Int. J. Chem. Sci.: 10(3), 2012, 1591-1600
ISSN 0972-768X
www.sadgurupublications.com

EFFECT OF DECOMPOSITION AND TREATMENT OF CARRIER MATERIALS WITH COW URINE ON RHIZOBIAL POPULATION OF INOCULANTS

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

S. D. Govt. College, Beawar, AJMER (Raj.) INDIA
[a]Govt. Polytechnique College, AJMER (Raj.) INDIA

ABSTRACT

Bio-fertilizers are vital components of sustainable agriculture. Rhizobia play an important role in nitrogen fixation. Previously peat was considered as an ideal carrier for Rhizobial inoculants, but good quality peat is not available in India. 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 Rhizobial growth. Their physico-chemical characteristics were compared with peat which is supposed to be an ideal carrier for Rhizobial inoculants. Particle density for S_D to S_M decreases but for G_D to G_M increases; organic matters percentage for both (S_D. to S_M; G_D to G_M) decreases on decomposition, as organic matter after decomposition produces CO_2 W.H.C. for both (S_D .to S_M; G_D to G_M) increases on decomposition which supported the view that the polysaccrides which are ultimate products of organic matter's decomposition will bind several particles together discrete structural units, which allow more water to infiltrates and less to run off (high permeability) 'N' content decreases for both (S_D. to S_M; G_D to G_M) but C/N ratio increases (S_D. to S_M; G_D to $G_{M)}$ i.e. availability of 'N' content increases P_2O_5 content increases on decomposition in both cases. Surprisingly for all, pH changes from acidic to alkaline range. On comparison with Kashmiri peat the overall order of suitability of carriers was $S_M > G_M > G_D >$. S_D W.H.C, C/N ratio and P_2O_5 content are the deciding factor. Population data of BK_2 isolate on different carriers of 80 mesh size at 28°C and 50% W.H.C was studied upto 150 days and found that the order of suitability is $G_M > S_M > S_D > G_D$ so it can be concluded decomposed product proves better carrier for Rhizobial inoculant than its undecomposed product. In set where cow urine is used for maintaining W.H.C the rhizobial population increases abruptly in G_D which is confirmed by pot experiments, number of nodules as well as plant weight is more in the plants of pot where seeds were inoculated by carrier material which is treated with cow urine for maintatance of W.H.C. Use of cow urine act as growth promoting as well as biopestisde. In cow urine urea, uric acid, allantoin and creatinine all are antibacterial, antimicrobial agents, free aminoacids and ammonia are source of nitrogen helpful in growth. The potential of improving Rhizobial inculants with cow urine treatement (disease control agent) is immense.

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

Key words: Cow urine rhizobia, Biofertilizer, Sustainable agriculture; *Abberivations* : Goat dropping (G_D); Sheep dropping (S_D); Goat manure (G_M); Sheep manure (S_M); Water holding capacity (W.H.C.), Farm yard manure (FYM), Yeast extract mannitol (YEM).

INTRODUCTION

Suitability of peat materials for Rhizobium inoculant production is not of very high order because of long distance transport involved and poor quality of peat material in India, so alternative cheap source of carrier material need to be developed, as tried by[1-4]. For this four carrier materials, sheep and goat droppings; sheep & goat manures were tried. Main objective is to compare nutrients availability of carrier of rhizobial inocluant and suitability of undecomposed and decomposed product. It was supported that decomposition of organic matter, providing complex elements more available to plants[5]. Organic matter % decreases upon decomposition[6] as CO_2 evolves. In general, high organic matter materials have a higher water holding capacity and a higher moisture content[7]. More work has to be done for explanation of increases of W.H.C. of decomposed products though its organic matter % decreases upon decomposition, nitrogen content of carrier material effect rhizobial growth as supported by work of some scientists[2,8]. “N” Content of decomposed products is more than undecomposed one[9], P_2O_5 content increases upon decomposition[10]. Organic phosphorus content in FYM (decomposed products) increases upto 7 weeks[11].

