Evaluation of Changes in Life Cycle and Biochemical Parameters of Earthworm *Eisenia fetida* due to Exposure to Some Selected Pesticides

Presented by

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Introduction

- The synthetic pesticides machinery has been rolling since early 1940s when Dichlorodiphenyltrichloroethane (DDT) was first introduced, bringing a novel paradigm in man's fight against pests and diseases.

- The role of pesticides in controlling harmful pests of crops cannot be denied. But continuous and indiscriminate use of all the kinds of pesticides since last few decades caused serious damages to ecosystems. As a result, concern for contamination of the environment gained tremendous importance.

- Detection of pesticide residue in fish, milk products, vegetables, food grains, meat, groundwater and even in human blood and breast milk became priority research.

- Scientists throughout the world are engaged in evaluating damages caused by the pesticides.

Importance and magnitude of pesticide use

•Globally, pesticides are widely applied in agricultural lands and are introduced into the soil environment due to runoff and leaching which is a great cause of concern.

•Pesticides are either directly applied to soil to control soil-borne pests or deposited on soil as runoff from foliar applications.

•With the increase of population during recent time demand of agricultural production has steadily enhanced, increasing the rate of pesticide application making it inevitable for protection and preservation of crops.

•The consumption rate of pesticide is lower as compared to the developed nations. But unregulated use of pesticides and lack of awareness often create acute environmental problems in India.

Adverse effects of pesticides on soil and soil organism

•Foliar application of many broad spectrum pesticides caused nearly total depletion of arthropod population thereby affecting the basic functioning of the soil ecosystem.

•Use of pesticides affect many non target soil organisms like isopods, millipeds, centipeds , collembola etc. and decrease the soil biodiversity.

•Nitrogen fixation, which is required for the growth of higher plants, is hindered by pesticides in soil.

•Inhibition of enzymatic activity of the soil including soil dehydrogenase, phosphomonoesterase and arginine deaminase activity are inhibited by pesticides thereby affecting the soil ecosystem.

