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RESEARCH ARTICLE

EFFECT OF ENDOSULFAN TOXICITY ON GLYCOGEN LEVELS IN BRAIN AND MUSCLE OF FRESHWATER FISH CHANNA STRIATUS (BLOCH)

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ABSTRACT

A large number of pesticides are commonly used to control various agricultural pests; however, their toxicological impact also extends to non-target species like fish. Fish is good indicator of aquatic contamination because its biochemical stress responses are quite similar to those found in mammals. The aim of the present study is to assess the glycogen content in brain and muscle of the fish *Channa striatus* exposed to sublethal concentrations of endosulfan 1/10th (high), 1/15th (medium) and 1/20th (low) of the 96 hour LC₅₀ values for the period of 7, 14 and 21 days. The fish exposed to endosulfan showed a decrease the glycogen level for 7, 14 and 21 days in brain and muscle. However, no information is on record concerning the three different sublethal concentration of endosulfan on the glycogen contents of fish. The objective of the present work was to observe the effect of endosulfan on glycogen levels in brain and muscle of freshwater fish, *Channa striatus*.

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INTRODUCTION

Environmental and chemical stress can interfere with physiological and biochemical functions such as growth, development, reproduction and circulatory system in fish. Numerous biochemical indices of stress have been proposed to assess the health of non-target organisms exposed to toxic chemicals in aquatic ecosystem (Fahmy, 2012). India is primarily an agro-based country with more than 60–70% of its population dependent on agriculture. However, 30% of its agricultural produce is lost owing to pest infestation. In the absence of a better alternative, deployment of pesticides becomes inevitable despite their known hazardous effects (Velmurugan *et al.*, 2009). Utilization of pesticides in India is about 3% of the total world consumption and is increasing at the rate of 2–5% per annum (Bhadbhade *et al.*, 2002). The aquatic environment is continuously being contaminated with toxic chemicals from industrial, agricultural and domestic activity (Begum, 2004). Pesticides are an integral part of present day agricultural technology. They are greatly contributing towards increasing world food supply by protecting the crop yield (Ganeshwade, 2012). Due to intensive development of agriculture in recent years and rapid growth of industrialization in our country, there has been a great increase in manufacture and utilization of fertilizers, pesticides, petrochemical products, detergent and other synthetic chemicals and agricultural wastes pose a serious threat to the water ecosystem and aquatic life (Burton and Sinsheimer, 1963). Fishes are very sensitive to a wide variety of toxicants

in water, various species of fish show uptake and accumulation of many contaminants or toxicants such as pesticides (Herger *et al.*, 1995). Due to accumulation of pesticides in tissues produces many physiological and biochemical changes in the fishes and freshwater fauna by influencing the activities of several enzymes and metabolites (Nagarathnamma and Ramamurthi, 1982). Pesticides are known to contaminate a number of inland water bodies closer to areas of pesticide applications. Although pesticides are needed for the management of pests, their harmful effects on non-target organisms cannot be ignored. Pesticides leave residues in water and mud even several days after being sprayed in the adjacent crop fields. Pesticides affect growth and nutritional value of fish, when their concentration in water exceeds the critical maximum limit (Arunachalam *et al.*, 1980; Stalin and Manohar Das., 2012). As fish fauna serves as a food source, it is essential to know the impact of water pollution on these organisms. Any change in the natural conditions of aquatic medium causes several physiological adjustments in fish (Black, 1955). The present investigation was to assess the glycogen contents in brain and muscle of *Channa striatus* exposed to three different sublethal concentration of endosulfan.

MATERIALS AND METHODS

The fish *Channa striatus* having mean weight 25-30 gm and length 22 – 24 cm were collected from PSP fish farm, at Puthur and acclimatized to laboratory conditions. They were given the treatment of 0.1% KMNO₄ solution and then kept in plastic pools for acclimatization for a period of two weeks. They were

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fed on rice bran and oil cake daily. The endosulfan was used in this study and stock solutions were prepared. Endosulfan LC₅₀ was found out for 96 h (6.82 µg/L) (Sprague, 1971) and 1/20th, 1/15th and 1/10th of the LC₅₀ values were 0.34, 0.45 and 0.68 µg/L respectively taken as sublethal concentrations for this study. Forty fish were selected and divided into 4 groups of 10 each. The first group was maintained in free from endosulfan and served as the control. The other 3 groups were exposed to sub lethal concentration of endosulfan 10 litre capacity aquaria. The 2nd, 3rd and 4th groups were exposed to endosulfan for 7, 14 and 21 days respectively. At the end of each exposure period, the fish were sacrificed and the required tissues were collected for glycogen estimation. The glycogen content of the tissues was estimated by the method of Kemp and Kits Van Heijninger (1954). The data so obtained were analyzed by applying analysis of variance DMRT one way ANOVA to test the level of significance (Duncan, 1957).

