



**Integrated Ecotoxicological Assessment of Pymetrozine and Cartap  
Hydrochloride: Acute, Chronic, Biochemical, and Temperature Mediated  
Toxicity in Pheretima posthuma**

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**Abstract:**

Acute, chronic, biochemical, and temperature mediated effects of Pymetrozine and Cartap hydrochloride were evaluated in *Pheretima posthuma*, a sentinel species for soil health. Dose dependent mortality was observed, with Cartap showing greater toxicity (LC50: 142 mg/kg soil) than Pymetrozine (185 mg/kg soil). Behavioral alterations including coiling, mucus secretion, and paralysis intensified with concentration, reflecting neuromuscular stress. Sub-lethal exposure significantly reduced growth and reproduction, with declines in body weight, cocoon production, and juvenile emergence, particularly under Cartap. Biochemical assays revealed marked depletion of protein, carbohydrate, and lipid reserves after 60 days, indicating metabolic disruption and energy exhaustion; lipid reduction was most pronounced. Elevated temperature ( $26 \pm 2$  °C) synergistically enhanced toxicity, increasing mortality, biomass loss, and reproductive suppression compared to lower temperature ( $20 \pm 2$  °C). Findings confirm that earthworms are highly vulnerable to combined chemical and thermal stressors, underscoring their role as bioindicators and highlighting ecological risks of pesticide residues under changing climatic conditions.

**Keywords:** Pymetrozine toxicity, Cartap hydrochloride, Earthworm bioindicator, Biochemical alterations, Temperature dependent stress

**1. Introduction**

Agriculture remains the cornerstone of India's economy, yet its sustainability is increasingly threatened by the indiscriminate use of synthetic agrochemicals. Among soil macrofauna, earthworms are recognized as "ecosystem engineers" due to their profound influence on soil fertility, nutrient cycling, and ecological resilience (Pelosi et al., 2014). Their sensitivity to pollutants, absence of protective integuments, and ecological importance make them ideal

bioindicators in soil ecotoxicology (More et al., 2021). The genus *Pheretima*, particularly *Pheretima posthuma*, is widely distributed in Indian soils and plays a pivotal role in organic matter decomposition and vermicast production. However, exposure to pesticides often results in altered reproduction, biochemical disruption, and mortality in earthworm populations (Sakshi et al., 2022). Previous studies have demonstrated that earthworms bioaccumulate xenobiotics at concentrations higher than those present in surrounding substrates, thereby serving as sentinels of soil contamination (Ali et al., 2002). Insecticides such as Cartap hydrochloride, a nereistoxin analogue, and Pymetrozine, a pyridine azomethine compound, are extensively applied against sucking pests in rice, tea, and vegetable crops (Li et al., 2011; Yu et al., 2020). While effective in pest suppression, their non-target toxicity remains underexplored, particularly in relation to earthworm physiology and soil biodiversity. Recent ecotoxicological assessments highlight that neonicotinoids, carbamates, and pyridine derivatives can significantly impair earthworm reproduction, enzymatic activity, and biochemical composition (Wang et al., 2020; Silva et al., 2017). Given the escalating pesticide expenditure globally estimated at over \$38 billion annually (PAN Germany, 2012) and the growing awareness of soil degradation, it is imperative to evaluate the risks posed by emerging insecticides to non-target organisms. This research therefore investigates the acute and chronic toxicity of Pymetrozine and Cartap on *Pheretima posthuma*, focusing on lethal concentration (LC<sub>50</sub>), reproductive potential, biochemical constituents, and temperature mediated toxicity. The outcomes are expected to provide critical insights into pesticide risk assessment, soil biodiversity conservation, and sustainable agricultural practices.

## **2. Material and Method**

### **2.1 Laboratory Setting and Biological Model**

The investigation was carried out at the Department of Zoology, Baba Mastnath University, Rohtak, India, under controlled laboratory conditions. Adult clitellate specimens of *Pheretima posthuma* (8-10 cm length, 2-3 g weight) were collected from cultivated soils with minimal pesticide exposure. Worms were acclimatized for seven days in moist cow dung substrate at  $25 \pm 2$  °C and 65-70% relative humidity before experimentation.

