RESEARCH PAPER

Comparative Histopathological Study of Honey Bee Species (*Apis mellifera*) Exposed To Various Insecticides

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Abstract

The current study was conducted at the histology laboratory in the Department of Zoology, Rawalpindi Women University, Rawalpindi Pakistan during 2025 against *Apis mellifera* species of the honey bee to analyse the histopathological changes and long-term surival of honeybees when exposed to various insecticides. A modeling approach regarding survival of bees in cages under chronic exposure to five insecticides (Round up, Lambda cyhalothrin, Cypermethrin, Imidacloprid and Chlorpyrifos) of different concentrations (1000, 500, 250, and 125 ppm). Cypermethrin has highest LT50 followed by Round up, Imidacloprid, Lambda-cyhalothrin, and Chlorpyrifos at all tested concentrations. Since, Chlorpyrifos showed more deadly effects with least LT50 as compare to other tested insecticides so, the results suggested that Chlopyrifos was extremely toxic insecticide to *Apis mellifera* at all tested concentrations. Histopathological analysis showed the effect and damage caused by insecticides to honeybees at cellular and tissue level. The results showed that the brain has extensive gaps in the neuropil as well as in the cellular bodies. The thorax and abdominal region exhibited significant morphological abnormalities, structural alterations, muscle fiber degeneration, disruption of tissue structures, malphagian tubule impairment, vacuolization in various region and necrotic effects. In summary, this study estimated the toxicity of diverse insecticides with chlorpyrifos, lambda-cyhalothrin and imidacloprid being the most harmful, that are declining the population of major crop pollinators.

Keywords: *Apis mellifera*, Chlorpyrifos, Imidacloprid, Cypermethrin, Histopathology, Toxicity

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Introduction

Pakistan is one of those countries that have rich naturally occurring flora and fauna. This environment encourages the flowering of numerous plant species, which serve as essential sources of nectar and pollen for bees. (Khan and Grahmn, 2023). So the country has a rich bee fauna given the wide variety of climatic conditions and habitat types (Qamer et al., 2021). For the reason that of the products produced in their hives and role as pollinators, honey bee species are important for medicine, the economy, and the environment (Shurjeel et al., 2019; Campani et al., 2025). Therefore they are regarded as the most lucrative pollinators from an economic perspective as significant contributor to both food security and the sustainability of biodiversity (Usman et al., 2022). About 90% of the world's food supply is made up of hundreds of plant species, of which 71 common crops are pollinated by managed bees as pollinators with amazing success (Khan et al., 2021; Khalifa et al., 2021; Usman et al., 2022). In terrestrial ecotoxicology, this honeybee specie is of particular interest (Stoyanova et al., 2025). Currently, in Pakistan almost more than one million colonies of *A. mellifera* are being managed by 12,000 beekeepers and annually produced more than 12,000 tons of honey (Khan & Grahmn, 2023).

In addition, the vitality of honey bees is affected by significantly detrimental factors include intensive agriculture with habitat loss, lack of suitable food, the emergence of new pathogens and long-term pesticides use (Farder-Gomes et al., 2021).The most researched insecticides in terms of pollinator side effects are neonicotinoids, with a focus on how they affect various bee species (Pervez & Manzoor, 2021). Pollinators may suffer adverse effect from the extensive use of pesticides, which could have unfavorable impact on crop productivity (Al- Dhafar et al. 2025). According to recent research, one potential mechanism for the non-target toxicity of pesticides is oxidative stress (Wachkoo et al., 2019). Organophosphorus insecticides were found to be comparatively toxic to *A. florea F*., *A. dorsata F*., and *A. indica F*. (Gauthier et al., 2018; Serra et al., 2023). Additionally, insecticidal residues on bee flora are another reason why bee dies (Stoyanova et al., 2025). Utilizing insecticides (Tracer 249SC, Chlorpyrifos 40EC, Advantage 20EC, and Confidor 200SL) and concentrations (1000, 500, 250 and 125 ppm), these insecticides bioassay revealed that imidacloprid and carbosulfan at high dose (1000ppm) were more toxic with LT50 of 4 hours (Husain et al., 2014; Bibi et al., 2024). Among the behavioral changes of honeybees caused by insecticidal residues includes the antennal signals alteration, loss of orientation and the cognitive working memory and compromised immune response due to which their ability to forage environmental resources decreases (Pervez & Manzoor, 2021; Favaro et al., 2023). Imidacloprid has harmful consequences on learning behaviors of bees (Shurjeel et al., 2020). The adult body size in bees has been reduced due to insecticidal exposure (Wintermantel et al., 2018). Histopathology is used widely in environmental studies to investigating the biological effects of contaminants (Lent et al., 2021) by causing microscopic tissue damage in vital organs. This technique helps to identify cellular and tissue-level alterations resulting from insecticide exposure and provides insight into the mechanisms of toxicity more accurately reflect the health status of the affected organisms (Wang et al., 2019; Gusso-Choueri et al., 2022; Tanabe et al., 2025). Therefore, the aim of present study is to investigate the histopathology of honeybee specie i-e *Apis mellifera*, when exposed to various insecticides.

