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TOXICITY OF AN ORGANOPHOSPHATE ETHION, A SYNTHETIC PYRETHROID BIFENTHRIN AND NEEM BASED FORMULATION NEEM CARE ON ZEBRAFISH, *DANIO RERIO*

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ABSTRACT
In the present study, the toxic effect of an organophosphate Ethion, a synthetic pyrethroid Bifenthrin and a neem based formulation Neem Care on zebrafish, *Danio rerio* was evaluated. Adult mature male and female zebrafish were randomly selected and exposed to these three pesticides. The mortality rate of zebrafish was monitored under laboratory conditions for the time period of 24, 48, 72 and 96 hours. Data obtained from the toxicity test were evaluated using the Stat plus® 2009 computer program. The 96-h LC_{50} of Ethion was 3.369µg/l, Bifenthrin was 2.334µg/l and for Neem care was 5.659µg/l. It was found that Bifenthrin was more toxic to zebrafish as compared to Ethion and Neem care. In this experiment it was also observed that the toxicity of these pesticides were time as well as concentration dependent. Zebrafish can be used as bioindicator to assess the pesticide generated pollution in aquatic environment.

***Keywords**: Zebrafish, Ethion, Bifenthrin, Neem care, Toxicity, Pesticide, Bioindicator*

INTRODUCTION
With increasing population, industrialization and agricultural farming use huge amount of pesticide, chemical fertilizers and other related chemicals originate from human activities and they are discharged into the water bodies directly or indirectly (Bansode and Patil, 2016). Uses of pesticides have become a necessity in developing countries like India where it is estimated that approximately 30% of its crop yield are lost due to pest attack each year (Tiwari and Ansari, 2014). Many laboratory and epidemiological studies have revealed that pesticides are responsible for carcinogenesis, neurotoxicity, behavioral alterations, reproductive diseases, endocrine abnormalities, developmental disabilities and respiratory dysfunction on non-target organisms. Pesticides can cause acute and chronic poisoning of fish and may damage their vital organs, skeletal deformities (Kumar and Ansari, 1984), reduced reproductive ability (Sharma and Ansari, 2010), and various biochemical alteration (Tiwari and Ansari, 2014). Fish has been reported to be deficient in enzymes that hydrolyze these insecticides. Therefore, fish have relatively slow metabolism and slow elimination of these compounds and thus show sensitivity towards pesticide (David *et al*, 2003).

The unrestricted, heavy use of synthetic pesticides and other similar compounds induced lethal and sub-lethal effect on various non-targeted organisms like fishes and insects in the aquatic environment and direct or indirect affect the users (Kumaresan *et al*., 2019). About two million tons of pesticides are currently utilized annually worldwide (Sharma *et al*., 2019). Commonly used organophosphoruses are malathion, Ethion, methyl parathion, dichlorvos, trichlorfon, chlorpyrifos, diazinon, quinalphos, fenitrothion and phosphamidon etc. (Sushma *et al*, 2023).

Ethion is an organophosphate pesticide. Ethion is used in agriculture, mainly to control insects on citrus trees, on cotton, fruits, nut trees and vegetables. It may also be used on lawns and turf grasses but it is not used in the home for domestic pest control. Ethion affects the nervous system by inhibiting acetylcholine-esterase. Many reports have indicated that Ethion harms fish. Therefore, monitoring the impact of these pesticides is necessary. Hence in this study Ethion (50% EC) is exposed to the freshwater fish *Danio rerio* to find out its toxicological effects.

Bifenthrin is a type -I pyrethroid used widely for the purpose of agricultural activity and public health applications (Sayed *et al.*, 2018). Synthetic pyrethroids prevent sodium channel transition from activated (non-conducting) state by extending the open state of sodium channels following an initial influx of sodium during depolarizing stage of action potential (Field *et al.*, 2017). Due to excellent insecticidal property and rapid degradation in agricultural field, pyrethroids are dominant insecticide over the world wide markets (Henault-Ethier, 2015). These are generally more demanding due to their very high insecticidal property, low cost and low mammalian toxicity (Gray *et al*., 2018). Due to global uses of bifenthrin, its residues are frequently detected in environmental media, residential areas and biota, causing potential risks to the health of human and other non-target animals. Bifenthrin exhibited high acute lethal toxicity to aquatic species likewise insect, amphibians and fishes. Additionally, bifenthrin causes neurobehavioral toxicity, developmental toxicity, immunotoxicity and endocrine disrupting effects (Yang *et al.*, 2018).

