ABSTRACT

The earthworm is adapted to the environment in decaying organic material, thrives in rotting vegetation, compost and manure. The earthworms are the best known of all soil inhibiting animals which posses cylindrical body with well marked external and internal segmentation and form over half of the weight of the soil. Earthworms are worldwide in distribution, excluding only the Arctic and Antarctic regions. They are readily commercially available, usually by weight primarily they are sold for vermiculture, owing to their remarkable ability to process organic matter into fertile compost, but they are also sold as bait. The composting process is known as vermiculture.

The Greek philosopher Aristotle referred to them as “Intestine of the earth” because of their habit of ingesting and ejecting the soil. Soil ecosystem engineers are those larger soil fauna that predominantly influence the development & maintenance of soil structure. Earthworms are identified as the most important soil ecosystem engineers in soils as the temperate region of the world. The India’s economy largely depends on agriculture hence the interest has increase progressively about the potential of exploiting beneficial role of earthworm through the process of vermicomposting. Earthworm has long, elongated narrow segmented body with distinct grooves between the segments. It is bilaterally symmetrical. A mature earthworm is about 15 cm in length and 3-5 mm in diameter. The earthworm is smooth in texture and dark brown in colour due to pigment porphyrine, in its body wall. The bodies of earthworm consist of about 100-120 small
ring like segments. In all the segments of the body except the first, last and clitellum there is a ring of chitinous setae lying embedded in the middle of each segment projecting backwardly. They help in locomotion by holding the soil.

Earthworms can play an important role in agro-ecosystem. Their feeding and burrowing habitat incorporate organic residues and amendments into the soil enhancing decomposition, humus formation nutrient recycling and soil structural developments (Kladivko et al, 1986) earthworm burrows persist as macros pores, which provides low resistance channels for root growth, water infiltration and gets exchange (Zachmann and linden, 1989) quality and placement of organic matter is main determinant of earthworms abundance in agricultural soil (Lofs and Holmin, 1983).

Charles Darwin said that, “All the fertile area of this planet has at least once passed through the bodies of earthworm”. Earthworms also help the growth of beneficial bacteria and actinomycetes by providing them optimum conditions of temperature and moisture to soil micro-organism. They also control parasitic nematodes and enhance the crop growth. After the death earthworm serves as food for microbial and animal scavenger and they are in important source of food for many predatory insects, birds and mammals. (Dash, M.C. and Senapati, B. K., 1986)

The pesticides are the substances which attract and destroy the pest. The pesticides commonly used for protecting plants from damaging influences such as weeds and insects in agriculture. It is also used in non-agriculture purposes. The pesticides have some benefits as well as drawback such as toxicity to human and soil desired species.
Pesticides can be classified as Insecticide, Fungicide, Herbicide, Nematocide and Molluscicide on the basis of on which pest they act. Pesticide a toxicant can be also classified on the basis of how they entered in the target organism. i.e. Stomach toxicant, contact toxicant, and systemic toxicant. Stomach toxicant entered in the body through the mouth and digestive tract where they are absorbed in the body. Contact toxicant entered in the body either exposure to water treated with the chemical or direct contact while systemic toxicant are absorbed through the organism via vascular system.

The food and Agriculture organization of the united nation (FAO) 2002, has defined Pesticide as any substance or mixture of substances intended for preventing, destroying or controlling any pest, including vector of human or animal disease, unwanted species of plants or animals, causing harm during or otherwise interfering with the production, processing, storage, transport or marketing of food, agricultural commodities, wood and wood products or animal feedstuffs, or substances that may be administered to animal for the control of insects, arachnids or other pest in or on their bodies. The term includes substances intended for use as a plant growth regulators, defoliants, desiccants, or agent for thinning fruit or preventing the premature fall of fruits. Also used as substances applied to crops either or before or after harvest to protect the commodity from deterioration during storage and transport.

