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# Toxic Effects of Methyl Parathion on Antioxidant Enzymes and Acetylcholinesterase Activity in Freshwater Fish, *Labeo rohita*

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The lethal concentration ($LC_{50}$) of methyl parathion and its effect on the lipid peroxidation and antioxidant enzyme systems are elucidated in *Labeo rohita* of size 75 ± 6 g at 96 h exposure. The probit analysis showed that the lethal concentration ($LC_{50}$) for 24, 48, 72 and 96 h were 15.5, 12.3, 11.4 and 10.2 mg $l^{-1}$ respectively. Besides lipid peroxides (LPO), the toxicity effect was followed from the activities of liver antioxidant enzymes catalase (CAT, EC 1.11.1.6), glutathione peroxidase (GPx, EC 1.11.1.9), superoxide dismutase (SOD, EC 1.15.1.1) and glutathione S- transferase (GST, EC 2.5.1.18). The effect of methyl parathion on tissue glutathione content and the inhibition of brain acetylcholinesterase (AChE, EC 3.1.1.7) activities were also studied. The LPO level and GST activity increased five folds and two folds respectively on exposure to methyl parathion at 10.2 mg $l^{-1}$. The SOD activity increased by seven fold compared to control. AChE activity was inhibited by 74% at a concentration of 1.8 mg $l^{-1}$ and 90% at 5.4 mg $l^{-1}$ signifying the effect of methyl parathion on the nervous system of fish. The study indicate that changes in antioxidant enzymes and decrease in the AChE activity can be used as biomarkers for monitoring toxicity due to methyl parathion exposure in aquatic organisms like fish.

**Key words: Methyl parathion, *Labeo rohita*, $LC_{50}$, oxidative stress, antioxidant enzymes, acetylcholinesterase**

Pesticides are substances, or mixtures used to control pests. Even though the use of pesticides has a very positive impact on food production, the associated risks include water contamination, livestock poisoning, death of beneficial insects, wildlife endangerment, pesticide tolerance and deterioration of human health (Gupta, 2004). Currently, the consumption of pesticide contaminated food is showing a slight declining trend, probably due to shift of farmers towards biopesticides, natural plant sources and other alternate methods (Das & Mukerjee, 2003).

Aquaculture areas are not exclusive areas of contamination and usually get exposed to influx from surrounding water bodies particularly during rainy seasons. The environmental toxic chemicals that are released in to the land eventually find their way to the rivers and sea as the final repository. Estuaries which are the links to the freshwater and marine systems, contain a variety of xenobiotics, having the potential to affect normal physiology of aquatic animals. Methyl parathion is an organophosphorus pesticide intended only for outdoor use and is classified under category I (*viz.*, most toxic) in USEPA. Methyl parathion is rapidly metabolized to biologically active methyl paraxon, which can chemically bind to acetylcholine esterase leading to a variety of clinical manifestations.

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Most of the toxicological studies in fish are confined to the effects of pesticides on fingerlings and not much study is available on growing/grown out fish. The intoxication by xenobiotics can happen at anytime in an open farm environment. Therefore, the objectives of the study were to determine the lethal concentration ($LC_{50}$) of methyl parathion in growing *Labeo rohita* (75 ± 6 g), an important candidate species in polyculture system and to evaluate its effect on acetylcholinesterase activity in brain and antioxidant enzymes in liver. The experiments were conducted in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India and with the approval of the Animal Ethic Committee of the Institute.

## Materials and Methods

Rohu *(Labeo rohita)* weighing about 75 ± 6 g and with mean body length 23 ± 5 cm were collected from a fish farm near Ernakulam, Kerala, India. The fish were brought to the laboratory and acclimatized for more than 15 days in plastic tank. The temperature and pH of water were maintained at 6.74 ± 0.4 and 32 ± 2°C respectively. The fishes were fed with commercial fish feed, the fish tanks were well aerated and the physical and chemical parameters were kept nearly constant. During the experimental period to supplement pesticide loss, if any 20% water in the tank was replaced on daily basis.

Methyl parathion-50% (O, O-dimethyl-O-4-nitrophenyl-Phosphorothioate-Bayer, Germany) a synthetic organophosphrous insecticide was obtained from market in Cochin. All other chemicals were purchased from Sigma (USA), Merck (Germany) and SRL (India).

