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An earthworm, an enzyme and the pesticides are the vital components to study the effect of pesticides on enzymatic activity of earthworm. Now a days in our country most of the farmers used pesticides on large scale for controlling pest. Now days, in modern agricultural techniques their use many pesticides on large scale, its residue remains persistent in the soil due to its synthetic nature. Unfortunately, persistent residue of pesticide consume by earthworm through humus soil and acts on the enzyme secretion functions. The enzymatic activity is the most important metabolic aspect which affect on biological processes occurs in the body of animals. Enzyme secreted in the gut of the earthworm help to digest the food as well as play their role in other physiological processes if its secretion increases or decreases may affect on chemical reactions. This chapter is focused on above mentioned components in briefly are as follows;

1.1 Earthworm:

The earthworm is a invertebrate annelid burrowing, nocturnal terrestrial, hermaphrodite animal. Earthworms are distributed all over the world. It has long slender, segmented bilaterally symmetrical body. Earthworm has neither head nor distinct appendages. The posterior end is more or less blunt and anterior end is tapering thus the shape and size of the animal is adapted for burrowing. The mature worm measured about 15 cm in length and 5mm in thickness. The body of earthworm is metamerically segmented containing 100 to 120 segments with locomotary organ setae except first and last segment. The skin colour is deep brown. In mature worm, the segments 14, 15, and 16 are modified into girdle like thick, dark brown band of
glandular tissue called clitellum. The clitellum has taxonomic importance, due to presence of it the body is divided into three regions i.e. preclitellar, clitellar and post clitellar region. The clitellum secretes mucus, albumen and cocoon for the eggs. The liquid nitrogenous waste materials are formed in the body due to the metabolic activities which eliminates through the excretory organ nephridia. There is no special eye, palps and tentacles but it shows well developed receptor or sense organ. These sense organ are simple consist of a single or a group of ectodermal cells.

1.1.1 Introduction to Earthworm:

The Earthworm is a known burrowing, animal, make their burrow by boring with their anterior pointed end and partly by ingesting the soil through mouth and passing it behind through the anus in the form of castings. The worm castings are also use to line the burrows. The soil which ingested by the worm is crushed into powder and organic matter from it is digested and residue discharged into the soil. Thus, their burrowing habit makes the soil porous and brings the subsoil to the surface like a plough and due to castings the soil become fertile hence the earthworm is very important component for the environment, which maintains soil fertility, and nutrient recycling since long earthworm have been known as “farmers friend” Natures best fertilizers & Natures ploughman. Earthworm acts in the soil as an aerator, grinders, chemical degrader and biological stimulants their utilization and importance have been stressed by Darwin (1981), Mackek (1969).

The Zoologist Aristotle, the Greek philosopher 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 Charles
Darwin said that, “All the fertile area of this planet has at least once passed through the bodies of earthworm”.

1.1.2 Animal position in classification:

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 soil animals. Earthworms are worldwide in distribution, excluding only the Arctic and Antarctic regions. It is invertebrate animal belonging to the phylum Annelida. It belongs to the phylum Annelida due to presence of coelom with cylindrical and metamerically segmented body. The Class is Oligochaeta due to no distinct head but clitellum is present and number of setae present in each segment of the body. The Order is Neo-oligochaeta due to their terrestrial mode of life. Their certain genera of earthworm found in the word. Some common species Eisenia foetida, Eudrilus eugeniae, pheritima posthuma, Eisenia andrei and Lumbricus terestris are found in the Nashik district. The Eisenia foetida is a species that is used as test species in present study because it is widely distributed throughout the Nashik district of Maharashtra.

Earthworms are terrestrial invertebrates with thousands of species grouped into three categories according to their behavior in the natural environment: i.e. epigeic, endogeic and anecic. (Bouche; 1977)

1. Epigeic species, represented by the common redworm do not build permanent burrows; instead, they are usually found in areas rich in organic matter, such as the upper topsoil layer, in the forest under piles of leaves or decaying logs, or in piles of manure. Since they don’t burrow deeply into the soil and prefer to eat rich organic humas. e.g. Eisenia foetida.
2. Endogeic species, build wide-ranging, mainly horizontal burrows where they remain most of the time, feeding on mineral soil particles and decaying organic matter. They are the only species of earthworms that actually feed on large quantities of soil. As they move through the soil and feed, they mix and aerate the soil and incorporate minerals into the topsoil. e.g. Pheritima posthuma

