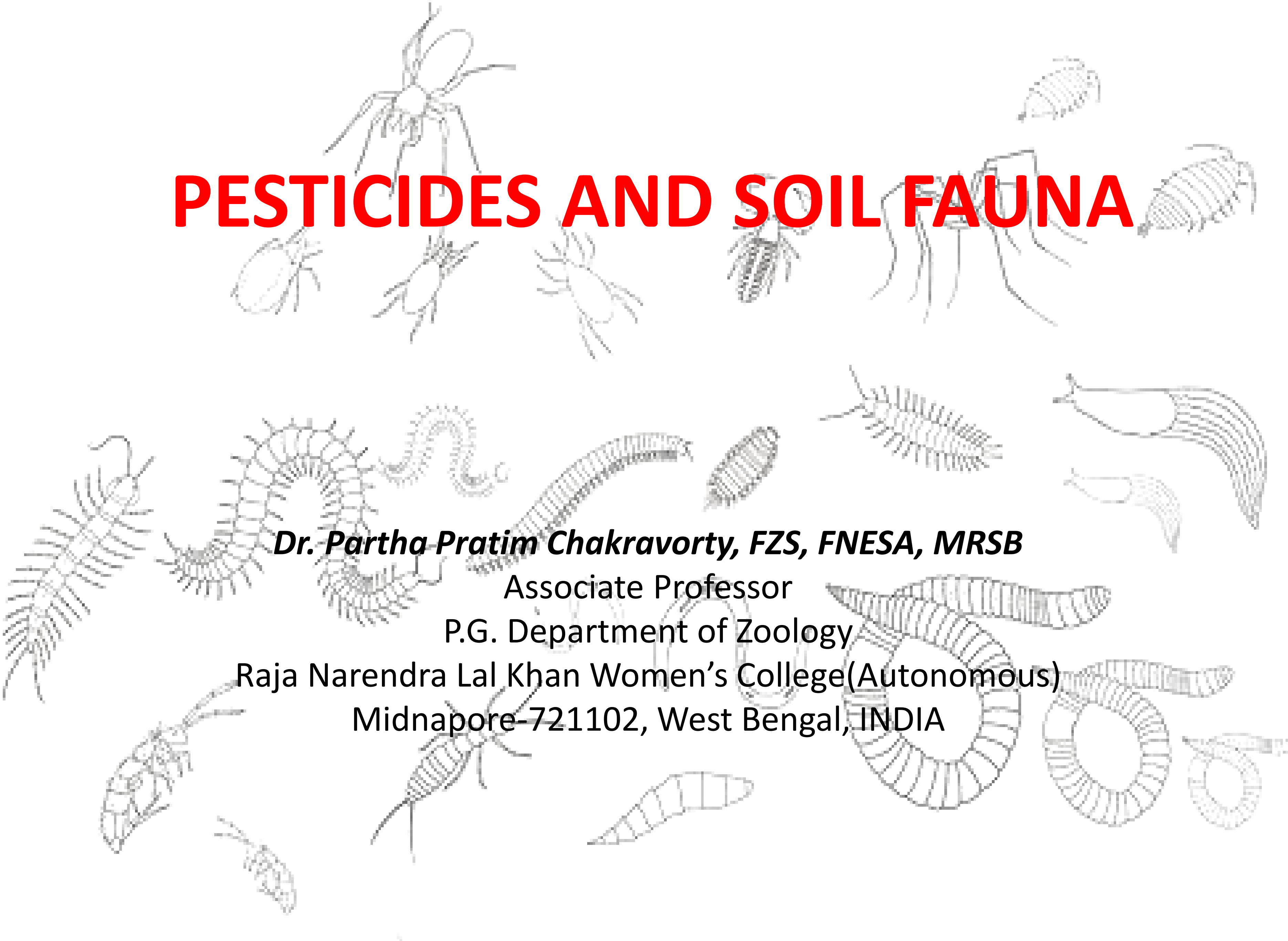


PESTICIDES AND SOIL FAUNA

Dr. Partha Pratim Chakravorty, FZS, FNESA, MRSB
Associate Professor
P.G. Department of Zoology
Raja Narendra Lal Khan Women's College(Autonomous)
Midnapore-721102, West Bengal, INDIA

Introduction

- The use of pesticides in agricultural systems is one of the most important factors contributing to the massive worldwide increase in food production and continuous exposure to these compounds may cause serious problems and stressful environment for non-target soil organisms.

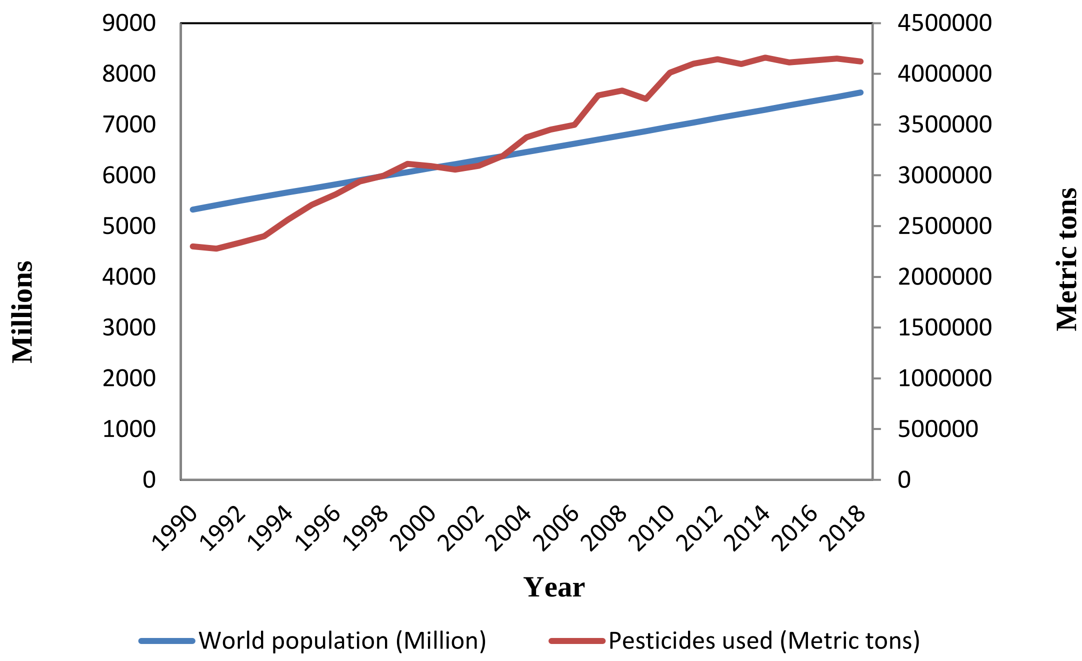
- This is inevitable, because alarming population growth throughout the globe necessitated production of more food and cash crops. High yielding varieties of crop were invented and application of collective farming and new engineering tools led to an era of modern agriculture inviting application of wide variety of agrochemicals.

