%, 40%, 60%, 80% 100% of feather compost extract medium. The optical density of the *B. megaterium* was high at 40% media replacement with feather compost extract. Turbidity of the growth medium was high at 40% and 20% replacement about 2.04 ±0.043 and 1.9133 ±0.032 respectively. Similarly pH of the growth medium was turn to alkali from neutral pH (Table-6.3), it was high at 40% and 20% supplement of feather compost extract. Replacement of media with feather compost was significantly influence the growth of *B. megaterium* (*P* < 0.01). In addition, the viable cell count also high in medium replaced with 40% compost extract. It was about 43.33 ±3.78 x 10^8 cfu/ml (20%) after 48 hours of growth followed by 40% extract replacement 3.99 ±0.27 x10^9 cfu/ml (Figure-6.3). The one way ANOVA analysis revealed that percentage supplementation of feather
compost extract significantly influence (P< 0.01) the growth of *B. megaterium* (Figure-6.3).

![Figure- 6.3: Growth of Bacillus megaterium. in different growth media. C = Control (Pikovskayas broth); 20% compost extract + 80% Pikovskayas broth; 40% compost extract + 60% Pikovskayas broth; 60% compost extract + Pikovskayas broth; 80% compost extract + Pikovskayas broth; 100% compost extract (alone).](image)

**Table- 6.3:** Growth of *Bacillus megaterium* in Pikovskayas broth growth medium supplemented with different concentration (%) of feather compost extract.

<table>
<thead>
<tr>
<th>Analysis</th>
<th>Control</th>
<th>20%</th>
<th>40%</th>
<th>60%</th>
<th>80%</th>
<th>100%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth*</td>
<td>1.85±0.0917</td>
<td>1.9133±0.032</td>
<td>2.04±0.043</td>
<td>1.66±0.089</td>
<td>1.2933±0.047</td>
<td>0.5233±0.078</td>
</tr>
<tr>
<td>Final pH</td>
<td>7.45±0.05</td>
<td>7.643±0.049</td>
<td>7.643±0.031</td>
<td>7.277±0.066</td>
<td>7.26±0.043</td>
<td>7.097±0.030</td>
</tr>
</tbody>
</table>

Note: Growth of *Bacillus megaterium* in different growth media. C = Control (Pikovskayas broth); 20% compost extract + 80% Pikovskayas broth; 40% compost extract + 60% Pikovskayas broth; 60% compost extract + Pikovskayas broth; 80% compost extract + Pikovskayas broth; 100% compost extract (alone). Values are mean with S.D. of three repeated experiments. One-way ANOVA analysis revealed that percentage supplementation of feather compost extract significantly influence the growth of *Bacillus megaterium.*
Figure- 6.4: Growth of *Bacillus megaterium* in different growth media. C = Control (Pikovskayas broth); 20% compost extract + 80% Pikovskayas broth; 40% compost extract + 60% Pikovskayas broth; 60% compost extract + Pikovskayas broth; 80% compost extract + Pikovskayas broth; 100% compost extract (alone). Values are mean with S.D. of three repeated experiments. One-way ANOVA analysis revealed that percentage supplementation of feather compost extract significantly influence the growth of *Bacillus megaterium*, except same * superscript.

**6.3.2.3 Growth of *Azotobacter chroococum* in compost extract medium**

The growth of *Azotobacter chroococum* in Azotobacter broth replaced with 20%, 40%, 60%, 80% 100% of feather compost extract medium. The optical density of the *A. chroococum* was high at 20% media replacement with feather compost extract. Compared with control medium, turbidity of the growth medium was high at 20% replacement and was about 1.8767 ±0.023. But pH of the growth medium was turn to alkali from neutral pH (Table- 6.4), it was high at control medium (7.45) and 20% supplement (7.43) of feather compost extract. Replacement of media with feather compost was significantly influence the growth of *A. chroococum* (P< 0.01). Further, the viable cell count also high in medium replaced with 20% compost extract. It was about 7.94 ± 0.53 x10⁹ cfu/ml (20%) after 48 hours of growth followed by control medium 1.95 ± 0.3 x 10⁹ cfu/ml.
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(Figure- 6.6). The one way ANOVA analysis revealed that percentage supplementation of feather compost extract significantly influence (P< 0.01) the growth of A. chroococum (Figure - 6.6).

