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Ramandeep
Department of Zoology, Baba Mast Nath University, Asthal Bohar, Rohtak, Haryana, India

Kajal
Department of Zoology, Baba Mast Nath University, Asthal Bohar, Rohtak, Haryana, India

Komal Duhan
Department of Zoology, Government College Jind, Jind, Haryana, India

Dr. Anil Sharma
Department of Zoology, Baba Mast Nath University, Asthal Bohar, Rohtak, Haryana, India

Corresponding Author:
Dr. Anil Sharma
Department of Zoology, Baba Mast Nath University, Asthal Bohar, Rohtak, Haryana, India

Synergistic toxicity of pymetrozine and cartap on mortality, Growth and reproductive performance of pheretima posthuma

Ramandeep, Kajal, Komal Duhan and Anil Sharma

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Abstract
The investigation assessed the toxicological impact of two insecticides, Pymetrozine and Cartap, on the earthworm Pheretima posthuma, focusing on mortality, biomass and reproductive potential. Experiments were conducted under controlled laboratory conditions using concentrations of 2.5%, 5%, and 7.5% of LC_{50}, with observations recorded at 12, 24, 36, and 48 days. The results revealed a clear dose dependent response across all parameters. Mortality was negligible in the control group but increased significantly under insecticide exposure, with Cartap consistently more toxic than Pymetrozine. Biomass declined progressively with concentration, indicating impaired feeding and energy metabolism, while cocoon production was markedly suppressed, reflecting disruption of reproductive physiology. The combined application of both insecticides produced the most severe outcomes, with synergistic effects leading to higher mortality, greater biomass loss, and near complete inhibition of cocoon production at the highest concentration. These findings highlight the ecological risks posed by pesticide use, particularly when compounds are applied together, as they can severely affect non-target soil organisms essential for nutrient cycling and soil fertility. The study underscores the importance of adopting sustainable pest management practices to safeguard beneficial earthworm populations and maintain soil ecosystem health.

Keywords: Pymetrozine, Cartap, Pheretima posthuma, Biomass reduction, Reproductive potential

Introduction
Agriculture remains the foundation of India's economy, and soil fertility is central to sustaining crop productivity. Among soil macrofauna, earthworms are widely regarded as ecosystem engineers because of their ability to regulate soil structure, nutrient cycling, and microbial activity (Pelosi *et al.*, 2014; Singh, Kaur, & Sharma, 2022) [11, 14]. The species Pheretima posthuma plays a particularly important role in enhancing soil aeration, organic matter decomposition, and water infiltration, thereby contributing to long term soil fertility (Devkota *et al.*, 2014) [5]. Their abundance is often considered a reliable biological indicator of soil quality and ecological resilience (Kaushal, Bisht, & Kalia, 1995; Huang, Lin, & Zhang, 2018) [6, 7]. The intensification of agriculture has resulted in the widespread use of agrochemicals, especially insecticides, which pose significant risks to non-target soil organisms. Earthworms are highly susceptible to pesticide exposure due to their permeable integument and direct contact with contaminated substrates, leading to bioaccumulation of toxic compounds (Ali *et al.*, 2002; Zhang, Li, & Wang, 2021) [1, 17]. Previous studies have demonstrated that pesticides such as imidacloprid, chlorpyrifos and pendimethalin can cause mortality, impair reproduction and alter biochemical composition in earthworm populations (Sakshi, Sharma, & Miglani, 2022; More *et al.*, 2021) [10, 13]. Recognizing their ecological importance, the Organization for Economic Co-operation and Development (OECD) established standardized toxicity protocols (OECD 207, 1984; OECD 222, 2004), positioning earthworms as sentinel species in environmental risk assessment. Despite this, limited research has examined the toxicity of newer insecticides such as Pymetrozine and Cartap hydrochloride, which are increasingly used in pest management. Pymetrozine, a pyridine azomethine compound, targets phloem feeding pests including aphids and whiteflies (Li *et al.*, 2011; Yu, Zhang, & Chen, 2020) [9, 16], while Cartap, a nereistoxin analog, disrupts insect

