



Original Research Paper

Sublethal Cypermethrin Toxicity in *Apis mellifera*: Integrated Biochemical, Oxidative Stress, Neurophysiological and Genotoxic Responses

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Abstract

The extensive use of synthetic pyrethroid insecticides in farming practices have ratcheted up the risk of unintentional exposure to invaluable pollinators. The current experiment explored the influence of cypermethrin on adult worker honeybees using a comprehensive approach consisting of acute toxicity, biochemical analyses, enzymatic assessments, neurophysiological examinations, and genetic analysis. The first step was to determine acute toxicity in adult bees via increasing dosages of cypermethrin. Probit method revealed a confidence interval between 8.18mg/L, the lethal concentration at 50 percent for the test organisms, ranging from 7.54–8.87 mg/L. Following that, sub-lethal doses led to biochemical changes in the tissue homogenate. Glucose levels before treatment was measured to be 86.42 mg/dl but dropped to 58.91 mg/dl at the highest concentration of cypermethrin. Total protein showed decreased values, from 61.84 mg/g of tissue to 40.52 mg/g at the lethal concentration. Alkaline phosphatase activity decreased as treatment time increased along with acid phosphatase activity, demonstrating an interference with metabolism and engulfment of membranes through lysosomal processes was initiated. The treatment also impacted lipid metabolism and caused an increase in cholesterol. Acetylcholinesterase activity decreased from an average of 8.94 $\mu\text{mol}/\text{min}/\text{mg}$ of protein to 4.83 $\mu\text{mol}/\text{min}/\text{mg}$ of protein showing that neurophysiology was also compromised. Antioxidation was decreased as the dosage increased with reductions in catalase, glutathione, and all other antioxidants. The increase in DNA damage was demonstrated by a number of comet parameters: comet length, the percentage of DNA in the comet, Olive tail moment, and the tail moment. These results conclude that the impact of cypermethrin in honeybees goes far beyond mortality to include various biochemical, physiological, and molecular processes. These biophysiological parameters can be helpful to monitor the stress caused by exposure to sub-lethal levels of pesticide in honey bees.

Keywords: *Apis mellifera*; cypermethrin; pyrethroid; biochemical biomarkers; oxidative stress; acetylcholinesterase; antioxidant defence; genotoxicity; honey bee

1. Introduction

Bees (*Apis mellifera*) are some of the most essential managed pollinators and play a huge role in the functioning of both agricultural and natural ecosystems. Bees facilitate plant reproduction, the functioning of ecosystems, and agricultural productivity as well (Klein *et al.*, 2007, Mittal *et al.*, 2021). Beekeeping also produces economically important products such as honey, beeswax, pollen, propolis, and royal jelly. In this regard, honey bee health-related issues can affect not only individual colonies but also agricultural production systems and processes within the environment.

Pollinators always face various factors that may affect them, such as habitat degradation, lack of nutritional resources, infectious diseases, and agricultural chemicals among other. Pesticide use is

of special concern because of the chance that honey bees collect pesticide residue while collecting nectar, pollen, water, and other things from the agricultural landscape (Potts *et al.*, 2010; Goulson *et al.*, 2015, Kumar, 2026).

Synthetic pyrethroids are widely used due to their fast insecticidal activity, broad range of effectiveness, and comparatively low toxicity in mammals. Cypermethrin is an effective Type II synthetic pyrethroid used predominantly to control many agricultural and household pests (Desneux *et al.*, 2007, Kumar & Maheshwari, 2025). The primary neurotoxic effect of pyrethroids is through action on the voltage-gated sodium channels. The alteration in kinetics of the sodium channels prolongs the influx of sodium as well as the excitation of neurons and may lead to hyperexcitability, tremors, incoordination, and

ultimately, paralysis and death (Soderlund *et al.*, 2002; Soderlund, 2012). Honey bees are at risk of pesticide exposure due to differences in their capacity for detoxification as compared with other groups of insects (Johnson *et al.*, 2010, Singh *et al.*, 2026). Routine assessments for the toxicity of pesticides have been dependent on assessment endpoints marked by mortality (i.e., LC₅₀ and other mortality measures). It is important to note that while mortality is necessary for defining acute toxicity, it does not correctly characterize the physiological effects of exposure at concentrations where death does not immediately occur. Sublethal exposure to pesticides can affect feeding, locomotion, navigation, learning, metabolism, and antioxidant regulation long before mortality is evident (Desneux *et al.*, 2007). The use of biochemical markers makes it possible to identify physiological changes due to toxic exposure. The changes in carbohydrates and proteins provide information on the disruption of energy metabolism and homeostasis of macromolecules whereas phosphatases provide data with respect to the disturbance of lysosomes and membranes. The activity of Acetylcholinesterase (AChE) determines the neurophysiological condition of the organism while the antioxidant markers (SOD, CAT, and GSH) communicate the ability of the cells to combat reactive oxygen species.

Oxidative stress adds more weight to the toxicity of the pesticides. When large amounts of reactive oxygen species are produced, the host antioxidant defenses are overwhelmed, leading to oxidative modification of proteins, nucleic acids, and membranes (Lushchak, 2011; Halliwell & Gutteridge, 2015). Oxidative damage can lead to the breaking of DNA resulting in genomic instability. The use of biochemical markers in addition to antioxidant markers and molecular markers provides more information on the stress induced by pesticides above and beyond a simple measure of mortality. Therefore, this study was undertaken to evaluate the effects of cypermethrin on adult worker *Apis mellifera* using the integrated biomarker approach. The acute toxicity of cypermethrin was established to provide information on its LC₅₀ and level of sublethal exposure. Measurements were also made for total protein, glucose, alkaline phosphatase, acid phosphatase, cholesterol, acetylcholinesterase, SOD, CAT, GSH, and DNA damage analysis. This integrated method was used to evaluate the relationship between the increasing exposure level of cypermethrin with the metabolic, neurophysiological, oxidative, and molecular changes in the honey bees.