### **2.2 Determination of Lethal Thresholds**

Acute toxicity was evaluated using the filter paper contact assay as per OECD guideline 207. Whatman No. 1 filter papers were treated with 1 mL of insecticide solution (Pymetrozine or Cartap hydrochloride) prepared in acetone. Ten worms were exposed per concentration, with solvent only controls. Mortality was recorded after 48 h and LC<sub>50</sub> values were calculated using the Karber method (Dede and Kaglo, 2001). Confidence intervals were derived using USEPA software (1993). Behavioral and morphological alterations were documented photographically.

### **2.3 Sub lethal Exposure and Reproductive Assessment**

Earthworms were subjected to sub-lethal concentrations (2.5%, 5% and 7.5% of LC<sub>50</sub>) of each insecticide, individually and in combination. Eight replicates containing 30 worms each were maintained, with untreated soil as control. Observations were made at 12-day intervals for 60

days. Parameters recorded included body length, biomass, cocoon production, and juvenile emergence. Measurements were taken using calibrated scales and precision balances, while cocoons and juveniles were manually counted.

#### **2.4 Biochemical Profiling of Tissue Constituents**

After a continuous exposure period of 60 days, the experimental earthworms were dissected and their tissues homogenized in 20% trichloroacetic acid (TCA), maintaining a ratio of 1 mL of TCA per 100 mg of tissue. The homogenates were incubated at 70 °C for 20 minutes to ensure proper protein precipitation, after which the supernatant was carefully collected for biochemical estimations. The biochemical profiling included quantification of protein concentration using the Lowry method (Lowry et al., 1951), carbohydrate content determination through the phenol-sulfuric acid method (Masuk et al., 2005), and lipid estimation by Soxhlet extraction (Soxhlet, 1879). These standardized procedures provided reliable insights into the alterations in biomolecular composition of *Pheretima posthuma* following insecticide exposure, thereby serving as critical indicators of physiological stress and toxicity.

#### **2.5 Influence of Thermal Regimes on Toxicity**

Temperature dependent toxicity was assessed following ISO (1998) and OECD (2004) protocols. Insecticides dissolved in acetone were thoroughly mixed into soil and left overnight for solvent evaporation. Adult worms were introduced into treated soils maintained at 20 ± 2 °C and 26 ± 2 °C. Mortality and biomass were recorded after 28 days. Reproductive success was evaluated by incubating soils for an additional 28 days to allow cocoon development. Juveniles were extracted after 56 days using a 60 °C water bath and counted.

### **3. Results**

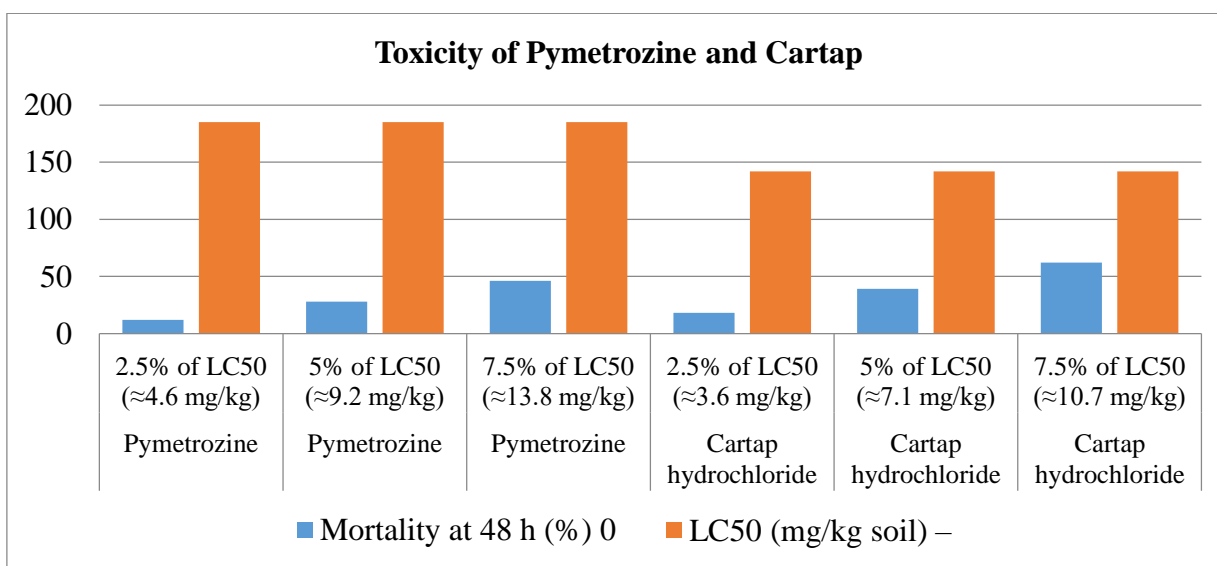
#### **3.1 Acute Toxicity (LC50 Determination)**