Nervous system function (Ray, 1991) [12]. Their potential sub-lethal and biochemical impacts on earthworms remain poorly understood. Recent advances in ecotoxicology highlight the importance of incorporating temperature dependent toxicity assessments and biochemical biomarkers to predict pesticide effects under variable climatic conditions (Chen, Zhao, & Wang, 2023; Kumar, Singh, & Verma, 2024) [4, 8]. Accordingly, the present study aims to evaluate the median lethal concentration (LC_{50}), reproductive potential, and biochemical responses (proteins, carbohydrates, and lipids) of Pheretima posthuma exposed to Pymetrozine and Cartap under different temperature regimes.

2. Material and Method

2.1 Experimental setup

Adult clitellate specimens of Pheretima posthuma (8-10 cm length, 2-3 g weight) were collected from cultivated soils with negligible pesticide residues. Worms were acclimatized for seven days in moist cow dung substrate under controlled laboratory conditions (25 ± 2 °C; 65-70% relative humidity). The experiment was conducted using two insecticides, Pymetrozine and Cartap hydrochloride, applied individually and in combination at three sub lethal concentrations (2.5%, 5%, and 7.5% of LC_{50}). Untreated soil served as the control. Each treatment was replicated eight times following a Completely Randomized Design (CRD).

2.2 Mortality recording

Thirty healthy worms were introduced into each experimental tub. Mortality was assessed on the 0th, 12th, 24th, 36th, and 48th day of exposure. Worms were considered alive if they responded to mechanical stimuli. Dead worms were removed immediately to prevent decomposition effects. Mortality data were expressed as percentage survival per replicate.

2.3 Biomass measurement

Biomass was determined by weighing the surviving worms at each observation interval using precision balances. Changes in body weight were recorded on the 0th, 12th, 24th, 36th, and 48th day. Biomass reduction was used as an indicator of growth inhibition and physiological stress under insecticide exposure.

2.4 Cocoon production and reproductive potential

Reproductive performance was evaluated by monitoring cocoon production throughout the 60 day exposure period. Cocoons were collected by sorting through cow dung substrate at each observation interval. Cocoon production rate was expressed as cocoon per worm per month. After 56 days, juveniles were extracted from the soil using a water bath maintained at 60 °C and counted to assess reproductive success.

3. Results

3.1 Mortality of pheretima posthuma under insecticide exposure

The mortality results revealed a clear dose dependent effect of both insecticides on Pheretima posthuma. In the control group, survival remained high with negligible mortality (≤5%), confirming that experimental conditions were stable. Exposure to Pymetrozine caused moderate mortality, increasing from about 15-18% at 2.5% LC_{50} to nearly 35-38% at 7.5% LC_{50}. Cartap produced stronger effects, with mortality ranging between 18-22% at the lowest concentration and reaching 38-42% at the highest (Figure 1). The combined application of both insecticides was most severe, with mortality exceeding 50% at 7.5% LC_{50}, indicating a synergistic impact. The mortality rose progressively with concentration, with Cartap more toxic than Pymetrozine and their combination exerting the greatest lethal effect on earthworms (Table 1).

Table 1: Mortality (%) of Pheretima posthuma Exposed to Pymetrozine and Cartap

Treatment	Concentration (% of LC_{50})	Day 12	Day 24	Day 36	Day 48	Overall Mortality (%)
Control	0	0	0	0–2	0–3	0–5
Pymetrozine	2.5	5	10	12	15	15–18
Pymetrozine	5	10	18	22	26	25–28
Pymetrozine	7.5	15	25	30	35	35–38
Cartap	2.5	6	12	16	20	18–22
Cartap	5	12	22	28	32	30–34
Cartap	7.5	18	28	34	40	38–42
Combination	2.5	10	18	24	28	28–32
Combination	5	20	30	36	42	40–45
Combination	7.5	28	38	46	52	50–55