2. Materials and Methods

2.1 Experimental organism and study design

The adult worker honeybees (*Apis mellifera*) were chosen as experimental organisms. The experiment adopted a completely randomized design (CRD). Four experimental groups consisting of untreated controls, and cypermethrin-treated groups were used. Three replicates were used in the biochemical studies. The study had two main phases; the acute toxicity phase which was done to determine the concentration-mortality relationships and median lethal concentration and the sublethal cypermethrin toxicity which consisted of biochemical, enzymatic, antioxidant and molecular biomarkers assessment.

2.2 Acute toxicity assessment

Adult healthy worker bees were given cypermethrin in a solution of 50% sugar at concentrations of 0.00, 2.50, 5.00, 7.50, 10.00, 12.50 and 15.00 mg/L. Each group received 30 worker bees. This study monitored the mortality level of the test subjects at the end of the exposure period. Mortality rate was adjusted using Abbott's formula and toxicological data were analyzed using probit analysis according to the toxicological method introduced by Finney. LC₁₀, LC₂₅, LC₅₀ and LC₉₀ were calculated.

2.3 Selection of sublethal exposure levels

Acute toxicity studies were used to establish the toxicology basis of sublethal exposure conditions chosen for further experiments. Similar to that, biochemical, antioxidant and molecular investigations were categorized into four experimental groups i.e control, low dose, medium dose and high dose which were used to test for concentration specific physiological responses apart from death.

2.4 Biochemical assessment

The biochemical evaluation made use of glucose, total protein as well as cholesterol. Glucose was used as the marker of carbohydrate/energy metabolism, while total protein was studied as the representative of protein homeostasis and general metabolic state.

The adult worker bees were homogenized, and biochemical assays were performed using the homogenate as described in the experimental section. Values were presented as mean $\pm$ standard deviation.

2.5 Phosphatase enzyme assessment

Activity levels of alkaline phosphatase (ALP) and acid phosphatase (ACP) were measured in order to assess changes that occurred in terms of membrane metabolism and lysosomal metabolism, respectively. ALP values were reported in IU/L and ACP was evaluated across the control, low-, medium- and high-dose treatment groups.

2.6 Acetylcholinesterase activity

We examined the activity of AChE as a biochemical marker of neurophysiological damage. AChE plays a critical role in stopping cholinergic signaling by hydrolyzing acetylcholine. The

activity of the enzyme was defined in $\mu\text{mol}/\text{min}/\text{mg}$ of protein. The predominant method for the quick measurement of AChE activity is based on the work of Ellman *et al.* (1961).

2.7 Antioxidant biomarkers

The antioxidant defense system was assessed through the use of SOD, CAT and GSH. SOD provides the first line of enzymatic defense against superoxide radicals while catalase catalyzes the conversion of the hydrogen peroxide into water and oxygen. Reduced glutathione plays a crucial role in intracellular redox homeostasis and in providing cellular protection against oxidative damage. The determination of catalase activity is done based on the biochemical principle provided by Aebi (1984), while the results from SOD and oxidative stress tests were interpreted based on available antioxidant literature (McCord & Fridovich, 1969; Fridovich, 1995; Halliwell & Gutteridge, 2015).

2.8 Assessment of DNA damage

DNA damage assessments were conducted through comet assay evaluation. Parameters evaluated on a molecular level were the tail length, % tail DNA, tail moment, and the Olive tail moment. Increasing levels of each of these parameters were interpreted as increasing damage of the strand of DNA and genomic instability. The comet assay approach to evaluation was interpreted based upon accepted principles for evaluating a single cell's DNA damage (Singh *et al.*, 1988; Tice *et al.*, 2000; Collins, 2004).

2.9 Statistical analysis

The biochemical and enzymatic data were recorded as mean $\pm$ SD, which was done in triplicate. The one-way ANOVA (analysis of variance) method has been used to determine the statistical significance of the differences between the control and low-/medium-/high-treatment groups, followed by the Tukey's HSD (honestly significant difference) multiple comparisons test. The results of the analysis were considered statistically significant at $p < 0.05$ and therefore, treatment means have been indicated with different superscript letters.

The statistical analyses were conducted with the help of IBM SPSS Statistics 26.0 for the experimental results, while acute toxicity analysis utilized Probit analysis.

3. Results

3.1 Acute toxicity and concentration–mortality relationship

Cypermethrin resulted in a significant mortality rate that increased with the concentration of the chemical. The untreated control had 0% mortality. At 2.50 mg/L cypermethrin, the mortality rate rose to 13.33% followed by 26.67% and 46.67% at 5.00 and 7.50 mg/L, respectively. Higher concentrations caused even larger numbers of deaths (63.33, 80.00 and 93.33% at 10.00, 12.50 and 15.00 mg/L cypermethrin).

Table 1. Acute toxicity of cypermethrin to adult worker *Apis mellifera*

Cypermethrin (mg/L)	Bees exposed	Dead bees	Mortality (%)
0.00	30	0	0.00
2.50	30	4	13.33
5.00	30	8	26.67
7.50	30	14	46.67
10.00	30	19	63.33
12.50	30	24	80.00
15.00	30	28	93.33

The results of probit analysis showed that the LC_{10} value was 3.12 mg/L, the LC_{25} value was 5.46 mg/L, the LC_{50} value was 8.18 mg/L, and the LC_{90} value was 14.72 mg/L. The 95% confidence limit for LC_{50} was found to be in the range of 7.54 mg/L to 8.87 mg/L. The regression equation was found to be $Y = 2.41 + 2.37X$, and the value of χ^2 was 2.31 which was within the acceptable limit of $p > 0.05$. The mortality effect was found to be associated with a gradual increase of behavioural effects. Initially, lower dosage led to a decrease in activity level along with some minor changes in feeding. But with an increase of concentration of exposure there were observed wing tremors, problems with coordination, and lethargy. At much higher dosages hyperactivity followed by loss of balance, inability to fly, paralysis and death.