The pH of decomposed products increases from acidic to alkaline range. For appropriate rhizobial growth, the necessary conditions are pH near about 7, good W.H.C. more nutrient availability[12]. Above conditions are fulfilled by decomposed product and so is observed in goat manure and sheep manure prove to be more efficient carriers than their undecomposed products i.e. goat and sheep droppings. The current global scenario firmly emphasizes the need to adopt eco-friendly agricultural practices for sustainable agriculture. Chemical agriculture has made an adverse impact on the health-care of not only soil but also the beneficial soil microbial communities and the plants cultivated in these soils. This eventually has lead to a high demand for organic product by the present-day health conscious society and sporadic attempts are being made by farmers all over the world to detoxify the land by switching over to organic farming dispensing with chemical fertilizers, pesticides, fungicides and herbicides. In India, organic farming was a well developed and systematized agricultural practice during the past and this ‘ancient wisdom’ obtained through Indian knowledge systems such as ‘Vedas’ specify the use of ‘panchagavya’ in agriculture for the health of soil, plants and humans i.e. cow dung, cow urine, cow milk, curd and ghee. Few farmers in the southern parts of India have used modified formulations of panchagavya and found them to enhance the biological efficiency of the crop plants and the quality of fruits and vegetables supported by work on biofertilizer[13] potential of

Traditional and Panchagavya. Utilizing panchagavya as biofertilizer was tested on the pulses and the cereal in soil amended with dried traditional and seaweed based panchagavya. Experimental seedling recorded higher rates of linear growth of both shoots and roots as compared to controls[14]. In the past three or four decades, the potential of seaweeds and their liquid extracts in agriculture used as a biofertilizer and a source of growth promoters.

EXPERIMENTAL

Methods and materials

(a) Sources of carriers materials: The materials used for the present investigations comprised sheep and goat dropping collected from S.K.N. College, Jobner (Rajasthan) sheep and goat manure College of Agriculture, Udaipur (Rajasthan) Kashmiri peat from Ganderwal (Kashmir).

(b) 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

(c) 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.

(d) Procedure for testing suitability of carriers:

(i) Inoculated 50 mL sterilized Y.E.M. borth with BK_2 isolates and incubates for 7 days.

(ii) 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 and Kashmiri peat were added 14 mL, 22 mL, 4.0 mL of N/10 H_2SO_4 respectively for, the purpose of neutralization.

(iii) Sterilized these carries at 15 lbs pressure. For 4 hrs. on three alternate days. For maintenance of 50% waters holding capacity of sterilized material of S_D, G_D, S_M, G_M and Kashmiri peat, 34.4, 32.0, 46.1, 37.7, 31.4 mL of broth mixed sterile water respectively. In which, there was 2.5 mL as inoculum of BK_2 isolate were added to each.

(v) 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^{o}C.

(vi) From 7th part of each withdrew one gm inoculated material and plated as usual for initial population of the inoculum.

(vii) At subsequent interval of 10, 20, 30, 60, 90, 150 days the enumeration of Rhizobial population is done using one packet of each at a time.

RESULTS AND DISCUSSION

As shown in Table 1 there is an increase in bulk density of 80 and 100 mesh size of decomposed product than their respective undecomposed material, it can be justified on the basis of organic matter % which decreases for decomposed product. As the organic matter % decreases the material becomes denser, which is supported by the study of earlier worker[1] who observed that bulk density decreases with organic matter[15].

(i) **Porosity percentage** can be correlated with organic matter percentage, as organic matter % decreases it decrease which is clear by porosity % of 80 and 100 mesh size samples of sheep and goat droppings & their manures.

(ii) **General trend regarding W.H.C.** is as organic matter % increases it increases but some literature is available which indicate that poly-saccrides – the product of organic matter decomposition-bind several soil particles together in discrete structural units which allow more water to infiltrate and less to run off (high permeability). It is observed W.H.C. of 80 and 100 mesh size samples of decomposed product is more than that of its respective undecomposed product though % of organic matter is in reverse order.

(iii) **The organic matter % of 80 and 100 mesh size samples -** It was found organic matter % decreases upon decomposition as CO_2 evolves. It is observed, organic matter decomposition produces ligands capable of complexing nutrient elements[6]. Complexed elements remain more available to plant roots. So it may that on decomposition of organic matter provide complexed element more available to rhizobial inoculant though organic matter % decreases on decompositions, as they observed organic manure (decomposed product), there was gain in soil physical health and associated enhancement in nutrient utilization[5].

