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TOXIC IMPACTS OF TWO ORGANOPHOSPHORUS PESTICIDES ON THE ACETYLCHOLINESTERASE ACTIVITY AND BIOCHEMICAL COMPOSITION OF FRESHWATER FAIRY SHRIMP *STREPTOCEPHALUS DICHOTOMUS*

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ABSTRACT

The 96 hrs LC_{50} values for malathion and glyphosate was determined to be 9.1 ppm and 5.5 ppb, respectively on the fairy shrimp, *Streptocephalus dichotomus* preadults. The 1/5th of 96 hrs LC_{50} of malathion (2.0 ppm) and glyphosate (1.1 ppb) respectively was chosen. The sub lethal toxic effect of each of these pesticides on the acetylcholinesterase activity (AchE) of *S. dichotomus* was assayed on days 0 and 15th of exposure, and found to be significantly decreased (0.13±0.006 nmol/mg/min) in malathion than in glyphosate when compared with control. Furthermore, the contents of selected biochemical constituents (total protein, carbohydrate and lipid) of *S. dichotomus* were estimated on '0', 15th and 30th days of exposures, and found to be decreased significantly on 30th day in both the pesticides when compared to control. The results indicated the fact that these pesticides are toxic to *S. dichotomus* at the tested concentrations.

KEYWORDS:AchE, Malathion, Glyphosate, *S. dichotomus*, Protein, Carbohydrate, Lipid.

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INTRODUCTION

Presently organophosphorus pesticides (Ops) have largely replaced organochlorine compounds in the agricultural activities. Ops have been widely used to control agricultural pests, but these are harmful to non-target aquatic organisms when frequently used, due to contamination of aquatic environment through run-off (Roche *et al.*, 2007; Joseph and Raj, 2011). Although at present usage of several pesticides contribute to increased agricultural production and to eradicate vector borne diseases, their harmful effects on the non-target animals are not ruled out and also concentrated in them more readily than to the terrestrial organisms (Nicholson and Hill, 1970; Edwards, 1973).OPs are known to inhibit acetylcholinesterase enzyme (AChE E.C 3.1.1.7), which plays an important role in neurotransmission at cholinergic synapses by rapid hydrolyzing the neurotransmitter acetylcholine to choline and acetate (Ozmen *et al.,* 1999; Fulton and Key, 2001). Some Ops are highly soluble in water and can therefore easily contaminate aquatic ecosystems, thereby increasing the exposure risk of aquatic flora and fauna (Agdi *et al.,* 2000). Malathion is commonly used to control mosquitoes and fruit flies. It is also used to eliminate fish ectoparasites. It could contaminate water supplies as aerial spraying to water bodies for controlling mosquitoes and flies, disposing waste materials and containers near water supplies or emptying the waste materials into sewer drains, or surface drifts from the agricultural area (Muirhead Thompson, 1971; Eto, 1974). Glyphosate is a broad-spectrum herbicide widely used to kill unwanted weeds both in agriculture and in nonagricultural landscapes. The indiscriminate use of this herbicide, careless handling, accidental spillage or discharge of untreated effluents into natural water ways have harmful effects on the crustacean populations and other aquatic organisms and may contribute to long term effects in the environment (Williams *et al.,*2000). The fairy shrimp, *Stretocephalus dichotomous* (Crustacea: Anostraca) are small freshwater fairy shrimps commonly found in seasonal vernal pools. They are subjected to risks of exposure to agrochemicals especially OPs and other inorganic chemicals. In this study, the sub lethal effects of malathion and glyphosate on AChE activity and concentrations of total protein, carbohydrate and lipid in *S. dichotomous* pre adults were studied.