Materials and Methods

The present research was performed at the laboratory for histology in the Zoology Department, Rawalpindi Women University, Rawalpindi Pakistan during 2025. *Apis mellifera* were collected from Shirkat-ul-Nahl Apiary, Islamabad, Pakistan. Around 50-60 workers of honey bees were collected, labelled and dated in perforated plastic jars to take them to lab. Bees were fed with 50% sucrose solution. Five insecticides viz., Round up (Glyphosate 41EC), Lambda- cyhalothrin (Demand® 2.5EC), Cypermethrin (10EC), Imidacloprid (Confidor® 25EC) and Chlorpyrifos (Lorsban® 40EC) were used at the concentrations (1000, 500, 250, and 125 ppm). To study the residual toxicity 5-7ml of insecticide concentration were poured in each 1L lidded glass jar, dried in air and then introduced 10 honeybees worker in each jar. These jars were perforated and placed at temperature 29±2°C and humidity 60±5% till the death of last bee worker. The mortality rate was determined after 3, 6, 12, and 24 hrs. Lethal time (LT50) and mortality was determined by Probit analysis.

elected insecticides with their common names, EC, trade names, type and suggested dose

Common Names	Emulsifiable Concentration	Trade Names	Type	Dose ml/acre
Round up	41EC	Roundup®	Glyophosate	1000
Lambda-Cyhalothrin	25EC	Demand®	Synthetic Pyrethroid	200

Cypermethrin	10EC	Chillas®	Synthetic Pyrethroid	220-300
Imidacloprid	25EC	Confidor®	Neonicotinoid	125
Chlorpyrifos	40EC	Lorsban®	Organophosphate	800-1000

After the determining the mortality rate, bees were dissected in transverse section and tissues from the head, thorax and abdomen region were processed for histology. These body parts were preserved in 10% formalin solution preventing its structure from damaging followed by tissue embedding in Paraffin wax to microtome designed for sectioning thin slices. Hematoxylin and eosin staining was accompanied examining under Light microscope to inspect and identified the changes in cellular structures and tissues of honey bees exposed to various insecticides (Hussain et al., 2014).

Results

1. Determination of Residual Toxicity

1) LT_{50} of *A. mellifera*

The LT_{50} value recorded for the control group was 96-103 hours. The lethal time required to kill 50% of honeybee sample (LT_{50} value) was found maximum (11 hours, 13.41 hours, 16.23 hours) by Cypermethrin followed by Round up (10.65 hours, 11.45 hours, 12.30 hours), Imidacloprid (7.5 hours, 10.64 hours, 11.44 hours), Lambda-cyhalothrin (6.32 hours, 8.59 hours, 10.64 hours) and Chlorpyrifos showed minimum LT_{50} of 5.32 hours, 6.91 hours and 8.80 hours at at the concentration of 1000 ppm, 500ppm and 250ppm, respectively. LT_{50} value was found maximum (18.39 hours) by Cypermethrin at the concentration of 125 ppm tailed by Imidacloprid (14.12 hours), Round up (13.53 hours), Lambda-cyhalothrin (12.29 hours) and Chlorpyrifos showed minimum LT_{50} of 10.39 hours to kill 50% of *A. mellifera* worker bees at same 125 ppm concentration. As LT_{50} value is the lethal time required to kill 50% of population, so the results suggested that Chlorpyrifos was more toxic to *A. mellifera* as it has least LT_{50} values at all concentrations (1000, 500, 250 and 125 ppm) followed by lambda- cyhalothrin, imidacloprid, round up and cypermethrin. The toxicity order of the tested insecticides was Chlorpyrifos > lambda-cyhalothrin > Imidacloprid > Round up > Cypermethrin.

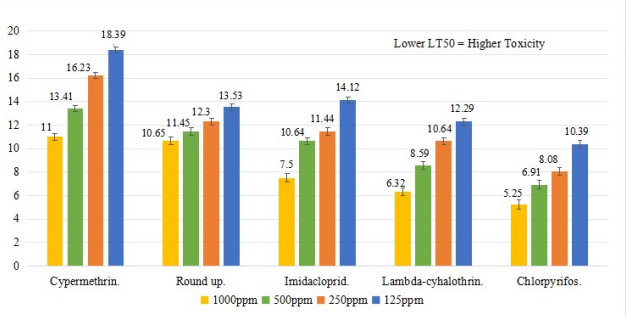


Fig 1. Graph showing LT_{50} with ±Standard error of *A. mellifera* worker bees at different concentrations of tested insecticides

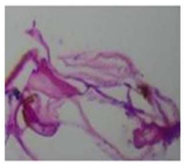
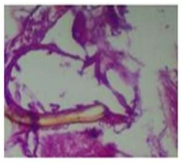
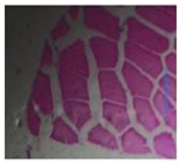
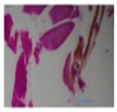
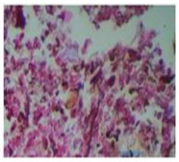
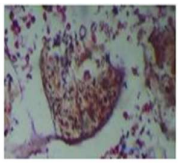
Table 2- LT_{50} of *A. mellifera* along with fiducial limit, P value and standard error at different concentrations

		LT_{50} (hours), Fiducial limit (95%), P value, Standard error															
Concentrations		**1000ppm**				**500ppm**				**250ppm**				**125ppm**			
Sr #	**Treatment**	**LT_{50}**	**F.L**	**P**	**S.E**	**LT_{50}**	**F.L**	**P**	**S.E**	**LT_{50}**	**F.L**	**P**	**S.E**	**LT_{50}**	**F.L**	**P**	**S.E**
1	Cypermethrin	11.00	10.41-11.58	.001	0.30	13.41	12.92-13.90	.000	0.25	16.23	15.74-16.72	.000	0.25	18.39	17.84-18.93	.000	0.28
2	Round up	10.65	10.06-11.24	.001	0.30	11.45	10.82-12.08	.002	0.32	12.30	11.73-12.86	.001	0.29	13.53	12.96-14.09	.000	0.29
3	Imidacloprid	7.50	6.81-8.18	.004	0.35	10.64	10.05-11.22	.001	0.30	11.44	10.83-12.04	.002	0.31	14.12	13.63-14.61	.000	0.25
4	Lambda-Cyhalotrin	6.32	5.67-6.96	.003	0.33	8.59	7.94-9.27	.003	0.33	10.64	10.05-11.22	.001	0.30	12.29	11.17-13.40	.001	0.29
5	Chlorpyrifos	5.25	4.50-5.99	.008	0.38	6.91	6.22-7.59	.005	0.35	8.08	7.41-8.74	.003	0.33	10.39	9.77-10.99	.001	0.31
6	Control	96	*	*	*	98.5	*	*	*	100.5	*	*	*	103	*	*	*

2. Histopathological Analysis

Results obtained from histopathological analysis of *A. mellifera* and controlled sample showed in Table 3.

