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Enzymatic activities of midgut of two Earthworm species of *Perionyx excavatus* and *Lampito mauritii*

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Abstract

The aim of the present study to analyzing the enzymatic activity of amylase, cellulase, protease, acid phosphatase and alkaline phosphatase in mid gut content of Lampito mauritii and Perionyx excavates were assessed. The experimental method was carried out for a period of 60 days of culture period. The collection of earthworms into the troughs i.e., at the initial day (1st day) and after an interval of middle (30th day) and final (60th day) the samples from mid gut of earthworms were collected and analyzed for the enzymes activity. The results of the present study, the enzyme an activity in mid gut content of *Lampito mauritii* and Perionyx excavates was increased with increasing composting periods and all the values obtained in the present experiments were showed significant changes. Interestingly, protease activity showed a negative correlation with all others in the case of Perionyx excavates.

Keywords: Earthworm, *Lampito mauritii, Perionyx excavates,* midgut, enzymatic activities

Introduction

Earthworms are known to inhabit at earth's soil since the Precambrian period about 600 million years ago (Pradip, 2005). Earthworms are invertebrates belonging to the Phylum Annelida and Class Oligochaeta (Sharique *et al*., (2006). Earthworms are found in all types of soil provided there is sufficient moisture and food (Julka, 1993). Earthworms have been associated with human civilization as friends of the farmer and they are found distributed throughout the world and hence, they are cosmopolitan in distribution. About 3,600 species of earthworms have been identified from the globe and several species of them are being successfully used for this purpose of vermin production. Among this about 500 species are present in India (Reynolds,1994). Earlier studies on several earthworm species Eudrilus euginae, Eisenia fetida, Perionyx excavatus, Lumbricus rubellus, Lampito mauritii are used for vermi composting of various organic wastes (Kale and Bano, 1988; Blanchart and Julka (1997); Subbarao, 2002). Digestive enzymes present in the gut of earthworms are responsible for the decomposition and humification of organic waste (Dash, and Senapati, 1986). The studies were carried out only in temperate earthworms of the Family Lumbricidae and quantitative studies have been limited to cellulase and chitinase (Urbasek,1990). The present study is an attempt to assess the activity of major digestive enzymes in the mid gut of the selected species of earthworms, *Perionyx excavates* and *Lampito mauritii* reared for 60 days.

Materials and Methods

Preparation of soil base Bedding Material (BM)

The soil base has been prepared exclusively for use in the present experiments in order to maintain uniformity. It is a mixture of soil and cow dung in 1:1 ratio by v/v. For this the soil and cow dung were collected separately and shade dried. They were then sieved using a wire mesh in order to get a uniform mixture. This mixture was used as the soil base for all experiments.

Collection and Storage of cow dung

The cow dung was obtained in the vermicomposting unit at animal house of our College, and this was used as an organic substrate in the present study. It was shade dried stored in large container and used as and when needed, both for the maintenance of the earthworm stock and in the preparation of soil base for the experiment in the present study.

Earthworm collection and Stock culture rearing

For the present study, two available earthworm species, namely *P. excavates* and *L. mauritii* which represented the most dominant species observed in the study area i.e., agricultural field of Mallipudhur village, Srivilliputtur taluk, were collected and the worms were transported safe along with native soil in wet gunny cloth and introduced into separate containers. This was maintained as a stock culture in animal house of our College. The stock culture rearing was done in separate earthen pots of uniform size under shade and protection from predators. The pots were filled uniformly with the bedding material, a mixture of sieved cow dung

(500g) and garden soil (500g). They were taken in such a way to get triplicates for the experimental rearing of each species separately. The moisture level in the culture media was maintained close to the field soil moisture by sprinkling water in required quantities on alterative days. Maximum average temperature of the ventilated room varied from 26 to 29° C (calculated from mean of actual maximum temperature) during study period.

Study period

The experimental process was carried out for a period of 60 days. Before introducing the earthworms into the troughs i.e., at the initial day (1st day) and after an interval of middle (30th day) and final (60th day) the samples from mid gut of earthworms were collected and analyzed for the enzymes activity.

Enzyme assay analysis

The enzyme assay analysis in the mid gut of two earthworm species were carried out considering the respective homogenates as the enzyme source that were prepared separately by adopting standard procedure.

Statistical Analysis

The results were statistical analysed Standard Error, ANOVA and Pearson's Correlation coefficient.

