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Full Length Article

Biochemical, Endocrine and Genetic Impairments in Response to Agrochemicals Intoxication in Common Carp (*Cyprinus carpio*)

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Abstract

Worldwide extensive use of agrochemicals in agricultural production poses potential ecotoxicological effects and disturbs aquatic biota, more specially fish. This study aims to evaluate comparative effects of selected agrochemicals such as profenofos, endosulfan and deltamethrin on biochemical, endocrine and genetic profiles of the common carp (*Cyprinus carpio* L.). Forty healthy carp (50 ± 7.45g and 15 ± 5.86 cm) were selected randomly and equally divided into four groups; control group labeled as E0 and three treated groups having 4 ppb of profenofos, deltamethrin and endosulfan, labeled as E1, E2, E3 respectively. Fish were exposed to chemicals for 96 h. Obtained results revealed that, significant changes were observed in biochemical parameters of treated groups in comparison with control group ($P < 0.05$): glucose, creatinine, serum amylase, alkaline phosphate, sodium and phosphorus levels increased significantly, while a significant ($P < 0.05$) reduction was recorded in serum protein, triglycerides, serum lipase and magnesium levels. Whereas significant increase in TSH and cortisol levels were found, while significant decrease ($P < 0.05$) in T3, T4 and insulin level were observed in experimental groups as compared to control group. Genetic parameters were also affected under the stressors and showed significant increase ($P < 0.05$) in micronuclei frequency in erythrocytes of treated fish compared to the control group. Toxicities of the three agrochemicals were: endosulfan > deltamethrin > profenofos. The obtained results provide solid evidence that unobservant use of such agrochemical causes a pernicious effect on nontarget organisms such as fish. © 2021 Friends Science Publishers

Keywords: Profenofos; Deltamethrin; Endosulfan; Fish; Health

Introduction

Agrochemical is a substance or mixture of substances that is use for preventing, abolishing, resisting or mitigating any pest for enhancement in crop yield. Agrochemicals are frequently applied to agricultural commodities in order to fulfill the increasing food demand and for protection of crops from pests, pathogen and weeds (Gupta *et al.* 2013). Along with beneficial effects these agrochemicals have some disadvantages such as odor and change in taste of water and lethality of non-target organisms in aquatic environment (Sathyamoorthi *et al.* 2019). Pesticides enter in aquatic environment through runoff from place of application and pose a serious threat to aquatic life (Al-Otaibi *et al.* 2019). Aquatic organisms face significant problems similar to terrestrial species analysis of fish and water samples from different aquatic environments shows 90% to have one or more than one toxicant (Hussain *et al.* 2020). On the basis of chemical nature and mode of action agrochemical are categorized into five major categories namely; oraganochlorine, organophosphate, carbamates, pyrethroids and neonicotinoids (Xiao *et al.* 2017).

Profenofos is broad spectrum organophosphate highly effective for controlling chewing and sucking insects and mites mostly present on cotton plants (Reddy and Rao 2008). Deltamethrin is a synthetic pyrethroid., Natural pyrethrins are from *Chrysanthemum cinerariaefolium* (Bradberry *et al.* 2005). It is used to control aphids, white flies, lice, tsetse flies, fleas, ticks, spiders, bees, cockroaches, ants, weevils, beetles and bedbugs (Costa 2015). Endosulfan is an organochlorine is preferred for pests management programs such as mosquitoes and tsetse fly public health control programs (Guo *et al.* 2008).

