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RESEARCH ARTICLE

Comparative valuation of the chlorpyrifos, acetamiprid, and lambda-cyhalothrin toxicity and their hematological and histopathological consequences in pigeons

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Abstract
Globally agrochemicals are widely used in the agricultural sectors, posing potential eco-toxicological risks and disrupting various lifeforms including birds. Thus, the current work was conducted to compare the acute toxic impacts of pesticides (e.g., chlorpyrifos, acetamiprid, and lambda-cyhalothrin) on the pigeon's health. In total 50 adult pigeons were purchased from a local market where these pigeons were fed on pollution-free food. Post adaptation period (15 days), the pigeons were arbitrarily separated into five distinct groups after having been identified in this manner by chance (each group containing 10 pigeons). Control group (group 1) was not treated with any pesticide while the remaining groups (groups 2, 3, and 4) were treated with 0.25-mg/kg body weight of chlorpyrifos, acetamiprid, lambda-cyhalothrin, and a mixture of all three pesticides (group 5), respectively. After 36 days of exposure, the groups that had been exposed to the pesticide showed a significant ($p < 0.05$) increase in both the total number of platelets and the number of white blood cells (WBCs), in comparison to the control group. On the other hand, the groups that were exposed to the insecticides had significantly lower levels of red blood cells (RBCs), hemoglobin (Hb), and packed cell volume (PCV) ($p < 0.05$). The value of mean corpuscular volume (MCV) was significantly ($p < 0.05$) reduced in acetamiprid-exposed group, while a significant increase was observed in other pesticide-exposed groups. Obvious histopathological changes were observed in the tissues of control group and no such changes were reported by control group. Necrosis, pyknosis, lymphocyte infiltration, congestion of blood, dissolution of plasma membrane, and vacuolation were observed in the livers of pesticide-treated pigeons. The intestinal study showed the formation of goblet cells, villi rupturing, degeneration of serosa, necrosis, and pyknosis in treated groups. Renal alterations, dilation of renal tubules, reduction of glomerulus tissue, and edema were observed. This study manifests that the uncontrolled use of pesticides impairs ecosystems and poses a substantial health risk to wildlife and ultimately to human.

Keywords Pigeon · Pesticides · Histopathology · Hematology · Red blood cell · Packed cell volume

Introduction

The rock dove, also known as domestic pigeon (*Columba livia*), is the most common and widely distributed bird species. Morphologically, it is the most diverse bird species with over 350 breeds and classified within the clade columbiformes, which includes nearly 44 genera and 315 species of pigeons (Young et al. 2017). Among diverse species of birds, doves have a long link with human culture. They are mostly kept for meat production and racing hobbies by small farms, and some houses in upper Egypt, as a source of protein for the local population (Moye and Pritsos 2010).

The use of pesticides in agricultural sector for the growth of crops is a common practice (Mahmood et al. 2016). The use of pesticides makes it possible to get rid of insects, bacteria, fungi, and other creatures that feed on crops and cause harm by devouring them. Since quite a few years ago, pesticides and insecticides have been utilized to a large degree for the goal of protecting crops from pests. Even while the use of pesticides is beneficial to the crops, there is still a chance that it will have a negative impact on the surrounding

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Extended author information available on the last page of the article environment. Insecticide toxicity is a global problem occurring especially in developing nations (Alla-Eldin et al. 2016). Recently, indiscriminate and widespread use of these chemicals in agriculture sector to meet increased food demand is alarming and getting more attention to address pesticides' potential eco-toxicity. Pesticide residues have been found in dairy, meat products, food products, soft drinks, and water (Soliman et al. 2015). The presence of potentially harmful compounds in the environment poses a threat to the viability of a wide variety of living organisms, including birds and aquatic life, which puts their ability to continue existing in a jeopardy. Concerns regarding the ability of the environment to maintain its balance and good health over the long term have been raised by a significant number of individuals in relation to the use of pesticides. An excessive use of pesticides is one of the primary causes of environmental pollution, which not only has short-term consequences but also has long-term repercussions on the ecology of the region that is damaged (Poudel et al. 2020).

Chlorpyrifos is a broad-spectrum organophosphate insecticide frequently used in controlling insects in domestic and agricultural sectors worldwide. It is class II insecticide, which is poisonous to most animal' species because the amino acid sequence of acetylcholinesterase is highly conserved in animals, while changes in toxicokinetic (adsorption, distribution, metabolism, and excretion) account for variances in sensitivity among taxa (Giesy et al. 2014). Other mode of action includes oxidative stress (Alla-Eldin et al. 2016). Birds, wild animals, animals used in agriculture, and pets and livestock kept in the home can all be harmed by chlorpyrifos. Acetamiprid is a member of neonicotinoid synthetic chlorinated insecticide family that has been recently introduced in the market (Annabi et al. 2019; Hedau et al. 2018). It acts as agonists on the nicotinic acetyl choline receptors and causes excitation through interruption of synaptic transmission (Annabi et al. 2019). Acetamiprid is used against insects that have gained resistance to organophosphate, carbamate, and synthetic pyrethroid (Annabi et al. 2019; Shengyun et al. 2005). Acetamiprid is considered less harmful to mammals, but due to low molecular weight and higher water solubility, can enter in plants and increased exposure to non-target organisms, which ultimately contaminate the food chain (Annabi et al. 2019). Lambda-cyhalothrin is type II synthetic pyrethroids (SPs) and used as a replacement for more toxic organophosphorous to control a wide range of insect for a variety of crops. SPs have high hydrophobicity and are distributed preferentially into lipid-rich internal tissues, especially brain and fat tissues, and may bioaccumulate in non-target organisms at higher trophic levels and cause toxic effects (Chang et al. 2016). Lambda-cyhalothrin disrupts the normal functioning of the nervous system in an organism, which leads to the paralysis or death of insect (Chang et al. 2016). The lambda-cyhalothrin kills variety of lepidoptera, hemiptera, diptera, and coleoptera. In Pakistan, it is generally used to treat *Chilo partellus* (Ahmad 2007).