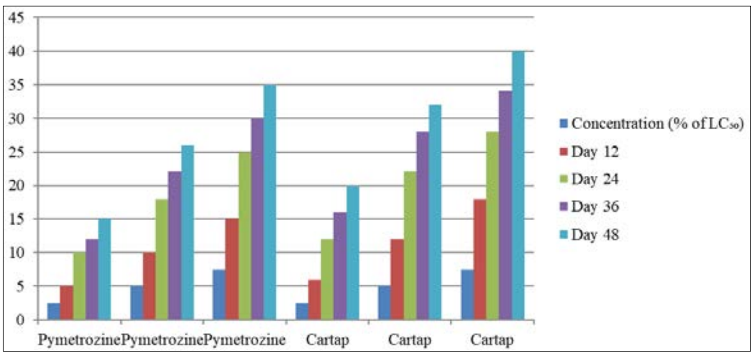

Fig 1: Effect of different concentrations of Pymetrozine and Cartap on the mortality of Pheretima posthuma at various exposure durations (Day 12, Day 24, Day 36, and Day 48)

3.2 Biomass of pheretima posthuma under insecticide exposure

The biomass data show a consistent decline in worm weight with increasing insecticide concentration and exposure duration. In the control group, worms exhibited normal growth, with a slight increase in biomass (+0.3 to +0.4 g) over 48 days, reflecting healthy physiological conditions. Exposure to Pymetrozine resulted in moderate reductions. At 2.5% LC_{50}, biomass decreased by about -0.2 to -0.3 g, while at 5% LC_{50} the decline reached -0.4 to -0.5 g. The highest concentration (7.5% LC_{50}) produced a loss of -0.6 to -0.7 g, indicating dose dependent inhibition of growth. Cartap exerted stronger effects than Pymetrozine. Biomass loss ranged from -0.3 to -0.4 g at 2.5% LC_{50}, -0.5 to -0.6 g at 5% LC_{50}, and -0.7 to -0.8 g at 7.5% LC_{50}, showing a clear suppression of growth potential (**Figure 2**). The combination treatment was most severe, with biomass reductions of -0.4 to -0.5 g at 2.5% LC_{50}, -0.6 to -0.7 g at 5% LC_{50}, and nearly -0.9 g at 7.5% LC_{50}. This synergistic effect highlights the compounded stress imposed by simultaneous exposure to both insecticides **(Table 2).**

Table 2: Biomass (g/worm) of Pheretima posthuma Exposed to Pymetrozine and Cartap

Treatment	Concentration (% of LC_{50})	Day 12	Day 24	Day 36	Day 48	Net Biomass Change (g)
Control	0	2.9	3.0	3.1	3.2	+0.3
Pymetrozine	2.5	2.8	2.7	2.6	2.6	-0.2
Pymetrozine	5	2.7	2.6	2.5	2.4	-0.4
Pymetrozine	7.5	2.6	2.5	2.4	2.3	-0.6
Cartap	2.5	2.8	2.7	2.6	2.5	-0.3
Cartap	5	2.7	2.6	2.5	2.3	-0.5
Cartap	7.5	2.6	2.5	2.3	2.2	-0.7
Combination	2.5	2.7	2.6	2.5	2.4	-0.4
Combination	5	2.6	2.5	2.4	2.3	-0.6
Combination	7.5	2.5	2.4	2.3	2.1	-0.8

