

Tissue-Specific Antioxidant, Digestive, and Neurophysiological Responses of *Luprops tristis* (Coleoptera: Tenebrionidae) to Sublethal Cypermethrin Exposure in Rubber Plantation Landscapes

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Abstract

The pyrethroid insecticides like Cypermethrin is widely used against agricultural pests, but its biochemical and tissue specific sublethal effects in non-model coleopteran pests has not been yet understood. This study tried to address the acute toxicity and time dependent biochemical responses of Mupli beetle (*Luprops tristis*), a major residential pest in rubber plantations in South India, to Cypermethrin. Mortality (LD₅₀) was estimated by probit analysis, and adults were then exposed to a sublethal concentration (1/10 LD₅₀) to assess oxidative stress, digestive metabolism, and neuro-detoxification in whole body, gut, and fat body over 24–96 h. Cypermethrin exposure caused a significant, time-dependent decline in antioxidant enzymes, including superoxide dismutase, catalase, peroxidase, glutathione peroxidase, and glutathione reductase ($p < 0.05$), with the most visible inhibition in gut tissues, indicating severe tissue-specific oxidative imbalance. Digestive enzymes (α -amylase, protease, and lipase) also showed progressive reduction, especially in the gut, reflecting impaired nutrient assimilation and metabolic disruption. Glutathione-S-transferase (GST) activity showed marked suppression in all tissues, most strongly in the gut, demonstrating reduced detoxification capacity, while acetylcholinesterase (AChE) activity was significantly inhibited in a time- and tissue-dependent manner, confirming neurophysiological impairment under sublethal exposure. Collectively, these findings show that Cypermethrin elicits sustained oxidative stress, metabolic dysfunction, and compromised detoxification and neural function in *L. tristis* in a distinctly tissue-specific manner and advocate the need for multi-tissue biochemical biomarkers for sublethal assessment of pesticides in plantation ecosystems.

Introduction

Synthetic insecticides remain important for global agricultural productivity and public health because they provide effective control of crop pests and disease vectors, thereby helping to protect food security and reduce vector-borne diseases (Sharma et al. 2024; Kumar et al. 2024). Despite the growing interest in biological control and Integrated Pest Management (IPM), chemical insecticides are necessary for safeguarding food security. However, repeated applications of these pesticides increase non-target exposure to sublethal concentrations, raising concerns about subtle biochemical and physiological effects beyond primary neurotoxicity. Pyrethroids are among the important group of pesticides, known for their strong insecticidal ability, fast knockdown, low mammalian toxicity than organophosphates and organochlorines, and environmental compatibility at recommended doses (Ruberti 2024). Cypermethrin, a widely used pyrethroid, exerts its toxic effects by binding to and disrupting voltage-gated sodium channels in nerve membranes, resulting in prolonged neuronal excitation, and ultimately causing paralysis death and death in insects (Soderlund 2012). Pyrethroids like Cypermethrin contaminate plantation soils and nearby residential areas, causing sublethal risks to non-target decomposers like *Luprops tristis* (Mupli beetle), with implications for ecosystem services and public health in tropical agro-landscapes. Insects exhibit tissue-specific responses to xenobiotics due to organ specialisation: the fat body serves as a metabolic detoxification hub, similar to the vertebrate liver, while the midgut provides a first barrier against ingested toxins (Toprak and Musselman 2021; Hou et al. 2025). Beetles possess

distinctive detoxification systems involving cytochrome P450s, yielding species-specific insecticide sensitivities (Nauen et al. 2022).

Even though the effects of pyrethroid pesticides are well-studied in model insects (Hodoşan et al. 2023), there is a lack of integrated approach to the time-dependent biochemical responses in non-model Coleopterans. *L. tristis* is a major pest found in residential areas near rubber plantations in South India causing public distress (Raju and Radhika 2023; Sebastian 2026). Massive infestation of these organisms occurs in residential areas near rubber plantations in South India (Mohammed et al. 2023). Cypermethrin controls these pest outbreaks, but its non-target risks to those organisms, including humans are a major concern (Rezende-Teixeira et al. 2022). Standard LD₅₀ assays mainly address mortality but do not generally detect progressive biochemical changes that can influence the long-term fitness of organisms (Bartling et al., 2024). Beyond neurotoxicity, pyrethroids trigger secondary metabolic disruptions (Mahanthesh et al. 2026). Detoxification enzymes like glutathione S-transferases (GSTs), carboxylesterases, and cytochrome P450s have the ability to metabolise pesticides but can produce reactive intermediates which induce oxidative stress (Amezian et al. 2021). This imbalance arises when reactive oxygen species (ROS- superoxide anions, hydroxyl radicals, and hydrogen peroxide) surpass the antioxidant defense mechanisms, damaging proteins, lipids, and DNA (Mahajan and Kaur 2025). Insects counter this using coordinated enzymes like superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), peroxidases (POD), and glutathione reductase (GR) (Messi et al. 2025). Insecticide-induced shifts in these markers signal sublethal stress (Rix and Cutler 2022). Pyrethroids also impair digestive physiology by inhibiting nutrient-processing enzymes (α -amylases and lipases), thereby disrupting energy metabolism and processes essential for growth and survival (Saadati and Mirzaei 2016). Sublethal exposure further compromises reproduction, feeding, immunity, and overall fitness, altering population dynamics (Guedes et al. 2026).

The study evaluates Cypermethrin's dose-dependent mortality and time-dependent effects (24–96 h) on antioxidant enzymes (SOD, CAT, GPx, peroxidase, GR), digestive enzymes (α -amylase, protease, lipase), and detoxification/neurophysiological markers (GST, AChE) in *L. tristis*. No prior tissue-specific, time-dependent data exists for these responses under realistic pyrethroid scenarios, nor their implications for plantation-adjacent pest control. We hypothesised that sublethal doses of Cypermethrin induces tissue-specific oxidative stress and dynamic digestive enzyme alterations in *L. tristis*, serving as biomarkers for pesticide pollution monitoring.

Despite the widespread use of pyrethroids in plantation landscapes, the sublethal biochemical and physiological impacts of Cypermethrin on non-model coleopteran pests remain poorly addressed, particularly at the tissue level. As a residential species that periodically swarms dwellings around rubber plantations, *L. tristis* represents a critical interface between plantation pest management and public well-being. However, existing toxicity assessments are restricted to acute toxicity endpoints and whole body responses, which overlook progressive, tissue-specific alterations in antioxidant, digestive, and neuro-detoxification systems that can compromise long-term fitness and population dynamics. This study addresses this gap by integrating acute toxicity bioassays with time-resolved measurements of key

biochemical markers in the whole body, gut, and fat body following sublethal Cypermethrin exposure, thereby establishing a multi-tissue biomarker framework for pyrethroid risk assessment in these kinds of beetles. To our knowledge, this is the first report that simultaneously evaluates antioxidant, digestive, and detoxification/neurophysiological enzyme responses across multiple tissues of *L. tristis* under realistic sublethal Cypermethrin exposure, providing novel insights into tissue-specific susceptibility and potential early-warning indicators of pesticide stress.

