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# EFFECT OF COMBINATION PESTICIDE ON ACETYLCHOLINE ESTERASE ACTIVITY IN FRESHWATER FISH *DANIO RERIO*

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## ABSTRACT

An aquatic system is mostly contaminated due to widely-used pesticides. The most popular classes of insecticides are organophosphates and synthetic pyrethroids. Chlorpyrifos and Cypermethrin are used around the world to control pest insects in agricultural, residential, and commercial buildings, although use in certain residential applications has been restricted in several countries. The effect of Chlorpyrifos 50% + Cypermethrin 5% EC on acetylcholine esterase activity in freshwater, *Danio rerio* was observed. AChE is an important enzyme that can be measured as an environmental bio-indicator. Group of fish were exposed to pesticide concentrations of 8 µg/L, 10 µg/L and 14 µg/L. Brain tissues were collected for analysis of the enzyme activity Acetylcholine Esterase (AChE). The inhibition of acetylcholinesterase activity increased with pesticide concentration and exposure period. Statistically significant change was calculated by ANOVA using SAS version 9.3.

**KEYWORDS: Aquatic, Pesticide, ACHE inhibition, Zebra fish.**

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# INTRODUCTION

Among all the chemical pollutants, pesticides are the major aquatic pollutants in many parts of the world[3]. Fishes and other aquatic organisms are sensitive to the pesticide contamination in water. Pesticide finds their way into natural water bodies and affect aquatic environment by leaching from soil or surface run-off. The dependence of modern agriculture on agrochemicals like pesticides heavily is emerging as a threat to the ecological balance of aquatic ecosystems. Synthetic pesticides like organophosphates used for controlling pests in agriculture are one of the major causes of aquatic pollution[11]. For effective control of *Spodoptera litura* and other agricultural insect pests synthetic pyrethroids and organophosphate insecticides have intensively been used[2]. Toxic conditions of the fish can be assessed by estimation of enzyme parameters enzyme activity of Acetylcholine Esterase (AChE) which is a neurotransmitter in the nervous system is very critical. Organophosphates (OP) inhibit AchE and it is critical for nerve function. OP cause irreversible blockage of AChE enzyme, causes acetylcholine accumulation which results in muscle overstimulation. It causes disturbances across the cholinergic synapses and can only be reactivated very slowly. Several extensively used organophosphorus ester insecticides are phosphorothionates. The oxon metabolites of phosphorothionates have long been known to be responsible for the acute cholinergic neurotoxicity associated with OP poisoning. There is now sufficient evidence to suggest that the oxon metabolites may also be directly responsible for the particular neurotoxicity that phosphorothionate insecticides, and especially chlorpyrifos (CP) are known to inflict on the developing organism. Cypermethrin is a pyrethroid ester insecticide, it modulates various ion channels, including sodium and chloride channels. The primary targets for cypermethrin is insect voltage-gated sodium channel, it is expected that mammalian sodium channels and receptors regulated by it can also act as primary targets for toxicity in humans. Other main channels and receptors, which are influenced by cypermethrin include, chloride channels, voltage-gated calcium channels, potassium channels, GABA receptors, glutamate receptors, acetylcholine receptors and ATPases[4]. In vitro data reveal that the oxons, which are present at increased levels in the developing brain, have the ability to directly disrupt, at toxicologically relevant doses, separately a number of neurodevelopmental processes, including those of neuronal proliferation, neuronal differentiation, gliogenesis and apoptosis[10]. Exposures to sub-lethal concentrations of pesticides can cause changes in fish enzyme activity. Zebrafish presents a unique case among vertebrates, because AChE is the only ACh-hydrolyzing enzyme in this organism. In addition, AChE of zebrafish is highly related to that of mammals[5]. The objective of this study was to assess the impact of pesticide Chloryprifos 50% + Cypermethrin 5% EC on cholinesterase activity of the freshwater fish, *Danio rerio*.