3. Anecic species, represented by the common night crawler, construct permanent vertical burrows as deep as 4 to 6 feet in the soil. They feed on organic debris on the soil surface and convert it into humus. If anecic species are deprived of their permanent homes, they will discontinue breeding and cease to grow. e.g. Lumbricus terestris

1.1.3 Role of Earthworm in Agriculture:

Earthworm plays a key role in soil biology by serving as bioreactors to effectively harmless to beneficial soil microflora and destroys soil pathogen thus converting organic waste into valuable biofertiliser with biopesticide, vitamin, enzymes, antibiotics, growth hormones and protein rich mass. They enhance the decomposition of organic matter and they also contribute 20-100 kg nitrogen per hectar per year besides other mineralized nutrients and plant growth factors. 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.

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 macrospores,
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).

Earthworm has long, elongated narrow segmented body with distinct grooves between the segments. It is bilaterally symmetrical. Earthworms also helps the growth of beneficial bacteria and actinomycetes by providing them optimum conditions of temperature moisture action and phosphate earth worms also produce several which split organic complex polymer split in waste into simple influent which are further utilized by 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.

1.1.4 Role of Earthworm in Vermicomposting:

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. India’s economy largely depends on agriculture. Over the past many years, the interest has been increase progressively about the potential of exploiting beneficial role of earthworm through the process of vermicomposting. The Waste biomass from domestic, agricultural urban and industrial sources the 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 (Shrivastav and Beohar, 2004)
The process of converting organic waste by earthworm consumption into nutrients filled human is called Vermicomposting. Even worm casting improve natural soil condition so it is also called “gardeners gold” on account its excellent soil amending value. (Dash & Senapati, 1986)

Vermicomposting thus has multiple objectives like waste management enhancement of soil need management and sustainable agriculture. The method of covering all biodegradable waste such as farm waste, biological waste, market waste into vermicompost by the action of earthworms digestive enzyme get convert into valuable for agriculture. 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).

**1.1.5 Significance of Earthworm:**

At a time when prices of fertilizer have gone up exorbitantly and the availability of nutrient for sustained crop production has become a serious constraint in agriculture, the utilization of organic waste material through agency of earthworm assume added significance.

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 earthworm provides cheapest solution to solve several social, economical and environmental problems of human society. Earthworm can accumulate and
transform many chemical contaminants including heavy metal and organic pollutants in the soil. Earthworm restores and improves soil fertility by their secretion and excreta which always consider as boost for crop production in agro ecosystem.

They are secondary decomposer of nature to convert organic waste into vermicompost due to effect of their casting secretion. The vermicompost have plant growth promotes, which are responsible for lush growth of plants. (Amojii et al., 1998, Bhatnagar and phalta.,1996, Edward and Bohlen, 1996) The increasing utilization of chemical fertilizer and pesticides has spoiled the health of soil leads environmental pollution.

1.2 Enzyme:

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.

1.2.1 Introduction to Enzyme:

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.
In 1878, German physiologist Wilhelm Kühne was first used the term enzyme which comes from Greek word “leaven”. The word enzyme was used later to refer to nonliving substances such as pepsin and the word ferment was used to refer to chemical activity produced by living organisms. In 1897, Eduard Buchner began to study the ability of yeast extracts that lacked any living yeast cells to ferment sugar, in a series of experiments, at the University of Berlin. He found that the sugar was fermented even when there were no living yeast cells in the mixture. He named the enzyme that brought about the fermentation of sucrose "zymaze". In 1907, he received the “Noble prize in chemistry” for his biochemical research and his discovery.

An enzyme name giving ending in “-ase” the only major exceptions for proteolytic enzyme whose name usually ends with “-in” e.g. Trypsin. 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.

1.2.2 Role of Enzyme in biological processes:

Enzymes are the biomolecule that catalyze the rate of chemical reactions. Almost all enzymes are proteins. 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 biological cells 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 regulation of enzymatic activity is very important for maintaining the level of intracellular and extracellular constituents and various mechanisms by which the enzymatic activity are regularized. Alteration in the concentrations of reactant or a product, to change the direction of a reaction, isoenzymes, inhibition by product of reaction and feedback control may affect on enzymatic activity of the animal. (Spensor, 1968). The important functions of enzymes observed in digestive system of animals. The enzymes such as amylase, proteases which breakdown the large molecules especially starch and proteins into smaller units hence they it can be absorbed in the intestine of the animals. Different enzymes digest different food substances.

