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Changes in Growth Rate and Activities of α Amylase and Cellulase of *P. excavatus*, an Epigeic Earthworm in Decomposing Leaf Litter of Five Tree Species

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Abstract:
Growth rate of epigeic earthworm Perionyx excavatus was recorded in the decomposing leaf litter of five tree species, namely Acacia auriculiformis, Shorea robusta, Anacardium occidentale, Eucalyptus citridora, Mangifera indica under laboratory conditions on 15th, 30th and 45th day. Biomass of earthworms increased significantly from the initial value in all the leaf litters tested but maximum weight gain indicating highest growth rate was observed in the litter of Anacardium occidentale (cashew) (4.15 ± 0.12 mg/worm/day) and minimum growth rate was observed in the litter of Eucalyptus citridora (eucalyptus) (2.92 ± 0.03 mg/worm/day) in 45 days. The digestive enzymes, i.e., α amylase and cellulase activities of P. excavatus were comparatively higher in Anacardium occidentale in comparison to Eucalyptus citridora during the experiment indicating the higher palatability of Anacardium sp (cashew) for P. excavatus.

Keywords*: Perionyx excavatus, leaf litter, growth rate, α amylase, cellulase*

1. Introduction

Earthworms constitute nearly 80% of invertebrate biomass in most soils (Sinha et al., 2003; Tondoh et al., 2007). Feeding and casting activities of earthworms significantly promote organic matter decomposition and enhance mineralization of soil organic matter in terrestrial environment. The major part of the net primary production enters through the detritus based food web litter. Earthworms have been known to breakdown leaf litter to release the nutrients content in the leaves. Litter decomposition is an important process regulating energy flow, nutrient cycles and structures of ecosystem. Many studies have shown that decomposition is influenced by different factors including litter quality and soil biota.

The role of earthworms in increasing decomposition and hence enhancing soil fertility and productivity is universally acknowledged (Owa et al., 2004). Earthworms have an in-house supply of enzyme such as amylase, cellulose, acid phosphatase and alkaline phosphatases. These enzymes biodegrade the complex biomolecules into simplier compounds and are responsible for the decomposition and humification of organic matter.

Quantitative differences in the activities of enzymes in the different regions of the gut of worms indicate regional specialization. Such differential activities of enzyme have been related to the types of food and rate of feeding of each species (Mishra & Dash, 1980 ; Mishra, 1993). However, our knowledge of the influence of different substrate media on the enzymatic activities of different earthworm species is limited (Baskaran et al., 1986).

The ecological suitability of different leaf litter types in improving biological diversity and micronutrient status in soil needs to be explored. In the present study, influence of five different leaf litters on the growth rate and enzyme activities of *Perionyx excavatus*, a major soil fauna known for its efficacy in enhancing decomposition and nutrient recycling has been studied. This has importance in understanding how the soil biota is affected by litter quality which in turn regulates entire process of nutrient recycling and fauna mediated mobilization of energy in natural ecosystems.

2. Materials and Methods

2.1. Collection and Culture of Test Specimens

Specimens of *Perionyx excavatus* were collected from the grasslands around Midnapore town (W.Bengal, India). Organically rich systems like composite pits, domestic and industrial organic waste deposit sites were chosen for specimen collection. Hand digging and sorting of soil method was used for collection of worms (Coleman et al., 2004).

The earthworms were cultured in the laboratory in earthen pots. Soil used for the medium was collected from the same grasslands from where specimens of *P. excavatus* were collected. Finely grounded soil was mixed with farmyard manure in the ratio 1:1 and was used as the culture medium for both the specimens (Ismail, 1997). The culture pots were covered with fine meshed iron nets and kept inside BOD incubators at 28 ± 0.5°C. An approximate level of 50- 60 % moisture for *P. excavatus* was maintained by adding distilled water into the medium. Dried and ground farmyard manure was added as feed every week during the entire period of culture.

2.2. Selection of Leaf Litter

Leaf litter of the following five different tree species that are common in Midnapore as well as in the state of West Bengal, India were used.

