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MICROBIAL SUCCESSION IN CASTS OF THE EARTHWORM, *EUDRILUS EUGENIAE*, FED UPON TENDU (*DIOSPYROS MELANOXYLON* ROXB.) LEAF RESIDUE

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ABSTRACT

Microbial succession in casts of *Eudrilus eugeniae* and in the control (compost without earthworms) was studied over a period of 120 days of incubation by maintaining optimum moisture conditions throughout the experiment. Total viable counts of different types of microorganisms such as total viable bacteria, total viable Gram negative bacteria, total phosphate dissolving bacteria, total fungi, total phosphate dissolving fungi, total actinomycetes, phosphate dissolving actinomycetes, total *Azotobacter* population and total nitrifying bacteria were noted as colony forming units per gram (CFUs/g) in casts and control using suitable growth media. In the cast material, there has been selectively favoured proliferation of certain beneficial types of microorganisms, particularly those which are significant in N and P transformations, as compared to the compost kept as control. However, the total viable bacteria, total fungi and total Gram negative bacteria also appear to follow, though in a diminished form, similar growth pattern in control like the casts. The components influencing growth of microbes, common to both cast and control, were adequate nutrients, moisture and aeration. It is, therefore, probable that certain gut-originated components may be responsible for favoured proliferation of selective beneficial microorganisms like those participating in N and P transformations.

INTRODUCTION

Vermicomposting is a process of composting organic waste material of diverse origin such as domestic, agricultural and industrial through the agency of suitable kind of earthworm species. The worms feed on organic materials, and fragment and digest them in their gut by their own enzymes and with the help of ingested microorganisms, and excrete nutritionally rich faeces or casts.

During this process, the nutritional elements in the organic matter are mineralized and concentrated into soluble and readily available forms through microbial action (Edwards 1995). The earthworm casts have been shown to contain increased quantities of important plant nutritional elements in the available form such as nitrogen, phosphorus and potassium together with the substances stimulating plant growth (Lee 1985). Moreover, it has been shown that worm casts contain increased populations of microorganisms as compared to surrounding soil or undigested organic matter (Parle 1963, Satchell 1967, Loquest et al 1977, Kale et al. 1992).

When earthworms feed on organic matter, they ingest many microorganisms, some of which are digested by the worms. However, the survived organisms, grow and flourish when the casts are excreted (Lee 1985, Dash et al. 1984, Edwards 1988, Tiwari et al. 1990). As the worm casts are rich in essential nutrients and possess adequate moisture and aerobic conditions, the growth and enzymatic activities of many microorganisms are enhanced in casts (Vinotha et al. 2000). However, a few

attempts have been made to investigate microbial processes associated with ageing of earthworm casts. Tiunov & Scheu (2000) studied the effect of gut passage, the age of the cast material and type of ingested substrate on microbial communities in earthworm casts. They reported increase in microbial respiration in fresh casts, but its subsequent decrease during cast incubation. This change may reflect both, a change in structure of the microbial community and the ratio between dormant and active stages of microorganisms. Thus, in spite of various studies on microorganisms in the casts, very little information is so far available on the succession of various microbial populations in the ageing casts. Hence, our aim was to study microbial succession in the casts incubated over an extended period under adequate moisture conditions at room temperature. It is hoped that the results will be useful for better understanding about dynamics of microbial processes during storage of vermicompost.

MATERIALS AND METHODS

Experimental Set-up

Adult, clitellate worms of *Eudrilus eugeniae* (Kinberg) were obtained from MPKV's Regional Dry Land Agricultural Research Center, Solapur. Prior to use in the study, the worms were adapted to partially decomposed leaf residues of tendu (*Diospyros melanoxylon* Roxb.) for two months at room temperature. Tendu leaf refuse was collected from a local beedi (a crude type of smoking stick with tendu leaf as the covering material) making unit and shredded into small pieces of about1 cm^2 and stored for further use.