Figure - 6.5: Growth of Azotobacter chroococcum in different growth media. C = Control (Azotobacter broth); 20% compost extract + 80% Azotobacter broth; 40% compost extract + 60% Azotobacter broth; 60% compost extract + Azotobacter broth; 80% compost extract + Azotobacter broth; 100% compost extract (alone).

Table - 6.4: Growth of Azotobacter chroococcum in azotobacter growth medium supplemented with different concentration (%) of feather compost extract.

<table>
<thead>
<tr>
<th>Analysis</th>
<th>Control</th>
<th>20%</th>
<th>40%</th>
<th>60%</th>
<th>80%</th>
<th>100%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth*</td>
<td>1.75 ±0.02</td>
<td>1.8767 ±0.023</td>
<td>1.6733 ±0.0152</td>
<td>1.36 ±0.03</td>
<td>0.9267 ±0.115</td>
<td>0.39 ±0.081</td>
</tr>
<tr>
<td>Final pH</td>
<td>7.45 ±0.05</td>
<td>7.433 ±0.141</td>
<td>7.177 ±0.070</td>
<td>7.117 ±0.006</td>
<td>7.027 ±0.015</td>
<td>7.017 ±0.015</td>
</tr>
</tbody>
</table>

Note: Growth of Azotobacter chroococcum in different growth media. C = Control (Azotobacter broth); 20% compost extract + 80% Azotobacter broth; 40% compost extract + 60% Azotobacter broth; 60% compost extract + Azotobacter broth; 80% compost extract + Azotobacter broth; 100% compost extract (alone). Values are mean with S.D. of three repeated experiments. One-way ANOVA analysis revealed that percentage supplementation of feather compost extract significantly influence the growth of Azotobacter chroococcum.
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6.3.2.4 Growth curve of biofertilizers in compost extract medium

Growth curve of *Rhizobium* sp. was studied with using 20% feather compost extract supplemented medium. Compared with control medium, the optimized media delay the multiplication of the *Rhizobium* sp. (Figure- 6.7), and it reached the stationary phase after 18 hours of growth in control liquid medium, but it was in 24 hours of growth in experimental medium. Stationary phase of the bacterial growth was extended up to 36 hours of growth in experimental medium.

Figure- 6.8 showed the growth curve of *B. megaterium*. Compared with control medium, the optimized media delay the multiplication of the *B. megaterium*. (Figure-6.8), and it reached the stationary phase after 24 hours of growth in control liquid medium and experimental medium (40%). Stationary phase of the bacterial growth was extended up to 36 hours of growth in experimental medium.
Figure - 6.7: Growth curve of *Rhizobium* sp. 0.1 % of *Rhizobium* inoculum was given in two different growth media of *Rhizobium* and the growth pattern of the bacteria was observed by viable plate count method. Standard medium = YEM broth; Compost extract medium = 40% compost extract + 60% YEM broth.

Figure - 6.8: Growth curve of *Bacillus megaterium*. 0.1 % of *B. megaterium* inoculum was given in two different growth media of phosphobacter and the growth pattern of the bacteria was observed by viable plate count method. Standard medium = Pikovskayas broth; Compost extract medium = 20% compost extract + Pikovskayas broth 80%.
Figure- 6.9 showed the growth curve of *A. chroococum*. Compared with control medium, the optimized media showed similar pattern of growth and it reached the stationary phase at 45\(^{th}\) hours of growth (Figure- 6.9), and there is no differences on decline phase in both media.

