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Research Article

BIOCONVERSION OF NEEM TREATED POULTRY WASTE INTO MICRO AND MACRO NUTRIENTS AT DIFFERENT INTERVALS BY EPIGEIC EARTHWORM, *EUDRILUS EUGENIAE* (KINEBERG, 1867)

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ABSTRACT

The efficiency of the earthworm *Eudrilus eugeniae* in the bioconversion of poultry waste of different proportions into micro and macro nutrients was assessed at different intervals. The earthworm maintained in the vermibeds, was utilized for experiment using pots with soil of different proportions of poultry waste such as 30g, 40g, 50g, 60g, 70g, and 90g which were prepared separately and control was also maintained simultaneously. One pot was mixed with the commercial neem to understand the impact of neem on the bioconversion of poultry waste into nutrients. The study was carried out for 20 days with interval of 10 days. The results revealed the potential nature of the earthworm in the biotransformation of poultry waste into micro and macro nutrients. In the neem treatment, the level of nitrogen was decreased when compared to control whereas the nutrients such as potassium and phosphorous were also increased. Similarly the analysis of Fe, Mn, Zn and Cu were also increased in the 70gm and 90gm of poultry wastes and in all other treatments showed lower levels of Fe whereas the Fe in the neem treated was same level as that of control value however in the neem treatment the level of nitrogen was decreased when compared to control. Histological observations were also simultaneously observed and studied the changes in the anatomy of the earthworm in relation to the adaptation to the bioconversion of poultry waste into nutrients.

INTRODUCTION

Vermicomposting, an eco-biotechnological process, transforms complex organic energy-rich substances into stabilized humus compost where the inoculated earthworms in turn maintain aerobic condition in the organic wastes and convert a portion of the organic material into worm biomass. In total, the potential of some epigeic earthworm recycle organic waste materials into value-added products (Benitz *et al*.1999). Vermicompost has been shown to have high levels of organic matter, organic carbon, total and available Nitrogen, Phosporous, Potassium, other micronutrients, microbial and enzyme activities, as well as plant growth regulators. Vermicomposting, a mesophilic process carried out by earthworms, involves ingestion, digestion, and absorption of organic waste followed by excretion of castings through the worms metabolic system which enhances the levels of plant-nutrients of organic waste during their biological activities. Utilization of earthworms for recycling of organic wastes is an important development in the biological sciences (Giraddi, 2000). In India, nearly 2000 million tons of animal wastes, 300MT of wastes, besides huge amount of agro-industrial and domestic sewage waste have been produced annually (Mishra, 2001) and therefore there is a tremendous scope for recycling of this waste using vermitechnologies so that quality organic manure is produced. Indeed it is the only bioprocess that involves one or other type of animal farming in which a multicellular animal is used in reactor systems to generate a product other than the animal offspring. Considerable work has been going on, in the science of vermicomposting, especially in terms of biotic and abiotic factors, which influence vermicast production, earthworm growth and fecundity. Studies are also being increasingly reported on the vermicompost ability of newer substrates and newer earthworm species. But the aspects of vermicomposting process design, control, operation, and optimization are by and large still unaddressed.

Among the animal droppings, with the exception of cow dung that is available in plenty, sheep and poultry droppings are the other animal droppings produced in large quantities. Poultry

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wastes are the excretory products of poultry birds, which are produced in large quantities in India . They contain good amount of nutrients required for plants growth. However, they are not suitable for direct application into the crop fields and therefore scientists tried to utilize these wastes in different ways. Composted poultry waste, on the other hand, can be an excellent resource for amending and fertilizing soils (Hart and Mc Neilam, 2000). In recent times, agricultural practices involves the utilization of plant derived compounds for the control of insect pest and particularly neem derived products are being used extensively for the management of agricultural pests. In this context, attempt has been focused to study the impact of neem on the bioconversion of poultry waste into nutrients by epigeic earthworm *Eudrilus eugenia.*