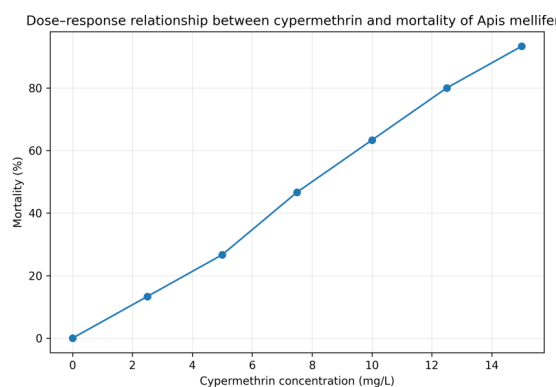


Figure 1. Dose–response relationship between cypermethrin concentration and mortality of adult worker *Apis mellifera*

The illustration indicates a clear concentration-dependent increase in the rate of mortality among adult worker honeybees of the species *Apis mellifera* associated with the level of exposure to cypermethrin. There was no occurrence of mortality in the control treatment groups; however, the mortality rate showed a progressive increase from 13.33% at a concentration of 2.50 mg/L to 93.33% at 15.00 mg/L. The high level of increase in the rate of mortality recorded at the higher concentration is an indication of the severe acute toxic effect of cypermethrin on honeybees. The probit analysis resulted in an LC_{50} value of 8.18

mg/L, confirming the fact that adult workers are very sensitive to the insecticide cypermethrin. The concentration–response relationship serves to provide the basis for the selection of sublethal concentrations for further biochemical, antioxidant, and molecular studies.

3.2 Effect on glucose metabolism

Cypermethrin significantly reduced glucose concentration in a dose-dependent manner.

Table 2. Glucose concentration following cypermethrin exposure

Treatment	Glucose (mg/dL; Mean $\pm$ SD)	Statistical group
Control	86.42 $\pm$ 3.18	a
Low dose	78.65 $\pm$ 2.94	b
Medium dose	69.83 $\pm$ 2.76	c
High dose	58.91 $\pm$ 3.05	d

The one-way ANOVA showed that the effect of the treatment was very significant ($F=74.91$; $p<0.001$). The glucose levels in the high-dose group were significantly less than those in the controls, with about 31.8% less glucose present in the high-dose group. Moreover, with the increased time period elapsed, this percentage started to decline more and more because of the process of disturbance occurring in carbohydrate metabolism and depletion of energy reserves.

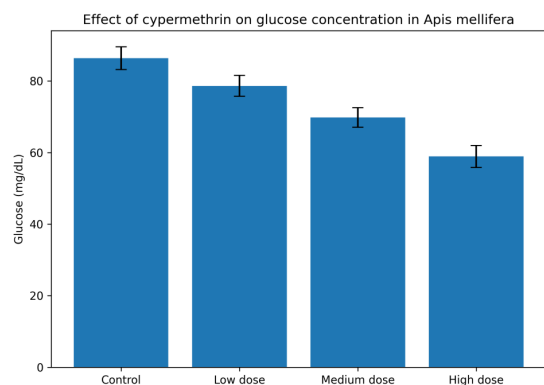


Figure 2. Effect of sublethal cypermethrin exposure on glucose concentration in adult worker *Apis mellifera*

The use of cypermethrin reduced glucose levels progressively from 86.42 $\pm$ 3.18 mg/dL in controls to 58.91 $\pm$ 3.05 mg/dL in the high-dose group while the low- and mid-dose groups attained a glucose concentration of 78.65 $\pm$ 2.94 mg/dL and 69.83 $\pm$ 2.76 mg/dL, respectively. Results from one-way ANOVA show that the treatment had a highly significant effect ($F = 74.91$, $p < 0.001$). Though there was a 31.8% decline from the control group to the high-dose group, it suggested some degree of disruption to carbohydrate metabolism. It is possible that the increase in glucose used when cypermethrin is applied could be attributed to

increased energy demands for detoxification and stress management.

3.3 Effect on total protein

Total protein similarly showed a progressive decline.

Table 3. Total protein concentration in *A. mellifera*

Treatment	Total protein (mg/g tissue; Mean $\pm$ SD)	Statistical group
Control	61.84 $\pm$ 2.26	a
Low dose	55.71 $\pm$ 2.08	b
Medium dose	48.63 $\pm$ 1.95	c
High dose	40.52 $\pm$ 2.11	d

The results of ANOVA revealed that there were differences in disorders caused by treatment ($F = 62.87$; $p < 0.001$). The concentration of treatment was the primary factor affecting the level of protein metabolism disturbance.

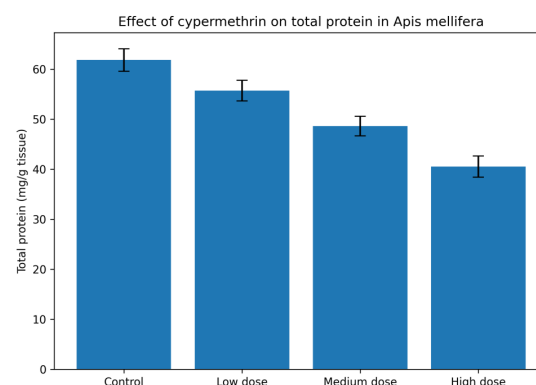


Figure 3. Effect of sublethal cypermethrin exposure on total protein content in adult worker *Apis mellifera*

The level of total protein was consistently lower as the degree of cypermethrin exposure increased. The control group had total protein levels of 61.84 $\pm$ 2.26 mg/g of tissue, whereas the low-, medium-, and high-dose treatment groups had total protein levels of 55.71 $\pm$ 2.08, 48.63 $\pm$ 1.95, and 40.52 $\pm$ 2.11 mg/g of tissue, respectively. The difference in total protein levels between treatment groups was highly significant ($F = 62.87$, $p < 0.001$). The decrease indicates that protein metabolism is likely impaired with increased cypermethrin levels and may be due to increased proteolysis and inhibited protein synthesis, and/or oxidative alteration of cellular proteins. Because proteins have structural, enzymatic, and regulatory functions, the decrease in total protein levels can be considered a sensitive indicator of the worsening physiological condition due to pesticide exposure.

