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Suitability of Vermicasts as Carrier Material for a Biofertilizer, *Azospirillum brasilense* (MTCC 4036)

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ABSTRACT

The vermicasts of an epigeic earthworm *Eudrilus eugeniae* Kinb was used as carrier material for a biofertilizer, *Azospirillum brasilense* (MTCC 4036). The study was conducted for a period of 10 months with the observation on the viable count of *A. brasilense* at regular intervals of 15 days. Results showed that about 1×10^7 g^{-1} viable cells of *A. brasilense* was found in some combinations of carrier materials (vermicasts: lignite) and that was significantly higher than experimental control i.e. lignite. The viable cells of *A. brasilense* showed a negative correlation with incubation period. A trend of increasing survival rate in *A. brasilense* in respect to increasing proportion of vermicasts in carrier material was observed. Results thus indicate the potential of vermicasts as carrier material for biofertilizers for sustainable crop production.

Keywords: earthworms, organic agriculture, vermicomposting, viability, worm cast
Abbreviations: BTB, bromo thymol blue; **CFU**, colony forming unit; **MTCC**, microbial type culture collection; **NPK**, nitrogen, phosphorus and potassium

INTRODUCTION

Biofertilizers are proven to have different beneficial roles in the soil-plant system including nitrogen (N) fixation, phosphate solubilization, nutrient uptake, etc. Biofertilizers are a cost effective supplement to chemical fertilizers and can help to economise the high investment needed for fertilizer use as far as N and phosphorus (P) are concerned (Motsara *et al*. 1995). The plant growth regulators produced by biofertilizers like *Azospirillum brasilense* could affect the growth of graminaceous plants like wheat and maize (Saikia and Jain 2007; Arancon and Edwards 2009).

Traditionally materials like lignite, charcoal and vermiculite are widely used as carrier materials for biofertilizers in India and these materials support the maintenance of 10^7 viable cells up to 4-6 months (Motsara *et al.* 1995). But recent studies have revealed that vermicompost could be a cost effective tool to carry biofertilizers in commercial crops. Recent evidences on the use of biofertilizers for enriching the vermicompost showed promising results on the enhanced quality of vermicompost (Kumar and Singh 2001; Kaushik *et al*. 2008). Recently, Kalra *et al.* (2008) reported that granular vermicompost supported the maintenance of 10^8 viable cells of *Rhizobium meliloti* Rmd 201 till 180 days when used as carrier material.

Vermicompost has been shown to have high levels of total and available nitrogen, phosphorous, potassium (NPK) and micro nutrients, microbial and enzyme activities and growth regulators (Parthasarathi and Ranganathan 1999; Chaoui *et al.* 2003; Prakash *et al.* 2008; Karmegam and Daniel 2009) and continuous and adequate use with proper management can increase soil organic carbon, soil water retention and transmission and improvement in other physical properties of soil like bulk density, penetration resistance and aggregation (Zebarth *et al*. 1999) as well as beneficial effect on the growth of a variety of plants (Atiyeh *et al*. 2002; Parthasarathi *et al.* 2008; Karmegam and Daniel 2008). The process of vermicomposting results in the increase of microbial diversity and activity dramatically and the vermicompost produced could be a definitive source of plant growth regulators produced by interactions between microorganisms and earthworms, which could contribute significantly to increased plant growth, flowering and yields (Arancon and Edwards 2009).

The aim of this study was to utilize vermicasts as carrier materials for the biofertilizer, i.e. *A. brasilense* with a focus on their survival rate and viability after incubation.

MATERIALS AND METHODS

Preparation of carrier material

Biogas slurry for the study was collected from the biogas plant situated at Kottagoundanpatty, Salem (Dt.), Tamil Nadu and used for the preparation of vermibed. Vermibeds were prepared in plastic trays of 45 × 30 × 15 cm size with 3 kg of biogas slurry (dry weight basis) in each tray. The vermibeds were moistened to hold 65-75% moisture content which was measured on the basis of oven dry weight of 5 g of vermibed materials. Then the vermibeds were allowed to stabilize for 24 h and 30 clitellate adult worms (*Eudrilus eugeniae* Kinberg, **Fig. 1**) were introduced into each tray. The moisture content (65-75%) of the vermibeds was maintained throughout the study by sprinkling tap water. The vermicasts found on the surface of vermibeds were collected from the 15th day onwards daily up to 30 days. The collected vermicasts (**Fig. 2**) were air dried, powdered and sieved through 100 µm sieve and stocked in polyethylene covers until use. Lignite was used as control carrier material in the study which was collected from a mineral separating industry situated at Salem (TN). The lignite and powdered vermicasts were mixed in various proportions (vermicast: lignite), 0: 1, 1: 1, 2: 1, 3: 1, 4: 1, 5: 1, 6: 1 and 1: 0. These mixtures were sterilized at 121°C in an autoclave for 3 h (Motsara *et al.* 1995) and allowed to cool at room temperature.

