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Comparative histological studies in the gut of earthworms exposed to chemical fertilizer and organic manure

KEYWORDS: Monocrotophos, *Ficus benghalensis, Ficus racemosa, Lampito mauritti, Eudrilus eugeniae,* Histological changes.

C.Esaivani
Department of Zoology, Sri Parasakthi College for Women, Courtallam, M.S.University, Tirunelveli, Tamilnadu, India

K.Vasanthi
Department of Zoology, Sri Parasakthi College for Women, Courtallam, M.S.University, Tirunelveli, Tamilnadu, India

K.Chairman
Department of Microbiology, Kamarajar Govt. Arts College MS University, India

ABSTRACT The present study has a strong focus on the practical aspects of the effect of chemical fertilizers and organic manure on the earthworms. The advent of Green revolution has lead to the use of chemical fertilizers and pesticides throughout the world to sustain high yielding crop varieties. Continuous and indiscriminate use of these chemicals has lead to the loss of soil fertility and soil organisms. Histopathological studies may signal a damaging effect of organisms resulting from prior or ongoing exposure to toxic agents. Monocrotophos is the most popular and widely used organophosphorus pesticide in agriculture. A large number of studies have reported general histological changes in earthworms. A fewer studies have reported more specific types of histopathological studies in *Eisenia foetida, Dendrodrilus rubidus, Lumbricus terrestris, Lumbricus rubellus* and *Octolasium traspandanum*. However none of the study was undertaken on earthworm *Lampito mauritti* which constitutes the dominant earthworm in crop fields of India. The earthworm *Eudrilus eugeniae* were reared in two different types of leaf litters namely *Ficus benghalensis* and *Ficus racemosa* mixed with cow dung in three different proportions such as 25%, 50% and 75% respectively. All parts of these plants are medicinally important. Large number of medicinal plants are claimed to possess anthelmintic activity. It has been demonstrated that all anthelmintics are toxic to earthworms and a substance toxic to earthworms is worthy for investigation as an anthelmintic. The aim of the study is to evaluate the changes in tissues of the earthworms that worked under field condition and also to compare the structural changes in tissues between worms exposed to chemical fertilizer and those worked in transforming the leaf litters into vermicompost. The findings of the present study clearly indicates that the changes observed in earthworms intestinal tract may have been caused by the exposure to over usage of pesticides and its residual availability in the soil may also be the case for the major alteration and damages observed in the tissues of earthworms exposed to pesticide monocrotophos at recommended field dose. The histology of earthworm which is subjected to various proportions of *ficus* leaf wastes and cow dung shows that increase in the concentration of litters gradually increase the damages of the tissues but the changes in tissues during vermicomposting were only minor damages when compared to that of the chemical fertilizer. Risk assessment is normally aimed at the protection of human health and the ecosystem, and the interrelationship of these two areas of protection is easy to perceive. The use of earthworms in risk assessment is to obtain more information on environmental quality and ensure environmental safety. There is an urgent need to test the chemicals causing toxicity to earthworms, and finding an alternative for the replacement of these chemicals is the need of the day, because earthworms play a major role in soil fertility as well as acting as a transferring route from the soil to the terrestrial ecosystem.

Introduction

Nowadays there is much usage of more number of chemical fertilizers to increase the productivity but it causes several damages to the ecology of the soil and their fertility. For health benefits agricultural practices are being modified with an organic farming (Kramer *et al* ., 2006). The use of biofertilizers is to increase the plant growth and development and it is also eco- friendly to the environment.

The cost of chemical fertilizers is very high and sometimes it is not available in the market for which the farmers fail to apply the chemical / inorganic fertilizers to the crop field in the optimum time. On the other hand, the organic manure is easily available to the farmers and is cost effective when compared to that of chemical fertilizers. In literature, some researchers have concluded the chemical fertilizers to be harmful for soil organisms but on the contradictory they have been supported too to be beneficial as far as their food supply is concern.

Organophosphorus pesticides are among the most widely used pesticides in agriculture and public health (Hodgson,2004). Monocrotophos is a liquid insecticide used on Paddy, Maize, Bengal gram, Sugarcane, Cotton and Bhendi for reducing the incidence of Jassid and fruit borer and for enhancing the yield of marketable fruit of Okra, as well as for achieving cost benefit ratio. In order to mitigate the losses due to pests, a huge quantity of pesticides is used in Okra. The excessive reliance on chemicals has lead to the problem of resistance, resurgence, environmental pollution and decimation of useful flora and fauna. The present study would like to focus on the deleterious effect of insecticides and increased usage of chemicals in the physiology of earthworm by undergoing histological studies.

