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IMPACT OF DICLOFENAC ON THE BIOCONVERSION OF FRUIT WASTE INTO MICRO AND MACRO NUTRIENTS BY EARTHWORM *EUDRILUS EUGENIAE*

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Abstract:

A study was made to understand the influence of Diclofenac on the efficiency of *Eudrilus eugeniae* in the bioconversion of fruit waste into micro and macro nutrients in our laboratory. The Fruit waste was collected from the market for the bioconversion study. In the present work, three experimental groups were maintained. One group was considered as control with cow dung alone and the second one was maintained with the fruit waste mixed with cow dung. The third group contained cow dung and fruit waste mixed with Diclofenac of 2mg. Ten earthworms were introduced into the three groups separately and this experimental set up was continued for 15 days and 30 days for the analyses of Nitrogen, Phosphorous, Potassium, Ferrous, Manganese, Copper and Zinc along with physico-chemical characters such as pH and Electrical conductivity. The analyses of Nitrogen, Potassium, Manganese, Zinc, Copper, on 15th day showed the decreased level in the third group where as Phosphorus level was increased. Among the various nutrients, phosphorus and ferrous were increased in the second group when compared with first group. The Electrical conductivity showed more or less same level and pH indicated slight increase in the third group. The experimental setup was maintained till 30th day for subsequent analysis of compost for the nutrients. The analysis indicated the decreasing trend of all nutrients except the manganese, Ferrous and copper. The pH was decreased in the third group. Electrical Conductivity remained the same. The second group showed increase of nutrients except Potassium and manganese. The results clearly shown that the diclofenac interfered the bioconversion of waste into micro and macro nutrients.

Key Words: *Eudrilus eugeniae*, Fruit Waste, Diclofenac and Vermicompost.

Introduction:

India and many other countries are facing from various problems particularly due to urbanization and solid waste management, which are a very rapid process and a worldwide phenomenon. Solid waste is the term now used internationally to describe non-liquid waste material arising from domestic, commercial, hospital, industrial, agricultural and mining activities. Solid waste comprises countless different materials such as dust, food waste, paper, metals, plastics, glass, discarded clothing, garden waste; etc. The disposal of solid waste should be properly managed. This study has been done for reducing the pollution problems due to solid waste by converting it into compost by using earthworms very successfully, economically and usefully.

Vermitechnology is an important aspect of biotechnology involving the use of earthworms for processing of various types of organic wastes into valuable resources. Vermicomposting helps to process wastes and simultaneously giving bio fertilizers and proteins (Prabha *et al*., 2005). Thus vermitechnology could successfully be used to clean the environment as it uses wastes as raw material to change polluted, costly chemical farming to sustainable agriculture (Lal *et al*., 2003).

Fruit wastes consist of fruits unsuitable for marketing due to bruising, inappropriate size, rotting, or insect attack, as well as marketable fruits that are discarded so as to raise their market prices. Wastes could be converted into a nutrient enriched biofertilizer and used for agricultural purposes or land restoration. Vermicomposting, a process involving the biostabilization of organic wastes by the joint action of earthworms and microorganisms, has proven to be a low- cost and rapid technique for the efficient management of vegetable wastes (Bansal and Kapoor, 2000).

DICLOFENAC:

In recent years pharmaceuticals have been seen as an emerging environmental protection problem due to their presence in water and soil ecosystems (Kummerer , 2010). Diclofenac (DCF, (2-[(2, 6-dichlorophenyl) amino] benzene acetic acid) is a synthetic non-steroidal anti-inflammatory drug (NSAD), mostly used as its sodium salt in medical care as an analgesic, anti arthritic, and ant rheumatic. The medicine has been applied in human medicine for over 30 years. Moreover, diclofenac may inhibit thromboxane-prostanoid receptor affecting arachidonic acid release and uptake, as well as inhibiting lipoxygenase enzymes, and activate the nitric oxide-cGMP antinociceptive (Gan, 2010).

Eudrilus eugeniae can be highly reproductive when living in ideal conditions. The life cycle extends from 43-122 days depending mainly on temperature. The maximum life expectancy of the worm is up to three years. The time requirement of *Eudrilus eugeniae* for maturity is 40 days and for cocoon incubation is 16 days. The average number of hatchlings is 2.7 earthworms cocoon /1 day (Dominguez *et al.*,2010).

