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Pyrethroid Ramifications in the Haematological Status, Tissue Protein and Lipid Content of *Anabus testudineus*

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ABSTRACT: Pyrethroids are popular insecticides used in agricultural fields and households for pest control. They are gaining their popularity because they are fast acting and required in very low doses. Cypermethrine is one such modern pyrethroid pesticide, which is a potent source of water pollution. Fishes are very vulnerable to a wide variety of such toxicants in water and the poisonous effects of pesticides on fishes can be easily established. Being lipophilic, it is easily absorbed by the fish gills and reaches the circulation where it affects the biologically active molecules, *viz*., carbohydrates, proteins, and lipids. Studies on the impact of two sub-lethal concentrations (0.12ug/l and 0.24ug/l) of Cypermethrin on the total tissue protein and lipid content of muscle and liver as well as certain hematological parameters of a labyrinth fish, *Anabus testudines* were evaluated. By summarizing the results using Analysis of Variance (ANOVA), it has been observed that the exposure decreased the total protein and lipid content significantly ($p<0.05$). Protein depletion was more in muscles than in the liver. In the case of hematology, RBC count and Hgb % decreased but contrastingly WBC count increased in both the pesticide-treated groups with increased time of exposure. Hence the study concludes that cypermethrine is highly pernicious to fish's physiological and biochemical conditions, even at low doses, although it is thought to be less harmful to birds and mammals directly. The most challenging part of the study was to unveil the fact that although cypermethrine is photodegradable and thought to be less toxic, but since it is seriously deleterious to fishes, it can be life-threatening to all the other carnivores that are connected to fishes in the food chain through biomagnifications. The present investigation contributes to a better understanding of the repercussions brought to animal society through the use of such hazardous synthetic pesticides.

Keywords: Biomolecules, Cypermethrine, Erythrocyte, Fish, Lymphocyte, Toxicity.

INTRODUCTION

The use of chemicals in agriculture as well as aquaculture has become an alarming threat to aquatic organisms. Conventional pesticide use in agriculture is a matter of concern because many of these pesticides are persistent in the environment. These pesticides are easily carried to water bodies either directly or indirectly leading to fish mortality and reduced fish productivity. Increased concentrations of unwanted chemicals in edible fish tissue become a health hazard to all of its consumers, especially human beings. Pollution of surface waters has been well documented worldwide and constitutes a major issue at local, regional, national, and global levels (Cerejeira *et al*., 2003; Spalding *et al*., 2003). To replace the conventional pesticides a new class of pesticides, the synthetic pyrethroid have been developed. They are found less toxic to birds and mammals since they are photodegradable (Moore and Waring 2001). Pyrethroids are synthetic analogs of the pyrethrins, which are extracts from the ornamental plant *Chrysanthemum cinerariaefolium*. They are found less toxic to birds and mammals, as they are photodegradable (Moore and Waring 2001). Since they are fast acting and required in very low doses, they easily became popular. Pyrethroids are strongly adsorbed on soil and sediments, and minimally eluted with water. However, fishes show extreme sensitivity to pyrethroid than corresponding values for mammals and birds. Due to its high toxicity and lipophilic nature, a pyrethroid pesticide causes a serious threat to the fish population (Bradbury *et al*., 1989). It can easily get into aquatic bodies by surface runoff waters and diffuse into the blood through their skin and gills (John and Prakash 2003). Fishes are directly exposed to environmental deadly impacts including harmful insecticides disclosure that affects their rearing ability as well as their profitable value (Fırat *et al*., 2011; Georgieva *et al*., 2014). The first step in the generation of toxic effects is the contact between the pollutants and biomolecules. Prediction of toxic effects that may occur at a higher level of biochemical organization and proper understanding of the alterations induced by the exposure to pollutants is necessary. Rosety and Jesus (2005) Cypermethrine has been classified as a Type II pyrethroid that act by blocking sodium channels and affects the function of GABA-receptors in nerve filaments. In mammals, type II pyrethroids trigger clinical symptoms known as the 'CS syndrome'

(Roberts and Hudson 1999). Tissues like the liver and muscle easily accumulate the pesticides that result in alternations of the biochemical parameters (Srivastava and Kaushik 2001).

