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Research Journal of Agricultural Sciences
An International Journal

P- ISSN: 0976-1675
E- ISSN: 2249-4538

Volume: 13
Issue: 02

*Res. Jr. of Agril. Sci.* (2022) 13: 406–411

CARAS

Res. Jr. of Agril. Sci. (Mar-Apr 2022)
13(2): 406–411

www.rjas.org

ISSN: 0976-1675 (P)
ISSN: 2249-4538 (E)

*Full Length Research Article*

# Analysis of Enzyme Activity during Vermicomposting of Organic Wastes Employing the Earthworm *Eudrilus eugeniae*

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Received: 02 Jan 2022 | Revised accepted: 28 Feb 2022 | Published online: 16 Mar 2022


## ABSTRACT

Vermicompost quality depends on the organic substrate acted upon by digestive enzymes in the gut of earthworms. Extent of enzyme activity varies depending upon the substrate and stage of vermicomposting operation. Keeping earlier observations in view, present study was undertaken to analyze enzyme activity in earthworm gut during various stages of vermicomposting of corn cob and cauliflower wastes using the earthworm species *Eudrilus eugeniae.* During the vermicomposting operation, the gut content of *Eudrilus eugeniae* was sampled at pre-determined intervals for enzyme analysis. Enzymes such as amylase, invertase and urease were estimated using standard protocols. Present investigation revealed that corn cob waste showed better enzyme activity as compared to cauliflower waste. Quantitative analysis of the enzymes recorded maximum amylase and invertase activity to be 11.2 and 9.6 μg $ml^{-1}$ $min^{-1}$ and 8.33 and 6.46 μg $ml^{-1}$ $min^{-1}$ on the 30$^{th}$ day; whereas, maximum urease activity was found to be 3.8 and 6.1 μg $ml^{-1}$ $min^{-1}$ on the 45$^{th}$ day; in gut content of *Eudrilus eugeniae* fed on corn cob and cauliflower waste, respectively. Organic wastes generated in large amounts, can be used as good organic substrates in vermicomposting using the earthworm species *Eudrilus eugeniae,* and thus be helpful in waste management.

***Key words*:** Vermicomposting, Corn cob, Cauliflower wastes, Earthworm gut, Enzymes, *Eudrilus eugeniae*

Earthworm population significantly contribute to soil formation, mineralization of organic matter, nutrient cycling, and water infiltration in terrestrial ecosystems [1-2]. Earthworms consume organic matter along with soil. This biological decomposition of organic matter has been described to be mediated by a variety of biochemical processes in which enzymes play a key role [3-4]. Enzymes in their digestive tracts carry out biodegradation of this partially decomposed content. The egested excreta called 'vermicast' contains plant growth hormones, various organic and nitrogenous compounds. The resultant humus-like material is called 'vermicompost', which enhances soil fertility and eventually promotes increase in plant production. Vermicomposting enhances soil biodiversity by promoting beneficial microbes, which in turn enhances plant growth directly by production of plant growth regulating substances (hormones and enzymes) and indirectly by controlling plant pathogens, nematodes and other pests, thereby enhancing plant health and minimizing the yield loss [5]. The rapid increase in the volume of waste is one aspect of the environmental crisis, accompanying global development [6]. On one hand tropical soils are deficient in all necessary plant nutrients and on the other large quantities of such nutrients are available in solid organic wastes [7]. Composting and vermicomposting are two of the best known-processes for the biological stabilization of a great variety of organic wastes [8].

A corncob is the central core of an ear of maize on which the kernels grow. It is made of pith surrounded by woody ring covered by chaff. During the processing of corn, a large volume of corncobs is generated as agricultural waste [9-10]. Most of this is either discarded or burnt, causing serious environmental pollution and no benefit [11-12]. The main components of corncobs are cellulose, hemicellulose, and lignin [13]. With respect to cauliflower, the floret is edible portion, whereas, upper stem, stalks, leaf midribs and the lower stem and leaves form the non-edible portion [14]. Leaves, stems and sprouts of cauliflower have high water content, but their dry matter contains high amounts of protein and sugars, and small proportions of low-lignified fiber [15]. Cauliflower has the highest waste index, i.e., ratio of non-edible to edible portion after harvesting [16] and thus generates a large amount of organic solid waste, which creates a foul odour on decomposition [14]. Cauliflower leaves are rich source of β carotene, iron and calcium but it has higher waste index [17]. Cauliflower waste contains appreciable amount of proteins and minerals [18]. Disposal of the non-edible portion of cauliflower remains a crucial problem [19].