# MATERIALS AND METHODS

*Danio rerio* procured from commercial fish farm quarantined and acclimated for 30 days to the laboratory conditions in 25 L glass aquaria. Fish were fed with commercial fish feed pellets. The water temperature was maintained in the range of 25.6 ˚C - 25.9˚C and pH 7.4 - 8.2. Concentrations were selected below the acute toxicity low concentration and the fish could survive the stress of the toxicant. Fifty fish were exposed to each pesticide concentration. Semi-static exposure was performed and water in the aquarium was replaced on alternate day. Chlorpyrifos 50% + Cypermethrin 5% EC was purchased from commercial market and used for the exposure. Fish were exposed for three different concentrations of 8, 10 and 14 µg/L. Control group was maintained. During the experiment the fish were observed for mortality and any toxicity sign with abnormal behavior. Three fish was periodically sacrificed on day 7, 14 and 21. The brain tissue was collected in 0.15 M KCl (cold buffer) and blotted and weighed. It was homogenized in 10% W/V in 0.1M, pH 7.4 Tris HCl buffer using glass homogenizer. The homogenate was centrifuged at 5000 rpm for 10 minutes and the supernatant was taken for

the estimation of enzyme activity. Estimation of AChE in the brain tissue of fish was performed[8]. 2.7 mL of phosphate buffer was added to 100 µL of supernatant and incubated for 37 $^{o}$C for 10 minutes and followed by the addition of 100 µl of Ellman's reagent (0.16 mM DNTB). The reaction was initiated by the addition of 100 µl of Acetylcholine iodide. The absorbance was recorded at 412 nm and the measurements were made in triplicate. Total protein was estimated with brain tissue homogenate (5 mg/mL) were prepared in 10% trichloroacetic acid (TCA)[12]. Bovine serum albumin was used as standard. Result was expressed as µg/mg.

V=∆A/min x 3/Protein x 1/14.3 µM/min/mg protein
∆A/min is the change in optical density
3 mL is the solution in the cuvette
14.3 molar extinction coefficient of DTNB M/min/mg protein

## STATISTICAL ANALYSIS

ANOVA (One-Way Analysis of Variance) was applied to compare the mean differences in the AChE levels between the concentrations using Student-Newman-Keuls Test[19].

## RESULTS

No mortality or toxicity signs were observed in the control and in the fish exposed to the concentrations of 8 µg/L, 10 µg/L and 14 µg/L. Brain tissues were collected for analysis of the enzyme activity Acetylcholine Esterase (AChE). Inhibition of AChE was more in 14 µg/L when compared to 10 µg/L and 8 µg/L. Statistically calculated significance and data are expressed as mean (n=5) ± standard deviation (SD). Means with the same letter are not statistically different at the level of 0.05 (Table 1).

**Table 1**

| Concentration µg/L | Brain tissue AChE (µM/min/mg protein) | | |
|---|---|---|---|
| | Day 7 | Day 14 | Day 21 |
| Control | 3.41$^{a}$ ± 0.22 | 3.54$^{a}$ ± 0.19 | 3.45$^{a}$ ± 0.27 |
| 8 | 2.66$^{b}$ ± 0.14 | 2.54$^{b}$ ± 0.06 | 2.42$^{b}$ ± 0.09 |
| 10 | 2.60$^{b}$ ± 0.17 | 2.52$^{b}$ ± 0.12 | 2.32$^{bc}$ ± 0.06 |
| 14 | 2.30$^{c}$ ± 0.14 | 2.33$^{b}$ ± 0.20 | 2.16$^{c}$ ± 0.13 |

***Means with the same letter are not significantly different***

## DISCUSSION

Conveniently, aquatic organism's fish serve as integrators of their total environment, and often respond to low concentrations of environmental contaminants[16]. Organophosphate irreversibly affects esterase's among which Choline esterase is most significant, but also inhibits other esterase's involved in hydrolyzing the ester bond in pyrethroid molecule. Thus Organophosphate noticeably lowers the $LD_{50}$ value for pyrethroid in combined exposure[13]. Primarily, chlorpyrifos interfere with signaling from the neurotransmitter acetylcholine. Chlorpyrifos-oxon a metabolite of chlorpyrifos, binds permanently to the enzyme acetylcholinesterase, preventing from deactivating acetylcholine in the synapse. Chlorpyrifos accumulates acetylcholine between neurons and irreversibly inhibits acetylcholinesterase. New molecules of acetylcholinesterase synthesis ensure that normal function of the nerves return. Chlorpyrifos mechanism of toxicity is well established for acute chlorpyrifos poisoning and lower-dose health impacts. Chlorpyrifos can also affect other enzymes, neurotransmitters, cell signaling pathways, at doses below that substantially inhibit acetylcholinesterase, which is a primary insecticidal mechanism. Acute symptoms of chlorpyrifos toxicity occur when excess acetylcholinesterase molecules are inhibited[6].Acetylcholinesterase activity of *Ctenopharyngodon idellus* exposed to