The range finding bioassay was conducted following APHA-AWWA-WPCF, (1975) and Reish & Oshida (1987) with fish exposed to a range of sequential concentrations (0.002, 0.02, 0.2, 2, 20 mg. $l^{-1}$) of methyl parathion. Mortalities were recorded at 24, 48, 72 and 96 h and dead fish were removed immediately. To determine the lethal concentration ($LC_{50}$), eight fish of approximately equal size were released into different fish tank, containing different concentrations (1.8, 3.6, 5.4, 7.2, 9.0, 10.8, 12.6, 14.4, and 16.2 mg $l^{-1}$) of methyl parathion. $LC_{50}$ was determined as per the methods of Reish & Oshida, (1987) and the mortality of fishes was recorded at 24, 48, 72 and 96 h. The dead fishes were removed immediately and kept frozen at -20°C pending analysis.

At the end of the experiments, fish were killed by decapitation. Liver and brain tissues were dissected, washed in physiological saline (0.9% NaCl), and kept at -20°C until analysis. The tissues were homogenized for 5 min in ice-cold 0.1M Tris-HCl buffer solution pH 7.2 (1:5 w/v) using Polytron homogenizer (PT3000, Kinematica, Switzerland) and centrifuged (Remi, India) at 8000 rpm for 30 minutes. Supernatant were used for determination of enzymes.

Lipid peroxide (LPO) (Ohkawa *et al.*, 1979), superoxide dismutase (SOD) (Misra & Fridovich, 1972), Catalase (Takahara *et al.*, 1960), Glutathione peroxidase (Pagila & Valentaine, 1967), total reduced glutathione (Ellman, 1959, Glutathione-s-transferase (Habig *et al.*, 1974) in the liver were estimated by standard methods. Acetylcholinesterase (AChE) activity in brain tissues was assayed by the method of Ellman *et al.* (1961).

Mean cumulative mortalities were calculated across the treatment duration for each of the three trials and one-way ANOVA was run using the *SPSS 10.0.* followed by Duncan's new multi-range test to calculate the significant difference between control and experimental mean (Daniel, 1987).

## Results and Discussion

The range finding test showed no mortality upto 2 mg $l^{-1}$ methyl parathion

concentration while at 20 $mgl^{-1}$ concentration, 100% mortality was observed (Table 1). The lethal toxicity study showed no mortality upto 5.4 $mgl^{-1}$. The exposure of fish to 96 h, at a concentration of 7.2 $mgl^{-1}$ of methyl parathion showed 10% mortality, while at a concentration of 16.2 $mgl^{-1}$ 100% mortality was noticed in 48 h (Table 2). The probit analysis showed that the $LC_{50}$ at 24, 48, 72, and 96 h were 15.5, 12.3, 11.4 and 10.2 $mgl^{-1}$ respectively, for *Labeo rohita* (Table 3). The $LC_{50}$ varies with size of fish and type of pesticide used. There are reports indicating $LC_{50}$ of 7.34 $mgl^{-1}$ for juveniles of *Labeo rohita* (Nair *et al.*, 2007). The $LC_{50}$ of the organophosphorus pesticide RPR-II was found to be 0.17 $mgl^{-1}$ for *Oreochormis mossambicus* of size 5 ± 1 g (Venkateswara Rao, 2006) while that of azinphosmethyl, parathion and carbaryl were 7.18, 6.46 and 13.86 $mgl^{-1}$ respectively for goldfish *(Carrassius auratus)* of size 2–5 g weight range (Ferrari *et al.*, 2004). For pyrethroid pesticide cypermethrin the 96 h $LC_{50}$ was reported to be 0.139 $mgl^{-1}$ for *Labeo rohita* of size 8.52 ± 2.54 g.