- More and more potent insecticides were invented and the application rate continued to increase leading to a rapid growth of pesticide market. The farmers got an immediate return with high yield. But the environment was stocked with huge amount of pesticides.

- Concern for contamination of the environment gained tremendous importance. Scientists throughout the world are engaged in evaluating damages caused by the pesticides.

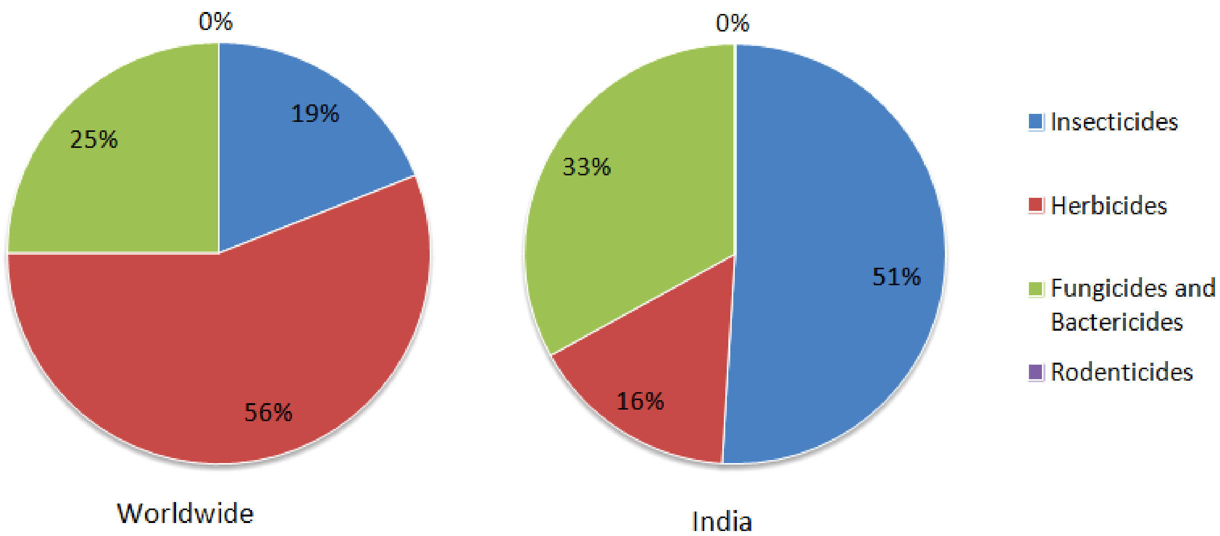
World Population vs Pesticide consumption

- The increase of world population in the 20th century would not have been possible without a parallel growth in food production, and this was achieved due to fertilizers and pesticides. The growth of human population and the worldwide pesticides use were significantly and positively correlated over the last century.



Pesticide use pattern in India

- Pesticide usage patterns in India differ from those in the world as a whole. Insecticides, fungicides, and herbicides are specially used in India. Insecticides account for the majority of the total. The present pesticide use pattern in India is insecticides > herbicides > fungicides + bactericides > other-pesticides, whereas the global pesticide use pattern is herbicides > fungicides + bactericides > insecticides > other- pesticides.



Soil Organisms

- Soil is the habitat of wide variety of organisms. Organisms spending at least one part of their life cycle in or on the soil may be defined as soil organisms measuring less than one micrometer to several centimetres in diameter.
- Soil fauna are classified on the basis of their size into micro fauna like Protozoa, Nematoda etc, mesofauna like Acari, Collembola, Enchytraedes etc. and macro fauna like Amphipods, Isopods, Centipeds, Millipeds, Insects, Molluscs, Earthworms etc.
- The ecological importance of these animals, particularly the role of earthworms in the organic breakdown and soil formation processes is well established. Activity of these organisms depends upon surrounding edaphic and climatic parameters, the environmental factors like temperature, moisture, pH etc. being most sensitive.

Ecological Importance of earthworms

- **The role of earthworms in turning over the soil was first pointed out by Aristotle who called them as "*The Intestines of the Earth*".**

- **Earthworms are most conspicuous non-target soil organism and worst victims of insecticide application in the agro-ecosystems. They constitute up to 92% of the invertebrate biomass in the soil and become easily susceptible to pesticides applied in agricultural fields. But presence of earthworms is beneficial to agro-ecosystems because of their contribution to complex processes such as litter decomposition, nutrient cycling and soil formation.**

- **Contributions of earthworms in soil ecosystem as recorded in these literatures include:**
  - **Physical participation by feeding, fragmentation of leaf litter, aeration turnover and dispersion.**
  - **Digestion of organic substances leading to chemical precipitation and nutrients enrichment of soil through dead tissue and metabolic by-products.**
  - **Grazing over micro flora and altering soil micro floral composition.**

- **In recent years it has been stressed that the role of the earthworms does not stop below ground. They also affect the above ground subsystem, especially plant performance including growth, development and plant community composition.**

EVALUATION OF ACUTE AND CHRONIC TOXICITY

Materials and methods

Test specimen

Perionyx excavatus, an indigenous species of indigenous epigeic earthworm, abundant in the grasslands of Midnapore district, West Bengal, was selected as test specimen. Grasslands, which were free from crop cultivation, i.e. uncontaminated from direct pesticide application, were chosen as the area of collection of the earthworms (Fig 1).