![Graph showing growth curve of *A. chroococum*.](image)

**Figure -6.9:** Growth curve of *Azotobacter chroococcum*. 0.1 % of *A. chroococcum* inoculum was given in two different growth media of *Azotobacter* and the growth pattern of the bacteria was observed by viable plate count method. Standard medium = *Azotobacter* broth; Compost extract medium = 20% compost extract + *Azotobacter* broth 80%.

### 6.3.3 Carrier stability of biofertilizers

#### 6.3.3.1 Physico-chemical properties of carrier materials

The physico-chemical properties such as pH (Table- 6.6, 6.7, 6.8), organic matters, C/N ratio, total nitrogen, total phosphorus, total Potassium and water holding capacity were estimated for the carrier materials like lignite and feather compost were used in this study and the results are presented in (Table- 6.5). Feather compost had superior nutritive properties than the lignite fly ash.
Figure- 6.10: Preparation of lignite (a) and cow dung + feather compost (b) as carrier material for biofertilizers.

Figure- 6.11 Carrier stability of biofertilizers in (i) Lignite fly ash (Standard) and (ii) Feather compost (test). a) *Rhizobium* sp., b) *Bacillus megaterium* and c) *Azotobacter chroococcum*.
Table 6.5: Chemical properties of carrier materials

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Lignite</th>
<th>Feather compost</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organic matter (%)</td>
<td>36.3</td>
<td>38.6</td>
</tr>
<tr>
<td>C/N Ratio</td>
<td>24.2</td>
<td>29.4</td>
</tr>
<tr>
<td>Total N (%)</td>
<td>0.48</td>
<td>0.62</td>
</tr>
<tr>
<td>Total P (%)</td>
<td>0.15</td>
<td>0.32</td>
</tr>
<tr>
<td>Total K (%)</td>
<td>2.4</td>
<td>2.8</td>
</tr>
</tbody>
</table>

Table – 6.6: pH change of carrier during the biofertilizer stability study (a) *Rhizobium* sp.

<table>
<thead>
<tr>
<th>Samples</th>
<th>0</th>
<th>15</th>
<th>30</th>
<th>45</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lignite fly ash</td>
<td>7</td>
<td>±0</td>
<td>7.17</td>
<td>±0.15</td>
<td>7.1</td>
</tr>
<tr>
<td></td>
<td>±0</td>
<td>±0.15</td>
<td>±0.03</td>
<td>±0.21</td>
<td>±0.15</td>
</tr>
<tr>
<td>Feather compost</td>
<td>7</td>
<td>±0</td>
<td>7.1</td>
<td>±0.1</td>
<td>7.23</td>
</tr>
<tr>
<td></td>
<td>±0</td>
<td>±0.1</td>
<td>±0.06</td>
<td>±0.06</td>
<td>±0.06</td>
</tr>
</tbody>
</table>

Table – 6.7: pH change of carrier during the biofertilizer stability study (b) *B. megaterium*.

<table>
<thead>
<tr>
<th>Samples</th>
<th>0</th>
<th>15</th>
<th>30</th>
<th>45</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lignite fly ash</td>
<td>7</td>
<td>±0</td>
<td>7.13</td>
<td>±0.06</td>
<td>7.3</td>
</tr>
<tr>
<td></td>
<td>±0</td>
<td>±0.06</td>
<td>±0.1</td>
<td>±0.05</td>
<td>±0.05</td>
</tr>
<tr>
<td>Feather compost</td>
<td>7</td>
<td>±0</td>
<td>7.13</td>
<td>±0.12</td>
<td>7.2</td>
</tr>
<tr>
<td></td>
<td>±0</td>
<td>±0.12</td>
<td>±0.1</td>
<td>±0.12</td>
<td>±0.06</td>
</tr>
</tbody>
</table>
Table – 6.8: pH change of carrier during the biofertilizer stability study (c) Azotobacter chroococcum.