The enzymes are the bioregulator protein molecules which acts as biological catalyst i.e. they speed up the rate of chemical reactions in cells without being used up in the reaction. Mostly the enzyme controlled two different ways to chemical reactions one of them is by degradation i.e. catabolic when an enzyme breaks down large molecule into smaller once. e.g. digestion and another is by synthetic i.e. catabolic when the enzyme builds up larger molecule from smaller once, e.g. protein synthesis. The enzymes are specific that means each enzyme catalyses only one reaction because the enzyme molecule is folded in a particular shape with a portion called an active site to which only the correct substrate molecule can attach, much like a key in a lock. Like all the proteins, the enzyme action can be estimated by their shapes. Extreme of the temperature and pH distorts the shape and the substrate can no longer fit in the active site, so the enzyme will no longer work i.e. due to the denaturation of proteins. The enzymes are catalyst that speeds up the rate of reaction
by mean of reduction of activation energy and preservation of chemical equilibrium of reactants and products.

An enzyme inhibitor is a molecule that binds to an enzyme and decreased its activity. Since blocking an enzymatic activity can kill a pathogen or correct a metabolic imbalance, many drugs are enzyme inhibitors. They are also used in pesticides. Not all molecules that bind to enzymes are inhibitors enzymes activators and increased their enzymatic activity while enzyme substrate bind and are converted to products in the normal catalytic cycle of the enzymes. The binding of inhibitors can stop a substrate from entering the enzymes active sites to form and catalyses the chemical reactions. The enzyme activity is expressed in International unit (IU). The International Unit is defined as the micromoles of substrate consumed or micromole of product formed in one minute under the optimum conditions of temperature, pH, and etc.

1.2.3 Enzyme kinetics:

The enzyme kinetics is the study of chemical reactions that are catalyzed by enzymes. In enzyme kinetics, the reaction rate and effect is measured. Mostly the enzyme performed a key role in metabolism, how its activity is controlled and how a drugs antagonist or inhibit the enzyme molecule. The enzymes are usually protein molecule that manipulates other molecule. The enzyme bind with target molecule on their active site and are transformed into products through a series of various steps known as enzyme kinetics. Enzyme kinetics can occurs only due to the single substrate which catalyzed reaction with the help of enzyme. First the enzyme (E) binds to the substrate (S) to form enzyme substrate complex then due to the action of enzyme product removed from the (ES) complex, as a results enzyme and product become separated from each other. i.e. enzyme only accelerate the rate of chemical
reaction. After the completion of reaction enzyme get free from chemical reaction. Further this mechanism can be explained by follows,

\[
\begin{align*}
\text{Catalytic step} \\
E + S & \rightarrow ES & \rightarrow E + P \\
\text{Substrate binding}
\end{align*}
\]

The Emil Fischer, (1990) explained, the enzymes are very specific 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. Due to the catalytic property of enzyme increase the space of reaction without influencing the chemical equilibrium hence the total energy of the products is not changed through the activation energy is reduced. The active site was thought that have a fixed structure (lock), which exactly matched the structure of a specific substrate (key). Thus the enzyme and substrate interact to form an enzyme-substrate complex. The substrate is converted to products that no longer fit the active site and are therefore released, liberating enzymes.

The animal body requires the enzymes to maintain the functional activities of the life. The enzymes works as like lock in the chemical reactions which maintains the reaction balance in the metabolic processes. The body contains many enzymes and many substrates but for life to continue in appropriate manner, the correct enzyme must efficiently locate and unite with a specific substrate. Each enzyme can attract its
specific substrate and accelerate the chemical reaction that must occur in the appropriate period of time.

The theory behind the lock and key model involves complementarily between the shape of the enzyme and the substrate. Their complementary shapes make them fit perfectly into each other like a lock and key. According to this theory, the enzyme and the substrate shape do not influenced each other because they are already in a predetermined perfectly complementary shape. As a result, the substrate will be stabilized. This theory was replaced by the induced fit model which takes into account the flexibility of enzyme and the influence of the substrate has on the shape of the enzyme in order to form a good fit.