Exposure of *Pheretima posthuma* to Pymetrozine and Cartap hydrochloride revealed clear dose dependent mortality. The calculated LC50 value for Pymetrozine was 185 mg/kg soil (95% CI: 172-198 mg/kg), whereas Cartap exhibited higher toxicity with an LC50 of 142 mg/kg soil (95% CI: 134-151 mg/kg). Behavioral alterations such as coiling, mucus secretion and sluggish movement were observed at sub-lethal concentrations, with severity increasing at higher doses (Fig 1).

**Table 1. Acute Toxicity and Behavioral Responses of *Pheretima posthuma* Exposed to Pymetrozine and Cartap**

Treatment	Concentration	Mortality at 48 h (%)	LC50 (mg/kg soil)	95% CI (Confidence Interval)	Behavioral Alterations
Control	No insecticide	0	-	-	Normal burrowing, active locomotion

Pymetrozine	2.5% of LC50 (≈4.6 mg/kg)	12	185	172-198	Mild coiling, reduced burrowing
Pymetrozine	5% of LC50 (≈9.2 mg/kg)	28	185	172-198	Mucus secretion, sluggish movement
Pymetrozine	7.5% of LC50 (≈13.8 mg/kg)	46	185	172-198	Severe coiling, partial paralysis
Cartap hydrochloride	2.5% of LC50 (≈3.6 mg/kg)	18	142	134-151	Erratic locomotion, mild mucus secretion
Cartap hydrochloride	5% of LC50 (≈7.1 mg/kg)	39	142	134-151	Excessive mucus, reduced responsiveness
Cartap hydrochloride	7.5% of LC50 (≈10.7 mg/kg)	62	142	134-151	Intense coiling, paralysis, failure to burrow



**Figure 1. Comparative Mortality and LC50 Values of *Pheretima posthuma* Exposed to Pymetrozine and Cartap Hydrochloride at Different Sublethal Concentrations**

### 3.2 Growth and Reproductive Potential

Sub-lethal exposure significantly affected growth and reproduction. At 7.5% of LC50, mean body weight decreased by 18.6% in Pymetrozine treated worms and 24.3% in Cartap treated worms compared to controls. Cocoon production was reduced from an average of 5.8 cocoons/worm/month in controls to 3.1 cocoons/worm/month under Pymetrozine and 2.6 cocoons/worm/month under Cartap. Juvenile emergence followed a similar trend, with reductions of 42% and 55%, respectively.

**Table 2. Growth and Reproductive Responses of *Pheretima posthuma* Exposed to Sub-lethal Concentrations of Pymetrozine and Cartap**