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Epigeic earthworms possess a diverse pool of digestive enzymes which enable them to digest bacteria, protozoa, fungi and partly decomposed plant debris [20]. Earthworms have an in-house supply of enzymes such as amylase, cellulase, acid phosphatase and alkaline phosphatases; these enzymes biodegrade the complex biomolecules into simpler compounds, and are responsible for the decomposition and humification of organic matter [21-22]. Vermicompost contains enzymes like amylase, lipase, cellulase and chitinase, which continue to break down organic matter in the soil and increase the levels of soil enzymes like dehydrogenase, acid and alkaline phosphatases and urease; leading to increase in soil fertility [23].

Decomposition of the organic matter is mediated by a variety of biochemical processes involving enzymes [24]. The study of enzyme activities has been shown to be a reliable tool for characterizing the state and evolution of the organic matter during vermicomposting [25]. Enzyme activities have been used widely as an index of soil fertility or ecosystem status because they are involved in the biological transformations of native and foreign compounds in soils [26]. Hence, quantification of the activity of enzymes is important for tracking the composting process. Knowledge of the influence of different substrate media on the enzymatic activities of different earthworm species is limited [27]. In the present study, the corn cob and cauliflower waste mostly comprised of discarded portions of the corn cob and cauliflower, which were collected from the local vegetable markets (10 kg each) and used for vermicomposting operation. Further, changes in the enzyme activity during vermicomposting of the corn cob and cauliflower wastes using the earthworm species *Eudrilus eugeniae* was analyzed.

## MATERIALS AND METHODS

Organic wastes in the form of corn cob and cauliflower wastes were collected from Ahmednagar City markets and were chopped into small pieces and weighed. Cowdung was collected from local sources for use as a manure. Earthworms belonging to the species *Eudrilus eugeniae* were procured from Loni (Pravara), District Ahmednagar. Standard and popular protocols were followed during the experimentation, as given below. Partial decomposition of both the wastes was carried out separately by using cow dung slurry in the ratio of 3:1 for 20 days. *E. eugeniae* is reddish brown in colour, its adult size is 5–7 mm × 80–190 mm. The earthworms introduced in the experimental setups were adults with mean size of 15.8 cm and mean weight of 2.58 gm. 50 no. of earthworms *Eudrilus eugeniae* were released into each of the experimental setup (Setup 'A': Corn cob waste and Setup 'B': Cauliflower waste).

Enhanced physiological stress level caused due to moisture deprivation could influence synthesis and storage of protein with enzyme activities [28]. The setups were covered with gunny bags to trap the moisture and protect the earthworms from predators. The limits and optimal temperature and moisture of *E. eugeniae* are 25°C (16°C-30°C) 80% (70%-85%), respectively [29]. Moisture content was maintained between 45-65% by sprinkling water. The wastes were periodically mixed/ turned in order to provide aeration and release out the initial heat produced during decomposition of organic matter. During the composting process, the temperature (°C) was observed to be in the range of 26 to 28°C in both the setups. The moisture content was estimated by Oven Dry Method [30]: 5 gm of vermicompost sample was taken in a pre-weighed petri dish. The petri plate containing the sample was kept in hot air oven maintained at 100±2°C for about 4 hours. The sample was cooled in a desiccator and weighed accurately. Moisture content was calculated as percentage loss in weight of the sample by the following formula:

$$\text{Moisture content (\%)} = \frac{x - y}{x - z} \times 100$$

Where;
z = weight of empty petri plate,
x = weight of petri plate + sample
y = weight of petri plate + sample after drying