As fish are in direct contact with water, they are exposed to the toxic chemical and accumulate raising concern about their biomagnification potential in the food

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webs (Zhang *et al.* 2020). Agrochemicals can disturb physiological functions and biochemical processes of organisms, so biochemical and endocrine parameters can be used as a biomarkers of stressor impacts (Isaac *et al.* 2017). Potential genotoxicity of pesticides are usually first monitored by using the micronucleus assay; a reliable, simple, easily performed bioassay (Talapatra and Banerjee 2007; Hemalatha *et al.* 2020). Induction of micronuclei in peripheral erythrocytes can be used as a biomarker for ecotoxicology, which can lead to damaging effects like retardation of growth and abnormal development in fish (Caliani *et al.* 2019). Common carp *(Cyprinus carpio* L.) is the third most frequently introduced important aquaculture species, and is source of 71.9% fresh water production, and a well-studied aquatic model organism in ecotoxicology when OECD standard bioassays are used (Rahman 2015). The aim of current study was to evaluate the comparative adverse effects of endosulfan, profenofos and deltamethrin on health status of the common carp during 96-h acute exposure.

Materials and Methods

Fish collection and acclimatization

Total one forty widespread freshwater omnivorous fish, Common carp (*Cyprinus carpio*) (50 ± 7.45g and 15 ± 5.86 cm) were collected from fish farm service road Kamra, Punjab Pakistan and brought to laboratory of Zoology department at Government Postgraduate College Haripur. Fish were allowed to acclimatize to lab condition for ten days prior to experiment, during this time period fish were fed with pelleted fish food and 70% of water was renewed on alternate days in order to prevent accumulation of toxic substances. The pH and temperature of laboratory water were maintained at 7.6-7.8 and 20-23°C, respectively.

Selected chemicals for exposure

Mmarketable commercial formulations of profenofos (40 EC), deltamethrin (60 EC) and endosulfan (37 EC) were selected for the study and purchased from local agrochemicals market of Peshawar Khyber Pakhtunkhwa, Pakistan.

Experimental setup

To investigate the possible toxic effect of Profenofos, Deltamethrin and Endosulfan, test solution of 4 ppb was prepared for each chemical from their commercial formulation. On the basis of fish mortality response and agrochemical concentration, the LC50 of profenofos, deltamethrin and endosulfan by Probit analysis (Finney 1980) was found to be 8.34 μg/L, 11.92 μg/L and 9.58 respectively for 96 h. Four glass aquaria of 60 L capacity were filled with 40 L of water and labeled as E0, E1, E2 and E3. E0 was control group having no chemical while E1 experiment one, E2 experiment two, E3 experiment three. Each aquarium contains 25 fish. Fish were exposed to the chemicals for 96 h (96-h acute bioassay).

Biochemical and endocrine analyses

After exposure of fish to agrochemicals for 96 h fish from each aquarium were collected and anesthetized with clove oil Blood samples were collected from the caudal vein of fish by using sterile syringes and immediately transmitted to a tube, allowed to clot. Collected blood samples were spun at 3000 rpm for 5 min to obtain blood serum. Sera were used for determination of biochemical parameters *i.e.*, serum protein, serum glucose, triglyceride, cholesterol, creatinine, serum amylase, serum lipase and alkaline phosphatase, Na, P, Mg and endocrine parameters *i.e.*, thyroid stimulating hormone (TSH), triiodothyronine (T3), tetraiodothyronine (T4), cortisol and insulin. Fully automated biochemical analyzer COBAS e 411 of Roche with Electro-Chemi-luminescence technology & CE approved 4th generation immunodiagnostic kits was used for the analyses.

Genotoxicity (Micronucleus assay)

For MN assay blood samples were taken from the caudal fin of fish by using disposable heparinized syringes and then transferred into EDTA tubes after that two drops of blood were applied immediately on clean grease free slides for making peripheral blood smears, two for each fish specimen. These slides were air dried for 24 h and then dipped into cold absolute methanol for 15 min and then air dried for one hour. Slides were stained with Giemsa dye in phosphate buffer for 30 min then slides were washed and dried. Prepared slides were observed under 100x oil emersion lens. Then total magnification becomes 10 × 100 = 1000) and frequency for MN were counted by using following formula:

$$\text{MN\%} = \frac{\text{Number of cells containing MN}}{\text{Total number of cells counted}} \times 100$$

Statistical analysis

The data for biochemical parameters, endocrine parameters and micronuclei frequency was statistically analyzed using SPSS® statistics software (version 24.0. Intergroup comparison was made by using one-way ANOVA while t-test was used for comparison between control and experimental group. The critical significance was set at 0.05.