Materials and methods

Insect Collection and Maintenance

Adult *L. tristis* were hand-collected from leaf litter and surrounding vegetation around a public building in Kalady, Ernakulam District, Kerala, India (10.197° N, 76.386° E), where they caused significant nuisance. Collections were done manually by hand-picking using forceps during early mornings and were transported to the laboratory in ventilated plastic containers containing leaf litter to minimize stress. Collections followed local regulations (no permissions required for nuisance pests). Specimens were identified based on morphological features like dark brown-to-black coloration and elongated oval bodies (8–10 mm) as per Sabu et al. (2014). Only healthy, uniform sized adults were selected. In the lab, the insects were maintained in aerated plastic containers at $27 \pm 2^\circ\text{C}$ and $65 \pm 5\%$ relative humidity, fed 2g fresh *Hevea brasiliensis* leaves daily (20 insects per container), replaced every 2–3 days and acclimatized for seven days prior to experimentation.

Acute toxicity tests

Analytical grade Cypermethrin stock ($\geq 99\%$ purity; Sigma-Aldrich, USA UNSPSC code-41116107) (1mg ml^{-1}) was prepared by dissolving 10 mg in 10 mL HPLC grade methanol ($\geq 99.9\%$ purity, Sigma-Aldrich, $\leq 1\%$ v/v final). Working solutions (10, 20, 25, 50, and $100\text{ }\mu\text{g ml}^{-1}$) were prepared by serial dilution. Groups of 10 adults ($n = 3$ replicates per concentration) were topically treated with treated insects were kept at $25 \pm 2^\circ\text{C}$ and $70 \pm 5\%$ relative humidity and observed for 24 hours. Insects were considered dead if they showed no movement or did not respond to a gentle touch stimulus. The LD_{50} was calculated via probit analysis in SPSS software (Abbott 1925), and normality was confirmed using Shapiro-Wilk analysis.

Biochemical analysis

Surviving adults were exposed to a sublethal dose ($0.55\text{ }\mu\text{g ml}^{-1}$, $1/10\text{th LD}_{50}$) at $25 \pm 2^\circ\text{C}$ and $70 \pm 5\%$ relative humidity. At each time point (24–96 hours), the surviving insects were anesthetized, dissected in saline and tissues (whole body, gut, fat body) isolated. For each treatment per time per replicate, 10 adults were pooled ($n = 3$ pools). Tissues were homogenized in 10 ml saline and centrifuged ($10,000 \times g$, 20 minutes, 4°C , Remi Electrotechnik India, C24-BL) and supernatants were used for the assays. All steps were carried out at 4°C to prevent enzyme degradation with normal saline as control. Total protein

content of the tissue homogenates was measured as per Lowry et al. (1951) using bovine serum albumin (BSA) standards. Absorbance at 660 nm (Shimadzu UV-1800 spectrophotometer) and enzyme activities were calculated from the BSA standard curve, expressed as mg ml⁻¹ protein.

Antioxidant and detoxification enzyme assays

Antioxidant enzyme activities were estimated in the tissue homogenates of *L. tristis* using spectrophotometry (Shimadzu UV-1800) in triplicate, and enzyme activities were expressed per mg of protein.

Superoxide dismutase (SOD) activity was estimated following the method of Marklund and Marklund (1974). The reaction mixtures were incubated at room temperature for 5 minutes, and absorbance was recorded at 420 nm using a UV-visible spectrophotometer (Shimadzu UV-1800). Catalase activity was determined according to the method of Luck (1965). The decomposition of hydrogen peroxide was monitored by measuring the decrease in absorbance at 240 nm using UV-visible spectrophotometer (Shimadzu UV-1800).

Peroxidase activity was estimated following the method of Reddy *et al.* (1995). The reaction mixture was incubated at room temperature for 5 minutes, and the increase in absorbance due to catechol oxidation was measured at 420 nm using a Shimadzu UV-1800 UV-visible spectrophotometer. Glutathione reductase (GR) activity was determined according to Mannervik and Carlberg (1985). The reaction was initiated by the addition of 0.1 mL NADPH, and the decrease in absorbance at 340 nm was recorded using a Shimadzu UV-1800 UV-visible spectrophotometer.

Glutathione peroxidase (GPx) activity was estimated following Rotruck *et al.* (1973). The reaction mixture was incubated for 5 minutes at room temperature, and the reaction was terminated by adding 0.5 mL trichloroacetic acid (TCA), followed by centrifugation at 3000 rpm for 10 minutes. The pellet was resuspended in 4.0 mL Na₂HPO₄ (0.1 M), followed by the addition of 1.0 mL DTNB. Absorbance was measured at 412 nm using UV-visible spectrophotometer.

Glutathione-S-transferase (GST) activity was estimated according to Habig *et al.* (1974). The reaction mixture contained 2.77 mL sodium phosphate buffer (0.1 M, pH 6.5), 50 µL of 1-chloro-2,4-dinitrobenzene (CDNB, 2 mM), and 150 µL of reduced glutathione (GSH, 10 mM). The reaction was initiated by adding 30 µL of enzyme extract and the change in absorbance was recorded at 340 nm using UV Visible spectrophotometer (Shimadzu UV-1800).

Acetylcholinesterase (AChE) activity was determined following Ellman *et al.* (1961). The reaction mixture consisted of 2.86 mL sodium phosphate buffer (0.1 M, pH 8.0) and 100 µL of enzyme extract. After pre-incubation for 5 min at room temperature, the reaction was initiated by adding 10 µL of 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB, 10 mM) and 30 µL of acetylthiocholine iodide (ATChI, 74 mM). The increase in absorbance was measured at 412 nm using a UV-Visible spectrophotometer (Shimadzu UV-1800).

Digestive enzyme assays

Digestive enzyme assays included estimation of α -Amylase, protease, and lipase.

α -Amylase activity was estimated using soluble starch as a substrate following Miller (1959). The reaction mixture containing starch solution and enzyme extract was incubated at room temperature for 5 minutes. The reaction was terminated by the addition of 3,5-dinitrosalicylic acid (DNS) reagent and heated in a boiling water bath. After cooling, absorbance was measured at 540 nm using a UV-visible spectrophotometer (Shimadzu UV-1800).

Protease activity was determined using azocasein as a substrate following Garcia-Carreno and Haard (1993). The mixture containing 2.0 mL azocasein solution and 0.5 mL enzyme extract was incubated at room temperature for 5 minutes. The reaction was terminated by adding trichloroacetic acid (TCA), followed by centrifugation at 3,000 rpm for 5 minutes. The absorbance of the clear supernatant was measured at 440 nm using a UV-visible spectrophotometer (Shimadzu UV-1800).