RESULTS

The glycogen levels in brain and muscle of *Channa striatus* exposed to low, medium and high sublethal concentration of endosulfan showed significant decrease when compared to control fish. The decrease in brain and muscle of *Channa striatus* glycogen levels were more pronounced at 21 days of exposure periods (Table 1 and 2).

Table 1. Glycogen (mg/g) in brain of *Channa striatus* exposed to sublethal concentrations of endosulfan

	7 Days	14 Days	21 Days
Control Brain	13.28 ± 1.01 ^c	14.36 ± 1.09 ^d	13.92 ± 1.05 ^d
Low concentration	12.94 ± 0.98 ^c	12.10 ± 0.92 ^c	11.35 ± 0.86 ^c
Medium concentration	11.72 ± 0.89 ^b	10.24 ± 0.77 ^b	8.74 ± 0.66 ^b
High Concentration	9.66 ± 0.73 ^a	7.55 ± 0.57 ^a	5.95 ± 0.45 ^a

All the values mean ± SD of six observations Values which are not sharing common superscript differ significantly at 5% (p < 0.05) Duncan multiple range test (DMRT)

Table 2. Glycogen (mg/g) in muscle of *Channa striatus* exposed to sublethal concentrations of endosulfan

	7 Days	14 Days	21 Days
Control Brain	21.36 ± 1.62 ^c	21.78 ± 1.65 ^d	22.46 ± 1.71 ^d
Low concentration	20.14 ± 1.53 ^c	19.52 ± 1.48 ^c	18.70 ± 1.42 ^c
Medium concentration	17.85 ± 1.36 ^b	16.24 ± 1.23 ^b	14.38 ± 1.09 ^b
High Concentration	14.22 ± 1.08 ^a	12.48 ± 0.94 ^a	8.15 ± 0.61 ^a

All the values mean ± SD of six observations Values which are not sharing common superscript differ significantly at 5% (p < 0.05) Duncan multiple range test (DMRT)

DISCUSSION

Endosulfan, an organochlorine pesticide, is used to control insects and mites infesting crops including vegetables, fruits, tea, coffee, cotton, rice and grains (Thangavel *et al.*, 2010). Excessive application of pesticides near agriculture field leads to found the surrounding aquatic medium through wind action and agriculture runoff. The non-target organisms like fish, crab, prawn and other aquatic animals are severely affected by the action of pesticides and reduction of the nutrients like carbohydrate, protein and lipids in the organisms. Carbohydrates are very important reserved food materials of all organs of living cells and sources of energy for fishes. Carbohydrates form an important organic constituent of animal

tissues. It is one of the important macromolecule, which comes first to reduce fish from enduring stresses caused by any xenobiotic by providing energy (Binukumari and Vasanthi, 2014). Carbohydrates are stored as glycogen in fish tissue and organs like the muscle and liver in order to supply the energy needs when there are hypoxic conditions, intensive stocking and a lack of food (Cicik and Engin, 2005; Wendelaar-Bonga, 1997). It has been demonstrated that liver glycogen levels decreased in *Oncorhynchus mykiss* as a result of the activation of glycolytic enzymes via catecholamines under lack of food and hypoxic conditions (Vijayan and Moon, 1992). The carbohydrate reduction suggests the possibility of active glycogenolysis and glycolytic pathway to provide excess energy in stress condition (Reddy *et al.*, 1993). Glycogen, a large and branched polymer of glucose, is the storage form of carbohydrate for virtually every organism from yeast to primates. The major glycogen stores in mammalian vertebrates exist in liver and muscle, smaller amounts of glycogen being present in kidney, intestine and several other tissues. Classically, it is thought that the glycogen stored in liver, kidney and intestine can be made accessible to other organs by virtue of their possession of an enzyme glucose-6-phosphatase (Vorananen *et al.*, 2011). Glycogen levels are found to be highest in liver, as it is the chief organ of carbohydrate metabolism in animals, followed by muscle. Liver glycogen is concerned with storage and export of hexose units for maintenance of blood glucose and that of muscle glycogen is to act as a readily available source of hexose units for glycolysis within the muscle itself. A fall in the glycogen level clearly indicates its rapid utilization to meet the enhanced energy demands in fish exposed to toxicant through glycolysis or Hexose Monophosphate pathway. It is assumed that decrease in glycogen content may be due to the inhibition of hormones which contribute to glycogen synthesis (Sobha *et al.*, 2007).

