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Evaluating the Effect of Diafenthiuron as Toxicological Agent on Blood Profile, Hepatorenal Performance and Immunity of Rabbit (*Oryctolagus cuniculus*)

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Abstract. Diafenthiuron is a thiourea derivative propesticide widely used in agriculture and forestry, involved in inhibition of oxidative phosphorylation and interrupts the mitochondrial ATP synthesis of the pest. Because there is no research regarding the toxicological effects of this chemical on non-target species. Hence the present study aim to assess the acute toxicity of this insecticide on rabbits (*Oryctolagus cuniculus*). A total of 48 rabbits were categorized into four groups, with 12 rabbits in each group. The groups were named as T0 (control), T1 (low dose), T2 (medium dose) and T3 (high dose). Rabbits in groups T1, T2 and T3 received oral administration of diafenthiuron at doses of 500 mg/Kg, 1000 mg/Kg, and 1500 mg/Kg, respectively which is based on their body weight. This administration continued for a period of 45 days. The T0 group served as the control group. Blood samples were collected at 15th, 30th and 45th day of experiment and analyzed for blood profile, hepatorenal performance and immunological analysis. Our results indicated that complete blood count was significantly ($P \leq 0.05$) reduced, except for platelets level which were significantly higher in dosage dependent groups as compared to control group. Furthermore, antibody titer test for immunity against Rabbit Hemorrhagic Disease (RHD) virus was also reduced in dosage dependent groups. However, hepatorenal parameters (bilirubin, alanine aminotransferase, aspartate transaminase, alkaline phosphatase, urea and creatinine) were significantly elevated upon exposure to the higher dosage of pesticide. Our study concluded that diafenthiuron has strong potential to cause toxicity even at sub lethal doses which in turn, affects the non-target organisms including human beings. Therefore, alternative ways must be used to avoid the accumulation of this pesticide in the environment.

Keywords: diafenthiuron, pesticide, *Oryctolagus cuniculus*, evaluation, blood profile, hepatorenal, immunity

Introduction

Pesticides are being used in large quantities globally due to their high efficacy of killing pests (Duke, 2017). Food and agricultural organization defined pesticide as any constituent used for controlling, damaging and destroying any harmful organism causing disease in both animals and human, undesirable types of creatures initiating damage and interference with the manufacturing and transportation of food, agrarian possessions, wood and wood products and animal feedstuffs or substances which may be directed to animals for the control of insects, arachnids or other pests in their bodies (Preetha *et al*., 2009; Park *et al*., 2009). Agriculturists utilize approximately 2.5 million tons of pesticides annually for pest control purposes (Jeschke, 2016; Qare, 2009). Although the use of these pesticides is helpful in reducing crop loss and to get better yield but on the other hand there are also some hazardous effects of these pesticides (Huber *et al*., 2000). Besides this, indiscriminate utilization of these chemicals not only pollutes the soil and water but also accumulates in the fruit and vegetable crops subsequently entering the food chain and body systems, blood and organs (Xavier *et al*., 2004). Moreover, pesticides also cause environmental pollution, loss of biodiversity and decay of common habitats (Cerejeira *et al*., 2003). Approximately 0.1% of pesticides, as estimated, effectively eliminate their intended organisms, leading to the accumulation of residual substances in the surrounding environment, thereby impacting non-target organisms (Rosell *et al*., 2008).

Diafenthiuron 1-tert-butyl-3-(2,6-diisopropyl thiourea) is a thiourea-derivative pesticide being extensively used in Pakistan for the control of sucking pests (Hameed *et al*., 2010). It has a distinctive mode of action, disrupting the respiratory activity of insects by blocking mitochondrial action, energy metabolism and molt inhibition, thereby causing paralysis of insect pests leading to their death within 2-3 days (Ishaaya *et al*., 2001). Photochemical activation of this insecticide is necessary for conversion into a carbodiimide derivative