Results

Physico-chemical parameters of water

The physico-chemical properties of laboratory water and experimental condition were regularly examined during the experiment. The reported values were as Mean ± SD for different parameters and are shown in Table 1.

Table 1: Physico-chemical parameters of laboratory water

Parameters	Data	Parameters	Data
Temperature	21± 1.19˚C	Calcium hardness	44 ± 1.55 mg/L
PH	7.5 ± 0.23	Total alkalinity	129±4.89 mg/L
Dissolve oxygen	6.4 ± 0.53	Conductivity	819±14.9 ms/L
Total dissolved solids	366 ± 10.81 mg/L	Chloride	18.5±2.1 mg/L

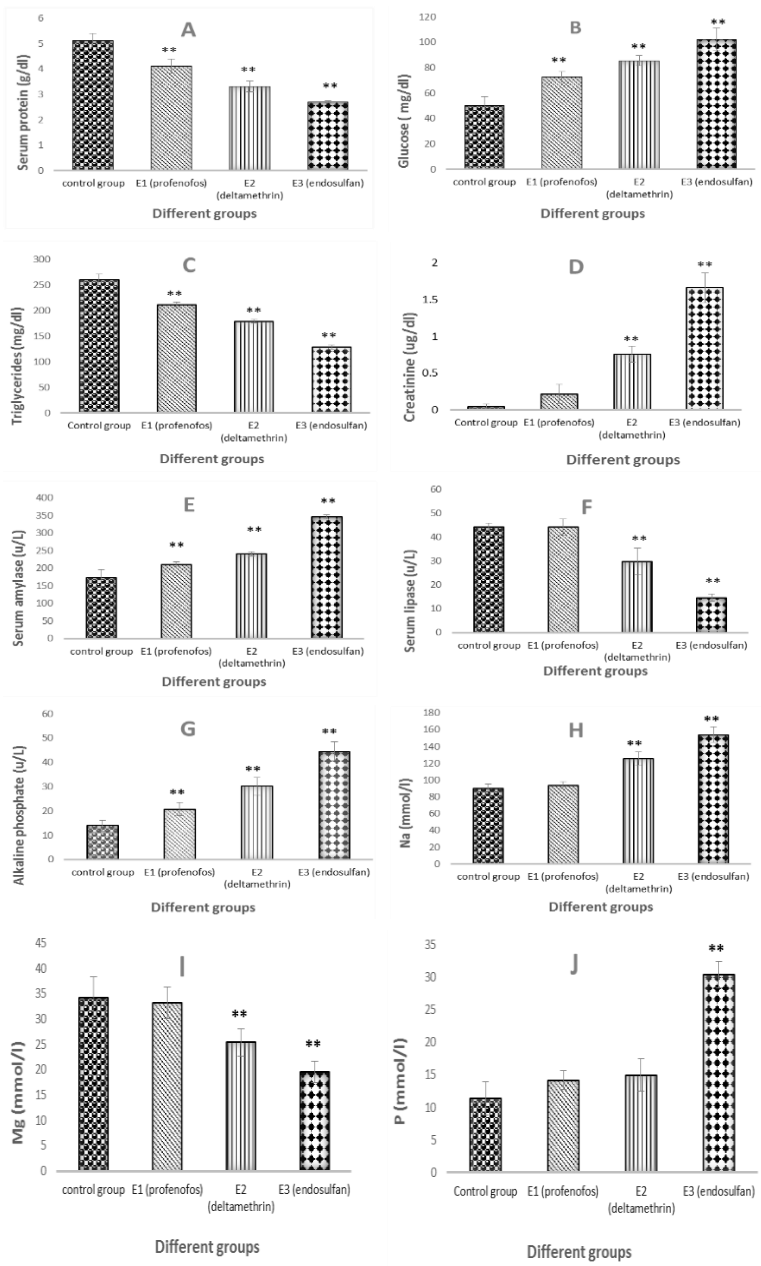

Fig. 1: A-J Serum protein, glucose, triglycerides, creatinine, serum amylase, serum lipase, ALP, Na, Mg and P levels of *C. carpio* after 96-h exposure to profenofos, deltamethrin and endosulfan. Sign (**) indicates significant difference (P < 0.05) to each other