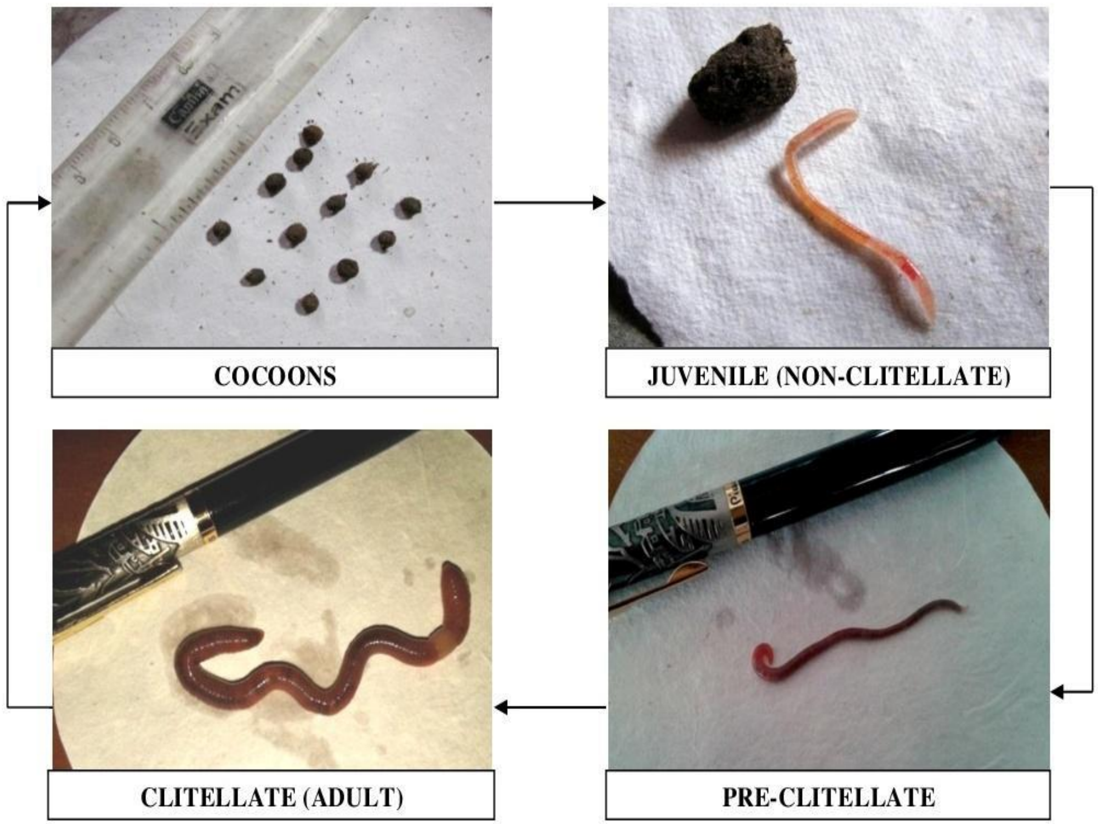


Fig 1 Stages of the life history of *Perionyx excavatus*

Culture and maintenance of test specimen

- After collection, age synchronized specimens were hand sorted and cultured in large cement vats (Fig 2).

- Finely grinded soil (collected from the collection site) and sun-dried farmyard manure mixed in the ratio of 1:1 was used as the culture medium. The culture vats were kept at shady and cool place and moisture content of the culture medium was maintained at an approximate level of 60-65% by adding water to it under regular supervision and thus the temperature is kept at around 28 ± 0.5ºC.

- Every week food was added in the form of sun-dried farmyard manure during the entire period of culture. The cocoons were hand sorted, cultured in separate culture pots and were later used as test specimens following guidelines for testing of chemicals for earthworm (OECD, 1984).

Fig 2 Culture Vat

Pesticides Used

Four pesticides from three different groups, used by the farmers, which are commercially and locally available, were used in the experiment. The name of the pesticides and their respective groups are given in Table 1.

Table 1. The pesticides used in the present study along with their commercial name and recommended agricultural doses

Sl. No	Pesticide Group	Technical Name	Commercial Name	Source of procurement
1	Herbicides	Pendimethalin	Dhanutop	Dhanuka Agritech Ltd, Gurgaon
2		Pretilachlor	Racer	Krishi Rasayan, Balasore
3	Organophsphate	Dimethoate	Rogorin	Plant Remedies Pvt. Ltd, Hazipur.
4	Synthetic Pyrethroid	Cypermethrin	Ustaad	United Phosphorus Ltd., Gujarat

Physicochemical parameters of the test medium

Natural soil, which was used as the experimental medium, was collected from the same grasslands from where the test specimens were collected. The physicochemical parameters of the test medium were evaluated and given in Table 2.

Table 2. Physiochemical parameters of the test soil media

Sl. No	Soil Parameters	Value
1	Organic carbon content	0.86%
2	pH	7.17
3	Moisture content	61.2%

Analytical method for chronic toxicity study

Acute toxicity i.e short term toxicity evaluation of **96 hours** and chronic toxicity i.e. long term toxicity bioassay for **28 days**, for cocoon production and specific activity of enzyme, of the selected pesticides on the test specimens was determined following the updated standard guidelines of **Organization for Economic Cooperation and Development (OECD), 2004**.

