Int. J. Chem. Sci.: 10(3), 2012, 1437-1444
ISSN 0972-768X
www.sadgurupublications.com

EFFECT OF *PSEUDOMONAS* ON VIABILITY OF MICROBIAL (*RHIZOBIAL*) INOCULANTS DURING STORAGE

M. ARYA, R. SHARMA* and D. DAS[a]

Department of Chemistry, S. D. Govt. College, BEAWAR (Raj.) INDIA
[a]Govt. Polytechnique College, AJMER (Raj.) INDIA

ABSTRACT

Two carriers of biological origin were taken and studied that are sheep dropping and goat dropping. Physico-chemical analysis of some carrier of biological origin, which used in *Rhizobium* culture prepration were done. On the basis of physico-chemical properties it can be concluded that S_D is more suitable carrier than G_D. For evaluation of growth of *Rhizobia* on carriers, two sets were taken *Rhizobia*l growth on 100 mesh size S_D and G_D carrirs at 28^{o}C and 50% W.H.C. *Rhizobia*l + *Pseudomonas* growth on 100 mesh size S_D and G_D carrirs at 28^{o}C and 50% W.H.C.

For first set, it was observed on 100 mesh size carriers *rhizobia*l population is comparative less for both carriers than their mixed inoculation set. The suitability order for 100 mesh size carriers is $S_D > G_D$, from Fig. 1 also similar trend could be seen. The other conclusion which can be drawn from second set observations is, *rhizobium* growth is more when a mixture *Rhizobium* & *Pseudomonas* was used as inoculants. It is indicated from second set's observations that G_D which gives less *rhizobia*l population in first set have comparative more *rhizobia*l growth as is clear from Fig. 2 that its efficiency increases. It may be + ive influence of Psuedomonas bacteria on population of *rhizobia*, which ultimately increase nodule numbers and weight of plant.

Key words: *Pseudomonas*, *Rhizobia*l, Inoculant.

INTRODUCTION

Symbiosis plays an important role in productive agriculture particularly in context of production of pulses and other leguminous crops. *Rhizobium* alone help the production agriculture since eternity. Utilization of atmospheric nitrogen by crops continue to help the humanity and in this century considerable improvement in the technology of utilization of atmospheric nitrogen has made Bio-fertilizer industry eventually to compete with fertilizer

*Author for correspondence; E-mail: avinash_1965@yahoo.co.in

industry. Bio-fertilizers are vital components of sustainable agriculture. *Rhizobia* play an important role in nitrogen fixation. Previously peat is considered as an ideal carrier for *Rhizobia*l inoculants, but good quality peat is not available in India. Survival of *Rhizobium* in coal based type of inoculants was done by various scientists[1-3]. Later improved type inoculants were developed[4]. So some carriers of biological origin (sundried, sheep and goat droppings, their manures) were tired as carriers. It is assumed that decomposed product of organic matter is less complex and easily available for *Rhizobia*l growth. Their physico-chemical characteristics were compared with peat which is supposed to be an ideal carrier for *Rhizobia*l inoculants.

Bio-fertilizer help to provide nutrients to soil microorganisms which are required for plant growth. These are of low cost, their application is easy and does not cause pollution. It serve as bio-pesticide, and its viability is maintained for longer period which may increase sustainable agriculture. Now special emphasis is given to new concept i.e. inoculation by group of associated bacteria. The inoculants should be compatible with each other and will be able to grow simultaneously without any inhibition in growth. Positive result of mixed inoculation was found on bean and pea[5]. There was an associative positive affect of *rhizobium* and PSB on yield and nutrient uptake by chickpea[6]. Co-inoculation on soyabean was also tried[7]. Combined inoculation was found to be superior than individual inoculation[8]. Here in Beawar (Rajasthan) most of farmer grow gram crop. *Rhizobium* and phosphate solubilizing bacteria are important to plant nutrition and play a significant role as plant growth promoting rhizobacteria (PGPR) in bio-fertilization of crops. It was found *Pseudomonas* enhance growth of mustard[9]. A study is proposed to conducted on a suitable sterilized carrier (100 mesh sheep and goat droppings) to investigate the effects on growth of *rhizobia*l isolates for gram and pea, if they are used in combination of *Pseudomonas* (P solubilizer bacteria).