Behavioral changes in *C. carpio*

Several changes in the behavior of treated group of *C. carpio* were observed throughout the experiment which varies from mild to severe depending upon the toxicity of pesticide. Various behavioral abnormalities shown by fish were, loss of body equilibrium, sinking to bottom, swimming at lateral side, darting swimming movement,

Table 2: MNi frequency in control and treated groups of *C. carpio*

Parameter	Control group E0	Profenofos (4 ppb)	Deltamethrin (4 ppb)	Endosulfan (4 ppb)
MN frequency (%)	0.19 ± 0.10	2.14 ± 0.51**$^{\Delta}$	3.16±0.56**$^{\Delta}$	4.22 ± 0.51**$^{\Delta}$

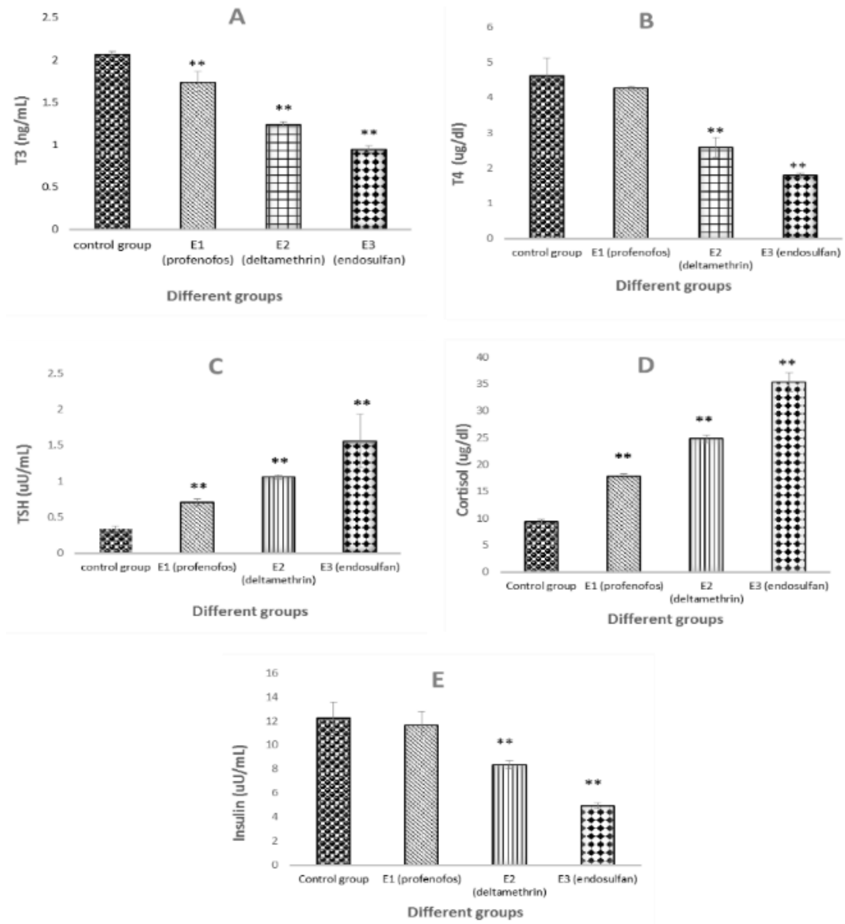

Fig. 2: (**A-E**) T3, T4, TSH, cortisol and insulin level of *C. carpio* against profenofos, deltamethrin and endosulfan exposure. Sign (**) indicates significant difference ($P < 0.05$) to each other

hyper-excitability, increase in movement of operculum, mucus secretions and due to stress some fishes tried to jump out of aquarium on the other hand no such type of alterations were noticed in control group of fish.