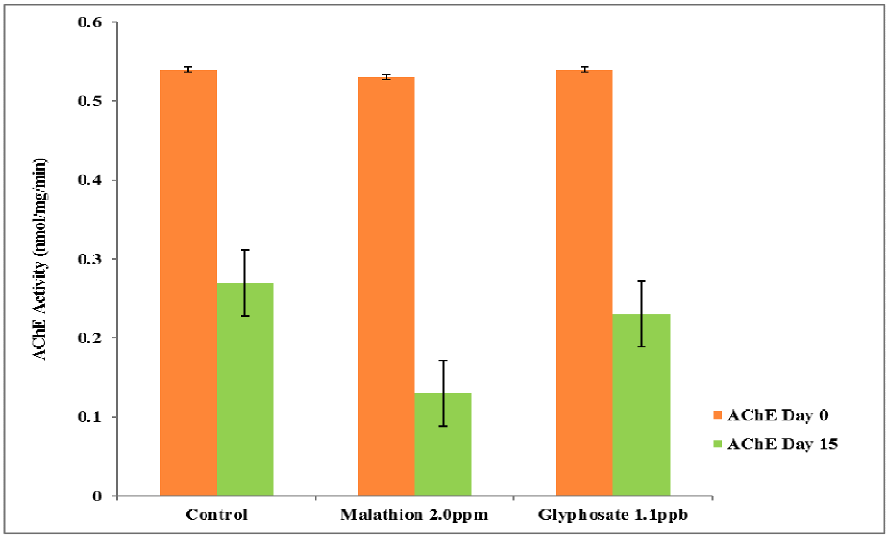
MATERIALS AND METHODS

S. dichotomous were originally collected from Putlur, Thiruvallur District (near Chennai) and Pennagaram, Dharmapuri District, Tamilnadu, India and were cultured under laboratory conditions for cyst (resting eggs) production. Fairy shrimps were hatched from cysts by following the procedure of Calleja *et al.* (1993). Toxicity tests were performed to determine the 96 hrs LC_{50} values of malathion and glyphosate and were found to be 9.1 ppm and 5.5 ppb respectively to the pre adults of *S. dichotomous* (Arun Kumar & Jawahar Ali in press). Based on the 96 hr LC_{50} value, a sub lethal concentration in each pesticide (1/5th of the 96 hr LC_{50}; malathion, 2.0 ppm; glyphosate, 1.1 ppb) was chosen for further studies. Preadults of *S. dichotomous* were exposed to sub lethal concentrations of malathion and glyphosate for a period of 30 days. Sampling was performed on the 15th day and 30th day of exposures. AChE activity was assayed on '0' and 15th days of exposure (Ellman *et al.,* 1961). The concentrations of biochemical constituents, such as total protein, carbohydrate and lipid were estimated on '0', 15th and 30th days of exposures in the whole body of *S. dichotomous* by following the methods of Bradford (1976), Hedge and Hofreiter (1962), and Van Handel (1985) and Inouye and Lotufo, (2006) respectively. One way analysis of variance (ANOVA) was performed to check the validity of data observed (George and Mallery, 2006).

RESULTS AND DISCUSSION

AChE activity in the fairy shrimp whole body at day 0 and day 15 after exposure to sub lethal concentration of malathion and glyphosate are given in Fig.1. The AChE activity was found to decrease in shrimps exposed to sub lethal concentrations of malathion and glyphosate on days '0' and 15 when compared with control. The decrease was higher in malathion exposed test shrimps than that of the glyphosate (P<0.05).

Figure 1
AChE activity in Fairy shrimp at Day 0 and Day 15 after exposure to Malathion and Glyphosate



Tables 1-3 depict the concentrations of total protein, carbohydrate, and lipid, respectively. There was a decrease in the levels of these biochemical constituents in test shrimps exposed to sub lethal concentrations of malathion and glyphosate on '0', 15th and 30th days of exposures when compared with control. On 30th day maximum decline was recorded. The decrease was higher in malathion exposed test shrimps than that of the glyphosate (P<0.05).