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in the occurrence of daylight and singlet oxygen, which will be much more powerful insecticide than itself (Ratnakar *et al*., 2017). This carbodiimide derivative is involved in the inhibition of mitochondrial respiration as well as in the inhibition of mitochondrial ATPase by selective and covalent binding to the proteolipids in the inner membrane and to porins in the outer membrane of the mitochondria (Plimmer, 2003; Kayser and Ellinger, 2001). Various analytical techniques have been described for residual analysis of diafenthiuron in food, soil and environmental samples including high performance liquid chromatography (HPLC), liquid chromatography–mass spectrometry (LC–MS), solid-phase extraction (SPE), solid-phase micro extraction (SPME) and supercritical fluid extraction (SFE). Many countries including Japan, China and India have set a maximum residual limit (MRL) for diafenthiuron residues in food particles in the range of 0.03–1.0 mg/Kg (Golge and Kabak, 2015; Wang *et al*., 2015; Brito *et al*., 2002).

Toxicological effects of diafenthiuron have been reported to induce toxicity in non-target organisms including disorders in honeybees (Stanley *et al*., 2014) biological malfunctioning in birds, mammals and fishes (Keum *et al*., 2008). In rats, extended exposure to this insecticide induces lung carcinogenicity and results in the formation of cavities within the lungs of this animal model. (Ishaaya *et al*., 2001). Fish was also found to be susceptible to diafenthiuron and causes harmful effects in common carp, *Cyprinus carpio* and *Labeo rohita* by killing the fish in 6 h (Riaz-ul-Haq *et al*., 2018).

Rabbits have similar physiological and metabolic processes to humans, which can make them suitable models for studying the effects of pesticides on living organisms. This similarity allows researchers to gain insights into how pesticides may affect humans and other mammals (Khelfa *et al*., 2012). Keeping these facts in mind, further research is required to investigate the possible effects of diafenthiuron on blood profile and hepatorenal parameters of rabbit and to estimate the immunological alterations upon exposure to this chemical.

Materials and Methods

Research animal and chemical. Adult male rabbits of specie *Oryctolagus cuniculus* were procured and immunized at the Veterinary Research Institute on Ghazi Road in Lahore, Punjab, Pakistan, two weeks prior to the study to facilitate their acclimation period. The procedures involving the handling, welfare and standard laboratory protocols for live rabbits in this research study received pre-approval during the Departmental Board of Studies meeting. Commercial grade diafenthiuron (® 50% WP) was purchased from local market. Rabbits weighing (1Kg-1.5Kg) was purchased and placed in wire cages with solid floor to provide adequate ventilation. They were given optimum light *i.e*. 12-h light-dark cycle and (23 ± 2 ºC) temperature (Bradbury and Dickens, 2016). Feed was given by oral method in the form of soaked bread and water.

Experimental design. The duration of the conducted experiment was 45 days. Male rabbits (n=48) were chosen randomly and then divided into four groups labelled as T_0, T_1, T_2 and T_3 respectively, containing 12 rabbits in each block. Groups T_1, T_2 and T_3 were subjected to three different doses of diafenthiuron *i-e* 500 mg/Kg BW, 1000 mg/Kg BW and 1500 mg/Kg BW whereas T0 was considered as control group (Su *et al*., 2023). All groups were allotted different cages. Accordingly, three different doses of diafenthiuron were orally given to rabbits along with water and soaked bread once per day. Dosage of the pesticide was designed according to $1/2^{nd}$, $1/3^{rd}$ and $1/4^{th}$ with respective to its LD_{50}. Furthermore, within each group, the corresponding dose was administered at 9 am and samples were promptly collected two hours later. (Shamsollahi and Asadi, 2014). Two blood samples, 3mL each, one for complete blood count and the other for hepatorenal analysis, were collected from each group at 15^{th}, 30^{th} and 45^{th} days of dose administration from jugular vein of specimen (Parasuraman *et al*., 2010). For effective sampling of blood, 5 mL syringes were used. Overbleeding were prevented by applying silver nitrate on affected site (Fang *et al*., 2009).The blood samples for CBC were taken in tube containing EDTA (Ethylenediaminetetraacetic acid) whereas for hepatorenal analysis, serum was separated from coagulated blood after ultra-centrifugation. The samples were categorized by labelling on tubes, stored them at 4 °C and examined within 6-10 h (Rauf and Sluss, 2008). Overbleeding were prevented by applying silver nitrate on affected site.