Susceptibility of Soil Sub-system to pesticides

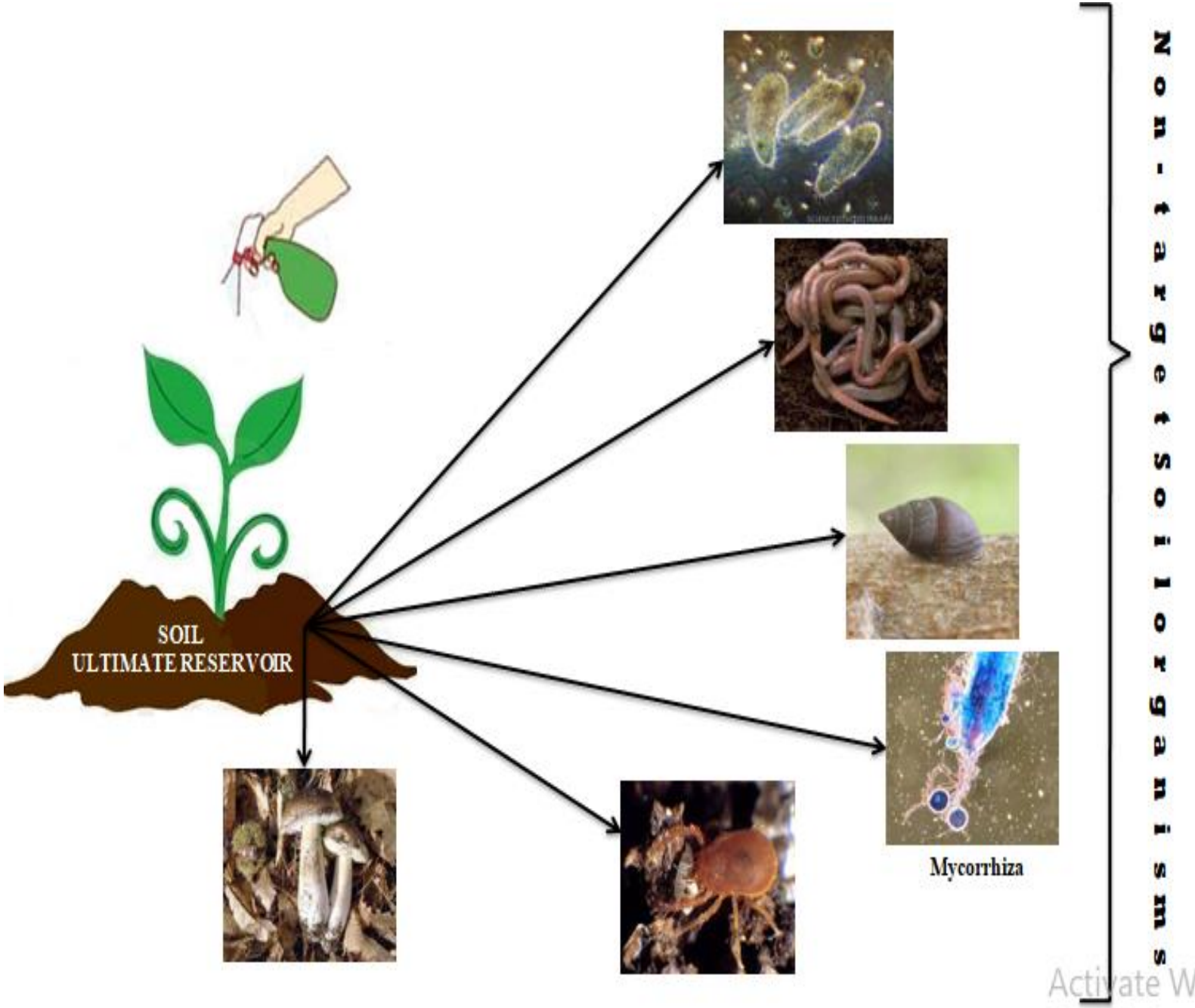
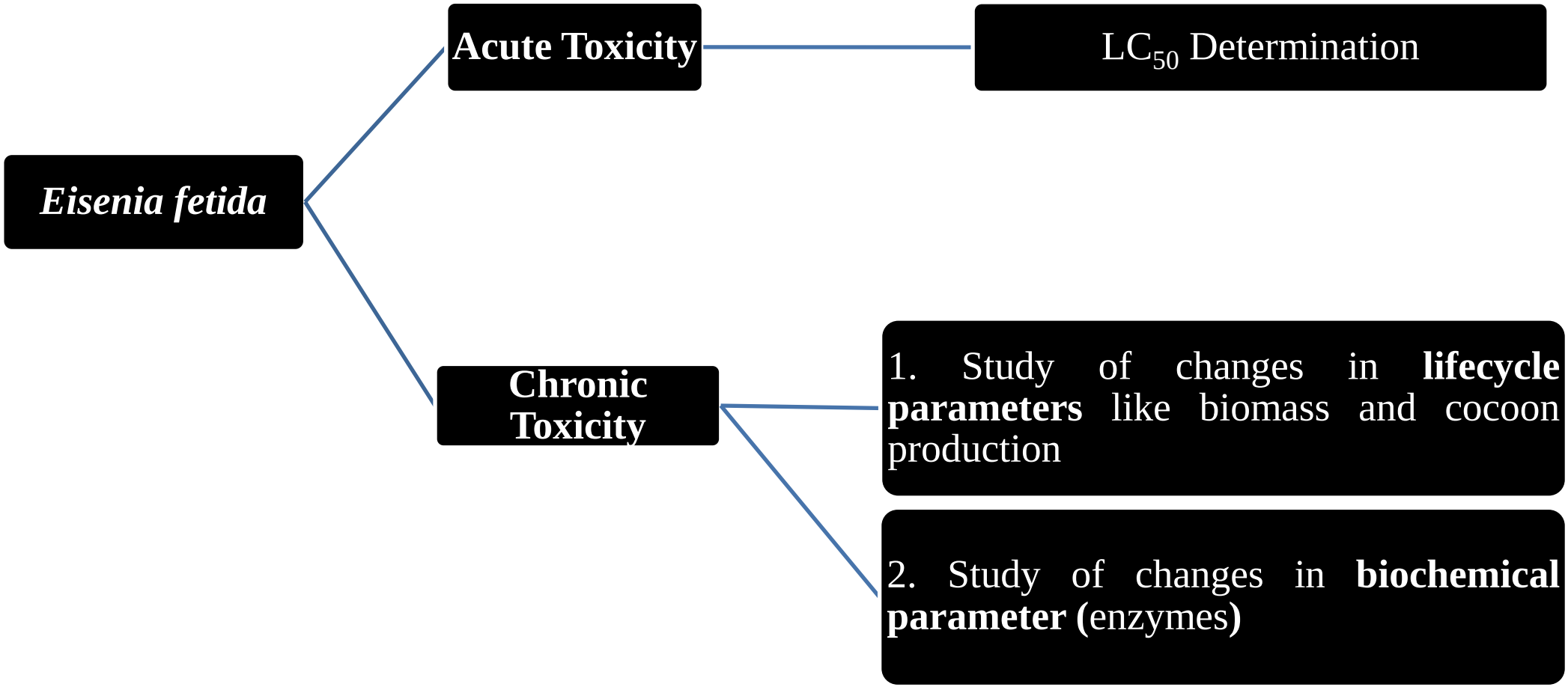