Enzymes are the highly specialized class of proteins. All the functions in the body depend directly or indirectly on the action of enzymes. The enzymes functions in the body as biological catalyst. In absence of enzyme, the reactions of metabolism would near at extremely slow rate. However, the enzymes, greatly increases the reaction rate at
normal body temperature and PH. The enzymes are among the most efficient catalyst known. Enzymes are the principle tools of living cell. Life may be expressed as an orderly functioning by enzymes disorder and hyper functioning of enzyme result in decrease all chemical reaction are necessary for existence growth, reproduction that catalyzed by various enzymes. Enzymes are biological catalysts. They increase the rate of chemical reaction taking place within living cells without themselves suffering any overall change. The reactants of enzymes catalyzed reactions are termed substrates and each enzyme is quite termed in character acting on a particular substrate to produce a products.

All enzymes are protein however without the presence of a non-protein component called cofactor many enzyme protein lack catalytic activity such inactive protein component of enzyme is termed as apoenzyme and active enzyme including cofactor called holoenzyme. Some enzyme binds cofactor more tightly than others when cofactor is bound so tightly that is difficult to remove without damaging enzyme it is sometimes called prosthetic group. Enzyme assay are laboratory methods for measuring enzymatic activity. It is important for the study of enzyme kinetics and enzyme inhibition.

Enzymes are the biomolecule which catalyses or increase the rate of chemical reactions. Almost all enzymes are proteins but all proteins are not enzymes. In enzymatic reactions, the molecules at the beginning of the process are called substrates and the enzyme converts them into different molecules, the products. Almost all processes in a biological cell need enzymes to occur at significant rates. Since enzymes are selective for
their substrates and speed up only a few reactions from among many possibilities, the set of enzymes made in a cell determines which metabolic pathways occur in that cell. The mechanism for a single substrate enzyme catalyzed reaction. The enzyme binds a substrate and produces a product. Reactants which are involved in an enzymatic reaction are referred to as substrate. The enzymes act on this substrate to form products. The reaction rate can be determined by either measuring the rate at which substrate is consumed or the rate at which product is formed. The enzyme activity is expressed in International unit (IU) One Unit is defined as the amount of the enzyme that produces a certain amount of enzymatic activity, that is, the amount that catalyzes the conversion of 1 micro mole of substrate per minute. Enzymes are very specific, and it was suggested by Emil Fischer in 1894 that this was because both the enzyme and the substrate possess specific complementary geometric shapes that fit exactly into one another. This is often referred to as the “lock and key” model. However, while this model explains enzyme specificity, it fails to explain the stabilization of the transition state that enzymes achieve. The "lock and key" model has proven inaccurate, and the induced fit model is the most currently accepted enzyme-substrate-coenzyme.

The Soil pollution is the major problem in agriculture caused due to indiscriminate use of pesticide, release of sewage, industrial effluents and domestic waste resulted in contamination ecosystem, causing several hazards to non target organisms. The pesticides have enormous value in agricultural field to control agricultural pest and diseases. The use of chemicals in the field increased along with overgrowing population, pesticides have become environmental contaminant. Today, there is need to increase
public awareness on environmental issues coupled with major disaster involving toxic
chemical has highlighted the need to identify regular toxic substances.

The Pesticides are non-biodegradable substances. Due to spraying pesticides its
residue remains in the soil, so soil become contaminate with pesticide. The soil is the
main source of food for earthworm. Earthworm ingest such a soil, the pesticide residue
enters in alimentary canal of digestive system of earthworm. The enzymes are catalytic in
function. Enzyme increases the rate of chemical reaction but due to action of pesticide
enzyme get malfunctioning or reduces its activity. The toxicity of particular pollutant
depends on many factors such as animal weight, time of exposure, temperature, PH and
hardness of water. The enzyme secretions are essential for the performing physiological
functions properly in the body of animals. The enzyme plays their role as a bioreactor, in
the physiology of various tissue and cells of the body to accelerate the rate of chemical
reactions. The malfunctioning of enzyme secretions by the tissue it is not to support
proper functions in the body systems of the animals.

Review of Literature:

Waste biomass from domestic, agricultural urban & Industrial source is one-man
course of environmental pollution in developing countries. It can be easily recycled by
the activity of the earthworm to produce remarkable Vermicompost. This is a good
substitute for chemical fertilizer has more NPK than normal heap of manure by
(Shrivastav and Beohar, 2004)
There are more than 3920 named species of earthworm so far reported worldwide. In India, so far, 509 species, referable to 67 genera and 10 families, have been reported (Kale, 1991). Certain species of earthworms such as Eisenia fetida, Aporrectodea tuberculata, Lumbricus terrestris, L. rubellus, Dendrobaena rubida, D. veneta, Eiseniella tetraedra, Allobophora chlorotica have been found to tolerate and remove wide range of chemicals from soil. Our study also indicates that E. fetida is most versatile chemical bio-accumulators.

