

## ***International Journal of Scientific Research and Reviews***

### **A Comparative Study of Different Carrier Materials on Rizobium Inoculant**

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#### **ABSTRACT**

The greatest hindrance in land use is the extreme pros and cons of using chemical fertilizers and manures. The safest way of replenishing soil is by using bio fertilizers. Bio fertilizers are microbial inoculants used for Nitrogen fixation, Phosphorous and Potash solubilisation. Among bio fertilizers Rhizobium is used for nitrogen fixation in pulses. Carrier based Rhizobium is supplied to farmers. The widely used popular carrier is processed lignite, which has some serious limitations. Hence, a suitable alternate carrier is the need of the hour.

Hence, four carriers viz. processed lignite, lignite with 0.1 per cent press mud, burnt rice husk and vermiculite were studied. All the four carriers under study were inoculated with Rhizobium inoculants. The population load, contamination level, pH, moisture content were studied at weekly intervals for four weeks. The study result indicates vermiculite to a better alternate for processed lignite.

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## **INTRODUCTION**

The boon of biotechnology is bio fertilizers. This bio fertilizer technology is highly practical, eco-friendly and is widely used by the elite farming community. The bio fertilizer is a low cost technology which can help to fix atmospheric nitrogen, solubilise phosphorous and potassium. Its inputs consist of living or latent micro organisms and they are renewable resources. In this way these bio fertilizers help to reduce the use of chemical fertilizers thereby saving huge amount of foreign exchange, energy, precious fossil fuels and also protecting the environment from heavy metals to produce harmless and healthy foods<sup>1</sup>. Hence, use of bio fertilizers is gaining momentum. The microbes are mixed in a carrier and supplied to farmers. The most popular carrier is processed lignite which has been selected due to its availability and low cost.

## **OBJECTIVES:**

1. To evaluate and identify a suitable alternate carrier for supply of bio-fertiliser viz Rhizobium spp.
2. To compare the performance of four carriers viz.
  - i. Processed lignite
  - ii. Processed lignite with press mud (0.1%)
  - iii. Burnt rice husk
  - iv. Vermiculite
3. To study the population load, contamination level pH, moisture in different carriers.
4. To choose the best carrier among the carriers under study

## **HYPOTHESIS**

The carriers under study are safe and non-detrimental to microbial multiplication.

The microbial multiplication is not greatly altered by the varying physical parameters of the carriers.

## **METHODOLOGY**

Rhizobium spp. was identified as per the guidelines detailed in Bergey's Manual of Determinative Bacteriology. All the four carriers under study were sterilized at 121°C for 3

hours using. After bringing to room temperature, the carriers were inoculated with Rhizobium culture.

It was serially diluted by serial dilution technique wherein 1g of sample was added to 9ml of sterile water. After thorough (mixture) mixing with cyclomixer 1ml from it was added to the second test tube containing 9ml distilled water. The process was repeated till  $10^8$  dilutions were obtained.

The medium used for plating is YEMA (Yeast Extract Mannitol Agar) with following composition.

Mannitol	-	10 gram
Magnesium sulphate	-	0.2 gram
Potassium hydrogen phosphate	-	0.5 gram
Sodium chloride	-	0.1 gram
Calcium carbonate	-	1.0 gram
Yeast extract	-	1.0 gram
Agar	-	18.0 gram
Distilled water	-	1000 ml

SOURCE: ISI, 1986

The plating was done in aseptic condition in laminar air flow chamber. The medium was added to sterilized petriplate, allowed to solidify; then 0.2 ml of required dilution viz. ( $10^6$ ,  $10^7$ ,  $10^8$ ) was plated.

The plates were then incubated in BOD Incubator at 25°C.

After three days the plates were counted using Lapis digital colony counter. The plate count was done in  $10^6$ ,  $10^7$ ,  $10^8$  dilution.

The population was calculated, using Fischer and Yates (1953) table modified by Date and Vincent (1962)<sup>2, 3</sup>.

The contamination level was studied at  $10^5$  dilutions.

The pH was measured by Elico pH meter. The pH was measured at 1:2 dilutions.

The moisture was measured by Gambaks hot air oven.

The above tests were repeated for all the four carriers at weekly intervals for four weeks.