3.4 Alkaline phosphatase activity

ALP activity declined markedly with increasing cypermethrin exposure.

Table 4. Effect of cypermethrin on ALP activity

Treatment	ALP (IU/L; Mean $\pm$ SD)	Statistical group
Control	148.62 $\pm$ 4.21	a
Low dose	136.48 $\pm$ 3.82	b
Medium dose	121.57 $\pm$ 3.46	c
High dose	102.34 $\pm$ 3.91	d

The treatment effect produced a statistically significant result ($F = 89.42$; $p < 0.001$). The continuing decline in ALP indicates a continuing disruption of membrane-associated enzymatic function and phosphate metabolism.

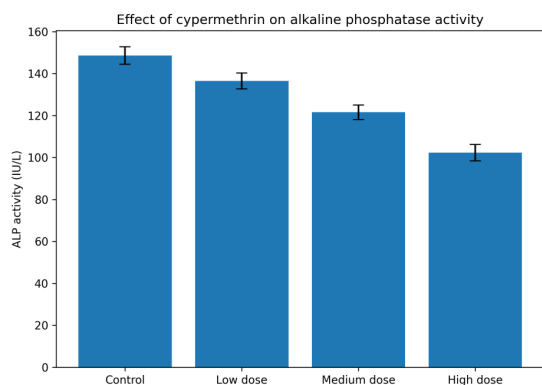


Figure 4. Effect of sublethal cypermethrin exposure on alkaline phosphatase (ALP) activity in adult worker *Apis mellifera*

A significant decline in ALP activity was demonstrated in a dose-dependent manner after exposure to cypermethrin. Activity decreased from control values of 148.62 ± 4.21 IU/L to 136.48 ± 3.82 , 121.57 ± 3.46 , and 102.34 ± 3.91 IU/L for the low, medium, and high dose groups, respectively. A one-way ANOVA showed significant differences between treatments ($F = 89.42$, $p < 0.001$). Since ALP is involved in the metabolism of phosphates, nutrient transport, and membrane-dependent cellular processes its steady decline indicates increasing levels of dysfunction in metabolism and cell membranes. These results provide evidence of the usefulness of ALP as a sensitive biochemical biomarker of cypermethrin-induced cellular distress in honeybees.

3.5 Acid phosphatase activity

In contrast to ALP, ACP activity increased with cypermethrin concentration.

Table 5. Effect on ACP activity

Treatment	ACP (IU/L; Mean $\pm$ SD)	Statistical group
Control	28.46 $\pm$ 1.21	a
Low dose	35.84 $\pm$ 1.46	b
Medium dose	44.92 $\pm$ 1.68	c
High dose	56.73 $\pm$ 2.05	d

The treatment effect was significant ($F = 98.37$; $p < 0.001$). The increase in ACP is consistent with the progressive lysosomal activation and intracellular decomposition associated with tissue injury.

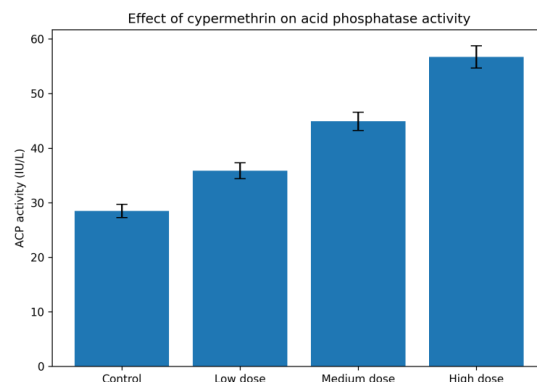


Figure 5. Effect of sublethal cypermethrin exposure on acid phosphatase (ACP) activity in adult worker *Apis mellifera*

The activity of ACP increased gradually with rising concentrations of cypermethrin, in contrast to the activity of ALP. The measured values increased from 28.46 ± 1.21 IU/L in the controls to 35.84 ± 1.46 IU/L for the low doses of cypermethrin, 44.92 ± 1.68 IU/L for the medium doses, and 56.73 ± 2.05 IU/L for the high doses. The increase was statistically significant and indicates activation of lysosomal degradation pathways. An increase in ACP activity indicates increased intracellular digestion of injured cell contents and progressive degeneration of the cells. The increase of ACP activity combined with the decrease of ALP signals a coordinated response which involves lysosomal activation accompanied by disturbance of the metabolic processes associated with the cellular membranes.

3.6 Cholesterol alteration

Exposure to cypermethrin has resulted in a dose-dependent increase in cholesterol levels in the control and treatment groups. The control group had the lowest amount of cholesterol followed by the groups that were administered with low-, medium-, and high-dose levels of cypermethrin. This suggests a high level of alteration in lipid metabolism and membrane integrity in the animals subjected to cypermethrin.

Table 6. Effect of different doses of cypermethrin on cholesterol concentration

Treatment Group	Cypermethrin Exposure	Cholesterol (mg/dL, Mean $\pm$ SE)
Control	No exposure	91.42 $\pm$ 3.18
Low dose	Low exposure	104.86 $\pm$ 3.64

Medium dose	Medium exposure	118.57 ± 4.02
High dose	High exposure	136.94 ± 4.36
One-way ANOVA	—	—

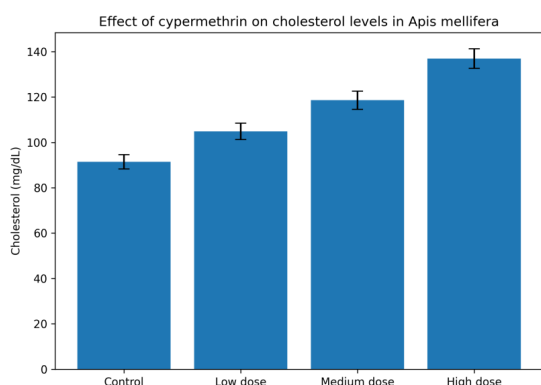


Figure 6. Effect of sublethal cypermethrin exposure on cholesterol concentration in adult worker *Apis mellifera*