MAETRIALS AND METHODS

Collection and maintenance of Earthworm

Eudrilus eugeniae, collected from Vallum, Tanjore District, and Tamil Nadu was brought to laboratory for acclimatization. It grows well at a temperature of more than 25ºC but best at 30ºC. It is locally available in and around South Tamil Nadu. Initially for the maintenance of earthworms, vermi bed was prepared as per the standard procedure. The earthworms were kept in an earthen pot, which was half filled with a mixture of loamy and humus soil supplemented with cow dung; poultry waste was moistened with tap water. During the period of acclimatization, the worms were fed with air dried cow manure. Earthworms of similar sizes were carefully selected from the earthen pots for further studies. The poultry waste was collected from Sri Venkateswara Poultry Farm near Vandavasi. Fresh poultry droppings were mixed thoroughly with soil. This mixture was placed in the form of heaps under shady place. Watering was done regularly twice in a day in order to maintain the optimum temperature and moisture. This set up was maintained for 30 days.

Experimental methods

In the experiment, Poultry waste was mixed with soil in eight different proportions separately i.e., 30g, 40g, 50g, 60g, 70g, 90g and one pot was mixed with commercial Neem product of 2ml/2kg soil along with poultry waste and introduced 10 worms. Similarly, control was maintained without the addition of neem. 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.

Micro and macronutrients analysis

Physico-chemical parameters such as pH, Electrical Conductivity (EC) (Davis and Freitas, 1970), and the macro and micro nutrients such as Total Nitrogen (N) (Black, 1965), Total Phosphorus (P) (Olsen and Sommers, 1982), Total Potassium (K) (Gosh *et al., 1983).* Total Manganese (Mn), Total Zinc (Zn), Total Copper (Cu), and Total Iron (Fe) (Pawluk, 1967) were estimated.

Histological studies

The histology of clitellar region of earthworm was studied by adopting the routine paraffin method. The control and experimental animals were blotted free of mucus, washed thoroughly in physiological saline, cut into pieces of clitellar region of desired size and fixed in Bouin's fluid immediately. 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. Tissue cross sections of 5-µm thickness were obtained using a rotary microtome. The section, thus obtained, were stained in Harris hematoxylene and eosin, dehydrated using alcohol, cleared in xylene and mounted using dihydroxy phthalate xylol. The stained slides were observed under Qasmo research microscope.

Statistical Analysis

The data obtained in this study were subjected to statistical analysis to understand the significance of the results by using student't' test and correlation matrix.

RESULTS

The conversion of poultry waste into vermicompost process was studied by using *Eudrilus eugeniae* in our laboratory on 10th day and 20th day in the control, neem treated poultry waste and in the different proportions of poultry waste (Figure: 1- 4). The compost was subjected to the analysis of Nitrogen (N), Phosphorus (P), Potassium (K), Iron (Fe), Manganese (Mn), Zinc (Zn), Copper (Cu), Electrical conductivity (EC) and pH.

Micro and macronutrients analysis

The analysis of nitrogen in the control showed 79% and this level was increased in the treatment of 50g, 60g, 70g and 90g of poultry waste and the increase of nitrogen was statistically significant at $P< 0.001$. In the neem treatment also, the level of nitrogen was increased on 10th day analysis however further analysis on 20th day, indicated the decrease of N level. The other nutrient, P was also increased to 30% which showed significance at $P<0.05$. The rest of the value was not showed significant except at 40g and 90g treatments on 10th day. The analysis of K on 10th day revealed gradual increase in all the treatments except neem treated poultry waste and 70g treatments. Statistical analysis also showed significance at $P<0.01$ level. The same experimental set up was kept for further analysis to study the efficiency of earthworm, on 20th day.

