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Aging of pressmud vermicasts of *Lampito mauritii (*Kinberg) and *Eudrilus eugeniae* (Kinberg) - Reduction in microbial population and activity

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Abstract: *Total microbial population (including fungi, bacteria and actinomycetes) and activity (dehydrogenase) in the pressmud vermicasts of Lampito mauritii and Eudrilus eugeniae (fresh, 15 and 30 days old) have been determined. The fresh pressmud vermicasts of both worms show relatively higher microbial population and activity than uningested pressmud. This enrichment are due to nutrient rich substrate concentration, optimal moisture, germination or multiplication of microbes after passing through the gut and large surface area of vermicasts. Increased microbial activity results in increased mineralization of micro and macro nutrients in the casts. But in 15 days old casts of both the worms there is a relatively reduced microbial population and activity and significantly in 30 days old casts ($p<0.05$). Such reduced microbial population and activity in aged casts are due to reduced moisture, nutrient content due to depletion, leaching and immobilization and inactivation of microorganisms. Therefore, the application of fresh pressmud vermicasts with increased microbial population and activity to the soil support better improvement of soil fertility.*

Key words: *Pressmud, Microbes, Dehydrogenase, Aging, Casts.*

Introduction

During vermicomposting process, when organic matter passes through the worm's gut it undergoes physical, chemical and biochemical changes by the combined effect of earthworm and microbial enzymatic activities. The role of microbial activity in the gut as well as in the casts is very essential for the degradation of organic waste and release of nutrients to plants (Flack and Hartenstein, 1984; James, 1991). Worm casts, compared to worm un-worked soil / organic wastes have been shown to have increased microbial and enzyme activities and micro (N,P,K) and macro (Zn, Fe, Mg, Mn, Cu etc) nutrients (Satchell and Martin, 1984; Edwards and Bohlen, 1996; Parthasarathi and Ranganathan, 2000). The reduction of these in aging casts is mainly due to the depletion of microorganisms, organic carbon and moisture (Parle, 1963; Scheu, 1987; Parthasarathi and Ranganathan, 2001). Since such studies on the aging effect on casts are very sparse, an attempt has been made on pressmud vermicasts produced by *Lampito mauritii* and *Eudrilus eugeniae.*

Pressmud (P), a major by-product of sugarcane processing, is produced approximately to the tones of 12 million annually in India. It contains rich organic matter, organic carbon, sugar, protein, enzymes, micro (N,P,K) and macro (Zn, Fe, Mg, Mn, Cu etc) nutrients and microbes (Yaduvanshi and Yadav, 1990; Ranganathan and Parthasarathi, 1999; Parthasarathi and Ranganathan, 1998, 1999, 2000). Because of its smell, cost involved in transport and fear that its application may lead to crust formation, pH variation, pollution problems farmers are reluctant to apply this pressmud to their land. Conventional composting of pressmud takes about six months, does not remove the bad smell completely, has less nutritive value and is compacted. Vermicomposting of pressmud alleviates its potential pollution problems and converts it into an eco-friendly, organic fertilizer or soil amendment. Our earlier studies have established that pressmud when vermicomposted by *L. mauritii* and *E. eugeniae* has removed completely its bad smell and has significantly enhanced micro and macro nutrients, enzyme activities and microbes (Parthasarathi and Ranganathan, 2000). The present study aims only at determining total microorganisms including fungi, bacteria and actinomycetes and microbial activity by estimating dehydrogenase activity in freshly deposited, 15 and 30 days old pressmud vermicasts of *L. mauritii* and *E. eugeniae*.

Materials and Methods

Sixty, sexually mature clitellate earthworms, *Lampito mauritii* (Kinberg) (70-80 days old) and *Eudrilus eugeniae* (Kinberg) (40-50 days old) were reared in separate cement tanks (50 x 35 x 30 cm), each containing 8 kg. of two month old dried pressmud (P) obtained from E.I.D. Parry Sugarmill at Nellikuppam, Tamilnadu with 60-70% moisture and 29 $\pm$ 2ºC (Thermo-Hydrometer, Germany). After regular feeding on P, freshly deposited casts (F-one day old), 15 days old casts (15d) and 30 days old casts (30d) of both worms were collected and air-dried by spreading in large trays. The moisture content of the substrates (P, F, 15 and 30d) was determined by ordinary dry-base method.

The total microbial population (fungi + bacteria + actinomycetes) from the substrates was determined by dilution plate techniques. Each substrate of 1g was suspended in 1 ml sterile saline in sterile test tube, shaken thoroughly in a vortex mixer and was used as inoculum. Using standard loop, 0.01 ml of each inoculum was inoculated on blood, nutrient and MacConkey agar plates for bacterial growth, Sabouraud's dextrose agar plates for fungal growth and actinomycetes agar plate for actinomycetes growth and incubated at 25ºC and 37ºC for 18-24 hr for bacteria, 5-7 days for fungi and 11-12 days for actinomycetes. The number of colony forming units (CFU) was

Table – 1: Determination of microbial population and activity* in the pressmud casts of *L. mauritii* (A) and *E. eugeniae* (B) ($p<0.05$).