Even though the pesticides are intended to kill insects, it is possible for non-target animals to get poisoned if they consume pesticide-contaminated insects or plant debris and/or devour exposed bait containers. Therefore, caution is required to be exercised while setting house baits since it is possible for children and pets to swallow pesticides (Nicolopoulou-Stamati et al. 2016). It is possible that a single encounter may have far-reaching consequences such as breathing difficulties, hypersalivation, miosis (constricted pupils), frequent urination, diarrhea, vomiting, and colic. Breathing difficulties are caused by increased bronchial secretions and bronchoconstriction. Tremors of the skeletal muscles can cause a variety of symptoms, including weakness, loss of coordination, and even convulsions in the affected animal (McGill et al. 2008). Acute poisoning can be identified by several symptoms, including difficulty breathing, unconsciousness, and paralysis of the muscles that control respiration. Moreover, exposure to OPs leads to brain and skeletal muscle damage, organ and system failure, and cardiovascular, pulmonary, hepatic, reproductive, developmental, and immunological system issues. Organophosphate insecticides have negative behavioral, reproductive, and developmental impacts on birds and slowing their growth. Other alterations seen in different birds include endocrine disturbance, nervous abnormalities, gait disorders, immune reaction deficiency, oxidative stress, and tissues lesions in various organs (Suliman et al. 2020).

Hematological profiles are an important indicator of an individuals' physiological state in both human and animal science. Several distinct hematological measurements include red blood cell (RBC) and white blood cell (WBC) counts, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hematocrit, as well as serum biochemical measurements of glucose, cholesterol, total protein, albumin, globulin, alanine aminotransferase (ALT), alkaline phosphatase (ALP), and aspartate aminotransferase (AST). It was determined that red blood cells (RBCs) and other parameters such as hemoglobin (Hb) and platelets of birds vary among species; other factors influencing the counts include breed, sex, and nutrition provided to the birds. Specially in birds, hematology assessment was being used for assessing the state of health and nutrition, identification of disease, prediction of the course of disease, and the efficacy of therapeutic intervention (Orakpoghenor et al. 2021).

Histopathology is a quick and accurate method for detecting pesticide effects in various animal tissues and organs. Most farmers in developing countries, such as Pakistan, are unconscious of pesticide hazards, and they commonly use such hazardous pesticides without following any safety measures or guidelines. Furthermore, used pesticide containers are dispose of in the environment, affecting non-target vertebrates and invertebrates (Boumezrag et al. 2021). This study was conducted (i) to investigate the comparative effects of chlorpyrifos, acetamiprid, and cyhalothrin on histopathological and hematological parameters and (ii) to assess the toxicity of selected pesticides on histology of liver, kidney, and intestine of pigeons.

Materials and methods

Experimental design

Studied pesticides such as chlorpyrifos, acetamiprid, and lambda-cyhalothrin used in this study were purchased from a local farm supply store. In total 50 healthy and mature pigeons (90 ± 10 days) were bought from a local market (34°20′2″N, 73°11′46″E) to evaluate the toxicity of chlorpyrifos, acetamiprid, and lambda-cyhalothrin to these birds. The pigeons were kept in neat and clean (washed with Dettol water) wooden wired cages under controlled conditions, vaccinated, and acclimatized for 15 days. During acclimatization no damage or death was recorded in pigeons. Prior to the commencement of the experimental work, their body weights (250 ± 15 g) were recorded. Thereafter, pigeons were randomly divided into five groups (1–5), each group containing 10 pigeons, and were kept in different cages. Group 1 birds were treated as control while remaining four groups, namely, group 2, group 3, group 4, and group 5, were treated with individual pesticides and a mixture of all three pesticides, respectively. The control group's pigeons did not have any contact with any pesticide that was being evaluated. Whereas 0.25-mg/kg body weight of chlorpyrifos, acetamiprid, and lambda-cyhalothrin was given to groups 2, 3, and 4, and mixture of all three pesticides at level of 0.25-mg/kg body weight was given to group 5. The insecticide exposure experiment was conducted for 36 days. Pigeons were fed on grains, seeds, and other items and were given access to clean drinking water following standard procedures. All the experimental works were performed according to the protocol approved by ethical committee of Hazara University.

Hematology

After 36 days of the trial, blood samples were collected from the pectoral vein of birds under observation. Pigeons were selected at random from each of the five groups to get blood samples from pectoral veins and collected blood samples were transferred to pre-labeled EDTA-containing tubes. Exactly, 2 mL of blood was collected using sterile disposable syringes coated with heparin. The hematological parameters of collected blood samples were determined following standard hematological techniques. Collected blood samples were examined to identify the red blood cells (RBCs), white blood cells (WBCs), hemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and platelet values using a hematological analyzer (Ghayyur et al. 2021).

Histopathology

Histological investigations (necrosis, pyknosis, lymphocyte infiltration, congestion of blood, dissolution of plasma membrane, vacuolation, formation of goblet cells, villi rupturing, degeneration of serosa, dilation of renal tubules, reduction of glomerulus tissue, and edema) were carried out for both control and pesticide-treated groups. The intestines, liver, and kidneys were immediately removed from pigeon body after slaughter and were rinsed with 0.5% NaCl solution to remove blood, mucus, waste, and other unwanted material. The washed tissues were fixed in 10% formaldehyde solution for 24 h (Ghayyur et al. 2021). Fixed tissues were rinsed and thereafter, dehydrated through a graded series of ethanol. The dehydrated tissues were cleared in xylene and then infused with paraffin wax. The waxed tissues were then sliced into 5-μm-thick sections using microtome (rotary microtome Rmt-SA390) and picked up on glass slides. The sections were then treated with xylene to remove paraffin wax, rehydrated through decreasing concentration of ethanol, stained with hematoxylin and eosin, dried, and fixed on slides using Canada balsam and cover slips. The stained tissues were studied under an Olympus optical microscope and photographed with an optika b3 digital camera at 40× and 100× magnification.

Statistical analysis

Statistical analyses were performed using SPSS 24.0 software (SPSS Inc., Chicago, IL). One-way ANNOVA test was performed to compare all the experimental groups, and the *t*-test was used to compare the control and experimental groups. The data was presented as mean ± SD and level of significance was $p < 0.05$.