Earthworms have been widely used in the breakdown of a wide range of organic waste including industrial, agricultural and municipal solid waste. The metabolic systems of earthworms transforming these waste into organic form as vermicompost accumulate some residues in their tissues, which cause damage or change in their organization and function of the same.

Ficus benghalensis is commonly known as Banyan tree or Vata tree in Ayurveda . It is a remarkable tree of India and is considered to be sacred in many places of India. *Ficus racemosa* commonly known as cluster fig is found throughout the greater part of India in moist localities. All parts of these plants (leaves, fruit, bark, latex and root) are medicinally important in the traditional system of medicine in India. A large number of medicinal plants are claimed to possess anthelmintic property in traditional system of medicine and are also utilized by the ethnic groups worldwide (Ravindra *et al*., 2008). Phytochemical analysis of *ficus benghalensis* has reported the presence of carbohydrates, flavonoids, aminoacids, steroids, saponins and tannins like phytoconstituents in their extracts and *ficus racemosa* has been reported to contain tannins, kaempferol, rutin , arabinose, bergapten, psoralenes, flavonoids, ficusin, coumarins and phenolic glycosides (Baruah and Gohain,1992) lupeol , quercetin and -sitosterol (Naghwa *et al*., 2005)

Among the reported compounds saponins bestow anthelmintic activity by causing vacuolization and disintegration of tegument (Prashant *et al*., 2011). Furthermore tannin rich plants and triterpenoids were said to exhibit beneficial anthelmintic activity. Tannin compounds disrupt the energy production of helminth by uncoupling oxidative phosphorylation or bind to the glycoprotein of

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parasitic cuticle, and thus leads to elimination of parasites (Yogesh *et al*., 2011). In view of its beneficial toxic effects, the safety and efficacy of these wastes during vermicomposting needs to be ascertained.

The earthworms were used as a model for studying anthelmintic activity of medicinal plant extracts in view of its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings (Khan Abedulla , 2008 ; Thorn *et al*.,1977) and also because of its easy availability. It has been demonstrated that all anthelmintics are toxic to earthworms and a substance toxic to earthworms is worthy for investigation as an anthelmintic.

Lokesh K Bhardwaj *et al* ., (2012) has reported the anthelmintic properties of *Ficus benghalensis* leaves extract against Indian earthworm *Pheretima posthuma*. (Bimlesh Kumar *et al*.,2011) have confirmed the invitro anthelmintic of aqueous and ethanolic extracts of leaves of *Clitoria ternatea* using *Eisenia fetida*. (Danquah *et al* .,2012) also performed an anthelmintic assay against *Lumbricus terrestris* indicating that the extracts of *Vernonia amygdaline* and *Alstonia boonei* have potent anthelmintic activity.

Earthworms are regarded as one of the most suitable animals for testing the toxicity of chemicals in soils and have been adopted as standard organisms for eco toxicological testing. Acute and chronic toxicity tests have been used traditionally to assess the toxicity of contaminants, with mortality and changes in biomass, reproduction rates and behavioural responses representing endpoints. The purpose for undergoing histological observation in the present study was based on the mortality and the change in biomass observed during the period of transforming the selected leaf wastes into vermicompost.

Wide literature survey shows, that so far no research has been undertaken to assess the effect of vermicompost obtained from *Ficus benghalensis* and *Ficus racemosa* and their impact on histology of the animal. Therefore, the objectives of present investigation was aimed to study the effect of selected leaf litters on the histology of the earthworm and also to compare the structural changes in tissues between worms exposed to inorganic and organic fertilizers ,with evidences of cross section of various regions of earthworms.

Materials and Methods

Collection of Earthworms

The earthworms were collected in groups directly from the field where conventional farming practices were carried out using inorganic fertilizers and pesticides, this serves as the control animal. Similarly, another group of worms exposed to litters of *Ficus benghalensis* and *Ficus racemosa* mixed with different proportions of cow dung was collected and this serves as the experimental animal.