At the end of the 20th day, an increase of the nutrients such as total N, P, and K was observed. It is obvious from the results that the vermicompost had comparatively higher quantities of total N, P, and K than the raw poultry waste. The correlation matrix showed in significant value where as P revealed significant correlation only in 90g treatment of poultry waste. In contrast to P, K showed insignificance when compared all other treatments. The analysis of Fe, Mn, Zn and Cu were increased only in the treatment with 70gm and 90gm of poultry wastes and all other treatments showed low levels of Fe.

Table 1 Analysis of correlation matrix between various treatments and mineralization process of poultry waste by *E. eugeniae* on 10th day.

	N	P	K	Fe	Mg	Zn	Cu	EC	pH
	1								
C	-0.73496*	1							
NT	-0.26611	-0.18216	1						
P1	-0.16553	-0.19878	-0.00809	1					
P2	-0.38013	-0.1356	0.302364	0.45318	1				
P3	0.170767	-0.12719	-0.32032	-0.13916	0.540148**	1			
P4	0.182995	0.131306	-0.092	-0.07387	0.113952	0.505219**	1		
P5	0.450456	-0.6217	0.291164	0.40841	0.31703	0.043787	0.172685	1	
P6	-0.25532	0.622799*	-0.084	-0.19485	-0.7122*	-0.74484*	-0.15333	-0.30715	1

Table 2 Analysis of correlation matrix between various treatments and mineralization process of poultry waste by *E. eugeniae* on 20th day.

	N	P	K	Fe	Mn	Zn	Cu	EC	pH
	1								
C	0.603606**	1							
NT	0.570306**	0.989967*	1						
P1	0.77415*	0.55117**	0.525309**	1					
P2	0.236304	0.672612*	0.645463*	0.558758**	1				
P3	-0.01156	-0.03128	-0.09619	-0.49167**	-0.33074	1			
P4	0.615178*	0.287262	0.361177	0.55394**	0.108201	-0.40643	1		
P5	-0.52943**	-0.3656	-0.28236	-0.2389	-0.00984	-0.622648	0.13644	1	
P6	0.047274	0	0.026944	-0.06561	-0.17782	0.386804	0.188445	-0.50232**	1

***Significant at P<0.01 level; ** Significant at P<0.05 level**

C = Control; NT=Neem Treated; P1=Poultry Waste (30g); P2=Poultry Waste (40g); P3=Poultry Waste (50g) P4=Poultry Waste (60g); P5=Poultry Waste (70g); P6=Poultry Waste (90G).

N=Nitrogen; P=Phosphorous; K=Potassium; Fe=Iron; Mn=Manganese; Zn=Zinc; Cu=Copper; EC=Electrical Conductivity; pH=Hydrogen Ion Concentration

The Fe in the neem treated and control value was same trend and showed statistical insignificance. Similarly Fe, Mn, Zn and Cu were also showed significance in 60g, 50g and 30g treatments. On 20th day analysis, Fe showed increase in all the treatments except in the neem treatment where the Fe level was less when compared to control. Mn also indicated the increase in all the treatments on 20th day. Similarly Zn and Cu were also increased significantly in the 40g, 50g and 70g treatments (Table 1, 2 and Fig.1, 2).

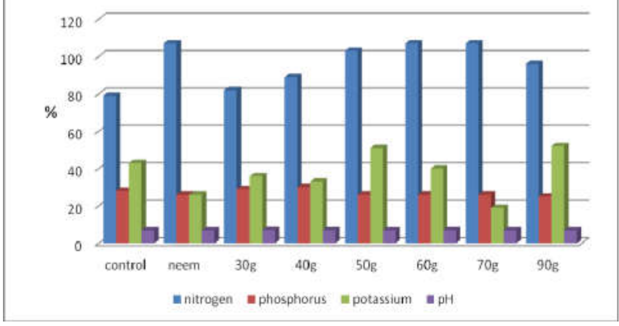

Fig.1. Nitrogen, phospohorus, potassium and pH of the vermicompost on 10th day analysis