Results

Behavioral study of pigeons

All birds were closely observed for behavioral changes as summarized in Table 1. Pigeons belonging to control group did not show any behavioral change throughout the experimental work. Birds of this group were quite active and had the maximum frequency of cooing sounds puffing

Table 1 Summary of sign and stress induced by selected pesticide treatment in pigeons

Symptoms	Control (G-1)	Chlorpyrifos (G-2)	Acetamiprid (G-3)	Lambda-cyhalothrin (G-4)	Mixture (G-5)
Drooping of wings	-	***	**	*	***
Diarrhea	-	**	**	*	***
Excessive salivation	-	*	*	**	***
Lethargy	-	**	**	*	**
Rate of mortality	-	***	**	-	***

- without any symptoms; * mild symptoms; ** moderate symptoms; *** severe symptoms

and preening. On the other hand, many pigeons that were treated with insecticides exhibited signs of anxiety. Additionally, other observed symptoms in insecticide-exposed pigeons were drooping wings, diarrhea, excessive salivation, and lethargy. Maximum of these symptoms were observed in pigeons exposed to the mixture of pesticides followed by chlorpyrifos, acetamiprid, and lambda-cyhalothrin.

Hematology and blood parameters

This study investigated hematological variations in pigeons after treating with studied insecticides at long-term (36 days) exposure. Significantly, varied hematological parameters were observed among various groups. These results depicted a rise in the number of total platelets as well as total leukocytes in bloods of the pigeons—exposed to individual insecticides and their mixture. All hematological parameters are summarized in Table 2 and Fig. 1. The highest red blood cell count was observed in control group, whereas group treated with mixture of insecticides had least RBC count. Similarly, hemoglobin and packed cell volume significantly decreased in treated groups compared with control group.

Total leukocyte count

The insecticide treatments displayed significant ($p < 0.05$) variations in total leukocyte count (TLC) with those reported for control group of pigeons (Fig. 1A). Leukocyte count significantly increased in the treated groups, compared with control group. The TLC increased in the following order: mixture of insecticides > chlorpyrifos > lambda-cyhalothrin > acetamiprid (Table 2).

Red blood cell count and hemoglobin

Selected pesticide treatment displayed significant variations ($p < 0.05$) in the erythrocyte number among various groups of studied pigeons. RBC values significantly reduced in pesticide-treated groups compared with the RBC values reported for control group (Fig. 1B). The least RBC count was observed in birds treated with a mixture of three pesticides and then for lambda-cyhalothrin-treated birds, and acetamiprid showed least effect on RBC count. The overall pesticides' effect on RBCs decreased significantly across the treated group as mixture of pesticides > lambda-cyhalothrin > chlorpyrifos > acetamiprid.

Table 2 A summary of hematological parameters of control and insecticide-treated pigeon groups

Parameters	Control	Chlorpyrifos	Acetamiprid	Lambda-cyhalothrin	Chlorpyrifos + acetamiprid + lambda-cyhalothrin
TLC (10^3/μL)	242.16 ± 16.15	316.63 ± 9.84^{**a}	277.13 ± 9.24^{*b}	304.53 ± 13.88^{**c}	356.13 ± 13.15^{**d}
RBCs (×10^6/μL)	6.12 ± 0.125	4.54 ± 0.41^{**a}	5.49 ± 0.36^{*b}	3.21 ± 0.22^{**c}	2.42 ± 0.30^{**d}
Neutrophils (%)	23.00 ± 1.00	42.00 ± 1.00^{**a}	31.00 ± 1.00^{**b}	31.00 ± 2.00^{**c}	40.33 ± 1.52^{**d}
Lymphocytes (%)	36.00 ± 1.00	17.00 ± 1.00^{**a}	28.00 ± 1.00^{**b}	22.00 ± 1.00^{**c}	18.33 ± 1.52^{**d}
Monocytes (%)	41.00 ± 1.00	41.00 ± 2.00^{a}	41.00 ± 1.00^{b}	47.00 ± 1.00^{**c}	41.33 ± 1.52^{d}
Hb (g/dL)	18.39 ± 0.84	11.72 ± 0.85^{**a}	13.55 ± 0.86^{**b}	14.43 ± 0.75^{**c}	10.72 ± 0.79^{**d}
PCV (%)	32.60 ± 3.08	25.53 ± 1.97^{*a}	22.66 ± 2.85^{b}	19.23 ± 1.42^{**c}	17.63 ± 2.01^{**d}
Platelets(×10^3/μL)	88.10 ± 6.81	136.30 ± 8.11^{**a}	89.26 ± 6.44^{b}	108.70 ± 7.90^{*c}	162.00 ± 8.51^{**d}
MCV (fL)	53.16 ± 4.06	58.56 ± 3.73^{a}	39.66 ± 4.14^{*b}	59.83 ± 1.78^{*c}	72.70 ± 3.29^{**d}
MCH (pg)	30.00 ± 0.80	25.80 ± 0.51^{**a}	23.73 ± 1.01^{**b}	45.13 ± 4.92^{**c}	44.33 ± 2.30^{**d}
MCHC (g/dL)	56.56 ± 3.16	44.10 ± 2.02^{**a}	60.19 ± 5.30^{b}	75.43 ± 9.56^{*c}	60.93 ± 2.83^{d}

Single asterisk (*) indicates a significant difference ($p < 0.05$) between the control and treatment groups while alphabets (a, b, c, and d) indicate significant difference among treated groups ($p < 0.05$)

The double asterisks indicate highly significant difference (**$p < 0.01$)