Lipase activity was assayed using p-nitrophenyl acetate as a substrate according to Tsujita et al. (1989). The reaction mixture consisted of 0.1 mL enzyme extract, 0.18 mL p-nitrophenyl acetate, and 1.72 mL sodium phosphate buffer. After incubation at room temperature for 5 minutes, absorbance was recorded using UV-visible spectrophotometer (Shimadzu UV-1800).

Statistical Analysis

Statistical analysis was performed using Jamovi statistical software (version 2.7.0). Differences between control and treated groups were analyzed using one-way analysis of variance (ANOVA) and post hoc comparisons were performed using Tukey's test. A probability level of $p < 0.05$ was considered statistically significant. All experiments were conducted in triplicate, and results were expressed as mean $\pm$ standard deviation (SD).

Results

Acute toxicity (24 h)

The acute toxicity of Cypermethrin against *L. tristis* following 24 h exposure demonstrated a clear concentration dependent increase in mortality. Probit analysis estimated the LD_{25} , LD_{50} , and LD_{90} (Table 1).

Table 1
Lethal dose (LD₂₅, LD₅₀, and LD₉₀) values of Cypermethrin against *L. tristis* after 24 h exposure

Parameter	Estimated Concentration ($\mu\text{g ml}^{-1}$)	95% Confidence Limits (Lower–Upper)
LD ₂₅	3.34	2.87–3.77
LD ₅₀	5.07	4.65–5.52
LD ₉₀	9.51	8.80–10.41

The probit slope (Suppl. Figure 1) showed a gradual increase in mortality with concentration.

Supplementary 1. Linear regression of concentration versus probit values showing a perfect fit between concentration of Cypermethrin and response of *L. tristis*

Pearson's chi-square goodness-of-fit ($\chi^2 = 9.172$; df = 4; p = 0.057) confirmed model fit. The subsequent analysis of the biochemical enzymes gave the following results.

Antioxidant enzymes of whole body, gut, and fat body

Cypermethrin exposure affected enzyme activities, and the changes became clearer with the increase in the exposure period. The activity of SOD showed a gradual decline, and by 96 h it was considerably lower than the control, reaching nearly a 3.5-fold reduction. CAT and POD activities also decreased, but their pattern was not exactly the same at each time point. Some variations were noticed between intervals, indicating that their response was not strictly uniform throughout the exposure period. For glutathione-dependent enzymes, GPx and GR did not initially show much change. However, as time progressed, their activities showed a noticeable decline. GST activity was lower in treated insects at all time intervals, and this reduction remained more or less similar across the exposure duration. AChE activity was also inhibited following treatment, with the reduction becoming more evident at longer exposure periods. Taken together, these results suggest that Cypermethrin disturbs antioxidant enzyme systems and may also interfere with normal neural function in *L. tristis* (Fig. 1).

The gut's antioxidant enzyme activities dropped more than the enzyme activities of the whole body. SOD and CAT activities decreased by about 83% and 55%, respectively, at 96 hours. POD, GPx, GR, and GST also showed a big drop over the exposure periods. Enzymes related to glutathione, especially GR, showed a big drop during the exposure periods. In addition, GST activity steadily decreased in the treated groups when compared to the control. AChE activity was strongly inhibited, especially after longer periods of exposure (Fig. 2).

The fat body of *L. tristis* showed a steady but moderate drop in enzyme activities. Over the course of the exposure periods, the activities of SOD, CAT, and POD showed reduction (Fig. 3).

The activities of GPx and GR also went down, which shows that glutathione-mediated redox regulation was less effective, but not as much as in gut tissues. GST activity steadily decreased over time,

indicating diminished detoxification efficacy. The activity of AChE went down over time, but the level of inhibition was less than those observed in gut tissues.

Digestive enzymes of whole body, gut, and fat body

All digestive enzyme activities in the whole-body homogenates exhibited a gradual decrease with increased duration of exposure. The α -amylase activity, protease activity, and lipase activity showed a steady reduction. These results show that Cypermethrin exposure affects the functioning of important digestive enzymes in *L. tristis* (Fig. 4).

Gut digestive enzymes exhibited a significant reduction following Cypermethrin exposure (Fig. 5).

α -amylase activity was significantly decreased (79%) after 96 hours. Protease activity also dropped sharply, and lipase activity showed considerable reduction, indicating that important digestive enzymes in gut tissues were suppressed over time.

The enzyme activities in the fat body showed a small drop compared to those in the gut (Fig. 6).

Even though the activities of α -amylase, protease, and lipase went down over time, the level of inhibition was much lower when compared to gut tissues.

Discussion

The study gives the finding that Cypermethrin exposure leads to the inhibition of antioxidant, detoxification, and neurophysiological enzyme systems throughout the entire body, including the gut and adipose tissue of *L. tristis*, leading to the initiation of severe oxidative stress. The enzyme inhibition differed across various tissues, indicating a tissue-specific susceptibility pattern. The gut showed the highest drop, followed by the whole body. The lower reduction showed by the fat body than others suggests that they have different physiological roles and are more able to adapt to toxicant stress. These results align with the research indicating that xenobiotic exposure interferes with redox homeostasis in insects, leading to oxidative damage to lipids, proteins, and nucleic acids (Gřešková and Petřivalský 2024; Olufemi-Salami et al. 2024).

We observed a clear decrease in SOD levels - an important antioxidant enzyme that helps remove superoxide radicals, particularly in the gut tissues, which suggests the increased formation of these radicals at the primary entry point for toxins. On the other hand, the fat body showed less inhibition, which might indicate a protective or adaptive response. This could be related to its role in metabolism and detoxification. A similar decline in SOD activity under pesticide stress has also been reported by Jamdhade et al. (2025). In summary, this reduction indicates a change in the balance between reactive oxygen species (ROS) production and antioxidant defenses. This imbalance can lead to oxidative stress and cellular damage (Hong et al. 2024).

Catalase (CAT), involved in the breakdown of hydrogen peroxide into water and oxygen, also showed inhibition in all the tissues studied, with the greatest reduction in the gut. This may be due to the accumulation of hydrogen peroxide and impairment of its removal, thereby increasing oxidative damage at the cellular level. The relatively smaller decline in the fat body further points to its possible role in coping with oxidative stress. Similar observations have been reported by Olaseni et al. (2025) in insects exposed to toxic environmental conditions. The role of ROS in such processes has also been widely discussed (Jomova et al. 2023).

Peroxidase (POD) activity followed a similar pattern, with the highest inhibition recorded in gut tissue. This indicates a reduced capacity to detoxify peroxides under Cypermethrin exposure. The combined reduction in CAT and POD activities suggests a weakening of the primary antioxidant defense system, which in turn limits the organism's ability to regulate reactive oxygen species effectively. This condition reflects an oxidative imbalance, where ROS generation exceeds the available antioxidant capacity. Comparable reductions in peroxidase activity under pesticide stress have also been documented (Wang et al. 2022).