Table 1
Protein content (µg/mg) in the tissue of Fairy shrimp (Streptocephalus dichotomus) exposed to sublethal concentration of malathion and Glyphosate, Mean±SD (N=3),*P<0.05

Exposure period days	Control Mean±SD	Malathion 2.0ppm Mean±SD	Glyphosate 1.1ppb Mean±SD
0	11.6±0.15	11.4±0.15	11.7±0.15
15	14.5±0.15*	5.26±0.15*	5.60±0.10*
30	12.4±0.15*	2.26±0.05*	2.76±0.15*

Table 2
Carbohydrate content (µg/mg) in the tissue of Fairy shrimp (Streptocephalus dichotomus) exposed to sublethal concentration of Malathion and Glyphosate, (Mean±SD) (N=3), *P<0.05

Exposure period in days	Control Mean±SD	Malathion 2.0ppm Mean±SD	Glyphosate 1.1ppb Mean±SD
0	4.73±0.15	4.56±0.20	4.73±0.15
15	5.06±0.11*	1.53±0.15*	1.63±0.30*
30	7.20±0.20*	0.40±0.10*	0.53±0.11*

Table 3
Lipid content (µg/mg) in the tissue of Fairy shrimp (Streptocephalus dichotomus) exposed to sublethal concentration of Malathion and Glyphosate, (Mean±SD) (N=3), *P<0.05

Exposure period in days	Control Mean±SD	Malathion 2.0ppm Mean±SD	Glyphosate 1.1ppb Mean±SD
0	23.9±1.59	23.5±0.88	22.7±0.98
15	27.8±1.60*	12.1±0.54*	16.1±1.00*
30	33.4±0.189*	5.26±0.04*	8.59±0.11*

Acetylcholine (AChE) is the most important neurotransmitter in most animals. It is resulted by a stimulated nerve cell into the synapse, or neuromotor junction with another nerve cell. Once Ach has been secreted into the synapse it binds to receptor sites on the next nerve cells, causing the latter to propagate the nerve impulse through the synapse, Ach secreted after the first impulse must be hydrolyzed by the AchE in the junction. Several studies on the mode of action and inhibition of AchE have been carried out. Indeed, inhibition of this enzyme is the focal target for most of the current synthetic pesticides. It has been established that the AChE enzyme unit consists of a negative subsite, which attracts the quaternary group of choline through both columbic and hydrophobic forces and an esterase subsite, where nucleophilic attack occurs on the acetyl carbon of the substrate Taylor (1985). The catalytic mechanism resembles that of other serine esterases (e.g. Alkaline phosphatases), where a serine hydroxyl group is rendered highly nucleophilic through a charge relay system involving the close apposition of an imidazole group and presumably a carboxyl group on the enzyme. The decrease in AChE level in this study was probably due to the impairment of neural transmission due to OPs inhibitory action. Key *et al.* (1997) reported decrease in AChE activity when grass shrimp exposed to sub lethal concentration of malathion. Modesto and Martinez, (2010) recorded decreased AChE activity in the brain and the muscle of *Prochilodus lineatus* when exposed to glyphosate (roundup). Inhibition of AChE resulted in abnormal accumulation of acetylcholine, which causes eventual paralysis of the muscle. Death may occur as a result of asphyxia caused by the paralysis of respiratory muscle (Koelle, 1975) in due course. Proteins are important organic substances required in tissue building and repair under extreme stress conditions, protein supplies' energy in metabolic pathways and biochemical reactions (Vishwaranjan *et al.,* 1988). In this study, the reduction of protein recorded may be due to proteolysis and increased metabolism under toxicant stress (Remia *et al.,* 2008). Marked decrease in protein content in the freshwater field crab *Pratelphusa hydrodromous* in response to the pesticide Malathion toxicity (Singaraju *et al.,* 1991) has been reported. Shebly and Kady (2008) recorded decrease of protein content in teleost fish *Oreochromis niloticus* treated with glyphosate. Reddy and Rao (1991) found a decrease in the protein level in the marine prawn *Metapenaeus monoceros* after the prawn was exposed to the pesticide methyl parathion. Geraldine *et al.