Partial decomposition of the shredded leaf residue was carried out in plastic containers by adding mixture of materials rich in microorganisms. For this, the microbe-rich mixture was prepared by mixing equal amounts of fertile soil, cow dung, pressmud from sugar industry and sewage sludge. Ten kg of shredded leaf material was inoculated with 10% microbe-rich mixture and incubated at room temperature for 30 days. A moisture level around 60-70% was maintained by sprinkling water when required.

Six plastic pots (size: 27 cm diameter and 16 cm height) were used for the experiment. Out of the six pots, three were used with 400 g partially decomposed material plus 10 mature earthworms, while the remaining three as control by filling them each with only 400 g of the feed material without earthworms. All the six pots were kept at room temperature maintaining 60-70% moisture level in the feed material. 100g additional feed material was added to the pots containing earthworms after every 15 days.

Microbial Analysis

The casts from the three pots containing earthworms were collected to maximum and mixed together to form a composite sample. Ten gramme each of the casts and compost (control) were used for microbial study to make viable counts of various bacteria and molds by serial dilution method using specific media. The first sample of casts was collected after 24 hrs and then after every 15 days till 120 days of incubation. Different media, used for the growth of different microorganisms, are given in Table 1.

The MPNs of nitrosofying and nitrifying bacteria were determined by adding 1 mL of the respective dilution into five tubes each containing 5 mL of ammonium-calcium carbonate medium and five tubes with nitrate-calcium carbonate medium respectively (Subbarao 1999). The tubes were incubated at room temperature for three weeks.

Table 1: Different general and selective media and conditions of incubation used for counting total viable numbers of different microbial types.

Type of microorganisms	Media used	Nutrient amendment	Antibiotic added	Incubation period (days)	Incubation temperature,°C
Total viable bacteria	Soil extract agar	-	100mg/L cyclohexamide	7	R.T.
Total phosphate dissolving bacteria	Soil extract agar	0.05% Y.E. + 1% glucose. 5mL 10% K_2HPO_4 + 10mL 10% $CaCl_2$	100mg/L cyclohexamide	7	R.T.
Total fungi	Soil extract agar	-	100mg/L chloramphenicol	7	R.T.
Total phosphate dissolving fungi	Katznelson and Bose medium	0.05% Y.E. + 1% glucose. 5mL 10% K_2HPO_4 + 10mL 10% $CaCl_2$	100mg/L chloramphenicol	7	R.T.
Total Actinomycetes	Chitin agar	-	-	10	R.T.
Phosphate dissolving actinomycetes	Chitin agar	5mL 10% K_2HPO_4 + 10mL 10% $CaCl_2$ added to 100mL	-	10	R.T.
Total gram negative bacteria	Plate count agar	10 mg/L Carystal violet	-	7	R. T.
Total *Azotobacter*	Ashby's mannltol agar	-	-	7	R. T.
MPN of Nitrosofying bacteria	Ammonium- calcium carbonate medium	-	-	21	R. T.
MPN Nitrifying bacteria	Nitrite-calcium carbonate medium	-	-	21	R. T.

R. T. = Room temperature; Y. E. = Yeast extract

RESULTS AND DISCUSSION

Microbial succession in the casts of *Eudrilus eugeniae* and in the compost (control) was studied over 120 days of incubation under conditions of adequate moisture. The temporal changes that occurred in total viable counts of different microbial populations in the casts and control are given in Tables 2 and 3 and Figs. 1-10.

Total microbial populations

The maximum counts of total viable microbial populations in the casts were found after 60 days of incubation. In general, the total viable counts of different microbial types in beginning were low in the casts as well as in control, but their numbers increased gradually afterwards in both; however, the rise in the counts being more in the casts. Nevertheless, the changes observed in the casts were also mirrored in a diminished form in the control (Fig. 1). Certain important microbial physiological

Table 2: Average total viable counts (CFUs/g × 10^5) of different microorganisms found in the casts and control on different days.