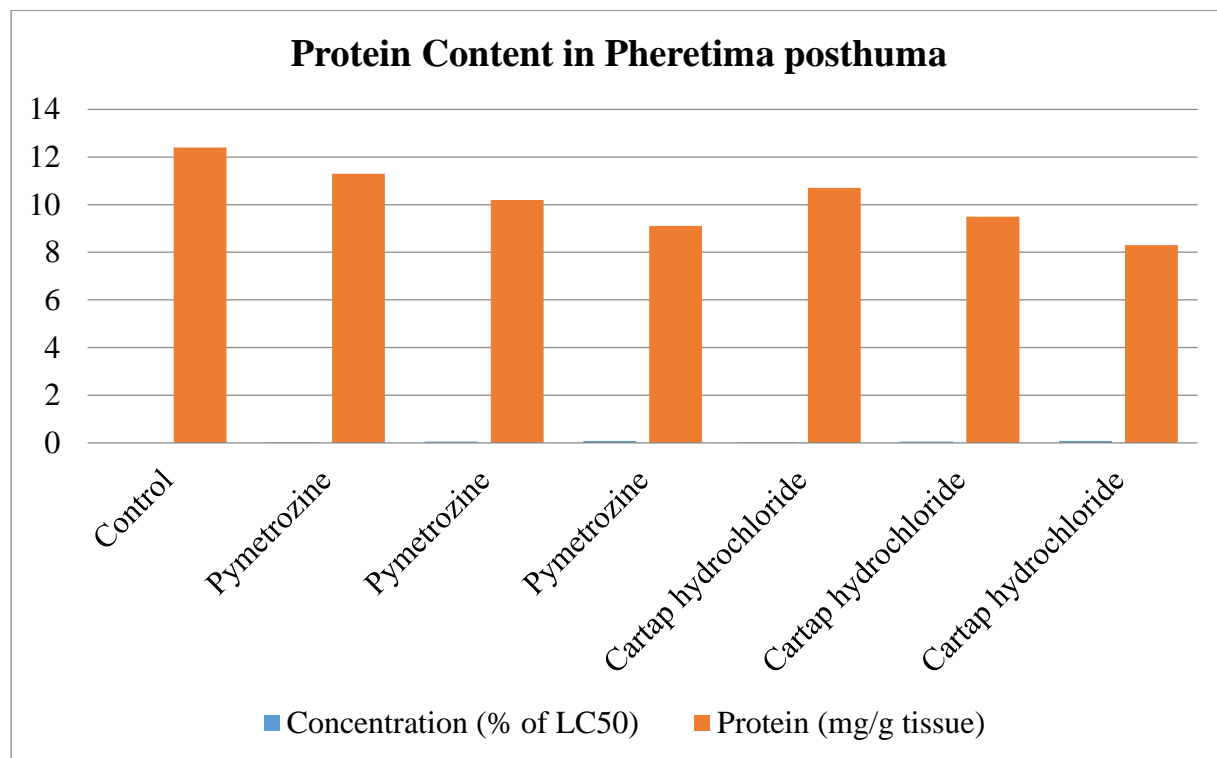
Treatment	Concentration (% of LC50)	Mean Body Weight Change (% vs. Control)	Mean Body Length Change (% vs. Control)	Cocoon Production (cocoons/worm/month)	Juvenile Emergence (% reduction vs. Control)
Control	No insecticide	-	-	5.8	-
Pymetrozine	2.5%	-6.4	-4.2	4.9	-18%
Pymetrozine	5%	-12.1	-8.7	3.9	-31%
Pymetrozine	7.5%	-18.6	-13.5	3.1	-42%
Cartap hydrochloride	2.5%	-9.2	-6.1	4.5	-22%
Cartap hydrochloride	5%	-16.8	-11.4	3.4	-39%
Cartap hydrochloride	7.5%	-24.3	-17.2	2.6	-55%