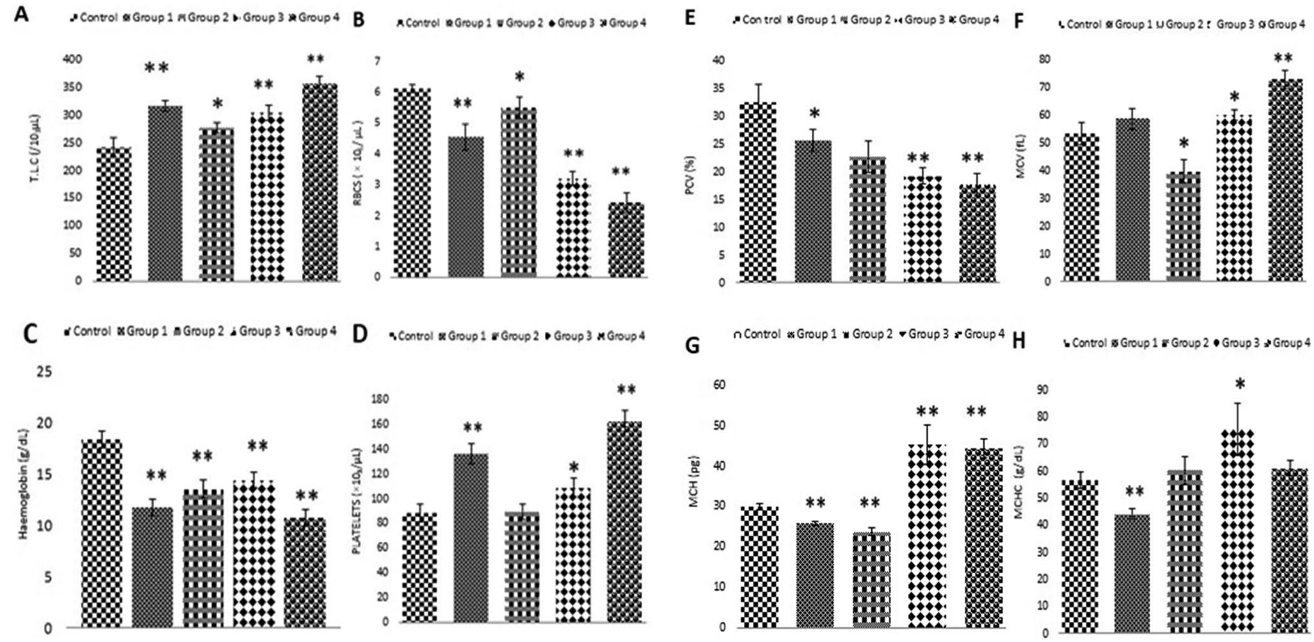

Fig. 1 **A–H** The comparative effect of three pesticides (chlorpyrifos, acetamiprid, and lambda-cyhalothrin) and distinction with control group on blood parameters of pigeon. Values are expressed as mean ± SE from triplicate groups. Bars with single asterisk (*) indicate significant (at $p < 0.05$) differences while bars with double asterisks (**) indicate highly significant differences between control and treated groups

The hemoglobin significantly ($p < 0.05$) reduced in treated birds compared with control group. The Hb varied from 10.72 ± 0.79 (treated with mixture of insecticides) to 18.39 ± 0.84 (control group). The striking decrease in Hb was recorded in birds exposed to a mixture of insecticides followed by chlorpyrifos, acetamiprid, and lambda-cyhalothrin (Fig. 1C).

Platelets

Selected insecticides' treatments displayed significant variations in the platelets number among the studied groups. With the exception of acetamiprid-treated birds, all other insecticides' treatments showed that platelet count increased significantly ($p < 0.05$) (Fig. 1D). The platelets were the highest (162.00 ± 8.51) in birds treated with a mixture of insecticides and the least (88.10 ± 6.81) platelet count was reported for control group.

Packed cell volume

Like many other hematological parameters, the PCV significantly ($p < 0.05$) varied among studied groups (Fig. 1E). However, in acetamiprid-treated birds, the PCV insignificantly decreased compared with birds that belong to control group. The highest decrease in PCV percentage was observed in birds that belong to a group exposed to a mixture of insecticides, and this decrease was almost equal to the decrease observed for lambda-cyhalothrin. The decrease in PCV percentage among treated birds was as follows: mixture of insecticides > lambda-cyhalothrin > acetamiprid > chlorpyrifos.

Mean corpuscular volume

Treatments with selected insecticides displayed comparatively insignificant (for chlorpyrifos) and significant (for lambda-cyhalothrin, acetamiprid, and mixture of insecticides; $p < 0.05$) variations in the MCV value (Fig. 1F). With exception of acetamiprid all other treated groups showed elevated levels of MCV compared with control group. The significant increasing level of MCV among treated groups was mixture > lambda-cyhalothrin > chlorpyrifos > acetamiprid.

Mean corpuscular hemoglobin

The MCH values of insecticide-treated groups significantly differed for each treated group and also were significantly different than those reported for control group (Fig. 1G). In case of lambda-cyhalothrin- and mixture of insecticide-treated groups, the MCH values significantly increased, whereas for chlorpyrifos and lambda-cyhalothrin, these values significantly decreased compared with control group. The MCH values at significance level ($p < 0.05$) were distributed as lambda-cyhalothrin > mixture of insecticides > chlorpyrifos > acetamiprid.

Mean corpuscular hemoglobin concentration

On treating with selected individual insecticides and their mixture the MCHC displayed both insignificant and significant variations compared with control group (Fig. 1H). The increase in MCHC values was observed in three treated groups, namely, acetamiprid, lambda-cyhalothrin, and mixture of insecticides, while chlorpyrifos-treated group showed decrease in comparison with control group. It was only chlorpyrifos- and lambda-cyhalothrin-treated groups which showed significant variation with control group, whereas other two treated groups presented insignificant variations. The MCHC decreasing order is as lambda-cyhalothrin > mixture of insecticides > acetamiprid > chlorpyrifos.

Histopathological alterations

A brief histological description of the abnormalities that may be observed in the pigeon's liver, kidneys, and intestines is shown in Table 3. A microscopic examination of the liver revealed polymorphic cells with fatty degeneration infiltrating them as well as congestion in the blood vessels. The intestinal tract had necrotic mucosa, and lamina propria included isolated aggregations of eosinophils amidst the necrotic intestinal glands. Similar observations somewhat corroborated in kidney.

Hepatic histopathology

Liver section of control group pigeons revealed hepatocytes containing spherical, large centrally placed nucleus with homogeneous cytoplasm. In treated birds most abundantly occurred alterations were hypertrophy of hepatocytes, necrosis, congestion of blood, and deterioration of hepatocytes (Table 3). In the liver tissues of chlorpyrifos-exposed pigeons, necrosis, disruption of the plasma membrane, pyknosis, lymphocyte infiltration, congestion of blood vessels, foamy cells, and cytoplasmic vacuolation were observed. It was discovered that the birds exposed to acetamiprid had undergone a variety of alterations such as blood congestion, necrosis, lymphocyte infiltration, foamy cell formation, and vacuolation. The hepatocytes of the lambda-cyhalothrin-treated birds showed the evidence of necrosis, pyknosis, cytoplasmic vacuolation, disintegration of the plasma membrane, and hypertrophy. The birds group exposed to a mixture of insecticides were effected markedly and showed the greatest number of changes, which included modifications,

Table 3 Concise histopathological variations in the liver, kidney, and intestine of pigeon