Days	Total Viable Bacteria		Phosphate Dissolving Bacteria		Total Fungi		Phosphate Dissolving Fungi		Total Actinomycetes		Phosphate Dissolving Actinomycetes	
	casts	cont	casts	cont	casts	cont	casts	cont	casts	cont	casts	cont
1	10.5	550.0	7.60	2.1	0.76	3.13	0.34	1.35	2.53	0.80	1.80	.57
15	787.0	1137.0	53.3	10.8	1.97	3.0	1.28	2.5	6.13	3.0	4.10	1.56
30	1674.0	3756.0	94.2	49.0	8.34	9.18	6.3	5.5	109.0	14.2	98.0	6.7
45	6152.0	3756.0	29.0	13.0	85.0	46.0	45.7	25.0	210.0	64.0	147.0	12.0
60	7321.0	3669.0	28.0	10.4	169.0	47.0	116.0	38.0	190.0	49.0	159.0	7.0
75	1139.0	2045.0	125.0	47.0	75.0	48.0	60.0	7.0	68.0	35.0	32.0	17.0
90	3602.0	487.0	86.0	39.0	42.0	7.0	18.0	6.0	40.0	24.0	36.0	12.0
105	423.0	430.0	183.0	51.0	47.0	15.0	24.0	9.0	59.0	39.0	46.0	17.0
120	507.0	370.0	315.0	103.0	58.0	18.0	12.0	3.0	100.0	59.0	47.0	8.0

Table 3: Average total viable counts (CFUs/g × 10^5) of different microorganisms found in the casts and control on different days.

Days	Total Gram negative Bacteria		Total Gram positive Bacteria		Total *Azotobacter*		MPN: Nitrosofying Bacteria		MPN: Nitrifying Bacteria		Total Microbial Population	
	casts	cont	casts	cont	casts	cont	casts	cont	casts	cont	casts	cont
1	0.44	472.0	10.06	78.0	1.59	0.17	0.022	0.014	0.011	0.011	15.413	552.6
15	29.0	470.0	758.0	667.0	2.69	0.67	1.5	0.35	0.013	0.011	799.22	1144.0
30	401.0	1859.0	1273.0	477.0	45.0	12.0	1.7	0.017	0.02	0.014	1838.0	2372.0
45	3536.0	1249.0	2616.0	2507.0	15.3	10.4	2.1	0.021	0.028	0.016	6465.0	3877.0
60	923.0	1249.0	6398.0	2420.0	5.7	2.4	3.5	0.064	0.12	0.049	7690.0	3768.0
75	231.0	114.0	908.0	1931.0	17.2	0.17	1.8	0.049	0.069	0.026	1300.0	2130.0
90	56.0	42.0	306.0	445.0	23.0	0.17	0.022	0.014	0.0046	0.00006	467.0	519.0
105	86.0	52.0	337.0	378.0	26.0	0.28	0.028	0.002	0.0079	0.00004	555.0	485.0
120	53.0	47.0	454.0	323.0	23.0	6.0	0.21	0.00045	0.018	0.00095	689.0	453.0

groups such as phosphate dissolving actinomycetes and fungi, *Azotobacter*, nitrosofying bacteria, and nitrifying bacteria, showed significantly higher numbers in the casts than the control. The trend of proliferation of many microbial populations in the cast environment and control did not differ much except a few microbial groups. The increased numbers of microorganisms in the casts, however, is generally assumed to be due to adequate moisture, better aeration and available nutrients. The total viable counts of different microbial groups, found in this study, are suggestive of selectively enhanced proliferation of certain physiological groups in casts as compared to control.

According to Devliegher & Verstraete (1995), who studied nutrient enrichment processes (NEP) and gut associated processes (GAP), NEP increased microbial biomass, microbial activity and the specific metabolic activity of the microbial biomass in the soil. GAP, on the other hand, reduced microbial biomass and microbial activity through feeding and digestion of microorganisms. In their studies, the plate count determinations showed higher increase in microbial numbers, which was assumed mainly due to NEP. During the present study, plate count determinations were made using casts, incubated under adequate moisture conditions over extended period, and compared with those in control compost incubated under similar conditions. The selectively enhanced proliferation of

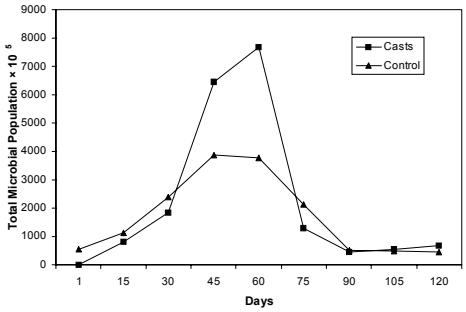

Fig. 1: Total viable microbial counts in casts and control.