Biochemical analyses

In the present study *C. carpio* exposed to profenofos, deltamethrin and endosulfan showed alterations in biochemical parameters in response to agrochemical exposure as presented in Fig. 1 A–J. The values of serum protein and triglycerides decreased significantly in all treatment groups, while values of serum lipase and magnesium insignificantly decreased in group E1 exposed to profenofos, while significantly decreased in group E2 and E3 exposed to deltamethrin and endosulfan respectively. The values of glucose, serum amylase and alkaline phosphatase increased significantly in all the treated groups while Na and creatinine increased slightly in treated group I and significantly in group II and III, P level increased slightly in treated group I and II while significantly in group III compared to control group. The order of toxicities was endosulfan > deltamethrin > profenofos.

Endocrine parameters

In the present study endocrine variations in *C. carpio* was assessed on exposure to Profenofos, Deltamethrin and Endosulfan for 96-h as presented in Fig. 2 A–E. Values of T3 decreased significantly ($P < 0.05$) in all the treated groups while T4 decreased slightly in group I exposed to profenofos while significantly in group II and III exposed to deltamethrin and endosulfan respectively. TSH and cortisol values increased significantly ($P < 0.05$) in all the treated groups while values of insulin decrease slightly in group I and significantly in group II and III. No such type of alteration was recorded in endocrine parameters of fish belonging to control group. The order of toxicity of agrochemical was Endosulfan > Deltamethrin > Profenofos.

Estimation of genotoxic effect through MN assay

After agrochemical exposure micronuclei induction was observed in all the treated groups as compared to control group (Fig. 3). The highest micronuclei frequency was recorded against treated group III (4.22%) exposed to endosulfan while lowest MN frequency was calculated against group I (2.14%) exposed to profenofos (Fig. 4), which shows that endosulfan is more toxic amongst all three selected agrochemicals. The Micronuclei frequency increased in order of Endosulfan > Deltamethrin> Profenofos as represented in Table 2.

Discussion

Current study was conducted to assess the biochemical, endocrine and genotoxic effect of selected agrochemicals on *C. carpio* as an experimental model. To our knowledge, the present study provides the first ecotoxicological assessment for selected agrochemicals by using a multi-biomarker approach in a *C. carpio*. Our results indicated that a low acute sublethal exposure to agrochemicals in *C. carpio* has significant effects on the fish physiology and behavior. During experiment fish in E0, control group, showed normal behavior while various behavioral abnormalities were shown by treated group of fish such as; loss of body equilibrium, sinking to bottom, swimming at lateral side, darting swimming movement, hyper-excitability, increase in movement of operculum, mucus secretions and due to stress some fishes tried to jump out of aquarium. Similar changes were noticed by (Ghelichpour *et al.* 2020) by exposing *C. carpio* to lufenuron and by (Ghayyur *et al.* 2020) in *C. mrigala* against chlorfenapyr, dimethoate and acetamiprid exposure.

Biochemical study plays a vital role in monitoring fish health and assessing toxic effect of pollutants on aquatic organism (Poopal *et al.* 2017). The findings of present study showed a significant decrease in serum protein and triglycerides of agrochemical exposed fish as compare to control group. This result was in agreement with that of (Khan *et al.* 2019) in *C. carpio* treated with endosulfan and *O. orientalis* exposed to Cypermethrin (Shruti and Tantarpale 2014). The hypoproteinemia in present investigation might be due to boosted proteolysis, proteolysis seems to offer a physiological mechanism in a bid to provide energy to deal with stressful condition created by toxicant exposure. Triglyceride are major energy reserve in fish, the decline in triglyceride level in present investigation represent liver dysfunction (Prakash and Verma 2020). Similar results were obtained by (Qadir *et al.* 2014) when they exposed *L. rohita* to imidacloprid. Glucose is the major source of energy and its higher level in plasma act as a stress indicator in present study significant increase in glucose level observed. Our results was in line with findings of (Khan *et al.* 2018) and (Ghayyur *et al.* 2019). Changes in creatinine level shows kidney dysfunction, in present study level of creatinine increased in fish exposed to agrochemical in comparison to control group which may be due to oxidative damage. Similar consequences were reported by (Prusty *et al.* 2011). Our result also show similarity with the findings of (Amin and Hashem 2012) they noticed an increase in creatinine level in *C. gariepinus* against deltamethrin exposure.