Blood profiling (complete blood count). After a 24 h sampling period, a Semi-Automatic Chemistry Analyzer from Guangzhoun Hekang Biotechnology Co. Ltd., in China (model HKTE0112) was employed to analyze various parameters of a complete blood count, including white blood cell count (WBCs) and red blood cell count (RBCs), hemoglobin (Hb), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular

hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and platelet count (Shah *et al*., 2007; Donovan and Brown., 2006).

Hepatorenal analysis. Microlab Chemical Analyzer diagnostic ELISA kit (BioTek 800 SN) to check the level of liver enzymes such as alanine transaminase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), bilirubin and kidney function by determining urea and creatinine level. The method of Waritani *et al*. (2017) were followed for liver enzyme detection. To reduce the error, each test was performed three times for each of the enzyme and then average values were taken. The results were calculated using following formula: Enzyme activity (IU/L) = /ΔA/min × Kinetic factor [k], K = 2712 for ALP whereas the value of K each for ALT and AST is 1780.

Determination of antibody titer. For immunological analysis, serum samples were centrifuged at 1600×g for 15 min at 4 °C and stored at -70 °C into serum capsules. Each serum sample was labelled properly and collected within 6 h to avoid changes. Serum samples were analyzed through standard hemagglutination inhibition (HI) method. In HI assay, rabbit heamorrhage disease (RHD) virus was used. Rows of 96-well microtiter plate were used for two-fold serial dilutions of serum then RHD virus was added into each well which was diluted upto 8 HA units and incubated for 30 min except last well in each row as a negative control. After that, RBCs were added and incubated for next 30 min. Agglutinated red diffuse wells showed low antibody concentration indicated by HI plate (Mondal *et al*., 2009).

Data analysis. Data analysis was performed using SPSS (Statistical Package for the Social Sciences) Version 21 from IBM Corporation. Hematological studies, hepatorenal performance and immunity of rabbits were assessed and the standard error mean (±S.E.M) was calculated. To examine variation between the groups, an ANOVA test was conducted, and the significance level (P=0.001) between the treated groups and control group was determined using post hoc testing (Tukey LSD).

Results and Discussion

Evaluation of diafenthiuron exposure on blood profile of rabbits. Table 1 shows the effect of different concentrations of diafenthiuron on complete blood count of rabbits. Results showed that all hematological parameters including white blood cells (WBCs), red blood cells (RBCs), hemoglobin (Hb), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and platelets were significantly lowered upon treatment with increasing concentration of diafenthiuron in a dose dependent manner. The platelets count (P≤0.001) was however, significantly increased in treated groups as compared to control group.

Evaluation of diafenthiuron exposure on hepatorenal performance of rabbits. In Table 2, shows the impact of various concentrations of diafenthiuron on the liver function and renal function tests in rabbits. The purpose of the study was to investigate how different doses of diafenthiuron affected specific markers associated with liver and kidney functions in these animals.

Upon analyzing the data, we observed noteworthy changes in the hepatorenal parameters. These parameters included bilirubin, ALT (alanine aminotransferase), AST (aspartate aminotransferase), ALP (alkaline phosphatase), urea and creatinine. Importantly, all of these markers exhibited significant increases in their levels, and this increase was found to be directly proportional to the dosage of diafenthiuron administered. Our findings revealed a dose-dependent relationship between the concentration of diafenthiuron and the observed alterations in the hepatorenal parameters.

Evaluation of diafenthiuron exposure on antibody titer test of rabbits. According to the results obtained from the analysis of the antibody titers of rabbits in response to RHDV (rabbit hemorrhagic disease virus), it was observed that there exists a significant disparity in the levels of antibodies between the control group and the groups treated with diafenthiuron. Additionally, it was found that the values of the antibody titers were notably lower in these groups compared to the untreated group.

