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ANALYSIS

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STUDIES ON ENZYME ACTIVITY OF EARTHWORM CAST FROM KITCHEN WASTE WITH COW DUNG

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ABSTRACT

The aim of this study was to assess the activity of enzymes during the course of decomposition of the substrate in the presence of epigeic earthworm species. Earthworm secretes enzymes namely amylase, cellulose and lipase, chitinase which degrade complex biomolecules into simple compounds utilizable by the symbiotic gut microflora. It is therefore easy to speculate that vermicast are rich in enzymes. The earthworms speed up the composting process and transform wastes into nutrient rich castings with help of these enzymes. The present study was taken up to find out the levels of amylase, cellulose, and lipase in vermicompost obtained different leaf litters and kitchen waste digestion by epigeic earthworm species at different time intervals (15, 30,45 days).

Key words: Epigeic earthworms, Vermicompost, Enzyme activity.

INTRODUCTION

Earthworms have an in-house supply of enzymes such as protease, lipase, amylase, cellulose and chitinase, which degrade complex biomolecule into simple compounds utilizable by the symbiotic gut microflora. The earthworms speed up the composting process and transform wastes into nutrient rich castings with the help of these enzymes. Castings are good fertilizer additive for agricultural crops (Kumar, 2004). The enzymes secreted by the earthworms alone and or in association with gut microflora are responsible for decomposition of complex organic materials and humification of soil organic matter (Dharmalingam, 2005). It is therefore easy to speculate that vermicasts are rich in enzymes which accelerated the mineralization rate and converted the wastes into organic fertilizer with higher nutritional value (Lakshmi Praba *et al.*, 2004).

Lakshmi Prabha et al., (2004) reported that significantly higher amylase, lipas and cellulose activities in vermicompost collected on 15th day of decomposition by the *Eisenia foetida* and *Eudrilus eugeniae* earthworms than in other samples. With increase in inoculation

time, the amylase, cellulose and lipase activities were decreased steadily for both species and a low value of amylase, cellulose and lipase activity was found on 45th day of inoculation time. Mishara (1993) reported that higher amylase activity be related to the presence of microorganisms in the gut capable of breaking down protein and starch. Higher activity of cellulase was reported in the worm casts by Edwards and Bohlen (1996). Mishra and Dash (1980) reported that the cellulose activity was less in *Perionyx millardi* in comparison to the activity recorded in other tropical worms.

The occurrence and activity of cellulase in the gut of clitellate stage indicates more utilization of carbohydrates by active clitellate worms (Parthasarathi, 2001). (Ranganathan and Vinotha, 1998) have recorded the occurance and activity of lipase in the gut of *Dichogaster bolani, Eudrilus eugeniae, Lampito mauritti, D.willis, Eutyphoes spp. and D.calebi.* Bansal and Kapoor (2000) studied vermicomposting with *E.foetida* of mustard residues and sugarcane trash mixed with cattle dung in a 90 day composting experiment. Vermicompost resulted in significant reduction in C:N ratio and increase in mineral N, after 90 days of composting. Microbial activity, as measured by dehydrogenase assay, increased upto 60 days and declined on further incubation.

MATERIALS AND METHODS

Collection and pre-decomposition of kitchen wastes and cowdung

The kitchen wastes were collected from college hostel. The collected kitchen wastes were chopped into small pieces and allowed to partial decomposition for 20 days. Then the waste was mixed with Cowdung in 3:2 ratio.

Epigeic earthworms used

The epigeic earthworms, *E. foetida, E. eugeniae* and *P. excavatus* were collected from Periyar maniammai University Vallam, Thanjavur, Tamil Nadu. The species were cultured at college laboratory, Government Arts College (Autonomous), Kumbakonam. Premises for six months.

Vermicomposting of kitchen wastes and cowdung

Totally four pots were maintained for the experimental purposes. The pots T1 was maintained as control for kitchen wastes and cowdung (without earthworm), T2, T3, T4, (with earthworm) were taken for composting of kitchen wastes and cowdung. In each pot a total of 3 kg of kitchen waste substrate and 2 kg of cowdung were taken and in T2, T3, and T4, pots the earthworm *E. foetida, E. eugeniae P. excavates earthworm* were released on the surface of the rate of 50 worms per pots except control. Care was taken to avoid light, rainfall and natural enemies. In control as well as in experimental pots, the compost sample was taken on 15th day,

30th and 45th day respectively for the analysis of enzymes. The activity of amylase, cellulase and lipase were estimated.