EXPERIMENTAL

Methods and materials

(a) **Preparation of carrier samples**: All the carriers sample sun dried on the roof top, then gently crushed with the help of wooden hammer, standard sieving were done of 80 and 100 mesh dimensions. The samples were kept in polythene bags

(b) **Methods of analysis:** Determination of physico- chemical properties namely pH, bulk density, particle density, W.H.C. percentage of organic matter, total Nitrogen, P_2O_5 content were carried out by adopting appropriate standard methodologies.

Procedure for testing suitability of carriers

- Inoculated 50 mL sterilized Y.E.M. borth with $R_{G,}$ (gram) or R_P (pea) isolate and incubates for 7 days.
- Took 20 g of each carrier in beaker. Add to S_D, G_D, 1.5 g of $CaCO_3$ each. To S_M, G_M were added 14 mL, 22 mL, of N/10 H_2SO_4 respectively for, the purpose of neutralization.
- Sterilized these carries at 15 lbs pressure. For 4 hours on three alternate days. For maintenance of 50% waters holding capacity of sterilized material of S_D, G_D, 32.0, 34.4 mL of sterile water mixed, respectively. In which there was 2.5 mL as inoculum of $R_{G,}$ (g) or R_P (pea) isolate was added.
- Mixed each material by separate sterile glass rods, each inoculated carrier was divided into 7 equal parts, out of which 6 part of each carrier were packed in polythene bags and placed at 28°C.
- From 7^{th} part of each withdrew one gm inoculated material and plated as usual for initial population of the inoculum. At subsequent interval of 10, 20, 30, 60 days the enumeration of *Rhizobia*l population is done using one packet of each at a time.

RESULTS AND DISCUSSION

Physico-chemical analysis of some carrier of biological origin carriers used in Rhizobium culture prepration

(i) As shown in Table 1 there is an increase in bulk density from G_D to $S_{D,}$ it can be justified on the basis of organic matter %, as the organic matter % decreases the material becomes denser[10].

(ii) Porosity percentage can be correlated with organic matter percentage, as organic matter % decreases it decreases, which is clear by porosity % of carriers shown in Table 1.

(iii) General trend regarding W.H.C., as organic matter % increases, it increases. It is observed W.H.C. of G_D $S_{D.}$

(iv) The organic matter % of G_D is less than S_D. as indicated in Table 1.

(v) Nitrogen percentage of G_D is less than S_D. But C/N ratio for G_D is more than; S_D. Positives effect of Nitrogen sources on N_2 fixation was observed[11]. Slow growing strains of *Rhizobium* were found to be stimulated by mixture of amino acids[12]. Addition of ammonium sulphate was found to improve *rhizobia*l counts in carrier materials[13]. It was also reported C/N ratio 17 to 50 is appropriate to *rhizobia*l growth.

(vi) P_2O_5 percentage is more G_D. for than S_D.

(vii) So on the basis of physicochemical properties it can be concluded that S_D (100 mesh size) is more suitable carrier than G_D 100 mesh size).

Table 1: Physico-chemical analysis of some carrier of biological origin carriers used in *Rhizobium* culture prepration

Parameter	Mesh size	G_D	S_D
Bulk density	100	0. 37	0.35
Particle Density	100	1.87	1.49
Porosity %	100	74.95	80.91
W.H.C. %	100	305.5	329.12
Organic matter %	100	61.0	77.5
N%	100	2.05	3.42
P_2O_5%	100	0.48	0.2
C / N ratio	100	17.3	13.17
Ash %	100	32.00	22.5
pH	100	6.1	6.0

Evaluation of growth of *Rhizobium* & *Rhizobium* + PS bacteria on cow urine treated & untreated sterilized carriers of biological origins to check the viability of *rhizobia* during storage of carriers

In Table 2 Population data of *Rhizobia*l isolate on S_D & G_D which were biological carriers of 100 mesh size at 28^{o}C and 50% W.H.C. has been given after 10, 20, 30, 60 days.

Comparison of wS_D & G_D up to 60 days S_D proves better than G_D, organic matter content, W.H.C. of S_D is more than G_D, simultaneously aeration is more in S_D which are essential requirement for suitable carrier as is seen in Fig. 1. One more drawback of goat dropping is its susceptibility to contamination, so *rhizobia*l population decreases abruptly.