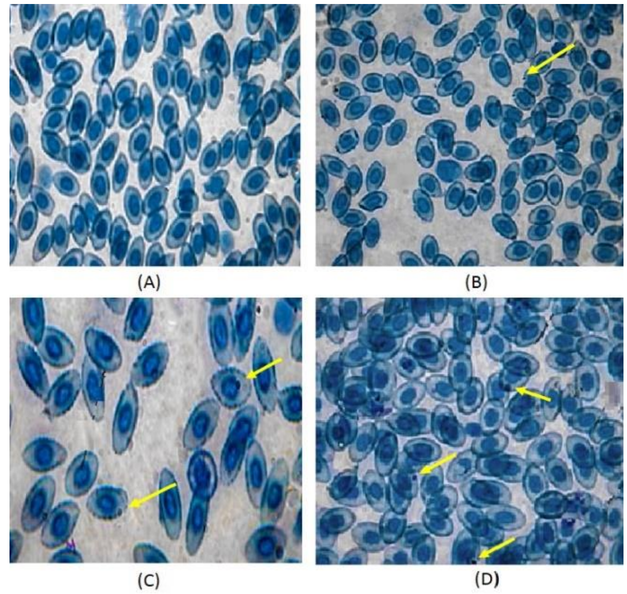

Fig. 3: MNi induction in erythrocytes of control, E0, (**A**) profenofos (**B**), deltamethrin (**C**) and endosulfan (**D**) treated groups

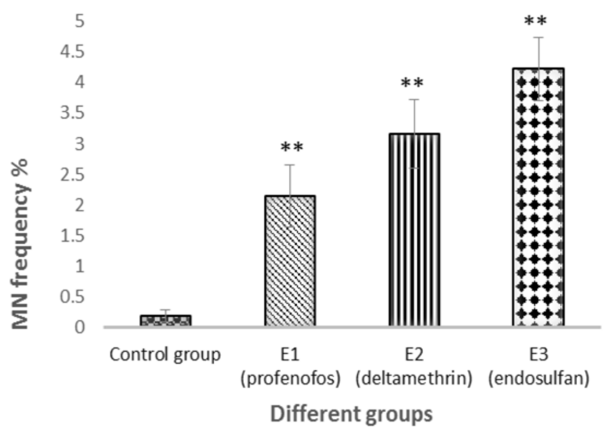

Fig. 4: Frequency (%) of MNi in erythroctes of *C. carpio* against Profenofos, Deltamethrin and Endosulfan exposure. Sign (**) indicates significant difference ($P < 0.05$) to each other

When toxicants enter different tissues are injured and damaged cells release specific enzymes, in this investigation treated fish revealed elevation in alkaline phosphatase.

Increase in ALP might be due to destruction of cell membrane of liver cells (Rahman *et al.* 2019). Similar increase in ALP was recorded by (Rahman *et al.* 2020) in *C. carpio*, by (Ghayyur *et al.* 2020) in *Cirrhinus mrigala* and by (Sancho *et al.* 2017) in *Anguilla anguilla*. Alteration in serum amylase indicates abnormality of digestive process in present study serum amylase increased in treated groups of fish, similar trend have been reported by (Lundstedt *et al.* 2004) and (Khan *et al.* 2019). Declined level of lipase due to agrochemical exposure may also be justified by the increased protein catabolism and reduced protein anabolism due to stress or may be due to decreased lipid levels. Similar reduction was reported in *C. carpio* exposed to malathion, chlorpyrifos and dimethoate (Rani *et al.* 2017).