Glutathione-dependent enzymes, including glutathione peroxidase (GPx) and glutathione reductase (GR), also showed reductions with maximum inhibition in the gut, followed by the whole body, while the fat body retained relatively higher activity. As these enzymes play a crucial role in maintaining intracellular redox balance through the glutathione cycle, their reduced activity suggests disruption of this system. Consequently, the ability to neutralize peroxides and free radicals becomes limited. The significance of glutathione-based detoxification has been well established (Vašková et al. 2023), and later studies have shown that impairment of these enzymes reduces the capacity to manage oxidative stress (Chai and Mieyal 2023). Glutathione-S-transferase (GST), another important detoxification enzyme involved in conjugating xenobiotics with glutathione, also showed inhibition, particularly in gut tissues. This reduction suggests reduced detoxification efficiency and a higher likelihood of toxicant accumulation. Similar reductions in GST activity under pesticide exposure have been reported in insects (Xu et al. 2025). Acetylcholinesterase (AChE), essential for nerve impulse transmission, was also inhibited in all tissues, with the greatest suppression again in the gut. This supports the neurotoxic nature of Cypermethrin, especially in tissues with direct exposure. Inhibition of AChE leads to the accumulation of acetylcholine at synapses, resulting in continuous nerve stimulation and eventual neuromuscular dysfunction. Such effects have been widely reported in pesticide-exposed organisms (Choi et al. 2024). These tissue-specific responses indicate that Cypermethrin persistence in plantation environments disrupts redox homeostasis, thereby increasing the pollution risks to detritivores, the main players in soil biogeochemical cycles.

The higher sensitivity of gut tissues may be due to their direct exposure to ingested toxicants and their high metabolic activity, which may increase ROS generation and oxidative damage, as evidenced by intestinal epithelial injury and oxidative stress responses reported in insects exposed to β -cypermethrin (Xu et al. 2024). On the other hand, the fat body showed relatively lower levels of enzyme inhibition, probably due to its central role in detoxification, storage, and metabolic regulation. Its ability to partially

manage oxidative stress has been discussed by Skowronek et al. (2021). Gut inhibition also indicates bioaccumulation through foliar residues, which in turn impair nutrient cycling in rubber litter ecosystems.

In short, the findings of the study indicate that Cypermethrin disrupts redox balance, detoxification pathways, and neural function in a tissue-specific manner in *L. tristis*. The higher susceptibility of gut tissues highlights their role as the primary site of toxicant-induced damage, while the comparatively stable response of the fat body suggests a certain level of adaptive capacity. These observations contribute to a better understanding of oxidative stress-related toxicity and support the use of antioxidant enzymes as reliable biomarkers of environmental stress in insects (Yang et al. 2025; Xiao et al. 2025).

The observations revealed a steady reduction in digestive enzyme activities with increasing duration of Cypermethrin exposure. The reduction in activities of α -amylase, protease, and lipase decreased in the whole body, gut, and fat body of *L. tristis*, indicating interference with normal nutrient metabolism. The reduction of α -amylase indicated less effectiveness in carbohydrate digestion, while protease and lipase activity reduction indicated low protein breaking efficiency and low lipid metabolism, respectively. However, the degree of reduction was not constant across tissues, with the gut exhibiting the highest decline, followed by the whole body, while the fat body showing relatively less suppression. This trend suggests that there is variation in tissue-specific sensitivity to toxic stress. Similar modifications in digestive enzyme activity under pesticide exposure have been commonly reported and are linked to disruptions in physiological and metabolic processes in insects (Liu et al. 2023; de Souza et al. 2025). α -amylase activity declined in all tissues, with the most pronounced reduction in the gut. As this enzyme plays a key role in carbohydrate digestion and is mainly active in the midgut, where complex carbohydrates are broken down into simple sugars (Bai et al. 2026), its reduction reflects impaired carbohydrate metabolism. This may result from damage to the midgut epithelial lining or decreased enzyme production under toxic stress. Comparable decreases in amylase activity have been observed in pesticide-treated insects (Chen et al. 2025; El-Morshedy et al. 2026). Protease activity followed a similar declining pattern, again showing the highest inhibition in the gut. Proteases are essential for protein digestion and supply amino acids required for various metabolic functions. Due to their sensitivity to environmental stress, their disruption can negatively impact insect growth and adaptability (Chamani et al. 2025). The significant inhibition observed may indicate structural and functional impairment of midgut cells responsible for enzyme secretion. Lipase activity also decreased progressively with longer exposure, suggesting disturbances in lipid digestion and energy metabolism. Since lipases break down lipids into fatty acids and glycerol, reduced activity can directly affect energy production and cellular functions. These enzymes are known to be particularly sensitive to chemical stress (Zhou et al. 2025).

The more substantial reduction in digestive enzyme activity within the gut can be attributed to its direct contact with Cypermethrin through ingestion, as well as its primary role in digestion and absorption. The insect gut also supports complex interactions between digestive enzymes and microbial communities, both of which are essential for proper digestion and detoxification. Disruption of these interactions by pesticides can lead to decreased enzyme activity and metabolic imbalance (Siddiqui et al. 2022). In

contrast, the fat body showed relatively moderate reductions, likely due to its main roles in storage, intermediary metabolism, and detoxification rather than direct participation in digestion. Its comparatively stable response to toxic stress has been noted in earlier studies (Nere et al. 2025; Staniszewska and Strachecka 2025). The overall decrease in whole-body enzyme activity likely reflects the combined effect of these tissue-specific responses, particularly the strong inhibition observed in the gut. Taken together, the decline in digestive enzyme activity suggests that Cypermethrin not only induces oxidative stress but also disrupts nutrient utilization and metabolic homeostasis. Such disturbances can ultimately affect growth, development, and survival, considering the crucial role of digestive enzymes in insect physiology (Bai et al. 2026; El-Morshedy et al. 2026).

The biochemical and physiological responses observed also point to potential ecological implications for *L. tristis*. The reduction in both antioxidant and digestive enzyme activities indicates that the insect's capacity to cope with stress and efficiently process nutrients is compromised. Since these processes are vital for growth, survival, and reproduction, their disruption may lead to reduced overall fitness. In particular, decreased activities of α -amylase, protease, and lipase are likely to impair feeding efficiency and nutrient assimilation, resulting in reduced energy availability and delayed development. At the same time, lower antioxidant enzyme activity may increase vulnerability to oxidative stress, thereby affecting cellular stability. These effects may extend beyond individual insects and influence population and ecosystem dynamics. Reduced energy reserves and increased stress conditions could lead to decreased reproductive success, a decline in population size, and potential alterations in population structure over time. Furthermore, *L. tristis* plays an important role in decomposition and nutrient cycling within its ecosystem. Any reduction in its population or functional efficiency may therefore have indirect consequences on soil quality and energy flow.

