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Haematological Alteration in Common Carp (*Cyprinus Carpio* L) Fish After Exposure for Tow Pesticides Goldate, Alexander and Their Mixture

Noor Yaseen Salih [1*] and Ahmed J. Mohammed Al-Azawi [2]
Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq.
*Corresponding Author.

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Abstract

The excessive and unwarranted use of pesticides at different stages of crop production can pose a great danger to the aquatic environment, and particularly to fish. Haematological parameters are indicated to be important for environmental pollution. Blood parameters respond to low doses of pollutants, so hematological changes in common carp fish are used for assessing the effects of contaminants. Toxicological effects of Goldate and Alexander individuals combined as a mixture were observed by monitoring hematological parameters in common carp fish. There were 3 concentration groups (1/10, 1/100, and 1/200) for every pesticide (Goldate, Alexander, and mixture of Goldate and Alexander) exposed to juvenile fish (60–80 gm. in weight) for 6 weeks. During the experimental period, the results showed a significant decrease in the red blood cell mean value (RBC), hemoglobin mean value (Hb), and total protein mean value (TP). While there was a significant increase in the white blood cell mean value (WBC) and albumin mean value (Al) after six weeks of exposure, It was found that these changes increase with increasing pesticide concentrations and exposure periods when they are single. However, these changes are more severe when the fish are exposed to a mixture of pesticides at the same time. This experiment found that these changes increase with increasing pesticide concentrations and exposure periods when they are single, but these changes are more severe when the fish are exposed to a mixture of pesticides at the same time, which is evidence of the synergistic action of these pesticides.

Keywords: Pesticides , Environment, Carp fish, Goldate, Alexander.

1. Introduction

A pesticide is a chemical or biological substance that is aimed at preventing, repelling, or destroying pests that may damage or disturb the growth and health of living organisms, which may be plants or animals. Pesticides are classified based on their origin, structure, or pests; they control the mode or site of action as insecticides, rodenticides, and fungicides [1-3]. Fish are one of the most broadly distributed organisms in an aquatic environment, and the fact that fish are susceptible to environmental contamination may reflect the degree of the biological effects of environmental pollution in waters. Monitoring of blood parameters, both cellular and non-cellular, may have considerable diagnostic value in assessing early warning signs of pesticide poisoning [4].

Several combinations of organophosphates were lethal at concentrations that were sublethal in single-chemical trials [5-7]. The acute toxicity of fenthion on *Cyprinus carpio* was highly toxic as compared to malathion, metasystox, and nuvan [8-10]. The acute toxicity of the insecticide mixture pointed out that the action of additional insecticides increases the toxicity level [11,12]. A method for calculating the Acute Insecticide Toxicity Loading was presented which accounts AITL method accounts for the total mass of insecticides used in the US, acute toxicity to insects using honey bee contact and oral LD 50 as reference values for arthropod toxicity, and the environmental persistence of the pesticides [13]. Among the synergetic actions of the pesticides selected, thiodan and fenthion were more toxic than fenthion and DDT [10]. The aim of this experiment was to study the chronic toxicity of gold, Alexander, and the mixture of them by assessing blood parameters.

2. Materials and Methods

The fish used in the present study were common carp (*Cyprinus carpio* L), juvenile fish, 60–80 g . in weight, from fish farms located in Al-Mahaweel City. Fish adapted to laboratory conditions for around one week. Throughout this period, fish were fed with 30% digestible protein and 3 kcal/g digestible energy. Aquariums (40-liter water capacity) used for this experiment were filled with de-chlorinated tap water prior to use and stocked with fish [14]. The aquarium was supplied with an air pump through a capillary system to provide constant air. The healthy fish were selected after isolating the weak and sick fish [15].Goldate is a trade name for herbicides that contain 240 mg of oxyfluorfen. Alexander is a trade name for insecticides that contain 15% SC indoxacarb. The LC50s of goldate, Alexander, and a mixture of them were 15.955, 4.627, and 7.642 mg/l, respectively.There were three concentrations (1/10, 1/100, and 1/200) for every pesticide (goldate, axander, and mixture of goldate and axander) **Table 1**, was exposed to fish for 6 weeks. The pesticide concentration was calculated depending on the following equation: In (µg/l) unit C1V1= C2V2