Fig 1. Other non-target soil organisms affected due to pesticide exposure

OBJECTIVES OF THE STUDY

The main objective of the present study was to assess ecotoxicological risk of the soil agro ecosystems rendered by intensive use of pesticides in the agricultural fields.



MATERIALS AND METHODS

Test Species- *Eisenia fetida*

Systematic position of the test specimens:

Phylum: Annelida

Class: Oligochaeta

Family: Lumbricidae

Order: Haplotaxide

Suborder: Lumbricina

Genus: *Eisenia*

Species: *fetida*

Collection of the test specimens

Specimens of *Eisenia fetida* was collected from a local vermicompost unit around Midnapore town (W. Bengal, India). The specimens were brought from the vermicompost unit in a plastic bag along with compost materials to the laboratory and used for culture.

Earthworms as model test organisms

- Earthworms are widely used to study the effect of agrochemicals on the soil fauna.
- Earthworm toxicity testing procedure recommended by OECD (1984) and USEPA (1996) are globally used in toxicological researches on earthworms.

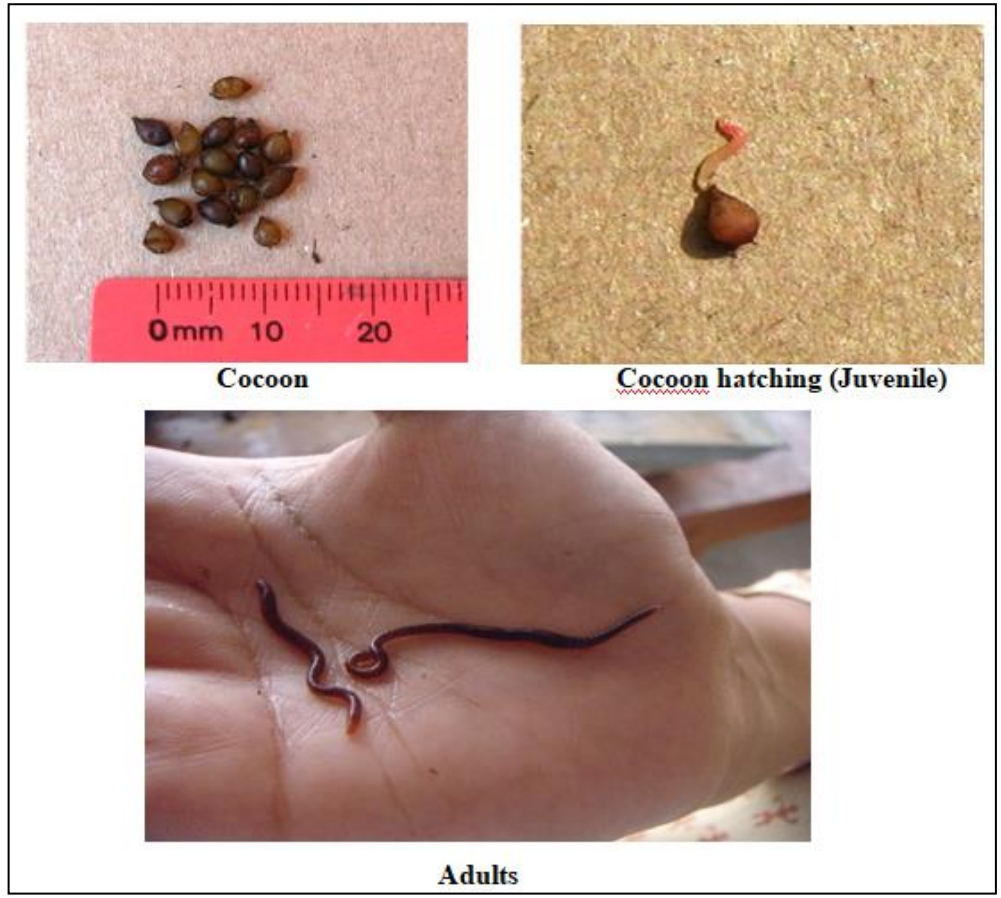


Fig 2. Life Cycle stages of *Eisenia fetida*

Pesticides used in the study

Insecticide group	Technical Name	Commercial Name	Source of Procurement
Organophosphate	Chlorpyrifos	Dursban 20% EC	Crystal Crop Protection Ltd. Gujarat, India
Pyrethroid	Cypermethrin	Ustaad 10% EC	United Phosphorous Ltd, Gujrat, India
Carbamate	Carbofuran	Fury 3% CG	Nagarjuna, Agrichem Ltd, West Bengal, India

Culture Medium & Period of Study:

Acute toxicity studies and the chronic toxicity studies were done for a period of 96 hours and 28 days respectively.

Fig 3. Finely ground uncultivated grassland soil + Dried Cowdung (1:1)

Temp
28±0.5^{0}C

Moisture
60-70%

Polyethylene Test Box
(16x12x1 cm^3)

Fig 4. Environmental Test Chamber

Methodologies

No.	Parameters	Methods used	References
1.	Acute Toxicity: LC_{50}	OECD* Guideline	OECD*, 1984
2.	Chronic Toxicity	OECD* Guideline	OECD*, 2004
3.	Life History parameters	OECD* Guideline	OECD*, 2004
4.	Biochemical parameters:		
	Acid phosphatase	Spectrophotometric	Walter and Schutt, 1974
	Alkaline phosphatase	Spectrophotometric	Walter and Schutt, 1974
	Acetylcholinesterase	Spectrophotometric	Ellman et. al., 1961
5.	Statistical Analysis	Software Analysis	Probit Analysis Software, USEPA, 1.5 SPSS V- 16

OECD*- Organisation for Economic Co-operation and Development


RESULTS

Acute Toxicity- LC_{50} determination

- The 96 hours LC_{50} value of the organophosphate pesticide Chlorpyrifos for *Eisenia fetida* was found as 0.072 mg/kg soil, for synthetic pyrethroid Cypermethrin was 0.020 mg/kg soil and for the carbaryl pesticide Carbofuran was found to be 0.010 mg/kg soil.

- Carbofuran was found to be most toxic among the pesticides which increases the susceptibility of the earthworms because lesser the LC_{50} value higher the toxicity of the chemical.