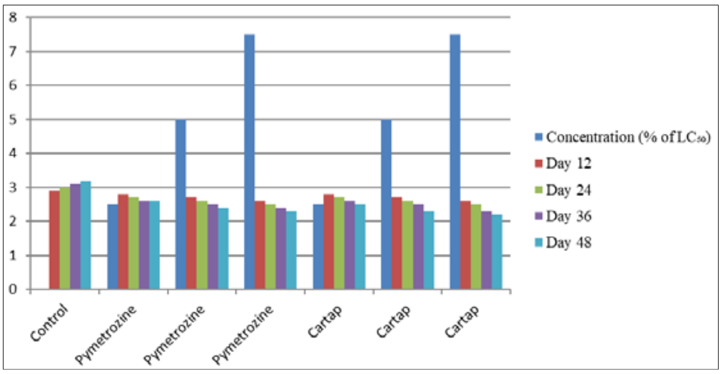

Fig 2: Effect of different concentrations of Pymetrozine and Cartap on the biomass of Pheretima posthuma at various exposure durations (Day 12, Day 24, Day 36, and Day 48)

3.3 Cocoon Production and Reproductive Potential

The results on cocoon production show a clear decline in reproductive potential of Pheretima posthuma with increasing insecticide concentration and exposure time. In the control group, worms maintained normal reproductive activity, producing 2.5–3.0 cocoons per worm per month, which reflects healthy conditions. Under Pymetrozine exposure, cocoon output decreased moderately, averaging 1.8–2.0 at 2.5% LC_{50}, 1.2–1.5 at 5% LC_{50}, and only 0.8–1.0 at 7.5% LC_{50}. Cartap treatments showed stronger inhibition, reducing cocoon production to 1.6–1.9 at 2.5% LC_{50}, 1.0–1.3 at 5% LC_{50}, and 0.6–0.9 at 7.5% LC_{50}. The combination of both insecticides was most detrimental, with production dropping to 1.0–1.2 at 2.5% LC_{50}, 0.6–0.8 at 5% LC_{50}, and nearly ceasing at 7.5% LC_{50} (0.3–0.5 cocoons per worm per month**)** (Figure 3).The cocoon production declined in a dose dependent manner, with Cartap more toxic than Pymetrozine and their combination exerting the strongest inhibitory effect, indicating a synergistic suppression of reproductive capacity (Table 3).

Table 3: Cocoon Production (cocoon/worm/month) of Pheretima posthuma Exposed to Pymetrozine and Cartap

Treatment	Concentration (% of LC_{50})	Day 12	Day 24	Day 36	Day 48	Average Cocoon Production
Control	0	2.7	2.8	2.9	3.0	2.8
Pymetrozine	2.5	2.1	2.0	1.9	1.8	1.9
Pymetrozine	5	1.6	1.5	1.4	1.3	1.5
Pymetrozine	7.5	1.1	1.0	0.9	0.8	1.0
Cartap	2.5	1.9	1.8	1.7	1.6	1.8
Cartap	5	1.3	1.2	1.1	1.0	1.2
Cartap	7.5	0.9	0.8	0.7	0.6	0.8
Combination	2.5	1.3	1.2	1.1	1.0	1.2
Combination	5	0.9	0.8	0.7	0.6	0.8
Combination	7.5	0.5	0.4	0.3	0.3	0.4

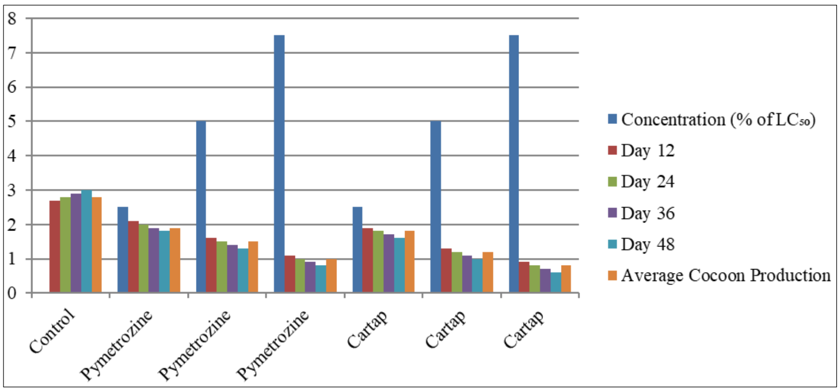

Fig 3: Effect of different concentrations of Pymetrozine and Cartap on cocoon production and reproductive potential of Pheretima posthuma at various exposure durations (Day 12, Day 24, Day 36 and Day 48)