Table 3: Comparison of body parts/tissues from control and insecticide exposed *A. mellifera* bees

Region/ section	Cellular structure/configuration in control/unexposed worker honey bees	Cellular structure/configuration in insecticide exposed worker honey bees
Head		
Thorax		
Abdomen		

Head Region

Normal head region of *A. mellifera* showed epithelial cells, cuticle, sensilla hairs, brain, neurophils, glial cells, mushroom bodies, sensory organ (antenna), compound eye and mouth part/hypopharangeal glands. However, when the same healthy honeybees were exposed to various insecticides showed various parts and cells of head region were damaged, deformation, fragmentation, cell lysis and vacuolization, like cypermethrin damaged epithelial cell lining and cuticles, round up caused degeneration of sensilla, epithelial cell and cutical damage, tissue fragmentation and necrosis. Similarly, imidacloprid induced cell lysis, necrosis, disruption and disorganization of neuronal tissues. In the same way, Lambda-cyhalothrin and chlorpyrifos affected and produced degeneration of antennal sensory receptors, degradation of cuticle layer, deformation of sensory hairs, epithelial cell vacuolization, gaps in neurophil cells along with the loss of pigmentation (fig 2 to 7).

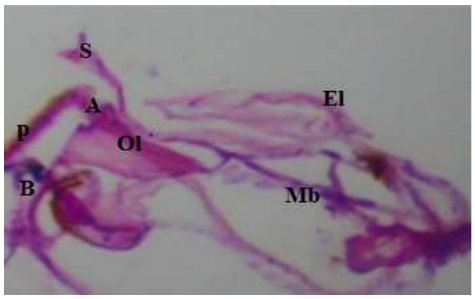


Fig. 2: Microscopic view of normal Head region of *A. mellifera* showing Brain (B), Antenna (A), Epithelial cell lining (El), Proboscis/ mouthpart (P), Sensory organs (S) Optic lobes (Ol) and Mushroom bodies(Mb)

(b)

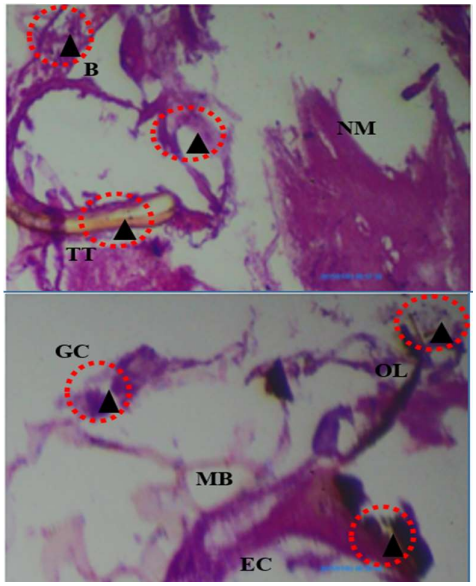
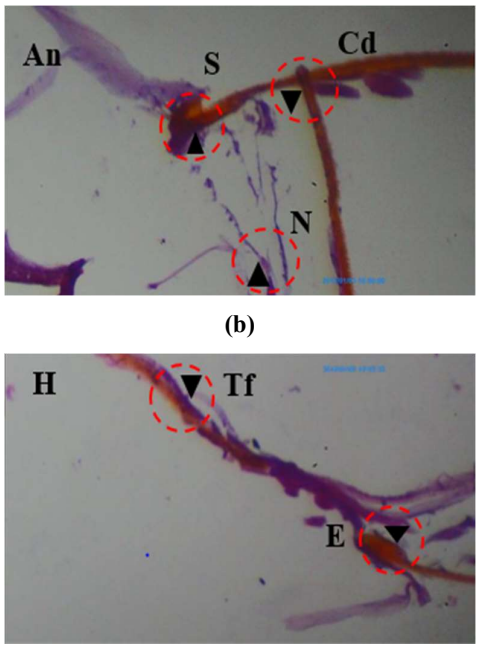
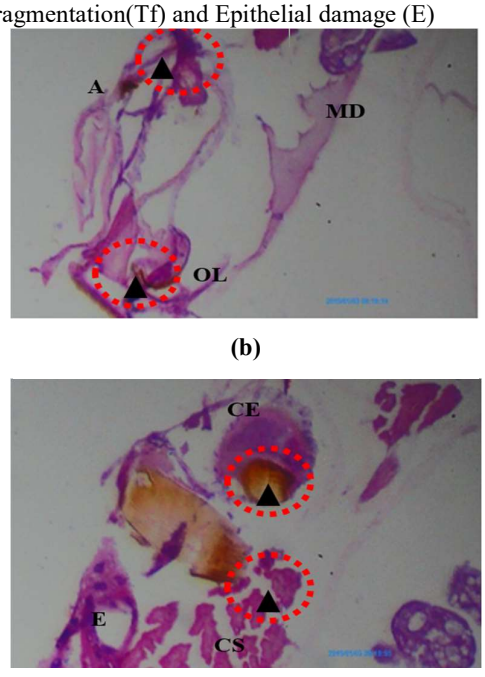


Fig. 3(a-b): Microscopic view of cypermethrin induced alterations in Head region (H) of *A.mellifera* showing nuclear condensation of brain tissues(B), Neural mass (NM), distortion of tracheal tube (TT), Optic lobes (OL), Mushroom bodies (MB), Cuticle (GC) and Epithelial damage (EC) and vacuolization



(b)