Rate of change in life history parameters of earthworms

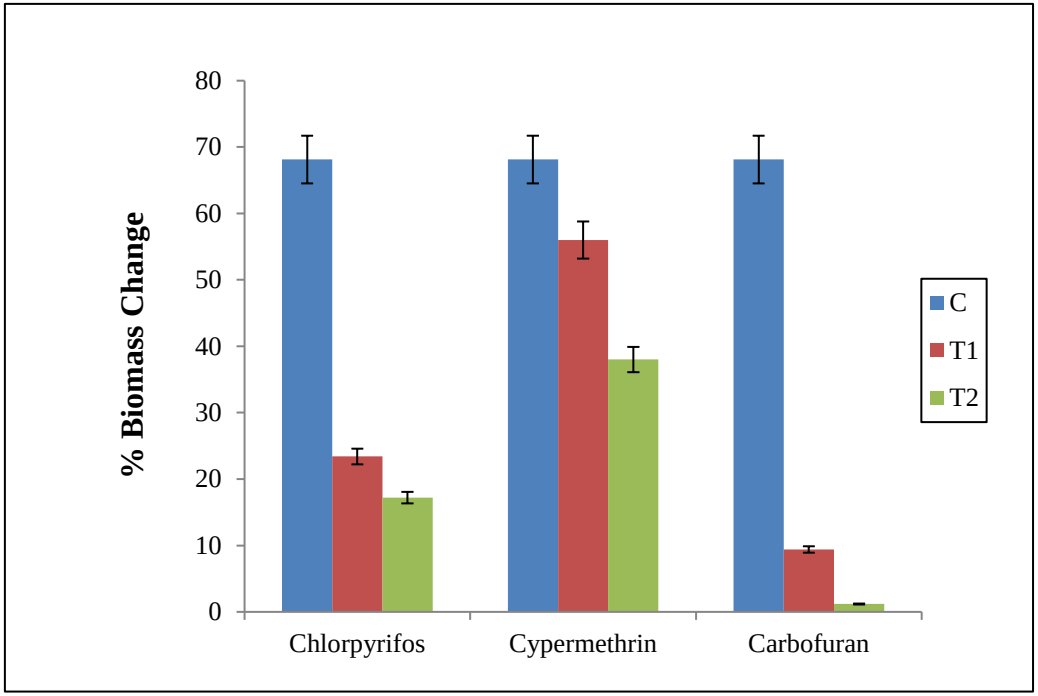


Fig: 5 Change in Biomass of *E. fetida* after 28 days exposure to sub-lethal doses (T1 and T2) of selected pesticides. C is the control value.

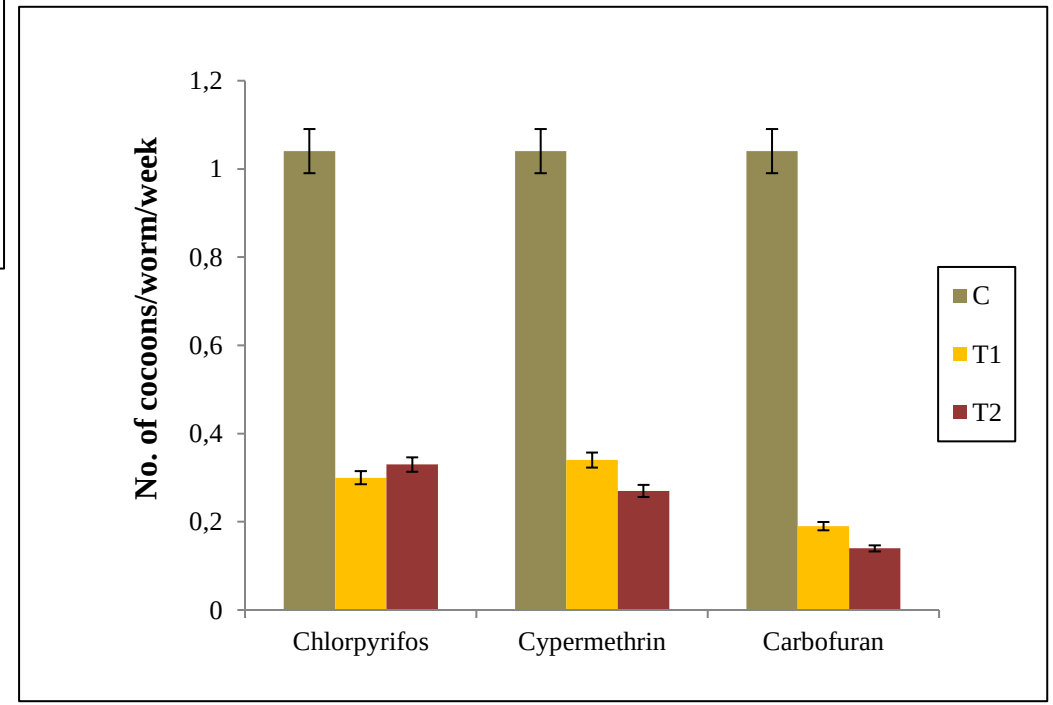


Fig: 6 Change in rate of cocoon production of *E. fetida* after 28 days exposure to sub-lethal doses (T1 and T2) of selected pesticides. C is the control value.