It is also important to consider that sublethal effects of Cypermethrin, such as disturbances in metabolism and enzyme function, may not cause immediate mortality but can progressively weaken individuals. This weakened state may increase their susceptibility to environmental stressors and predation. Overall, the findings suggest that the impact of Cypermethrin extends beyond direct toxicity, affecting not only individual fitness but also population stability and broader ecological processes.

Conclusion

This study presents the first comprehensive, time-dependent assessment of Cypermethrin induced sublethal biochemical alterations in *L. tristis*, integrating antioxidant, digestive, detoxification, and neural enzyme systems across multiple tissues. Unlike previous research that primarily addressed acute toxicity or individual enzyme responses, our findings demonstrate that prolonged sublethal exposure (0–96 h) causes coordinated and progressive suppression across interconnected physiological pathways. Antioxidant enzymes (SOD, CAT, and POD) displayed temporal decline, an indication of oxidative stress overload. Digestive enzymes (α -amylase, protease, and lipase) were consistently inhibited, reflecting impaired nutrient metabolism and energy imbalance. Detoxification capacity, evidenced by reduced GST activity, was progressively compromised, while suppression of AChE activity revealed concurrent

neurophysiological disturbances. Collectively, these results highlight a systemic mode of biochemical impairment rather than isolated enzymatic effects. Although this study offers important insights regarding pesticide exposure, it has a few limitations. The analysis was primarily restricted to biochemical and enzymatic parameters and thus may not encompass the full range of physiological responses in *L. tristis* under Cypermethrin exposure. Furthermore, as the experiments were conducted under controlled laboratory conditions, the findings may not completely represent natural environmental situations, where organisms are subjected to multiple interacting stressors. In addition, the study focused on short-term exposure, and the potential impacts of long-term or chronic exposure remain to be investigated. Cypermethrin causes tissue-specific biochemical toxicity in *L. tristis*, thus highlighting the need for multi-biomarker monitoring in pyrethroid-polluted plantation landscapes so as to mitigate the effects on non-target organisms and thereby supporting Integrated Pest Management. Future research should therefore aim to adopt a more comprehensive approach by including long-term assessments and broader ecological perspectives, which would provide a deeper understanding of how pesticide exposure affects insect survival, adaptation, and ecological fitness.

Declarations

Ethical Approval

All authors have read, understood, and have complied as applicable with the statement on "Ethical responsibilities

of Authors" as found in the Instructions for Authors.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors have no competing interests to declare.

Clinical trial number

Not applicable

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Authors' contributions

Sajini S Menon has collected the insect samples, analysed the samples, and written the manuscript and interpretation. Lakshmi Gopakumar has supervised the manuscript writing: framed the manuscript structure, data analysis and interpretation, guided the writing, reviewed, edited, and corrected the manuscript for publication. Vijesh Varghese has guided the lab work, and Rajesh Kumar Sivakumar has supervised Sajini S Menon.

Availability of data and materials

Data will be made available on reasonable request.

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Figures

Figure 1

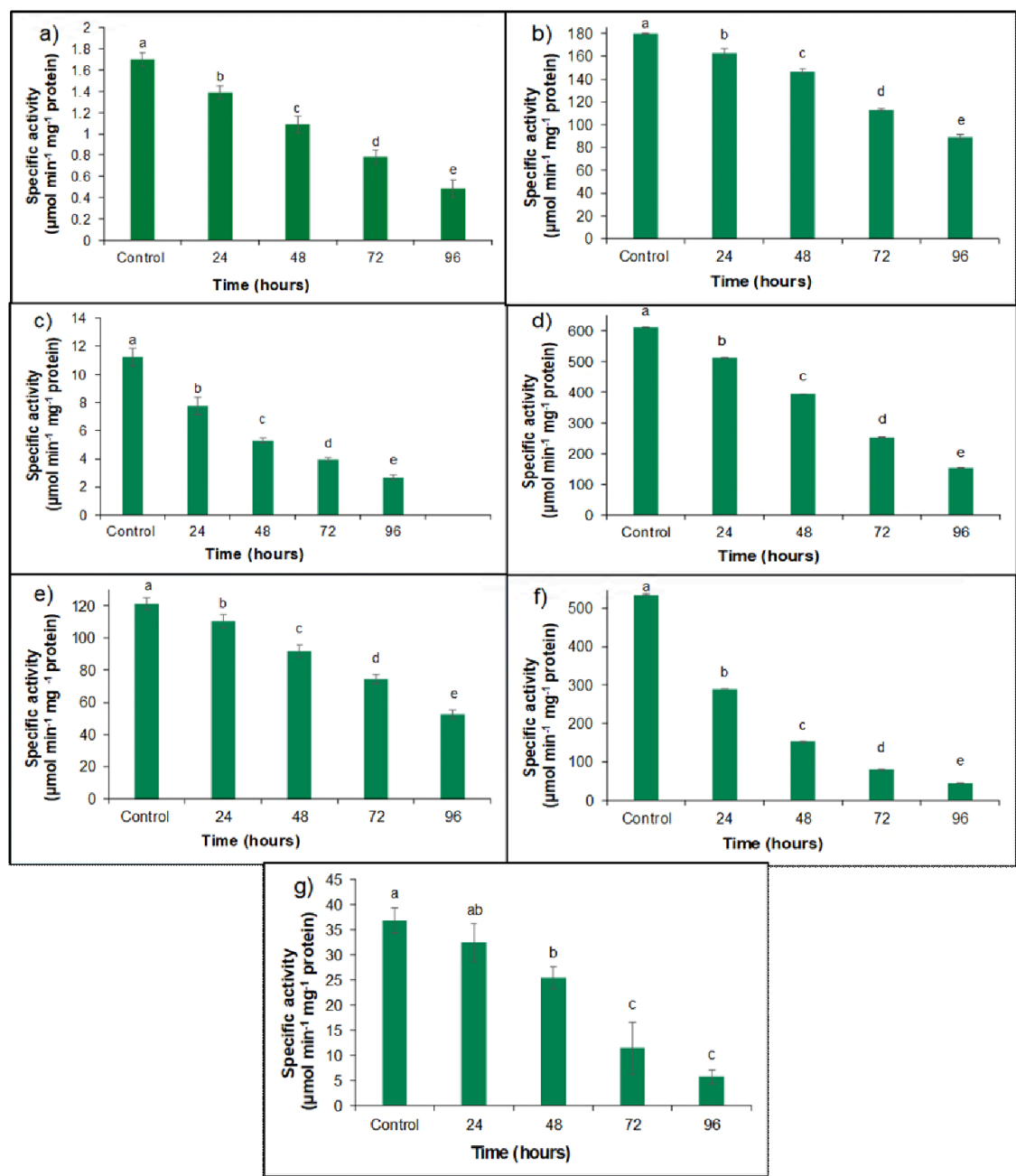


Figure 1

Antioxidant enzyme activities [a) SOD, b) CAT, c) POD, d) GR, e) GPx, f) GST, g) AChE] in the whole body of *L. tristis* following Cypermethrin exposure. Values are expressed as mean $\pm$ SD (n = 3). Lowercase letters above the bars indicate significant differences at $p < 0.05$.