Fig. 4(a-b): Microscopic view of Round up induced alterations in *A.mellifera* head region (H) showing Antenna region (An), Sensilla degeneration (S), Cuticle damage (Cd), Necrosis (N), Tissue fragmentation(Tf) and Epithelial damage (E)



(b)

Fig. 5(a-b): Microscopic view of imidacloprid induced alterations in head region (H) of *A.mellifera* showing Antenna region (A), Mushroom bodies (Mb), structural alteration in optic lobes (Ol), Compound eye structure (CE), Chitinous exoskeleton (CE), Cuticle structure (CS), Epithelial lining(El)

(a)

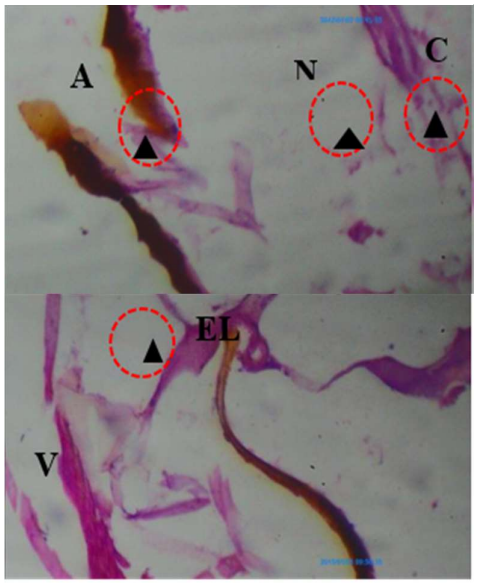
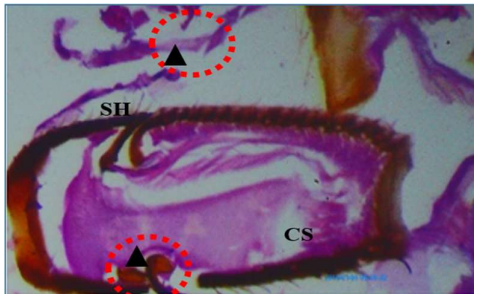


Fig. 6(a-b): Microscopic view of Lambda-cyhalothrin induced alterations in Head region (H) of *A.mellifera* showing abnormalities in Antennal region (A), Cuticle (C) damage, Necrosis (N), damage in epithelial cells linning (El) and vacuolization (V)



(b)

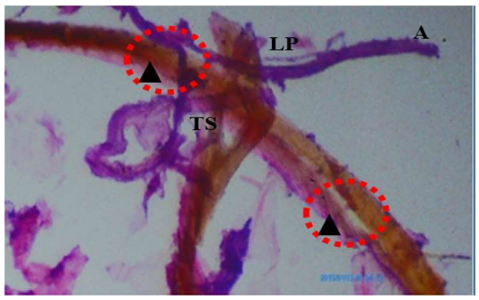


Fig. 7(a-b): Microscopic view of chlorpyrifos induced alterations in head region (H) of *A.mellifera* showing degeneration of sensory hairs (SH), Disruption of chitinous and cuticle structure (CS), tarsal setae (TS), Antenna (A), labial palps (LP) and epithelial cell vacuolization (V)

Thorax Region

Although thorax is usually the rigid and strongest part of any insect's body, neverthless affected by insecticides. Normal healthy thorax did not show any disruption, deformation or disarticulation of direct and indirect flight muscles, wings, legs or tracheal tube. Cypermethrin created tissue lysis, swelling of muscle fibers and vacuolization in the thorax region of *A. mellifera.* Similarly, disorganization of muscle fiber, disruption in direct and indirect flight muscle and pigment deposition caused by insecticide named roundup. Imidacloprid induced fragmentation and loss of alignment in muscle fibers and erosion of cuticle layers. Lambda cyhalothrin and chlorpyrifos produced muscle fiber degeneration, vacuolization, structural alterations in exoskeleton and cuticle layers, vacuolization of epithelial lining along with necrosis (fig 8-13).

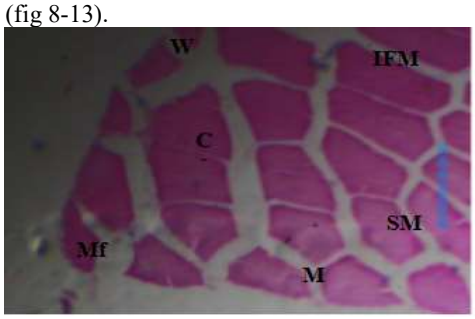
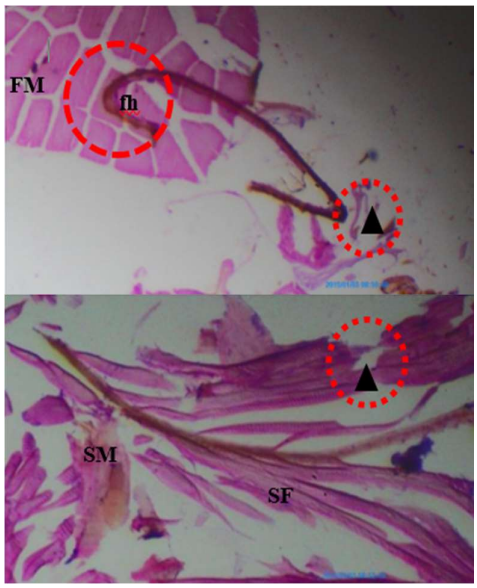


Fig. 8: Microscopic view of normal Thorax region of *A. mellifera* showing direct and indirect flight muscles (IFM), muscle fibers (Mf), Wings articulation (W), Cytoplasm (C), myocytes (M) and Striated Muscle fibers (SM)



(b)

(c)