For S_D upto 10 days there is an abrupt increase in *rhizobia*l population, then gradually it decreases. After 60 days it proves even better than goat dung as is clear in Table 2. The suitability order is $S_D > G_D$ from Fig. 1 similar trend could be seen finally it may be concluded from the above observations that the sheep dung of 100 mesh size is more suitable carrier and can be used efficiently as *rhizobia*l inoculant.

Table 2: Population data of *Rhizobia*l isolate on different biological carriers of 100 mesh size at 28°C and 50% water holding capacity.

Population (after days)

Carriers	0	10	20	30	60
Sheep dropping	5.0 x 10^5	6600 x10^5	4000 x 10^5	65 x 10^5	15 x 10^5
Goat dropping	2.5 x 10^5	3000 x 10^5	1820 x 10^5	40 x 10^5	4 x 10^5

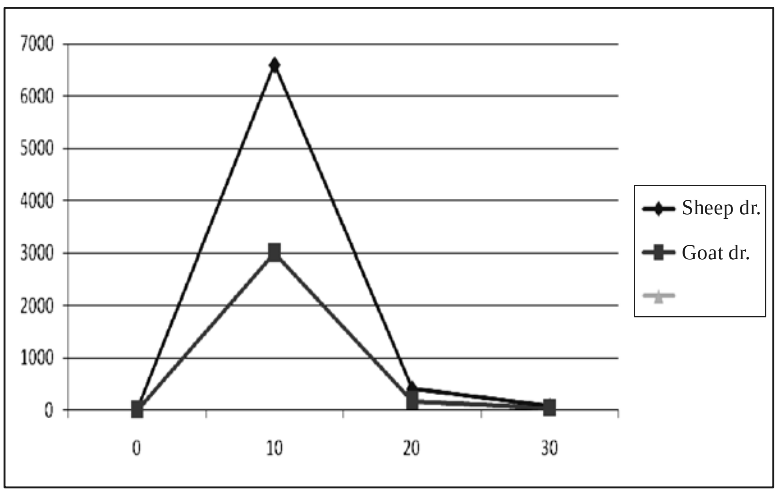

Fig. 1: Population data x 10^5 of *Rhizobia*l isolate on different biological carriers of 100 mesh size at 28°C and 50% water holding capacity

Table 3 indicates population data of *rhizobia*l isolate in which inoculant is a mixture of *rhizobia*l isolate + *pseudomonas* isolate on sterilized sheep dropping and goat dropping as carriers materialsof 100 mesh size at 28°C and 50% water holding capacity is maintained by sterized water. Data shows after 10 days there is sudden increase in population of *rhizobia* 10^4 to 10^8, both in S_D and G_D which is maintained upto 20 days. Then after 30 days it decreases upto 10^7. Carriers in which only *Rhizobium* inoculant is used it was observed though they are inoculated with high population of *rhizobia* there is gradual decrease in population more rapidly than of mixed inoculation. It indicates -

Table 3: Population data of *Rhizobia*l isolate + *Pseudomonas* isolate on different biological carriers of 100 mesh size at 28°C and 50% water holding capacity.

Population (after days)

Carriers	0	10	20	30	60
Sheep dr.	3.0 x 10^4	85000 x 10^4	40000 x 10^4	6500 x 104	1000 x 10^4
Goat dr.	3.5 x 10^4	45000 x 10^4	12000 x 10^4	4000 x 10^4	420 x 10^4

- Mixed inoculation works which was confirmed by pot experiments, number of nodules as well as plant weight is more in the plants of pot where seeds were inoculated by mixed culture's broth. It may be of + ve influence of Psuedomonas bacteria on population of *rhizobia* which ultimately increase nodule numbers and weight of plant.
- Phosphate solublizing effect of Psuedomonas bacteria play an important role in rise of *rhizobia*l population.