<table>
<thead>
<tr>
<th>Samples</th>
<th>0</th>
<th>15</th>
<th>30</th>
<th>45</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lignite fly ash</td>
<td>7</td>
<td>±0</td>
<td>7.23</td>
<td>±0.06</td>
<td>7.4</td>
</tr>
<tr>
<td>Feather compost</td>
<td>7</td>
<td>±0</td>
<td>7.3</td>
<td>±0</td>
<td>7.47</td>
</tr>
</tbody>
</table>

6.3.3.2 Survival of *Rhizobium* sp. *Bacillus megaterium Azotobacter chroococcum* during storage at room temperature

Carrier stability of biofertilizers was studied with control lignite carrier and the feather compost carrier. Initial moisture content of the carrier bioinoculum was maintained as 40% level. The viable count of the biofertilizer was studied by standard plate count method. Viability of *Rhizobium* sp. was enumerated by using YEMA with congoored indicator. Viable cell count was increased up to 45th day of incubation and the total viable count was firmly maintained up to 60th day of incubation. Compared with control carrier viability of *Rhizobium* sp. was high (Figure – 6.12a), similarly, *B. megaterium* was also highly stable on 60th day of storage, which was enumerated by using Pikovskayas agar medium (Figure – 6.12b). Similar trend was observed when *Azotobacter chroococcum* was maintained in the experimental carrier (Figure – 6.12c). There is no significant difference on viable count during carrier stability study.

Figure - 6.11 shows the moisture content of the carrier bioinoculants during stability study for 0 days period. Every 15 days known amount of sample was taken for pH and moisture analysis. There was no significant differences observed on pH of the carrier and moisture up to 45th day of carrier stability study (Figure – 6.13 a,b,c). But little variation was observed on 60th day sample.
Figure 6.12: Effect of feather compost on viability of biofertilizers; (a) *Rhizobium* sp. (b) *Phosphobacter* sp. and (c) *Azotobacter* sp., during storage at room temperature (32 ± 3°C) for the period of 60 days. Every 15 days known amount of sample was taken for viability of biofertilizer bacteria. The viability of (a) *Rhizobium* sp. was enumerated on YEMA medium with congored indicator; viability of (b) *Phosphobacter* sp. was estimated by using Pikovskayas agar medium and the (c) *Azotobacter chroococcum* was estimated by using Azotobacter agar medium.
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Figure – 6.13: Effect of feather compost on maintenance of moisture during biofertilizer stability study (a) *Rhizobium* sp. (b) *B. megaterium* and (c) *Azotobacter chroococcum* during storage at room temperature (32 ± 3°C) for the period of 60 days. Moisture content was determined by standard protocol.
6.3.3.3 Nodulation efficiency of \textit{Rhizobium} sp.

Nodulation ability of the \textit{Rhizobium} sp was examined by seed inoculation study used with groundnut seeds. Total nodules of the individual plant were enumerated on 45\textsuperscript{th} day of the ground nut plant growth. Highest number of nodulation was accounted on 30\textsuperscript{th} day carrier stability samples (Table- 6.9). Compared with uninoculated control seed inoculated plants showed significant amount of nodules (P< 0.01) on their root (Figure- 6.14). The one way ANOVA analysis of the results of both carrier inoculants showed no significant variation, but little difference was observed on feather compost carrier inoculants (Table- 6.9).

Table- 6.9: Effect of carrier on stability of \textit{Rhizobium} sp. during different days of storage.

<table>
<thead>
<tr>
<th></th>
<th>Nodulation efficiency of Rhizobium in different storage days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Control</td>
<td>18.2 ±1.924</td>
</tr>
<tr>
<td>Lignite carrier</td>
<td>35.8 ±4.147</td>
</tr>
<tr>
<td>Feather compost carrier</td>
<td>44 ±2.236</td>
</tr>
<tr>
<td>F-value</td>
<td>100.4</td>
</tr>
<tr>
<td>P-value</td>
<td>&lt; 0.01</td>
</tr>
</tbody>
</table>

All values are the average of three individual experiments with standard deviation. One way ANOVA analysis F and P value are given in the table.
Figure- 6.14: Effect of carrier on stability of *Rhizobium* sp. nodulation during different days of storage. a) 0 days, b) 15\textsuperscript{th} day of carrier stability, c) 30\textsuperscript{th} day of carrier stability, d) 45\textsuperscript{th} day of carrier stability, and e) 60\textsuperscript{th} day of carrier stability. 1) control; 2) lignite carrier; 3) feather compost carrier.