*, 1999 reported protein depletion in the freshwater prawn *Macrobrachium malcolmsonii* in response to dichlorovos exposure. Umminger, (1970) observed decrease in protein content in the fish *Fundulus beterolitus* and stated that the aquatic inhabitants exposed to toxic conditions utilized protein as energy source. Carbohydrate is an important biochemical constituent of an animal tissue; they not only act as building blocks of cell but also serve as a reservoir of chemical energy to be increased or decreased according to organismal need. In this study, the decrease in carbohydrate content indicates the fact that it was extremely utilized due to energy demand occurred in test shrimp exposed to pesticides. It may result in impairment of carbohydrate metabolism in long run (Thenmozhi *et al.,* 2010). Decrease in carbohydrate content has been reported in the marine prawn *Metapenaeus* following exposure to the pesticide methyl parathion (Reddy and Rao, 1991). The carbohydrate reduction suggests the possibility of active glycogenolysis and glycolytic pathways to provide excess energy in stress condition. Jabakumar *et al.* (1990) studied the sublethal exposure of the Cypermethrine in the freshwater fish *Labeo thermilis* and reported decrease in the carbohydrate content. Somanath (1991) found reduction of carbohydrate level in the fish *Labeo rohita* due to the effect of sublethal concentration of tannic acid toxicity. Govindan *et al.* (1994) studied the effect of phosphomidon in *Gambusia affinis* and found a decrease in carbohydrate level. Fahmy (2012) observed decreased carbohydrate content in the telost fish *Oreochromis niloticus* exposed to malathion. Lipid content is an essential organic constituent of the tissues of all animals, and plays a vital role in energy metabolism. Lipids are the best energy producers of the body next to carbohydrates. The present investigation showed that the lipid content decreased significantly which indicates its necessity to meet out required high energy demand due to pesticides toxicity in test shrimp. Villalan *et al.* (1990) observed declining of lipid content in *Macrobrachium idella* due to cadmium toxicity. Nagabushanum *et al.* (1972) reported decrease in lipid level in the hetopancreas of the freshwater prawn *Macrobrachium kristensis* in response to pesticide exposure. Bhavan and Geraldine (1997) observed reduction in lipid content in the prawn *Macrobrachium malcomsonii* exposed to endosulfan and suggested that the accelerated hydrolysis of lipid might be to cope up with the increased energy demand occurring due to the pesticide toxicity. Manohar Patil and Kulkarni (1995) found the reduction of lipid content in the freshwater fish *Channa punctatus* when it was exposed to the pesticide, summach. Susan *et al.* (1999) reported decrease in total lipid content when *Catla* exposed to pyrethriod, fenvalerate. Senthilkumar *et al.* (2007) found the reduction of lipid content in the freshwater crab *Spiralathelphusa hydrodroma* when it was exposed to a pesticide, chlorpyrifos. Frontera *et al.* (2011) observed decline in lipid content in heptopancreas of freshwater crayfish *Cherax quadricarinatus* following exposure to glyphosate.In this study, malathion and glyphosate were toxic and caused metabolic distress in *S. dichotomus* preadultsat the tested concentration of respective pesticides. Therefore, their levels need to be checked in various water bodies situated adjacent to agricultural fields and other point sources of their contaminations.

