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# Enzyme Analysis and Histological Studies of Earthworms in the Treatment of Different Organic Wastes

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**ABSTRACT**

The study aims at the deleterious effect of the pollutants on the earthworm especially the gut epithelium cells. The study also focuses the levels of various enzymes and their activities. The two earthworms species namely *Eudrillus eugeniae* and *Eisenia fetida* were selected for our study. The enzyme activity and histopathological effect of the industrial waste, agricultural and domestic wastes to the earthworm species *Eudrillus eugeniae* and *Eisenia fetida* were studied under laboratory conditions. Earthworm species were exposed to different organic waste (Vegetables and fruits peels, Coffee grounds, Newspaper, Textile dye waste, Endosulfan) for 40 days and the mortality was recorded every 10 days. In addition, one sections of the worm were made after 96 hours for microscopic examination. The overall maximum enzyme activity was observed in a *Eisenia fetida* are compared to *Eudrilus eugeniae*. Result showed that the vermicompost quality was improved in enzyme activity. The histopathological manifestations that textile dye waste and endosulfan waste gradually increase damages of tissue. The soft peritoneal membrane lining intestinal epithelium is more affected. It was concluded that composting of organic waste by vermicomposting promote humification, increased microbial activity and enzyme production, which in turn increases the aggregate stability of soil particles resulting in better aeration with pollution free technique. The result of the study showed that both mortality and histopathology data could be used in environmental risk assessment of textile dye waste and endosulfan.

***Key words**: Eudrillus eugeniae, Eisenia fetida, Endosulfan and organic waste, Vermicomposting.*

## Introduction

Concerns about the state of the environment and problems of solid waste disposal are issues that are increasingly demanding attention globally. In Iran, serious environmental problems and economic concerns have arisen from a lack of appropriate technology and facilities, urban diseases, solid waste disposal in unsanitary and undesirable ways, and open dumping of waste in and around cities (Aksakal E.L *et al*., 2016). Thus, research into possible solutions to the problems of urban waste management by the production of compost fertilizer from biodegradable waste could reduce environmental problems and

constitutes a cost-effective step towards a more effective waste management system (Hu *et al*., 2016 and Hussain *et al*., 2017). Organic waste residues reach the soil in a variety of ways, causing toxicity to earthworm and they will suppress or nearly eliminate earthworm population, they enter the environment through industrial and agricultural activities, reaching the earthworm room soil and water (Garcia – Gil *et al*., 2000). Globally earthworms are used as biomarkers for evaluating chemical environmental pollution. Hence, in present study the different types of wastes were selected for the assessment of their toxic effect on two epegic earthworm species *Eudrillus eugeniae* and *Eisenia fetida* was chosen as an indicator species for assessment of agro ecosystem contamination because of its widespread occurrence in aerable and fasture lands and its consequent vulnerability to surface applied pesticides. In recent years, through a number of studies for assessing the toxicity of wastes to earthworm mortility, growth and reproduction were carried out (Solol *et al*., 2019). A very few studies have been reported on the histopathological effects of wastes on earthworms (Ryals *et al*., 2013). Histology is the most useful tool for determining the influence of agricultural pesticide, industrial pollutants, organic wastes etc., at tissue level of an organism as it provides useful information concerned with the growth, damage and disorganization of tissues (DeLonge *et al*., 2013). Hence this study was undertaken with view to investigating the enzyme analysis and toxicity of different organic waste to two earthworm species, *Eudrillus eugeniae* and *Eisenia fetida* as well as histopathological effects.

## Materials and Methods

The soil collected from agriculture land in kundrathur, Chennai, Tamil Nadu. The soil was air-dried, ground and screened through a nylon fiber sieve to remove stones, plant root and other large particles. Two type of earthworm species were collected from Hand in Hand organization in Puthuperungulathur, Chennai, Tamil Nadu. All earthworms were kept in a moist soil mixed with cow dung. Distilled water was given to reach 60% of maximum water holding capacity. Soil was changed every four weeks and earthworms were maintained until required for experimentation. Five different type of waste was collected from different areas in and around Chennai. The collected waste were chopped into small size and allow to decomposition for 15 days.