6.3.3.4 Phosphate solubilizing efficiency of *B. megaterium* and *A. chroococcum*

The present result of the phosphate solubilizing efficiency of *B. megaterium* in different carrier such as carrier based (lignite and feather compost) on the Pikovskaya’s agar plates production of area of clear zone formation was shown in Figure- 6.15. Table-6.10, showed the efficiency of phosphate solubilization of the *B. megaterium* isolated from the carrier materials (both lignite carrier and feather compost carrier), which was the average of five different isolates from the same day of carrier stability. There was no significant variation (P<0.01) on phosphate solubilization between the lignite carrier and the feather compost carrier up to 45\textsuperscript{th} day of the experiment, but it was significantly varied on 60\textsuperscript{th} day of carrier stability (Table – 6.10).
Table -6.10: Phosphate solubilization ability of *Bacillus megaterium* during storage in carrier stability

<table>
<thead>
<tr>
<th>Phosphate solubilization index during carrier storage days</th>
<th>0</th>
<th>15</th>
<th>30</th>
<th>45</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lignite carrier</td>
<td>1.424</td>
<td>1.484</td>
<td>1.434</td>
<td>1.584</td>
<td>1.38</td>
</tr>
<tr>
<td></td>
<td>±0.195</td>
<td>±0.15</td>
<td>±0.182</td>
<td>±0.118</td>
<td>±0.164</td>
</tr>
<tr>
<td>Feather compost carrier</td>
<td>1.7</td>
<td>1.95</td>
<td>1.85</td>
<td>1.95</td>
<td>1.75</td>
</tr>
<tr>
<td></td>
<td>±0.209</td>
<td>±0.209</td>
<td>±0.224</td>
<td>±0.209</td>
<td>±0.177</td>
</tr>
<tr>
<td>F- value</td>
<td>4.65</td>
<td>16.38</td>
<td>10.42</td>
<td>11.59</td>
<td>11.75</td>
</tr>
<tr>
<td>P - value</td>
<td>0.063</td>
<td>0.004</td>
<td>0.012</td>
<td>0.009</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Results are mean of three repetitive experiments with an average of 5 different isolates with ± standard deviation. Index value was calculated by = zone of solubilization/ colony size.

Figure- 6.16, showed the phosphate solubilizing efficiency of *A.chroococcum* in different carrier such as carrier based (lignite and feather compost) on the Pikovskayas agar plates production of area of clear zone formation. Table- 6.11 showed the efficiency of phosphate solubilization of the *A.chroococcum* isolated from the carrier materials (both lignite carrier and feather compost carrier), which was the average of five different isolates from the same day of carrier stability. There was a significant variation (P value is given in the Table- 6.11) on phosphate solubilization between the lignite carrier and the feather compost carrier up to 60\(^{th}\) day of the experiment.
**Figur-6.15**: Phosphate solubilization (zone) during carrier storage of a) 0 days, b) 15th day of carrier stability, c) 30th day of carrier stability, d) 45th day of carrier stability, and e) 60th day of carrier stability. *Bacillus megatherium* grown on Pikovskayas Agar medium. After 96 hours of growth the zone of phosphate solubilization was accounted. Values are their in table 6.10.