Amylase estimation

1.0 g of vermicompost sample was extracted with 10ml ice-cold 10 mm calcium chloride solution, kept for 3h at room temperature and centrifuged the extract at 54,000 g at 4 C for 20minutes. The activity of amylase was estimated by the method of Peter Bernfield (1995).

Cellulase estimation

1.0 g of vermicompost sample was dissolved in 5.0 ml of citrate buffer of pH 5.0 and centrifuged at 10000 rpm in a centrifuge for 15 minutes at room temperature. The supernatant was used for the assay. The activity of cellulase enzyme was done according to the method described by Dension and Koehn (1977).

Lipase estimation

1.0 g of vermicompost sample was homogenized with twice the volume of ice-cold acetone. Filtered and washed the sample successively with acetone, acetone : ether (1:1) and ether and air dried the sample and extracted 1.0 g of the sample in 20 ml ice-cold water. Centrifuged at 15,000 rpm for 10 minutes and used the supernatant as enzyme source. The activity of lipase enzyme was estimated by the method of Jayaraman (1981).

The results obtained were analyzed statistically and treatment means were separated using Duncan Multiple Range Test (DMRT).

Result

Activity of enzymes during the course of decomposition of the substrate in the presence of earthworms were determined

The earthworms secrete enzymes namely amylase, cellulase and lipase which bring about rapid biochemical conversion of the proteinaceous, cellulosic and lipid materials in a variety of organic wastes.

Amylase

The activity of amylase in the vermicompost at different time intervals (15, 30 and 45 days) by *P. excavatus, E. eugeniae and E. foetida* is given in table 1 and fig 1.The results revealed a significantly (P>0.01) higher amylase activity in vermicompost collected on 15th day of decomposition by all the three species of earthworms than in (control) other samples. With increase in inoculation time, the amylase activity had decreased steadily for all the three species and a low value of amylase activity was found on 45th day of inoculation time. The amylase activity decreased significantly as the composting period increases. Among the three selected species, amylase activity was higher in *P. excavatus* when compared to other species, *E. eugeniae* and *E. foetida*.

Cellulase

The activity of cellulase in the vermicompost at different time intervals (15, 30 and 45 days) by *P. excavatus, E. eugeniae and E. foetida* is represented in table 1 and fig 2.

In the present study, the highest cellulase activity (0.232) was noted in kitchen wastes and cowdung decomposted by *P. excavatus* on 15th day of inoculation time and the low activity of cellulase (0.092) was observed in vermicompost collected on 45th day of inoculation time by *E. foetida.* High substrate content in kitchen waste and cowdung at initial stage of decomposition night have induced the synthesis of cellulase on 15th day of earthworm inoculation as compared with other treatments. The level of cellulase was found to be significantly decreased as the composting period increases.

The enzyme cellulase acts upon the substrate degrades the complex cellulase material into simple compounds which is utilized by the gut microflora. In *P. excavates* the level of cellulose activity was more when compare to the level of cellulase in *E. eugeniae* and *E. foetida*. Cellulase activity was found to be decreased significantly at 1% level on 15, 30 and 40th day of composting *P. excavatus, E. eugeniae* and *E. foetida.*

Lipase

The activity of lipase in the vermicompost at different time intervals (15, 30 and 45 days) by *P. excavatus, E. eugeniae* and *E. foetida* is represented in Table 1 and fig 3.

Lipase is the enzyme involved in the catabolic degradation of fat. Similar to amylase and cellulase, lipase was also maximum in vermicompost collected on 15th day of inoculation, and the least was found in vermicompost collected on 45th day of inoculation. However, the contrast to amylase and cellulase, the activity of lipase was more *in E. eugeniae* when compared was found to be dcreased significantly 1% level on 15th and 30th day of composting by *P. excavatus, E. eugeniae and E. foetida* species.