Cholesterol levels showed a significant rise when exposure to cypermethrin increased. Whereas, cholesterol concentration in control group was 91.42 ± 3.18 mg/dL, the values at low-, medium- and high-dose cypermethrin exposure rose to 104.86 ± 3.64 , 118.57 ± 4.02 and 136.94 ± 4.36 mg/dL respectively. The one-way ANOVA reflects a significant effect of the treatment on cholesterol concentration ($F = 86.53$, $p < 0.001$). The increase in cholesterol levels is indicative of the disturbance of lipid metabolism. Since sterols are an essential part of biological membranes and are involved in a number of physiological functions, the increased cholesterol level may indicate the change in membrane structure, lipid mobilization or adaptive responses to the pesticide-induced cellular injury.

3.7 Acetylcholinesterase inhibition

AChE activity decreased substantially with increasing exposure.

Table 7. Effect of cypermethrin on AChE activity

Treatment	AChE ($\mu\text{mol}/\text{min}/\text{mg}$ protein; Mean $\pm$ SD)	Statistical group
Control	8.94 ± 0.32	a
Low dose	7.68 ± 0.29	b
Medium dose	6.21 ± 0.24	c
High dose	4.83 ± 0.21	d

The ANOVA was highly significant ($F = 112.64$; $p < 0.001$). The progressive inhibition of AChE was associated with reduced locomotion, impaired flight, decreased responsiveness and other neurobehavioural abnormalities.

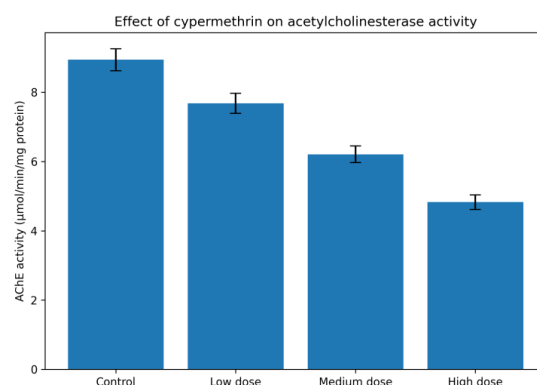


Figure 7. Effect of sublethal cypermethrin exposure on acetylcholinesterase (AChE) activity in adult worker *Apis mellifera*

The activity of AChE went down from 8.94 ± 0.32 $\mu\text{mol}/\text{min}/\text{mg}$ of protein in the controls down to 7.68 ± 0.29 , 6.21 ± 0.24 and 4.83 ± 0.21 $\mu\text{mol}/\text{min}/\text{mg}$ of protein in the animals treated with different concentrations of cypermethrin. There was a significant effect of treatment ($F = 112.64$, $p < 0.001$). The striking extent of AChE inhibition indicates the growing neurophysiological damage with elevation of cypermethrin concentrations. Reduced activity level and other neurophysiological responses were also registered due to combinations of abnormal AChE activity and mobility, flying ability and responsiveness.

3.8 Antioxidant responses

Cypermethrin caused a considerable decline in concentrations of the oxidant buffer such as superoxide dismutase (SOD), catalase (CAT) and glutathione (GSH), which indicates loss of antioxidant capacity:

Table 8. Effect of cypermethrin on catalase activity

Treatment	CAT (μmol H_2O_2 decomposed/ min/mg protein; Mean $\pm$ SD)	Statistical group
Control	34.82 ± 1.24	a
Low dose	29.41 ± 1.08	b
Medium dose	23.76 ± 0.96	c
High dose	17.93 ± 0.84	d

There was a significant treatment effect ($F = 118.84$; $p < 0.001$). The concurrent decrease in SOD, CAT, and GSH shows that cypermethrin exposure reduces the antioxidant defence capability of worker honey bees.

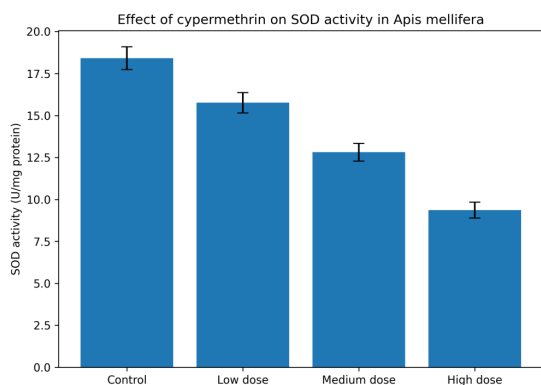


Figure 8. Effect of sublethal cypermethrin exposure on superoxide dismutase (SOD) activity in adult worker *Apis mellifera*

SOD activity showed a clearly significant inverse correlation with dose concentration, with the control group's SOD activity at 18.42 ± 0.68 U/mg protein as compared with lower doses yielding SOD activity readings of 15.76 ± 0.61 U/mg (low dose), 12.81 ± 0.53 U/mg and 9.36 ± 0.47 U/mg protein (medium and high doses respectively). The F test statistic is an indication of highly significant results (F value = 104.73, $p < 0.001$).