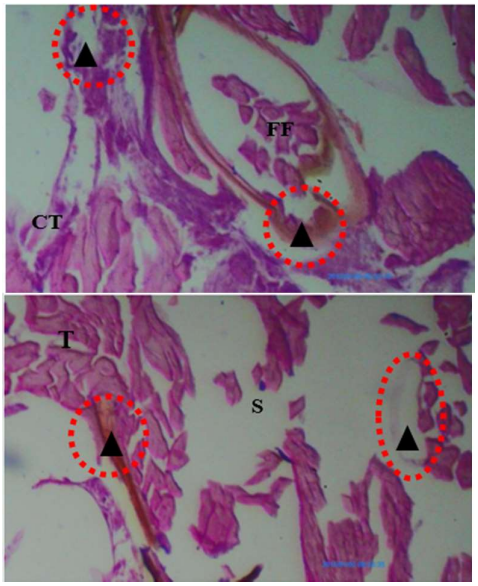
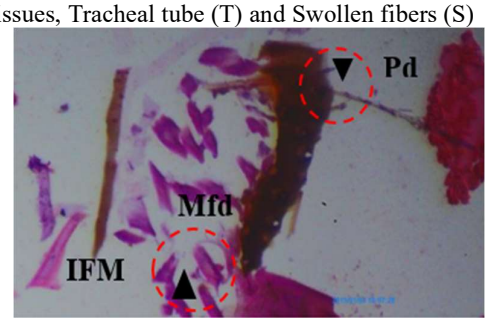


Fig. 9(a-d): Microscopic view of cypermethrin induced alterations in Thorax region of *A.mellifera* showing Flight muscle (FM), Fungal hypha (Fh),

Vacuolization (V), Striated muscle (SM), Striated fibers (SF), Fragmented fibers (FF), Connective tissues, Tracheal tube (T) and Swollen fibers (S)



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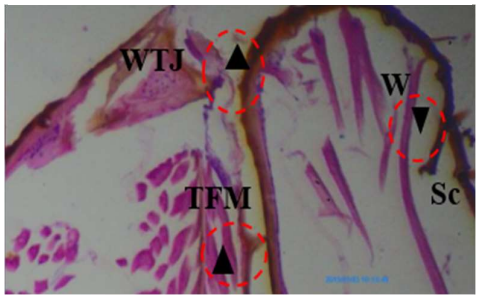
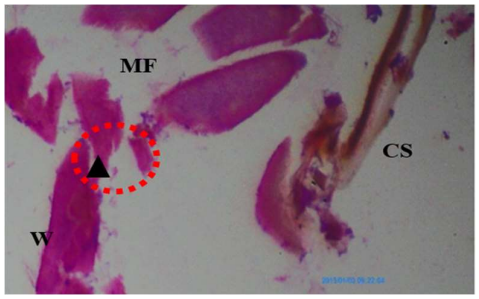


Fig. 10(a-b): Microscopic view of Round up induced alterations in *A.mellifera* thorax region showing Muscle fiber disorganization (Mfd), Pigment deposition (Pd), Indirect Flight Muscles (IFM), Wings-Thorax junction (WTj), Wings (W), Sclerotized cuticle (Sc) and Thoracic Flight Muscle (TFM)



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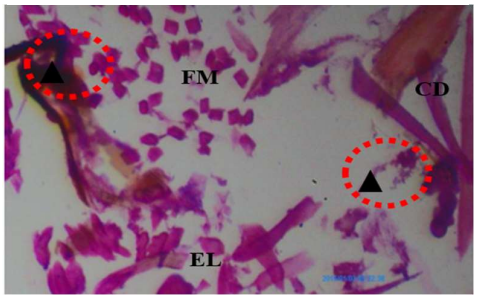
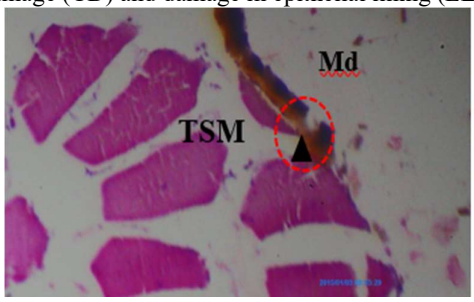


(a)

Fig. 11(a-b): Microscopic view of imidacloprid induced alterations in thorax region of *A. mellifera* showing muscle fiber (MF), cuticle structure (CS), wings (W), fragmentation of muscles (FM), cuticle damage (CD) and damage in epithelial lining (EL)



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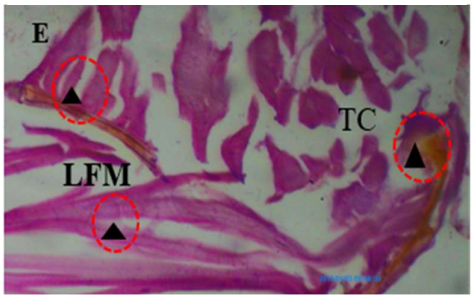

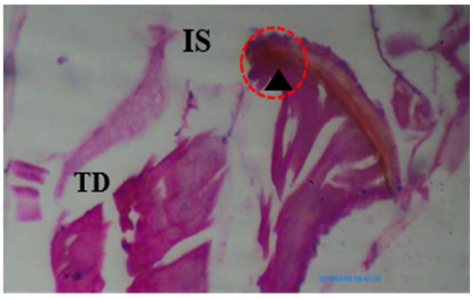

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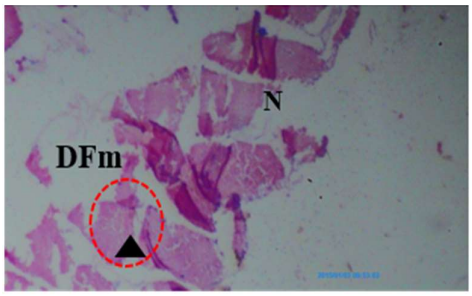