Biochemical responses of aquatic organisms to contaminants usually represent the first measurable effects of contaminant exposure, and accordingly are advantageous for use in monitoring programs (Hinton, 1994). The results of the present findings showed a significant decrease in glycogen levels in brain and muscle of *Channa striatus* exposed to sublethal concentrations of endosulfan at 7, 14 and 21 days. The decreased level of glucose and glycogen contents in the liver, muscle, intestine, kidney and brain of *Channa punctatus* exposed to phenyl mercuric acetate (Karuppasamy, 2000). Many workers reported a similar trend of decrease in carbohydrate (Venkatramana *et al.*, 2006; Saradhamani and Selvarani, 2009). Reduction of muscle glycogen was observed in *Anguilla anguilla* exposed to fenitrothion (Sancho *et al.*, 1998) in *Clarias batrachus* exposed to insecticide rogor (Begum and Vijayaraghavan, 1999) in *Brycon cephalus* exposed to folidol 600 (Aguiar *et al.*, 2004) in *Leporinus obtusidens* exposed to glyphosate herbicide (Gluszczak *et al.*, 2006) in silver catfish (*Rhamdia quelen*) exposed to glyphosate herbicide (Gluszczak *et al.*, 2007) in *Rhamdia quelen* exposed to clomazone herbicide (Crestani *et al.*, 2006) and in *Labeo rohita* exposed to malathion (Thenmozhi *et al.*, 2011). A fall in the glycogen level clearly indicates its rapid utilization to meet the enhanced energy demands in fish exposed to toxicants through glycolysis or hexose monophosphate pathway. It is assumed that decrease in glycogen content may be due to the inhibition of hormones which contribute to

glycogen synthesis. Carbohydrate content decreased in heptachlor intoxicated fish *Tilapia mossambica* and stated this may be due to the rapid utilization of carbohydrates by the tissue possibly to overcome the pesticides induced stress (Radhaiah *et al.*, 1987). Mishra and Srivastava (1984) and Ganeshwade, (2011) reported that depletion of glycogen in fish under the organophosphorus toxicity. Rawat *et al.* (2002) observed that continuous decrease in quantity of glycogen in the liver of *Heteropneustes fossilis* exposed to endosulfan. Venkataramana *et al.* (2006) reported that depletion of glycogen in heart muscle of gobiid fish, *Glossogobius giurus* when exposed to malathion. Bantu *et al.* (2013) reported that glycogen content was decreased in the muscle of freshwater fish exposed to chlorantraniliprole. Glycogen level was decreased in liver and muscle of *Oreochromis niloticus* exposed to malathion (Al-Ghanim, 2012). Adeyem and Adewale (2013) addressed that the glycogen content was decreased in liver and muscle of African cat fish, *Clarias gariepinus* was exposed to lead and cypermethrin. In conclusion, thus the endosulfan intoxication has disturbed the normal functioning of cells with alterations in the fundamental biochemical mechanism in fish. This study showed that endosulfan altered the carbohydrate metabolism in *Channa striatus* by affecting the levels of glycogen in brain and muscle due to impairments in energy requiring vital processes.