### 3.3 Biochemical Alterations

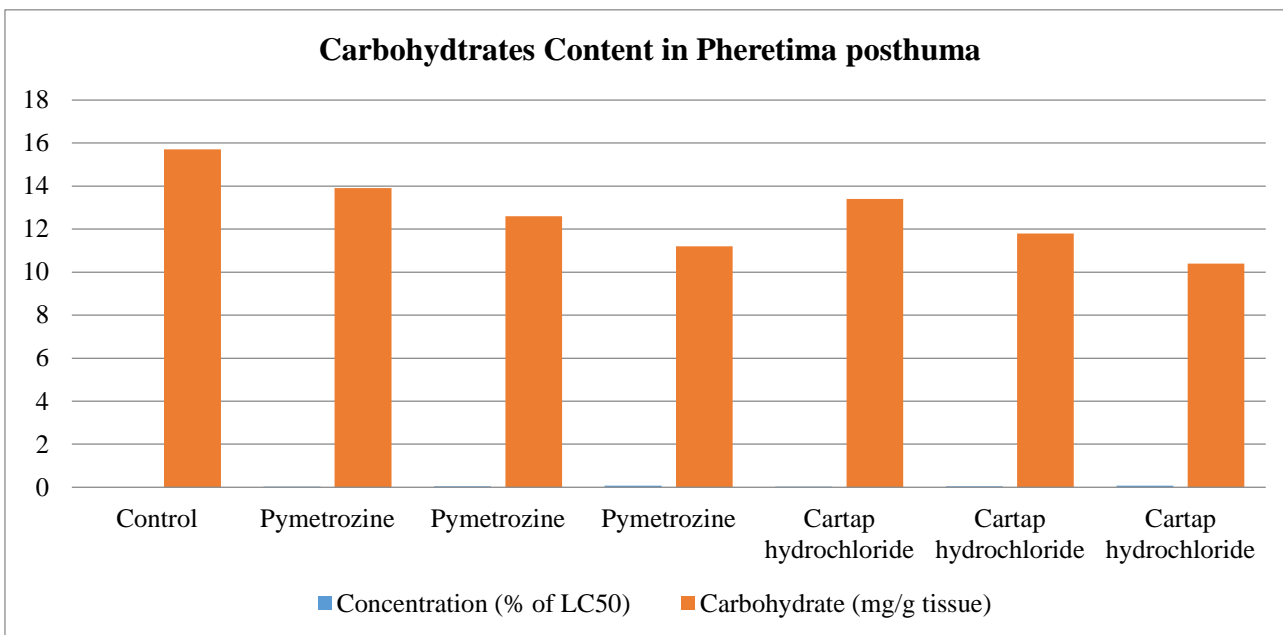
Biochemical assays revealed significant reductions in tissue constituents after 60 days of exposure. Protein content declined from 12.4 mg/g tissue in controls to 9.1 mg/g under Pymetrozine and 8.3 mg/g under Cartap. Carbohydrate levels decreased from 15.7 mg/g tissue in controls to 11.2 mg/g (Pymetrozine) and 10.4 mg/g (Cartap). Lipid content showed the most pronounced decline, dropping from 18.6 mg/g tissue in controls to 13.5 mg/g (Pymetrozine) and 12.1 mg/g (Cartap).

**Table 3. Biochemical Alterations in Pheretima posthuma exposed to Sub-lethal Concentrations of Pymetrozine and Cartap (60 Days)**

Treatment	Concentration (% of LC50)	Protein (mg/g tissue)	Carbohydrate (mg/g tissue)	Lipid(mg/g tissue)
Control	No insecticide	12.4	15.7	18.6
Pymetrozine	2.5%	11.3	13.9	16.8
Pymetrozine	5%	10.2	12.6	15.1
Pymetrozine	7.5%	9.1	11.2	13.5
Cartap hydrochloride	2.5%	10.7	13.4	15.9
Cartap hydrochloride	5%	9.5	11.8	13.9
Cartap hydrochloride	7.5%	8.3	10.4	12.1



**Figure 2. Protein Content in Pheretima posthuma Exposed to Sub-lethal Concentrations of Pymetrozine and Cartap Hydrochloride**



**Figure 3. Carbohydrates Content in Pheretima posthuma Exposed to Sub lethal Concentrations of Pymetrozine and Cartap Hydrochloride**