Histopathological alteration	Control group	Chlorpyrifos	Acetamiprid	Lambda-cyhalothrin	Mixture
Liver					
Necrosis	-	***	**	**	***
Pyknosis	-	**	**	*	**
Lymphocyte infiltration	-	**	***	-	*
Plasma membrane dissolution	-	**	-	*	**
Vacuolation	-	*	*	**	**
Congestion of blood	-	***	***	-	***
Deterioration of hepatocytes	-	**	**	*	***
Hypertrophy of hepatocytes	-	***	***	***	***
Intestine					
Formation of goblet cell	-	***	***	*	***
Villi rupturing	-	***	**	*	***
Necrosis	-	**	***	*	**
Pyknosis	-	**	*	*	**
Vacuolation		**	*	*	-
Destruction of serosa	-	-	***	-	-
Kidney					
Congestion of blood	-	*	***	**	**
Dilation of renal tubules	-	***	**	*	***
Edema	-	**	**	*	*
Vacuolation	-	**	**	*	**
Reduction of glomerulus tissue	-	**	***	*	**

Rare (*), frequent (**), abundant (***), absent (-)

severe blood congestion, necrosis, lymphocyte infiltration, pyknosis, vacuolation, plasma membrane collapse, and degeneration of hepatocytes. In addition, the birds exposed to a mixture of insecticides were the one that had the greatest number of changes followed by chlorpyrifos = acetamiprid and lambda-cyhalothrin (Fig. 2).

Kidney histopathology

A microscopic examination of renal tissue revealed that the structure of nephron had not changed in control group (Fig. 3). The renal nephron comprises glomerulus, podocyte, uriniferous tubule, and cellular features of inter-renal tubules. Kidney tissues of pigeons, which were exposed to chlorpyrifos solution, showed blood congestion, vacuolation, and dilation of renal tubules. In acetamiprid-exposed birds, the histopathological changes observed in kidney tissue were blood congestion, vacuolation, cell degeneration with edema, reduction of glomerulus tissue, and dilation of renal tubules. Histopathological changes observed in lambda-cyhalothrin group were blood congestion, reduction of glomerulus tissue, edema, and dilation of renal tubules. In a mixture of insecticide group, the observed histopathological changes were hypertrophy, severe cell disintegration, severe degeneration in uriniferous tubules, blood congestion, and dilation of renal tubules.

Intestine histopathology

Pigeon intestinal tissues were subjected to a microscopic inspection, and the results showed that the villi structure in the control group did not change (Fig. 4). Villi rupturing, development of goblet cell, vacuolation necrosis, and pyknosis were some of the alterations that were seen in the intestinal tissues of pigeons that were exposed to chlorpyrifos. Variations that were seen in the acetamiprid-exposed pigeons' group included the production of goblet cells, the destruction of serosa, vacuolation, necrosis, and psychosis. Villi rupturing, development of goblet cells, necrosis, and pyknosis were some of the alterations that were seen in the lambda-cyhalothrin group. Histopathological changes such as villi rupturing, necrosis, pyknosis, and fusion of villi and development of goblet cell were also observed in the mixture group.

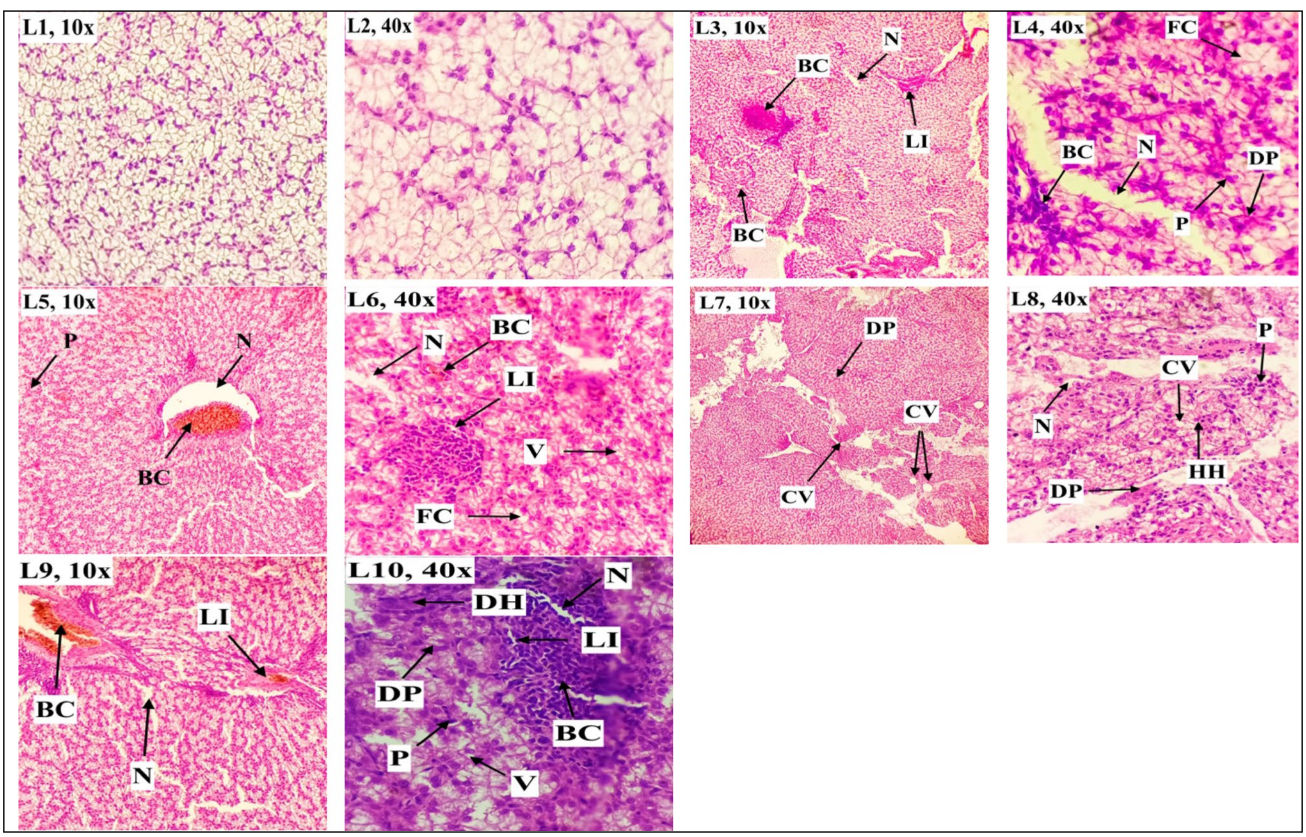

Fig. 2 L1 and L2 Pigeon hepatic tissue of control group. L3 and L4 Pigeon hepatic tissue treated to chlorpyrifos. L5 and L6 Pigeon hepatic tissue treated to acetamiprid. L7 and L8 Pigeon hepatic tissue treated to lambda-cyhalothrin. L9 and L10 Pigeon hepatic tissue treated to mixture: necrosis (N), plasma membrane dissolution (DP), infiltration of lymphocytes (LI), pyknosis (P), congestion of blood (BC), hyperplasia of hepatocytes (HH), cytoplasmic vacuolation (CV), and deterioration of hepatocytes (DH)