The studies against pesticidal effects, done in the recent past show the intense magnitude of their hazardous impact on the living world. Pesticides like malathion, diazonin, and carbofuran damage to vital organs were studied through the histopathological alterations reported in different fish species like *L. rohita*, *Heteropneustes fossilis*, *C. carpio*, *Channa punctatus*, *O. mossambicus*, Nile tilapia *(O. niloticus*), and *Cirrhinus mrigala* (Deka and Mahanta 2017; David and Kartheek 2014; Mohammed *et al.*, 2019; Khafaga *et al.*, 2020), Banaee *et al*. (2011); Ullah and Zorriehzahra (2015) demonstrated that pesticides adversely alters the hematology of many freshwater fishes. Additionally, reports also explain that some well-known organophosphates, such as malathion and endosulfan, create undesirable effects on the enzyme activity, i.e., L-Keto acid-activated–glutaminase, lactate dehydrogenase (LDH) level, citrate-synthase (CS), glucose 6-phosphate phosphate dehydrogenase (G6-PDH) in the brain, liver, skeletal muscles, and the gills of *C. batrachus* and *L. rohita* (Mastan and Shaffi 2019; Thenmozhi *et al.*, 2011).

Earlier several investigations were also done on finfish and shellfish species to study the impact of pyrethroids on the nervous system. Its action on the ion exchange process and mitochondrial membranes were studied by Lutnicka *et al.* (2009); Carcamo *et al.* (2017); Wang *et al.* (2017). The genotoxic and oxidative stress in the treated *Danio rerio* (Farag *et al*., 2021), immunotoxic impacts in *Cyprinus carpio* (Soltanian and Fereidouni 2017), deformities during early development in *Labeo rohita* (Dawar *et al.*, 2016) and hepatotoxicity in the *Catla catla* (Sharma and Jindal 2020) are good shreds of evidence of toxicity impacts of cypermethrine.

Cypermethrine is classified as a weak carcinogen by the US EPA due to its ability to promote tumor-initiating potentiality in mouse skin as evaluated by Shukla *et al*. (2002). The DNA-damaging potentiality of cypermethrine in the organs and tissues of mice was evidenced in a comet assay made by Patel *et al.* (2006). Singh *et al*. (2012) reviewed the cypermethrin-induced neuro damage in animals. Few recent studies support that Cypermethrine can also become an air pollutant and adversely affect our vital organs and cause neurodegenerative impacts. Sheikh *et al.* (2014) witnessed histopathological lung and liver damage in mice on exposure to pyrethroid inhalation. Eyitayo *et al*. (2022) witnessed coagulation profiles of rabbits on inhalation of cypermethrine. Daewood *et al.* (2020) tried to mitigate the immune, histopathological, and inflammatory implications of deltamethrine with dietary B-glucan

A very recent study made by Wenping *et al.* (2022), on wild fishes proved that pyrethroid bioaccumulation varies according to their geographic distribution as well as feeding habit. But since very little work was done on the response of locally available small fishes of Meghalaya towards pyrethroid pesticide, an effort was made through the present investigation to sentient pesticidal impact on fishes, since it is an integral part of the human food chain.

MATERIALS AND METHODS

This research was conducted in the Department of Zoology, University of Science and Technology Meghalaya.

Test species and laboratory care

Live matured specimens of average total length (TL) of 22-.25 cm (mean ± SD, 24.88 ± 1.39) and body weight (BW) of 90-100.00 g (98.52 ± 5.08) were purchased from a local market and brought to the laboratory. Fishes were kept in a glass aquarium for 3 days for acclimatization. They were then divided into three groups (each group included 5 live fishes) in three different aquaria(capacity 100 L). Acute toxicity tests were conducted to find out the 96 hr Lc_{50} value of Cypermethrine in *Anabus* which was considered as 2.4ug/l. However the Lc50 being 0.67 µg/L for *Heteropneustes fossilis* (Saha and Kaviraj 2003), 2.60 µg/L for *Cyprinus carpio* (Saha and Kaviraj 2003), 3.14ug/l for rainbow trout and 2.9ug/l for carps ((Dobsikova *et al.*, 2006). Fishes were exposed to 2 sub-lethal concentrations, i.e. 5% and 10% of 96hr Lc 50 value, i.e. 0.12ug/l and 0.24ug/l respectively for 14,21, and 28 days.

1. Control-Fishes kept in plain water without treatment

2..Group I-Fishes were treated with 0.12ug/l.of cypermethrine.