Result and Discussion

The present study, results of enzyme activities of both earthworm species *Perionyx excavates* and *Lampito mauritii* were represented in the table-1 and 2. These enzyme activities of both species of P. excavates and *L. mauritii* were observed that increase in the composting period. The activity of amylase of *L. mauritii* was seen to increase from 23.12 ± 3.28, 53.30 ± 6.03 and to 95.36 ± 6.74 IU/g (dw) on the 1st day, 30th day and 60th day respectively. In the case of cellulase, the initial value was 15.17 ± 3.18 on1st day and this was increased to 45.36 ± 7.69 and 73.30 ± 6.81 IU/g (dw) on30th day and 60th day respectively. The activity of protease was seen to increase from 10.0 ± 1.53, 20.0 ± 1.53and to 31.47 ± 1.20 IU/g (dw) on the 1st day, 30th day and 60th days respectively. In the case of acid phosphatase, the initial value was 12.47 ± 1.45 on1st day and this was increased to 27.23 ± 2.73and 45.68 ± 1.20 IU/g (dw) on30th day and 60th days respectively. The activity of alkaline phosphatase was seen to increase from 9.0 ± 1.15, 19.57 ± 1.20and to 29.37 ± 0.88 IU/g (dw) on the 1st day, 30th day and 60th day respectively (Table- 1).

The activity of amylase of *Perionyx excavates* was seen to increase from 30.13 ± 3.18, 80.30 ±4.73 and to 118.23 ± 8.99 IU/g (dw) on the 1st day, 30th day and 60th days respectively. In the case of cellulase, the initial value was 17.36 ± 5.36 on1st day and this was increased to 56.43 ±4.06 and 82.13 ± 5.93 IU/g (dw) on 30th day and 60th days respectively. The activity of protease was seen to increase from 20.13 ± 0.88, 32.0 ± 1.73 and to 47.35 ± 0.88 IU/g (dw) on the 1st day, 30th day and 60th days respectively. In the case of acid phosphatase, the initial value was 21.37 ± 0.67 on1st day and this was increased to 37.27 ± 1.20 and 65.36 ± 2.33 IU/g (dw) on 30th day and 60th days respectively. The activity of alkaline phosphatase was seen to increase from 13.47 ± 0.88, 27.43 ± 1.20 and to 43.67 ± 1.20 IU/g (dw) on the 1st day, 30th day and 60th days respectively (Table -2).

Table-1. Different enzyme activities in the midgut of *Lampito mauritii* reared for 60 days.

Enzymes	Initial (1st day)	Middle (30th day)	Final (60th day)
Amylase (IU/g dw)	23.12 ± 3.28 (0)	53.30 ± 6.03 (131)	95.36 ± 6.74(312)
Cellulase (IU/g dw)	15.17 ± 3.18 (0)	45.36 ± 7.69 (199)	73.30 ± 6.81(383)
Protease (IU/g dw)	10.00 ± 1.53(0)	20.00 ± 1.53 (100)	31.47 ± 1.20(215)
Acid phosphatase (IU/g dw)	12.47 ± 1.45(0)	27.23 ± 2.73(118)	45.68 ± 1.20 (266)

Each value represents as mean (Mean ± SE) of three observations. Values within parenthesis indicate the percentage of change from initial value.

Table -2: Different enzyme activities in the mid-gut of earthworm species, Perionyx excavates reared for 60days.

Enzymes	Initial (1st day)	Middle (30th day)	Final (60th day)
Amylase (IU/g dw)	30.13 ± 3.18(0)	80.30 ± 4.73(167)	118.23 ± 8.99(292)
Cellulase (IU/g dw)	17.36 ± 5.36(0)	56.43 ± 4.06(225)	82.13 ± 5.93(373)
Protease (IU/g dw)	20.13 ± 0.88(0)	32.0 ± 1.73 (59)	47.35 ± 0.88(135)
Acid phosphatase (IU/g dw)	21.37 ± 0.67(0)	37.27 ± 1.20 (74)	65.36 ± 2.33(206)
Alkaline phosphatase (IU/g dw)	13.47 ± 0.88(0)	27.43 ± 1.20 (104)	43.67 ± 1.20(224)

Each value represents as mean (X ± SE) of three observations. Values within parenthesis indicate the percentage of change from initial value.

The activity of amylase and cellulase were prominent than by acid phosphatase, protease, and alkaline phosphatase. Earlier study, Loquat and Vinceslas (Vinotha *et al*., (2000) reported that cellulase activities of gut walls. The activity of the enzymes, cellulase, amylase, invertase, protease, peroxidase, urease, phosphatase and dehydrogenase in the worm casts have been reported (Venkateshwaralu,1995). However, based on the single experiment, exclusively conclusive opinion cannot be framed. Elaborate studies are needed to arrive a cut and ride conclusion. In this context, it was to be known that certain earthworms such as *Eisenia fetida* (Venkateshwaralu, 1995) *Millsonia anomala* and *Hormo gasterelisae* (Lattaud *et al*., 1997) had a strong mutualistic earthworm and micro flora digestive system for hydrolyzing the different organic wastes. It was also reported that the earthworms did not have their own commensally micro flora (Alauzet *et al*., 2001). The role of microbes in the synthesis of enzymes for the effective digestion of the substrates was reported to depend on a symbiotic cellulolytic micro flora (Urbasek, 1990). The present results suggest that gut wall of the earthworm or the indigenous microbes present in the gut might be responsible for the activities of the all the enzymes.

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