At pre-determined time intervals (i.e., 15, 30, 45 and 60 days), 2-3 earthworms were removed from each experimental set-up, washed with sterile distilled water so as to get rid of mud, dirt and microbes attached to the surface of body. Thereafter, the earthworms were sacrificed by chilling. The worms were kept under refrigeration for three to four hours in order to kill them without causing any harm or alteration to the microbial and digestive enzyme activities of the gut [31]. Each specimen to be dissected was pinned down horizontally on sterilized dissecting board with the dorsal part downward. The ventral part was cut open longitudinally along the earthworm using sterilized dissecting kits [32]. The earthworms were dissected to expose the digestive tract. The gut was dissected and its contents were collected in two separate sterile petri plates. The gut contents were homogenized prior to analysis of enzyme activity.

Various enzymes such as amylase, invertase and urease; involved in the degradation of complex organic material into simple compounds, which were estimated in the present study. *Standard Methods of Biochemical Analysis* by Thimmaiah [33] was referred for the analysis of enzyme activity in the earthworm gut content samples. Standard graph of glucose was prepared for amylase and invertase estimation; whereas, ammonium sulphate graph was prepared for urease estimation. The amylase, invertase and urease activity at specified time intervals (i.e., 15, 30, 45 and 60 days) was calculated using the standard graphs and the following formula:

$$\text{Enzyme activity } (\mu g\ ml^{-1}\ min^{-1}) = \frac{\text{Amount of product}}{\text{Incubation time} \times \text{Vol. of enzyme used}}$$

## RESULTS AND DISCUSSION

Vermicompost is the microbial composting of organic wastes through earthworm activity to form organic fertilizer which contain higher level of organic matter, organic carbon, total and available N, P, K and micronutrients, microbial and enzyme activities [34-36]. Enzyme activity, of the various factors is influenced by type of food [37]. Availability of nutrients in the gut could have been a result of digestion of ingested materials either by microbial enzymes produced within the gut or by the gut wall of these earthworms [38]. During their passage through the gut, the population of gut microflora increases, this may be responsible for the increased enzyme activities [39].

Earthworms speed up the composting process and transform wastes into nutrient rich castings with the help of enzymes [40]. Vermicasts are rich in enzymes which accelerate the mineralization rate and convert the wastes into organic fertilizer with higher nutritional value [41]. Various hydrolytic enzymes are believed to control the rate at which various substrates are degraded. We can get a fair idea of the quantum of organic matter degradation by analyzing the enzyme activity at a particular time. The present investigation demonstrated the relation between the type of substrate and enzyme activity in the gut of the earthworm during the composting process.

Earthworms stimulate biochemical activity and nutrient cycling by 40-45% contributing to the reduction of period of degradation of agricultural wastes resulting in maturation of vermicompost by 28th day [42]. Benitez *et al*. [43] observed changes in hydrolytic enzyme activities throughout vermicomposting. Jayakumar *et al*. [44] analyzed the enzyme activity in vermicasts collected from three different earthworm species, *E. eugeniae, L. mauritii* and *P. ceylanensis*, which showed increased nutrients, microbial population and enzyme activities viz., amylase, cellulase, invertase, phosphatase and protease. Enzymatic analysis of the gut content of the earthworms was conducted at specified time intervals. The concentration of enzymes namely: amylase, invertase and urease, in terms of its optical density was measured using spectrophotometer and calculated using standard graphs. The enzyme activity at specified time intervals (i.e., 15, 30, 45 and 60 days) was calculated as given in (Table 1-3), respectively. Higher enzyme activity was observed during the initial stages of vermicomposting, which may be due to the availability of organic substrates present in the form of corn cob and cauliflower wastes. Enhanced enzymatic activities in the casts is due to the microbial activity [45]. Devi *et al*. [42] observed maximum enzyme activities (cellulase, amylase, invertase, protease and urease) during 21-35 days of vermicomposting.