Tissue Fixation

The histology of gut of earthworm was studied adopting the routine paraffin method (Humason, 1979). Agar serves as a good medium for empting the gut contents .50ml jars were filled with 30ml of 1.5% agar gel prepared with deionized water (Pokarzherskii et al., 2000). After cooling and solidifying, this gel in the jars was taken out and cut into small pieces. The earthworms were then transferred into the agar pieces containing jars and kept for 24 hrs to remove all the gut contents. After removing the gut contents, 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 hr, after which the tissues were transferred to 70% alcohol. The tissues were then dehydrated by passing through different grades of alcohol, then cleared using xylene, and finally embedded in paraffin wax (58°C MP).

Tissue Sectioning

Tissue section of 5- m 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 dihydroxyphthalate xylol (DPX). The stained slides were observed under Axio-2 Plus research microscope.

Results and Discussion

Histological studies of various gut regions of the worms exposed to chemical fertilizers and litters of Ficus benghalensis **and** Ficus racemosa **mixed with different proportions of Cow dung**

Foregut

Histology of the tested foregut reveals that the epidermis has suffered damages, showing large intercellular spaces, and loss of structural integrity, when compared with that of organisms exposed to organic manure. The epidermis displayed severe signs of degradation almost exposing the circular muscle. Some regions showed an almost complete disintegration of the epithelial cell lining whereas the foregut region of the worms worked in 25% of the leaf litters shows that the cuticle and epidermis are untouched with the damage. The circular and longitudinal muscles are well formed and show no marks of damage. The ventral and dorsal blood vessels are very much unaffected. This indicates that 25% of *Ficus benghalensis* and *Ficus racemosa* does not affect the histology of earthworm.

Midgut

In the midgut region the circular muscles showed extensive loss of structural organization and loss of staining properties when compared to organisms exposed to vermicompost an organic fertilizer. This muscular layer was almost completely destroyed, suffering extensive necrosis. The longitudinal muscle displayed atrophy and was completely altered in its structural integrity. Cells showed necrosis and severe changes on their shape and consequently, muscle fibres were completely disintegrated. On the other hand the tissues of the worms worked in vermicompost containing 50% of the litters reveals that the cuticle is damaged in some areas. The epidermis has eroded completely in few areas. Light destruction is visible in some parts of circular muscles. Longitudinal muscles are unaffected. The chloragogen cells are spread through the coelom. The intestinal wall is also damaged severely. In general, the effect of 50% of *Ficus racemosa* is much more visible than in other combinations whereas the tissue of the experimental animal were not affected when *Ficus benghalensis* were mixed with cow dung in equal proportion.

Hindgut

The tissues of worms exposed to chemical fertilizer indicated that the epithelial lining were totally obliterated and fused with development of cavitations. The nuclei were swollen with weak staining when compared to the experimental worms. The size of chloragogen tissue was reduced and the nerve cord was severely affected. The intestinal epithelium is injured beyond recognition. Cells completely lost their shape, compromising the structural integrity of the chloragogenous tissue. The atrophy of the intestinal epithelium was evident, as it became very thin, when compared to that of earthworms exposed to organic manure. The intervilli spaces disappeared and the villi were fused, forming a continuous and thin layer of cells. The tissues of the worms worked in vermicompost containing 75% of litters showed that the cuticle of the earthworm hind gut region, epidermis under it is faintly visible. The circular muscles have shown the signs of damage in several areas. The longitudinal muscle has shown the signs of cracks in major parts of the regions. The dorsal and ventral blood vessels are seen faintly. The intestinal epithelium is also injured. In short, the increased proportion of *Ficus benghalensis* has a telling effect on the histology of the earthworm whereas the clitellar region of 75% *Ficus racemosa* exposed earthworms is severely affected.

Structural changes of tissues exposed to chemical fertilizer and organic manure

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In the present study, extensive alterations were observed in the tissues of the body wall and intestinal tract of the organisms exposed to inorganic fertilizer and pesticides when compared to that of organisms exposed to organic manure.

The histological changes observed in earthworm's body wall may have been caused by the exposure to over usage of pesticides and its residual availability in the soil, since one of the main exposure routes of earthworm is through dermal contact (Vijver *et al* .,2005). Mohssen, (2000) have concluded that the recommended field dose of glyphosate is detrimental to *Pheretima elongata*, causing severe histopathological lesions and histochemical changes in the intestinal lining and causing atleast 50 % mortality in the earthworm population. Therefore, it seems feasible that this may also be the case, for major alterations and damages observed in the tissues of earthworms exposed to pesticide monocrotophos at recommended field dose.