Serial No.	Common Name	Scientific Name
1.	Acacia	Acacia auriculiformis
2.	Shal	Shorea robusta
3.	Cashew	Anacardium occidentale
4.	Eucalyptus	Eucalyptus citridora
5.	Mango	Magnifera indica

Table 1: Different leaf litters used in the study

2.3. Collection and Preservation of Leaf Litter

Freshly fallen senescent leaves of the above mentioned tree species were collected in large quantities during the months of January to March, representing the period of highest litter fall. These were properly air dried and stored in labelled polythene container.

2.4. Determination of Growth Rate of Test Organism

100 gm of different leaf litter was taken in small inert plastic containers (16x12x1cm; total area, 192 cm^2) with plastic lids pierced by aeration holes. Five juveniles (one week old) *Perionyx excavatus* each in the range of 9.9 – 14.6 mg fresh weight, were collected from stock culture in laboratory. These worms were pre-weighed and inoculated in experimental containers. Each experiment was replicated three times. The earthworms were counted and weighed every 15 days up to 45 days.

2.5. Estimation of activity Digestive Enzyme

The gut homogenate of test specimen were used as the enzyme source. The specimens were put to starvation for at least 2 hours to clean the gut of food materials and faces. quantitative estimation of digestive enzyme viz α amylase (Bernfield, 1955) and cellulase (Sadasivam & Manickam, 2006) were estimated.

2.7. Statistical analysis

The data were analyzed using Excel (version 2007) and SPSS (version 10).Data were subjected for analysis of variance (ANOVA) followed by Duncan's multiple ranged tests to differentiate the statistical difference between results of earthworm growth in different culture material.

3. Results and Discussion

On the 15th day of the experiment, *Perionyx excavatus* showed maximum gain of biomass in the litter of mango (1.253 ± 0.132 mg/worm/day) followed by cashew, shal, acacia and eucalyptus (0.893 ± 0.20). On the 30th day, maximum growth rate was observed in the litter of cashew (3.890±0.010) followed by shal (3.823 ± 0.007), mango (3.963 ± 0.093), acacia (3.877 ± 0.027) and eucalyptus (2.843 ± 0.045). At the 45th day, maximum growth rate of *P. excavatus* was registered with litter of cashew (4.153±0.121) followed by shal (3.820 ± 0.035) and acacia (3.433±0.096) whereas minimum growth rate was observed in the leaf litter of eucalyptus (2.927 ± 0.038).

Leaf litter	Initial(mg)	after15 days(mg)	Growth rate*	After 30days(mg)	Growth rate*	After 45 days (mg)	Growth rate*
Cashew	166.33±2.60	329.66±17.70	1.05±0.14^{ab}	1335.00±3.78	3.890±0.10^{a}	2037.33±56.59	4.15 ±0.12^{a}
Mango	177.66±10.26	366.00±26.63	1.25± 0.132^{a}	1368.66±34.26	3.96±0.93 ^{a}	2013.33±20.9	4.07± 0.02^{a}
Shal	162.33±1.45	318.00±9.23	1.03±0.07^{ab}	1310.00±1.155	3.82±0.00 ^{a}	1883.33±16.70	3.82± 0.03^{b}
Acacia	167.66±4.33	339.33±12.97	1.14±0.07^{ab}	1331.33±12.11	3.87± 0.02^{a}	1714.33±46.92	3.43± 0.09^{c}
Eucalyptus	173.3±3.52	308.00±3.46	0.89±0.02 ^{c}	1027.33±17.14	2.84± 0.04b	1491.66±19.65	2.92± 0.03^{d}

*Table 2: Trend of change of biomass (mg)(mean SE; n=3) of P. excavatus in different phases of experimental study (0-45 Days) in different leaf litters, * (mg worm-1 day-1)*

The mean values followed by different letters are statistically different (ANOVA, Duncan's multiple ranged test; p< 0.05)