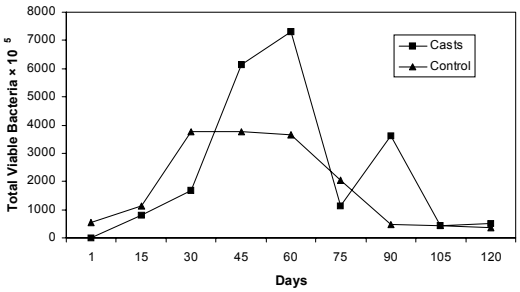

Fig. 2: Total viable bacterial counts in casts and control.

certain physiological groups like phosphate dissolving actinomycetes and fungi, *Azotobacter*, nitrosofying, and nitrifying bacteria suggests the possibility of certain components from gut associated processes (GAP) to be involved in proliferation of these microorganisms in the earthworm casts.

Total viable bacterial population

The total viable bacterial count closely followed the trend as shown by total microbial population (Fig. 2). In control the total viable bacterial counts initially after 1, 15, 30 days of incubation were about 52, 1.4 and 1.4 times higher than those in the cast respectively. The total viable bacterial populations later proliferated faster in the casts as compared to control. Thus, the total viable bacteria in the casts increased by 75, 159, 586, 697 times of the numbers on day-1, after 15, 30, 45, 60 days, while those in control only by 1.5, 3.0, 6.8 times after 15, 30, 45 days of incubation respectively. The total viable bacteria in casts reached their maximum numbers (7321 × 105CFUs/g) after 60 days of incubation, while those in control (3756 × 105CFUs/g) after 45 days. The counts declined continuously after 60 days in casts and 45 days in control till the end of the experiment.

The total viable count of Gram positive bacteria in the casts and control increased about 64-fold and 31-fold after 60 days of incubation with maximum numbers of 6398×10^5 and 2420×10^5 CFUs respectively. The maximum numbers of total viable Gram negative bacteria in the casts and control were found after 45 days of incubation (3536×10^5CFUs/g and 1249×10^5CFUs/g respectively) with a continuous decline thereafter. The major contribution to total bacteria has come from continued proliferation of Gram positive bacteria, especially during the later part of the experiment after 45 days.

The Gram positive bacteria contributed 95% of total viable bacteria and 91% of total microbial populations in the casts after 60 days of incubation as against 80% of total viable bacteria and 79% of total microbial populations in the control incubated for the same period. Remarkably, the total viable count of Gram positive bacteria in the casts after 45 days incubation was only 42% of total viable bacteria and 40% of total microbial populations, while in control it was 67% and 65% respectively. Thus, it seems that there may be a great shift in bacterial population in the casts during incubation at optimum moisture. The overall trend and bacterial succession show the possibility of deleterious effects on certain Gram negative bacteria in the casts, while there may be selective proliferation and survival or rejuvenation of Gram positive bacteria in the cast environment. Actinomycetes population may have certain role in inhibition of Gram negative bacteria through production of antibiotic like substances. The present data showed maximum total viable count of actinomycetes in the casts after 45 days of incubation coinciding with the start of decline in Gram negative bacterial counts.

Tiunov & Scheu (2000) noted an increase in microbial respiration in fresh casts with its subsequent decrease later during cast incubation. This may reflect a change in the structure of microbial community and in the ratio between dormant and active stages of microorganisms. It seems that these possibilities are reflected in the current results of plate count determinations.