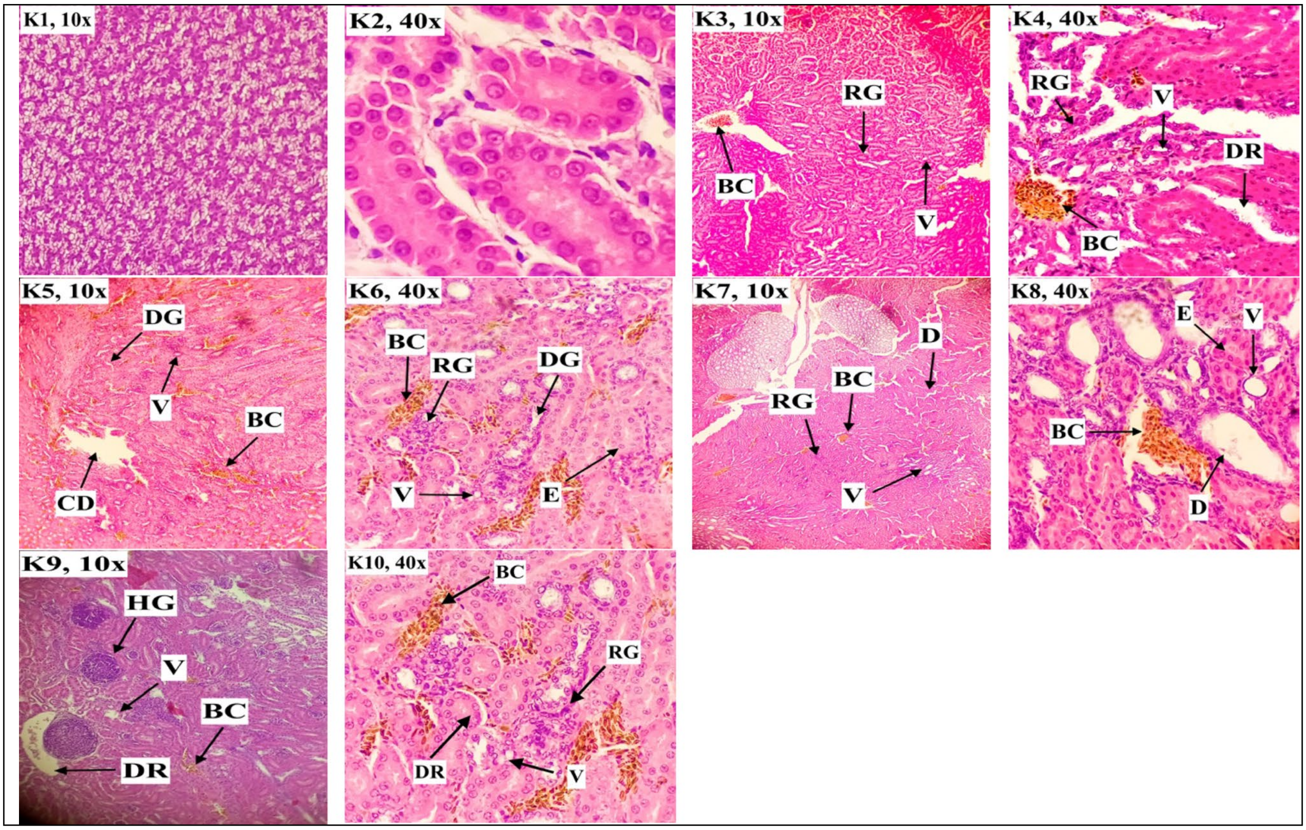

Fig. 3 K1 and K2 Pigeon kidney tissues of control group. K3 and K4 Pigeon kidney tissue exposed to chlorpyrifos solution. K5 and K6 Pigeon kidney tissue exposed to acetamiprid solution. K7 and K8 Pigeon kidney tissue exposed to lambda-cyhalothrin. K9 and K10 Pigeon kidney tissue exposed to mixture solution: blood congestion (BC), dilation of renal tubules (DR), reduction of glomerulus tissue (RG), vacuolation (V), edema (E), and cell degeneration (CD)

Discussion

A variety of agrochemicals including insecticides and herbicides are frequently used in agricultural fields to protect the cereals and vegetables which present potential risk to the farmers, environment, and all other livings present in the same ecosystem. This study investigated the effects of selected insecticides, namely, chlorpyrifos, acetamiprid, lambda-cyhalothrin, and a mixture of all these insecticides, on behavior, hematology, and histopathology of pigeons. Evaluation of toxicological and safety effects of pesticides is of vital importance because these chemicals caused male infertility, gonadotoxic effects, reduce testosterone level, chromosomal aberrations, fetal malformations, and cancers (Hussain et al. 2014). The pigeons in the control group were observed to be normal and no mortality was recorded in these birds, whereas in insecticide-exposed pigeons several abnormalities were observed. The observed symptoms after exposure were drooping wings, diarrhea, depression, decreased frequency of cooing, excessive salivation, and lethargy. The chlorpyrifos converts to chlorpyrifosoxon inside the body of living organisms. This oxon metabolite elicits AChE inhibition, leading to the acetylcholine buildup in the cholinergic receptors, hence cause cholinergic toxicity (Eaton et al. 2008). Thus, the clinical and behavioral signs observed in the current study could, therefore, be linked to the cholinergic toxicity caused by metabolites of the selected pesticide. Our results were in line with those previously reported for Japanese quill of exposure to pesticides (Hussain et al. 2014). Similar behavioral changes were also testified earlier in the male Japanese quill of exposure to lambda-cyhalothrin and chlorpyrifos (Boumezrag et al. 2021; Suliman et al. 2020). Previously, feeding of chlorpyrifos and acetamiprid also caused behavioral abnormalities in fish species (Ghayyur et al. 2021).