Significant difference at $p<0.05$

Change in specific activity of Acid and Alkaline Phosphatase

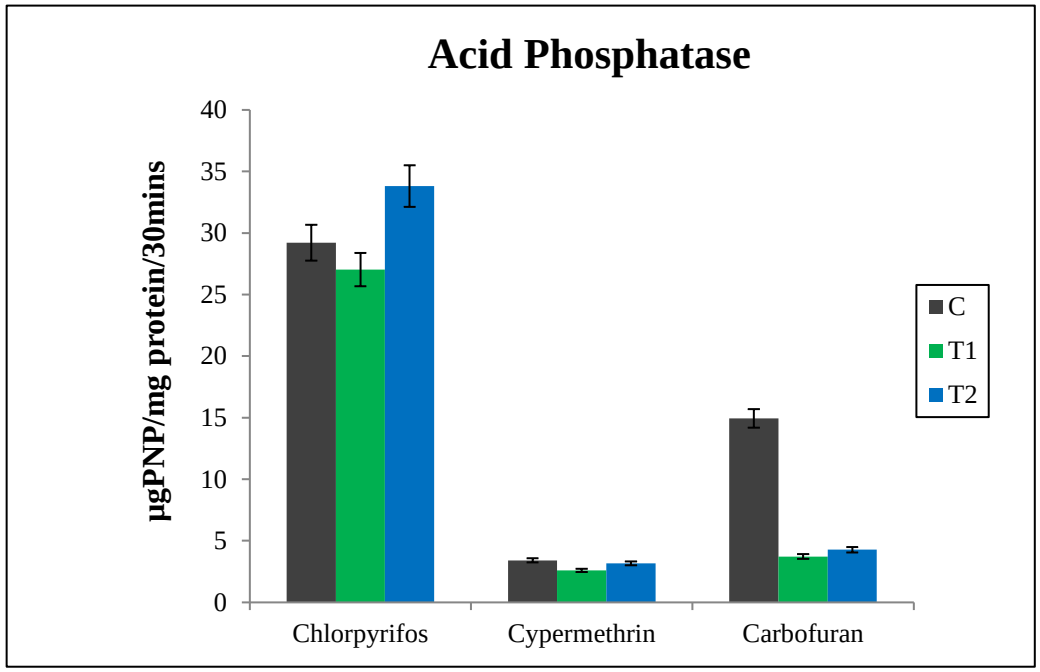


Fig: 7 Change in specific activity of acid phosphatase of *Eisenia fetida* exposed to sub-lethal doses (T1& T2) of selected pesticides. C is the control value..