The total actinomycetes reached their maximum numbers early after 45 days of incubation (Fig. 3). The early stimulation of actinomycetes might be due to availability of characteristic substrates like chitin. Damage to fungal hyphal cell walls during gut passage might enrich the casts with chitin (Cooke & Rayer 1984) and also other nutrients. Chitin can be effectively utilized only by actinomycetes. Actinomycetes maintained their numbers relatively at high level until completion of the experiment, which is suggestive of their importance in the microbial ecology of casts.

Total fungal population

The total fungal population reached maximum (169 × 105CFUs/g) in the casts after 60 days of incubation remaining almost four times higher than the control (47 × 105CFUs/g). However, the fungal population in control up to 30 days of incubation was higher than the casts. This is mainly due to preferential feeding and initial damage to fungal hyphae by earthworms. The total fungal count after 60, days declined gradually in both, casts and control (Fig. 4).

Total phosphate dissolving microbial populations

The total counts of phosphate dissolving fungi in the casts were much more higher than the control, even though the total fungal population was slightly greater in control initially up to 30 days of incubation (Fig. 5). This is suggestive of shifting of fungal populations to beneficial ones selectively in the casts.

The phosphate dissolving bacteria initially grew slowly up to 30 days and then showed a considerable dip after 45 and 60 days of incubation (Fig. 6). However, during this period total counts of phosphate dissolving actinomycetes (Fig. 7) and phosphate dissolving fungi were considerably higher. Later, the total viable counts of actinomycetes and fungi decreased, but phosphate dissolving bacterial counts gradually increased. Thus, there seems to be a temporal shift in the phosphate dissolving microbial populations in the casts. The process of phosphate dissolution is dominated initially by actinomycetes and fungi and later taken over by bacteria.

Microbial populations active in nitrogen transformations

The total *Azotobacter* counts in the casts increased rapidly up to 30 days of incubation (45 × 105 CFUs/g) and afterwards up to 105 days showing a temporary decline in between. In general, the *Azotobacter* counts were higher in the casts than the control (Fig. 8).

The MPN values for nitrifying and nitrosofying bacteria in the casts were significantly higher than the control. The maximum MPN values for nitrifying and nitrosofying bacteria in the casts were recorded after 60 days of incubation (0.12 × 105 and 3.5 × 105 respectively). A peculiar trend of proliferation was noticed with the populations of *Azotobacter*, nitrifying and nitrosofying bacteria, i.e., when the *Azotobacter* counts declined after 30 days of incubation, the MPN values of nitrosofying bacteria started increasing. When the MPN values of the nitrosofying and nitrifying bacteria were maximum after 60 days, *Azotobacter* population was minimum (Figs. 9 and 10).

The *Azotobacter* populations appear to be sensitive to the activities of other microorganisms and also to changing environmental conditions in the casts. This was evident from the observation that

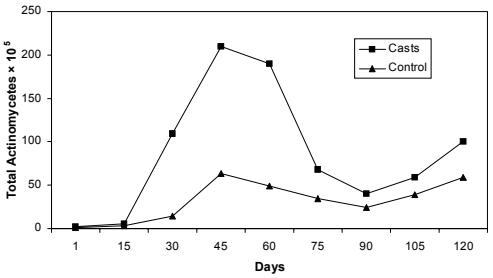

Fig. 3: Total actinomycetes counts in casts and control.

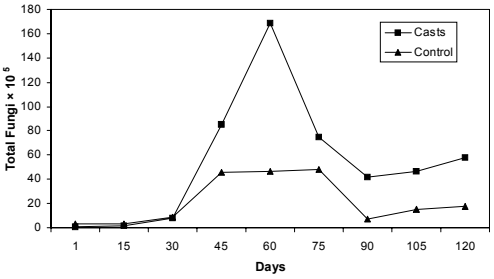

Fig. 4: Total fungal counts in casts and control.

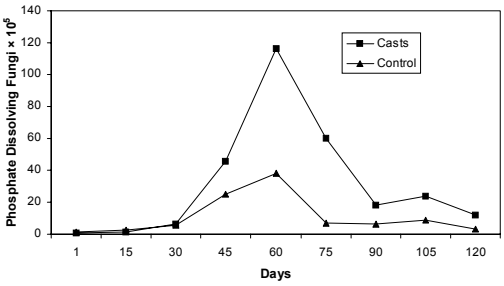

Fig. 5: Total phosphate dissolving fungal counts in casts and control.