Mass multiplication of biofertilizers

The cultures of *Azospirillum brasilense* (MTCC 4036) were procured from Microbial Type Culture Collection (MTCC), Chandi-

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Research Note

Fig. 1 The earthworm species used in the study, clitellate *E. eugeniae*, in groups.

Fig. 2 Vermicasts of *E. eugeniae* used in the study.

garh and used for the present study. The organism was revived in suggested broth medium and sub-cultured in Bromo Thymol Blue (BTB) medium. A loopful of *A. brasilense* was transferred to 100 ml of BTB broth medium and incubated at 37°C for 72 h. After incubation, 10 ml of the inoculum was transferred to 1000 ml of BTB broth and kept in a shaking incubator at 60 rpm for four days.

Preparation of biofertilizer inoculum

The mass multiplied cultures of *A. brasilense* were then mixed with each ratio of carrier material as mentioned above. Each carrier material (165 g) was mixed with 35 ml of mass multiplied liquid culture holding 1×10^9 inoculants/g of carrier material. The different ratios of carrier materials with inoculum were packed in (7.6 × 12.7 cm) (transparent inner cover and white non transparent poly bags; **Fig. 3**) under sterile conditions as per the standard method described by BIS (Bureau of Indian Standards). Each combination of carrier material with inoculum was kept at room temperature (27 ± 2°C) in sealed bags with six replicates. The pockets with *A. brasilense* in carrier materials were subjected to viability analysis by using the spread plate technique as described by Benson (2002). The viability count of *A. brasilense* was individually carried out once every 15 days for a total period of 10 months. Data were subjected to analysis of variance (ANOVA) followed by

Fig. 3 Carrier materials (vermicasts: lignite) with *A. brasilense* in packets under storage.

Duncan's multiple range test to differentiate the significant difference between different treatments at the probability level of $P<0.05$ using SPSS® computer software for Windows (version 9.05). The relationship between viable cell counts in different carrier materials and incubation days was measured using correlation coefficient.

RESULTS AND DISCUSSION

The 10-months' study conducted on the viability of *A. brasilense* in different carrier materials upon incubation is shown in **Table 1**. The viable cells of *A. brasilense* were observed up to 4 and 6 months respectively in 0: 1 and 1: 1 combination of vermicasts and lignite. Though the number of viable cells decreased in subsequent months, the carrier material mix with higher proportion of vermicasts (3: 1, 4: 1, 5: 1, 6: 1 and 1: 0) sustained more than 1×10^7 g^{-1} cells indicating that the carrier materials with high proportions of vermicasts and vermicasts alone are able to support the survival of *A. brasilense*. After 135, 180 and 270 days of incubation of 0: 1, 1: 1 and 2: 1 vermicast: lignite carrier materials, there were no viable cells observed in the 10^{-7} dilution. The difference in the number of CFU counts in lignite was significantly different ($P<0.05$) than experimental control. The relation between viable counts of *A. brasilense* with reference to incubation period shows a negative correlation (**Fig. 4**). The correlation between CFU of *A. brasilense* in the carrier materials (vermicast: lignite), 0: 1, 6: 1 and 5: 1 and incubation period was highly significant ($P<0.01$).

The effect of N-fixing *Azotobacter chroococcum* strains, *Azospirillum lipoferum* and the phosphate-solubilizing *Pseudomonas striata* on N and P contents of the vermicompost clearly showed that the inoculation of N-fixing bacteria into vermicompost increased N and P contents (Kumar and Singh 2001). It is evident from the experiment conducted by Kaushik *et al*. (2008) that *A. chroococcum*, *A. brasilense* and *Pseudomonas maltophila* helped to increase the availability of N and P. Traditionally, materials like lignite, charcoal and vermiculite are widely used as carrier materials for biofertilizers in India and these materials support the maintenance of 10^7 viable cells up to 6 months (Motsara *et al.* 1995). Kalra *et al.* (2008) reported that maximum viable population of rhizobia could be recovered in granular vermicompost followed by charcoal and FYM after 6 months of incubation. In the present study, the required number of viable cells (1×10^7 g^{-1}) of *A. brasilense* was not observed in lignite in the 5th month of incubation (1: 0). It is evident from the present study that the vermicasts of *E. eugeniae* support the survival of *A. brasilense* until the end of the 10th month, which is longer than observed in lignite (4 months). The present observation supports the findings of Kalra *et al*. (2008), who reported similar results in vermicompost extract carrier material. Their report showed that a population of 1.0×10^6 - 2.1×10^6 CFU g^{-1} could be maintained even after one year in vermicompost carrier material. Results