(iv) **Nitrogen percentage** of 80 and 100 mesh size samples of sheep and goat droppings and their manures, there is a decrease of N % from undecomposed to decomposed product but C/N ratio increases upon decomposition. It may also be suggested that availability of nitrogen increases on decomposition which ultimately produce positives effect on N_2 fixation[9]. Slow growing strains of

Rhizobium were stimulated by mixture of amino acids[8]. Addition of ammonium sulphate improve rhizobial counts in carrier materials[2]. It was also reported C/N ratio 17 to 50 is appropriate to rhizobial growth.

(v) **P_2O_5 percentage** increases upon decomposition, as we observe in the given data of 80 and 100 mesh size samples in Table 1. N and P content increases after incubation[10]. During the incubation the cattle dung. I.P. was doubled in second week over initial content[16].

O.M. content in FYM (decomposed product) increased upto 7 weeks[11].

Table 1: Physico-chemical analysis of some carrier of biological origin and of Kashmiri peat which are used in Rhizobium culture prepration

Parameter	Mesh size	S_D	S_M	G_D	G_M	Kashmiri peat
Bulk density	80	0.33	0.38	0.29	0.41	0.46
	100	0.37	0.4	0.35	0.53	0.59
Particle Density	80	1.47	0.99	1.84	1.76	1.16
	100	1.49	1.32	1.87	2.75	2.28
Parasity %	80	77.56	72.10	84.24	76.4	74.40
	100	74.95	69.6	80.91	73.1	70.30
W.H.C. %	80	369.9	486.06	345.0	402.54	339.2
	100	329.12	338.2	305.5	339.65	328.10
Organic matter %	80	81.00	66.7	68.0	65.25	58.42
	100	77.5	59.63	61.0	51.26	53.12
N%	80	3.66	1.47	2.08	1.82	0.91
	100	3.42	1.94	2.05	2.09	2.32
P_2O_5%	80	0.46	0.58	0.21	0.9	1.73
	100	0.48	1.80	0.2	2.11	1.39
C/N ratio	80	12.86	26.38	19.00	20.84	37.32
	100	13.17	17.78	17.3	14.25	13.31
Ash %	80	19.00	22.7	19.00	34.74	40.87
	100	22.5	36.3	32.00	48.74	50.92
pH	80	6.1	7.8	6.1	8.2	7.6
	100	6.0	8.1	6.1	8.4	7.5

In Table 2, population data of BK_2 isolate on different carriers of 80 mesh size at 28 C and 50 % W.H.C. has been given after 10, 20, 30, 60, 90 and 150 days.

(i) After 10 days rhizobial population reaches upto 1010. Gradual decrease is observed thereafter upto 5th month. If we compare SD GD and SM GM upto 90 days SM, GM proves better than SD, GD, i.e. decomposed products serves as better carriers.

(ii) Though rhizobial population after one and two months on SD was more than SM but reverse case was observed after three and five months as is evident in Table 2.

(iii) GM and SM continuously upto 5th months prove themselves as better carriers than GD and SD, respectively.

(iv) On comparison with Kashmiri peat the overall order of suitability of carriers is SM > GM > GD >. SD W.H.C, C/N ratio and P_2O_5 content were the deciding factors.

(v) Population data of BK_2 isolate on different carriers of 80 mesh size at 28^{o}C and 50% W.H.C is studied 150 days and found that the order of suitability is GM > SM > SD > GD

(vi) So it can be concluded decomposed product proves better carrier for Rhizobial inoculant than its undecomposed product.

Table 2: Population data of BK_2 isolate on different biological carriers and Kashmiri peat of 80 mesh size at 28^{o}C and 50% water holding capacity

Carriers	Population (after days)						
	0	10	20	30	60	90	150
Sheep dropping	3.5 x 10^4	9.0 x 10^8	8.2 x 10^8	5.9 x 10^8	4.2 x 10^7	8.9 x 10^5	2.1 x 10^5
Sheep manure	4.4 x 10^4	8.5 x 10^9	9.5 x 10^8	8.0 x 10^7	1.5 x 10^7	9.5 x 10^6	2.7 x 10^6
Goat dropping	3.0 x 10^4	1.5 x 10^{10}	4.0 x 10^8	6.5 x 10^7	1.0 x 10^7	8.5 x 10^6	2.8 x 10^6
Goat manure	4.0 x 10^4	5.0 x 10^{10}	7.8 x 10^8	7.0 x 10^8	4 x 10^8	2.3 x 10^8	8.0 x 10^7
Kashmiri peat	2.6 x 10^4	9.2 x 10^{10}	2.8 x 10^9	8.6 x 10^8	6.8 x 10^8	9.2 x 10^7	4.5 x 10^7

Fig. 1, Population data of BK_2 isolate on different biological carriers and Kashmiri peat of 80 mesh size at 28°C and 50% water holding capacity after 10 days it was observed suitability order was K. peat > G_M > G_D >. S_M > S_D.