The specific activity and enzyme inhibition of acetylcholinesterase enzyme was evaluated following the process of **Ellman et al., (1961)** using the first 7 segmental tissue of earthworm, acetylthiocholine as substrate and DTNB. The centrifuged supernatant was taken to carry out the spectrophotometric method.

Statistical Methods

Probit analysis was done to calculate the total mortality of the test specimens obtained after 96 hours of exposure by **EPA probit analysis program, version 1.5 (US EPA, 2006)** to determine LC_{50} value and 95 % confidence limit of each insecticide.

The data of the entire experimental study for each insecticide was analyzed for single factor ANOVA followed by Least Significance Difference (LSD) test to evaluate significant variation between control and treatments at 5 % level of probability using **SPSS 16.0** statistical software.

Experimental procedure

- Both acute and chronic toxicity study were performed with age synchronized specimens of 250–300 mg weight.
- Experiments were conducted in small inert polythene boxes (Fig 3) having **192 cm^2 area (16 X 12 X 1 cm)** containing **200gm** (for acute toxicity) and **500gm** (for chronic toxicity) of dried, grinded and sieved soil having particle size of 0.25 mm, collected from the same grasslands from where the test specimens were collected, as the test medium.
- The moisture content of the soil was measured by Infrared Torsion balance moisture meter. Lastly, the experimental boxes were kept inside an **Environmental Chamber** (Fig 4) at a controlled and constant temperature of **28 ± 0.5°C and 60-65%** relative humidity.
- The test specimens were released in the boxes after contaminating the soil with respective doses of pesticides.

Fig 3 Test Box

Fig 4 Environmental Chamber

Results

ACUTE TOXICITY

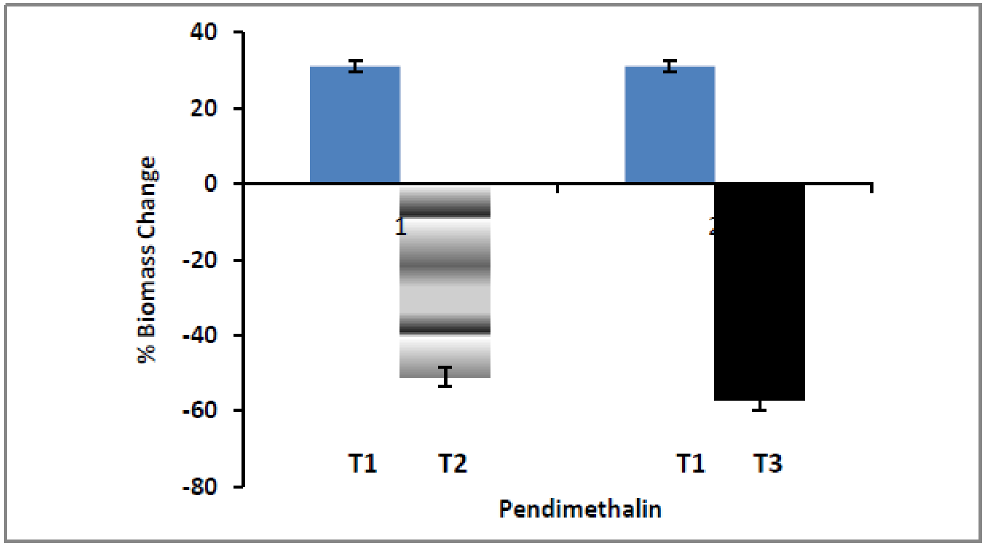
The 96 hours LC_{50} values of the

- herbicides Pendimethalin and Pretilachlor for P. excavatus were found as 0.016 and 0.052 mg/kg soil.
- organophosphate insecticide Dimethoate was found 0.017 mg/kg soil.
- Synthetic pyrethroid Cypermethrin was 0.012 mg/kg soil.
- The degree of toxicity was found to be

  Cypermethrin>Pendimethalin>Dimethoate>Pretilachlor

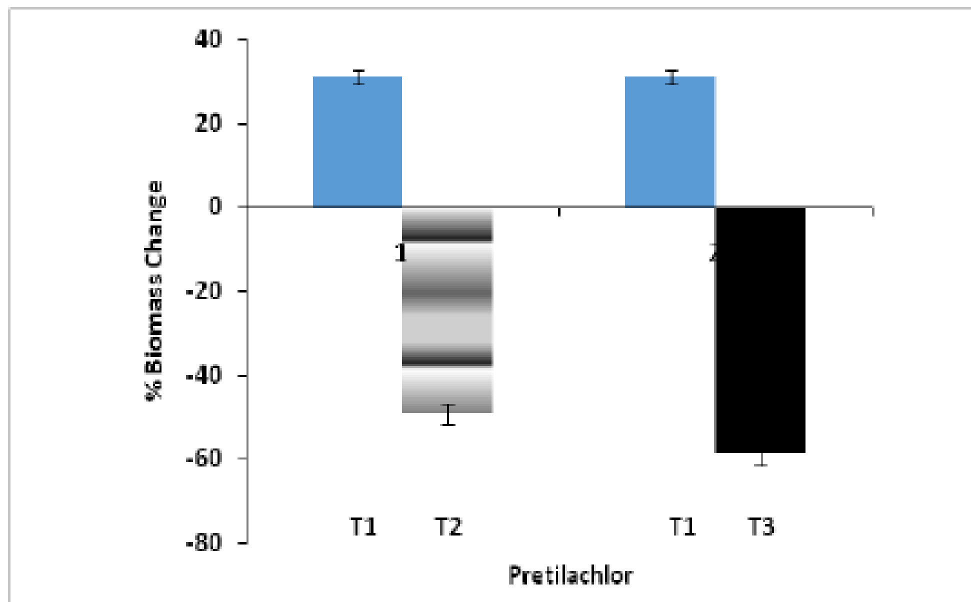
Synthetic pyrethroid was found to be the most toxic pesticide for the earthworms