Since, earthworm *Eudrilus eugniae* are considered as bio indicator, the present study is aimed to select the Earthworm and attempted to study the efficiency of earthworm in the bio conversion of Fruit waste mixed with Diclofenac by analyzing the micro and macro nutrients and also focused to Study the histology of earthworm to understand the impact of Diclofenac on the anatomy of earthworm.

Materials and Methods:

Collection of Earthworm:

Eudrilus eugeniae the elusion earthworm species was collected form Periyar Maniyammai University, Vallam, Tanjavur Dt, Tamil Nadu and the collected earthworms were brought to laboratory for acclimatization.

Experimental Methods:

The animals were grouped into three categories. The first category (group) contained cow dung and the second group contained cow dung mixed with pre digested fruit waste. The third group was prepared by taking cow dung, fruit waste mixed with diclofenac. The experimental set up was maintained in the laboratory with the introduction of earthworm *Eudrilus eugeniae.*The waste mixtures were allowed to pass through earthworm guts for vermicomposting. The moisture content of the organic substrates in each pot was maintained between 60% and 65% throughout the study period by sprinkling water after every 24 hours. The experiment was conducted by randomized design with three replications. The experimental set up was maintained till 15 and 30 days. After that the compost material was analyzed for the micro and macro nutrients.

Results and Discussion:

The conversion of Fruit waste into vermicompost process was observed by using *Eudrilus eugeniae* in our laboratory at various intervals. In the three groups the macro and micro nutrients were analyzed separately on15th day and 30th day. The analysis of Nitrogen (N), Phosphorus (P), Potassium (K), Iron (Fe), Manganese (Mn), Zinc (Zn), Copper (Cu), Electrical conductivity (EC), and pH, were carried out on 15th and 30th day, in the control, Fruit waste and diclofenac treated Fruit waste. The results obtained were represented in the table 1-2 and fig 1-2.

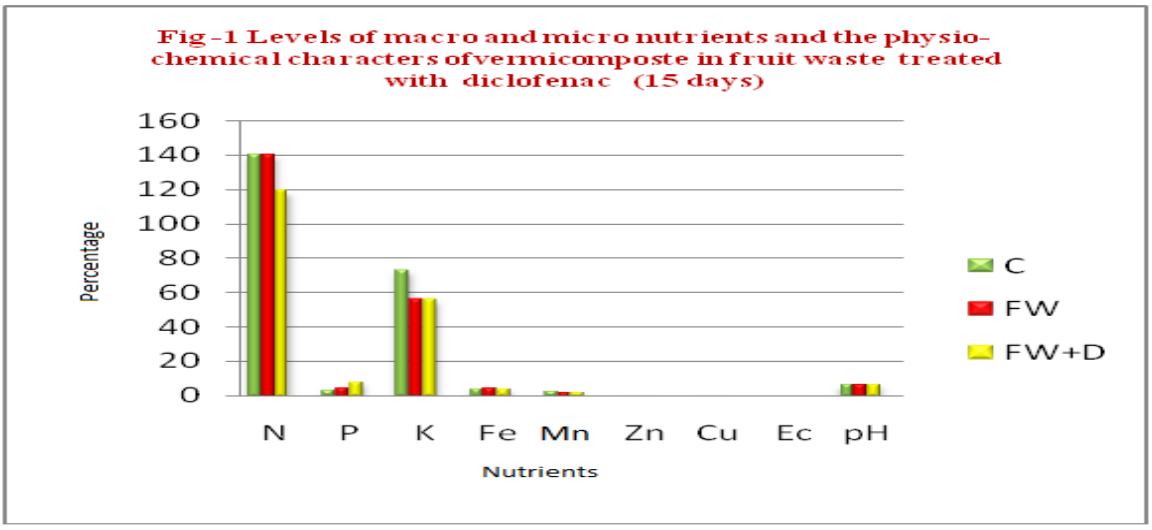
Table 1: Levels of macro and micro nutrients and the physico-chemical characters of vermicompost in fruit waste treated with diclofenac (15 days)