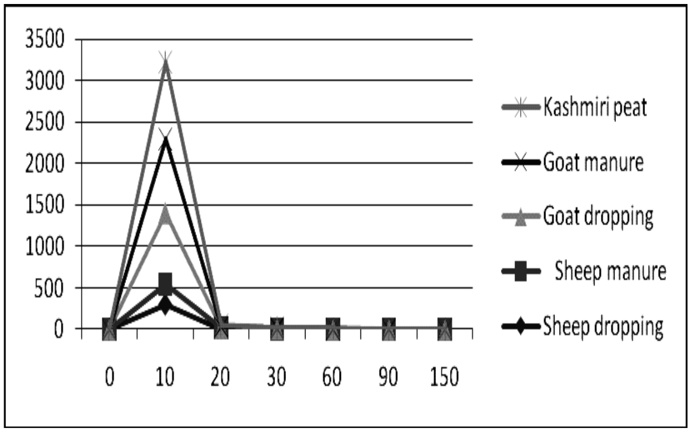

Fig. 1: Population data of BK_2 isolate on different biological carriers and Kashmiri peat of 80 mesh size at 28°C and 50% water holding capacity after 10 days

(i) GM's shelf life was more or less equivalent to Kashmiri peat, which is an ideal carrier for rhizobial inoculants

(ii) After one month there was a gradual decline in rhizobial population as seen in Fig. 2.

(iii) After 150 days it proves even better than Kashmiri peat as is clear in Table 2. The suitability order is GM > SM > GD > SD from Fig. 2 similar trend could be seen finally, it may be concluded from the above observations that the decomposed products are more suitable carriers and can be used efficiently as rhizobial inoculant.

(iv) In set where cow urine is used for maintaining W.H.C the rhizobial population increases abruptly in GD which is confirmed by pot experiments, number of nodules as well as plant weight is more in the plants of pot where seeds were inoculated by carrier material which is treated with cow urine for maintatance of W.H.C.

(v) It can be concluded, use of cow urine act as growth promoting as well as biopestisde. In cow urine urea, uric acid, allantoin and creatinine all are antibacterial, antimicrobial agents, free aminoacids and ammonia are source of nitrogen helpful in growth. The potential of improving Rhizobial inculants with cow urine treatement (disease control agent) is immense.

(vi) Though suitability order remains GM > SM > GD > SD but rhizobial population on SM and GD becomes more or less equal which indicate cow urine act as growth promoting as well as biopestisde.

(vii) Rhizobial population on all carriers were found to be in range of 108, which is a positive response.

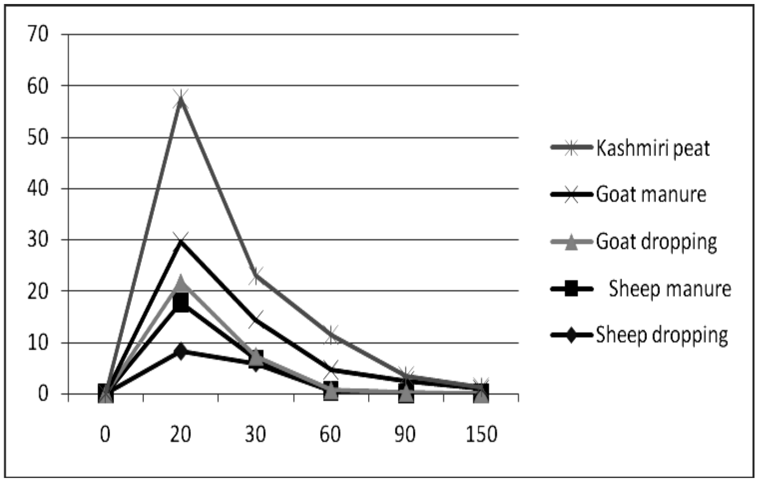

Fig. 2: Population data of BK_2 isolate on different biological carriers and Kashmiri peat of 80 mesh size at 28^{o}C and 50% water holding capacity upto 5th months