Earthworm's chloragogenous tissue and intestinal epithelium also revealed histopathological changes on organisms exposed to pesticides. The chloragogenous tissue presented clear changes, as cells began to lose their shape, while no major effects were observed in their intestinal epithelium. The histological findings throw light on epithelial tissue damage especially fusion of intestinal villi that leads to the reduction of nutrient absorption area from food. Simultaneously, intoxication process consumes reserve energy from chloragogen tissue which would lead to reduced production of biomass and cocoon production.

It most group of animals, histopathological changes have been shown to cause tissue damage and it can be taken as a marker of toxicants exposure and in certain case provide precise information about the toxicant causing such damages (Hinton *et al*., 1992). (Gupta and Sundararaman, 1988) have found that carbaryl induced changes in chloragocytes of *Pheretima posthuma* where the nuclei become swollen and rounded because of the effect of carbaryl toxication. Morgan *et al*., (1992) have recognized the use of earthworm as biological monitor to determine the accumulated concentrations of pollutant in their tissue.

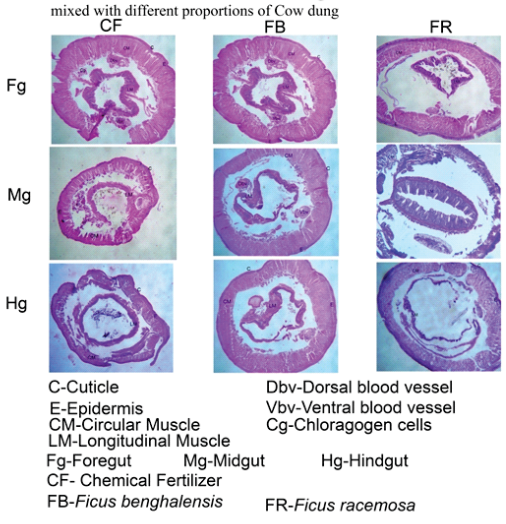
The tissue specimen of the worms worked in vermicompost containing appropriate quantity of cow dung and litters of *Ficus benghalensis* and *Ficus racemosa* , showed the clear evidence of nerve cord. Clear chloragogen tissue layer was observed with no vacuole formation in the vermicompost worked worm. In the chemical fertilizer exposed earthworms, the disintegration of nuclei and the enlargement of epithelial layer was visualized. A similar observation was noticed by (Morowati, 2000) on exposure of *P.elongata* to herbicide and found that the inner epithelial layer underwent necrosis with disrupted cell membrane. The bioavailability of the chemical to the earthworm can be modified dramatically by soil physical or chemical characteristics (Lanno *et al*., 2004). The effect of various pesticides and heavy metals on the earthworm brings down the risk of entry of these pollutants into plant system and into sequential food chain.

Researches have reported that the passage of organics through the earthworm's gut significantly alters the physical structure of the material. Large particle are broken down into numerous smaller particles, with a resultant of enormous increase in surface area. Soil pollution bio-indicators are essential to establish environmental standards. The objective of culturing earthworms in *ficus* leaf wastes revealed that the earthworm could live in these wastes. In the present investigation earthworms was subjected to various combinations of *ficus* leaf wastes and cow dung. It has been observed that even though these leaf waste materials possess certain compounds which are toxic to earthworms, the changes in tissues observed during vermicomposting were only minor damages. The histology of earthworm, which is subjected to various proportions of leaf wastes and cow dung, shows that increase in the concentration of litters gradually increase the damages of the tissues.

Conclusion

The feasibility of using earthworms for leaf waste management is dependent on the fundamental knowledge of basic parameters governing the survival and growth of earthworm species. The present set of investigation is aimed in this direction and the results are encouraging. Soil pollution bio-indicators are essential to establish environmental standards. The objective of employing earthworms for vermicomposting leaf wastes of *f.benghalensis* and *f.racemosa* revealed that the earthworm could live in these wastes when they are mixed with appropriate proportion of cow dung as supporting medium. Since, histological based results provide more information about earthworm toxicology. The present study clearly stresses that vermicompost are a boon to the agriculture and they are ecofriendly with less cost effectiveness without affecting the soil and their ecosystem. Earthworm's are farmer's friend so replacements of the chemical fertilizer by the organic manure namely vermicompost is an adaptive way for today's world. Thus treating the wastes with appropriate quantity of cow dung would help to reduce the major environmental risk posed due to leaf litter accumulation without affecting the physiology of earthworm.

Histological studies of various gut regions of the worms exposed to Chemical Fertilizer and litters of *Ficus benghalensis* and *Ficus racemosa* mixed with different proportions of Cow dung



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