**Extraction of Earth worm Paste**

Earthworm *Eudrillus eugeniae* and *Eisenia fetida* were obtained from 40 day samples. The species were washed with tap water and left in a tray containing paper for gut clearance for 10 h. The worms were washed with distilled water and kept it in a plastic tray. Finally the worms were exposed to the sun for two days to kill them. Mucus and coelomic fluid that oozed out digested the dead worms forming a brown coloured paste; Earthworm paste obtained was diluted in 10 % DMSO for evaluation of enzyme activity.

The amount of enzymes presents in the treated earthworm as well as control was estimated individually. Aliquot of 5 ml of sample were dissolved in respective buffers for respective enzyme assay and kept on the shaker for 30 minutes. Then the samples were centrifuged at 10000 x g and the supernatant was used for enzyme assay.

**Pectinase activity assay**

Tubes of 25 ml with mixed solutions of 2 ml 1% citrus pectin–acetum buffer (pH 4.8) and 0.5 ml coarse enzyme solution were kept at 50 °C for 30 min (Sherman and Ansari, 2010; Das *et al.,* 2012). Then, tubes were removed and cooled quickly, 2.5 ml DNS reagent was added, and the solutions were seethed for 5 min. After that, the solutions were mixed with 10 ml distilled water. Finally, the OD540 nm of mixed solutions was measured and the corresponding D-galacturonic content was determined from the standard d-galacturonic calibration curve. The following was adopted as the definition of pectinase activity: with pectin as the degrading chemical, under the conditions of pH 4.8 and constant temperature of 50 _C for 30 min, the quantity of pectinase hydrolyzing pectin needed to form 1mg of d-galacturonic acid per 60 min was 1U where U ¼ mg d-galacturonic acid $h^{-1}$:

**Laccase activity assay**

Laccase activity was determined in duplicate as described previously (Raj, 2017 and Baldwin, 2013) by monitoring oxidation of syringaldazine ($\varepsilon$max = 6.4 × 104M″1 cm″1; Sigma, St. Louis, MO) at 530 nm. The reaction mixture (3 ml) which contained 0.1 ml of the enzyme sample and 2.9 ml of 20 _M syringaldazine in 50mM sodium phosphate buffer,

pH 7.0 was incubated at 30 °C for 10 min. Enzyme activity was expressed in units; 1U being defined as the amount of enzyme causing the formation of 1 _mol of product per minute under the assay conditions used.

**Lipase activity assay**

Lipase activity was also measured using various *p*-nitrophenyl esters as described by Dominguez *et al*., 2010 with some modifications. One volume of a 10m Msolution of each substrate in 2-propanol was mixed with 9 volumes of 100mM Tris–HCl buffer (pH 8). When long acyl chain *p*-nitrophenyl esters (C14–C18) were used, PVA 0.25% (w/v) was also incorporated into this buffer. This mixture was then pre-warmed at 40 °C in a water bath and immediately distributed (1 ml) into 1.5 ml cells. The reaction was started by adding 0.5 ml of enzyme solution at an appropriate dilution in 10mM Tris–HCl (pH 8). The absorbance at 410 nm of the assay against a blank without any enzyme was continuously monitored for 2–5 min using a UV–vis spectrophotometer (ShimadzuUV-160A, Shimadzu Corporation, Kyoto, Japan). The reaction ratewas calculated from the slope of the curve absorbance versus time, using a molar extinction coefficient of 12,750 cm″1 M″1 for *p*-nitrophenol. One enzyme unit was defined as the amount of protein releasing 1 _mol of *p*-nitrophenol per minute under the above conditions.

**Histological activity**

The histology of gut of earthworm was studied adopting the routine paraffin method (Jacoby R *et al*., 2017 and Wroclaw Kabala *et al*., 2019). Gut of earthworm, dissected out from the control and experimental animals, were blotted free of mucus, washed thoroughly in physiological saline, cut into pieces of desired size and fixed in Bouins fluid fixative immediately after autopsy. Fixation was carried out at room temperature for 24 hrs, after which the tissues were transferred to 70% alcohol. Several changes of 70% alcohol were given until the yellow colour disappeared from the tissues. The tissues were then dehydrated by passing through ascending grades of alcohol, cleared in xylene, infiltrated with molten paraffin, and finally embedded in paraffin wax (58°C MP). Tissue section of 5-µm thick transverse and longitudinal sections were obtained using a rotary microtome (Leica, Germany). The sections, thus obtained, were stained in Harris hematoxylene and eosin, dehydrated using alcohol, cleared in xylene and mounted using dihydroxy phthalate xylol (DPX). The stained slides were observed in a Carl Zeiss (Germany) Axio-2 Plus research microscope (Kolbe *et al*., 2019 and Kundan *et al*., 2019)