Table 3. revealed a significant decrease in the antibody titers in a dosage-dependent manner among the groups that were exposed to different concentrations of diafenthiuron. In comparison to the untreated group, the observed decline in the antibody titers was statistically significant (P≤0.05).

These findings indicate that the administration of diafenthiuron had a negative impact on the production or maintenance of antibodies against RHDV in rabbits. The reduced antibody titers in the diafenthiuron-treated groups suggest that the rabbits exposed to this compound exhibited diminished immune responses to the viral infection.

Table 1. Effect of different concentration of diafenthiuron administered for a period of 45 days on blood profile of rabbit

Blood profile parameters	Duration (in days)	Concentration of diafenthiuron (mg/Kg)			
		0	500	1000	1500
Hb (g/dL)	15	12.8 6± 0.333^{a}	11.30±0.057^{b}	10.80±0.100^{c}	8.16± 0.033^{d}
	30	12.46 ± 0.333^{a}	10.76±0.333^{b}	10.30±0.057^{c}	7.86± 0.033^{d}
	45	12.76 ± 0.384^{a}	10.80±0.057^{b}	9.70±0.057^{c}	7.66± 0.088^{d}
RBCs (x 10^{12} g/L)	15	6.81 ± 0.008^{a}	6.11±0.005^{b}	5.10±0.000^{c}	4.80± 0.05^{d}
	30	6.68 ± 0.090^{a}	6.23±0.120^{b}	4.66±0.333^{c}	4.16± 0.033^{d}
	45	6.66 ± 0.033^{a}	5.13±0.066^{b}	4.40±0.057^{c}	3.86± 0.033^{d}
HCT (%)	15	40.46 ± 0.290^{a}	39.16±0.284^{b}	32.50±0.400^{c}	28.40± 0.057^{d}
	30	40.13 ± 0.120^{a}	33.30±0.300^{b}	30.93±0.517^{c}	24.86± 0.578^{d}
	45	40.03 ± 0.120^{a}	32.30±0.200^{b}	31.36±0.317^{c}	24.50± 0.400^{d}
MCV (fL)	15	64.33 ± 0.066^{a}	60.00±1.126^{b}	57.76±0.611^{c}	54.10± 0.100^{d}
	30	64.20 ± 0.152^{a}	60.10±0.100^{b}	12.47±0.404^{c}	50.60± 0.152^{d}
	45	62.83 ± 0.266^{a}	58.46 ±0.033^{b}	12.93±0.152^{c}	50.06± 0.033^{d}
MCH (pg)	15	21.03 ± 0.366^{a}	19.40 ± 0.115^{b}	18.86±0.033^{b}	17.56± 0.437^{d}
	30	20.76 ± 0.371^{a}	19.40 ± 0.115^{b}	18.40±0.057^{b}	16.36± 0.523^{d}
	45	20.26 ± 0.753^{a}	17.73 ± 0.600^{b}	17.86±0.033^{b}	16.50± 0.305^{d}
MCHC (g/dL)	15	33.63 ± 0.088^{a}	30.23 ±0.285^{b}	29.02±0.433^{b}	26.03± 0.133^{d}
	30	29.06 ± 4.083^{a}	29.90 ± 0.066^{b}	27.50±0.503^{b}	25.86± 0.484^{d}
	45	32.36 ± 0.811^{a}	29.17 ± 0.088^{b}	27.20±0.152^{b}	23.20± 0.300^{d}
Platelets (x10^{9}/L)	15	433.00 ± 0.577^{a}	606.33 ± 6.893^{b}	661.66±1.666^{c}	708.66± 0.666^{d}
	30	432.66 ± 0.666^{a}	654.66 ± 0.666^{b}	662.66±0.333^{c}	719.66± 2.603^{d}
	45	433.00 ± 0.577^{a}	658.33 ± 0.333^{b}	673.00±1.527^{c}	755.44± 2.215^{d}
WBCs (x10^{9}/L)	15	8.70 ± 0.000^{a}	7.70 ± 0.057^{b}	6.83±0.066^{c}	5.46± 0.260^{d}
	30	8.73 ± 0.033^{a}	6.30 ± 0.057^{b}	5.06±0.033^{c}	4.63± 0.033^{d}
	45	8.80 ± 0.057^{a}	5.86 ± 0.033^{b}	4.86±0.033^{c}	3.86± 0.033^{d}

All data are expressed as mean ± standard deviation. Means with different superscripts in a row have significant difference among them (P≤0.05) Tukeytest.