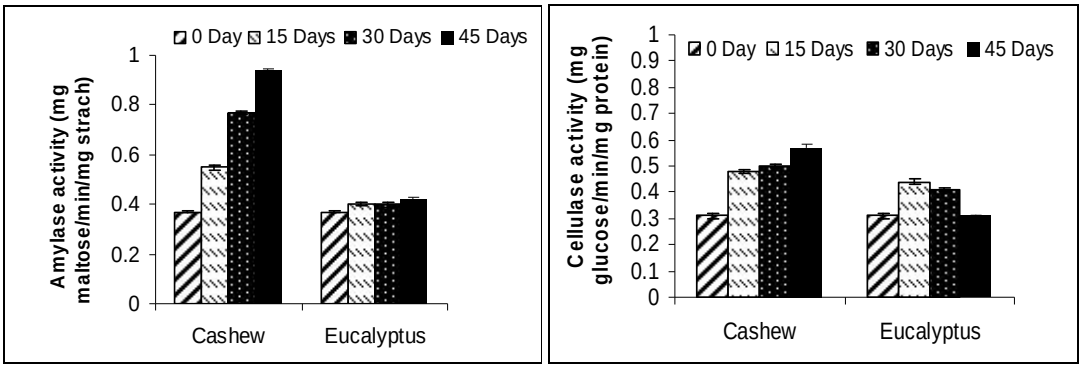

Figure 1: Activity of α amylase and Cellulase of P.excavatus in the most and least preferred leaf litter in different phases of experimental study (0 -45 days.)

Activities of the enzyme α amylase was higher in the litter of cashew in comparison to eucalyptus where the enzyme activity increased from 0.377 ± 0.003 to 0.966 ± 0.028 mg maltose/min/mg starch in 45 days. The activity of cellulase was also found to be higher in cashew in comparison to eucalyptus where the enzyme activity increased from 0.310 ± 0.006 to 0.560 ± 0.001 mg glucose/min/mg protein in 45 days. In eucalyptus, activity of cellulase increased initially to 0.45 ± 0.000 in 15 days and again was found to decrease to 0.313 ± 0.003 mg glucose/min/mg protein.

In the present study a relationship between litter characteristics and their palatability for *P excavatus*, a major soil fauna was found which was reflected in the growth rate of the worms and activities of certain digestive enzymes of the test specimen. Earlier studies have shown that litter of cashew has higher content of reducing sugar, total carbohydrates and protein whereas lowest nutrient content for all the parameters were found in the leaf litters of eucalyptus (Pattanayak et. al., 2014). Again, in case of anti-nutrient parameters, highest content of tannin and polyphenol was found in eucalyptus. Experiments of decomposition in litter bags and litter food selection carried out previously also exhibited maximum colonization of *P. excavatus* in litter of cashew than in comparison to eucalyptus (Pattanayak et. al., 2014). Kale et al., (1992) reported that the growth of earthworms depended on the quality of the available food. Highest and lowest growth rates of *P. excavatus* in cashew and eucalyptus respectively thus can be related to the chemical constituents and consequent palatability of the leaf litter by the test specimens. Researchers have reported that higher growth of earthworm can be related to the availability of more nutrients and N content in the leaf litter (Banu et al., 2005; Loh et al., 2005 and Shekal et al., 2005).

The digestive enzymes of the litter feeding aniimals, particularly oligochaetes, are responsible for decomposition and humification processes and their activity is directly related to many factors including litter type and its palatability (Parthasarathi & Ranganathan, 2000). Comparatively higher enzyme activity for both the enzymes of *P. excavatus* in cashew in comparison to eucalyptus indicated the higher palatability of cashew for the test specimen which could be assimilated more efficiently consequently enhancing the growth rate of *P. excavatus*.

4. Conclusion

It was concluded from the present study that higher palatability of cashew can be related with its litter characteristics and was reflected in increased growth rate and higher rates of enzyme activity of the test specimen. Thus cashew can be considered as suitable tree species in afforestation practices for enhancing nutrient recycling and for environmental preservation and sustainability.

5. References

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