DISCUSSION

Organic farming enhanced soil organic carbon, available phosphorus content and microbial population/enzymic activity of soil/vermisubstrate thus making it sustainable for organic crop production (Singh *et al*., 2007). Some species like *P. excavatus, E. eugeniae and E. foetida* play significant role in decomposting organic matter, mineral cycling and the activity of selected enzymes during the course of decomposition of the substrate (Edwards and Lofty, 1972; Edwards and Bohlen, 1996). Vermicomposting of cowdung, fruit wastes and kitchen wastes are more effective and less expensive and it yields a fully stabilized, composted materials which release essential nutrients for plant growth (Lakshmiprabha *et al.*, 2004; Umamaheswari *et al.*,

2004). Earthworms have an in-house supply of enzymes such as protease, lipase, amylase, cellulase and chitinase, which degrade complex biomolecules into simple compounds utilizable by the symbiotic gut microflora. The earthworms speed up the composting process and transform wastes into nutrient rich castings with help of these enzymes (Kumar, 2004). The enzymes secreted by earthworms alone and / or in association with gut flora are responsible for decomposition of complex organic materials and humification of soil organic matter (Dharmalingam, 2005). It is therefore easy to speculate that vermicasts are rich in enzymes which accelerated the mineralization rate and converted the wastes into fertilizer with higher nutritional value. The present study was taken up to find out the levels of amylase, cellulase and lipase in vermicompost obtained from different cowdung and kitchen wastes digestion by *P. excavatus, E. eugeniae* and *E. foetida* at different time intervals (15, 30 and 45 days).

The earthworms secrete enzymes namely amylase, cellulase and lipase which being about rapid biochemical conversion of the proteinaceous, cellulosic and lipid materials in a variety of organic wastes.The results revealed a significantly ($P>0.01$) higher amylase, cellulase and lipase activities in vermicompost collected on 15th day of decomposition by all the three species of earthworms than in other samples. With increase in inoculation time, the amylase, cellulase and lipase activities was decreases steadily for all the three species of this study and a low value of amylase, cellulase and lipase activities decreased significantly as the composting period increases. The might be due to the fact that amylases act upon the substrate, namely kitchen waste and cowdung and aids in the degradation of proteins and starch present in the kitchen waste results in decreased level of them that might be the reason for low level of amylase as composting time increases (Lakshmi praba *et al* ., 2004). Mishra and Dash (1980) reported that higher amylase activity might be related to the presence of microorganisms in the gut capable of breaking down protein and starch.

Higher activity of cellulase was reported in the worm casts by Edwards and Bohlen (1996) and Lakshmi praba *et al.*, (2004). Mishra and Dash (1980) reported that cellulase activity was less in *P.millardi* in comparsion to the activity recorded in other tropical worms. In this study, the cellulase activity was more in *P. excavatus* when compared to the level of cellulase in *E. eugeniae* and *E. foetida*. Cellulase activity was found to be decreased significantly at 1% level of on 15, 30 and 45 days of composting by *P. excavatus, E. eugeniae* and *E. foetida*. The occurrence and activity of cellulase in the gut of clitellate stage indicates more utilization of carbohydrates by active clitellate worms (Parthasarathi, 2001).

Lipase is the enzyme involved in the catabolic degradation of fat. Lipase was also maximum in vermicompost collected on 15th day of inoculation. However, in contrast to amaylase and cellulase, the activity of lipase was more in *E. eugeniae*, when compared to *P.*

excavatus and *E. foetida* species. Lipase activity was found to be decreased significantly at 1% level on 15th and 30th day of decomposting by *E. eugeniae, P. excavatus,* and *E. foetida.*

Ranganathan and vinotha (1998) have recorded the occurrence and activity of lipase in the gut of *Dichogaster bolaui, E. eugeniae, Lampito mauritti, Eutyphoeus spp*. and *D. calebi*. Similar to this study Lakshmi Prabha *et al*., (2004) also reported the lipase activity was found to be more in *E. eugeniae* when compared to *E. foetida* species. Among the three species selected for the study, the levels of amylase and cellulase were higher in *P. excavatus* than *E. eugeniae* and *E. foetida*. In contrast to this, the level of lipase was more in *E. eugeniae* than *P. excavatus* and *E. foetida.*

Table 1

Level of amylase, cellulase and lipase in the kitchen wastes and cowdung at different time intervals (15, 30 and 45 days) of composting by *P.eugeniae* and *E.foetida*

Enzymes*	Earthworms species	Control (without earthworms)	Decomposition time		
			15 days	30 day	45 days
Amylase	*Perionyx excavatus*	0.099	0.280**	0.224**	0.184**
	Eudrilus eugeniae	0.099	0.154**	0.128**	0.109 NS
	Eisenia foetida	0.099	0.145**	0.122	0.105 NS
Cellulase	*Perionyx excavatus*	0.084	0.232**	0.134**	0.094 NS
	Eudrilus eugeniae	0.084	0.226**	0.128**	0.091 NS
	Eusenia foetida	0.084	0.197**	0.097	0.092 NS
Lipase	*Perionyx excavatus*	0.092	0.346**	0.248**	0.202*
	Eudrilus eugeniae	0.092	0.382**	0.284**	0.214**
	Eisenia foetida	0.092	0.312**	0.212**	0.190 NS