Earthworms have been found to bioaccumulate heavy metals, pesticides and lipophilic organic micropollutants like the polycyclic aromatic hydrocarbons from the soil. E. fetida was used as the test organisms for different soil contaminants and several reports indicated that E. fetida tolerated 1.5 % crude oil (containing several toxic organic pollutants) and survived in this environment. (Safwat et al., 2002). Eisenia foetida, E. andrei, Eudrilus euginae, Lumbricus rubellus and Perionyx excavatus are major waste eater and biodegrader earthworm species. They are used worldwide for waste degradation and are found to be very successful functionaries for the ecological management of organic municipal wastes (Edwards, 1988). E. euginae and P. excavatus are believed to be the more versatile waste managers (Graff, 1981).

The earthworm forms major components of the soil system & these organisms have been efficiently ploughing the land for millions of the year and recycling the organic materials for efficient growth of plants. Although many species of earthworms are suitable for waste processing two species namely Eisenia foetida and Eudrilus eugenie have been taken into consideration for vermiculture. Their growth productivity & activity to transform organic work like sludge have been widely reviewed (Edward, et.al, 1998).
The use of vermicompost is the best way to improve structure of soil and soil fertility. The technology of vermicompost is simple, economical, environmental and socially best. An appropriate vermicomposting technology will make the recycling of organic waste rapidly feasible which can help to provide renewable source of plant and to minimize the environmental pollution. (Edward et. al., 1985). The pesticides are widely used in the world to control pest which are harmful to the crops. The pesticides may be used for a number of purposes against a variety of different organisms thus pesticides are indispensable in many ways (Ogleshy, 1958)

The behavior of the organisms as a sensitive and relevant parameter for toxicity tests has been suggested and used by different authors (Yeardley et al. 1996; Schaefer 2003; Natal-da-Luz et al. 2004; Loureiro et al. 2005; Garcia et al. 2008). Tisler and Erzen (2006) could indicate low environmental risk. Nonetheless, studies have demonstrated their hazardous effects on non-target aquatic and terrestrial organisms (Halley et al. 1993; Sun et al. 2005; Tisler and Erzen 2006; Jensen et al. 2007; Diao et al. 2007; Kolar et al. 2008; Novelli et al. 2012a, b).

**Aim of the Study:**

To Study Effect of Pesticides on Enzymatic Activities and Behaviour in Earthworm from Nashik District

**Objectives of the Study:**

1. To find out acute toxicity LD$_{50}$ value of selected pesticides used in agriculture.

2. To find which pesticide is more toxic and affect on enzyme secretion in earthworm.
3. To evaluate and compare enzymatic activity of various enzymes secreted by earthworms.

4. To suggest policy guidelines for farming practices.

**Hypothesis of the Study:**

The pesticide is a synthetic material which could not degraded into soil, its residue remains in the soil, unfortunately it enters in the gut of earthworm by their feeding habit because humus soil is its natural food ultimately these residue critically affect on tissue and reduces its enzyme secretary activity.

1. The enzymatic activity is more in control earthworm than treated earthworm with insecticide, fungicide, herbicide, and Nematocide and Molluscicide pesticides.

2. Insecticide and fungicide pesticide are more toxic than herbicide, Nematocide and Molluscicide pesticide

**Research Methodology:**

**Selection of animal:** The earthworms were collected from vermicompost unit and brought to the laboratory acclimatize in vermibox under laboratory condition in laboratory for month. Healthy and approximately same sized earthworms were selected for the experiment.

**Selection of pesticide:** -Insecticide:-Nuvan, Fungicide:-Bavistin, Herbicide:-Roundup, Nematocide: - Furadan, Molluscicide:-Snailkill pesticides were selected for the experiment because it is used farmers on large scale in their agriculture.
Preparation of toxicant solution: - (Stock solution)

Solutions were prepared using above commercial grade Insecticide:-Nuvan, Fungicide:-Bavistin, Herbicide:-Roundup, Nematocide:-Furadan, Molluscicide:-Snailkill separately. All standards were prepared in tap water. 5 ml of pesticide per liter gives 1 ppm and different dilutions were prepared by adding required amount of tap water. Stock was then added in tray containing 1kg soil. By means of micropipette.