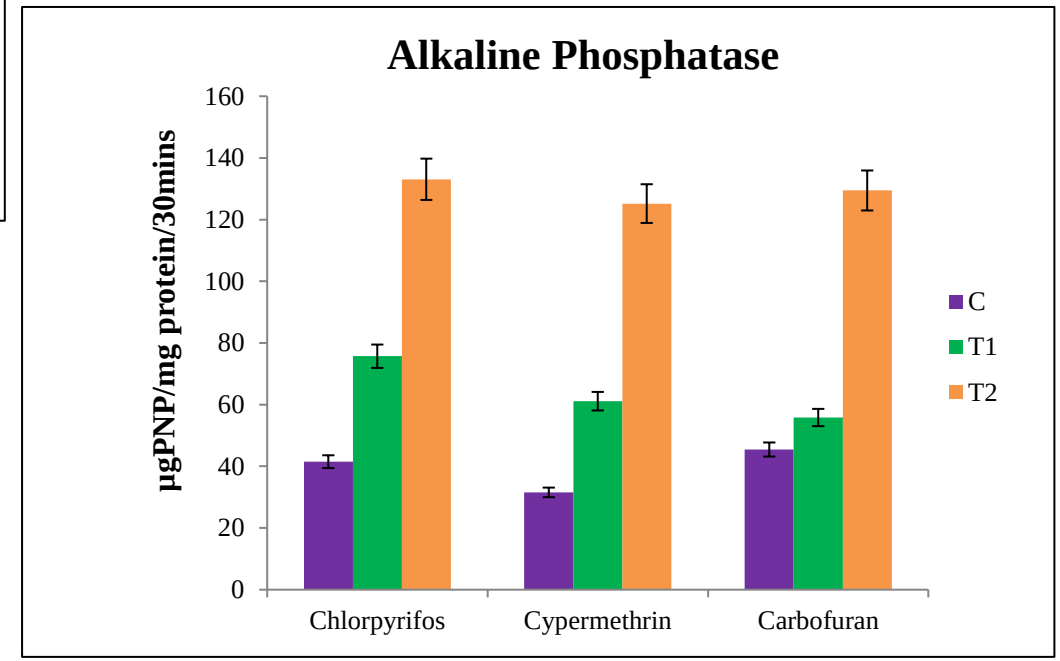


Fig: 8 Change in specific activity of alkaline phosphatase of *Eisenia fetida* exposed to sub-lethal doses (T1& T2) of selected pesticides. C is the control value.