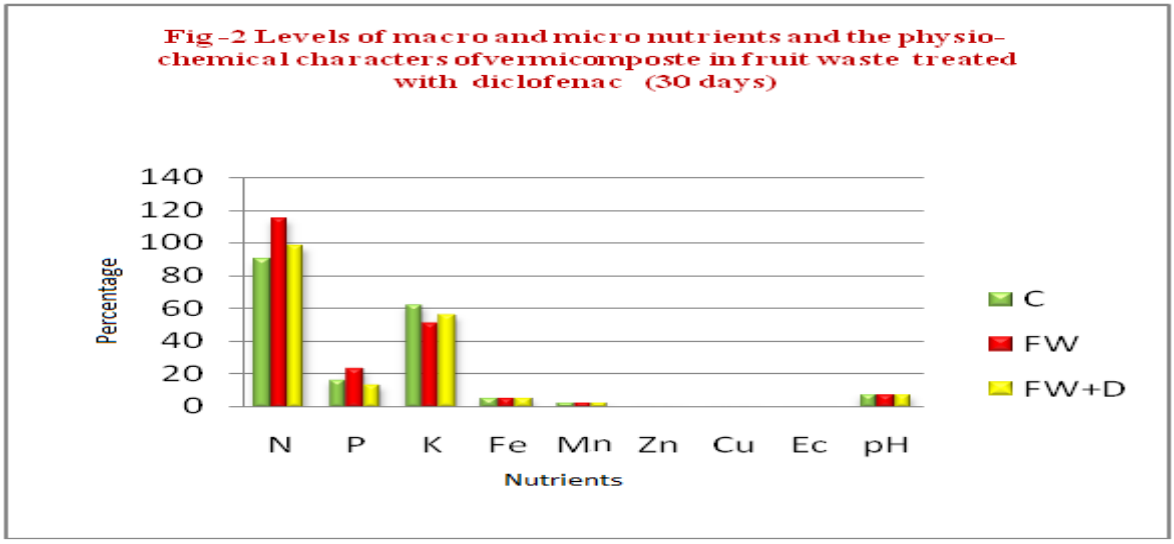
T	Macro Nutrients			Micro Nutrients				Physico-Chemical Characters	
	(N)	(P)	(K)	(F)	(Mn)	(Zn)	(Cu)	(Ec)	pH
C	140 ± 10.2	3.1 ± 1.3	73 ± 6.3	4.16 ± 1.8	2.20 ± 1.8	0.59 ± 0.02	0.66 ± 0.05	0.21 ± 0.03	6.5 ± 0.08
F W	140 ± 11.3	4.4 ± 2.1	56 ± 1.2	4.32 ± 2.3	2.16 ± 2.3	0.55 ± 0.08	0.61 ±0.03	0.30 ± 0.04	6.8 ± 0.09
F W+D	119 ± 8.6	8.1 ± 1.4	56 ± 9.3	4.17 ± 1.6	2.09 ± 1.9	0.55 ± 0.06	0.53 ± 0.01	0.20 ± 0.01	6.8 ± 0.13

Table 2: Levels of macro and micro nutrients and the physico-chemical characters of vermicompost in fruit waste treated with diclofenac (30 days)

T	Macro Nutrients			Micro Nutrients				Physico-Chemical Characters	
	(N)	(P)	(K)	(F)	(Mn)	(Zn)	(Cu)	(Ec)	pH
C	89.6 ± 11.2	16.2± 2.8	62 ± 9.5	5.07 ± 1.0	2.09± 0.8	0.56 ± 0.02	0.65 ± 0.02	0.3 ± 0.01	7.4 ±1.3
FW	114.8 ±10.1	22.9 ± 1.9	51 ± 1.2	5.26 ± 1.2	2.05 ± 0.4	0.75 ± 0.06	0.75 ± 0.06	0.34 ± 0.04	7.2 ±1.2
FW+D	98.0 ±10.5	13.2 ± 0.9	56 ± 1.1	5.08 ± 1.1	2.18 ± 0.9	0.52 ± 0.01	0.69 ± 0.04	0.3 ± 0.09	7.2 ±1.1

T - Treatment, N - Nitrogen, P - Phosphorus, K - Potassium, Zn - Zinc pH - Hydrogen ion concetration, Mn - Manganese, Cu - Copper, F - Ferroue, Ec - Electrical coductivity, C - Control, FW – Fruit Waste, FW+D – Fruit Waste and Diclofenac