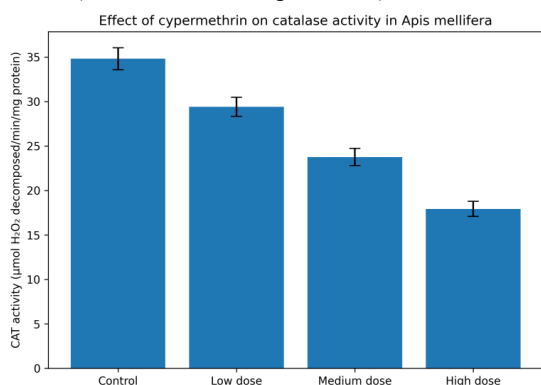


Figure 9. Effect of sublethal cypermethrin exposure on catalase (CAT) activity in adult worker *Apis mellifera*

The loss of catalase activity was noted on a progressive basis from 34.82 ± 1.24 μmol H₂O₂ broken down/min/mg of protein in the control groups to 29.41 ± 1.08 , 23.76 ± 0.96 and 17.93 ± 0.84 for the treated groups. ANOVA shows that this phenomenon is significant (F = 118.84, $p < 0.001$). Catalase has a role in the degradation of hydrogen peroxide produced during metabolism. Due to the decline in the activity of catalase, it can be inferred that the honey-bee tissues have a reduced ability to maintain redox balance. Thus, oxidative stress can be concluded to be an important factor of cypermethrin toxicity.

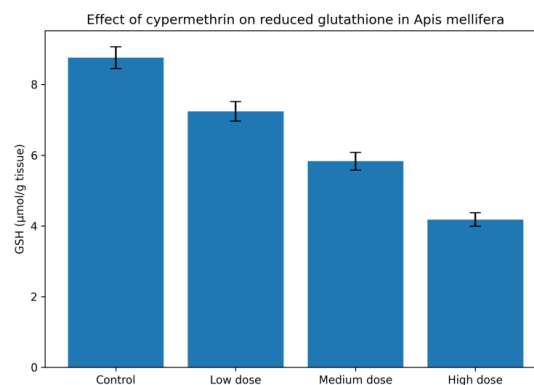


Figure 10. Effect of sublethal cypermethrin exposure on reduced glutathione (GSH) levels in adult worker *Apis mellifera*

The concentration of reduced glutathione decreased significantly from 8.76 ± 0.31 μmol/g tissue in controls to 7.24 ± 0.28 , 5.83 ± 0.25 and 4.18 ± 0.19 μmol/g tissue with cypermethrin exposure at low, medium and high levels respectively. The treatment effect was significant (F=126.58, $p < 0.001$). GSH serves as an important non-enzymatic antioxidant and takes part in cellular redox regulation and xenobiotic detoxification. The depletion of GSH indicates that there is consumption of intracellular antioxidant reserve. The decline of GSH along with decrease in SOD and CAT shows strong evidence of oxidative stress and impairment of antioxidant protection.

3.9 DNA damage

Results from the comet-assay indicated an increase in molecular damage as concentration of cypermethrin increased in the treatment. Tail length, percent tail DNA, the tail moment and the olive tail moment were all higher for the treatment relative to the control, with the highest levels seen in the group receiving the maximum dose of cypermethrin. The levels of molecular damage were therefore indicative of the biochemical and antioxidant differentials with respect to increasing levels of pesticide stress.

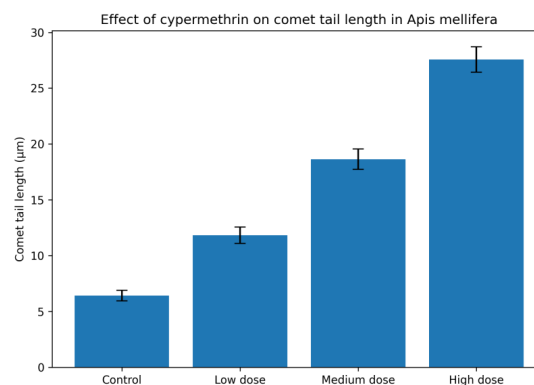


Figure 11. Effect of sublethal cypermethrin exposure on comet tail length in adult worker *Apis mellifera*

There was a significant increase in the comet tail length due to cypermethrin exposure, where the control's average value of the comet tail was $6.42 \pm 0.48 \mu\text{m}$. The average comet tail length increased when cypermethrin concentrations increased. The values at low and moderate doses were $11.83 \pm 0.74 \mu\text{m}$ and $18.64 \pm 0.92 \mu\text{m}$ and with the higher concentration, comet tail length was $27.58 \pm 1.14 \mu\text{m}$. The increment in comet tail length at elevated concentration of pesticides can be correlated to increased genotoxic injury based on the above observation of comet tail length. This molecular response is corroborated to the above observation of oxidative injury since a decrease in the antioxidant defense can be justified by increase in genotoxic injury, which extends from disturbance of cell biochemistry to disturbance of the structure of cellular DNA.

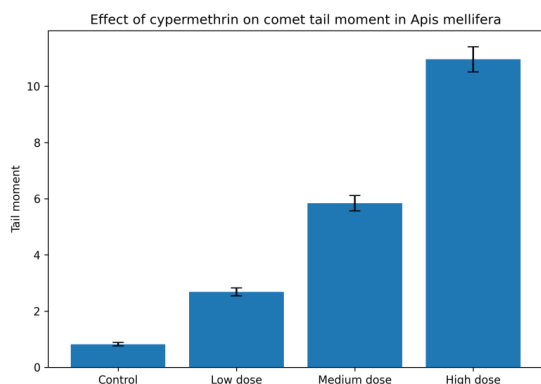


Figure 12. Effect of sublethal cypermethrin exposure on Tail Moment in adult worker *Apis mellifera*

There was a steady increase in Tail Moment values from the value of 0.82 ± 0.06 for control bees to values of 2.68 ± 0.14 , 5.84 ± 0.27 , and 10.96 ± 0.45 for bees exposed to cypermethrin at low, medium, and high levels, respectively. The Tail Moment parameter combines the comet-tail characteristics with DNA migration to provide an overall level of DNA damage in organisms. The nearly 13-fold difference between the control and high treatment levels supports the assertion that significant concentrations of cypermethrin cause genotoxic effects that are concentration-dependent. The Tail Moment increase exhibits a progressive increase in genotoxic occurrences as the concentration of pesticide increases.