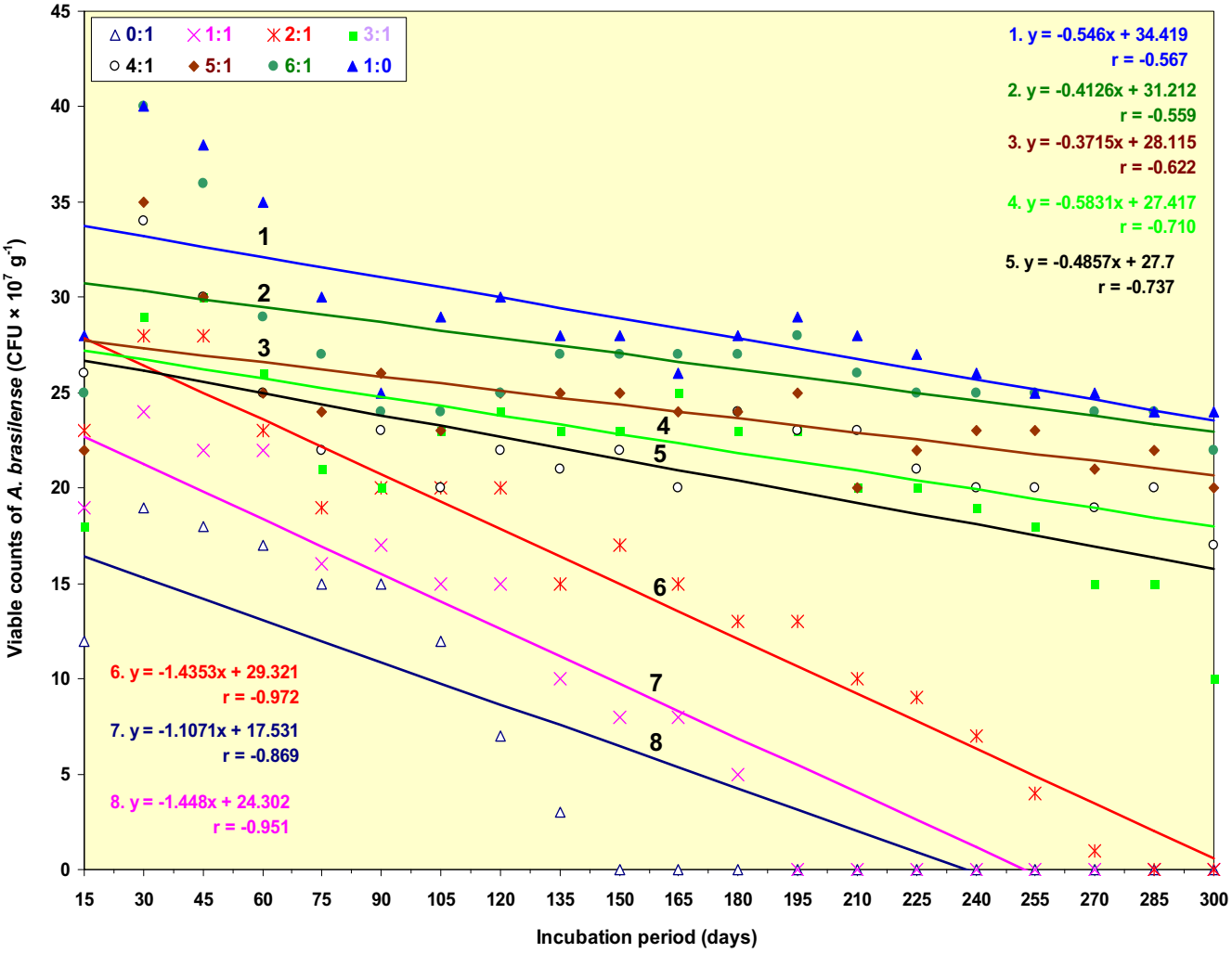

Fig. 4 Correlation between incubation days and colony forming units of *A. brasilense* (300 days) in different combination of carrier material (vermicasts: lignite). Numbers given over regression lines correspond to respective equation and correlation coefficient (r).

Table 1 Survival rate of *A. brasilense* in different ratio of carrier materials (vermicast: lignite, 300 days). Values are rounded of mean values of three replicates.

Incubation period (days)	Viable cells of *A. brasilense* (CFU × 10^7 g^{-1}) in different carrier materials used (Vermicast: Lignite)							
	0:1	1:1	2:1	3:1	4:1	5:1	6:1	1:0
30	19 a	24 ab	28 ab	29 ab	34 bc	35 bc	40 c	40 c
60	17 a	22 ab	23 ab	26 ab	25 ab	25 ab	29 b	35 bc
90	15 a	17 ab	20 ab	20 ab	23 ab	26 b	24 ab	25 b
120	7 a	15 b	20 bc	24 bcd	22 bcd	25 cd	25 cd	30 d
150	0 a	8 b	17 c	23 cd	22 cd	25 cd	27 d	28 d
180	0 a	5 a	13 b	23 c	24 c	24 c	27 c	28 c
210	0 a	0 a	10 b	20 c	23 c	20 c	26 c	28 c
240	0 a	0 a	7 b	19 c	20 c	23 c	25 c	26 c
270	0 a	0 a	1 a	15 b	19 bc	21 bc	24 bc	25 c
300	0 a	0 a	0 a	10 b	17 bc	20 c	22 c	24 c

The values with same letters between columns are not significantly different at 5% level (P<0.05) by Duncan's Multiple range test.

thus indicate a trend of increasing viability of biofertilizers with increasing proportion of vermicast in carrier material.

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