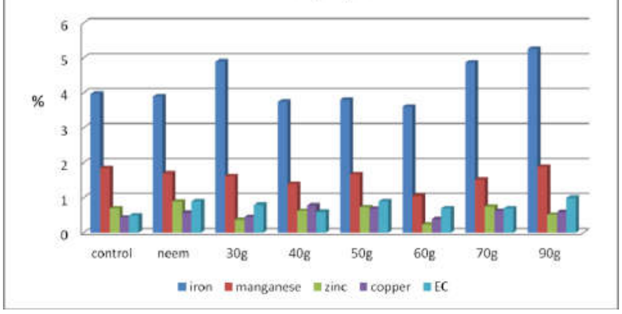

Fig.2. Iron, manganese, zinc, copper and electrical activity of the vermicompost on 10th day analysis

Physico - Chemical Properties

Physico-chemical properties of the soil mixed with different weights of poultry waste were analyzed on 10th and 20th day along with control and experiment. The electrical conductivity (EC) was significantly increased in the 30g to 90g treatments including neem treated poultry waste. On 10th day, the pH value was normal and no change was observed in all other treatments and indicated statistically insignificance. In contrast to the analysis on 10th day, the value was increased in the treatments after 20th day. The statistical analysis showed insignificance including correlation matrix (Table 1, 2 and Fig.1, 2).

Histology

The cross section of clitellar region of control, neem treated and poultry waste treatments of higher proportion were taken to study the anatomical features of *E. eugeniae*. In the control animal, the epidermis of the *E. eugeniae* consists of an epithelium and an overlying fibrous cuticle. Below the epidermis, circular and longitudinal muscles were intact and form the body wall. The coelom was clearly seen. Along with this, luman, blood vessels chloragogan cells were seen clearly. The pigment cells were numerous.

In the neem treated section, clitellar region showed less change when compared to control. The size of the blood vessel was reduced. The cuticle, epidermis and circular muscles were not changed in the experiment. However, the coelom was enlarged. The pigment cells were reduced to a greater extent. In the poultry treated clitellar region, very little changes were noticed in the luman, blood vessels and pigment cells. Inter vascular space was much reduced. Only in certain regions the thickness of the body wall was reduced. The poultry waste and neem were not much impact on the anatomy of the *E. eugeniae* (Plate 1 and 2).

PLATE 1.HISTOLOGICAL SECTIONS OF CLITELLAR REGION OF THE EARTHWORM *EUDRILUS EUGENIAE* (4x)

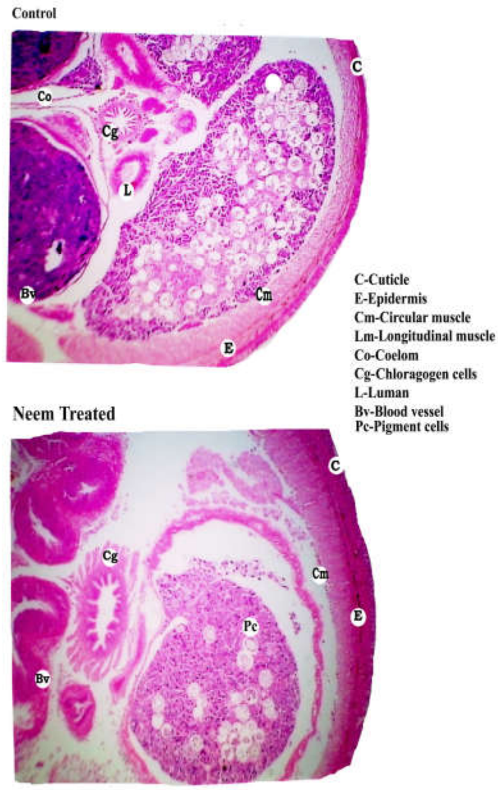

PLATE 2.HISTOLOGICAL STUDIES OF CLITELLAR REGION OF THE EARTHWORM *EUDRILUS EUGENIAE* (4x)