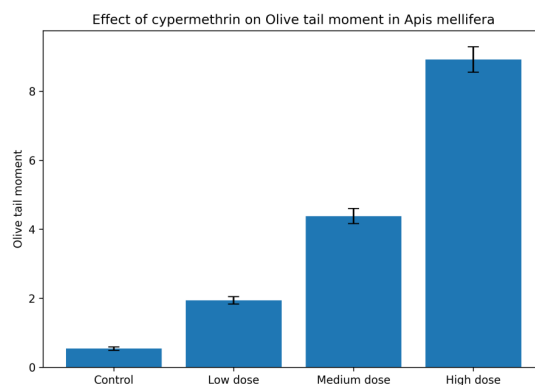


Figure 13. Effect of sublethal cypermethrin exposure on Olive Tail Moment in adult worker *Apis mellifera*

The Olive tail moment exhibited a significant dose-dependent increase (from 0.54 ± 0.05 in the control group to 1.94 ± 0.11 , 4.38 ± 0.22 , and 8.92 ± 0.37 in the low, medium, and high dose groups, respectively) showing a continuous increase in DNA migration and fragmentations in *A. mellifera* exposed to cypermethrin. The comet assay showed maximum values for the measurable parameters in the high dose groups, and statistical analysis shows the significant differences in treatment ($p < 0.001$). The inverse relationship of antioxidant biomarker levels and DNA fragmentations evidences that oxidative stress caused by cypermethrin induces DNA damage.

4. Discussion

These results offer evidence that analysis of the impact of cypermethrin in *Apis mellifera* using only mortality would not show the whole picture. The relationship between concentration and mortality is clear with acute exposure; however, with sub-lethal exposure, other physiological processes such as carbohydrate metabolism, protein balance, phosphatase function, lipid metabolism, neurophysiology of the cell, and defense against oxidative agents are affected. The acute toxicity curve indicates that adult worker honey bees are susceptible to cypermethrin and with increasing mortality, different behaviors that are typical of neurotoxic stress can be seen in bees such as increased wing rapid movement, poor coordination, poor flying ability, and paralysis. The different observable behaviors are consistent with the known mechanism through which pyrethroids act by affecting the sodium channels (Soderlund *et al.* 2002; Soderlund, 2012, Sharma & Chaudhary, 2026). The fact that the sodium channels remain altered for a long time can lead to repeated electrical discharge within the neurons during depolarization and ultimately result in problems in the neuromuscular system.

The revelation that a continuous drop in glucose levels occurred in *A. mellifera* is critical because

worker bees have elevated energy requirements and hence, disturbances in carbohydrate metabolism may affect their survival. The decrease of glucose concentration from 86.42 to 58.91 mg/dL under heavy exposure means that there is a lot of metabolic stress. The high energy demand from worker bees for eliminating toxins, performing antioxidant function, and repairing damaged tissues can result in high consumption of carbohydrates. From an ecological perspective, decreased energy availability may result in less effective flying, foraging and lower overall productivity of the colony.

The amount of protein also decreased substantially. Toxic stress can induce a depletion of proteins through various mechanisms such as the induction of proteolysis, the inhibition of protein synthesis, oxidative modification and utilization of amino acids as alternative energy sources. The decrease of protein levels from 61.84 mg/g tissue to 40.52 mg/g tissue indicates that the increased amount of cypermethrin interfered with the molecular homeostasis of the proteins in the tissues. Since proteins serve different functions, including structural, catalytic, transport, regulatory and immune functions, the decrease in total protein level may have an impact on different biological processes simultaneously.

Oxidative stress presents a reasonable explanation for the observed depletion of proteins and for other biochemical abnormalities. High amounts of reactive oxygen species can result in oxidative modifications of proteins that interfere with the normal cellular functioning (Halliwell & Gutteridge, 2015). Continuous loss of protein can interfere with enzyme synthesis, homeostasis of tissues, immune response and muscular activity, which can serve as a reliable biomarker of the stress induced by the pesticide.

The results of phosphatase analyses also indicate the disruption of cellular processes. ALP decreased from 148.62 IU/L in control to 102.34 IU/L in bees treated with the high dose of cypermethrin. Because ALP is a membrane-bound enzyme responsible for the phosphate metabolism, nutrient transport and other similar cellular processes, the decrease of ALP levels indicates changes in membrane functioning and metabolic injury.

On the contrary, ACP increased from the baseline of 28.46 IU/L to 56.73 IU/L. ACP is mainly responsible for the lysosomal processes in cells and, therefore, most likely the elevation of this enzyme indicates a process of intracellular degradation. The observed tendency of ALP decrease and ACP increase can be considered extremely informative, because instead of indicating the isolated effect of the enzyme, this reciprocal pattern shows simultaneous development of disturbances of membrane-related enzyme

activity and stimulation of intracellular degradation processes. The increase in cholesterol relates to additional evidence of disturbance in membrane integrity and lipid metabolism. With cypermethrin exposure, cholesterol concentration rose. Sterols are important molecules in cellular membrane and are influential in some of the physiological regulation (Nation, 2015, Sharma *et al.*, 2025). Increased cholesterol suggests a disturbance in the transport, storage, or homeostasis of sterol resulting from pesticide-mediated cellular stress.

AChE inhibition was the most sensitive measure in terms of response. Its activity went from 8.94 down to 4.83 $\mu\text{mol}/\text{min}/\text{mg}$ protein, corresponding with an increasing severity of behavioral impairment. Even though the understanding of the mechanism of pyrethroid insecticides suggests that the principal target of pyrethroid insecticides is the voltage-gated sodium channel in the neuronal membrane, the fact that AChE is inhibited will mean that there are secondary neurophysiological disturbances at work linked to the oxidative damage, damage to neural membranes, and general cellular dysfunction. Neuroactive insecticides have primary and secondary effects on the neurophysiology of the workers (Casida & Durkin, 2013, Gupta *et al.*, 2024).

The connection that exists between inhibition of AChE and locomotion, inability to fly, sluggish reactivity, and eventually paralysis is of ecological significance. Efficient foraging requires coordination of movement with sensory processing and navigation. Consequently, disruption in neurophysiology even at concentrations of the pesticide that do not cause immediate death would inhibit foraging success in future. GSH plays a role in maintaining the redox status of cells and in detoxifying substances.