Table 1. Test for range finding of *Labeo rohita* exposed to methyl parathion

| Sl. No | Concentration ($mgl^{-1}$) | Percentage of mortality |
|---|---|---|
| 1 | 0.002 | 0 |
| 2 | 0.02 | 0 |
| 3 | 0.2 | 0 |
| 4 | 2 | 0 |
| 5 | 20 | 100 |

The abnormal behavioural changes viz., opercular movement, dullness, loss of equilibrium, stopping of food intake, erratic and hysteric swimming, swimming at the water surface, circling movement and gasping were noticed immediately in fish exposed to 15.0 and 20.0 mg $l^{-1}$ concentrations of methyl parathion. Prior to death, the fish became less active or inactive, remained hanging vertically in the water or lay down on their sides at higher concentrations. The clinically observed toxic signs such as darkening of the body surface, erosion or rotting of fins and tails, loss of fish scales and haemorrhagic patches on the body surface are similar to earlier reports on pesticide poisoning (Rao *et al.*, 1967; Anees, 1975)

The specific level of lipid peroxides (LPO) on methyl parathion exposure during lethal toxicity exposure for 96 h was higher than that of a control fish (Table 4). At 10.2 $mgl^{-1}$ ($LC_{50}$), the hepatic LPO increased almost 5 times ($p<0.05$). There are reports indicating the generation of ROS in the presence of organic contaminants like organophosphorus pesticides in aquatic animals (Monserrat *et al.*, 2003; Dorval *et al.*, 2005). LPO has been reported as a major

Table 2. Test for lethal toxicity of *Labeo* rohita exposed to methyl parathion

| Sl. No | Conc. ($mgl^{-1}$) | log Con | No. of fish taken | Percentage of mortality 24 h | 48 h | 72 h | 96 h |
|---|---|---|---|---|---|---|---|
| 1 | 07.2 | 1.9741 | 8 | 0 | 0 | 0 | 10 |
| 2 | 09.0 | 2.1972 | 8 | 10 | 20 | 20 | 30 |
| 3 | 10.8 | 2.3795 | 8 | 20 | 30 | 40 | 40 |
| 4 | 12.6 | 2.5337 | 8 | 30 | 30 | 50 | 60 |
| 5 | 14.4 | 2.6672 | 8 | 30 | 60 | 100 | 100 |
| 6 | 16.2 | 2.7850 | 8 | 40 | 100 | 100 | 100 |

24 h R=0.990 R Square=0.979 S.E of Est. 2.111E-02 Y=0.861 + 7.064E-03X ; 48 h R=0.947R Square=0.896S.E of Est. 4.730E-02Y=0.894 + 7.064E-03X ; 72 hR=0.964R Square=0.930S.E of Est. 3.886E-02Y=0.881 + 3.163E-03X ; 96 hR=0.987R Square=0.974S.E of Est. 2.390E-02Y=0.823 + 3.666E-03X

Table 3. Lethal toxicity value of methyl parathion in *Labe rohita*

| Sl. No | Exposure Time (h) | $LC_{50}$ ($mgl^{-1}$) | 95% confidence limit Upper limit | Lower limit |
|---|---|---|---|---|
| 1 | 24 | 15.5 | 26.74 | 13.30 |
| 2 | 48 | 12.3 | 13.91 | 11.03 |
| 3 | 72 | 11.4 | 12.65 | 10.57 |
| 4 | 96 | 10.2 | 11.48 | 08.83 |

contributor to the loss of cell function under oxidative stress conditions (Storey, 1996; Hermes-Lima *et al.*, 1995). LPO disintegrates the biomembrane rich in polyunsaturated fatty acids (PUFA) which are susceptible to oxidation (Rady, 1993). The results suggested that the exposure to methyl parathion enhanced ROS synthesis in the liver of *Labeo rohita* and that antioxidant defences were not totally able to effectively scavenge them, thus leading to lipid peroxidation.

The SOD activity in the liver of *Labeo rohita* after methyl parathion exposure was higher than that in the control (Table 4). There was significant ($p<0.05$) increase in SOD activity with increase in the concentration of methyl parathion, and a 11 fold increase was noticed at a methyl parathion concentration of 10.2 $mgl^{-1}$ compared to control. Increase in SOD compares well with increase in LPO and induction of SOD could occur during the increased production of superoxide anion radical.

The catalase (CAT) activity increased (61%) significantly ($p<0.05$) with the increasing concentration of pesticide upto 5.4 mg $l^{-1}$ followed by a decrease at higher concentrations (Table 4). The increase in catalase activity in liver after 96 h appears to be due to the increased generation of ROS. At higher concentrations, however, the cells are unable to increase the production of catalase probably due to cell damage and are limited by their inability to counter the effect of methyl parathion toxicity. The increased SOD and CAT levels in *Labeo rohita* indicate an elevated antioxidant status attempting to neutralize the impact of the ROS. This result supported the statement (Alves *et al.*, 2002), that the exposure to pesticides elicits pro-oxidant conditions that trigger adaptive responses such as increase in the activity of the antioxidant enzymes.