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REFERENCES

1. Agdi, K, Bouaid, A, Esteban, A.M, Hernando, PF, Azmani, A, Camara, C, 2000. Removal of atrazine and four organophosphorus pesticides from environmental waters by diatomaceous earth remediation method. J. Environ. Monit. 2(5), 420-423.
2. Bradford, MM 1976. A rapid and sensitive for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry 72:* 248-254.
3. Calleja, MC, Persoone, G, Geladi, P. 1994. Comparative acute toxicity of the first 50 multicenter evaluations of in vitro cytotoxicity chemicals to aquatic non-vertebrates. Archives Environmental Contamination Toxicology 26: 69-78.

4. Edwards, C.A., 1973. In Environmental pollution by pesticides. Plenum press p.542.
5. Ellman, G.L., K.D. Courtney, V. Andres. JR and R.M. Featherstone, 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol., 7: 88-90.
6. Eto, M, 1974. Organophosphorus pesticides organic and biological chemistry. CRC press, Cleveland Ohio. pp.387.
7. Fahmy, G.H. 2012. Malathion toxicity: Effect of some metabolic activities in *Oreochromis niloticus*, the Tilapia fish. Int.J.Bio.Biochem and Bioinf. 2(1):52-55.
8. Frontera, J.L., Vatnick, I, A. Chavlet and E.M. Rodriquez. 2011. Effects of Glyphosate and Polyethylenamine on Growth and Energitic Reserves in the Freshwater Crayfish *Cherax quadicarinatus*. Arch. Environ. Contam.Toxicol 61: 590-598.
9. Fulton, MH, Key, PB. 2001. Acetylcholinesterase inhibition in estuarine fish and invertebrates as an indicator of organophosphorus insecticides exposure and effects. Envion.Toxicol.Chem 20: 37-45.
10. George, D, Mallery, P. 2006. SPSS for windows Step by Step a Simple Guide and Reference 13.0 update sixth Edition Pearson Education, Inc.
11. Geraldine. D., P.S. Bhavan, T. Kalimurthy and Z. Zyzpragassarzan, 1999. Effect of dichlorovos intoxication in the freshwater prawn, *Macrobrachium malcolmsonii*. J.Environ.Bio., 20(2): 141-148.
12. Govindan, V.S., L. Jacon and R. Devika, 1994. Toxicity and metabolic changes in *Gambusia affinis* exposed to phosphomidon. J. Ecotoxicol. Environ.Monit., 4(1): 1-6.
13. Hedge, J.E. and Hofreiter, B.T. 1962. In: *Carbohydrate Chemistry,* 17 (*Eds.* Whistler R.L. and Be Miller, J.N.), Academic Press, New York.
14. Inouye L.S., Lotufo G.R. 2006. Comparison of macro-gravimetric and micro-colorimetric lipid determination methods. Talanta 70(3): 584-587.
15. Jabakumar, S.R.D., S.D.J. Flora and R.M. Ganesan, 1990. Effect of short –term sublethal exposure of Cypermethrine on the organic constituents of the freshwater fish. J. Environ. Biol., 4(2): 203-209.
16. Joseph, B and S.J. Raj, 2011. Impact of pesticide toxicity on selected biomarkers in fishes. Int.J.Zool.Res., 7: 212-222.
17. Key, P.B., M.H. Fulton, 1993. Lethal and sublethal effects of chloripyrifos exposure on adult and larval stages the grass shrimp, *Palaemonetes pugio*. J. Environ.Sci. Health B28, 621-640.
18. Key, P.B., M.H. Fulton, G.I Scott, S.L. Layman E.F. Wirth, 1998. Lethal and sublethal effects of Malathion on three life stages of the grass shrimp, *Palaemonetes pugio*. Aquatic Toxicol. 40: 311-322.
19. Koelle, G.B., 1975. Anticholinesterase agents. In “The Pharmacological basis of therapeutics” (Ed. L.S Goodman and A. Gillman), MacMillan publishing Co., New York; pp 404-466.
20. Manohar Patil., P and R.S. Kulkarni, 1995. Effect of Summach ovarian and hepatic biochemical contents in the freshwater fish, *Channa punctatus* under pesticide treatment. J. Natcon., 7(2): 167-169.
21. Modesto, K.A, Martinez, C.B.R. 2010. Effects of Roundup Transorb on fish: hematology, antioxidant defences and acetylcholinesterase activity. Chemosphere 81: 781-787.
22. Muirhead Thompson, RC, 1971. Pesticides and freshwater fauna. Academic press. Vol.33 pp.248.
23. Nagabhushanam, R., J. Despande and R. Sarojini. 1972. Effect of some pesticides on the biochemical constituents of freshwater prawn *Macrobrachium kistneis.* Proc. Nat. Symb.Ecotoxi, 73-84.
24. Nicholson, H.P. and D.W. Hill., 1970. Pesticides contaminants in water and their environmental impact. In relationship of agricultural to soil and water pollution. Conference on Agriculture Waste Management, Cornell University, New york.