Table 1. The Goldate, Alexander and mixture of them concentration that used in acute toxicity test of common carp

Groups	Goldate (G) mg/l	Alzander (A) mg/l	Mixture (M) mg/l
1 (1/10)	1.595	0.463	1.840+0.460
2 (1/100)	0.159	0.046	0.184+0.046
3 (1/200)	0.079	0.023	0.092+0.023

Haematological changes Blood was sampled from five fish from each control and treated groups during each exposure period of 3 and 6 weeks. It was collected by heart puncture using a sterile disposable insulin syringe (previously washed with heparin to avoid clotting of fish blood due to fish blood clots rapidly), and almost all samples always require anticoagulant treatment, then quickly placed in EDTA tubes to determine hematological tests and Eppendrof tubes to determine AL and TP [16,17]. Physical and chemical conditions of Aquaria are given in **Table 2**.

Table 2. Physical and chemical conditions of Aquaria

Physical and chemical properties	Range
Temperature (°C)	20 - 28
Dissolved oxygen (D.O) (mg/L)	5.5 – 7.9
Hydrogen ion concentration(pH)	6.9 – 7.6
Electrical conductivity (µs/cm)	850 - 1220

3. Results and Discussion

Blood assessment is an essential technique for determining the physiological health of fish. Haematological measurements can be used to detect changes in a characteristic that exceed its usual homeostatic limitations [18].Red blood cell (RBC) results showed a significant decrease compared with control (C = 0.935) in the mean RBC value after 3 weeks in groups G1, G2, G3, A1, A2, and A3 with values of (0.745, 0.835, 0.810, 0.750, 0.745, and 0.815) 1012/L, respectively. As well, there was a greater decrease in groups M1, M2, and M3, with values of (0.690, 0.705, and 0.710) 1012/L, respectively. Also, the results found a significant decrease in the mean RBC value after 6 weeks in groups G1, G2, G3, A1, A2, and A3, with values of 0.0.695, 0.740, 0.730, 0.670, 0.685, and 0.745 µg/L, respectively. As well, there was a greater decrease in groups M1, M2, and M3, with values of (0.625, 0.640, and 0.635) 1012/L, respectively. The results showed significant differences in the value of RBCs in group M1 only with a concentration of 1/10 of the mixture of pesticides (M1) when comparing the differences in exposure times between 3 and 6 weeks see **Table 3**.

Table 3. The Effect of Group and Period in RBCs

Groups	Mean ± SE of RBCs 10^{12}/L				LSD value
	3 Weeks		6 Weeks		
Control	0.935 ±0.04		0.935 ±0.04		
	A	a	A	a	0.273 NS
G1	0.745 ±0.02		0.695 ±0.06		0.287 NS
	BC	a	BCDE	a	
G2	0.835 ±0.02		0.740 ±0.01		0.115 NS
	B	a	BC	a	
G3	0.810 ±0.02		0.730 ±0.03		0.185 NS
	AB	a	BCD	a	
A1	0.750 ±0.01		0.670 ±0.02		0.096 NS
	BC	a	BCDE	a	
A2	0.745 ±0.03		0.685 ±0.02		0.185 NS
	BC	a	BCDE	a	
A3	0.815 ±0.02		0.745 ±0.02		0.091 NS
	B	a	B	a	
M1	0.695 ±0.01		0.625 ±0.02		0.068 *
	C	a	E	B	
M2	0.705 ±0.05		0.640 ±0.04		0.292 NS
	C	a	CDE	a	
M3	0.710 ±0.02		0.635 ±0.02		0.107 NS
	C	a	DE	a	
LSD Value	0.906 *		0.102*		---

Means of RBCs with different big-letters in the same column and small-letters in the same row are significantly different. * ($P \leq 0.05$).