The N value was decreased in the diclofenac treated experiment where as in the other treatments, the level of N was same as that of control value. All the values were statistically significant at P<0.01. The 89.6 % of nitrogen level was noticed in the fruit waste on 30th day analysis. In the diclofenac treatment the level of nitrogen was increased by 119 % on 30th day. The study indicated that the optimum levels of mineralization takes nearly 15days. Similarly P was also increased only in the treatment where the level was 8.1 ± 1.4 and showed significance at P<0.05 (Table-1and Fig-1). The rest of the value was also showed significant on 30th day and the analysis on 30th day, indicated the gradual increase of nutrients in all treatments except diclofenac treated group. Statistical analysis also showed significance at P<0.01 level. The increase in P content during vermicomposting could have been induced by the mineralization and the mobilization of phosphorus through bacterial and faecal phosphatase activity of the earthworms. The direct action of worm gut enzymes and the stimulation of micro flora could increase phosphorus. Le Bayon *et al*.,(2006) who observed an increase in phosphorus during the vermicomposting of paper waste sludge and organic substrates. The significant increase in the total nitrogen on 30th day analysis is probably due to mineralization of the organic matter. The increase in the available phosphorous is due to the passage of ingested material through earthworms and due to the stimulation of microbial flora (Edwards and Bohlen, 1996). The microbial flora also increases the available potassium in the final product. Microorganisms in the feed mixture produce acids which convert the insoluble potassium into soluble one (Atiyeh *et al*., 2000).

The same experimental set up was kept for further analysis to study the efficiency of earthworm, on 30th day where the nutrients were increased. The statistical analysis revealed significant at P<0.01 level (Table-2 and Fig-2). Similar trend of K also noticed on 30th day. The level observed on 30th day was lesser then the level observed on 15th day. The earthworm biomass had increased in all the treatments. An increase in the values was observed in the following parameters such as total N, P, and K on 15th and 30th day in Fruit waste treated when compared to control. It is obvious from the results that the vermicompost had comparatively higher quantities of total N and P than the Fruit waste. The analysis of macro and micro nutrients at 30 days showed decreasing level (Table-2). The analysis of Fe, showed an increase only in the fruit waste and all other treatments showed more or less equal. The Fe in the diclofenac treated and control value was same trend and showed statistical insignificance. Similarly Fe, Mn, Zn and Cu were also showed insignificance. On 15th day analysis, Fe showed an increase in all the treatments. Mn also indicated the slight decrease in all the treatment on 15th day. Similarly Zn and Cu were also decreased significantly at P<0.05 level.

Physico- Chemical Properties:

Physico-chemical properties of the three experimental groups were analysed on 15th and 30th day along with control and experiments. The Electrical Conductivity (EC) was increased by 0.30 ± 0.04 in Fruit waste treatment where as in diclofenac treated Fruit waste the level was decreased. The increased values were statistically significant at P<0.01 level. On 15th day, pH value was slightly increased and no change was observed in rest of the treatments and indicated statistical insignificance. In contrast to the analysis on 15th day, the value was increased in the treatments after 30th day. The significant value was observed only in the diclofenac treated experiment. The pH of the present study was increased on 15th day whereas on 30th day it was decreased when compared to the control of 30th day analysis. The bioconversion of organic material into intermediate substances of organic acids and the volatilization of ammonia could also account for the decrease in pH. Similar observations were reported by Garg *et al*., (2006). The soil pH is an important because it affects the availability of nutrients in the soil. Many plant nutrients are not readily available to plants in highly alkaline or acidic soils. Vermicompost is potential organic manure rich in plant nutrients compared to farmyard

manure or other organic manures in respect to supply of N, P, and K fertilizers The activities of dehydrogenase, nitrogenase, phosphatase, and urease were found higher in the process of vermicomposting. It is fast growing in popularity as a tool of reclamation of waste and is used for reclamation of waste land, (Sinha *et al*., 2005).A decrease in pH was recorded in all treatments during vermicompostingand ranged between 6.5 ± 0.08 and 7.4 ± 1.3. The shift in pH during the study could be due to microbial decomposition during the process of vermicomposting (Elvira *et al*., 1998).

Histological Studies of Anterior, Clitellar, Middle and Posterior Regions of the Earthworm *Eudrilus eugeniae* treated with Diclofenac: (Plate 1&2)

The cross sections of control and diclofenac treated were taken to study the anatomical features of *Eudrilus eugeniae*. In the control animal, the epidermis of the *Eudrilus eugeniae* consists of an epidermal epithelium and an overlying fibrous cuticle. Below the epidermis, circular and longitudinal muscles were intact. The coelom was clearly seen. Along with this, luman, blood vessels, chloragogen cells were seen clearly. Below the epidermis, the pigment cells were numerous

PLATE 1: Cross Sections of Anterior and Clitellar regions of Earthworms, *Eudrilus eugeniae* exposed to Diclofenac