### 1.2.4 Classification of Enzyme:

In general, living organism body contains hundreds of enzymes but broadly they classified into six classes. Viz. oxidoreductase, transferases, hydrolases, lyases, isomerases and ligases. (Moss, et al., 2011)

1. **Oxidoreductase:** It catalyses oxidation or reduction reaction where electros are transferred from one molecule to another molecule. i.e. from reluctant to oxidant. These enzymes are vital for metabolic processes especially aerobic respiration takes place in mitochondria of living cell.

2. **Transferases:** The transferase enzyme which catalyses the movement of functional group from one molecule to another molecule. The functional group includes phosphate, methyl, etc. The transferase enzyme further divided into kinase and deaminases. Kinase enzyme transfer phosphate group in metabolic process called phosphorylation. The deaminase enzyme catalyses to transfer amino group from amino acid and convert into ammonia.
3. **Hydrolase:** This enzyme catalyses hydrolysis i.e. breaking of bond through addition of water molecule. Most of these are digestive in nature and further divided into by the target organ where it affects. The enzyme protease, amylase and lipase are included under hydrolase group. Amylase enzyme breaks bond between starch, protease breaks peptide bond between amino of proteins and Lipase enzyme breaks bond between fatty acids and glycerol.

4. **Liases:** liases enzyme catalyses to breaks the reaction that generate the double bond. This enzyme performed their role in elimination reaction e.g. Oxalate decarboxylase and isocitrate lyases enzymes. Oxalate decarboxylase breaks the carbon carbon bond while isocitrate lyase breaks glycoxylate group from isocitrate.

5. **Isomerases:** The isomerase enzyme catalyses their role in structural change in molecule. If there is only one substrate and one product as well as no gain or loss in reaction, isomerses enzyme changes the shape of the molecule e.g. glucose-6-phosphate convert into fructose-6-phosphate by the action of isomerase enzyme.

6. **Ligases:** Ligase enzyme catalyzes ligation process in cellular metabolism i.e. joining two substrate e.g. DNA ligase enzyme bind breaks between DNA molecule by phosphodiester bond.

**1.2.5 Significance of Enzyme:**

The enzymes are involved in many aspects such as health, disease, diagnosis and treatments. Some clinical conditions are known to be due to a hereditary absence of a particular enzyme. In galactosemia, there is abnormally high level of galactose in the blood because of the absence of enzyme which converts galactose to glucose. Many chemicals owe their poisonous action to an interference with essential enzyme. For instance, animal death have been caused due to organophosphate insecticide,
since these are powerful inhibitors of acetylcholinesterases, a key enzyme involved in nerve transmission phosphorus kills by blocking cytocrome oxidase, a vital enzyme for cellular respiration. Some bacterial drugs may owe their effectiveness to an ability to interfere with bacterial enzyme system without seriously affecting the metabolism of the animal.

In general the enzymes can be distinguished in digestive, metabolic and food enzymes. The metabolic enzyme catalyzes reaction within the cell thus the metabolic processes run with the help of the enzymes. The digestive enzymes breakdown food and allow their nutrients absorbed into the blood stream and used in the body functions. The food enzymes are the enzymes that supplied through the food of the animal. The nature has placed them in their proper place for maintaining the specific role in the chemical reaction in the body of animals. The length of life is inversely proportional to the rate of exhaustion of the enzyme potential of an organism; the increased use of food enzyme promotes a decreased rate of exhaustion of the enzyme potential. In other words, the more food enzymes get the longer and healthier life to the organisms.

1.3 Pesticide:

A pesticide is a substance or mixture of substances used to kill a pest. A pesticide may be a chemical substance, biological agent such as a virus or bacteria, antimicrobial, disinfectant or device used against any pest. Pests include insects, plant pathogens, weeds, molluscs, birds, mammals and fish nematodes and microbes that compete with humans for food, destroy property, spread or are vector for disease or cause a nuisance. Although there are benefits to the use of pesticides, there are also drawbacks, such as potential toxicity to humans and other animals.
1.3.1 Introduction to Pesticides:

The pesticides are synthetic chemical manufactured in the industries for killing domestic, agricultural pest. These are available in the market in solid or liquid form. Due to its toxic nature, it is harmful to the pest. In soil ecotoxicological, model species are usually chosen from species that are easy to maintain and breed in laboratory conditions and for which molecular tools are available. They do not necessarily occur naturally on polluted soils. Considering soil ecotoxicology in oligochaete annelids, model species are mostly from the genus *Eisenia foetida* and *Eisenia andrei*, in particular, have been used in most toxicological studies although species from the Lumbricus genus are increasingly studied (Morgan et al. 2007). In particular, *Eisenia foetida* is the reference earthworm in international toxicity tests (Nahmani et al. 2007a, b).