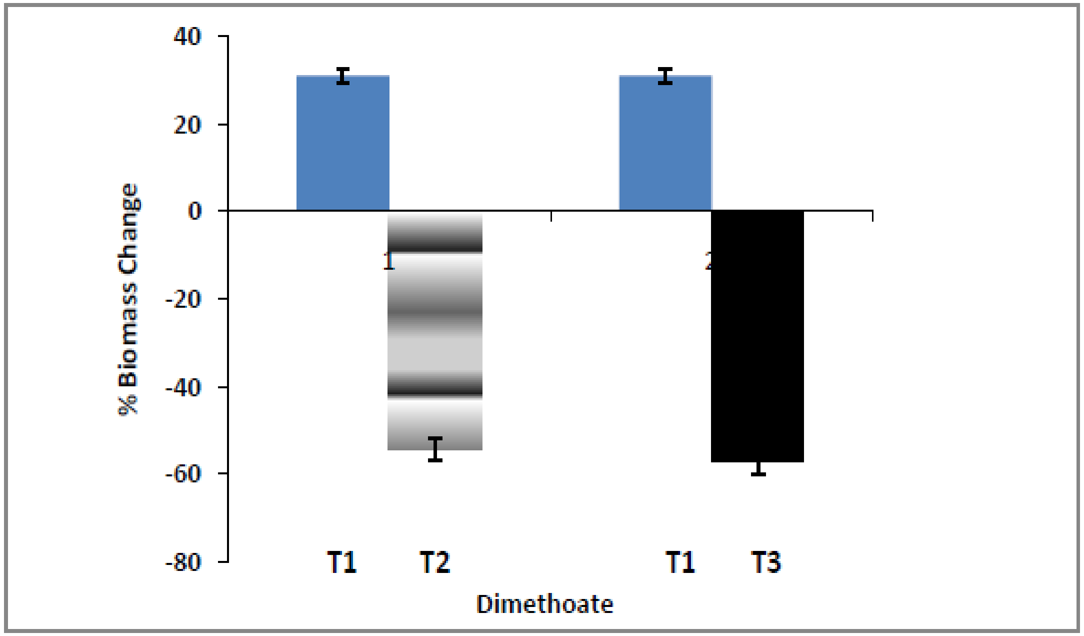
CHRONIC TOXICITY



P. excavatus exposed to sub-lethal doses of the herbicides showed significant alteration in biomass in case of both the herbicides compared to their control values. In T2 dose, i.e 25% of LC_{50} value, pendimethalin showed maximum reduction in biomass but in case of T3 dose,i.e. 50% of LC_{50} values, pretilachlor showed maximum reduction in earthworm biomass.