In view of the environmental problems caused by synthetic chemicals due to growing need for alternative source of pest control that minimize this damage, there has been extensive research on pest control by natural plant products. Azadirachtin derived from neem (*Azadirachta indica* A. Juss), is very effective and considerably used in various neem based formulations. Azadirachtin acts as antifeedant, repellent, repugnant agent and induces sterility in insects by preventing ovipositor and interrupting sperm production in males (Suman *et al* 2017). It has been reported that neem based pesticides are target specific and comparatively less toxic. Recently, some neem based formulations were found toxic to the adult zebrafish as well as their embryos and fingerlings. Moreover, they also affected its reproductive ability (Ahmad *et al*, 2011; Ansari and Sharma; 2009, Ansari and Ahmad, 2010a; Ansari and Ahmad, 2010b; Sharma and Ansari, 2010 and Ahmad and Ansari, 2011).

All kind of pesticides (synthetic as well as natural) are being used extensively in the control of crop pest, mosquitoes and vector borne disease. With rapid industrialization and increase in human population, the pollution of water bodies has become a universal phenomenon in the control of various insect pests because they can be applied whenever and wherever needed. They are economical and most important thing is the reliability of control method. Hence, the production and consumption of pesticides has greatly increased in recent years. The contribution of pesticides to increase agricultural production cannot be denied, but synthetic pesticides have also caused unknown ecological damage, also induced serious health hazard among workers during manufacture, formulation and field applications (Dixit and Dutta, 1973; Anonymous, 1975; Kashyap, 1984; Ansari and Kumar, 1988).

Zebrafish was selected as the test species because they are the model organism (Spitsbergen and Kent, 2003), for developmental biology and toxicology research and also recommended by International Organization for Standardization and the Organization for Economic Co-operation and Development (OECD, 2010, 1992). The zebrafish is one of the models of choice for research on developmental biology because of its fecundity and its genetic and physiological similarity to mammals. These advantages have led the use of the zebrafish as a model in drug discovery and toxicological screening (Peterson et al, 2008). Further, small body size reduced the supplies required and costs to conduct experiment and also zebrafish can easily reproduce in laboratory and observe its complete life cycles and different stages can easily be observed for the experimental and developmental studies.

The present study was carried out to determine and compare the acute toxicities of three pesticides; Ethion, Bifenthrin and Neem care to establish relationship between toxicity of three different classes of pesticides i.e. organophosphate and synthetic pyrethroid and neem-based formulation.

MATERIALS AND METHODS

Zebrafish, *Danio rerio* were reported from Uttar Pradesh (Ansari and Kumar, 1982). Zebrafish were collected from the local ponds, stocked and acclimatized for a time period of 10-15 days under the laboratory conditions in glass aquaria containing de-chlorinated water. The water of the aquarium was aerated continuously through stone diffusers connected to a mechanical air compressor. Water temperature maintained between 25 ± 2℃ and the pH was maintained between 6.6 and 8.5. Fish were fed twice daily alternately with raw chopped goat liver, brine shrimps, spirulina, tubifex worm and blood worm fish food purchased from local markets.

For the toxicity test, Zebrafish, *Danio rerio* of similar age group, maturity and adultness were procured from the laboratory breed general culture. Toxicity tests were performed in laboratory to determine 24, 48, 72 and 96-h LC_{50} values using five different concentrations of Ethion (2.5, 3.0, 3.5, 4.0, and 4.5µg/l), five concentrations of Bifenthrin (1.5, 2.0, 2.5, 3.0, and 3.5µg/l) and five concentrations of Neem care (3.5, 4.5, 5.5, 6.5, and 7.5µg/l) previously diluted in acetone. Six replicates of ten fishes for each concentration of pesticides were performed accompanied by its respective control having the same volume of acetone without the pesticide. The randomization of fish in test aquaria was done according to the method prescribed by the U.S. Federal water pollution control administration, 1968.

Mortalities of Zebrafish were recorded for different concentrations. After every 24-h up to 96-h the water was changed. A fish was considered dead when its gill movements ceased and it did not respond to gentle prodding. Dead fish were removed from the aquaria to avoid deterioration. Ethion, Bifenthrin and Neem care was purchased from the local market.

The result was computed by StatPlus® version 2009 computer software purchased from Analyst soft, Vancouver, Canada. The LC_{50} values, upper and lower confidence limits (UCL and LCL), slope, Chi-square values were calculated.