Even though there is a large variation in the conventional values of RBC, WBC, and platelet count depending on the quality of the diet, age, weight, and other environmental factors, avian hematology is quite significant when it is used for diagnostic purposes. Pesticides especially organophosphate are one of the most toxic pesticides to birds, as it elevated the risk of mortality (Etterson et al. 2017). Hematological findings in the present study showed significant decrease in erythrocyte counts, hemoglobin concentration packed cell

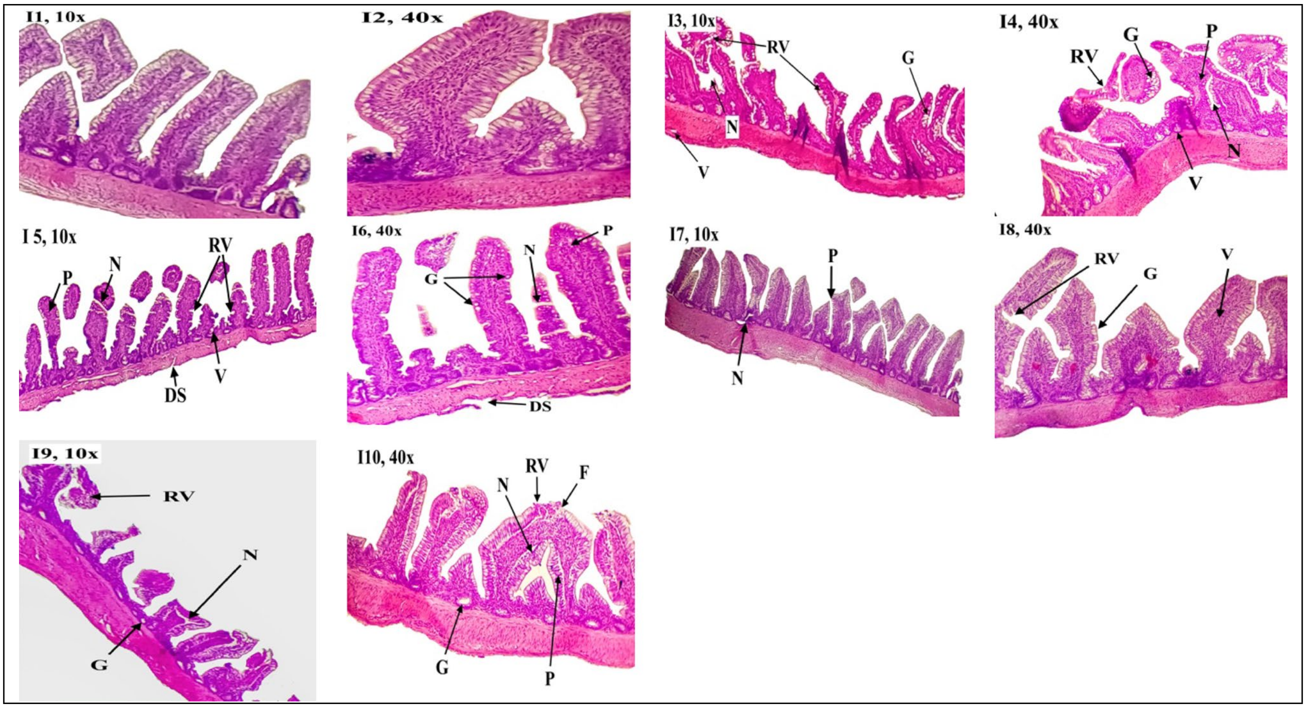

Fig. 4 I1 and I2 Pigeon intestine tissue of control group. I3 and I4 Pigeon intestine tissue of chlorpyrifos group. I5 and I6 Pigeon intestine tissue of acetamiprid group. I7 and I8 Pigeon intestine tissue of lambda-cyhalothrin group. I9 and I10 Pigeon intestine tissue of mixture group: rupturing of villi (RV), necrosis (N), pyknosis (P), goblet cell formation (G), vacuolation (V), fusion (F), and destruction of serosa (DS)

volume (PCV), mean corpuscular hemoglobin, and hematocrit percent. The decline in erythrocyte counts could be due to the toxic effects of insecticides on circulating erythrocytes and the blood-forming tissues. Hemoglobin concentration was also decreased, which could be attributed to reduction in feed intake and deficiency of iron. Similar results have also been reported in Japanese quill (Hussain et al. 2014). The decrease in hemoglobin levels could be due to toxic effects of insecticides on maturating erythrocytes and inadequate iron supply (Hussain et al. 2011; Karanthi et al. 2004) while decrease in hematocrit values could be due to increase destruction of erythrocyte or decrease in size (Rahman and Siddiqui 2006). In the current work, significant reduction in the value of Hb and RBCs were observed, which could be attributed to reduced heme production in the bone marrow, an elevation in the rate of destruction, or a fall in the rate of RBC creation (Ahmad et al. 2015). The reduction in RBC and Hb content could potentially be attributed to pesticides disrupting the erythropoietic tissue, affecting the viability of the cells (Fadina et al. 2017; Zhang et al. 2010). RBCs along with Hb play a key part in providing oxygen to various parts of the body, while PCV is the percentage of RBCs in the blood which depend upon the number as well as size of RBCs. Therefore, any change in the size and number of RBCs or Hb change the PCV percentage. Previously, decreased RBC count, Hb levels, and PCV were observed in chlorpyrifos- and acetamiprid-treated fish (Ghayyur et al. 2019; Ghayyur et al. 2021). The decrease in hematological parameters may also be due to reduced hemopoietin production, erythropoiesis, and impaired proliferation of hemopoietic progenitor cells or direct RBC lysis (Hussain et al. 2011; Salih et al. 2010).

In this study total leukocyte (WBC) and platelet counts significantly increased in insecticide-treated birds with maximum increase in birds treated with a mixture of insecticides. The rise in WBCs and platelets could be attributed to increased formation of antibodies, which help in survival of the pigeon exposed to different insecticides (Malik and Maurya 2014). Previously, insecticide-treated cockerels showed increased number of leukocytes to control tissue injuries induced by these insecticides (Hussain et al. 2021). Additionally, increased WBC and platelet counts were reported in Swiss albino mice on exposure to acetamiprid (Bagri et al. 2013). The hematological alterations in mean cell hemoglobin (MCH), mean cell volume (MCV), and mean cell hemoglobin concentration MCHC values also change because the calculation of these parameters depend on RBCs, Hb, and PCV values. The selected pesticides tested in this study had significantly increased MCH and MCV values. The increase in MCV and MCH values on treatment with pesticides designates that decrease in RBC count may be resulted from destruction of RBCs or their lessened synthesis in bone marrow. Our results are in line with those reported for Swiss albino mice and cockerels when exposed to acetamiprid and other trichlorfon (Bagri et al. 2013; Hussain et al. 2021). Similar observations were reported in *Oreochromis mossambicus* exposure to chlorpyrifos (Ghayyur et al. 2019) and *Cyprinus carpio* exposed to silver nanoparticles (Vali et al. 2020).