**Table – 6.11**: Phosphate solubilization ability of *Azotobacter* sp during storage in carrier stability

<table>
<thead>
<tr>
<th></th>
<th>Phosphate solubilization index during carrier storage days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Lignite carrier</td>
<td>1.288 ±0.060</td>
</tr>
<tr>
<td>Feather compost carrier</td>
<td>1.718 ±0.182</td>
</tr>
<tr>
<td>F-value</td>
<td>25.13</td>
</tr>
<tr>
<td>P-value</td>
<td>0.001036</td>
</tr>
</tbody>
</table>

Results are mean of three repetitive experiments with an average of 5 different isolates with ± standard deviation. Index value was calculated by = zone of solubilization/ colony size.
**Figure- 6.16:** Phosphate solubilization (zone) during carrier storage of a) 0 days, b) 15\textsuperscript{th} day of carrier stability, c) 30\textsuperscript{th} day of carrier stability, d) 45\textsuperscript{th} day of carrier stability, and e) 60\textsuperscript{th} day of carrier stability. *Azotobacter chroococcum* grown on Pikovskayas Agar medium. After 96 hours of growth the zone of phosphate solubilization was accounted. Values are their in table

### 6.4 Discussion

Biofertilizer is a substance which contains living microorganisms which, when applied to the seed, plant surfaces or soil colonizes the rhizosphere or the interior of the plant and promotes growth by increasing the supply or availability of primary nutrients to the host plant. Wastes are the substrate for number of useful products. In this point, poultry waste material like feathers are used as valuable substrate for compost manure production and this manure would be the substrate for biofertilizer growth and stability, has been studied.

### 6.4.1 Growth of Biofertilizers

In this present study, biofertilizer bacteria such as *Rhizobium* sp., *Bacillus megaterium* and *Azotobacter chroococcum* were used. For mass cultivation of bacteria, they required growth media. Synthetic complex media such as yeast extract manitol salt agar for *Rhizobium* sp, Pikovskayas Agar for *Bacillus megaterium* and azotobacter agar

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for *Azotobacter chroococcum* has been used for mass production of biofertilizers. For industrial production of biofertilizers, instead of using synthetic commercial media, natural or industrial waste materials are used as substrate for carbon and nitrogen source. Molasses and corn steep liquor are commonly used raw material for industrial production of various biotechnological valuable products.

In this research, is an attempt to investigate extract of feather compost as nutrient media for growth and mass production of biofertilizers. 20% v/v, supplementation of feather compost extract showed higher growth than the synthetic media used for cultivation. It may be due to availability of mineral nutrients and enriched amino acids derived from feather keratins. Usually microorganism utilizes different carbon sources for their energy requirements. Rhizobia are moderately easy to culture and not particularly fastidious in their nutrient requirements (Graham and Parker, 1964). All rhizobia utilize monosaccharides and disaccharides readily and to a lesser extent trisaccharides, alcohols and acids. In addition, organic nitrogen also supports fast growth of *Rhizobium* sp. In this study, feather compost contains keratin hydrolysate which supplies free aminoacids to microbial growth. Not only for *Rhizobium* growth, would be a suitable supplement for the growth of *B. megaterium* and *A. chroococcum*. Results on growth curve of the biofertilizers in the control media and formulated feather extract media also revealed the nutrient value of the feather compost extract.

### 6.4.2 Carrier stability

In the present study data obtained was suggested that feather compost can be used as carrier for bioformulations, similar results were also reported by various researchers, Jayaraj *et al.* (2005) reported that the formulations of *B. subtilis* and *P. fluorescens* are commercially available. Those of *Bacillus* are very stable due to the ability of this bacterium to form spores (Emmert and Handelsman, 1999) that are long lived, and resist heat and desiccation (Kloepper, 1991). Several materials have been appraised as carriers for microbial inoculants serving various purposes (Stella and Sivasakthivelan, 2009; Selvi, 2013). Arora *et al.*, (2008) successfully assessed sawdust as carrier material for bacterial inoculants. Coconut-shell powder, coffee husk, press-mud or combination of such materials with soil and/or charcoal has also been found suitable as carriers for
phosphate solubilizing bacteria (Han and Lee 2005; Tilak and Subba Rao, 1978), used for improving shelf life of the microbial inoculants. Brahmaprakash and Sahu, (2012) suggested that liquid inoculants and alginate based granular formulations are two important new inoculant formulations which are an alternative to peat/lignite based ones.