RESULTS AND DISCUSSION

After the exposure of these pesticides, the zebrafish showed behavioral changes, they aggregated at one corner of the aquarium, resting at the bottom and frequently come to the surface followed by the heavy breathing with stronger opercular movement loss of equilibrium. Also, over secretions of mucus was observed from the body surface. their body colour also darkened, pectoral and pelvic fins got expended and the fish rolled vertically prior to death. However, the behavioral changes were more prominent for Bifenthrin than Ethion than that of Neem care.

The results of the toxic effects are illustrated in table **1**. It is visible that the LC_{50} values decreases, with the increase in exposure period. It means that the toxicity of these pesticides increases with the increase in time period. In other words, the mortality of fish was concentration as well as time dependent. From the given table, it is also evident that Bifenthrin is more toxic than Ethion and Neem care. The concentration of Bifenthrin required for killing the fish is lower than that of the concentration of Ethion and Neem care. It was observed that during the exposure of Bifenthrin, the LC_{50} value after 24-h was 4.635µg/l which decreased to 2.334µg/l after 96-h of exposure. The 24-h LC_{50} value of Ethion was 4.986µg/l which decreased to 3.369µg/l after 96-h of exposure. On the other hand, the 24-h LC_{50} value of Neem care was 9.532µg/l which decreased to 5.659µg/l after 96-h of exposure. The 96-h LC_{50} of Bifenhrin is 2.334µg/l whereas for Ethion and Neem care is much higher 3.369µg/l, 5.659µg/l. No mortality was observed in control sets. This indicates the less toxic nature of neem pesticide and organophosphate than the synthetic pyrethroid on the fish. All these three pesticides showed dose and time-dependent action.

The slope value shown in the table is steep. The LC_{50} values of the pesticides showed a significant ($p<0.05$) negative correlation with exposure time. The chi-square values were not significant, indicating that the fish population used in the experiment was homogeneous.

Bifenthrin was found to cause zebrafish mortality at very low concentrations. Bifenthrin is a synthetic pyrethroid and one of the most widely used all over the world. These classes of pesticides are mainly absorbed through the dermal, oral and respiratory routes and their metabolic degradation occurs at many sites (Miyamoto, 1976). Due to their Lipo-philicty, pyrethroids have a high rate of gill absorption, which it turn would be a contributing factor in the sensitivity of fish to pyrethroid exposures (Polat *et al.*, 2002). It is not only toxic for fishes only but also toxic to many aquatic invertebrates' species (Paul and Simonin, 2006). Synthetic pyrethroids causes neurotoxicity by interacting with Na^+ and K^+ permeability of nerve membrane resulting in repetitive discharges at the synapse and neuromuscular junction, which slows down both the activation and deactivation properties of the channels, leading to over excitation (Shafer *et al*., 2005; Shashikumae *et al*., 2010; Idris *et al.*,2012). This diverse mode of action, rapid absorption of pyrethroid might explain its extreme toxicity to fishes. The present 96-h LC_{50} value of bifenthrin to zebrafish, *Danio rerio* (2.334µg/l) is much lower from the results of earlier workers. Shubhajit and Nimai, 2021, worked on *Clarias batrachus* 96-h LC_{50} value was 3.464µg/l. Liu *et al*., (2005), stated the 96-h medial leathal toxicity values of 2.08 and 0.80 µg/l for common carp and tilapia (Tilapia spp.) respectively. The differences in their results may be due to differences of test medium, age, size and body weight of fishes, health and species variation. Dobsikova *et al.*, 2006 and Velisek *et al.*, 2007 observed that the 96-h LC_{50} for fish is below 30µg/l for the bifenthrin which belongs to such a highly toxic groups of chemicals for the fish and other aquatic organisms.

Ethion is a well-known organophosphate pesticide has been in agricultural use over the last few decades for controlling pest of cotton, tobacco, paddy, sugarcane and vegetables plants. It has been classified as a moderately hazardous pesticide by the World Health Organization (WHO, 2007). It has a moderate order of acute toxicity following oral and dermal administration. The acute toxic action of Ethion is the inhibition of the enzyme acetylcholine esterase (AChE) activity resulting in neuro toxicity to aquatic vertebrates and also humans. Ethion is moderately to highly persistent in soil. Its potential for biodegradation is decreased by its hydrophobicity and strong adsorption to organic matter and soil particles (Howard, 1991).