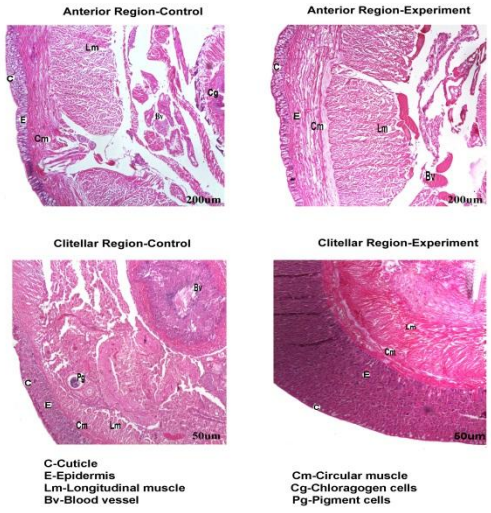
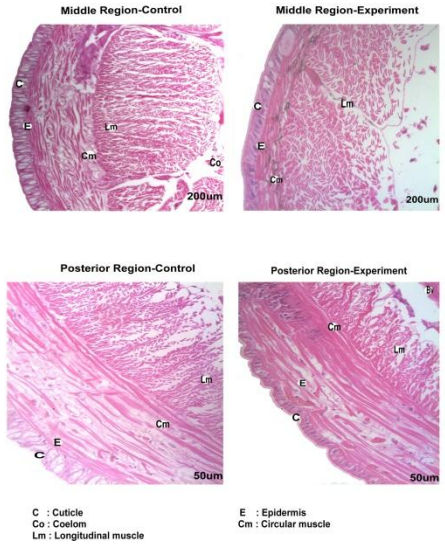


PLATE 2: Cross Sections of Middle and Posterior regions of Earthworm *Eudrilus eugeniae* exposed to Diclofenac



Anterior Region:

Histology of the anterior region reveals that the cuticle is not much damaged and not much spoilage in the area of circular muscles but in some part of the longitudinal muscle signs of injury is seen. Detachment of peritoneum from longitudinal muscles is seen in some of the areas. The lumen of the intestine is not much damaged. The ventral nerve cord is damaged to a lesser extent. The dorsal and ventral blood vessels ruined largely.

Clitellar Region:

In the control, the cuticle, epidermis, longitudinal, circular muscle are very intact where as in the experimental, the intact nature is disturbed. The circular muscles are loosened. Number of pigment cell are absent in the experiment. Blood vessels are not seen clearly. Coelom is much reduced in the experimental clitellar region.

Middle Region:

In middle region the cuticle and the epidermis are intact. The circular and longitudinal muscles are also unaffected. The peritoneum shows the signs of disconnect at several places. The typhlosole and the nephridiopore are clearly visible. The chloragogen cells crumble to a considerable extent. The ventral nerve cord is not much damaged where as the dorsal blood vessel and ventral blood vessel have shown the signs of damage. The nephridia are feebly visible. Generally, the tissue of the test individuals were not much affected in this concentrations.

Posterior Region:

The cuticle of the posterior region is faintly visible. Light destruction is visible in some parts of circular muscles. However, in the longitudinal muscles more damage and fissures are seen in few areas. The peritoneum covering the intestine is injured largely. The intestinal epithelial lining and the lumen of the intestine are also

damaged. Greater degree of injury to the dorsal and ventral blood vessel have been observed. Ventral nerve cord is damaged beyond recognition.

Conclusion:

Vermicomposting technique converts decomposable organic wastes into valuable vermicompost through earthworm activity in a faster and better process when compared with the conventional methods of composting. The accumulation of toxic chemicals and its effects on the growth, reproduction and life cycle of a dominant earthworm species was described by many researchers in Indian crop fields. Hence in the present study it could be concluded that *Eudrilus eugeniae* exhibited by a way of showing minor pathological symptoms and observed that the contaminant reduced the vermicomposting ability of the earthworm. Analysis of various physico chemical parameters during composting at different time intervals showed favorable changes in pH, electrical conductivity, and nitrogen which are proved to be an important criteria for monitoring the efficient and quick degradation of waste into high quality organic manure, thus vermicomposting by earthworm which promises to play a significant role both in cleaning the environment and building up of soil fertility for sustainable agriculture. The species of *Eudrilus eugeniae* exhibit better in converting the waste and increasing the percentage of NPK. Vermicomposting could be applied as an alternative technology for the management of waste. The suitability of the vermicompost produced as an organic fertilizer was confirmed by the analysis of micro and macro nutrients.

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