Blood electrolytes are bio-indicator of physiology and health Status of fish. In present investigation values of sodium and phosphorus increased while magnesium level decreased. Increase in sodium and phosphorus was also reported by (Borges *et al.* 2007) when fish was exposed to Cypermethrin. Similar to our findings increase in sodium was reported by (Akhtar *et al.* 2019) in *Schizothorax esocinus* exposed to Cypermethrin. Our result shows disagreement with findings of (Atamanalp *et al.* 2002) who reported decrease in phosphorus after exposure of fish to cypermethrin.

Many fishes respond to stressors by showing endocrine shifts. In present study *C. carpio* showed decline in plasma T3 and T4 while significant increase in plasma TSH level. This result was in agreement with that reported by (Thangavel *et al.* 2010) in *S. mossambicus* exposed to endosulfan and by (Ghayyur *et al.* 2020) in *Cirrhinus mrigala.* Reduction in plasma T3 occur possibly because of diminution of T4 secretion or production (Li *et al.* 2008). Cortisol is an adrenocortical hormone involves in ion regulation and energy metabolism and used as a primary indicator of stress; it showed a significant increase in present study. Our results are in line with findings of other researchers e.g. *C. carpio* exposed to trichlorfon (Woo and Chung 2020), *C. carpio* exposed to cyfluthrin (Sepici-Dinçel *et al.* 2009) and *Salmo salar* exposed to atrazine (Waring and Moore 2004). In present study decline in level of insulin was recorded, the toxicant may have injured the islets of Langerhans which reduce the insulin secretion from β-cells (Thoker 2015). Similar to our outcomes, decline in insulin level was reported in *Danio rerio* (Ahmad *et al.* 2018), due to inhibition of β-cells by toxicant. Our result shows dissimilarity with the findings of (Shah *et al.* 2019) in which they reported an increase in insulin level of fish against dimethoate exposure.

MN assay is mostly used to detect biological impacts of aquatic pollutants on aquatic organisms, micronuclei induction in peripheral erythrocytes is due to genotoxic effect of pollutants (Tripathy 2020). Present study revealed genotoxicity of agrochemical in the peripheral erythrocytes of *C. carpio* as indicated by MN assay. Genetic damage increased in treated groups of fish as compare to control group, represented by formation of MN in erythrocytes. Our results show agreement with the findings of (Naqvi *et al.* 2016) when they treated *O. mossambicus* with different pesticides and (D'Costa *et al.* 2018) in *Danio rerio* exposed to monocrotophos. Similar genotoxic effects were also reported in erythrocytes of Grass carp on exposure to chromium, lead and copper (Shah *et al.* 2020). Likewise thiamethoxam administration also result in significant increase in micronuclei frequency in *L. rohita* (Ghaffar *et al.* 2020). Similar increase in micronucleus frequency was also reported in *O. mykiss* treated with fipronil (Ucar *et al.* 2020).

Conclusion

Present investigation clearly confirmed the harmful impacts of selected agrochemicals on biochemical, endocrine and genetic profile of *C. carpio* which showed that genetic, biochemical and endocrine parameters can be used as efficient biomarker for ecotoxicology. On the basis of current investigation, we can conclude that agrochemical pose serious threat to aquatic life in order to minimize poisonous effects of agrochemicals its use should be minimized and environment friendly agrochemical should be formulated having fast degrading ability and more target specificity.

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Author Contributions

Sanaa: Perform experiment, Shehzad Ghayyur: Designed the study, Sadia Tabassum & Shumaila Noreen: Review the article, Sajid Mahmood: Analyzed the data, Mujadad Ur Rehman & Bashir Ahmad: Help in sample collection, Muhammad Kabir & Muhammad Sajid: Write the article, Muhammad Fiaz Khan: Supervised the study

Conflict of Interest

The auther have no conflict of interest.

Data Availability

Data presented in this study are available on fair request to the corresponding author.

Ethics Approval

All the experimental work was conducted in accordance with the ethical guideline approved by the ethical committee of Hazara University, Mansehra, Pakistan.

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