## Results and Discussion

The pectinase (Table 1) enzyme activity (µg/ml) showed endosulfan treated worms showed lowest pectinase activity which was 1.71 ± 0.23 and 1.55 ± 0.10 for *Eudrillus eugeniae* and *Eisenia fetida* respectively. The maximum enzyme activity showed in Vegetable and fruits peel treated worms which was 1.84 ± 0.05 and 1.89 ± 0.09 with *Eudrillus eugeniae* and *Eisenia fetida* respectively. Kandeler (1988) reported pectinase are endo-acting hydrolytic enzymes that digest pectate into oligogalacturonic acids. The high yield of pectinase was observed in sugarcane bagasse by both species compare to other treatments. The maximum Laccase (Table 2) enzyme activity of 17.90 ± 1.86 and 17.07± 2.65 by *Eudrillus eugeniae* and *Eisenia fetida* respectively. The minimum enzyme activity was found in textile dye waste treated worms of 7.21 ± 1.58 and 7.42 ± 1.96 by *Eudrillus eugeniae* and *Eisenia fetida* respectively. Total laccase content was increased in *Eisenia fetida* compared to *Eudrillus eugeniae*. While Lazcano C (2010) reported that particle size, porosity and chemical composition of the substrates play crucial role in enzyme production. The lipase (Table 3) enzyme activity by *Eudrillus eugeniae* treated worms showed maximum lipase content compared to *Eisenia fetida*. The Lipase activity increased in News paper waste which was 0.46 ± 0.04 for *Eudrillus eugeniae* and Vegetable and fruits peel treated worms was 0.44 ± 0.03 by *Eisenia fetida*. Fats and oils require digestive action before absorption occurs. In the present study the lipase activity was slightly increased in all treatment in both species. Lim *et al*., (2015) reported that lipase in earthworms is the primary digestant that breaks down oils and fats into smaller molecules.

Use of specific herbicides, fungicides and insecticides in the agricultural field can be highly toxic to earthworms and they will suppress or nearly eliminate earthworm population (Lim *et al*., 2016 and Margalef *et al*., 2017). The earthworm gut was damaged in all endosulfan treatment. These observations agree with Tautges (2019) who reported similar pathological lesions in gut of Desi fowls that in-

**Table 1.** The Pectinase content (µg/ml) of Earthworm on various treatment

| DAYS AFTER INTRODUCTION OF EARTHWORM | | | | | | |
|---|---|---|---|---|---|---|
| SAMPLE | 10 | 20 | 30 | 40 | F-VALUE | P-VALUE |
| *EUDRILLUS EUGENIAE* | | | | | | |
| Control | 1.57 ± 0.15 | 1.61 ± 0.23 | 1.77 ± 0.12 | 1.81 ± 0.23 | 35.941 | 0.081 |
| Vegetable and fruits peel | 1.68 ± 0.12 | 1.74 ± 0.17 | 1.78 ± 0.07 | 1.84 ± 0.05 | 2.936 | 0.099 |
| Coffee grounds | 1.27 ± 0.06 | 1.67 ± 1.10 | 1.84 ± 0.03 | 1.80 ± 0.08 | 41.920 | <0.001** |
| News paper waste | 1.39 ± 0.15 | 1.46 ±0.23 | 1.66 ± 0.10 | 1.84 ± 0.17 | 2.825 | 0.107 |
| Textile dye waste | 1.29 ± 0.10 | 1.33 ± 0.13 | 1.57 ± 0.22 | 1.77 ± 0.12 | 4.596 | 0.038* |
| Endosulfan | 1.33 ± 0.13 | 1.34 ± 0.09 | 1.36 ± 0.32 | 1.61 ± 0.23 | 1.202 | 0.370 |
| *EISENIA FETIDA* | | | | | | |
| Control | 1.56 ± 0.41 | 1.63 ± 0.18 | 1.69 ± 0.21 | 1.94 ± 0.04 | 1.397 | 0.432 |
| Vegetable and fruits peel | 1.66 ± 0.11 | 1.73 ± 0.07 | 1.78 ± 0.30 | 1.89± 0.09 | 3.986 | 0.052 |
| Coffee grounds | 1.46 ± 0.10 | 1.63 ± 0.10 | 1.69 ±0.02 | 1.86 ± 0.08 | 10.454 | 0.004** |
| News paper waste | 1.45 ± 0.26 | 1.53 ± 0.10 | 1.61 ± 0.10 | 1.70 ± 0.06 | 1.497 | 0.288 |
| Textile dye waste | 1.35 ± 0.13 | 1.43 ± 0.29 | 1.48 ± 0.25 | 1.50 ± 0.16 | 0.308 | 0.819 |
| Endosulfan | 1.25 ± 0.05 | 1.29 ± 0.11 | 1.32 ± 0.05 | 1.45 ± 0.10 | 3.349 | 0.076 |