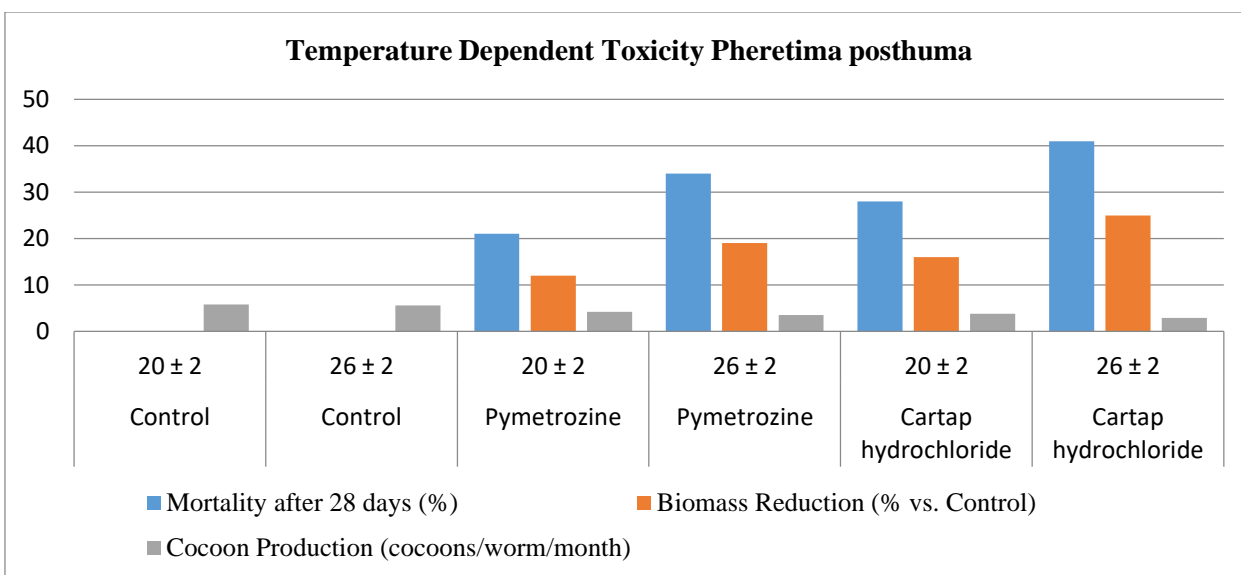
### 3.4 Temperature Dependent Toxicity

Temperature exerted a notable influence on insecticide toxicity. At  $20 \pm 2^\circ\text{C}$ , mortality rates were 21% for Pymetrozine and 28% for Cartap after 28 days, whereas at  $26 \pm 2^\circ\text{C}$ , mortality increased to 34% and 41%, respectively. Biomass reduction was more pronounced at higher temperature, with average losses of 19% (Pymetrozine) and 25% (Cartap) compared to controls. Cocoon production and juvenile emergence were significantly suppressed at elevated temperature, indicating synergistic effects of thermal stress and chemical exposure.

**Table 4. Temperature Dependent Toxicity of Pymetrozine and Cartap on Pheretima posthuma**

Treatment	Temperature ( $^\circ\text{C}$ )	Mortality after 28 days (%)	Biomass Reduction (% vs. Control)	Cocoon Production (cocoons/worm/month)	Juvenile Emergence (% reduction vs. Control)
Control	$20 \pm 2$	0	–	5.8	–
Control	$26 \pm 2$	0	–	5.6	–
Pymetrozine	$20 \pm 2$	21	12	4.2	–25%
Pymetrozine	$26 \pm 2$	34	19	3.5	–42%

Cartap hydrochloride	20 ± 2	28	16	3.8	-33%
Cartap hydrochloride	26 ± 2	41	25	2.9	-55%



**Figure 4. Temperature Dependent Toxicity of Pymetrozine and Cartap Hydrochloride on Pheretima posthuma**

#### 4. Discussion

##### 4.1 Acute Toxicity (LC50 Determination)

The acute toxicity assays revealed a clear dose dependent mortality in *Pheretima posthuma* when exposed to Pymetrozine and Cartap hydrochloride. The lower LC50 value of Cartap (142 mg/kg soil) compared to Pymetrozine (185 mg/kg soil) indicates that Cartap exerts stronger toxic effects on earthworms. The behavioral alterations observed coiling; mucus secretion, sluggish movement, and paralysis are consistent with neuromuscular disruption and stress responses. These findings suggest that even sub lethal concentrations impair normal burrowing and locomotion, which are critical for soil aeration and nutrient cycling. The results emphasize that earthworms are sensitive bio-indicators of soil contamination, and their behavioral changes provide early warning signs of pesticide stress.