Our findings revealed that the physiological well-being of male rabbits was negatively impacted by the exposure to diafenthiuron. A comprehensive analysis of the blood profile demonstrated a significant decrease in all parameters following the administration of increasing doses of diafenthiuron in the treated groups, as compared to the untreated group.

Specifically, the reduced number of red blood cells (RBCs) observed in the treated groups was attributed to the adverse effects of oxidative stress and the development of a hypoxic environment induced by the pesticide (George and Shukla, 2013). Furthermore, the hematocrit level was found to be diminished due to the decreased quantity of RBCs. Additionally, the mean corpuscular volume and hemoglobin levels were also lower, indicating the presence of anemia caused by the pesticide. (Zorriehzahra *et al*., 2010). WBCs level were lowered in dose dependent groups because of the toxicity caused by the chemical. On the other hand, Platelet count were increased significantly due to thrombocytosis which had been produced due to inflammation of prostaglandins and production of free radicals that had been resulted due to the disturbance and toxicity stress of non-specific tissues (Varadarajan *et al*., 2013: Saka *et al*., 2011).

Moreover, hepatorenal performance of rabbits showed a significant increase in dose-dependent groups as compared to control group. Similar results were reported by Khan *et al*. (2012) and showed a raised level of liver enzymes upon exposure to pyrethroids on fish and mammalian species. This was due to leakage of liver enzymes from hepatocytes due to higher detoxification rate in pesticide treated groups (Tuzmen *et al*., 2008). Our results in renal function is similar to those of Firat *et al*. (2011) who showed higher level of creatine, bilirubin, urea and decrease in the albumin and globulin proteins upon exposure to pesticide under stress condition and indicated a renal failure and acute hepatic necrosis.

Table 2. Effect of different concentration of diafenthiuron administered for a period of 45 days on hepatorenal performance of rabbit

Hepatorenal analysis	Duration (in days)	Concentration of diafenthiuron (mg/Kg)			
		0	500	1000	1500
Bilirubin (mg/dL)	15	72.33± 0.333^{a}	86.66±0.333^{b}	92.00±2.516^{c}	97.55± 0.333^{d}
	30	72.66± 0.333^{a}	100.66±1.201^{b}	110.66±2.185^{c}	122.00± 1.154^{d}
	45	72.66± 0.333^{a}	111.66±1.666^{b}	127.00±0.577^{c}	145.66± 0.666^{d}
ALT (u/L)	15	72.33± 0.333^{a}	86.66±0.333^{b}	92.00±2.516^{c}	97.66± 0.333^{d}
	30	72.66± 0.333^{a}	100.66±1.201^{b}	110.66±2.185^{c}	122.00± 1.154^{d}
	45	72.66± 0.333^{a}	111.66±1.666^{b}	127.00±0.577^{c}	145.66± 0.666^{d}
AST (u/L)	15	30.33± 0.333^{a}	24.00±0.577^{b}	20.33±0.333^{c}	14.66± 0.333^{d}
	30	30.66± 0.333^{a}	18.00±1.527^{b}	14.00±0.577^{c}	9.66± 0.333^{d}
	45	30.00± 0.000^{a}	12.33±0.666^{b}	9.33±0.666^{c}	8.66± 0.333^{d}
ALP (u/L)	15	12.33± 0.333^{a}	18.33±0.333^{b}	22.00±1.154^{c}	25.66± 0.333^{d}
	30	11.66± 0.333a	21.33±0.881^{b}	26.00±1.154^{c}	30.66± 0.666^{d}
	45	12.00± 0.000^{a}	24.00±0.577^{b}	30.66±1.201^{c}	33.66± 0.333^{d}
Urea (mg/dL)	15	24.66± 0.333^{a}	26.66±0.333^{b}	29.66±0.333^{c}	31.66± 0.333^{d}
	30	24.66± 0.333^{a}	30.33±0.333^{b}	32.66±0.333^{c}	34.66± 0.333^{d}
	45	24.66± 0.333^{a}	34.66±0.333^{b}	37.66±0.333^{c}	41.66± 0.333^{d}
Creatinine (mg/dL)	15	0.66± 0.033^{a}	.83±0.033^{b}	1.13±0.066^{c}	1.46± 0.033^{d}
	30	0.66± 0.033^{a}	.86±0.033^{b}	1.50±0.000^{c}	1.96± 0.033^{d}
	45	0.73± 0.033^{a}	1.46±0.033^{b}	2.06±0.066^{c}	2.46± 0.033^{d}