DISCUSSION

Over the last few decades, production of broilers has been on a constant rise and as a result large amount of poultry waste is being produced every day. Poultry droppings are the excretory products of poultry birds, which contain good amount of nutrients required for plants' growth. However, they are not suitable for direct application into the crop fields hence the waste has been converted into compost in different ways. In this study for the benefit of farmers, environmentalists, etc., the waste has been converted into composted using *E. eugeniae* and efficiency of the earthworm has also been assessed by analyzing the macro and micro nutrients. Vermicomposting accelerates the mineralization process and subsequently enriches the end product with more available form of soil nutrients. The present analysis showed the increase of nitrogen in the various treatments and suggested that nitrogen increase in vermicomposted material was directly related to the physico-chemical properties of the initial substrates. Benitez *et al.* (1999) suggested that the hydrolytic enzyme production plays an important role in C and N cycle in waste decomposition system and is drastically influenced by the availability of easily degradable organic compounds in vermibeds.

It is obvious from the results that the vermicompost material had greater nitrogen content. It is suggested that the earthworm enhances the nitrogen level of the substrate by adding its excretory products, mucus, body fluid, enzymes and even through the decaying tissues of dead worms in vermicomposting sub-system (Suthar, 2007). The increasing trend of N in the vermicomposts produced by the earthworm species in the present study corroborated with the findings of earlier reports (Balamurugan *et al*., 1999). The enhancement of N in vermicompost was probably due to mineralization of the organic matter containing proteins and conversion of ammonium nitrogen into nitrate which could be attributed directly to the species specific feeding preference of individual earthworm species and indirectly to mutualistic relationship between ingested microorganisms and intestinal mucus (Suthar and Singh, 2008).

The gizzard and the intestine, work as a 'bioreactor' where only 5-10 percent of the chemically digested and ingested material is absorbed into the body and the rest is excreted out in the form of fine mucus coated granular aggregates called 'vermicastings' which are rich in nitrates, phosphates and potash. The analysis also showed significant increase of phosphorous and potassium in the treatments and the mineralization process may be increased in the gut due to enzymatic actions of microbes. Earthworms accelerate the growth of beneficial decomposer microbes in waste biomass which indirectly helpful in the bioconversion. According to Lee (1992) the passage of organic residue through the gut of earthworm, results in the conversion of Phosphorous which are available to plants. The release of phosphorous in the available form is performed partly by earthworm gut phosphatases, and further release of P might be attributed to the P-solubilizing microorganisms present in the worm casts.

The results obtained in this study are similar to those by Delgado *et al.* (1995) who demonstrated higher potassium concentration in the end product obtained from the sewage sludge. Earthworm processed waste material contains high concentration of exchangeable K, due to enhanced microbial activity during the vermicomposting process, which consequently enhances the rate of mineralization. The analysis of Fe, Mn, Zn and Cu were also increased in the treatment with 70g and 90g whereas in rest of the treatments low levels of nutrients were observed. The increased nutrients may due to the action of symbiotic microbes and enzymatic actions in the gut regions of the earthworm. Apart from the micro and macro nutrients, an increase in the pH was recorded at the end of the experiment. The variability in pH could be due to the production of CO_2 and organic acids during organic waste decomposition.

According to Ndegwa *et al*. (2002) the shifting level of pH could be attributed to the mineralization of nitrogen and orthophosphates and bioconversion of organic materials into intermediate products such as organic acids. The near-neutral pH of vermicompost may be attributed by the secretion of NH^{+}_{4} ions that reduce the pool of H^{+} ions (Haimi and Huhta,1987) and the activity of calciferous glands in earthworms containing carbonic anhydrase that catalyzes the fixation of CO_2 as $CaCO_3$, thereby preventing the fall in pH (Kale *et al*. 1982). The increased trend of pH in the vermicompost is in consistence with the findings of Tripathi and Bhardwaj (2004) which was due to higher mineralization, whereas the present findings are in contradiction to the findings of Haimi and Huhta (1987) and Ndegwa *et al*.(2002) who reported lower pH. The increased pH during the process was probably due to the degradation of short-chained fatty acids and ammonification of organic nitrogen. Fares *et al.* (2005) found the increased pH at the end of the composting process, which was attributed to progressive utilization of organic acids and increase in the mineral constituents of waste.