25. Ozmen, M, Sener, S, Mete, A, Kucukbay, H. 1999. In vitro and in vivo acetylcholinesterase- inhibiting effect of new classes of organophosphorus compounds. Environ Toxicol Chem 18: 241-246.
26. Reddy, P.S and K.V.R. Rao, 1991. Methyl parathion induced alternations in the tissue carbohydrate catabolism of marine prawn *Metapenaeus monoceros*. Bull. Environ. Contam. Toxicol., 47: 925-932.
27. Remia, K.M, L. Logaswamy, K. Logankumar and D. Rajmohan. 2008. Effect of an insecticide Monocrotophos on some biochemical constituents of the fish *Tilapia mossambica*. Poll.Res.27 (3): 523-525.
28. Roche, H.A. Tidov and A. Persic, 2007. Organochlorine pesticides and biomarker responses in two fishes *Oreochromis niloticus* (Linnaeus, 1758) and *Chrysichthys nigrodigtatus* (Lacepede, 1803) and an invertebrate, *Macrobrachium vollenhoveniil* (Herkot, 1857), from the lake Taabo (Cote'd Ivoire). J. Applied.Sci., 7:3860-3869.
29. Sarvana Bhavan, P and P. Geraldine, 1997. Alternations in concentrations of protein, carbohydrates, glycogen, free sugar and lipid in prawn *Macrobrachium malcolmsonii* exposed to sublethal concentration of endosulfan. Pesticide Biochem. Physiol., 58: 89-101.
30. Senthilkumar, P., K. Samyappan, S. Jayakumar and M. Deecaraman 2007. Effect of chloripyrifos on the nutritive value in a freshwater field Crab. *Spiralotherlphusa hydrodroma.* Res. J. Agi and Bio. Sci., 3(6): 760-766.
31. Shebly El. A.A. and M.A.H.El Kady. 2008. Effects of Glyphosate Herbicide on Serum Growth Hormone (GH) Levels and Muscle Protein Content in Tilapia (*Orechromis Niloticus* L.) Res. J. Fisheries and Hydrobiology, 3(2): 84-88.
32. Singaraju, R., M.A. Subramanian and varadaraj, 1991. Sublethal effects of Malathion of the protein metabolism in the freshwater field crab *Pratelphusa hydrodromous*. J. Ecotoxicol. Environ.Monit., 1(1): 41-44.
33. Somanath, B., 1991. Effect of acute sublethal concentration of tannic acid on the protein, carbohydrate and lipid level in the tissue of the fish *Labeo rohita* J.Environ.Bio., 12(2): 107-112.
34. Susan, T., K. Anita Veeriah and K.S. Tilak, 1999. Biochemical and enzymatic changes in the tissues of *Catla catla* exposed to the pyrethroid fenvalerate. J. Ecobiol., 11(2): 109-116.
35. Taylor, P., 1980. Anticholinesterase agents. In “The Pharmacological basis of therapeutics” (Ed. A.S Gillman, T.W. Roll and F. Murad (Eds), MacMillan publishing Co., New York; pp 404-466.
36. Thenmozhi, C., V. Vignesh, R. Thirumurugan and S. Arun. 2010. Impacts of Malathion on mortality and biochemical changes of freshwater fish *Labeo rohita*. Iran. J. Environ. Health. Sci. Eng 8(4):189-198.
37. Umminger, B.L., 1970. Physiological studies on *Fundulus beterolitus* III. Carbohydrate metabolism and survival of sub-zero temperature. J.Exp.Zool. 170: 76-81.
38. Van Handel E 1985. Rapid determination of total lipids in mosquitoes. J Am Mosq Control Assoc 1:302-304.
39. Villalan, P., K.R. Narayanan and K.S. Ajmal, 1990. Biochemical changes due to short term cadmium toxicity in the prawn *Macrobrachium idella*. Progress in pollution Research Proc.Nt. Young scientists Sem. Environ. Pollut., 138-140.
40. Vishwaranjan, S., S. Beena and A. Palavesam, 1988. Effect of tannic acid on Protein, Carbohydrate and Lipid level in the tissue of the fish *Oreochromis mossambicus*. Environ. Ecol., 6: 289-292.
41. Williams, Gm, Kroes, R, Munro, IC. 2000. Safety evaluation and risk assessment of the herbicide roundup and its active ingredient, glyphosate, for humans. Regul. Toxicol. Pharmacol, 31(1), 117-165.