All data are expressed as mean ± standard deviation. Means with different superscripts in a row have significant difference among them (P≤0.05) Tukeytest.

Table 3. Effect of different concentration of diafenthiuron administered for a period of 45 days on blood profile of rabbit

Duration (in days)	Concentration of diafenthiuron			
	0	500	1000	1500
15	13.17 ± 0.376^{a}	12.63 ± 0.285^{b}	12.97 ± 0.328^{c}	13.30 ± 0.265^{d}
30	13.30 ± 0.400^{a}	12.90 ± 0.404^{b}	12.47 ± 0.088^{c}	13.13 ± 0.291^{d}
45	13.03 ± 0.384^{a}	13.37 ± 0.296^{b}	12.93 ± 0.318^{c}	13.00 ± 0.473^{d}

All data are expressed as mean ± standard deviation. Means with different superscripts in a row have significant difference among them (P≤0.05) Tukeytest.

Antibody titer of rabbits against RHDV shows that there is significant difference in the level of antibody for control group as well as diafenthiuron-treated groups, moreover, the value of antibody is lower. This lower level of antibody was due to elevated level of pesticide in dose dependent groups. Clumping of RBCs in titer plate showed the lower level of antibody in the respective treated groups whereas standard plate showed less agglutination and considered as control group.

Conclusion

It was concluded that exposure to diafenthiuron resulted in a dose-dependent reduction in the blood profile, as indicated by statistically significant differences (P=0.05). Additionally, there was a notable increase in hepatorenal performance in the dosage groups compared to the untreated groups. Furthermore, the administration of increasing doses of diafenthiuron led to a significant decrease in immunity, as evidenced by a reduction in the antibody titer test against RHD virus.

These results provide clear evidence of the adverse impact of diafenthiuron on the physiology of rabbits. In light of these findings, it is highly recommended that measures be taken to prevent the accumulation of diafenthiuron in the environment and the food chain.

These preventive measures are crucial for minimizing the potential negative consequences associated with the exposure to diafenthiuron, which have been clearly demonstrated in this study.

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Conflict of Interest. The authors declare they have no conflict of interest.

References

Bradbury, A.G., Dickens, G.J.E. 2016. Appropriate handling of pet rabbits: a literature review. *Journal of Small Animal Practice*, **57:** 503-509.

Brito, N.M., Navickiene, S., Polese, L., Jardim, E.F. G., Abakerli, R.B., Ribeiro, M.L. 2002. Determination of pesticide residues in coconut water by liquid–liquid extraction and gas chromatography with electron-capture plus thermionic specific detection and solid-phase extraction and high-performance liquid chromatography with ultraviolet detection. *Journal of Chromatography A*, **957:** 201-209.

Cerejeira, M.J., Viana, P., Batista, S., Pereira, T., Silva, E., Valério, M.J., Silva-Fernandes, A.M. 2003. Pesticides in Portuguese surface and ground waters. *Water Research*, **37:** 1055-1063.

Donovan, J., Brown, P. 2006. Blood collection. *Current Protocols in Immunology*, **73:** 1-7.

Duke, S.O. 2017. Pesticide dose–a parameter with many implications. In: *Pesticide Dose*: *Effects on the Environment and Target and Non-Target Organisms*, chapter 1, (pp. 1-13). American Chemical Society, Washinton DC, USA.