Fig. 12(a-d): Microscopic view of Lambda-cyhalothrin induced alterations in Thorax region of *A.mellifera* showing thoracic skeletal muscle (TSM), Muscle fiber degeneration (Md), Exoskeleton (E) with thoracic cuticle (TC), Longitudinal flight muscle (LFM), Direct flight muscles(DFM), Necrosis (N), Irregular staining and

(d)

Tissue disorganization (TD)

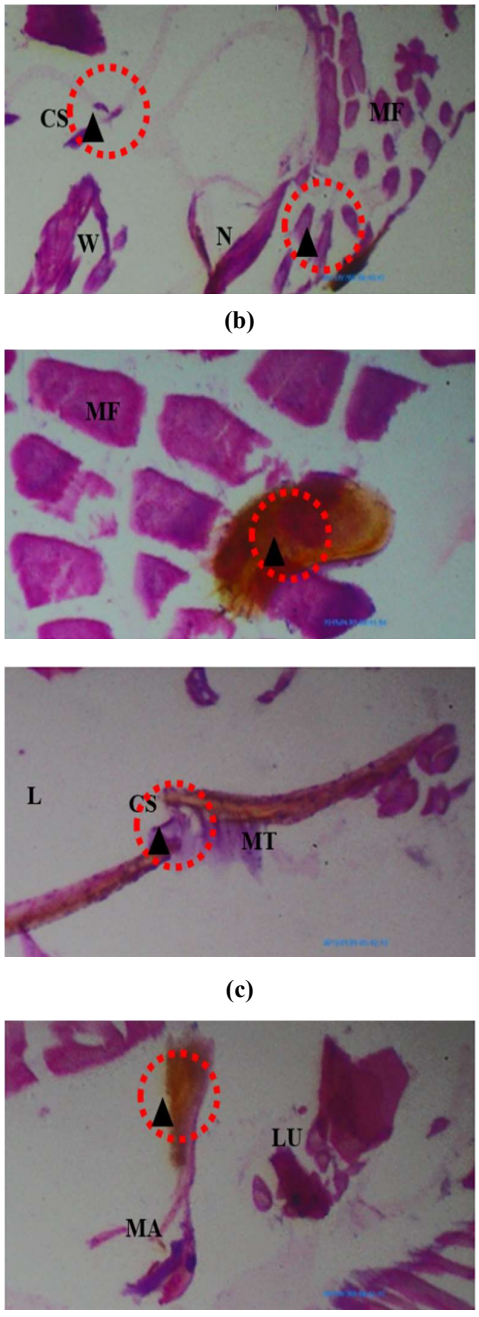


Fig. 13(a-d): Microscopic view of chlorpyrifos induced alterations in thorax region of *A. mellifera* showing disruption of cuticle structures(CS), Wings (W), Muscle fiber degeneration(MF), Necrosis (N), Disruption of muscle fiber (MF), Damage to cuticle structure (CS), vacuolization, Lumen (L), mandibular area (MA)

Abdomen Region

It is the soft part of insect's body. It contains all the important and visceral organs including reproductive system and fat bodies covering these organs. Therefore, this can be the most effected part of body by various types of insecticides such as, breaking up and vacuolization of malphagian tubules by cypermethrin. Round up induced damage in epithelial cells, disorganization of tracheal lining, impairment of malphagian tubules, nuclear degeneration of midgut epithelial cells and necrosis. Similarly, disruption of neural control and oxidative stress, severe disintegration and fragmentation of epithelial lining, cytoplasmic vacuolization, cell lysis and necrotic debris accumulated in gut lumen caused by imidacloprid. Lambda- cyhalothrin created defragmentation of fat tissues and cuticle lining, vacuolization, alteration in midgut epithelial cells, collapse in tracheal structural and necrosis. Whereas, chlorpyrifos caused cellular degradation, digestion impairment, infiltration of hemocytes, gut lumen degradation, neural alterations, loss of tissue architecture, disruption of tissue structure, necrosis and cytoplasmic vacuolization (Fig 14 to 19) illustrated these results.

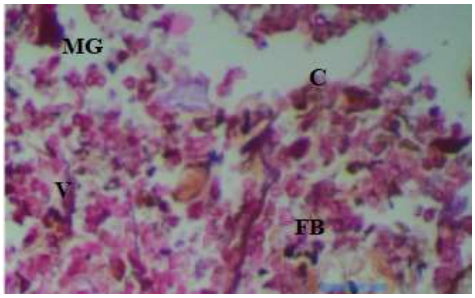
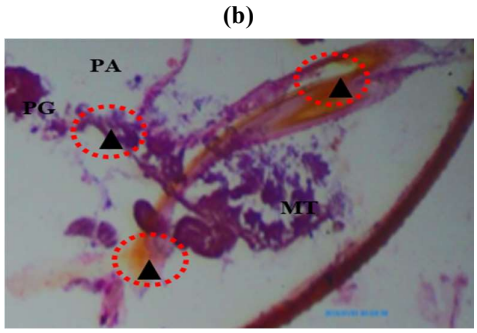
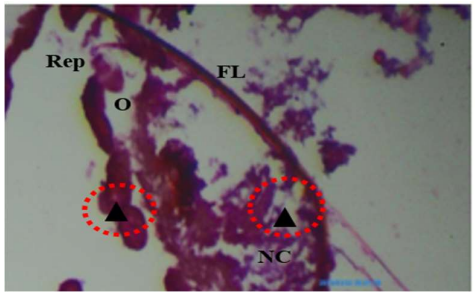
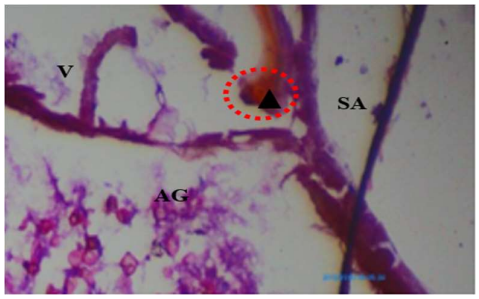