Table 1 Amylase enzyme activity in gut content of *Eudrilus eugeniae*

| Time interval | Starch | Enzyme | Incubation | DNS | Boiled for 5 min., added 1 ml potassium sodium tartarate solution, cooled under running tap water and read the absorbance at 530 nm | Enzyme activity Set 'A' | Set 'B' |
|---|---|---|---|---|---|---|---|
| Blank | 1 ml dw | | | | | 0.00 | 0.00 |
| 15 days | 1 ml Starch | 1ml Dil. Enzyme | At 27°C for 15 min | 2 ml | | 9.33 | 7.33 |
| 30 days | | | | | | 11.2 | 9.06 |
| 45 days | | | | | | 8.33 | 8.00 |
| 60 days | | | | | | 7.33 | 7.00 |

Table 2 Invertase enzyme activity in gut content of *Eudrilus eugeniae*

| Time interval | Enzyme solution | DNS reagent | Boiled the tubes in boiling water bath for 5 min. and read the absorbance at 530 nm | Enzyme activity Set 'A' | Set 'B' |
|---|---|---|---|---|---|
| Blank | 1 ml dw | | | 0.00 | 0.00 |
| 15 days | 1 ml enzyme solution | 1 ml | | 4.73 | 3.29 |
| 30 days | | | | 8.33 | 6.46 |
| 45 days | | | | 7.13 | 6.12 |
| 60 days | | | | 4.33 | 4.62 |

Table 3 Urease enzyme activity in gut content of *Eudrilus eugeniae*

| Time interval | Enzyme solution | Distilled water | Phenate solution | NaOCl | Mixed the contents and allowed to stand for 20 min. Diluted to 50 ml with water and read the absorbance at 630 nm | Enzyme activity Set 'A' | Set 'B' |
|---|---|---|---|---|---|---|---|
| Blank | 1 ml | 10 ml | | | | 0.0 | 0.0 |
| 15 days | 1 ml | 9 ml | 4 ml | 3 ml | | 2.8 | 4.7 |
| 30 days | | | | | | 3.2 | 4.4 |
| 45 days | | | | | | 3.8 | 6.1 |
| 60 days | | | | | | 1.3 | 3.2 |

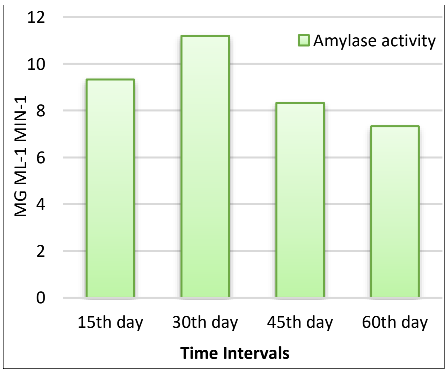


Fig 1 Amylase activity in gut content of *Eudrilus eugeniae* fed on corn cob waste

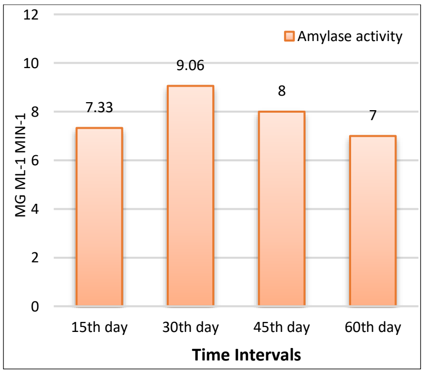


Fig 2 Amylase activity in gut content of *Eudrilus eugeniae* fed on cauliflower waste

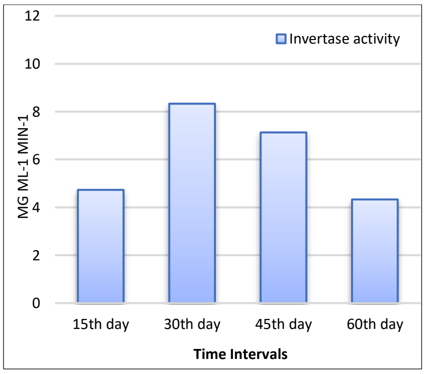


Fig 3 Invertase activity in gut content of *Eudrilus eugeniae* fed on corn cob waste