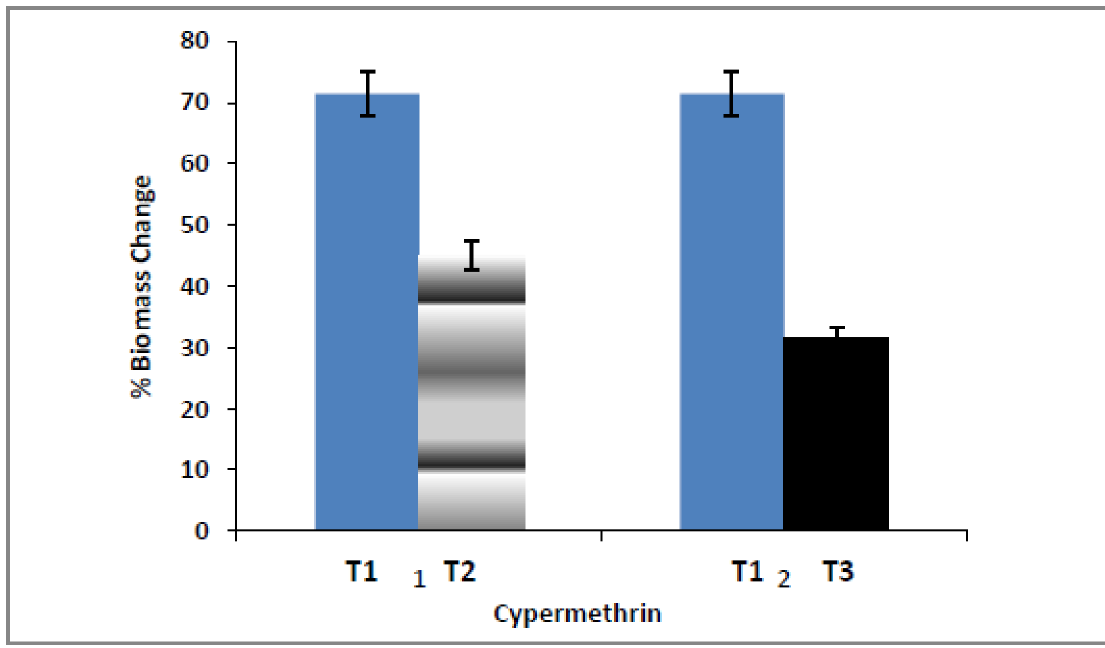
Change in Biomass





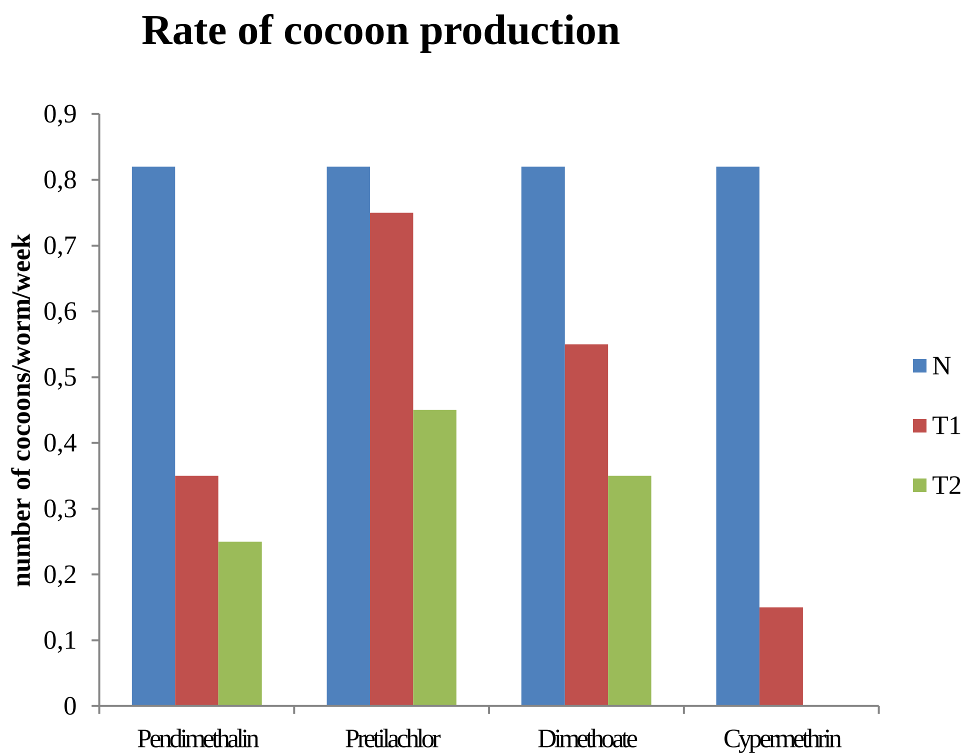
P. excavatus exposed to sub-lethal doses of the organophosphate pesticide Dimethoate and synthetic pyrethroid Cypermethrin showed significant alteration in biomass compared to their control values.