The hepatic glutathione peroxidase (GPx) activity in the liver of fish increased

Table 4. Effect of different methyl parathion concentrations on LPO, liver specific antioxidant enzymes and AchE in brain in *Labeo rohita* after 96 h exposure

| S. No | Conc (mg $l^{-1}$) | LPO | SOD | CAT | GPX | GSH | GST | AChE |
|---|---|---|---|---|---|---|---|---|
| 1 | Control | 0.69 ± 0.1$^{a}$ | 1.7 ± 0.4$^{a}$ | 7.9 ± 1.1$^{a}$ | 1.6 ± 0.2$^{a}$ | 1.5 ± 0.3$^{a}$ | 1909.5 ± 583$^{a}$ | 147 ± 15$^{a}$ |
| 2 | 1.8 | 0.87 ± 0.2$^{a}$ | 2.6 ± 0.2$^{a}$ | 7.1 ± 0.8$^{ab}$ | 4.3 ± 1.2$^{b}$ | 2.9 ± 0.1$^{b}$ | 2310.3 ± 309$^{a}$ | 39.0 ± 6$^{b}$ |
| 3 | 3.6 | 1.09 ± 0.3$^{a}$ | 4.2 ± 0.8$^{b}$ | 10.4 ± 1.1$^{c}$ | 7.2 ± 0.6$^{c}$ | 3.2 ± 0.6$^{b}$ | 2973.3 ± 118$^{b}$ | 21.9 ± 3$^{bc}$ |
| 4 | 5.4 | 1.54 ± 0.3$^{ab}$ | 3.8 ± 0.6$^{b}$ | 12.9 ± 0.3$^{d}$ | 11.3 ± 2.2$^{d}$ | 3.9 ± 0.6$^{bc}$ | 3129.2 ± 180$^{bc}$ | 14.7 ± 4$^{c}$ |
| 5 | 7.2 | 1.98 ± 0.6$^{b}$ | 11.3 ± 0.6$^{c}$ | 7.2 ± 0.5$^{ab}$ | 4.1 ± 1.8$^{b}$ | 4.5 ± 0.9$^{cd}$ | 3648.0 ± 198$^{c}$ | 13.9 ± 4$^{c}$ |
| 6 | 9.0 | 2.34 ± 0.3$^{b}$ | 11.8 ± 1.1$^{c}$ | 6.2 ± 0.3$^{b}$ | 3.8 ± 0.3$^{b}$ | 5.1 ± 0.8$^{d}$ | 4660.7 ± 249$^{d}$ | 12.9 ± 3$^{c}$ |
| 7 | 10.2 | 3.53 ± 0.9$^{c}$ | 12.4 ± 0.7$^{c}$ | 7.9 ± 0.2$^{a}$ | 3.6 ± 0.4$^{b}$ | 5.2 ± 0.3$^{d}$ | 4733.7 ± 292$^{d}$ | 11.2 ± 1$^{c}$ |

Results are given as mean ± SD (n = 3). Values that have a different superscripts (a,b,c,d) differ significantly (P<0.05, Duncan's multiple range test); Activities of enzymes represented as follow: LPO - nmol malonaldehyde ; SOD- one unit is amount of protein required to give 50% inhibition of epinephrine autoxidation; CAT- nmol $H_2O_2$ $min^{-1}$ mg $l^{-1}$ Protein; GPX - nmol $min^{-1}$ mg $l^{-1}$ Protein; GSH- μ mol $g^{-1}$meat; GST- μ mol min-1 mg $l^{-1}$ protein; AChe-n mols min-1 mg $l^{-1}$ protein

($p<0.05$) after methyl parathion exposure upto a concentration of 5.4 $mgl^{-1}$ followed by a decrease at higher concentration (Table 4). The GPx inhibition observed in the present study reflects a possible antioxidant defence failure, which is also reflected in the increased LPO levels and decreased catalase activity leading to cell damage. GPx plays an important role against the LPO, since it is mainly involved in the removal of organic xenobiotics and, to a small extent, hydrogen peroxides. GPx activity increases on exposure to chemical contaminants (Almedia *et al.*, 2002; Sayeed *et al.*, 2003; Zhang *et al.*, 2004). The decrease in GPx could be attributed to the negative feedback from excess of substrate or damage by oxidative modification (Tabatavaie & Floyd, 1994). Reductions in GPx activity indicate that antioxidant capacity of the tissue was surpassed by the amount of hydrogen peroxide (Remacle *et al.*, 1992).