sublethal concentrations of chlorpyrifos in liver, kidney and gills. An increase in the acetylcholine content and its consequent accumulation at the synapses of neurons causes inhibition of AChE activity. Accumulation of acetylcholine leads to prolonged excitatory postsynaptic potentials causing hyperstimulation of the receptor[9]. The 28 day Wistar rat study that cholinesterase was depressed to a different degree in plasma and brain of animals receiving Chlorpyrifos alone or in combination with Cypermethrin. Cholinesterase is involved in the signal transmission at neuromuscular junctions and is expressed in the organism nervous system[23]. Chlorpyrifos produced a significant long term inhibition of AChE activity in the field crab *Barytelphusa guerini*[14]. Inhibition of AChE enzyme activity in the brain of *Gambusia affinis* exposed to sub-lethal exposure of Chlorpyrifos[22]. The effects of organophosphorus pesticide diazinon on acetylcholinesterase activity in brain tissue of freshwater fish, *Clarias gariepinus* exposed to 0.66 and 1.32 ppm sublethal concentrations and reported AChE activity in brain significantly decreased[1].sub-lethal doses of cypermethrin after 96h significantly alters the levels of total protein in muscle and liver tissues and the activity of enzyme acetylcholinesterase (AChE) of the freshwater teleost fish *Colisa fasciatus* in time and dose dependent manner[20]. Exposure to sulethal doses of cypermethrin for 24 h and 96 h exposure period caused significant change with time and dose dependent alterations in total protein, acetylcholinesterase, and cytochrome oxidase in liver and muscle tissues of fish *Labeo rohita*[21]. Cypermethrin inhibits AChE activity in the brain of *Labeo rohita* fingerlings[7]. Maximum inhibition of acetylcholinesterase activity of cypermethrin at sublethal concentrations content in the brain tissue of juvenile and adult freshwater fish *Tilapia mossambica*[18]. The toxic effect of cypermethrin on acetylcholinesterase and acetylcholine activity was investigated and the inhibition of acetylcholinesterase (AChE) in the brain was high compared to gill and liver tissues of freshwater fish *Cyprinus carpio*[17].The present data collected from the experiment conducted with the combination pesticide correlates with the individual exposure of Chlorpyrifos and Cypermethrin pesticides to fish where inhibition of AChE has been reported by several authors[17, 18,21,22]. Chlorpyrifos 50% + Cypermethrin 5% EC are highly toxic to freshwater fish *Poecilia reticulata* and users selecting both Chlorpyrifos and Cypermethrin from aquaculture point of view should take consideration of both safe or harmless and safe dischargeable concentrations for better management of aquaculture[15].

## CONCLUSION

The results of the investigation document the inhibitory effect of the combination pesticide on Choline esterase activity of *Danio rerio.* Continuous presence of low levels of pesticide in the aquatic environment may induce long term stress effects in fish. The measurement of AChE activity can be used as a reliable monitoring tool for hazard assessment in the aquatic environmental system.

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**CONFLICTOFINTEREST**

Conflict of interest declared none.

## REFERENCES

1. Adedeji O.B, Response of Acetylcholinesterase Activity in the Brain of *Clarias gariepinus* to Sublethal Concentration of Diazinon. Journal of Applied Sciences in Environmental Sanitation, 6 (2): 137 141, (2011).
2. Ahmad Munir, Sufian Saif Bhatti, Kamran Yousaf, Muhammad Naeem, Pyrethroids and new chemistry insecticides mixtures against *Spodoptera litura* (noctuidae: *lepidoptera*) under laboratory Conditions. Asian J Agri Biol, 1(2):45-50, (2013).
3. Amrita Mullick, Anand M Jha, Om Prakash Sharma, Cytotoxic and Chromotoxic effects of herbicide Stomp Xtra on air breathing teleost *Channa*

*Punctatus* (Bloch), Int J Pharm Bio Sci; 5 (3) : (B) 543 – 552, July (2014).