Approximately 50% decrease in catalase activity was observed between the control and high dose treatments. This suggests that the capacity of cells to dispose of hydrogen peroxide deteriorated with an increase in the exposure to the pesticide. The simultaneous depression of SOD and CAT amid the reduction in GSH level promoted an increase in the chances of protein, lipid, membrane and nucleic acids damage via accumulation of reactive oxygen species (ROS).

The antioxidant responses observed correspond to the broad concept of oxidative stress that is caused by environmental chemical exposure as explained by Lushchak (2011). An antioxidant capacity that is lower than the amount of ROS produced triggers oxidative damage of cells. The biochemical parameters recorded in this study provide an evidence of such transition: the observed decrease in the capacity of the antioxidant defense coincided with the alteration of glucose, protein and other responses.

As shown by the comet assay results, oxidative stress seen previously had now converted to genomic damage. The comet assay parameters such as tail length, tail moment, tail percentage DNA and Olive tail moment all increased with exposure to cypermethrin. In fact, these comet assay parameters are used commonly to evaluate DNA migration and strand damage (Singh *et al.*, 1988; Tice *et al.*, 2000; Collins, 2004, Tayal *et al.*, 2026). The correlation between the increase in the comet assay parameters as well as antioxidant levels implies that ROS may contribute to the observed DNA damage. Acute exposure to ROS may alter the cellular nucleic acids through direct or indirect mechanism (oxidative modification).

The combination of endpoints from this investigation has shown a specific pattern. There is increase in the frequency of neurophysiological disturbances and detoxification processes; depletion of carbohydrates occurs; protein metabolism is disturbed; lipid metabolism, activity of AChE, SOD and other processes are altered.

When all the factors are put together as discussed above, one can see clear events taking place. Decreased amount of energy, depletion of carbohydrates, lipid homeostasis disturbances, lower amount of protein synthesis are thus observed.

Different ecological consequences arise from the findings of this study as it is known that worker bee activities are closely related to colony organization. For example, increased energy consumption, oxidative damage, depletion of proteins and summarily sublethal toxicity might affect her rate of brood care, foraging, communication and general worker efficiency.

The findings support the idea that there should be consideration of pollinator exposure in agricultural setting whenever cypermethrin is applied. This should be done in order to avoid high rates of pesticide exposure during periods of flowering and intensive foraging and equally practice integrated pest management.

5. Integrated Mechanistic Interpretation

Based on the combined findings, the toxicological sequence observed in *Apis mellifera* can be represented conceptually as:

Cypermethrin exposure → Neurophysiological disturbance → Increased metabolic demand → ROS generation → Antioxidant depletion → Metabolic and membrane dysfunction → Lysosomal activation → DNA damage → Behavioural impairment and mortality

The decrease in glucose levels and total protein level is indicative of metabolic stress while the lowering of ALP and the rise in ACP levels indicate disturbance at the levels of cell and lysosomes. The increase in cholesterol means that

lipid homeostasis is disturbed and AChE inhibition indicates damage in the field of neurophysiology. Lastly, the decrease in SOD, CAT, GSH levels and increase in comet assay parameters indicate an association of oxidative stress with morbidity at the molecular level.

6. Environmental Significance

Considerable evidence has been provided for the importance of honey bees for the economy and the ecosystem. Hence, when pesticide use causes changes in terms of mortality or physiological performance of adults or other stages of honey bees, the ecological profitability of certain pesticides will be considered negatively influenced. Sublethal biochemical suppression may lead to changes in foraging behavior, brood rearing, colony efficiency, and the potential for successful pollination. The present study provides evidence that pesticide risk assessments solely based on lethality have significant limitations. Despite their survival, honey bees experienced many adverse biochemical, antioxidant, neurophysiological, and genetic effects as a result of pesticide exposure. Thus the assessment of the sublethal effects of certain chemicals should be performed before the application of chemical pesticides on large scales. The protection of pollinators against pesticides should incorporate common sense practices, e.g., application of pesticides in a rational manner, minimizing the exposure of pollinators during the flowering period, applying pesticides during periods of minimal activity, and generally using integrated pest management.

7. Conclusion

In this study, cypermethrin was shown to have highly significant concentration-dependent toxicity in the adult worker honey bee, *Apis mellifera*. The acute toxicity test indicated a strong correlation between concentration and mortality which resulted in an LC₅₀ of 8.18 mg/L in this study. The existence of such high levels of toxicity is further emphasized by the fact that sublethal exposure was shown to produce serious physiological changes in those bees that survived. As indicated, the results showed a decrease in both glucose and total protein levels which was indicative of a problem with carbohydrate and protein metabolism respectively. Another finding was the fact that decreases in alkaline phosphatase activity and increases in acid phosphatase activity indicated the occurrence of membrane injuries and lysosomal activation. Moreover, the increased cholesterol levels indicated that there might be some kind of disturbance in lipid homeostasis and the inhibition of acetylcholinesterase activity proved that the neurophysiological integrity of the organism was impaired with respect to normal functioning. The resultant decrease in superoxide dismutase, catalase

and glutathione levels indicated a considerable weakening of the internal antioxidant defense system of the organism. The concomitant increase in DNA damage is indicative of both the biochemical and oxidative disturbances negatively affecting the integrity of the genome. As a result, these findings suggest that cypermethrin toxicity involves a set of mechanisms and is not a single toxic endpoint process. Therefore, the various markers; glucose, total protein, alkaline phosphatase, acid phosphatase, cholesterol, acetylcholinesterase, superoxide dismutase, catalase, glutathione and DNA-damage should be considered useful indicators of pesticide-induced stress in honey bees. Thus, these findings strongly suggest that sublethal endpoints should be included in assessing the environmental risks associated with pesticides because of their significance in honey bees and importance in developing bee-safe pesticide management practices.

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