Table 1: Toxicity of an Organophosphate Ethion, a Synthetic Pyrethroid Bifenthrin and a Neem Based Formulation Neem Care on Zebrafish, *Danio rerio* (Cyprinidae)†.

Pesticides	Exposure Period (h)	LC_{50} values (µg/l)	Confidence limits		Slope	Chi-square values
			LCL	UCL		
Ethion	24	4.986	4.464	5.790	1.237	3.019
	48	4.386	3.917	4.887	1.303	0.159
	72	3.874	3.460	4.371	1.385	0.072
	96	3.369	3.046	3.821	1.330	0.322
Bifenthrin	24	4.635	3.629	5.085	1.695	0.415
	48	3.519	2.855	3.992	1.780	0.396
	72	2.777	2.224	3.460	1.921	0.172
	96	2.334	1.983	2.885	1.612	0.366
Neem Care	24	9.532	8.074	11.436	1.436	1.889
	48	9.093	7.303	10.006	1.589	1.336
	72	6.326	5.351	7.421	1.649	1.571
	96	5.659	4.677	6.973	1.707	0.056

† Six batches of ten fishes were taken for the each pesticide treatment. The slope value shown in the table is steep. The LC_{50} values of the pesticides showed a significant ($p<0.05$) negative correlation with exposure time. The chi-square values were not significant, indicating that the fish population used in the experiment was homogeneous. **LCL** represents lower confidence limits and **UCL** represents upper confidence limits.

In the result of the present study 24-h and 96-h LC_{50} value of Ethion for zebrafish were 4.986µg/l and 3.369µg/l respectively, and results were compared with other researchers. Sushma *et al., (2023)* observed that the LC_{50} values for 24-h and 96-h were 4.9µg/l and 2.8µg/l during the study on the fish *Catla catla* . LC_{50} of an organophosphate Dimethoate to fresh water fish *Colisa fasciatus* for 24-h and 96-h LC_{50} were found to be 22.15 mg/l and 21.65 mg/l respectively (Singh RN, 2013). Srivastava *et al.*, (2010), observed 96-h LC_{50} value in *Heteropneustes fossilis* was 100 mg/l when exposed to Roger. Singh and Ansari, (2016) observed that 24-h and 96-h LC_{50} 0.548 µg/l and 0.388 µg/l during the study of profenofos on zebrafish, *Dnio rerio*.

In the result of the present study 24-h and 96-h LC_{50} value of Neem care for zebrafish were 9.532 µg/l and 5.659 µg/l respectively. Literatures on the toxic effect of neem based formulations to fish especially *Danio rerio* are limited. In 2009 Ansari and Sharma was reported that Achook, a neem based pesticide to be toxic to zebrafish. *Ahmad et al. (2011)* observed that 24-h and 96-h LC_{50} value in zebrafish during the study of Neemgold on the fingerlings of zebrafish, which were 0.52 µg/l and 0.46 µg/l. With the present study it concluded that the "safe" neem based products are not so safe to the aquatic animals.
The toxicity of a pesticide varies from species to species and the variation may be due to the size, age, health and physiological parameters of water and differential tolerance of animal's exposure.

CONCLUSION

The present study concludes that Bifenthrin was more toxic to zebrafish as compared to Ethion and Neem care. In this experiment it was also observed that the toxicity of these pesticides were time as well as concentration dependent. Zebrafish can be used as bioindicator to assess the pesticide generated pollution in aquatic environment. Therefore, these pesticides should be used with great caution and in a sustainable way so that it may not be hazardous to aquatic environment and human beings. Moreover, extensive investigation should be done for their safe use in aquaculture.

REFERENCES

Ahmad MK and Ansari BA (2011). Toxicity of Neem based pesticide Azacel to the embryo and fingerlings of Zebrafish, *Danio rerio* (Cyprinidae). *World Journal of Zoology,* **6,** 47-51**.**

Ahmad MK, Sharma DK, Ansari S and Ansari BA (2011). Comparative Study of Synthetic Pyrethroid Lambda-cyhalothrin and Neem based Pesticide Neemgold on the Fingerlings of Zebrafish *Danio rerio* (Cyprinidae). *Research Journal of Chemical Sciences* **1**(6) 91-94.

Anonymous (1975). The Epa and the regulation of pesticides, staff Report to the subcommittee on administrative practices and procedure on the committee on the judiciary of the US senate. 32-34.