The findings of this study, which aimed to conduct a histological examination of tissue sections obtained from a variety of organs, proved beyond a reasonable doubt that the insecticides were responsible for at least some of the abnormalities that were observed in pigeons. Significant changes were brought about in several organs that are a part of the digestive and excretion systems because of exposure to the chemical compounds chlorpyrifos, acetamiprid, and lambda-cyhalothrin, either individually or in a mixture. These organs consist of the liver, intestine, and kidneys. The histopathological results demonstrated that the histopathological changes were mildly manifested in the birds treated with individual insecticides compared with changes observed in birds exposed to a mixture of these insecticides. Liver plays a significant role in many body functions such as homeostasis, detoxification, enzyme production, and metabolism, therefore serving as a reliable biomarker organ for eco-toxicological studies (Ghayyur et al. 2021; Sharma et al. 2019). The observed histopathological changes were necrosis, plasma membrane dissolution, lymphocyte infiltration, pyknosis, congestion of blood, hyperplasia of hepatocytes, cytoplasmic vacuolation, and deterioration of hepatocytes. Besides, hypertrophy, increased diameter of central duct, and an increased diameter of the pigeon liver were observed. These alterations were visible as a direct result of the long-term exposure of pigeons to selected insecticides and their mixture. These histopathological results are in agreement with those reported previously (França et al. 2017; Oliveira 2017). This study depicted that the combination of chlorpyrifos, acetamiprid, and lambda-cyhalothrin did, in fact, have a synergistic effect on liver of pigeons. Because of its role in the processes that are associated with detoxification and biotransformation, the liver is an organ that is frequently targeted by xenobiotic substances and gets affected adversely. Saoudi et al. (2021) reported the lethal effects of chlorpyrifos on liver of pigeon which included hypertrophy of hepatocytes, increased diameter of central duct, vacuolation, and deterioration of hepatocytes. Similar histopathological changes were also reported in male rates on exposure to lambda-cyhalothrin (El-Saad and Abdel-Wahab 2020). The degenerative changes of hepatocytes observed in current investigation are in agreement with those obtained for *C. carpio* and in female albino mice and rabbit on treatment with imidacloprid (El-Aziz et al. 2020; Saoudi et al. 2021; Tsompana and Buck 2014).

The kidney is the principal organ in the body that is responsible for the regulation of osmoregulatory activities (Gross and Garrard 1988). In addition to the management of water and salt, these duties also encompass the regulation of a variety of minerals, including sodium, calcium, phosphorus, and potassium. According to the findings of this study, the renal histopathological changes that took place included vacuolation, peritubular inflammatory infiltrates, vacuolation, extensive degeneration with tubular epithelium, and dilation of renal tubules with cast deposits. These morphological changes might have resulted the lethargy and initial illness in pigeons (Hussain et al. 2021). Most of these alterations might be due to pesticides' ability to produce reactive oxygen species, which leads to oxidative stress and ultimately cause damage of cell membrane, lipids, carbohydrates, and nucleic acid (Hussain et al. 2011; Suliman et al. 2020). Previously, structural alterations observed in the kidney of Japanese quills on exposure to chlorpyrifos were congestion, extensive presence of pyknotic nuclei in the tubular epithelial cells, degenerated tubules, glomeruli, and Bowman capsules (Suliman et al. 2020). Most of the renal symptoms observed in our study were also reported previously in *Clarias batrachus*, rabbits, and *Cyprinus carpio* on exposure to pesticides like chlorpyrifos, acetamiprid, and lambda-cyhalothrin (Mottalib et al. 2018; Chowdhury et al. 2022; Shameena et al. 2021).

The digestive tract is one of the most susceptible portions of the body to any pollutants that may enter the body along with food since it is the first organ that comes into direct contact with contaminated food. Digestive gland of control specimens exhibited normal morphology of the digestive tubules and surrounding connective tissue. The epithelial cells of the digestive tubules were generally normal without any histoarchitecture loss. The histological alterations that occurred in the gastrointestinal tract included the development of goblet cells, the rupturing of villi, the vacuolization of enterocytes, necrosis, pyknosis, and damage to the serosa. Additionally, the serosa was shown to have been damaged. Because so few research had been conducted on the subject, there was a paucity of information on the effects that pesticides had on the digestive systems of birds (Boumezrag et al. 2021). This resulted in a lack of understanding. As a result of this, the modifications were congruent with the study that was done in zebra fish as well as in broilers when they were exposed to a range of pesticides. This research was conducted in both species (Haq et al. 2016; Lall 2003).

Conclusions

Pigeons have been proposed as ideal model organisms for the risk assessment of birds exposed to agrochemicals. According to the findings of this study, orally fed pesticides like chlorpyrifos, acetamiprid, and lambda-cyhalothrin revealed adverse impacts on the hematology and histopathology of pigeons. Clinical signs and hematological and histological alterations concluded that chlorpyrifos, acetamiprid, and lambda-cyhalothrin are potential toxic compounds that can induce different levels of toxicity to non-target organisms like birds. These results elucidated that selected insecticides are hepatotoxic and nephrotoxic compounds and also, they induce hematological and histopathological alterations in blood and tissues. These compounds can even be toxic for human as it is located at the top level of food chain. This study depicted that a mixture of selected insecticides caused severe damages compared with individual insecticides. It is therefore recommended for the regulatory agencies of the country to raise awareness, strengthen the laws, and take regulatory measures regarding the safe use of these insecticides. These regulatory agencies must ensure the avoidance of over, needless, and improper use of such compounds.

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Data Availability The datasets generated during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical approval The experimental animals were kept under conducive environment and every possible effort were made to minimized stress upon the animals following ethical guidelines of animal ethical committee, Department of Zoology, Hazara University Mansehra, Pakistan.

Consent to participate Not applicable.

Consent for publication The authors agreed to publish in the Environmental Science and Pollution Research.

Competing interests The authors declare no competing interests.

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