In the present study, the enzyme activity viz., amylase, invertase and urease in the gut content of *Eudrilus eugeniae* initially increased and then decreased gradually. The activity of amylase in the gut content of *Eudrilus eugeniae* fed on corn cob and cauliflower wastes at specified time intervals is depicted in (Fig 1-2), respectively. Amylase activity was found to be maximum on the 30th day i.e., 11.2 and 9.6 µg ml$^{-1}$ min$^{-1}$ and minimum on the 60th day i.e., 7.33 and 7 µg ml$^{-1}$ min$^{-1}$; of enzyme extracted from the gut sample. Similar results were observed by Mechery and Prabha [46] wherein, increased amylase activity was noticed on 30th day of vermicomposting, after which, it decreased steadily with increase in incubation time up to 40th day of vermicomposting of tea wastes.

Invertase activity was found to be maximum on the 30th day i.e., 8.33 and 6.46 µg ml$^{-1}$ min$^{-1}$ and minimum on the 60th day i.e., 4.33 µg ml$^{-1}$ min$^{-1}$ and on the 15th day i.e., 3.29 µg ml$^{-1}$ min$^{-1}$, of enzyme extracted from the gut content of *Eudrilus eugeniae* fed on corn cob and cauliflower wastes, respectively (Fig 3-4) after which it decreased steadily up to 45th day and thereafter at 60th day of vermicomposting. Likewise, Mechery and Prabha [46] observed increased activity of invertase in leaf litter waste on the 30th day of vermicomposting which decreased significantly up to 45th day.

Further, urease activity was found to be maximum on the 45th day i.e., 3.8 and 6.1 µg ml$^{-1}$ min$^{-1}$ and minimum on the 60th day i.e., 1.3 and 3.2 µg ml$^{-1}$ min$^{-1}$ of enzyme extracted from the gut content of *Eudrilus eugeniae* fed on corn cob and cauliflower wastes, respectively (Fig 5-6). High activity of urease in the gut content of the worms from both the composting sets and decrease at the later stages was observed. According to Benitez *et al*. [47], this may be due to the continuous accumulation of extracellular enzymes in humic matter, which become stabilized and resistant to physical and microbial degradation. Between composting methods, higher urease activity was observed in vermicomposting, which could be due to increased biomass of earthworms and microbes which increases the urease activity [48]. Blessy and Lakshmi [49] found higher enzyme activities of enzymes namely amylase, cellulase and invertase on the 25th day of composting of Lady's finger waste using the earthworm *Eudrilus eugeniae*. Whereas, during the present investigation, the amylase and invertase activity in the gut content of earthworms from both the vermicomposting set ups was found to be maximum on the 30th day; and the urease activity was found to be maximum during the later period i.e., 45th day. Further, the activity of enzyme amylase and invertase was found maximum in the gut content of earthworm fed on corn cob waste, while urease activity was maximum in the gut content of earthworm fed on cauliflower waste. Devi *et al*. [42] observed that amylase activities in vermicomposting were increased by 28th day and reached the peak values 825 µg reducing sugar g$^{-1}$ hr$^{-1}$ and similarly the invertase and urease activities reached to peak values of 876 µg reducing sugar g$^{-1}$ hr$^{-1}$ and 197 µg $NH_4$ + g$^{-1}$ hr$^{-1}$ on 35th day, respectively. Enzyme activities decreased after 35th day as composting proceeded and they stated that this is probably due to a decrease in microbial population, and available nutrients in the organic matter.