**Table 2.** The Laccase content (µg/ml) of Earthworm on various treatments

| DAYS AFTER INTRODUCTION OF EARTHWORM | | | | | | |
|---|---|---|---|---|---|---|
| SAMPLE | 10 | 20 | 30 | 40 | F-VALUE | P-VALUE |
| *EUDRILLUS EUGENIAE* | | | | | | |
| Control | 5.23 ± 1.42 | 10.12 ± 1.67 | 14.45 ± 1.49 | 17.52 ± 1.31 | 10.875 | 0.056 |
| Vegetable and fruits peel | 7.64 ± 1.10 | 12.36 ± 2.02 | 15.31 ± 2.32 | 17.97 ± 2.78 | 11.901 | 0.003** |
| Coffee grounds | 6.50 ± 1.30 | 8.68 ± 1.71 | 10.86 ± 1.47 | 13.72 ± 2.96 | 6.588 | 0.023 * |
| Newspaper waste | 5.08 ± 1.56 | 6.91 ± 1.19 | 8.25 ± 1.43 | 9.45 ± 1.34 | 4.785 | 0.059 |
| Textile dye waste | 4.41 ± 1.33 | 4.66 ± 1.21 | 5.09 ± 1.65 | 8.21 ± 1.58 | 4.311 | 0.153 |
| Endosulfan | 6.30 ± 1.41 | 11.11 ± 1.76 | 14.00±1.55 | 18.90 ± 1.86 | 28.200 | 0.001** |
| *EISENIA FETIDA* | | | | | | |
| Control | 5.45 ± 1.56 | 8.31 ± 1.52 | 11.71 ± 1.81 | 15.43 ± 1.41 | 19.941 | 0.002** |
| Vegetable and fruits peel | 6.42 ± 1.15 | 12.69 ± 2.12 | 15.52 ± 1.71 | 17.33 ± 1.80 | 21.238 | 0.001** |
| Coffee grounds | 6.41 ± 1.80 | 10.78 ± 1.49 | 13.79±2.34 | 17.43 ± 1.79 | 16.722 | < 0.001** |
| News paper waste | 3.65 ± 1.08 | 5.87 ± 1.06 | 8.34 ± 1.53 | 9.76 ± 0.73 | 12.416 | 0.004** |
| Textile dye waste | 3.15 ± 0.23 | 3.38 ± 1.07 | 6.71 ± 1.22 | 8.42 ± 1.96 | 10.056 | 0.006** |
| Endosulfan | 6.29 ± 1.92 | 11.21 ± 1.97 | 15.66 ± 2.34 | 18.07± 2.65 | 17.190 | 0.001** |