Exposure system: - Acute toxicity was calculated by bioassay studies carried out to determine the potency of pesticide. Static but renewal type of bioassay method of Sprague (1971) was adopted in the present investigation to estimate LD$_{50}$ value of the respective pesticides. Soil used for acclimatization and conducting experiment was clear tap water.

Screening test: - In order to fix concentration a preliminary range finding or screening test was conducted. The test was conducted by static method. A group of 10 earthworm were exposed to five widely spread trays for different concentration for 24 hrs. Repeated above experiment range of five concentration was fixed which gives mortality in the range of 10% to 90%. The main aim of this test is to save time and labors.

Assessment of LD$_{50}$: - (50% lethal dose) the experts were carried out for finding the range of concentration for confirmation evaluation. The mortality of animal was recorded at 24, 48, 72 and 96 hrs of exposure to different pesticides and corrected for natural response by Abbott’s formula. (Abbott, 1952). The corrected mortality data were analyzed following the method of Finney, (1971) to determine the LD$_{50}$ value. LD$_{50}$
value were obtained by probit regression line taking log concentration and corresponding mortalities (probit kill) respectively.

**Estimation of Enzymatic activity:**

The enzymatic activity can be estimated by using protocol given in the reference book *Biochemical Methods: S. Sadasivam and A. Manickam, 3rd edition* New Age International Publisher, New Delhi.


**Conclusions:**

The humus soil which is the natural food consume by earthworm, If earthworm consumes such pesticide-contaminated humus soil, the persistent residues get concentrated in the tissue of animal and due to their lipo-proteinous nature of pesticide. The concentrated residue affected on tissue and results decreased enzymatic activity of the tissue of the earthworm. The change in enzymatic activity may alter the metabolic
processes of the animal because of reduction in enzymatic activity of earthworm the
worm might not be able to utilize or digest organic matter properly which is their natural
food, thus earthworms unable to produce enough of vermicompost. In the present
investigations, following interpretations are found.

1. There is significantly decreased the content of amylase, protease (≤ 0.01) and urease
enzyme (≤ 0.05) due to effect of insecticide residue in earthworm while no significant
difference observed in the content of cellulase and lipase enzyme secretion in
earthworm.

2. There is significantly decreased the content of amylase, cellulase, protease, lipase and
urease enzyme at (≤ 0.01) due to effect of fungicide residue in earthworm.

3. There is significantly decreased the content of amylase, lipase, urease enzyme (≤ 0.01)
and cellulase, (≤ 0.05) due to effect of herbicide residue in earthworm while no
significant difference observed in the content of protease enzyme secretion in
earthworm.

4. There is significantly decreased the content of amylase, cellulase, protease, lipase and
urease enzyme (≤ 0.01) due to effect of nematocide residue in earthworm.

5. There is significantly decreased the content of amylase, protease (≤ 0.01) and
cellulase, urease enzyme (≤ 0.05) due to effect of molluscicide residue in earthworm
while no significant difference observed in the content of lipase enzyme secretion in
earthworm.
Limitations of the study:

The present research work is limited for the Nashik district only and hence it is one of the limitations for the study. For the present study, the specific enzymes and pesticides were selected for the studies. As far as the research is concern the above mentioned parameters and their effects was essential for the studies but due to limitations of specific area and animal selection it was limited. The effects of fertilizers are also affecting on earthworm but it is not estimated by me due the depth of present work it is not precise in the study for consideration.

Implications of the study:

The earthworm is a friend of farmer as their functioning role is to making soil porous and improving its fertility. The earthworm is most important organism in terrestrial ecosystem, its population and eliminating nitrogenous waste into the soil activity remains to construct the soil posture for good health of crop in the field. The farmers try to understand the importance of the earthworm, forms major components of the soil system & these organisms have been efficiently ploughing the land for millions of the year and recycling the organic materials for efficient growth of plants. The present research work is applied to the agriculture and the agriculture is a backbone of the Indian economy. It is essential to aware the people mostly farmers and cultivators about the pesticidal effect on earthworm and soil.