Figure 2

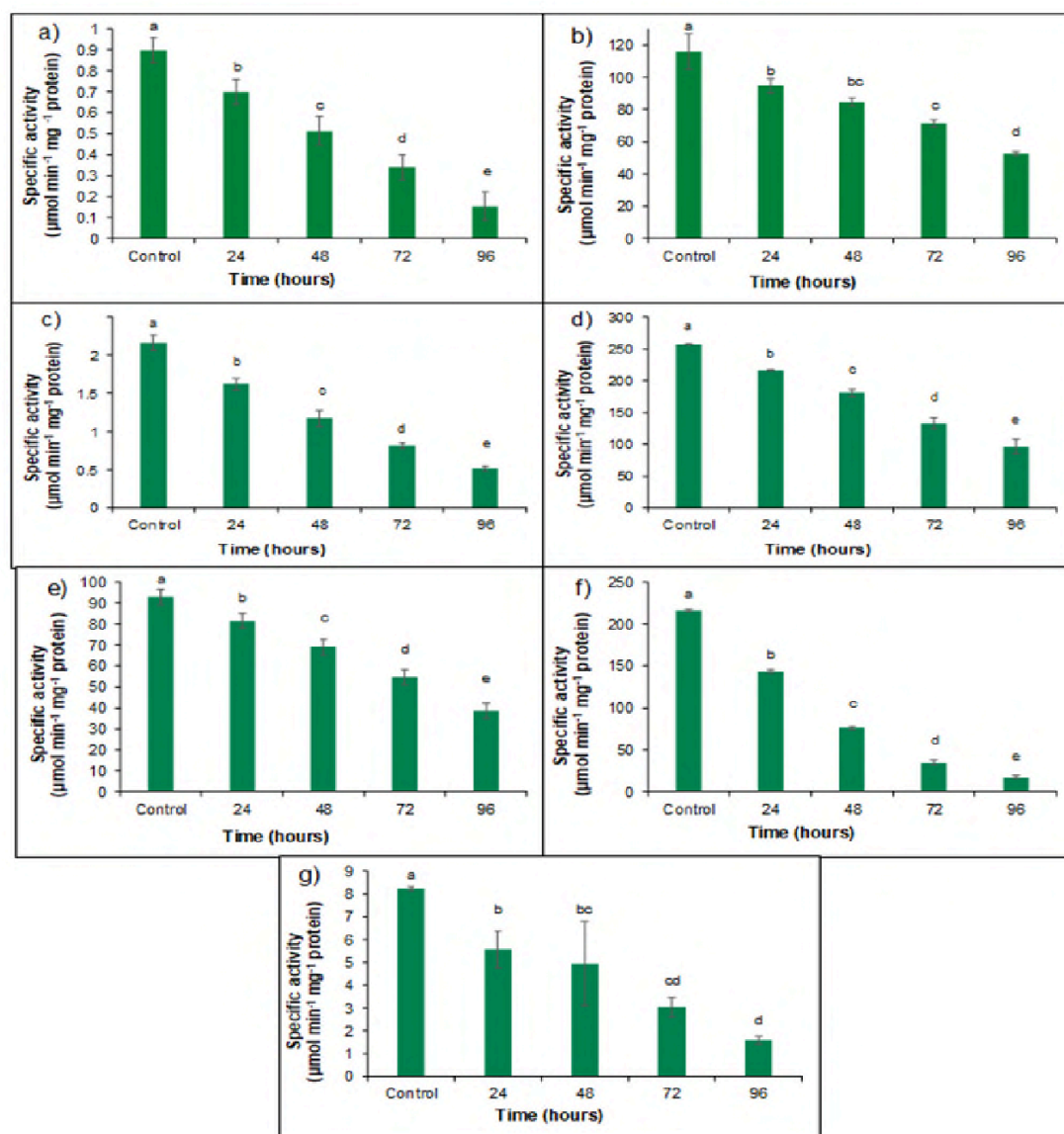


Figure 2

Antioxidant enzyme activities [a) SOD, b) CAT, c) POD, d) GR, e) GPx f) GST g) AChE] in the gut of *L. tristis* following Cypermethrin exposure. Values are expressed as mean $\pm$ SD ($n = 3$). Lowercase letters above the bars indicate significant differences at $p < 0.05$