3. Group II-. Fishes were treated with 0.24ug/l of cypermethrine.

Fishes from each of the above groups were then analyzed for their protein and lipid content at an interval of 14,21 and 28 days. They were fed with fish feed mostly zooplankton. After the treatment period, fish were anesthetized with chloroform. At the end of each day, blood was extracted from the caudal vein in a 1 ml heparin-treated disposable syringe for hematological studies and was then sacrificed to take out muscle and liver tissue.

Hematological parameters

Hemoglobin (Hb) estimation. Hemoglobin **%** was assessed by the Sahlis method (1962.) At first, the marked hemoglobin tube was loaded with 0.1N HCl up to mark 10. Then slowly by capillary motion, the blood was filled up to mark 20 cu. mm in the capillary tube. After that, the blood was poured into a hemoglobin tube already containing 0.1N HCl. After leaving for 10 min, the mixture was diluted with drop-by-drop addition of distilled water and stirred continuously with a glass rod till the color matches that of the standard brown glass rod present on the side tubes of the Sahlis haemometer. The level in the hemoglobin tube, at which all the tubes show similar color is the correct reading that denotes the hemoglobin percentage.

Total count of RBC

The erythrocyte count was performed using Neubauer's improved double hemocytometer (Fein–optic blankernburg, GDR) taking Hayem's solution as RBC diluting fluid. Blood was diluted 1:200 with Hayem's fluid (Mishra *et al*., 1977). Erythrocytes were

counted in the loaded hemocytometer chamber in mm^3 $\times 10^3$ (Wintrobe and Maxwell 1974).

Total count of WBC

Total count of WBC: Total white blood cells (WBC) were counted using a Neubauer hemocytometer WBC diluting fluid was used to dilute blood in the ratio 1:20 and placed in four big (1sq mm) corners squares of the hemocytometer. The total number of WBC was calculated in mm^3 $\times 10^3$.

Biochemical parameters.

Protein Analysis. The soluble protein was first extracted from the liver and muscle tissue by the differential centrifugation method**.** The total soluble protein was then calculated by Lowry's assay (1951).

Lipid Analysis: Total lipid content was estimated by using the Folch method (Folch *et al.*, 1956). 1±0.1 g of fish muscle was taken and oven-dried. The powdered oven-dried tissue was then mixed with 5ml of chloroform: methanol (2:1) mixture and covered with aluminum foil. After keeping it for 24 hrs at room temperature, it was filtered with Whatman no. 1 filter paper and the filtrate was taken in a pre-weighed Petri dish and oven-dried. The Petri dish was weighed with lipids and the difference in weight was taken as total lipid content and the percentage was calculated.

Statistical Analysis. In this study for a result, five different counts from each sample were taken and the standard deviation was calculated using MS Excel. The Way Analysis of Variance was also done in MS Excel.

RESULTS AND DISCUSSION

Effect on Protein and Lipid Content. The biochemical parameters i.e protein and the lipid content of the muscle and liver tissues of the treated *Anabus* fishes show significant variations (ANOVA, $P<0.05$) concerning the sublethal doses of Cypermethrin over 14, 21 and 28 days which are listed in Table 1 and 2.

The protein content in the control group remained invariably the same for 28 days.

The muscle protein of the Group I(0.12ug/l) fish decreased gradually with increasing time as compared to its control value (Table 2). The lowest protein value was 07.42±0.27mg/ml recorded on the 28th day.

Similarly, the muscle protein of Group II fishes decreased gradually to its lowest value of 03.34±0.03mg/100g on the 28th day.

In the case of liver protein in the control group remained invariably the same during 28 days.

Liver protein of the Group I (0.12ug/l) fishes decreased with increasing time to its lowest value of 08.54±0.12mg/100g on the 28th day.

Similarly, in Group II fishes it decreased gradually to 05.21±0.02mg/100g on the 28th day. It is also evident that protein content decreased with increasing concentration of Cypermethrine on the 14th 21st and 28th day in both the liver and muscle tissues. However, in muscle tissues, the rate of protein degradation was more than in liver tissues.