It seems clear that increased pesticide doses and the period of exposure had significantly affected the number of R.B.C. in examined common carp fish. The changes in blood parameters may cause by destruction or haemolysis of RBC by the effect of the pesticides that cause anemia and decrease in haemoglobin, the decrease in haemoglobin may be caused by the effect of the herbicide on the metabolic enzyme of haeme that was mention by the researcher [19], also haemodilution is another cause for decrease haemoglobin that occur to maintenance the

respiration and gas exchange, the secretion of catecholamine under stress condition increase flow of blood to gills with an increase of permeability of gills tissue, also increase taking water by the fresh water fish (haemodilution) that was mention by the researchers [20, 21].Analysis of variance of the Haemoglobin (Hb) results showed a significant decrease compared with control (C=8.45 g/dl) in the means Hgb value after 3 weeks in group G1 which had a value of 7.85 g/dl and more decrease in groups A1, M1 and M2 with value (7.80, 6.70 and 7.25) g/dl, respectively. While after 6 weeks the results found a significant decreas compared with control (C=8.45 g/dl) in the mean Hb value in groups G2, G3, A2, A3 and M3 that had values (7.70, 7.50, 7.65, 7.80 and 7.45) g/dl, respectively. So there was a greater decrease in the means Hb value of groups G1, A1, M1 and M2 which had values (6.95, 6.65, 6.20 and 6.75) g/dl, respectively. The results showed significant differences in the value Hb in groups G1, G3, A1 and M3 when comparing the differences in exposure times between 3 and 6 weeks **Table 4**. They found a decrease in the red blood cells (RBC) and haemoglobin (Hb) in common carp fish after exposure to Dursban [21].

Table 4. Effect of Group and Period in Hb

Groups	**Mean ± SE of RBCs 10^{12}/L**		**LSD value**
	3 Weeks	**6 Weeks**	
Control	8.45 ±0.05	8.45 ±0.05	
	A a	A a	0.273 NS
G1	7.85 ±0.05	6.95 ±0.05	0.304 *
	BC a	C b	
G2	8.20 ±0.10	7.70 ±0.10	0.608 NS
	AB a	B a	
G3	8.20 ±0.10	7.50 ±0.10	0.608 *
	AB a	B B	
A1	7.80 ±0.10	6.65 ±0.15	0.775 *
	C a	C b	
A2	8.25 ±0.15	7.65 ±0.15	0.912 NS
	A a	B a	
A3	8.30 ±0.20	7.80 ±0.10	0.962 NS
	A a	B a	
M1	6.70 ±0.10	6.20 ±0.10	0.608 NS
	E a	D a	
M2	7.25 ±0.15	6.75 ±0.25	1.253 NS
	D a	C a	
M3	8.25 ±0.05	7.45 ±0.15	0.680 *
	A a	B b	
LSD Value	0.362 *	0.418 *	---

Means of Hb with different big-letters in the same column and small-letters in the same row are significantly different. * ($P \leq 0.05$).

Regarding white blood cells after 3 weeks, this study has found a significant increase compared with control (C = 205.15 109/L) in groups G1, G2, G3, A3, and M1, which had values of 236.05, 230.75, 229.15, 186.40, and 230.00 109/L, respectively. While there was a significant decrease compared with the control in group A3 (186.40109/L), After 6 weeks, analysis of variance showed a significant increase in all groups of pesticides (G1, G2, G3, A1, A2, A3, M1, M2, and M3), which had values of 276.20, 267.55, 259.80, 250.65, 233.25, 224.15, 287.15, 271.40, and

262.25 109/L, respectively. The results showed significant differences in the value of WBCs in all groups of pesticides except for A2, A3, with concentrations of 1/100 and 1/1000 of Alexsander pesticides when comparing the differences in exposure times between 3 and 6 weeks **Table 5.** The initial increase in the WBCs count might be the result of direct stimulation of immunological defense due to the presence of toxic substances or may be associated with induced tissue damage [22]. WBCs are involved in the regulation of immunological function in many organisms, and the increase of WBCs in stressed animals indicates a protective response to stress [23].