Fig. 14: Microscopic view of Abdomen region of *A. mellifera* showing Midgut (MG) ventriculus (V), Fat body tissues(Fb), Cytoplasm(C), Malphagian tubules (Mt) and reproductive organs







(d)

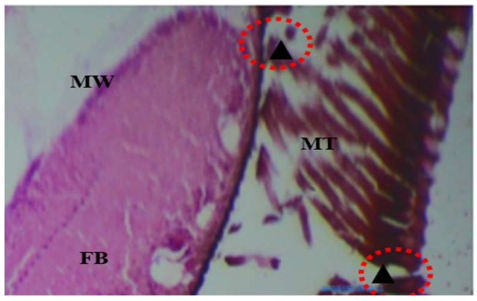
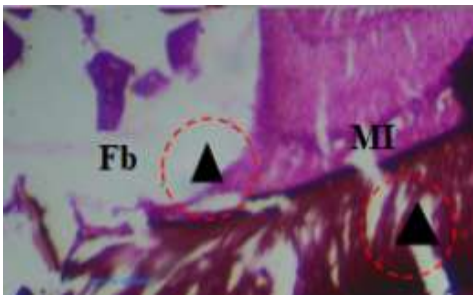


Fig. 15(a-d): Microscopic view of cypermethrin induced alterations in Abdominal region of *A.mellifera* showing Posterior abdomen (PA), Poison gland (PG), Muscular tissue, damage to reproductive organs (Rep), reduced oocyte cells (O), degeneration of follicular layer (Fl), Nurse cells, Venom(V), Sting apparatus (SA), Accessory glands, Midgut wall, Fat body cells (FB) and Malphagian tubules (M)



(b)

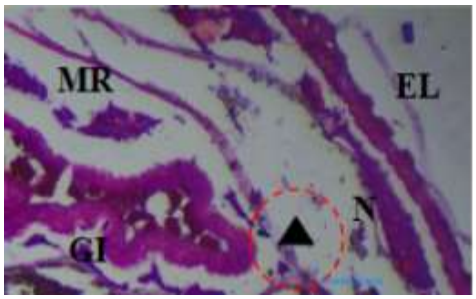
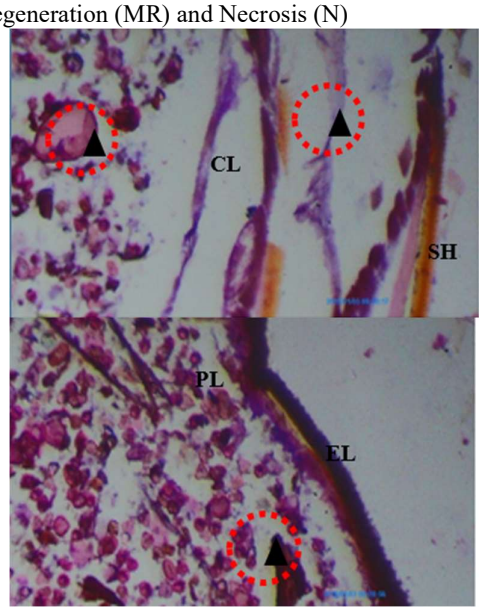
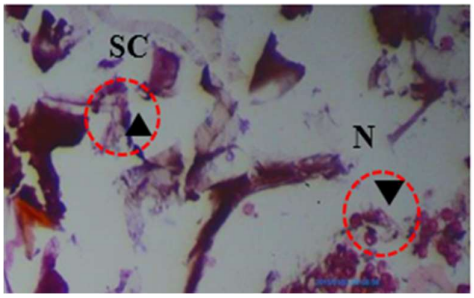


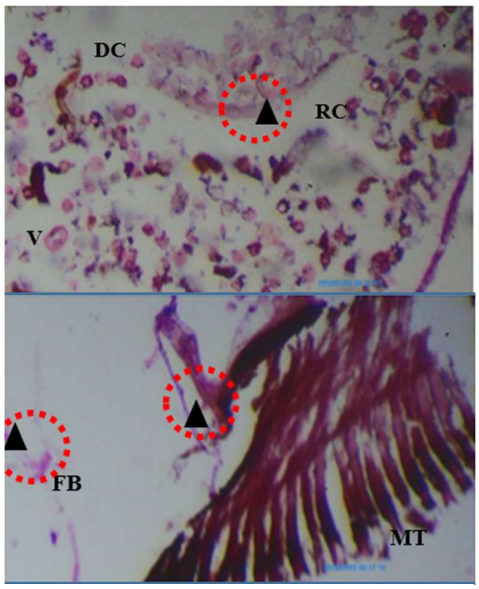
Fig. 16(a-b): Microscopic view of Round up induced alterations in *A.mellifera* abdominal region showing Posterior Abdomen (PA), Hindgut rectum (HG), Epithelial lining damage (Ed), Tracheal lining disorganized (Tl), Fat cells (Fc), Fat bodies (Fb), Malphagian tubules Impairment (MI), Epithelial lining damage, Gut lumen (GL), Muscle fiber regeneration (MR) and Necrosis (N)



(a)

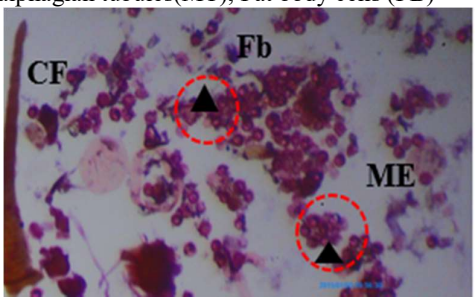


(c)