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REFERENCES

- Adeyem, J.A and Adewale, O.O., 2013. The effects of exposure to multiple stressors of Lead (Pb) and Cypermethrin on biochemical profiles of African catfish, *Clarias gariepinus*. *International Journal of the Nigerian Society for Experimental Biology*. 25 (2):26 – 30.
- Aguiar, H.L., Moraes, G., Avilez, M.I., Altran, E.A., Correa, F.C., 2004. Metabolical effects of folidol 600 on the neotropical freshwater fish matrinxã, *Brycon cephalus*. *Environ. Res.* 95, 224–230.
- Al-Ghanim, K.A., 2012. Malathion toxicity in Nile tilapia, *Oreochromis niloticus* – A haematological and biochemical study. *African Journal of Agricultural Research*. 7(4): 561-567.
- Arunachalam, S., Jeyalakshmi, K. and Aboubucker, S., 1980. Toxic and sublethal effects of carbaryl on a fresh water Cat fish, *Mystus vittatus*. *Archives of Environmental Contamination Toxicology*, 9: 307-316.
- Bantu, N., V.R. Vakita and S. Karra, 2013. Effect of chlorantraniliprole on biochemical and certain biomarkers in various tissues of freshwater fish *Labeo rohita* (Hamilton) *Environment and Ecology Research* 1(4): 205 – 215.
- Begum, G., 2004. Carbofuran insecticide induced biochemical alterations in liver and muscle tissues of the fish *Clarias batrachus* (Linnaeus) and recovery response. *Aquat. Toxicol.* 66, 83–92.
- Begum, G., Vijayaraghavan, S., 1999. Effect of acute exposure of the organophosphate insecticide rogor on some biochemical aspects of *Clarias batrachus* (Linnaeus). *Environ. Res.* 80, 80–83.
- Bhadbhade, B. J.; Sarnaik, S. S.; Kanekar, P. P., 2002. Bioremediation of an industrial effluent containing monocrotophos. *Curr. Microbiol.*, 45: 346–349.
- Binukumari, S and J. Vasanthi., 2014. Toxicity of the pesticide Dimethoate 30% EC on the Carbohydrate content of the Freshwater fish, *Labeo rohita*. *J. Chem. Bio. Phy. Sci. Sec. B*, 4(1): 220-223.
- Black, E.C., 1955. Blood levels of haemoglobin and lactic acid in some freshwater fishes following exercise, *J. Fish. Res. Bd.* XII 9117.
- Burton, A and R.L. Sinsheimer, 1963. Insecticides effects on cutthroat trout of repeated exposure to DDT. *Science*, 42: 958.
- Cicik, B and Engin, K., 2005. The Effects of Cadmium on Levels of Glucose in Serum and Glycogen Reserves in the Liver and Muscle Tissues of *Cyprinus carpio* (L., 1758) *Turk J Vet Anim Sci*, 29 : 113-117.
- Crestani, M., Menezes, C., Gluszczak, L., Miron, D.S., Lazzari, R., Duarte, M.F., Morsch, V.M., Pippi, A.L., Vieira, V.P., 2006. Effects of clomazone herbicide on hematological and some parameters of protein and carbohydrate metabolism of silver catfish *Rhamdia quelen*. *Ecotoxicol. Environ. Saf.* 65, 48–55.
- Duncan, B.D., 1957. Multiple range tests for correlated and heteroscedastic means. *Biometrics*, 13: 359-364.
- Fahmy, G.H., 2012. Malathion toxicity: Effect on some metabolic activities in *Oreochromis niloticus*, the tilapia fish. *International Journal of Bioscience, Biochemistry and Bioinformatics*, 2 (1): 52 – 55.
- Ganeshwade R M, 2012. Effect of dimethoate on the level of cholesterol in freshwater *Puntius ticto* (Ham). *Science Research Reporter* 2(1): 26 – 29.
- Ganeshwade, R.M., 2011. Biochemical Changes Induced by Dimethoate in the Liver of Freshwater fish *Puntius ticto* (Hamilton). *Biological Forum. An International Journal*, 3(2): 65-68.
- Gluszczak, L., Miron, D.S., Crestani, M., Fonseca, M.B., Pedron, F.A., Duarte, M.F., Vieira, V.L.P., 2006. Effect of glyphosate herbicide on acetylcholinesterase activity, metabolic and hematological parameters in piava (*Leporinus obtusidens*). *Ecotoxicol. Environ. Saf.* 65, 237–241.
- Gluszczak, L., Miron, D.S., Moraes, B.S., Simoes, R.R., Schetinger, M.R.C., Morsch, V.M., Loro, V.L., 2007. Acute effects of glyphosate herbicide on metabolic and enzymatic parameters of silver catfish (*Rhamdia quelen*). *Comp. Biochem. Physiol.* 146C, 519–524.
- Herger, W., Jung, S.J and Peter, H., 1995. Acute and prolonged toxicity to aquatic organisms of new and existing chemicals and pesticides. *Chemosphere*, 31: 2707 - 2726.
- Hinton, D.E., 1994. Cells, cellular responses, and their markers in chronic toxicity of fishes. In: Malins, D.C., Ostrander, G.K. (Eds.), *Aquatic Toxicology: Molecular, Biochemical, and Cellular Perspectives*. CRC Press, Boca Raton, FL, pp. 207–239 Chapter 4.
- Karupphasamy, R. 2000. Effect of phenyl mercuric acetate on carbohydrate content of *Channa punctatus* Uttar Pradesh *J. Zool.* 20(3): 219-225.