**Table I: Population studies**

CARRIERS	Population Load in CFU/ml											
	I week			II week			III week			IV week		
	10 <sup>6</sup>	10 <sup>7</sup>	10 <sup>8</sup>	10 <sup>6</sup>	10 <sup>7</sup>	10 <sup>8</sup>	10 <sup>6</sup>	10 <sup>7</sup>	10 <sup>8</sup>	10 <sup>6</sup>	10 <sup>7</sup>	10 <sup>8</sup>
Processed lignite	50	42	36	55	47	40	61	52	48	65	58	50
Processed with lignite & 0.1% press mud	52	45	38	58	49	42	63	54	50	68	60	51
Burnt rice husk	55	49	41	60	51	45	65	57	52	70	63	53
Vermiculite	75	63	58	82	72	69	93	77	72	104	95	89

**Table II: Contamination Study**

CARRIER	Contamination level in CFU/ML			
	I Week	II Week	III Week	IV Week
	10 <sup>5</sup>	10 <sup>5</sup>	10 <sup>5</sup>	10 <sup>5</sup>
Processed lignite	Nil	Present	Present	Present
Processed with lignite & 0.1% press mud	Nil	Nil	Nil	Present
Burnt rice husk	Nil	Nil	Nil	Present
Vermiculite	Nil	Nil	Nil	Nil

**Table III: Moisture Estimation**

CARRIER	Moisture%			
	I Week	II Week	III Week	IV Week
Processed lignite	40	37	35	34
Processed with lignite & 0.1% press mud	40	38	35	35
Burnt rice husk	40	39	36	35
Vermiculite	40	40	40	40

**Table IV: pH Estimation**

CARRIER	pH			
	I Week	II Week	III Week	IV Week
Processed lignite	7.0	7.0	7.1	7.3
Processed with lignite & 0.1% press mud	7.0	7.1	7.4	7.5
Burnt rice husk	7.0	7.8	8.2	8.5
Vermiculite	7.0	7.2	7.5	7.7

## DATA ANALYSIS

There are totally four cases considered for analysis of the data collected on the growth of Rhizobium in the different carriers.

### ***Case I: Analysis of population***

In each dilution between the populations keeps reducing, considering the week's tally the population increases gradually by time.

### ***Case II: Analysis of the level of contamination***

According to ISI standards of contamination level,  $10^5$  dilution is said to be contamination free. Until the 1st week, all the 4 carriers were contamination free. Vermiculite was only carrier which was contamination free till the 4<sup>th</sup> week.

### ***Case III: Analysis of moisture***

ISI standards for moisture are 40%. All the carriers were equally maintained for the first week and then the carriers gradually began to dry. But vermiculite was never left to a state of dryness.

### **Case IV: Analysis of pH**

In the 1st week, all the carriers were neutral except burnt rice husk which was with 7.7 pH (very weak base, approximately neutral). The observation of 4<sup>th</sup> week ended with all the carriers changing from neutral to base (weak base).

Processed lignite	7.3
Processed lignite with 0.1% press mud	7.5
Vermiculite	7.7
Burnt rice husk	8.5

## **RESULTS**

The study of the Rhizobial count in different carriers for four weeks revealed the following facts. Among the four carriers under study vermiculite supported a higher population load in all the dilutions for all the four weeks. It was followed by burnt rice husk, processed lignite with 0.1% press mud and processed lignite. (Refer table I).

The contamination study revealed the following. Upto one week, no contamination was noticed in all the carriers, while vermiculite was contamination free for all the four weeks. (Refer Table II)

Vermiculite supported a higher moisture level in all the four weeks (Refer Table III)

The pH rise was very high in Burst rice Husk followed by vermiculite, processed Lignite with 0.1% press mud and processed lignite (Refer Table IV).

From the study results the following aspects were concluded. Among the four carriers under study, the best carrier identified was vermiculite in terms of supporting higher population load, contamination free environment for microbes, ideal moisture and ideal pH.

It was attributed to the sterile nature of vermiculite which is a type of clay roasted to 1600°C. This has ensured a complete aseptic nature of the carrier. Further the innate absence of organic carbon has helped in non support of any contamination before addition of inoculum.

## **CONCLUSION**

Supply of nitrogen through chemical fertilizers has several environmental demerits. It damages the soil fertility.

Bio fertilizers replenish the soil with micro-organisms like rhizobium which fix atmospheric gases in the soil.

Vermiculite as a carrier is considered better from this study as it is contamination free and has good population density, considerable moisture content and pH value. Vermiculite also gives a golden yellow and shiny appearance which will attract buyers.

Thus vermiculite can be a better carrier than others and will produce a positive impact among society at large.

The only disputing factor with the use of vermiculite is its high cost. When it is commercially used in a large scale then the cost of raw material will come down.

With the advent of new organic sources like polyacrylamide gel, carboxynethyl cellulose could be thought of as better carriers than the existing ones which further needs a detailed evaluation.

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