**P, 0.01; NS – Not Significant;

Units: * Amylase – mg of maltose / min / mg starch

Cellulase – mg of glucose / min / mg protein

Lipase – mill is equivalent / min / mg protein

Level of amylase, cellulase and lipase in the kitchen wastes and cowdung at different time intervals (15,30 and 45 days) of composting by *P. excavatus, E. eugeniae* and *E. foetida*.

Fig .1. Amylase

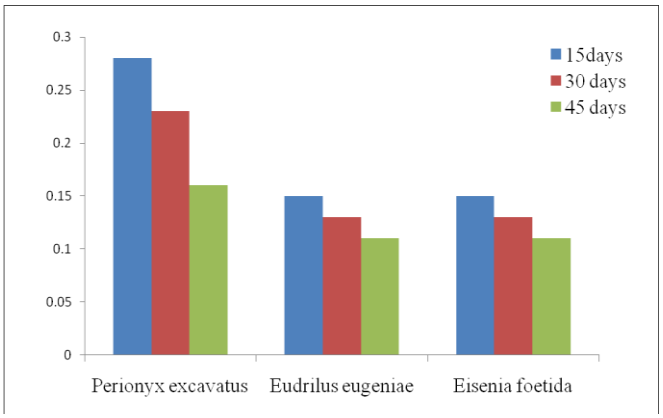


Fig . 2.Cellulase

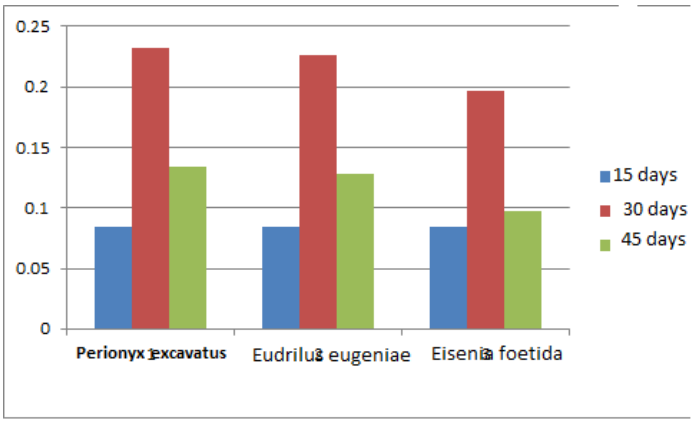
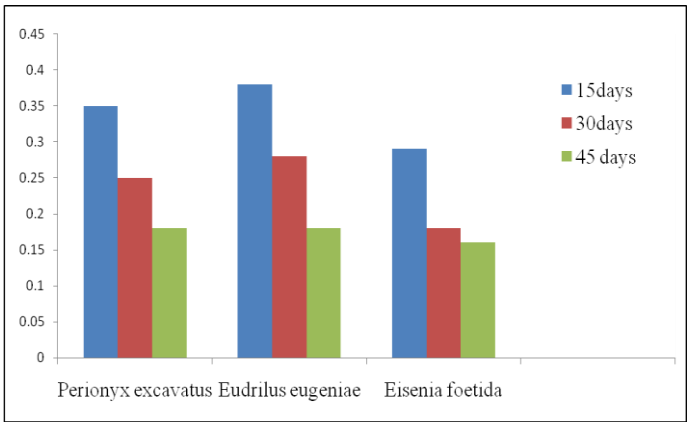


Fig . 3. Lipase



CONCLUSION

The activity of amylase, cellulase and lipase in the vermicompost at different time intervals (15, 30 and 45 days) by *P. excavatus*, *E. eugeniae* and *E. foetida* revealed a significantly (P>0.01) higher amylase, cellulase and lipase activity in vermicompost collected on 15th day of decomposition by all the three species of earthworms.

The amylase, cellulase and lipase activity decreased significantly as the decomposing period increases.

In contrast to amylase and cellulase, the activity of lipase was more in *E. eugeniae* when compared was found to be decreased significantly 1% level on 15th and 30th day of decomposing by *P. excavatus, E. eugeniae and E. foetida* species.

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