Microbial population	Pressmud	Fresh casts		15 days old casts		30 days old casts		Analysis of variance			
	(60-70%M)	A (43%M)	B (46%M)	A (25%M)	B (31%M)	A (10%M)	B (13%M)	W	X	Y	Z
Fungi (CFU x 10^4 g^{-1})	136.5	304.8	346.5	234.7	292.7	175.5	215.6	1840.3	5651.3	468.1	8.3
Bacteria (CFU x 10^6 g^{-1})	365	823.7	1758.6	706.6	1313.8	420	1018	21139.2	53534.3	7069.6	10.8
Actinomycetes (CFU x 10^5 g^{-1})	13	34.5	66.8	27.5	45	17.3	28.3	154.1	517.4	56.8	6.2
Total microbial population (CFU x 10^6 g^{-1})	514.5	1162.7	2169.8	968.3	1652	613.3	1262	25649.7	156636.4	7825.7	12.5
Dehydrogenase*	8.1	29	34.9	21.6	25.5	15.5	18.5	19303.1	69519.6	4954.6	0.59

%M – Percentage of moisture; w – F – substrates (days); y – F – (interaction) - level of significance at 0.05
* µH (hydrogen)/5 g substrates; x – F – (species); z – critical differences

expressed as CFU x 10^4 g^{-1} for fungi, CFU x 10^6 g^{-1} for bacteria, CFU x 10^5 g^{-1} for actinomycetes and CFU x 10^6 g^{-1} for total microbial population, respectively according to the method described by Baron *et al.* (1994). The dehydrogenase activity from the substrates was determined according to the method of Stevenson (1959) and the activities were expressed in µl H (hydrogen) /5g substrates. The data were analysed statistically by using analysis of variance (ANOVA).

Results and Discussion

Total microbial population and activity in the ejested pressmud vermicasts of *L. mauritii* and *E. eugeniae* (F, 15 and 30 d) are represented in the Table-1. Wormcasts form a suitable base for free living beneficial microbes whose activity is essential for release of nutrients in available form to plants (James, 1991). The size of microbial population in wormcasts mainly depends on the type and quality of ingested soil and plant materials (Edwards and Bohlen, 1996). Microbial biomass and activity were found to be more in the casts than underlying soil. The high microbial population and activity in the casts is believed to be caused by selective food consumption with high concentration of microbes (Scheu, 1987; Lavelle and Martin, 1992; Pedersen and Hendriksen, 1993; Parthasarathi and Ranganathan, 1999). In the present study P vermicasts of both *L. mauritii* and *E. eugeniae* had been shown to have significantly ($p<0.05$) higher microbial population (2.3 and 4.2 fold, respectively) and activity (3.6 and 4.3 fold, respectively) than uningested P. Such enhancement are due to nutrient rich substrate concentration, optimal moisture, germination/ multiplication of microbes after passing through the gut and the large surface area of P vermicasts are ideally suited for better feeding and multiplication of microbes. Our previous study has shown that pressmud vermicasts are the `hot spot' of fungi and bacteria (Parthasarathi and Ranganathan, 1998). Microbial biomass and activity was found to be decreased in aged casts (Scheu, 1987; Parthasarathi and Ranganathan, 2001). Reduced microbial and enzyme activities and micro and macro nutrients in aged casts were mainly due to reduced moisture, organic carbon and microorganisms (Scheu, 1987; Tiwari, 1996; Parthasarathi and Ranganathan, 2000). The present study shows that aged P vermicasts of *L. mauritii* and *E.eugeniae* (15 and 30d, respectively) have significantly reduced microbial population (1.2 and 1.9 and 1.3 and 1.7 fold, respectively) and activity (1.3 and 1.8 and 1.4 and 1.9 fold, respectively) ($p<0.05$) than F due to reduced moisture, nutrient content (Parthasarathi and Ranganathan, 1999) and may be due to leaching and immobilization and inactivation of microorganisms (Mulongoy and Bedoret, 1989).

Enhanced microbial population and activity in the fresh pressmud vermicasts (upto 15 days age old) would lead enhanced enzyme activities and nutrient mineralization. But in age old pressmud vermicasts (more than 30 days) there was reduced microbial population and activity that may leads reduced enzyme activities and nutrient mineralization. Thus in conclusion, the application of fresh pressmud vermicasts to the soil may helps to build and sustain soil condition and fertility.

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