- Kemp, A. and Kitsvan Heijhinger, J.M., 1954. A colorimetric micromethod for the determination of glycogen in tissues. *Biochem. J.*, 56: 646-648.
- Mishra J. and Srivatava A.K., 1984. Effects of chlordane on the blood and tissue chemistry of a teleost fish, *Heteropneustes fossilis*, *Cell and Molecular Biology*. 30: 519-523.
- Nagrathamma, S and R. Ramamurthi, 1982. Metabolic depression in the freshwater teleost *Cyprinus carpio* exposed to an organophosphate pesticide. *Curr. Sci.*, 51 (B): 668 - 669.
- Radhaiah V., Girija M. and Jayantha Raok., 1987. Changes in selected biochemical parameters in the kidney and blood of the fish *Tilapia mossambica* (peters) exposed to heptachlor. *Bulletin of Environmental Contamination Toxicology*. 39: 1006-1011.
- Rawat D.K., Bais V.S. and Agrawal N.C., 2002. A correlative study on liver glycogen and endosulfan toxicity in *Heteropneustes fossilis* (Bloch). *Journal Environmental Biology*. 23(2): 205 - 207.
- Reddy, M.M., Kumar, V.A., Reddy, S.N.L., 1993. Phenol induced metabolic alteration in the brain and muscle of fresh water fish *Channa punctatus* during sublethal toxicosis. *J. Ecotoxicol. Environ. Monit.*, 3(1):7-1.
- Sancho, E., Ferrando, D.M., Fernandez, C., Andreu, E., 1998. Liver energy metabolism of *Anguilla Anguilla* after exposure to fenitrothion. *Ecotoxicol. Environ. Saf.* 41, 168-17.
- Saradhamani, N. and B.J. Selvarani, 2009. A study on the effect of herbicide metribuzin on the biochemical constituents of the freshwater fish, *Tilapia mossambica* Peters (Pisces: Cichlidae). *Curr. Biotica*, 3: 220-231.
- Sobha, K., Poornima, A., Harini, P and Veeraiah, K., 2007. A study on biochemical changes in the fresh water fish, *Catla catla* (Hamilton) exposed to the heavy metal toxicant cadmium chloride. *Kathmandu University Journal of Science, Engineering and Technology*. 1(4): 1-11.
- Sprague, J.B., 1971. Measurement of pollutant toxicity to fish. III. Sublethal effects and Safe concentrations. *Water Res.* 5:245-266.
- Stalin.S. I and S. S. Manohar Das., 2012. Biochemical changes in certain tissues of *Cirrhina mrigala* (Hamilton) (Cyprinidae: Cypriniformes) exposed to fenthion. *International Journal of Environmental Sciences*. 2(3) : 1268 – 1277.
- Thangavel P., Sumathirai K., Maheswari S., Rita S. and Ramaswamy M., 2010. Hormone profile of an edible, freshwater teleost, *Sarotherodon mossambicus* (Peters) under endosulfan toxicity, *Pestic. Biochem. Physiol*, 97(3) , 229 – 234.
- Thenmozhi, C., V. Vignesh, R. Thirumurugan and S. Arun., 2011. Impacts of malathion on mortality and biochemical changes of freshwater fish *Labeo rohita*. *Iran. J. Environ. Health. Sci. Eng.*, 8 (4) : 387- 394.
- Velmurugan, B., M. Selvanayagam, E.I. Cengiz and E. Unlu., 2009. Histopathological changes in the gill and liver tissues of freshwater fish, *Cirrhinus mrigala* exposed to dichlorvos. *Braz. Arch. Biol. Technol*, 52 (5) : 1291-1296.
- Venkataramana, G.V., P.N. Sandhya Rani and P.S. Murthy., 2006. Impact of malathion on the biochemical parameters of gobiid fish, *Glossogobius giuris* (Hamilton). *Journal of Environmental Biology*, 27(1) : 119-122.
- Vijayan, M.M and Moon, T.W., 1992. Acute handling stress alters hepatic glycogen metabolism in food-deprived rainbow trout (*Oncorhynchus mykiss*). *Can. J. Fish Aquat. Sci.*, 49: 2260-2266.
- Vornanen, M., Asikainen, J and Haverinen, J., 2011. Body mass dependence of glycogen stores in the anoxia-tolerant crucian carp (*Carassius carassius* L.). *Naturwissenschaften*, 98: 225- 232.
- Wendelaar-Bonga, S.E, 1997. The stress response in fish. *Physiol. Rev*, 77: 591-625.