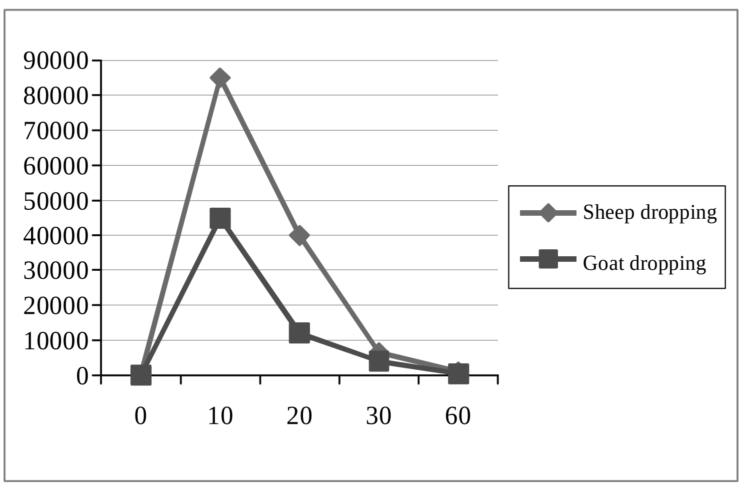

Fig. 2

- If we compare experiments, which were conducted without Psuedomonas bacteria, and the population data of *rhizobia*l isolate in which inoculant was a mixture of *rhizobia*l isolate + *pseudomonas* isolate on sterilized sheep dropping and goat dropping as carriers materials of 100 mesh size at 28^{o}C and 50% water holding capacity, we found more promising results as seen in Fig. 2. Even in GD there is abrupt rise in *rhizobia*l population upto two months.

- It may be + ve influence of Psuedomonas bacteria on population of *rhizobia* which ultimately increase nodule numbers and weight of plant in which co-inoculation was carried out.

REFERENCES

1. M. W. Paczkowski and D. L. Berryhill, Survival of Rhizobium Phaseoli in Coal-Based Legume Inoculants, Appl. Environ. Microbiol., **38**, 612-615 (1979).
2. S. D. Sparrow and G. E. Ham, Survial of Rhizobium Phaseoli in Six Carrier Materials, Agron. J., **75**, 181-184 (1983).
3. R. J. Kremer and H. L. Peterson, Effects of Carrier and Temperature on Survival of Rhizobium Spp. in Legume Inocula, Development of an Improved Type of Inoculant, Appl. Environ. Microbiol., **44**, 1790-1794 (1983).
4. N. S. Subbarao, Phosphate Solubilizing Micro-Organism, In: Biofertilizer in Agriculture and Forestry, Regional Biofert. Dev. Centre, Hissar, India (1988) pp. 133-142.
5. T. Natarajan, T. Thangaraj and G. Oblisami, Effect of Combined Inoculation of Rhizobium and Phosphobacteria on French Beans and Pea, Paper Presented in National Symposium on Biological Nitrogen Fixation in Relation to Crop Production, TNAU, Coimboture, Jan., 23-25 (1980).
6. R. Alagawadi and A. C. Guar, Associative Effect of Rhizobium and Phosphate Solubilizing Bacteria on the Yield and Nutrient up take of Chickpea, Plant Soil, **105**, 241-246 (1988).
7. L. M. Pant, G. V. Raghuram and A. K. Katiyar, Coinoculation of Phosphorus Solubilizing Bacteria with Bradyrhizobium Japonicum on Soyabean, J. Indian Soc. Sci., **43**, 691-692 (1995).
8. B. Anandaraj and Leema Rose Delapierre, A Studies on Influence of Bioinoculants (*Pseudomonas* Fluorescens, Rhizobium Sp., Bacillus Megaterium) in Green Gram J. Biosci. Tech., **1(2)**, 95-99 (2010).
9. P. Pandey, S. C. Kang, C. P. Gupta and D. K. Maheshwari, Rhizosphere Competent *Pseudomonas* Aeruginosa GRC1 Produces Characteristic Siderophore and Enhances Growth of Indian Mustard (Brassica Campestris), Epub. Oct. 5 (2005).

10. Antony Joesph, Madras Agric. J., **64(4)**, 211-217 (1977).

11. H. S. Jhonson and D. Hume, Rhizobium, News Letter, **18(2)** (1973).

12. H. Bhalla, Science & Culture, **39**, 4 (1973).

13. V. Iswarn, Curr. Sci., **41(8)**, 299 (1972).

Accepted : 06.05.2012