Table 5. Effect of Group and Period in WBCs 109/L

Groups	**Mean ± SE of WBCs 10^9/L**		**LSD value**
	3 Weeks	**6 Weeks**	
Control	205.15 ±9.15 CD a	205.15 ±9.15 F a	55.67 NS
G1	236.05 ±5.15 A b	276.20 ±0.10 AB a	22.16 *
G2	230.75 ±1.95 AB b	267.55 ±2.75 ABC a	14.50 *
G3	229.15 ±3.35 AB b	259.80 ±1.40 BC A	15.62 *
A1	203.70 ±4.60 CDE b	250.65 ±2.45 CD a	22.42 *
A2	188.60 ±3.90 DE a	233.25 ±9.75 DE a	45.18 NS
A3	186.40 ±4.80 E a	224.15 ±13.95 EF a	63.47 NS
M1	230.00 ±5.80 AB b	287.15 ±5.25 A a	33.67 *
M2	213.85 ±3.85 BC a	271.40 ±10.50 ABC b	48.12 *
M3	217.35 ±9.25 BC b	262.25 ±4.45 BC a	44.16 *
LSD Value	17.78 *	23.295 *	---

Means of WBCs with different big-letters in the same column and small-letters in the same row are significantly different. * ($P \leq 0.05$).

Albumin, After 3 weeks, this experiment has found a significant increase in the albumin mean value compared with the control (C = 2.66 g/dl) in groups G3, A1, A2, and M3, with values of 4.39, 4.30, 4.85, and 4.46 g/dl, respectively. While there was no significant difference in the albumin mean in groups G1, G2, A3, M1, and M2, they had values of 3.16, 3.99, 4.75, 3.80, and 4.10 g/dl, respectively. After 6 weeks, this experiment has found a significant increase in the albumin mean value compared with the control (C = 2.66 g/dl) in groups G2, G3, A1, A2, A3, M2, and M3, which had values of 4.90, 5.06, 4.74, 5.05, 5.45, 4.74, and 5.01 g/dl, respectively. While there was no significant difference in the albumin mean in groups G1 and M1, they had values of 3.89 and 4.02 g/dl, respectively. The results showed significant differences in the albumin mean value in groups G1 and G3 with concentrations of 1/10 and 1/200, respectively, of herbicide (Goldate) when comparing the differences in exposure times between 3 and 6 weeks **Table 6**. The present results confirm impairments in protein metabolism as a result of pesticide

exposure. Serum protein concentrations may be altered because of the toxic effects of pesticides through damage to protein synthesis by hepatocytes and disturbance of kidney function [24, 25].

Table 6. Effect of Group and Period in Albumin

Groups	Mean ± SE of Total protein (g/dl)		LSD value
	3 Weeks	6 Weeks	
Control	2 .66 ±0.56	2.66 ±0.56	2.23 NS
	C a	C a	
G1	3.16 ±0.06	3.89 ±0.01	0.261 *
	BC b	BC a	
G2	3.99 ±0.93	4.90 ±0.84	5.39 NS
	ABC a	a	
G3	4.39 ±0.05	5.06 ±0.05	0.334 *
	AB B	a	
A1	4.30 ±0.10	4.74 ±0.11	0.639 NS
	A a	AB a	
A2	4.85 ±0.35	5.05 ±0.43	2.40 NS
	A a	AB a	
A3	4.75 ±0.45	5.45 ±0.34	2.42 NS
	ABC a	A a	
M1	3.80 ±0.50	4.02 ±0.48	2.98 NS
	ABC a	BC a	
M2	4.10 ±0.60	4.74 ±0.19	2.71 NS
	ABC a	AB a	
M3	4.46 ±0.14	5.01 ±0.11	0.779 NS
	AB a	AB a	
LSD Value	1.544 *	1.39 *	---

Means of Al with different big-letters in the same column and small-letters in the same row are significantly different. * ($P \leq 0.05$).