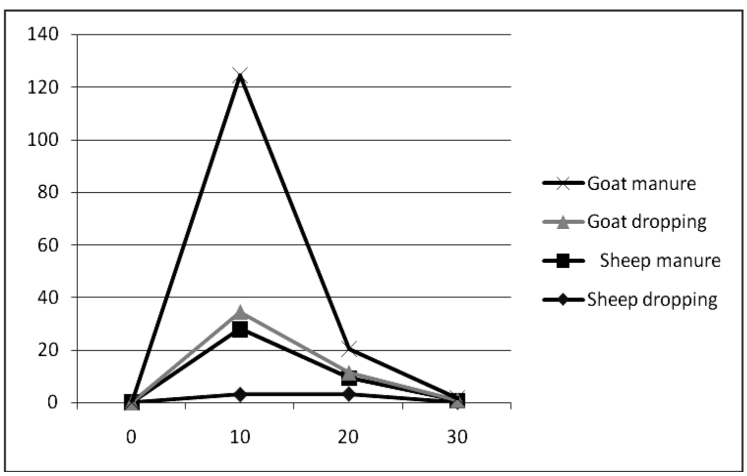

Fig. 3: Population data of BK_2 isolate on different biological carriers and Kashmiri peat of 80 mesh size at 28^{o}C and 50% water holding capacity (maintained by cow urine) upto one month

Table 3: Population data of BK_2 isolate on different biological carriers and Kashmiri peat of 80 mesh size at 28°C and 50% water holding capacity (maintained by cow urine) upto one month

Carriers	Population (after days)			
	0	10	20	30
Kashmiri peat	6-5 x 10^3	9.2 x 10^{10}	9.8 x 10^9	8.6 x 10^8
Sheep dropping	3.0 x 10^3	3.0 x 10^9	3.2 x 10^9	1.9 x 10^8
Sheep manure	2.4 x 10^3	2.5 x 10^{10}	6.1 x 10^9	5.0 x 10^8
Goat dropping	1.0 x 10^3	6.5 x 10^9	2.0 x 10^9	2.5 x 10^8
Goat manure	8.0 x 10^3	9.0 x 10^{10}	9.1 x 10^9	8.0 x 10^8

REFERENCES

1. P. D. Bajpi, B. R. Gupta and Ram Bali, Indian J. Agric. Res., **12(1)**, 39 (1978).
2. V. Iswarn, Curr. Sci., **41(8)**, 299 (1972).
3. K. S. Jauri, R. S. Bhatnagar and V. Iswaran, Curr. Sci., **48(4)**, 20 (1979).
4. P. Somasegaran, Report on Expert Consultation on Legume Inoculants Production and Quality Control, Food and Agricultural Organization Rome, (1991) pp. 87-106.
5. R. Lal and B. T. Kang, Trans., 12th Inter. Cong. Soil. Sci., New Delhi (1982) pp. 567.
6. N. S. Randawa and F. E. Broadbent, Soil. Sci., **99**, 362 (1965).
7. M. A. Khan, Science Journal, **44**,149-162 (1999).
8. H. Bhalla, Science & Culture, **39**, 4 (1973).
9. H. S. Jhonson and D. Hume, Rhizobium. News Letter, **18(2)** (1973).
10. P. J. Khankhane and B. R. Yadav, J. Indian Soci. Soil Sci., **48(4)**, 793 (2000).
11. N. K. Tomar, S. S. Khanna and A. P. Gupta, Indian J. Agric. Cam., **19(2)**, 25 (1986).
12. R. J. Roughly and D. Vinicent, J. Aus. Ind. Agric. Sci., **24**, 239 (1967).
13. V. Sangeetha and R. Thevanathan, Biofertilizer Potential of Traditional and Panchagavya Amendedwith Seaweed Extract, J. American Sci., **6(2)**, 61 (2010).

14. K. Tharmaraj, P. Ganesh, R. Suresh Kumar, A. Anandan and K. Kolanjinathan, A Critical Review on Panchagavya – A Boon Plant Growth Int. J. Pharm. Biol. Archives, **2(6)**, 1611 (2011).

15. Antony Joesph, Madras Agric. J., **64(4)**, 211 (1977).

16. S. S. Khanna, N. K. Tomar and A. R. Gupta, Proc. 3rd Inter. Cong. on Phosphorus Compounds Brusels Oct., 4-6 (1984).

Revised : 03.05.2012 *Accepted : 06.05.2012*