**Table 3.** The Lipase content (µg/ml) of Earthworm on various treatments

| Days After Introduction of Earthworm | | | | | | |
|---|---|---|---|---|---|---|
| SAMPLE | 10 | 20 | 30 | 40 | F-VALUE | P-VALUE |
| *EUDRILLUS EUGENIAE* | | | | | | |
| Control | 0.18 ± 0.06 | 0.23 ± 0.09 | 0.30 ± 0.03 | 0.40 ± 0.06 | 41.985 | < 0.001** |
| Vegetable and fruits peel | 0.22 ± 0.02 | 0.25 ± 0.03 | 0.35 ± 0.05 | 0.46 ± 0.02 | 33.715 | 0.001** |
| Coffee grounds | 0.23 ± 0.02 | 0.25 ± 0.03 | 0.42 ± 0.03 | 0.45 ± 0.04 | 45.423 | 0.001** |
| News paper waste | 0.17 ±0.03 | 0.24 ± 0.03 | 0.31 ±0.01 | 0.46 ± 0.04 | 57.632 | 0.001** |
| Textile dye waste | 0.16 ±0.03 | 0.24 ± 0.03 | 0.35 ±0.03 | 0.35 ±0.03 | 30.707 | 0.001** |
| Endosulfan | 0.16 ±0.01 | 0.21 ± 0.01 | 0.36 ± 0.04 | 0.37 ± 0.03 | 56.308 | 0.001** |
| *EISENIA FETIDA* | | | | | | |
| Control | 0.18 ± 0.06 | 0.20 ± 0.04 | 0.28 ± 0.06 | 0.40 ± 0.03 | 20.638 | 0.005** |
| Vegetable and fruits peel | 0.26 ± 0.03 | 0.26 ± 0.04 | 0.34 ± 0.02 | 0.44 ±0.03 | 23.747 | 0.001** |
| Coffee grounds | 0.22 ± 0.01 | 0.26 ± 0.04 | 0.33 ± 0.02 | 0.36 ± 0.04 | 18.547 | 0.001** |
| Newspaper waste | 0.17 ± 0.03 | 0.23 ± 0.01 | 0.26 ± 0.03 | 0.34 ± 0.03 | 25.654 | 0.001** |
| Textile dye Sludge | 0.15 ± 0.02 | 0.19 ±0.01 | 0.27 ± 0.02 | 0.32 ± 0.07 | 12.038 | 0.003** |
| Endosulfan | 0.17 ± 0.01 | 0.18 ± 0.02 | 0.25± 0.02 | 0.33 ± 0.04 | 31.379 | 0.001** |

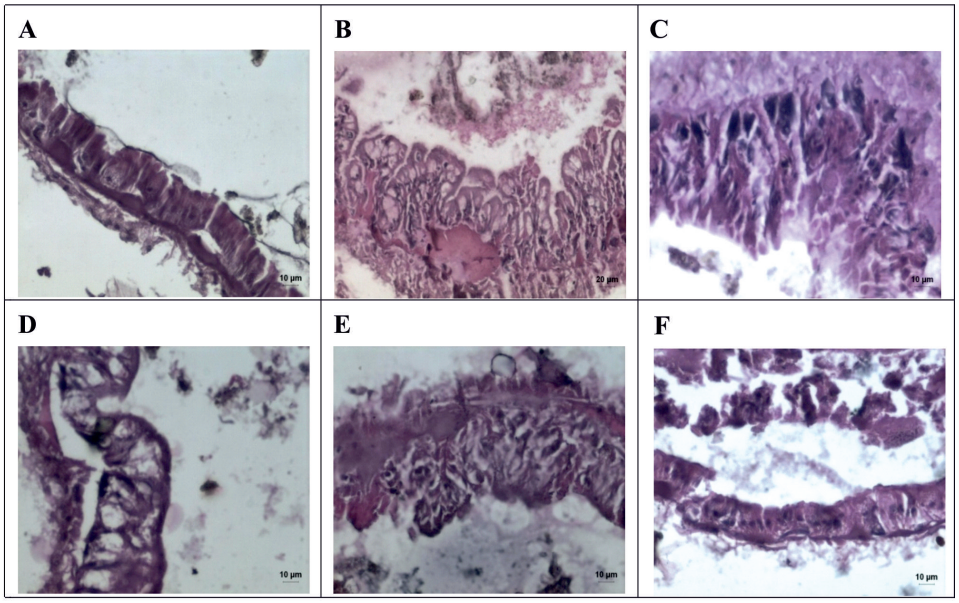


**Fig. 1.** Histological studies in Gut region of the Earthworm *E. Euginea* treated with different organic wastes.
A) *E. eugeniae* - control - No abnormalities detected
B) *E. eugeniae* - vegetables and fruits peel waste - Increased goblet cells
C) *E. eugeniae* - Coffee grounds - Pyknotic nucleus seen on gut epithelial cells
D) *E. eugeniae* - Newspaper waste - Swollen gut epithelial cells and mucosal epithelial degeneration
E) *E. eugeniae* - Textile dye waste - Necrotic gut epithelial cells
F) *E. eugeniae* - Endosulfan - Pyknotic nucleus seen on gut epithelial cells