After 3 weeks, this study has found a significant decrease in the total protein mean value compared with the control (C = 12.48 g/dl) in groups M2 and M3, which had values of 8.00 and 8.01 g/dl, respectively. While there was no significant difference in the total protein mean in groups G1, G2, G3, A1, A2, A3, and M1, which had values of 13.01, 9.97, 9.10, 9.00, 9.65, 10.65, and 9.40 g/dl, respectively, After 6 weeks, this study has found a significant decrease in the total protein mean value compared with the control (C = 12.48 g/dl) in groups M2 and M3, which had values of 7.31 and 7.39 g/dl, respectively. While there was no significant difference in the total protein mean in groups G1, G2, G3, A1, A2, A3, and M1, which had values of 10.76, 10.49, 8.73, 8.67, 8.97, 10.02, and 9.03 g/dl, respectively, The results showed no significant differences in the total protein mean value of all groups when comparing the differences in exposure times between 3 and 6 weeks see **Table.7**. They used total protein, albumin, and globulin tests to monitor the course of diseases such as immune disorders, liver dysfunction, and impaired kidney activity [17]. Toxicants cause severe pathological alterations in fish, mainly gill lesions and protein level changes, which are indications of exposure to pesticides [27-29]. protein reduction, Al, and globulin concentrations because liver function may be decreased and no longer produce Al or proteins. The total protein was reported as an index of liver disturbance [30].

Table 7. Effect of Group and Period in Total protein

Groups	Mean ± SE of Total protein (g/dl)		LSD value
	3 Weeks	6 Weeks	
Control	12.48 ±2.72	12.48 ±2.72	10.70 NS
	AB	A	A	a	
G1	13.01 ±0.69	10.76 ±0.33	3.29 NS
	A	A	AB	a	
G2	9.97 ±0.52	10.49 ±0.44	2.94 NS
	AB	A	AB	a	
G3	9.10 ±0.70	8.73 ±0.51	3.71 NS
	AB	A	AB	a	
A1	9.00 ±0.50	8.67 ±0.37	2.67 NS
	AB	A	AB	a	
A2	9.65 ±1.25	8.97 ±0.03	5.38 NS
	AB	A	AB	a	
A3	10.65 ±0.95	10.02 ±0.58	4.80 NS
	AB	A	AB	a	
M1	9.40 ±0.40	9.03 ±0.60	3.12 NS
	AB	A	AB	A	
M2	8.00 ±1.60	7.31 ±0.09	6.89 NS
M3	8.01 ±0.30	7.39 ±0.07	1.351 NS
LSD Value	4.973 *	4.49 *	---

Means of TP with different big-letters in the same column and small-letters in the same row are significantly different. * ($P \leq 0.05$).

4. Conclusion

This experiment found a decrease in red blood cells (RBC), hemoglobin (Hb), and total protein (TP). While there was an increase in white blood cells (WBC) and albumin (Al) after six weeks of exposure, It was found that these changes increase with increasing pesticide concentrations and exposure duration when they are single. However, these changes are more severe when the fish are exposed to a mixture of pesticides at the same time, which is evidence of the synergistic action of these pesticides.

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Conflict of interest

The authors declare that they have no conflicts of interest.

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References

1. Lakshmipathy, K.,; Sindhu, S.; Singh, A.,; Chikkaballapur Krishnappa, S.; Duggonahally Veeresh, C. A review on pesticides degradation by using ultraviolet light treatment in agricultural commodities, *eFood*. **2024**, *5(1)*, e129. https://doi.org/10.1002/efd2.129.