The quantity of electrical conductivity (EC) of vermicompost depends on the raw materials used for vermicompost and their ion concentration. The increased EC during the period of the vermicomposting processes is in consistence with that of earlier workers (Jadia and Fulekar, 2008), which was probably due to the degradation of organic matter releasing minerals such as exchangeable Ca, Mn, K, and P in the available forms, that is, in the form of cations in the vermicompost. The vermicompost thus prepared is found to possess desirable nutrients at desired levels such as pH and total nitrogen which can be used for the crop production and maintenance of soil fertility. In the present study, the time taken for the bioconversion of poultry waste into vermicompost was nearly 20 days and it can be verified further studies with analysis of nutrients by allotting more time. The present study revealed that all vermicomposts prepared from the organic waste possessed considerably higher levels of nutrients such as P, K, and Mn which was probably due to the coupled effect of earthworm activity as well as a shorter thermophilic phase (Tognetti *et al.* 2007). The vermicompost thus prepared is found to possess desirable nutrients at desired levels such as pH, total nitrogen and hence it can be used for the crop production and maintenance of soil fertility.

The histological studies revealed the anatomical changes of the earthworm which indicate the quality and nature of the toxicants. To understand the impactness, the structural details are indicators of the impactness of the unwanted pollutants prevailing in the environment. In the present study also clitellar regions of the *E. eugeniae* have been studied in the neem treated and poultry waste treated regions of the clitellum. Follicle cells are numerous in the control sections. In the neem treated, not many variations have been found. The present study has shown that neem has not significantly decreased the thickness of body wall and epidermis however in some regions there may be increased in the thickness of intestinal epithelium and such change has not been well observed in other sections. Similar changes to the intestine have been reported in the earthworm *Lumbricus terrestris* exposed to volcanic soil with high Cu and Fe content (Amaral *et al.* 2006). The increased intestinal epithelial thickness may be interpreted as an adaptation of the earthworms to increased exposure to toxicants including neem, to protect the gut lining.

According to Morgan *et al*. (2002), morphological alterations in *Dendrodrilus rubidus* intestinal epithelium are a way coping with exposure to high metal concentrations. Collectively, such changes would affect the health of the earthworms including changes to the absorption and digestion of nutrients. Similar changes have also been reported in earthworms exposed to tetraethyl lead and lead oxide (Rao, 2003) and the organophosphorous pesticide profenofos (Reddy and Rao, 2008) whereas in the present study the poultry waste has not altered much in the clitellar regions of *E.eugeniae.* The number of follicle cells have been reduced and supposed to be the pigment cells are also reduced in numbers. These results suggest that the morphological biomarkers like morphometric and histological changes are helpful in assessing the toxicity of neem as well as poultry waste to earthworms.

CONCLUSION

The present study suggested that the addition of neem and poultry wastes based vermicompost contributed to the enrichment in total and available N, P, K and Ca. The treatment of 20^{th} days enhanced the nutrient contents in vermicomposting; hence, value was added to the quality of the vermicompost. The physical character like the EC was significantly increased in the 30g to 90g treatments including neem treated with poultry waste. On 10^{th} day, the pH value was normal and no change was observed in all other treatments and indicated statistically insignificance. In contrast to the analysis on 10^{th} day, the value was decreased in the treatments after 20^{th} day. The significant value was observed only in the neem treated experiment alone. The final vermicomposts were homogenous, rich in important plant nutrient macro and micronutrients which indicated their agricultural value as a soil conditioner.

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