Ansari BA and Ahmad MK (2010 a). Toxicity of synthetic pyrethroid Lambda-cyhalothrin and Neem based pesticides Neemgold on Zebrafish, *Danio rerio* (Cyprinidae), *Global Journal of Environtal Research* **(4)**3, 151-154.

Ansari BA and Ahmad MK (2010b). Toxicity of pyretheroid Lambda-cyhalothrin and Neemgold to the embryo of Zebrafish, *Danio rerio* (Cyprinidae), *Journal of Applied Biosciences*, **36**(1), 163-165.

Ansari BA and Sharma DK (2009). Toxic effect of synthetic pyrethroid Deltamethrin and Neem Based formulation Achook on Zebrafish, *Danio rerio, Trends in Biosciences*, **2**(2), 18-20**.**

Ansari BA and Kumar K (1988). Cypermethrin toxicity effect on the carbohydrate metabolism of the Indian catfish, *Hetropneustes fossilis*. *The Science Total Environment* **72** 161-166.

Ansari, BA, Kumar K (1982). A record of Zebrafish Danio (*Brachydanio rerio*) (Cyprinidae) from Uttar Pradesh with notes on sexual dimorphism. *Journal of Advanced Zoology*. **3**(1) 88-89.

Bansode SB and Patil RD (2016). Relative toxicity of bifenthrin and carbosulfan fresh water fish *Gara mullya (*sykes). *Biolife* **4**(2) 482-484.

David M, Shivakumar H, Shivakumar R, Mushigeri SB and Ganti BH (2003). Toxicity evaluation of cypermethrin and its effect on oxygen consumption of the fresh water fish, *Tilapia mossambicas*. *Indian Journal of Environmental.Toxicology,* **13** 99-102.

Dikshit TSS and Dutta KK (1973). Endrin induced cytological chandes in albino rats. *Bulletin Environmental Contaminations of Toxicology*. **9** 65-69.

Dobsikova R, Velisek J, Wlasow T, Gomulka P, Svobodova Z and Novotny L (2006). Effects of cypermethrin on some haematological, biochemical and histopathological parameters of common carp (*Cyprinus carpio* L). *Neuroendocrinology Letters*, **27** 101-105.