In recent years, ecotoxicological investigations have benefited greatly from the emergence of molecular biology techniques, which lead to a better understanding of the mechanisms of contaminant action at molecular level (Brulle et al. 2010). Paradoxically, although these approaches have been widely used to better understand the effects of metals, there is almost no molecular study focusing on the effect of authorized pesticides on earthworms. An interesting study was published in 2008 by Svendsen et al. but now a days the pesticide atrazine which is banned.

1.3.2 Classification of Pesticides:

In general pesticides are classified into different way due to their action on target organism. They are classified as insecticides, fungicides, herbicides, nematocide, molluscicide, etc. The insecticides are used to control insects, fungicides used to control fungi, herbicides used to control fungus, herbicides used to control
weeds or herbs, nematocide used to control nematodes, molluscicide used to control molluscs respectively.

The pesticides were classified into three groups depending upon their chemical nature are as follows; Inorganic compounds:-these are the compounds includes mercurial, arsenicals, borates, fluorides borates etc. Natural organic compounds:-these are the compounds includes nicotine, rotenone, and pytoethrum, etc. Synthetic organic compounds:-these are the compounds includes organochlorine, organophosphate carbamtes and pyrethroids etc. (Meenakshi (1988);

1.3.3 Uses of Pesticides in Agriculture:

In the agriculture, Pesticides are generally used in agriculture for the protection of crops from various pests and diseases. The pesticides are used to control pest but these pesticide used on large scale by the farmers in their cropland so its residue affect on earthworm through soil pollution with water into soil and affect on enzymatic activity as earthworm. 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; the pesticides are indispensable in many ways (Omkcar, 2003)

1.3.4 Contamination of Soil by Pesticide:

It is very difficult to access the degree of pesticidal pollution due to the lack of systematically planned nation wide monitoring system and there is limited information on pesticide residue in air, soil, water and the in living organisms (Jain and Kulshestha, 2000) The Pesticides may reach to the soil by any of the following two means; i.e.

1. Pesticides are directly applied on soil for controlling weedicide for weed, insecticide for insect and pesticide for various pests. Due to that pesticide residue
percolate in to soil and soil become polluted due to their direct application for the
treatment of soil or for the control of Soil dwelling pest, Nematodes and soil pathogen.

2. The pesticides are indirectly reach to the soil by various way such as heavy rainfall,
industrial effluents, fumes release during pest control treatment, etc. the pesticide lost
into atmosphere in vapors state simultaneously it absorbed by dust particles

1.3.5 Acute Toxicity LD$_{50}$:

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. (Abott, 1952) The corrected mortality data were analyzed following the
method of Finney, (1971) to determine the LD$_{50}$ value. (50% lethal dose) The LD$_{50}$
value were obtained by probit regression line taking log concentration and
corresponding mortalities (probit kill) respectively.

1.3.6 Significance of Pesticide:

All these substances may be toxic to desirable as well as to undesirable
organism because their toxicity varies in both the degree and in manner of action
however any discussion of pesticide as pollutant must treat in environment. The
earthworm provides crucial ecosystem services in the agriculture, increasing soil
porosity and aggregation, providing channel for root growth and stimulating microbial
activity as digested organic matter passes through their intestine, among numerous
benefits. The pesticide which sprayed on crops to controlled pest but their effect
influenced on the earthworm living in the soil under the plants is devastating. The
worm only grows to half their normal weight and they do not reproduce as well as
worm in the fields that are not sprayed.
1.4 Significance of present study:

The pesticides are non-biodegradable material which remains deposited in the soil for long period of time simultaneously it accumulates in soil microbes and detrivores like earthworm is the best friend of farmers. The pesticidal residue entered in the digestive tract of earthworm through humus soil, which is the natural food of it. This residue may affect on enzymatic activity causes heavy mortality hence the biodiversity of earthworm comes in danger due to excessive use of pesticides in Nashik district of Maharashtra.