4. Anand Kumar Singh, Manindra Nath Tiwari, Om Prakash, Mahendra Pratap Singh, A Current Review of Cypermethrin-Induced Neurotoxicity and Nigrostriatal Dopaminergic Neurodegeneration Current Neuropharmacology, 10, 64-71, (2012).
5. Behra M, Etard C, Cousin X & Strähle U, "The Use of Zebrafish Mutants to Identify Secondary Target Effects of Acetylcholine Esterase Inhibitors," Toxicological Sciences, vol. 77, pp. 325-333, Oct, (2004).
6. Christensen K, Harper B, Luukinen B, Buhl K, Stone D, Chlorpyrifos Technical Fact Sheet; National Pesticide Information Center, Oregon State University Extension Services. (2009), Acessed on 20th November 2014. http://npic.orst.edu/factsheets/chlorptech.pdf.
7. Das B. K & Mukherjee S. C, "Toxicity of cypermethrin in *Labeo rohita* fingerlings biochemical, enzymatic and haematological consequences" Comparative Biochemistry and Physiology C, vol. 134, no. 1, pp. 109 -121, (2009).
8. Ellman G. L, Courtney K.D, Andres V. Jr. & Featherstone R.M, A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol. 7, 88-95, (2009).
9. Jindal R. & Kaur M, Acetylcholinesterase inhibition and assessment of its recovery response in some organs of *Ctenopharyngodon idellus* induced by chlorpyrifos. International Journal of Science, Environment and Technology, Vol. 3, No 2, 473- 480, (2014).
10. John Flaskos, The developmental neurotoxicity of organophosphorus insecticides: A direct role for the oxon metabolites. Toxicology Letters, 209, 1, 86-93. https://sciencescape.org/paper/22155227 [Abstract], (2012).
11. Lakshmaiah G & Indira P, Investigation of acute toxicity of Phorate (an organophosphate) on fresh water fish *Cyprinus carpio*. Int J Pharm Bio Sci 5(4): (B) 104 - 110, Oct, (2014).
12. Lowry O.H, Rosebrough N.J, Farr A.L. & Randall R.J, Protein measurement with folin phenol reagent. *J. Bol. Chem.* 193, 265-275, (1951).
13. Martin T, Ochou O. G, Vai ssayre M, Fournier D, Organophosphorus insecticides synergize pyrethroids in the resistant strain of cotton bollworm, *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae) from West Africa. J. Econ. Entomol. 96-468, (2003).
14. Madhusudan Reddy Narra, Rudra Reddy Regatte, Rajender Kodimyala, Sub-acute toxicity effects of chlorpyrifos on acetylcholinesterase activity and recovery in the freshwater field crab *Barytelphusa guerini*. International Journal of Environmental Sciences Volume 3, No 1, (2012).
15. Nageshwar Wast, Gupta A.K, Prakash M.M, Gaherwal S, Acute Toxicity of Chlorpyrifos 50% + Cypermethrin 5% EC to the Guppy, *Poecilia reticulata* (Peters, 1859) Global Veterinaria, 12 (3): 393-398, (2014).
16. Ogueji Emmanuel Okechukwu1, Ibrahim Baba, Usman Auta Jehu, Investigation of acute toxicity of chlorpyrifos-ethyl on *Clarias gariepinus* - (Burchell, 1822) using some behavioural indices. International Journal of Basic and Applied Sciences, 2 (2) 176-183, (2013).
17. Pugazhendy K, Meenambal M, Vasantharaja C, Venkatesan S, Jayachandran K, Attenuating Property of *Delonix elata* Plant against Inhibition of Acetylcholinesterase due to Cypermethrin Toxicity on Fresh Water Fish *Cyprinus carpio* (Linn). International Journal of Pharmaceutical & Biological Archives; 3(3): 654-658, (2012).
18. Reddy AT, Ayyanna K, Yellamma K, Sensitivity of brain cholinesterase to cypermethrin toxicity in freshwater teleost *Tilapia mossambica*. Biochem Int Mar; 23(5):959-62, (1991).
19. SAS version 9.3, SAS Institute Inc., Cary, NC, USA, 2002-2010.
20. Shailendra Kumar Singh, Sunil Kumar Singh, Ram P. Yadav, Toxicological and Biochemical Alterations of Cypermethrin (Synthetic Pyrethroids) Against Freshwater Teleost Fish Colisa fasciatus at Different Season. World Journal of

Zoology 5 (1): 25-32, (2010).
21. Sudhanshu Tiwari, Richa Tiwari, Ajay Singh, Impact of Cypermethrin on Fingerlings of Common Edible Carp (*Labeo rohita*). The Scientific World Journal Volume 2012 (2012), Article ID 291395.http://dx.doi.org/10.1100/2012/ 291395.
22. Venkateswara Rao J, Ghousia Begum, Pallela R, Usman P. K, Nageswara Rao R, Changes in Behavior and Brain Acetylcholinesterase Activity in Mosquito Fish, *Gambusia affinis* in response to the Sub-Lethal Exposure to Chlorpyrifos. Int. J. Environ. Res. Public Health, 2(3), 478-483, (2005).
23. Wielgomas B & Krechniak J, Effect of α-Cypermethrin and Chlorpyrifos in a 28 Day Study on Free Radical Parameters and Cholinesterase Activity in Wistar Rats. Polish J. of Environ. Stud. Vol. 16, No. 1 91-95, (2007).