Change in Biomass



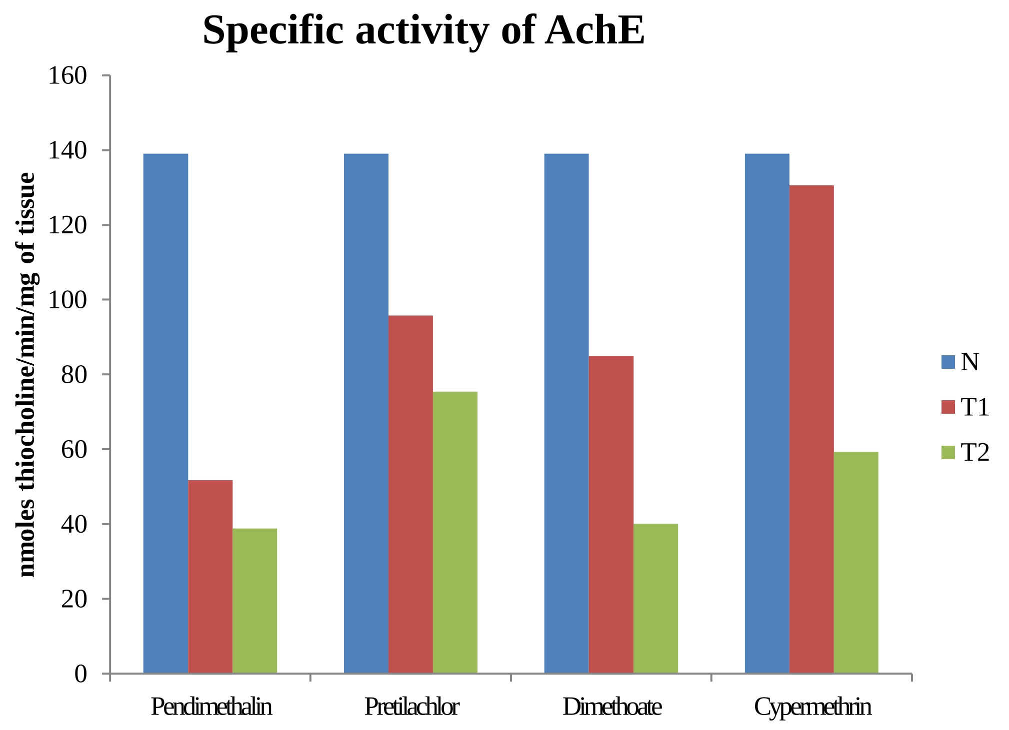
Change in Rate of cocoon production

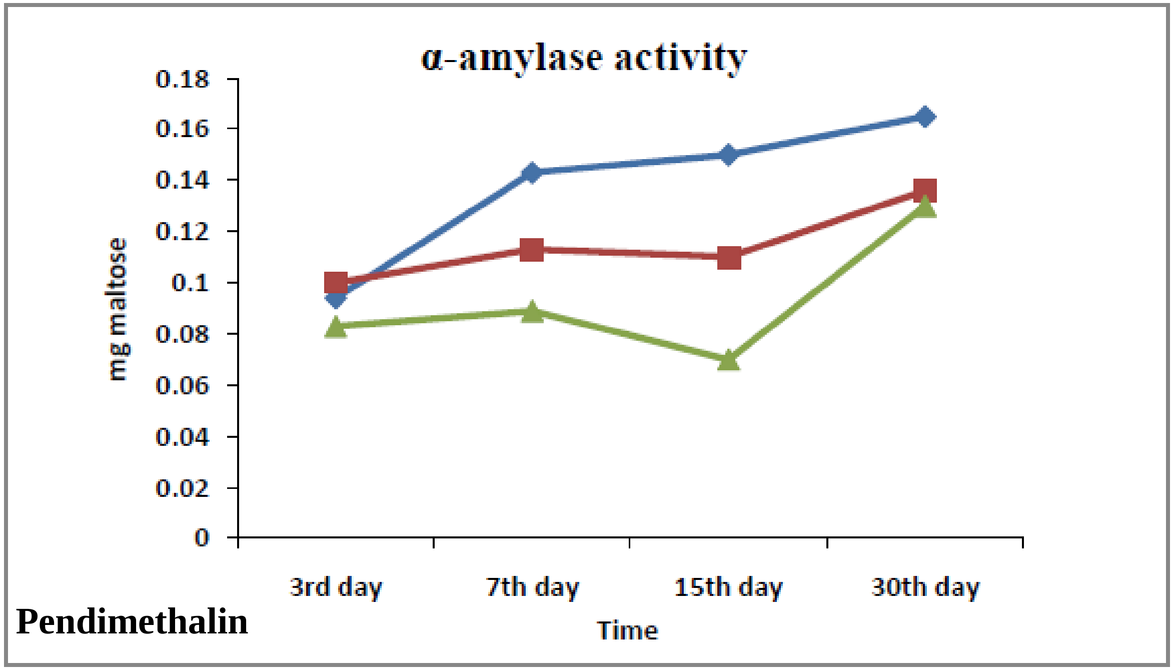
- *P. excavatus* cocoon production was significantly reduced in case of all the four pesticides even at the lowest dose (T1) tested (25% of LC_{50}), while increased sublethal dose (T2) of pesticide (50% of LC_{50}) led to the severity of reduction of cocoon production. Cypermethrin showed the highest toxicity among the pesticides with no cocoon produced in T2 dose