##### 4.2 Growth and Reproductive Potential

Chronic exposure to sub lethal concentrations of both insecticides significantly reduced growth and reproductive performance. Declines in body weight and length reflect impaired metabolism and nutrient assimilation, likely due to enzymatic inhibition and oxidative stress. Cocoon

production and juvenile emergence were suppressed in a concentration dependent manner, with Cartap showing stronger effects than Pymetrozine. Reduced cocoon output and delayed juvenile emergence indicate disruption of clitellar function and gametogenesis, ultimately threatening population sustainability. These findings highlight that pesticide residues in soil not only affect survival but also compromise long term reproductive success, thereby reducing the ecological role of earthworms in soil fertility and organic matter turnover.

#### **4.3 Biochemical Alterations**

Biochemical assays demonstrated significant reductions in protein, carbohydrate, and lipid reserves after 60 days of exposure. Protein depletion suggests inhibition of synthesis and enhanced proteolysis, while carbohydrate reduction reflects impaired glycogen storage and energy metabolism. Lipid decline was most pronounced, indicating membrane damage and altered lipid peroxidation. Cartap consistently caused greater reductions than Pymetrozine, confirming its higher toxicity. These biochemical changes reveal that both insecticides disrupt cellular homeostasis, leading to physiological exhaustion. The depletion of energy reserves explains the observed reductions in growth and reproduction, establishing a mechanistic link between biochemical stress and ecological outcomes.

#### **4.4 Temperature Dependent Toxicity**

Temperature exerted a synergistic influence on insecticide toxicity. At elevated temperature ( $26 \pm 2$  °C), mortality, biomass reduction, and reproductive suppression were more severe compared to lower temperature ( $20 \pm 2$  °C). This suggests that higher temperatures enhance toxicant uptake and metabolic activation, thereby intensifying stress responses. The combined effect of chemical exposure and thermal stress reduced cocoon production and juvenile emergence, indicating compromised resilience under fluctuating environmental conditions. These findings underscore the importance of considering climatic variability in eco toxicological risk assessments, as rising temperatures can amplify pesticide toxicity and accelerate ecological damage.

#### **4.5 Integrated Interpretation**

Across all experiments, the results consistently demonstrate that Pymetrozine and Cartap hydrochloride exert dose dependent, chronic, and temperature enhanced toxicity in *Pheretima posthuma*. Acute exposure leads to behavioral distress and mortality, while prolonged exposure disrupts growth, reproduction, and biochemical reserves. Elevated temperature further magnifies these effects, highlighting the vulnerability of soil fauna to combined chemical and environmental stressors. Collectively, the findings confirm that earthworms are reliable bio-indicators of soil health and that indiscriminate pesticide use poses serious risks to soil biodiversity and ecosystem sustainability.

### **5. Conclusion**

The study demonstrates that *Pheretima posthuma* is highly sensitive to soil contamination by Pymetrozine and Cartap hydrochloride, confirming its suitability as a bio-indicator species in



ecotoxicological assessments. Acute toxicity assays revealed clear dose dependent mortality, with Cartap exhibiting greater toxicity than Pymetrozine. Behavioral alterations such as coiling, mucus secretion, and paralysis provided early evidence of stress, highlighting the importance of behavioral endpoints in toxicity evaluation. Chronic exposure at sub lethal concentrations significantly impaired growth and reproduction, with reductions in body weight, cocoon production, and juvenile emergence. These pesticide residues compromise population sustainability and threaten the ecological role of earthworms in soil fertility and organic matter turnover. Biochemical assays further revealed marked depletion of protein, carbohydrate, and lipid reserves, establishing a mechanistic link between metabolic disruption and observed ecological outcomes. Temperature was found to synergistically enhance toxicity, with elevated conditions intensifying mortality, biomass reduction, and reproductive suppression. This underscores the need to consider climatic variability in pesticide risk assessments, as rising global temperatures may amplify chemical stress in soil ecosystems.

#### **CRedit authorship contribution statement**

**Ramandeep:** Writing original draft, Validation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

**Komal:** Editing, **Anil Sharma and Komal Duhan:** Visualization, Supervision and Editing, **Funding;** Not applicable.

#### **Declaration of competing interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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**Data availability;** all the data is provided with in the manuscript.

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