2. Faruque A.; Fakhruddin A.; Abdulrahman A. ; Abdullah M. ; Khursheed M.; Saati, W.; Ehab Y. Elbendary K., Mohamed H. Abdelrahman H. Pesticides impacts on human health and the environment with their mechanisms of action and possible countermeasures. *Heliyon*. **2024**, *10(7)*, e29128. https://doi.org/10.1016%2Fj.heliyon.2024.e29128.
3. Environmental Protection Agency. *Worker protection standard website: American Public Health Association*. http://www.epa.gov/oppfead1/safety/workers/amend.htm, **2013**.
4. Pant, J.; Tewari, H.; Gill, T.S. Effects of Aldicarb on The Blood and Tissues of a Freshwater Fish. *Bull. Environ. Contam. Toxicol*. **1987**, *38*, 36-41. https://doi.org/10.1007/bf01606554.
5. Kaur, S.; Chowdhary, S.; Kumar, D.; Bhattacharyya, R.; Banerjee, D. Organophosphorus and carbamate pesticides: Molecular toxicology and laboratory testing. Clinica Chimica Acta; *International Journal of Clinical Chemistry*, 2023, *551*, 117584. https://doi.org/10.1016/j.cca.2023.117584.
6. Pandey A.; Pandey G. Hazards of organophosphate and carbamate nanoparticles to fish species. *International Journal of Universal Pharmacy and Bio Sciences* **2013**, *2(3)*, 17-27.
7. Cathy, A.; Baldwin, D.H.; Collier, T.K.; Hebert, V.; Stark, J.D.; Scholz, N.L. The Synergistic Toxicity of Pesticide Mixtures: Implications for Risk Assessment and The Conservation of Endangered Pacific Salmon. *Environmental Health Perspective* **2009**, *117(3)*, 348–353 https://doi.org/10.1289/ehp.0800096.
8. Tasneem S.; Yasmeen R . Biochemical changes in carbohydrate metabolism of the fish – *Cyprinus carpio* during sub -lethal exposure to biopesticide Derisom. *Iranian Journal of Fisheries Sciences.* **2020**, *19(2)*, 961–973. https://doi.org/10.22092/ijfs.2018.116876.
9. Amaeze, N.H.; Komolafe, B.O.; Salako, A.F.; Akagha, K.K.; Briggs, T.D.; Olatinwo, O.O.; Femi, M.A. Comparative assessment of the acute toxicity, haematological and genotoxic effects of ten commonly used pesticides on the African Catfish, *Clarias gariepinus* Burchell 1822. *Heliyon*, **2020**, *6(8):* e04768. https://doi.org/10.1016/j.heliyon.2020.e04768.
10. Muralidharan, L. Acute Toxicity and Synergetic Action of Some Pesticides on *Cyprinus Carpio*. *International Journal of Advanced Research* **2014**, *2*, 27-33.
11. DiBartolomeis, M.; Kegley, S.; Mineau, P.; Radford, R.; Klein, K. An assessment of acute insecticide toxicity loading (AITL) of chemical pesticides used on agricultural land in the United States. *PLoS One*. **2019**, 14(8), e0220029. https://doi.org/10.1371/journal.pone.0220029.
12. Verena T.; Arnd W.; Christian M., Markus E.; Steven L.; Ana C.; Daniel S.; Zhenglei G.; Ismael R. Acute toxicity of pesticide mixtures to honey bees is generally additive, and well predicted by Concentration Addition, *Science of The Total Environment*. **2023**, *857(3)*, 159518, https://doi.org/10.1016/j.scitotenv.2022.159518.
13. Holme, T. Connecting Chemistry Education and Insects. *Journal of Chemical Education*. **2022**, *99(4)*, 1545–1546. https://doi.org/10.1021/acs.jchemed.2c00233.
14. Ambreen, F.; Javed, M. Assessment of Acute Toxicity of Pesticides Mixtures for *Cyprinus carpio* and *Ctenopharyngodon idella*. *Pakistan Journal of Zoology*. **2015**, *47(1)*, 133-139.
15. Environmental Protection Agency (EPA). Ecological effects test guidelines OPPTS 850. 1075 fish acute toxicity test, freshwater and marine. *Office of prevention, pesticides and toxic substances (7101)*. U.S. EPA **1996**, 712-C-96-118.
16. Witeska, M.; Wargocka, W. Disodium EDTA Used As Anticoagulant Causes Hemolysis in Common Carp Blood. *Turkish Journal of Veterinary and Animal Sciences* **2011**, *35*, 99-104. doi: 10.3906/vet-0908-51.
17. SAS. Statistical Analysis System, User's Guide. Statistical. Version 9.6th ed. SAS. Inst. Inc. Cary. N.C. USA. **2018**.
18. Witeska, M.; Kondera, E.; Ługowska, K.; Bojarski, B. Hematological methods in fish–Not only for beginners. *Aquaculture* **2022**, *547*, 737498. https://doi.org/10.1016/j.aquaculture.2021.737498
19. Abdul-Ahad, S.A. *The Effect of Danitol on Common Carp Fishes*. M.Sc. Thesis, College of