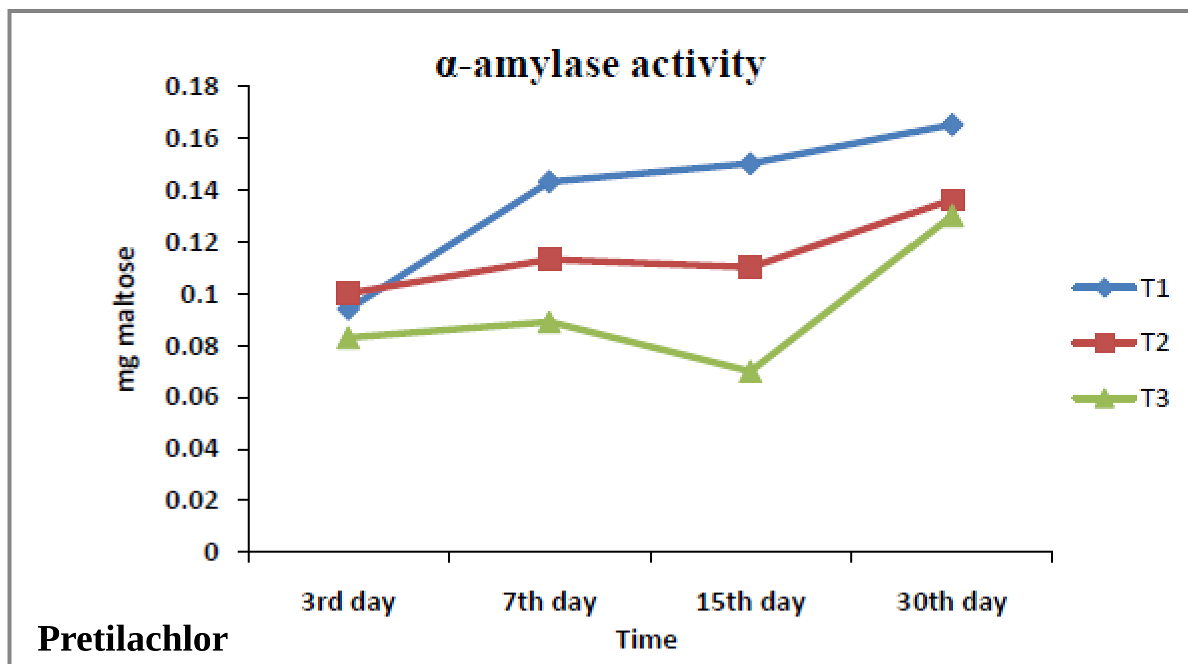
Change in the Specific activity of AchE

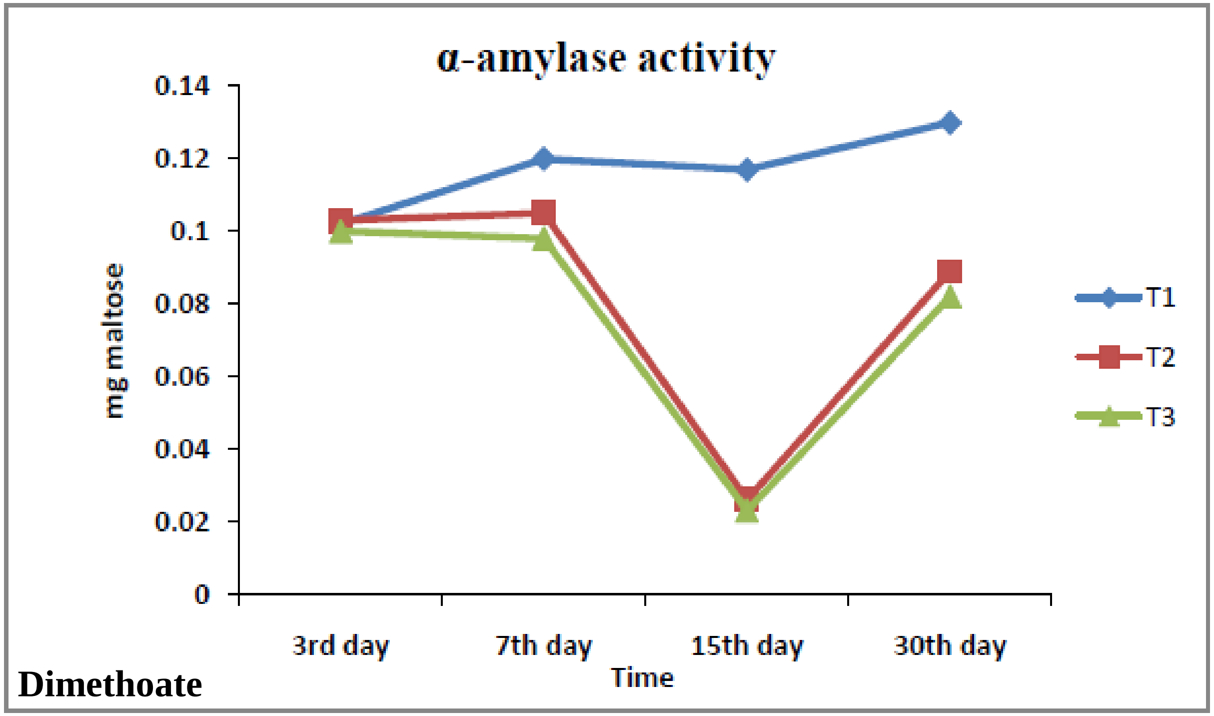
The specific activity of acetylcholinesterase (AchE) in *P. excavatus* was significantly reduced in case of all the selected pesticides in the sublethal doses (T1 and T2) compared to the control (N) value. Pendimethalin showed the maximum inhibition of specific enzyme activity of AchE in both the sublethal dose exposed earthworms, i.e T1 and T2 (Fig 3). The percentage inhibition of AchE as compared to control was 63.2% and 72.4% for T1 and T2 respectively.



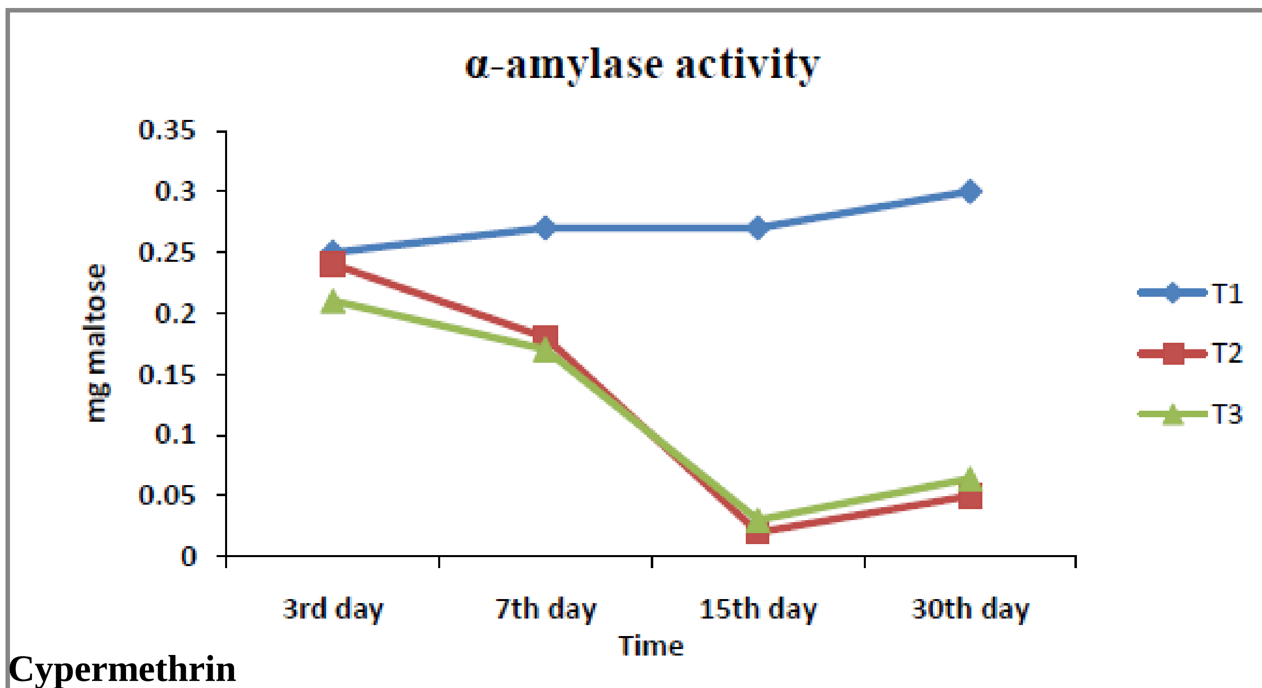


The α-amylase activity in the test specimens decreased after 7th day of the experiment in both the doses, i.e, T2 (Red) and T3 (Green) doses, and showed a maximum drop on 15th day in case of both the herbicides when compared to Control (Blue).





The α-amylase activity in the test specimens decreased after 7th day of the experiment in both the doses, i.e, T2 (Red) and T3 (Green) doses, and showed a maximum drop on 15th day in case of both organophosphate and pyrethroid pesticides when compared to Control (Blue).



Conclusions

The following conclusions were drawn from the results-

- To evaluate ecological risk of application of an insecticide in agro-ecosystems, LC_{50} value alone is not adequate. The LC_{50} values of the pesticides for non-target soil organisms must be compared with their Recommended Agricultural Dose (RAD) to evaluate the ecological safety of the pesticides.

- The chronic toxicity studies show that life history parameter like biomass and reproductive parameter like cocoon production can be used to detect pesticide pollution in agro-ecosystems.

- Activities of the enzyme like acetylcholinesterase and digestives enzyme like α-amylase show significant changes in response to the sublethal doses of the pesticides. Thus, these parameters can be used as potential biomarkers to detect pesticide pollution in agro-ecosystems.


THANK YOU