Lignites are commonly used carrier, so it was used for control carrier of this study. Compare to this, cowdung-feather compost has stably maintained the viability of all three biofertilizers, even little growth of biofertilizers was also observed. The stability was not much significant to the control carrier, but it has some nutritive property, that’s why the microbial inoculants have some growth in this carrier, it was clearly observed through viable count method. Muniruzzaman and Khan, (1992) found that viable counts of two indigenous rhizobial strains (SB-1 and JJS-1) remained more than $10^8$ g-1 up to 75 days in many carrier materials including charcoal. Low-grade coal can also be used as a carrier for rhizobia inoculants (Singh and Tilak, 1977). Many researchers have also suggested that Fly-ash alone and in combination with other materials is a promising carrier for bio-formulation of *Rhizobium* (Kumar and Gupta, 2008) and *Trichoderma viride* and *T. harzianum* (Kumar et al., 2012).

Moisture content and the pH of the carrier greatly influence the stability of bioinoculants. Van Schrevan, (1970) reported the effect of moisture content of the carrier-based culture upon the number of rhizobia. On the basis of several reports, it has been noted that the optional moisture in sterilized carriers lies in the range of 40 -60%. Previous studies have suggested that high temperature could cause growth and increase of the bacterial population; it produced wastes that were not only toxic for bacteria, but also changing the pH of medium that could be the reason of population reduction and death of bacteria (Dearmon et al., 1962). Mendez and Videira, (2005) stated that bacterial maintenance at 28°C for 41 days caused an increase in number of viable bacterial cells on all carriers so that the population reached nearly $10^9$ bacteria per gram of carrier. In this study, 40% moisture was maintained, it was almost maintained during the entire study period, but the pH of the carrier was little varied, because of microbial activity during storage.
The study by Kalra et al., (2008) that the granular vermicompost as a carrier is capable of holding $10^8$ viable bacteria after 180 days. Sekar and Karmegam, (2010) reported that vermicasts as a carrier material which supports the survival of more than $1 \times 10^7$ g-1 viable cells of *A. chroococcum*, *B. megaterium* and *R. leguminosarum* till the end of 10th month which is longer than observed in lignite (a commercial carrier material). Saleh et al., (2001) reported that the population of *Azotobacter vinelandii* A1 in rice husk carrier rise up to 128% from the initial population after storing at 30°C. At 30 days after storage, the bacterial population increased slightly even stored at 5°C. The findings of the present study also showed similar results when the feather compost used as carrier for the survival of the biofertilizer of *Rhizobium.sp*, *B. megaterium* *A. chroococcum* inoculants for period of 60 days during storage than the lignite as a carrier.

Suitable carrier should be cheap, easily used, mixable, package able, and available. Also, the carrier must permit gas exchange, particularly oxygen, and has high organic matter content and high water holding capacity as well (Bashan, 1998; Ben Rebah et al., 2002). It is worth mentioning that sterilized carriers generally support higher populations and display much longer shelf lives (Kalra et al., 2010).

The results of this chapter clearly pointed out that material like lignite, and the feather compost has maintained the viability as well as their biological activities. Biofertilizer biological activities of the bioinculants such as nodulation, nitrogen fixation, phosphate solubilization, growth hormone synthesis and maintain their population constantly. Several researchers find the biological property of these inoculants during storage was studied. Valid differences in nodulation among groundnut were reported by Duggar, (1935). The data on nodule weight and nitrogenase activity were analyzed by the Scott- Knott procedure (Gates et al., 1978). Studies indicated that variation in nodulation could be due to low rhizobial density, incompatibility of the rhizobia and edaphic factors that hinder the effectiveness of the rhizobia (Zaharan, 1999; Slattery and Pearce, 2002; Kiros Habtegebrail and Singh, 2006). Different researchers also isolated rhizobial strains with different nodulating ability in Ethiopian soils (Desta Beyene and Angaw Tsigie, 1987; Aynababa Adamu et al., 2001). In our study, the Rhizobia stored with feather compost carrier have higher efficiency than it stored in lignite carrier. This indicates, the
feather compost carrier would be maintaining the biological property of the \textit{Rhizobium} sp. However, Aynabeba \textit{et al.}, (2001) observed some variation in their morphological and physiological characters of \textit{R.leguminosarum} var viceae when nodulation in faba bean. Rate of nodulation is the factor that the inoculants have retained its biological activity. If the strain loss its nodulation property that could loss its nitrogen fixing ability. Inoculation of efficient \textit{Rhizobium} sp. may improve the nodulation and increasing the yield (Lupwayi and Mkandawire, 1996; Aynababa Adamu \textit{et al.}, 2001; Kiros Habtegebrail and Singh, 2006).