Field LM, Davies TGE, O'Reilly AO, Williamson MS, Wallace BA (2017). Voltage-gated sodium channels as targets for pyrethroid insecticides. *European Biophysics Journal*, **46** 675-679.
Gray L, Florez SD, Barreiro AM, Vadillo-Sánchez J, González-Olvera G, Lenhart A, Manrique-Saide P, Vazquez-Prokopec GM (2018). Experimental evaluation of the impact of household aerosolized insecticides on pyrethroid resistant *Aedes aegypti*. *Science Reporter* **8** 12535.
Hénault-Ethier L (2015). Health and environmental impacts of pyrethroid insecticides: what we know, what we don't know and what we should do about it. Executive summary and scientific literature review. *Prepared for Équiterre. Montreal*. 68.
Howard PH (1991). Handbook of Environmental Fate and Exposure data for Organic Chemicals-Pesticides. Vol. 3. Lewis Publishers, Chelsea, MI, USA, 5-13.
Idris SB, Ambali SF, Ayo JO (2012). Cytotoxicity of chlorpyrifos and cypermethrin: the ameliorative effects o antioxidants. *African Journal of Biotechnology*, **11**(99) 16461-16467.
Kashyap SK (1984). Report of PTI: International workshop on pesticide formulation, held at New Delhi.
Kumar K, Ansari BA (1984). Malathion Toxicity: Skeletal deformities in Zebrafish, (*Brachydanio rerio*). *Pesticide Science*, **15** 107- 111.
Kumaresan, V, Pasupuleti M, Arasu MV, Al-Dhabi NA, Arshad A Nurul Amin SM., Yusoff F Md and Arockiaraj J (2019). Acomprative trancriptome approach for identification of molecular changes in Aphanomyces invadans infected *Channa striatus. Molecular Biology Reports.*, **45** 2511-2523.
Liu TL Wang YS and Yen JH (2005). Separation of bifenthrin enantiomers by chiral HPLC and determination of their toxicity to aquatic organism. *Journal of Food and Drug Analysis* **12** 357-360.
Miyamoto, J (1976). Degradation, metabolism and toxicity of synthetic pyrethroids. *Environmental health Perspectives*, **14** 15-28.
Organization of Economic Cooperation and Development; Guidelines for testing of chemicals, Guideline 2010. "fish, Early- life stage Toxicity Test." Adopted July 17, 1992.
Paul EA, Simonin HA (2006). Toxicology of Three Mosquito Insecticides to Crayfish. *Bulletin Environmental Contaminations of Toxicology*,**76** 614-621.
Peterson RT, Nass R, Boyd WA, Freedman JH, Dong K, Narahashi T (2008). Use of non-mammalian alternative models for neurotoxicological study. *Neurotoxicology*. **29** 546-555.
Polat H, Erkoc FU, Viran R and Knock O (2002). Investigation of acute toxicity of betacypermethrin on guppies Poecilia reticulate. *Chemosphere*, **49**(1) 39-44.
Raina R, Belzer W, Jones K (2009). Atmospheric concentrations of captain and floret in the lower Fraser Valley agricultural region of Canada. *Air Soil Water Res*; **2**:41–9.
Singh R N, (2013). *Acute toxicity of an organophosphate, dimethoate to an air breathing fish Colisa fasciatus* (Bl. And Schn*). Indian Journal of Scientific Research.* **4**(1) 70-75.
Shafer TJ, Meyer A, Crofton KM (2005). Development neurotoxicity of pyrethroid insecticides: critical review and future research needs. *Environment Health Prospects*. **113** 123-136.
Sharma A, Kumar V, Shahzad B, et al. (2019). Worldwide pesticide usage and its impacts on ecosystem. *SN Applied Sciences***.1**(11): 1-16. doi: 10.1007/s42452-019-1485-1.
Sharma DK and Ansari BA (2010). Effect of the synthetic Pyrethroid Deltamethrin and the neembased pesticide Achook on the reproductive ability of Zebrafish, *Danio rerio* (Cyprinidae), *Archive Polish Fishery* **18,** 157- 161.
Shashikumar S, Rajini PS (2010). Cypermethrin induced alteration in vital physiological parameters and oxidative balance in *C. elegans*. *Pesticide Biochemistry and Physiology*. **97** 235-242.
Saha S and Saha NC(2021). Study on Acute Toxicity of Bifenthrin to *Clarias batrachus* (Linn.). *Indian Journal of Ecology*, **48(2)**: 545-548.
Singh CB and Ansari BA (2016). Bioassay of Profenofos and Cypermethrin on Zebrafish, *Danio rerio*. *Scholars Academic Journal of Biosciences*. **4**(7) 578-582.
Spitsbergen JM, Kent ML (2003). The state of the art of the Zebrafish model for toxicology and toxicologic pathology research-advantages and current limitations. *Toxicological Pathology*. **31** 62-87.

Srivastava AK, Mishra D, Shrivastava S, Srivastava SK and Srivastav, AK (2010). Acute toxicity and behavioural responses of *Heteropneustes fossilis* to an organophosphate insecticide, dimethoate. *International Journal of Pharma and Bio Sciences*. **1** 359-363.
Chaudhary Suman, Rupinder K Kanwar, Sehgal Alka, David M Cahill, Colin J Barrow, Sehgal Rakesh and Jagat R Kanwar (2017). Progress on *Azadirachta indica* based Biopesticides in Replacing synthetic Toxic Pesticides. *Frontiers in Plant Science*. **8** 610.
Sushma M, Lalitha V, Venkata Rathnamma V (2023). Determination of LC_{50} and Behavioral studies of freshwater fish *Catla catla* (Hamilton) Exposed to Ethion (50% EC). *YMER* **22(**11) 1266-1277.
Syed F, Awasthi KK, Chandravanshi LP, Verma R, Rajawat NK, Khanna VK, John PJ, Soni I (2018). Bifenthrin-induced neurotoxicity in rats: involvement of oxidative stress. *Toxicology Research (Camb)*. **7**(1) 48–58.
Tiwari P, Ansari BA (2014). Comparative study of acute toxicity of endosulfan, chlorpyrifos and permethrin to zebrafish *Danio rerio* (Cyprinidae). *Scholars Academy Journal of Biosciences* **2**(7) 404-409.
Velisek J, Svobodova Z and Piackova V (2009). Effects of acute exposure to bifenthrin on some haematological, biochemical and histopathological parameters of rainbow trout (*Oncorhynchus mykiss*). *Veterinarni Medicina* **54**(3) 131-137.
Yang Y, Wu, N. and Wang, C. (2018). Toxicity of the pyrethroid bifenthrin insecticide. *Environmental Chemistry Letters*, **16** 1377-1391.