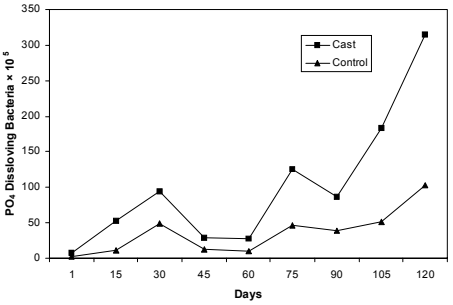

Fig. 6: Total phosphate dissolving bacterial counts in casts and control.

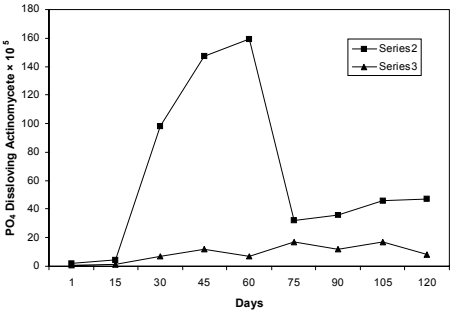

Fig. 7: Total phosphate dissolving actinomycetal counts in casts and control.

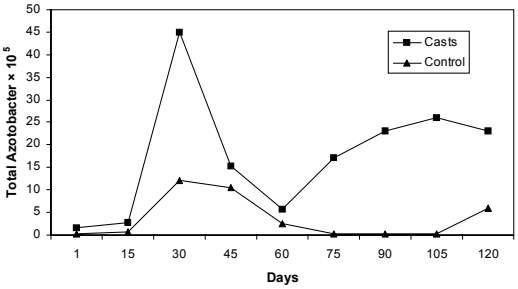

Fig. 8: Total *Azotobacter* counts in casts and control.

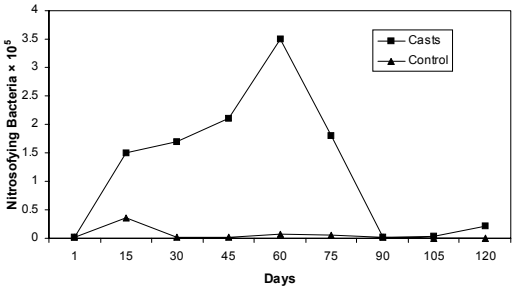

Fig. 9: Total nitrosofying bacterial counts in casts and control.

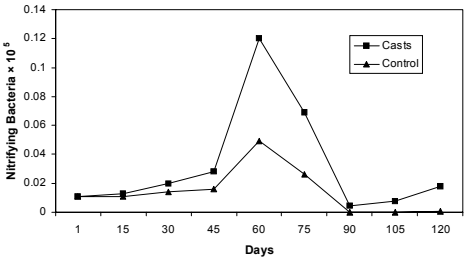

Fig.10: Total nitrifying bacterial counts in casts and control.

when nitrosofying and nitrifying bacteria reached their maximum numbers, the *Azotobacter* counts were relatively low. When *Azotobacter* counts increased again after 60 days incubation, the MPN values of nitrosofying and nitrifying bacteria declined. However, total viable counts of *Azotobacter*, MPNs of nitrosofying and nitrifying bacteria were much more in the casts than in the control compost.

CONCLUSIONS

The following conclusions can be drawn from the present studies.

1. The microbial succession in the casts incubated under optimum moisture levels might lead to favoured proliferation of certain physiological groups such as phosphate dissolving actinomycetes and fungi, nitrifying and nitrosofying bacteria, and *Azotobacter* as compared to compost (control).
2. There may be a shift in microbial types during cast incubation so as to favour the growth of certain kinds, and there may be elimination of certain microorganisms over an extended incubation under optimum moisture conditions.
3. As maximum viable counts of many beneficial microorganisms are found within 60 days incubation of casts, it is suggested that the vermicompost be used within 60 days of its formation, if stored, and handled at optimum moisture levels.

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