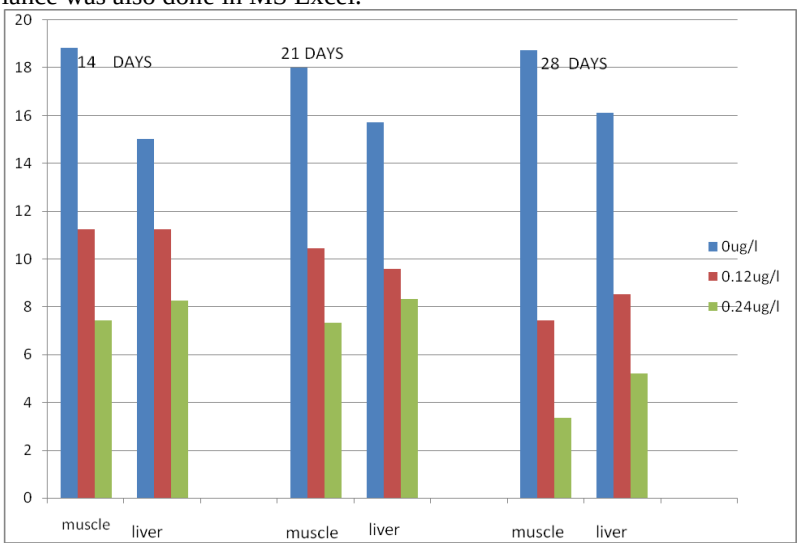

Fig. 1. Graphical representation of the effect of Cypermethrine on the Protein content of different tissues of *Anabas testudineus*. (g/100g). Values are reported as mean ± standard deviation of five replicates.

Table 1: Protein content of Cypermethrine treated different tissues of Anabas testudineus (g/100g). Values are reported as mean ± standard deviation of five replicates.

Biochemical Parameter	Number of Days	Tissue	Concentration of Cypermethrine		
			0 ug/l	0.12ug/l.	0.24ug/l
Protein	14	Muscle	18.83±0.11	11.24±0.04	07.02±0.08
		Liver	15.03±0.05	11.26±0.07	08.27±0.24
	21	Muscle	18.02±0.23	10.44±0.01	07.33±0.08
		Liver	15.73±0.42	9.57±0.46	08.34±0.06
	28	Muscle	18.37±0.36	07.42±0.27	03.34±0.03
		Liver	16.12±0.34	08.54±0.12	05.21±0.02

Table 2: Effect of Cypermetrin on the Lipid content of different tissues of *Anabas testudineus*. (g/100g). Values are reported as mean ± standard deviation of five replicates.

Biochemical parameter	No. of Days	Tissue	Concentration of Cypermethrine		
			0	0.12ug/l.	0.24ug/l
LIPID	14	Muscle	07.02±0.97	04.13±0.54	02.24±0.22
		Liver	06.84±0.04	05.27 ± 0.22	03.21± 0.18
	21	Muscle	08.25±0.12	03.12 ± 0.36	01.28 ± 0.17
		Liver	07.11±0.11	04.21 ± 0.67	02.25 ± 0.23
	28	Muscle	08.68±0.25	01.26±0.34	00.24±0.01
		Liver	07.11±0.36	02.21±0.17	01.74±0.04

Cypermethrine also has a strong impact on the lipid content of muscle and liver tissues. It can be seen from Table 2, that lipid content decreased significantly with increased sublethal doses in all the experimental days, i.e. 14th, 21st day and 28th day. Moreover lipid content also decreased in both Groups with their increased time of exposure. The lowest lipid value 07.42±0.27 mg/100g, is seen in muscle lipid on the 28th day of Group I. In the case of Group II fishes, the lowest level is recorded in muscle lipid to be 03.34±0.03 mg/100g on the 28th day. Similar results were also observed by Atamanalp *et al.* (2002) in the case of pyrethroid-treated rainbow trout. Lipids store energy in the form of glycerol ester and circulate in the blood. To moderate the toxic stress, these glycerol esters are channelized to fill up the extra energy demand. A similar finding was given by Patil and Patole (2012) in their findings of the impact of malathion and cypermethrin on lipid constituents of freshwater fish *Lepidocephalicchthys guntea*. They observed that the level of lipid content decreased significantly in all treated groups. Stalin and Das (2012) observed a decrease in lipid levels in the liver tissues of *Cirrhina mrigala* exposed to fenthion. The reduction in the cholesterol level may be attributed to the inhibition of cholesterol biosynthesis in the liver or because of poor cholesterol absorption from the diet. Remia *et al*. (2008); Ganeshwade (2011), reported that there was a decrease in cholesterol content in the liver and muscle tissues of freshwater fish *Punctius ticto* and *Tilapia mossambica* on their exposure to pesticides. Choudhary and Gaur (2001) observed the same pattern of cholesterol decrement in the liver and muscle tissue of *Tor tor* (Ham.) due to dimethoate toxicity. They opined that it was caused due to the damage caused by dimethoate on the liver tissue, which is the site of lipid storage. Shinde *et al*. (2002) observed a reduction in ovarian lipids of *Notopterus notopterus* (Pallas) when exposed to heavy metals. Similarly, *Gambusia affinis* exposed to a pesticide, phosphamidon showed a significant decline in the level of total lipids in the muscle, liver, and brain (Govindan *et al.*, 1994). The findings of Ram and Sathyanesan (1984) on *Channa punctatus* intoxicated with mercuric chloride also supported the findings of the present study. Cholesterol is an important normal body constituent used in the structure of cell membrane synthesis of bile acid and synthesis of steroid hormones. Loss of lipids may be a consequence of the inhibition of lipid synthesis and mobilization of stored lipids (Verma and Panigrahi 1988). The decrease in tissue lipids and proteins might be partly due to their cell repair and tissue organization with the formation of lipoproteins, which are important cellular constituents of cell membranes and cell organelles present in the cytoplasm.