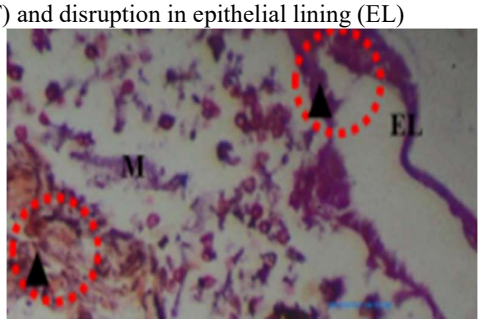
(d)

Fig. 17(a-d): Microscopic view of imidacloprid induced alterations in abdominal region of *A. mellifera* showing Midgut (MG), ventriculus (V), disintegration of epithelial lining (EL), separation of peritrophic membrane (P), chitinous layer (Cl), Sensory hairs (SH), leg region(L), appendage cuticle (AC), digestive cells(DC), regenerative cells(RC), Malphagian tubules(MT), Fat body cells (FB)

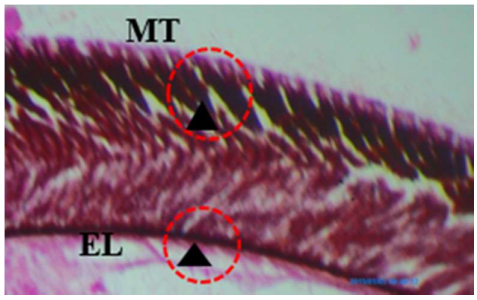


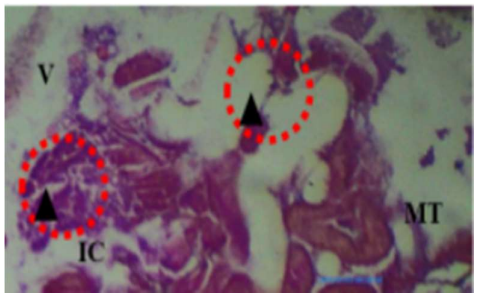
(a)

Fig. 18(a-b): Microscopic view of Lambda-cyhalothrin induced alterations in Abdominal region of *A.mellifera* showing Cuticle fragment (CF), Fat body tissue (Fb), Midgut epithelium (ME), Tracheal structure (TS), Vacuolization (V), Structural collapse (SC), Necrosis (N), Malphagian tubules (MT) and disruption in epithelial lining (EL)



(b)





(c)

(d)

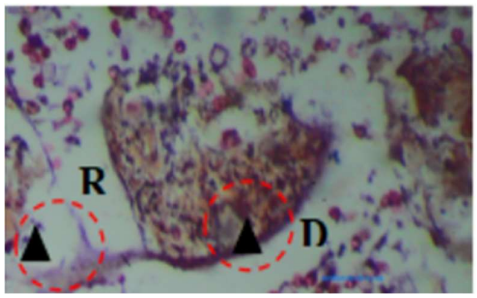


Fig. 19(a-d): Microscopic view of chlorpyrifos induced alterations in abdomen region of *A. mellifera* showing midgut (M), epithelial lining disruption, Striated muscles (SM), Vacuolization (V), Immune cell infiltration (IC), Malphagian tubules (MT), Damage to reproductive organs(R) and degradation of gut lumen (D)

Any small invisible damage to honeybee's body by these poisons in environment eventually effect its survival, lessen its immune system and affected overall loss of pollinators in nature and reduced yield of natural food i.e. honey.

Discussion

Hussain et al. (2014) found that the LT50 values for imidacloprid and chlorpyrifos were 4 and 6 hours at 1000 ppm concentration. According to Saad et al. (2023), emamectin benzoate had the highest level of toxicity (LC50=0.247ppm), followed by imidacloprid, chlorpyrifos, indoxacarb, thiophanatemethyl, and lambda- cyhalothrin (LC50 values of 2.43, 10.22, 14.4, 96.9, and 829.4 ppm). Glyphosate had the lowest level of toxicity (LC50 = 6861.151 ppm). When compared to other pesticides, chlorpyrifos is comparatively toxic to bees. According to earlier research by Sanchez-Bayo and Goka (2014), and Dai et al. (2019), chlorpyrifos has a high acute oral toxicity to honeybees. Similar histopathological changes were also reported by Ibrahim et al. (2023) in the brain of *A. mellifera* treated with sulfoxaflor that showed, most neuronal cells had nuclear pyknosis, cuticle and epithelial cell degeneration, vacuolization, and neuronal loss. According to Pervez and Manzoor (2021), *A. mellifera*'s muscles and nerve activity have been significantly impacted by insecticide exposure. Serra et al., 2021 examined the midgut region of honeybees that is of extreme importance in study of ecotoxicology, imidacloprid and lambda-cyhalothrin exposed midgut region showed disintegration of epithelial cells, cytoplasmic vacuolization and breakdown of peritrophic membranes. Moreira et al. (2025) found that samples from the midgut epithelial cells of *A. mellifera* showed a high degree of pathological alterations, including nuclear degeneration, oxidative stress, and cytoplasmic changes, when examined ultrastructurally. The current study's findings are consistent with those of Oliveira et al. (2024), who found that the midgut epithelium displays high levels of vacuolization, spherocrystals, apocrine secretion, nuclear pyknosis, cell fragments released into the organ lumen, loss of cell-cell contact, and damage to the peritrophic matrix and regenerative cell nests.

Conclusion

It can be concluded from the present research work that all the tested insecticides proved to be lethal to honeybee specie; *Apis mellifera*. The results showed that Chlorpyrifos insecticde was more toxic to *Apis mellifera* at all tested concentrations. Whereas, Cypermethrin was proved to be probably less toxic insecticide to *Apis mellifera*. Histopathological analysis revealed a clear impact of toxicity of tested insecticides on the cells and tissues of brain, thorax and abdominal region of *Apis mellifera*. Structural abnormalities, degenerative changes and lessions were observed in result of insecticides exposure that contribute to decline in population of honeybees.

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