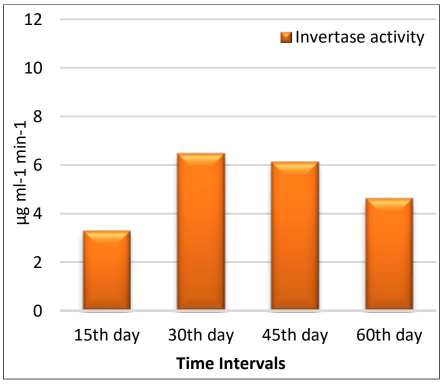


Fig 4 Invertase activity in gut content of *Eudrilus eugeniae* fed on cauliflower waste

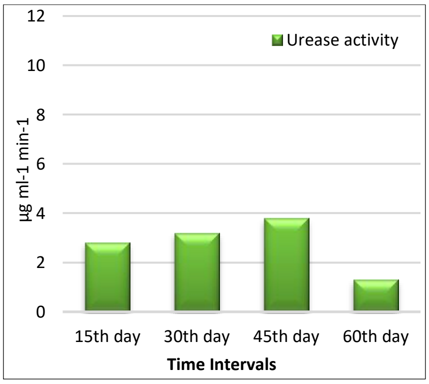


Fig 5 Urease activity in gut content of *Eudrilus eugeniae* fed on corn cob waste

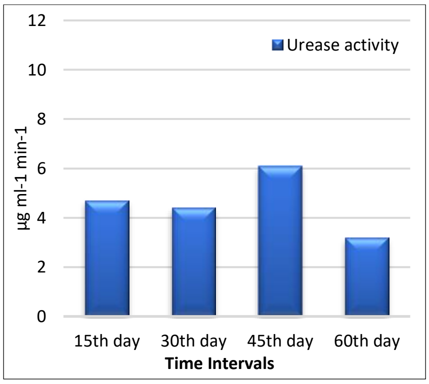


Fig 6 Urease activity in gut content of *Eudrilus eugeniae* fed on cauliflower waste

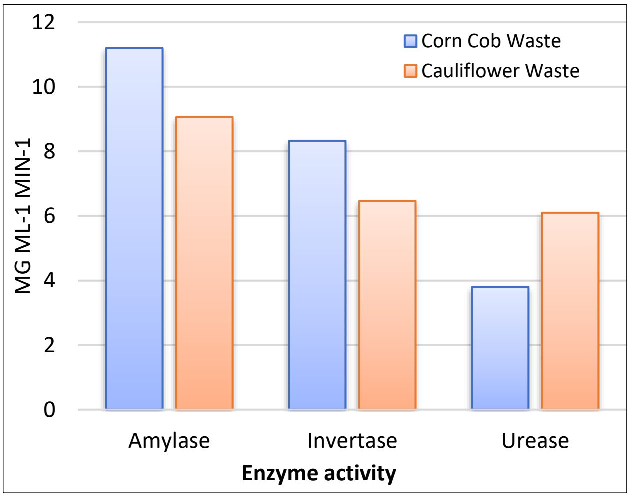


Fig 7 Variation in enzyme activity in gut content of *Eudrilus eugeniae* fed on 2 different substrates

Comparing the three enzymes, amylase activity was found to be greater than invertase and urease activity, irrespective of the substrate used (Fig 7). In this light, Achsah and Lakshmi [50] stated that increased amylase activity in vermicompost is due to the presence of rich starch in the organic substrate.

## CONCLUSION

Solid wastes of plants in the form of the inedible portions of corn cob and cauliflower are generated in large volumes, but are left untreated. These wastes can be wisely and efficiently utilized by subjecting them to vermicomposting. Various species of earthworms can be used for this operation. Estimation of enzyme activity during the vermicomposting process reveals the ability of earthworm species to breakdown organic matter. Therefore, in the present study, analysis of enzyme activity of namely amylase, invertase and urease; in the gut of the earthworm *Eudrilus eugeniae* during various stages of vermicomposting of corn cob and cauliflower wastes was conducted; wherein, corn cob waste showed better enzyme activity as compared to cauliflower waste. Thus, it can be concluded that corn cob and cauliflower wastes can be used as good organic substrates for conversion into a nutrient-rich biofertilizer, re-establishing the fact that the earthworm species *Eudrilus eugeniae* can be employed in the vermicomposting of organic wastes and render their bioremediation services in the waste management practices.

***Acknowledgments***

*The authors are grateful to the Principal and Head of Department of Zoology, Ahmednagar College, Ahmednagar (M.S.) for providing laboratory and library facilities*.

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