Significant difference at $p<0.05$

Inhibition of Acetylcholinesterase

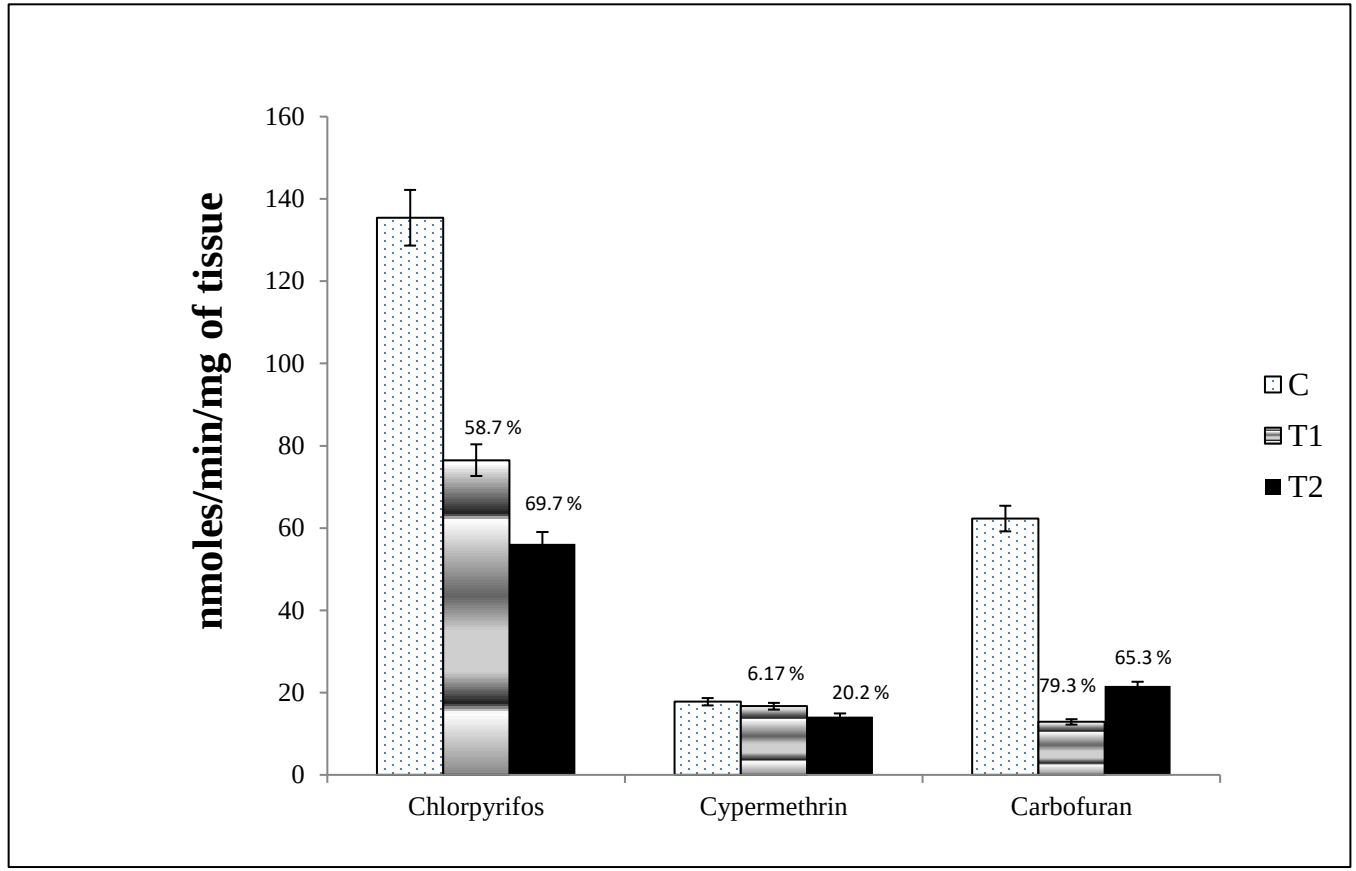


Fig: 9 Acetylcholinesterase (AchE) inhibition of *Eisenia fetida* exposed to sub-lethal doses (T1& T2) of selected pesticides. C is the control value.

Significant difference at $p<0.05$

Conclusion

The following conclusions were drawn from the results of the present experimental study:

- The three pesticides tested in the acute toxicity experiments exhibited low LC_{50} values and it can be concluded from this observation that the pesticides are harmful to earthworms.

- The chronic toxicity studies show that life history parameter like biomass, cocoon production and biochemical parameters like activities of the enzymes acid and alkaline phosphatase, acetylcholinesterase of the earthworms show significant changes in response to the sublethal doses of the pesticides and thus can be used as biomarkers and can be considered as potential tools to detect pesticide pollution in agro-ecosystems.

- These pesticides have the potential to affect the earthworms adversely. So, it is recommended that a proper scientific management protocol, regarding the application of pesticides can be developed to control indiscriminate use of toxicants in the agricultural fields and reduce environmental degradation.

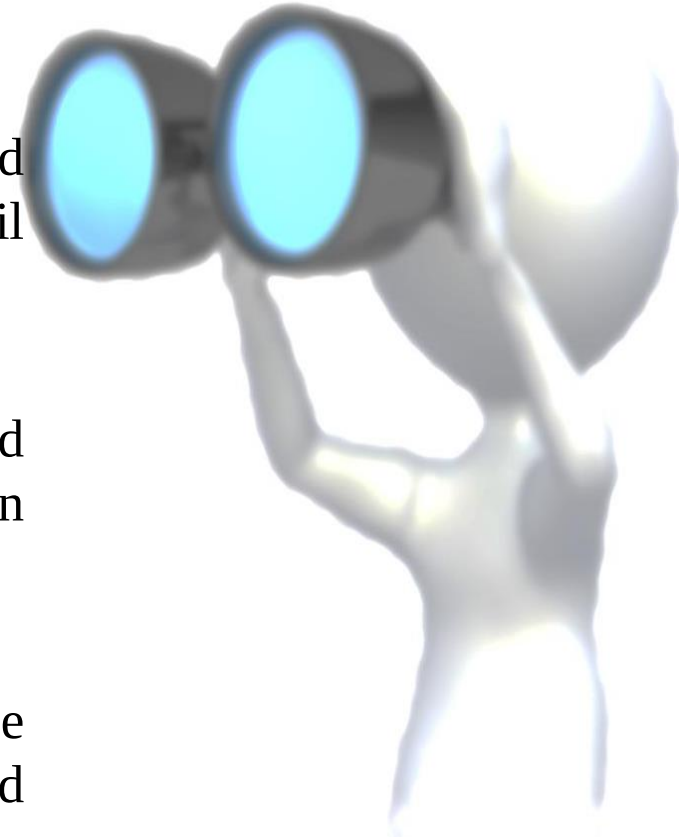
Future Scope

- By conducting the above experiments and from the results generated we have tried to establish an ecotoxicological model for the soil organisms taking earthworm as model specimen.

- The above methodologies can be implemented in practical field conditions to determine the effects of any toxicant on soil organisms and the parameters studied.

- In future we would like to explore the changes in the genetic and molecular level as a result of pesticide contamination in earthworms.

- Proper management for the application of pesticides can be generated and the farmers i.e. the end users should be educated through proper awareness campaigning and workshops.



thank you!