Fang, Z.F., Gai, H., Huang, Y.Z., Li, S.G., Chen, X.J., Shi, J.J., Wu, L., Liu, A., Xu, P., Sheng, H.Z. 2006. Rabbit embryonic stem cell line derived from fertilized, parthenogentic or somatic cell nuclear transfer embryos. *Experimental Cell Research*, **312:** 3669-3682.

Fýrat, O., Cogun, H.Y., Yüzereroðlu, T.A., Gök, G., Fýrat, Ö., Kargin, F., Kötemen, Y. 2011. A comparative study on the effects of a pesticide (cypermethrin) and two metals (copper, lead) to serum biochemistry of *Nile tilapia*, *Oreochromis niloticus*. *Fish Physiology and Biochemistry*, **37:** 657-666.

George, J., Shukla, Y. 2013. Emptying of intracellular calcium pool and oxidative stress imbalance are associated with the glyphosate-induced proliferation in human skin keratinocytes HaCat cells. *Internationally Scholarly Research Notes Dermatology*, **2:** 30-38.

Golge, O., Kabak, B. 2015. Evaluation of QuEChERS sample preparation and liquid chromatography–triple-quadrupole mass spectrometry method for the determination of 109 pesticide residues in tomatoes. *Food Chemistry*, **176:** 319-332.

Hameed, A., Aziz, M.A., Aheer, G.M. 2010. Susceptibility of *Bemisia tabaci* Gen. (Homoptera: Aleyrodidae) to selected insecticides. *Pakistan Journal of Zoology*, **42:** 295-300.

Huber, A., Bach, M., Frede, H. G. 2000. Pollution of surface waters with pesticides in Germany: modeling non-point source inputs. *Agriculture, Ecosystems and Environment*, **80:** 191-204.

Ishaaya, I., Kontsedalov, S., Mazirov, D., Horowitz, A.R. 2001. Biorational agents in IPM and IPM programs for controlling agricultural pests. *Universiteit Gent*, **66:** 363-374.

Jeschke, P. 2016. Propesticides and their use as agrochemicals. *Pest Management Science*, **72:** 210-225.

Kayser, H., Eilinger, P. 2001. Metabolism of diafenthiuron by microsomal oxidation: procide activation and inactivation as mechanisms contributing to selectivity. *Pest Management Science*, **57:** 975-980.

Keum, Y.S., Liu, K.H., Seo, J.S., Kim, J.H., Kim, K., Kim, Y.H., Kim, P.J. 2008. Dissipation of foliar residues of diafenthiuron and its metabolites. *Bulletin of Environmental Contamination and Toxicology*, **68:** 845-851.

Khan, A., Ahmad, L., Khan, M.Z. 2012. Hemato-biochemical changes induced by pyrethroid insecticides in avian, fish and mammalian species. *International Journal of Agriculture & Biology*, **14:** 834-842.

Khelfa, D.E., El-Ghany, W.A., Salem, H.M. 2012.

Serological and molecular typing of *Clostridium perfringens* and its toxins recovered from weaned Rabbit is flocks in egypt. *Life Science Journal*, **9:** 2263-2271.

Mondal, S., Ghosh, R.C., Mate, M.S., Karmakar, D.B. 2009. Effects of acetamiprid on immune system in female Wistar Rats. In: *Proceedings of the Zoological Society*, **62:** 109-117. 26 January 2010.

Parasuraman, S., Raveendran, R., Kesavan, R. 2010. Blood sample collection in small laboratory animals. *Journal of Pharmacology & Pharmacotherapeutics*, **1:** 87-93.

Park, H., Ko, D.H., Kim, J.Q., Song, S.H. 2009. Performance evaluation of the piccolo press point-of- care-chemistry analyzer, *The Korean Journal of Laboratory Medicine*, **29:** 430-438.

Plimmer, J.R. 2003. Herbicides, alphabetical classification. *Encyclopedia of Agrochemicals*, Published by Wiley Online Library, USA.