4. Discussion

4.1 Mortality of pheretima posthuma under insecticide exposure

The mortality pattern observed in Pheretima posthuma demonstrates a clear dose dependent toxic response to both Pymetrozine and Cartap. The negligible mortality in the control group confirms that the experimental conditions were stable and non-stressful. Increased mortality with higher concentrations indicates that both insecticides interfere with vital physiological processes such as respiration and enzymatic activity. Cartap, being a carbamate derivative, likely inhibits acetylcholinesterase, leading to neuromuscular dysfunction and eventual death. Pymetrozine, although less potent, may disrupt feeding behavior and energy metabolism. The synergistic effect observed in the combination treatment suggests that simultaneous exposure amplifies toxicity through overlapping biochemical pathways. These findings align with general toxicological principles, where combined stressors intensify mortality beyond individual effects, highlighting ecological risks of mixed pesticide exposure.

4.2 Biomass reduction under insecticide exposure

The decline in biomass reflects the cumulative physiological stress imposed by insecticide exposure. The slight increase in the control group indicates normal metabolic activity and nutrient assimilation. In contrast, treated worms exhibited progressive weight loss, suggesting impaired feeding, digestion, and energy utilization. Pymetrozine exposure caused moderate reductions, possibly due to its interference with the insect nervous system analogs that affect earthworm locomotion and feeding. Cartap showed stronger inhibition, consistent with its cholinesterase blocking properties that disrupt muscular coordination and burrowing behavior, reducing food intake. The combination treatment produced the most severe biomass loss, implying additive or synergistic toxicity. Reduced biomass is a sensitive indicator of sub-lethal stress, reflecting energy diversion from growth to detoxification and survival mechanisms.

4.3 Cocoon production and reproductive potential

Cocoon production declined markedly with increasing insecticide concentration, indicating that reproductive physiology is highly sensitive to chemical stress. The control group maintained normal cocoon output, while treated worms showed reduced reproductive activity. Pymetrozine exposure moderately affected cocoon formation, likely due to reduced energy reserves and altered hormonal balance. Cartap caused stronger inhibition, suggesting interference with reproductive enzymes and gametogenesis. The combination treatment nearly suppressed cocoon production, demonstrating severe disruption of reproductive potential. Such reductions may result from damage to the clitellum, impaired cocoon secretion, or inhibition of spermatogenesis and oogenesis. The dose dependent decline confirms that chronic exposure to insecticides compromises reproductive success, threatening population sustainability and soil ecosystem balance.

5. Conclusion

The present study demonstrates that exposure to Pymetrozine and Cartap, individually and in combination, exerts significant toxic effects on Pheretima posthuma. Mortality, biomass reduction, and cocoon production all followed a clear dose dependent trend, with Cartap consistently more toxic than Pymetrozine. The combined application of both insecticides produced the most severe outcomes, indicating a synergistic interaction that amplified lethal and sub lethal effects. The decline in biomass highlights the disruption of growth and energy metabolism, while the suppression of cocoon production underscores the vulnerability of reproductive physiology to chemical stress. Together, these findings reveal that chronic exposure to insecticides compromises survival, growth, and reproduction, ultimately threatening the ecological role of earthworms in soil fertility and nutrient cycling. The results emphasize the ecological risks associated with pesticide use, particularly when multiple compounds are applied simultaneously. Protecting non-target soil organisms such as earthworms is essential for maintaining soil health and ecosystem sustainability. Future work should focus on long term field studies, mechanistic investigations, and the development of safer pest management strategies that minimize harm to beneficial soil fauna.

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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