Hence it is observed (Table 1 and 2) that in both muscle and liver tissue, the protein and lipid content diminished with increased time of exposure to the toxicant as well to its increased concentration. Similar findings were observed by Majumdar *et al.* (2017) in the fish *Oreochromis niloticus*, and Pawar *et al*. (2021) in fish, *Channa gachua* exposed to organophosphate pesticide. The investigation is also supported by the findings of Singh *et al*. (1988) and Singh & Singh (2014) who witnessed the decrease of serum lipids and proteins of pesticide sprayer farmers. These changes might have occurred due to several possible causes such as increased protein degradation or decreased rate of synthesis. It may also be due to poor amino acid polymerization into a polypeptide chain. In the lack of glucose during stressful conditions, ketoacids are fuelled for gluconeogenesis. Sometimes amino acids are also utilized in the maintenance of osmotic and ionic regulation, thereby hindering protein synthesis and hence less protein count (Schmidt and Nielson 1975; Jenkins *et al.,* 2003). The oxidation of amino acids provide energy to cope with the stress. The destruction or necrosis of cellular function and consequent impairment in protein synthetic machinery can also decrease the protein level (Moore and Waring 2001). The toxic intolerance results in the conversion of tissue protein into an easily utilizable form of soluble protein fraction. The body suffers from energy deficiency and brings about altered enzymatic activities to compensate for the energy loss and hence the protein 0level declines (Atamanalp *et al.*, 2002). However the impact of toxicity is dose-dependent, and also the duration of its exposure in the tissue. (Pickering and Henderson 1964). The time-dependent and tissue-specific response in the present study could be attributed to the concentration of cypermethrin in the tissue. Lipids are an integral part of the cell and maintain cell structural integrity and also regulate cell permeability. They are not only the fuel for energy production but also regulate many metabolic processes.

Effect on Haematological Parameters. Treatment of cypermethrine at the sublethal doses of 0.12ug/l. and 0.24ug/l caused significant alternations in the hematological values. In the control group, the Hgb % remained invariably the same throughout the experimental days, except for a slight increase on the 28th day. In Group I, the Hgb % was found to decline sharply. The lowest value was recorded as 1.97±0.04 on

the 28th day. In Group II also a clear impact of the sublethal dose was observed. The lowest value of 0.06±0.11 was observed on the 28th day. In both the Groups (Table 2) the Hgb % declined gradually with their increased time of exposure. Moreover, it can also be seen that the highest dose showed a severe impact.

Similarly for the RBC count maximum degradation of RBC was recorded in Group I on the 28 day 2.84×10^6 mm^3 which was significantly low than its control level, which was 4. 13×10^6 mm^3.In Group II, the least count was 1.08±0.04 which was observed on 28 th day of the treatment.

Contrastingly, the WBCs showed an elevated count with an increased concentration of Cypermethrin. The highest count was recorded on the 21st day which accounts for $11.02 \pm 0.04 \times 10^6$ mm^3.

Table 3: Effect of Cypermethrin on the Haematological parameters.