Phosphate solubilizing bacteria (PSB) plays a major role in the solubilization and uptake of native and applied soil P (Krishnaveni, 2010). Phosphate solubilization is one of the biological activities of \textit{B. megaterium} and \textit{A. chroococcum}. If the strain has less efficient, should not have phosphate solubilizing activity, in this research, \textit{B. megaterium} and \textit{A. chroococcum} were recovered from both carrier materials at different storage period. The total viability and the efficiency of the strains were supported that feather compost is suitable carrier for maintaining the bioinoculants such as \textit{Azotobacter chroococcum} and \textit{Bacillus megaterium}.

The major mechanism associated with the solubilization of insoluble phosphate is the production of organic acids, accompanied by acidification of the medium (Puente \textit{et al.}, 2004). From the study results the following aspects were concluded, among the two carriers under study, the best carrier identified was feather compost in terms of supporting higher population load, ideal moisture and ideal pH. Gyaneshwar \textit{et al.} (1999) also reported that the colonies with clear halo zones are considered to be PSB. Most P-solubilizing bacteria and fungi (Achal \textit{et al.}, 2007; Aseriet \textit{et al.}, 2009; Yadav and Tarafdar, 2011) were isolated from the rhizosphere of various plant and are known to be metabolically more active than those isolated from sources other than rhizosphere. The major mechanism associated with the solubilization of insoluble phosphate is the production of organic acids, accompanied by acidification of the medium (Puente \textit{et al.}, 2004). The numbers of the viable cells were significantly declined in Fly-ash bio-formulations throughout the incubation period (Kumar, 2014). A decline in population on
prolonged incubation may be attributed to the depletion of nutrients, moisture and autolysis of cells (Gaind and Gaur, 2003).

Statistically, studies suggested that there were no significant differences in cfu counts among the formulations at the end of storage period (60 days). All the strains were managed to retain the viable cell count in both formulations. Muniruzzaman and Khan (1992) found that viable counts of two indigenous rhizobial strains (SB-1 and JJS-1) remained more than $10^8$ g-1 up to 75 days in many carrier materials including charcoal. Results of Survival of *Bacillus megaterium* in different inoculant formulations had revealed that the survival of *Bacillus megaterium* in liquid inoculant was found to be superior compared to nutrient glucose broth and lignite inoculant after 6 months of storage (Sridhar *et al.*, 2004). Brahmaprakash and Sahu, (2012) suggested that liquid inoculants and alginate based granular formulations are two important new inoculant formulations which are an alternative to peat/lignite based ones. Today, advances in inoculant technology are concerned with improving quality, extending useful shelf life and developing new formulations for use under less favorable conditions.

The biofertilizers selected in this study were found to be potent nitrogen fixer, and a phosphate solubilizer. The numbers of the viable cells were significantly maintained in all the two bio-formulations throughout the incubation period. The maintenance of viable bacteria on incubation may be attributed to the availability of nutrients, moisture and pH of the carrier may suitable for viability of bacteria. The results of the present studies showed that, lignite and feather compost can be used as carrier materials for biofertilizers strains. The data obtained in respect to feather compost as carrier materials are comparable to other commercially available carrier materials. Although, feather compost is basic in nature and it has good mineral content.