Veterinary Medicine, University of Baghdad. **1996** (In Arabic).

20. Trot, L.; Torres, P. The Effect of Subleathal Concentration of Cadmium on Haematological Parameters in The Dogfish *Scyliorhinus canicula*. *Journal of Fish Biology*. **1988**, *32(2)*, 277-282. https://doi.org/10.1111/j.1095-8649.1988.tb05361.x .
21. Showy, B.G.; Rabee, A.M. Hematological Alternation In Common Carp Fish (*Cyprinus Carpio* L. 1758) After Exposing to Dursban. *Iraqi Journal of Science* **2019**, *60(3)*, 448-452. https://ijs.uobaghdad.edu.iq/index.php/eijs/article/view/650.
22. Kumar, A.A. Endosulfan induced biochemical and Pathophysiological Changes in Freshwater Fish, Clarias Batrachus . Ph.D dissertations, Osmania University, Hyderabad, Andhra Pradesh, India. **1994**.
23. Witeska, I. The Effect of Toxic Chemicals on Blood Cell Morphology in Fish. *Fresen. Environ. Bull*. **2004**, *13(12A)*, 1379-1384. https://doi.org/10.3390%2Fani13162625.
24. Arafa, A.; Afify, M.; Nervana, S. Evaluation of Adverse Health Effects of Pesticides Exposure [Biochemical And Hormonal] Among Egyptian Farmers. *Journal of Applied Science Research*. **2013**, *9(7)*, 4404-4409.
25. Mostafalou, S.; Abdollahi, M. Pesticides and Human Chronic Diseases: Evidences, Mechanisms, and Perspectives. *Toxicology and applied pharmacology* **2013**, *268(2)*, 157-177. https://doi.org/10.1016/j.taap.2013.01.025.
26. Mochida, K.; Lou, Y.H.; Hara, A.; Yamauchi, K. Physical Biochemical Properties of IgM From a Teleost Fish. *Immunology* **1994**, *83*, 675–80.
27. Peebua, P.; Kruatrachue, M.; Pokethitiyook, P.; Singhakaew, S. Histopathological Alterations of Nile Tilapia, *Oreoechromis niloticus*, *Tilapia zillii*and *Synodontis schall* From Ei Salam Canal, Egypt. *Egyptian Journal of Aquatic Biology and Fisheries* **2008**, *87*, 99–138.
28. Kaoud, H. A.; El-Dahshan, A.R. Bioaccumulation and histopathological alterations of the heavy metals in *Oreochromis niloticus* fish. *Nature and Science* **2010**, *8*, 147-156.
29. Maurya, P. K.; Malik, D.S. Accumulation and Distribution of Organochlorine And Organophosphorus Pesticide Residues in Water, Sediments And Fishes, *Heteropneustis Fossilis* and *Puntius ticto* From Kali River, India. *Journal of Toxicology and Environmental Health Sciences* **2016**, *8*, 30–40. https://doi.org/10.5897/JTEHS2016.0367.
30. Yang, J. L.; Chen, H. C. Effects of Gallium on Common Carp (*Cyprinus Carpio*): Acute Test, Serum Biochemistry, And Erythrocyte Morphology. *Chemosphere*, **2003**, *53(8)*, 877–882. https://doi.org/10.1016/S0045-6535(03)00657-X.