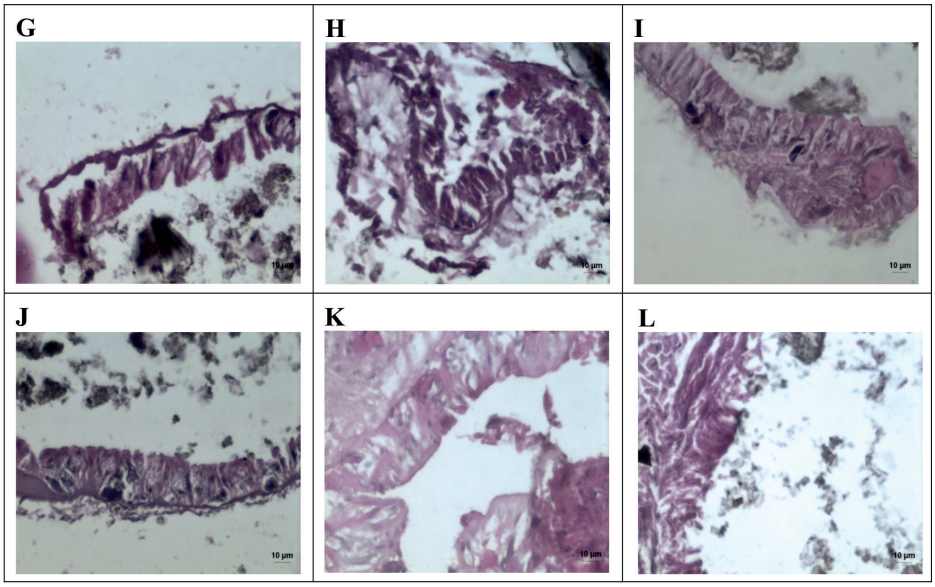


**Fig. 2.** Histological studies in Gut region of the Earthworm *E. Fedita* treated with different organic wastes
G) *E. fetida* - control - No abnormalities detected
H) *E. fetida* - vegetables and fruits peel waste - Necrotic gut epithelial cells
I) *E. fetida* - Coffee grounds - Necrotic gut epithelial cells
J) *E. fetida* - Newspaper waste - Pyknotic nucleus seen on gut epithelial cells and increased goblet cells
K) *E. fetida* - Textile dye waste - Necrotic gut epithelial cells
L) *E. fetida* - Endosulfan

fected with *R. echinobothrida*, as well as, Brar *et al*., (2013) who showed desquamation of epithelium, congestion, cellular infiltration, hemorrhagic exudates and desquamation of submucosal lands especially in duodenal of earthworm (Guenet *et al*., 2020). Cell death or necrosis was observed in this study. According to (Martinez – Balanco *et al*., 2013) cell death is not a single entity but heterogenous structure, mechanism and biological function. Cell death or necrosis is characterized by pyknotic nuclei, cytoplasmic swelling and mitochondrial damage which results from failure in osmotic regulation caused by loss of cellular energy supplies (Lugato *et al*., 2018). By the 40th day of exposure there was recovery of the epithelial lining. According to (Lazcano *et al*., 2013) recovery could be brought by the chloragogen cells. These cells are known to migrate to the wound or lost tissue and regenerate them. It is well known fact that earthworms have a great power of regeneration (Ling *et al*., 2016).

## Conclusion

Many researches revealed that earthworms can improve soil fertility by stimulating physical, chemical, and biological characteristics of the soil. They can also change soil ecology by suppressing plant pathogens and promoting the growth of soil microflora and fauna. The overall study concluded that the *E. eugeniae* and *E. fetida* could be used to convert various waste into vermicompost. Through vermitechnology large amount of waste could be converted into organic manure, which in turn not only reduced pollution but also enhanced plant growth. It showed transporting the waste into vermicompost, traveling through the region of the gut, *E. eugeniae* and *E. fetida* were more effective by converting the environmental waste into vermicompost. Earthworms are beneficial to farmers in breakdown of organic matter, increasing soil fertility, maintaining soil structure and aeration of soil. This results in higher crop yield without polluting the environment. Farmers are forced to use herbicide and pesticide to increase crop yield. Though the practice is successful in immediate response in long term response it reduces the soil micro flora and earthworms which have destructive impact on the environment and could cause irreversible damage. It is very important to take the earthworm for a safer environment.

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