Figure 3

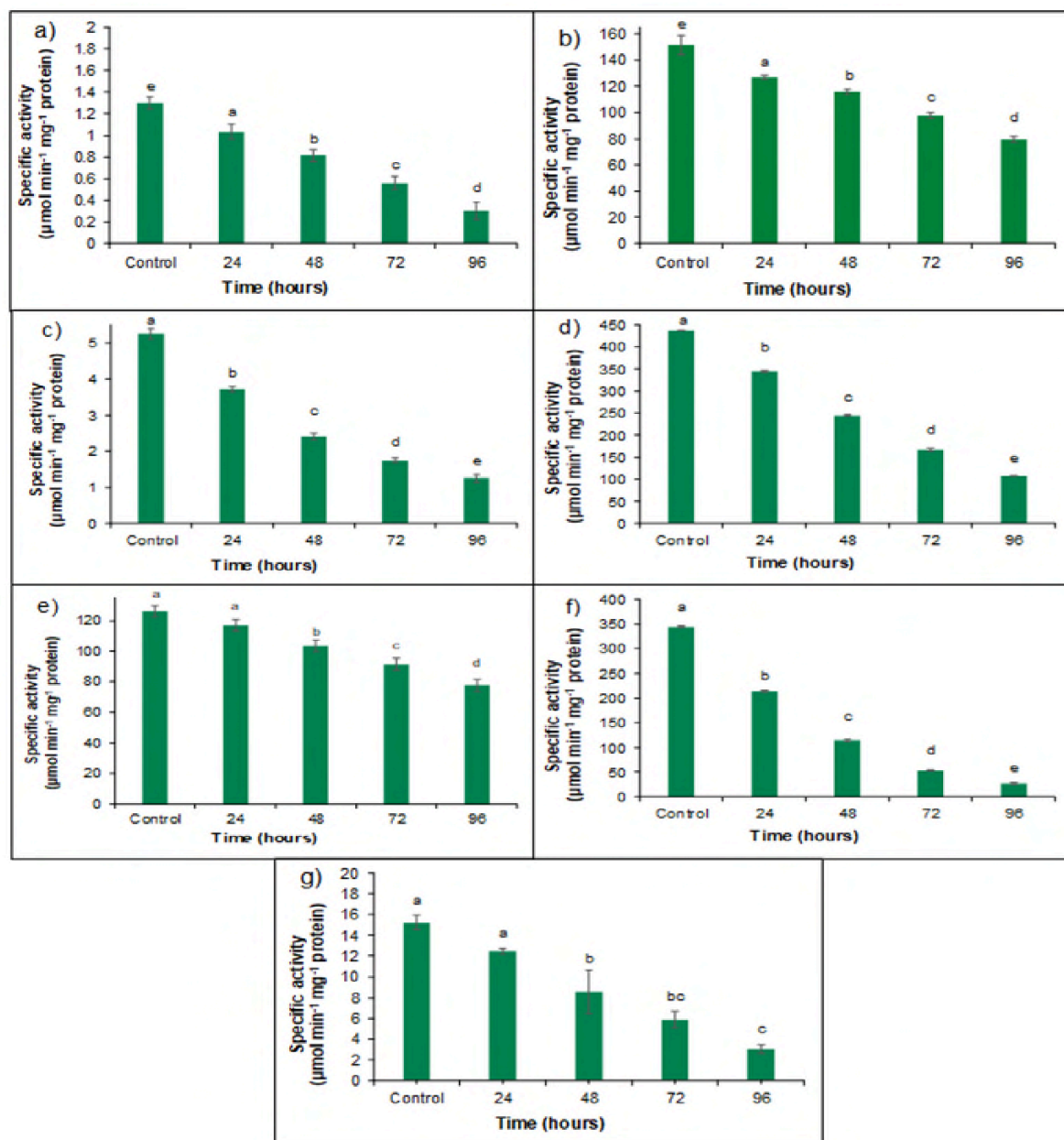


Figure 3

Antioxidant enzyme activities [a) SOD, b) CAT, c) POD, d) GR, e) GPx f) GST g) AChE] in the fat body of *L. tristis* following Cypermethrin exposure. Values are expressed as mean $\pm$ SD (n = 3). Lowercase letters above the bars indicate significant differences at p < 0.05.

Figure 4

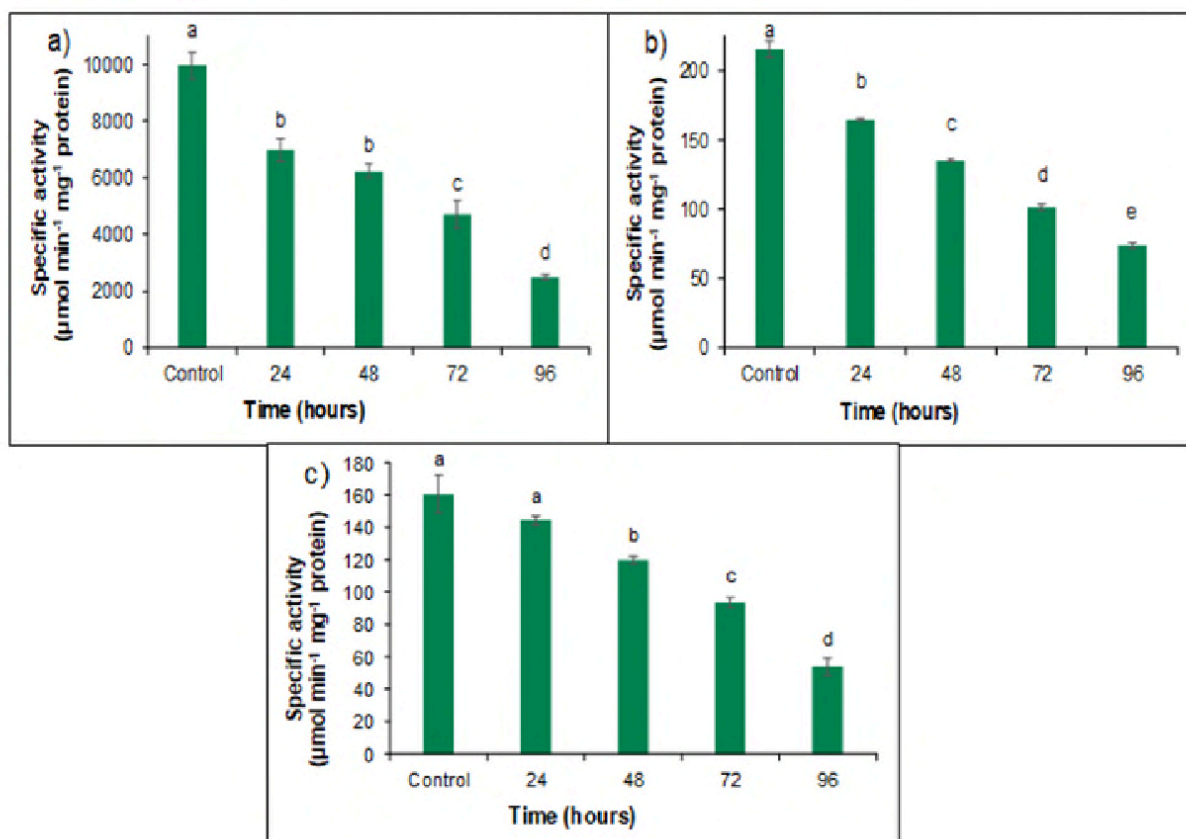


Figure 4

Digestive enzyme activities in the whole body of *L. tristis* following Cypermethrin exposure (a- α -amylase, b-protease, c- lipase). Values are expressed as mean $\pm$ SD (n = 3). Lowercase letters above the bars indicate significant differences at $p < 0.05$.