Haematological Parameter	Conc .of Cypermethrine(ug/l)	Duration of exposure(Days)		
		14 DAYS	21 DAYS	28 DAYS
Haemoglobin (g %)	0	7.27± 0.38	7.98 ± 0.27	8.04 ± 0.72
	0.12ug/l	5.17±0.01	3.28±0.08	1.97±0.04
	0.24ug/l	3.85±0.04	1.64±0.07	0.06±0.11
RBC($x10^6$ mm^3)	0	4.34 ±0.07	4.13±0.39	4.39±0.12
	0.12ug/l	3.58±1.25	2.84±0.28	1.5±0.04
	0.24ug/l	2.56±0.11	2.03±0.04	1.08±0.04
WBC($x10^6$ mm^3)	0	6.75 ±1.94	6.84±0.28	6.32±0.29
	0.12ug/l	7.76±0.47	8.03±1.68	9.72±0.12
	0.24ug/l	8.06±0.17	9.56.0±0.93	11.02±0.04

The present investigation reveals a direct influence of cypermethrine on the blood parameters. The levels of hemoglobin and red blood cells decreased with increasing Cypermethrin (Table 3). Similar results were reported for Cypermethrin treatment in carp *Cyprinus carpio* (Dorucu and Girin, 2001), in *Labeo rohita* (Das and Mukherjee 2003), in rainbow trout *Oncorhynchus mykiss* (Nuri and Girgin, 2003) and air breathing teleost *Channa punctatus* (Saxena and Seth 2002) and for Diazinon treatment in *Cyprinus carpio* Svoboda **(**2001). The reason for decreased levels of hemoglobin can be either its increased destruction or slowing down of the rate of its synthesis (Reddy and Basamohidden 1989).

In the present study in comparison to Hgb% and total RBC count, contrastingly TLC seemed to increase. Similar findings have also been reported in the case of other toxicants like Eldrin and endosulfan in the air-breathing teleost *Channa punctatus* by Mahajan and Juneja (1979). A similar change in blood parameters was also observed by Ullah *et al.* (2022) in their experiment on hematological changes brought about in fish *Ctenopharyngodon idella* on its exposure to Cypermethrine. Mahanta *et al.* (2023) observed an Altered blood cell count, an increased value of aberrant cell count, and an increased amount of micronuclei formation due to pyrethroid treatment in *Heteropneustes fossilis*. Hundekari *et al.* (2013) also observed leucocytosis, i.e. increased TLC in patients suffering from organophosphate poisoning. Since leucocytes are immune cells, they play a defensive role in stress conditions. As the toxic antigens diffused into the blood, it created a stimulatory effect and led to leukocytosis, releasing the fish from a stressful state. These alternations in blood cell counts can also be caused by the non-specific immunity of the fish.

Thus, it was observed by the present work that the biochemical profiles of blood, total protein, and total lipid contents underwent a significant depletion of varying degrees in the tissues of the pollution-affected fish. However, the extent of damage caused indicated differential sensitivity of the tissues against the toxicant effectiveness. On the whole, it can be stated that high energy demand creates the stressful condition. This energy may be obtained from organic constituents such as carbohydrates, proteins, and/or lipids (Ganesan, 2010).

CONCLUSIONS

Thus, the current investigation concludes that cypermethrine exposure has a strong potential to alter the hematology and biochemical constituents in various tissues of *Anabus testudines*. Proteins and lipids being vital biochemical constituents for growth and development are directly affected by subsequent exposure to cypermethrine. The toxicant caused hematological disturbance which could lead to impairment of the fish's ability to combat diseases, reducing its chances for survival and potential for growth and reproduction. These effects of Cypermethrine even at low doses are severe. It can be attributed to the excessive demands under toxic stress. Since fishes are a part of the food chain of many higher animals, further study to demonstrate the possible effect on higher animals including humans is required. In addition, more research on microbial degradation of pesticide need further attention. An in depth knowledge and understanding of the environmental and the ecological potential impacts of pyrethroid pesticides in soil is required (Braganca *et al.*, 2016).

FUTURE SCOPE

The present piece of work limelight only on the biochemical and hematological deteriorations brought about by cypermethrin. However, more research is required to study its impact on different organs as well as on DNA damage. Emphasis should be also given to find out ways to mitigate the harmful effects of the pyrethroids.

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