Preetha, G., Stanley, J., Chandrasekaran, T., Kuttalam, S. 2009. Toxicity of imidacloprid and diafenthiuron to *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) in the laboratory conditions. *Journal of Plant Protection Research*, **49:** 290-296.

Qare, A.A. 2009. Potential leaching of pesticides to groundwater: a look at the most common use pesticides in palestine. In: *Proceedings of the 2nd International Conference on Water: Values and Rights*, pp. 13-15.

Raff, P.M., Sluss. 2008. Pre-analytical issues for testosterone and estradiol assays. *Steroids*, **73:** 1297-1304.

Ratnakar, V., Sridevi, D., Vidyasagar, B. 2017. Sublethal lethal exposure of certain newer insecticides molecules to honeybee, *Apis mellifera* Linnaeus. *International Journal of Pure Applied Biosciences*, **5:** 641-646.

Riaz-ul-Haq, M., Javeed, R., Iram, S., Rasheed, M.A., Amjad, M., Iqbal, F. 2018. Effect of diafenthiuron exposure under short and long-term experimental conditions on hematology, serum biochemical profile and elemental composition of a non-target organism, *Labeo rohita*. *Environmental Toxicology and Pharmacology*, **62:** 40-45.

Rosell, G., Quero, C., Coll, J., Guerrero, A. 2008. Biorational insecticides in pest management. *Journal of Pesticide Sciences*, **33:** 103-12.

Saka, W.A., Akhigbe, R.E., Azeez, O.M., Babatunde, T.R. 2011. Effects of pyrethroid insecticide exposure on haematological and haemostatic profiles in rats. *Pakistan Journal of Biological Sciences*, **14:** 1024.

Shah, M.K., Khan, A., Rizvi, F., Siddique, M. 2007. Effect of cypermethrin on clinico-haematological parameters in rabbits. *Pakistan Veterinary Journal*, **27:** 171-175.

Shamsollahi, M., Asadi, F. 2014. Neurotransmitter effect of galanin on the mean plasma concentrations of growth hormone, triiodothyronine, thyroxine and milk protein in dairy goats sannan. *Endocrinology*, **2:** 793-801.

Stanley, J., Chandrasekaran, S., Preetha, G., Kuttalam, S. 2010. Toxicity of diafenthiuron to honeybees in laboratory, semi-field and field conditions. *Pest Management Science*: *Formerly Pesticide Science*, **66:** 505-510.

Su, M., Bao, R., Wu, Y., Gao, B., Xiao, P., Li, W. 2023. Diafenthiuron causes developmental toxicity in zebrafish (*Danio rerio*). *Chemosphere*, **323:** 138253.

Tuzmen, N., Candan, N., Kaya, E., Demiryas, N. 2008. Biochemical effects of chlorpyrifos and deltamethrin on altered antioxidative defense mechanisms and lipid peroxi comdation in rat liver. *Cell Biochemistry and Function*, **26:** 119-124.

Varadarajan, R., Jose, J., Hari Sankar, H.S., Philip, B. 2013. Haematological and ionic alterations in *Oreochromis mossambicus* (peters) induced by phenolic compounds. *Journal of Aquatic Biology of Fish*, **1:** 90-96.

Wang, L., Ge, H., Wang, Y., Ge, B. 2015. Determination of diafenthiuron and its metabolites residue in apple by QuEChERS-high performance liquid chromatography-tandem mass spectrometry. *Journal of Food Safety and Quality*, **6:** 436-441.

Waritani, T., Chang, J., McKinney, B., Terato, K. 2017. An ELISA protocol to improve the accuracy and reliability of serological antibody assays. *MethodsX*, **4:** 153-165.

Xavier, R., Rekha, K., Bairy, K.L. 2004. Health perspective of pesticide exposure and dietary management. *Malaysian Journal of Nutrition*, **10:** 39-51.

Zorriehzahra, M.J., Hassan, M.D., Gholizadeh, M., Saidi, A.A. 2010. Study of some hematological and biochemical parameters of rainbow Trout (*Oncorhynchus mykiss*) fry in western part of Mazandaran province, Iran. *Iranian Journal of Fisheries Science*, **9:** 185-198.