Figure 5

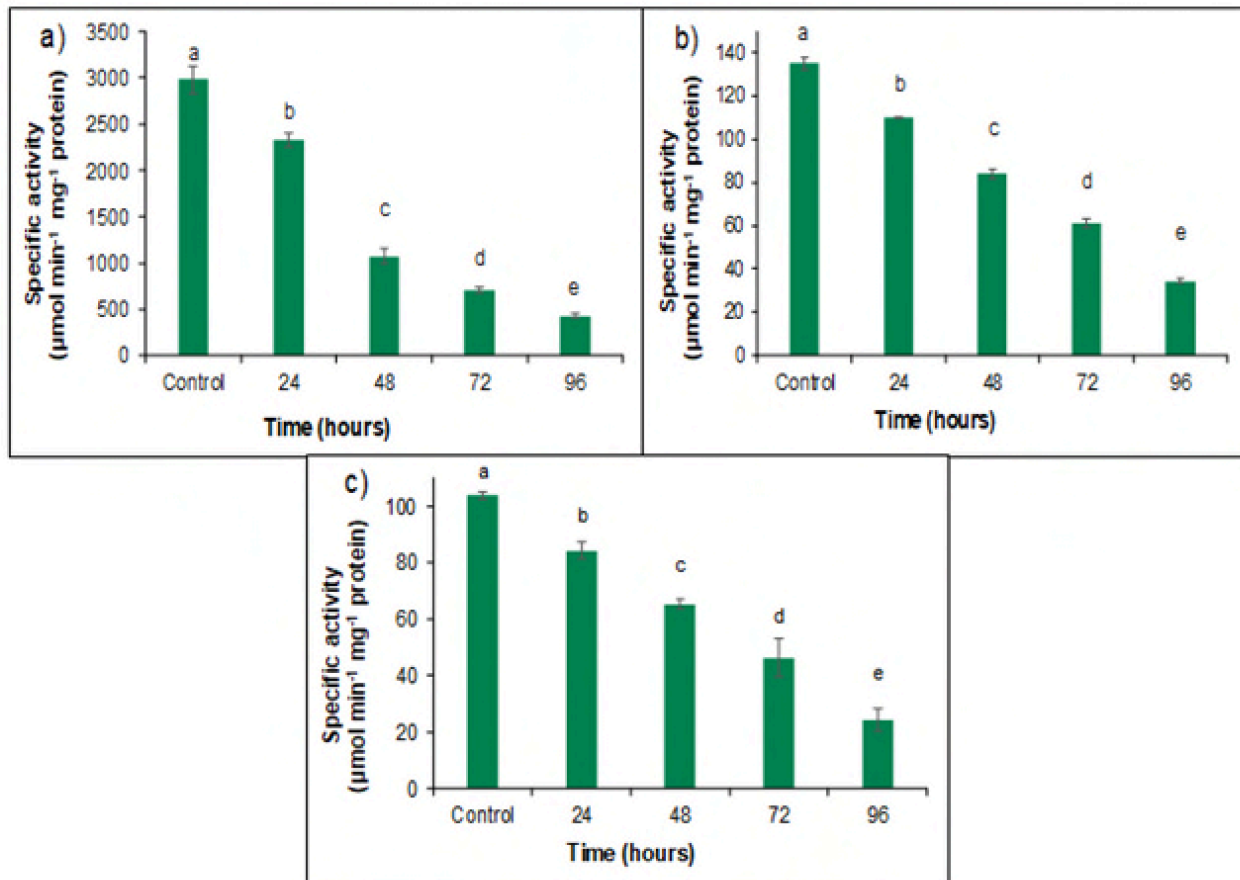


Figure 5

Digestive enzyme activities in the gut of *L. tristis* following Cypermethrin exposure (a- α-amylase, b- protease, c- lipase). Values are expressed as mean ± SD (n = 3). Lowercase letters above the bars indicate significant differences at p < 0.05.

Figure 6

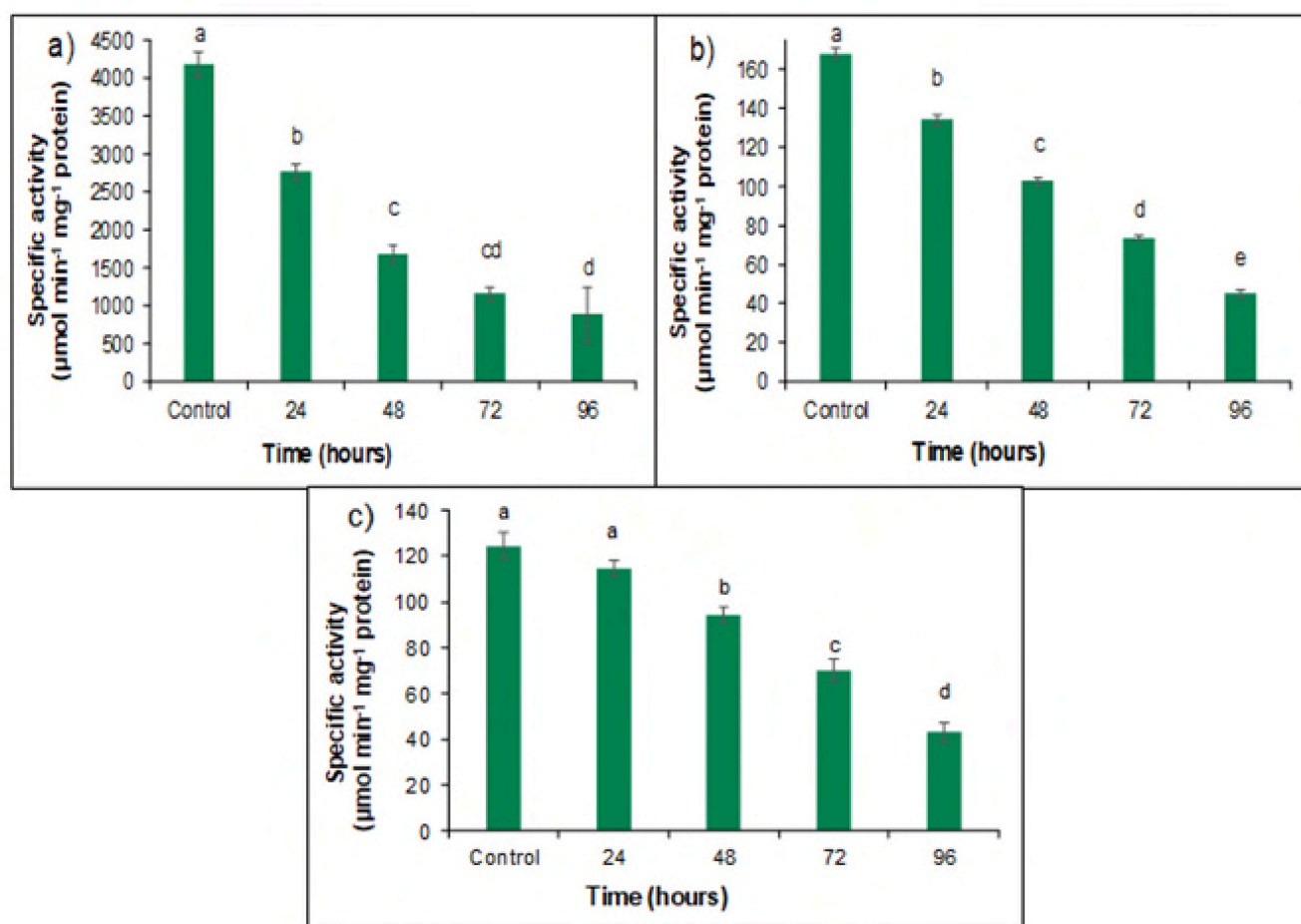


Figure 6

Digestive enzyme activities in the fat body of *L. tristis* following Cypermethrin exposure (a- α-amylase, b- protease, c- lipase). Values are expressed as mean ± SD (n = 3). Lowercase letters above the bars indicate significant differences at p < 0.05.

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