The total reduced glutathione (GSH) content increased ($p<0.05$) gradually with increase in the concentration of methyl parathion compared to control (Table 4), and a 3.5 fold increase was noticed at 10.2 mg $l^{-1}$. During a moderate oxidative stress, the glutathione levels increased as an adaptive mechanism due to increased synthesis; however, a severe oxidative stress suppresses glutathione levels due to the impairment of the adaptive mechanism (Zhang *et al.*, 2004).

Considerable decline in GSH could be due to an increased utilization of tissue GSH (Monterrio, *et al.*, 2006). In this study, however, there was no decrease in GSH up to the final concentration of 10.2 $mgl^{-1}$ indicating the effectiveness of the cell to replenish GSH at this high level of pesticide concentration. GSH depletion may reduce the ability to scavenge free radicals raising the general oxidative potential in the cell (Elia *et al.*, 2003).

The glutathione-s-transferase (GST) activity in the liver of *Labeo rohita* after methyl parathion exposure increased ($p<0.05$) with increase in concentration of methyl parathion (Table 4). The activity increased by two fold on exposure to methyl parathion at 10.2 $mgl^{-1}$ concentration. GST plays an important role in protecting tissue from oxidative stress (Fournier *et al.*, 1992; Banerjee *et al.*, 1999). The increased GST activity in liver observed in the present study after exposure to methyl parathion suggests the increase in detoxification processes in *Labeo rohita.*

Acetylcholinesterase (AChE) activities in brain tissues were gradually inhibited ($p<0.05$) with increase in the concentration of methyl parathion, after 96 h exposure (Table 4). AChE is the major neurotransmitter in the nervous system and was inhibited by 74% at a methyl parathion concentration 1.8 $mgl^{-1}$ itself and 90% of the activity was inhibited at concentration 5.4 $mgl^{-1}$. Further increase in methyl parathion concentration did not inhibit AChE activity significantly. Weiss (1961) and Zinkl *et al.* (1991) proposed that depression of 70-90% brain AChE activity occurs in response to organophosphorus pesticide at the $LC_{50}$. The inhibitory effect on AChE activity indicates that insecticides might interfere in vital processes like energy metabolism of nerve cells (Nath & Kumar, 1999).

The present study reveals that methyl parathion toxicity in *L. rohita* is caused by oxidative stress which the fish counter by increased production of antioxidants. Further, inhibition of AChE in brain causes the ultimate death of the fish at higher concentrations. Therefore antioxidant status including the level of antioxidant enzymes, higher antioxidant potential in response to methyl parathion and significant drop in acetylcholinesterase activity can be used as good biomarkers for monitoring methyl parathion toxicity.

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## References

Almeida, J. A., Diniz, Y. S., Marques, S. F. G., Faine, L.A., Ribas, B. O., Burneiko, R. C. and Novelli, E. L. (2002) The use of oxidative stress responses as biomarkers in Nile tilapia (*Oreochromis niloticus*) exposed to *in vivo* cadmium contamination, *Environ. Int.* **27**(8), pp 673-679

Alves, S. R., Severino, P. C., Ibbotson, D. P., de Silva, A. Z., Lopes, F. R., Saenz, L. A. and Bainy, A. C. (2002) Effects of furadan in the brown mussel *Perna perna* and in the mangrove oyster *Crassostrea rhizophorae, Mar. Environ. Res.* **54** (3-5), pp 241-245

Anees, M. A. (1975) Acute toxicity of four organophosphorus insecticides for a freshwater teleost *Channa punctatus* (Bloch), *Pak. J. Zool.* 7(2), pp 135-141

APHA-AWWA-WPCF. (1975) Bioassay for aquatic organisms: *Standard methods for estimation of water and waste water,* 19th edn., pp 800-869 American Public Health Association, Washington.

Banerjee, B. D., Seth, V., Bhattacharya, A., Pasha, S. T. and Chakraborty, A. K. (1999) Biochemical effects of some pesticides on lipid peroxidation and free radical scavengers, *Toxicol. Lett.* **107** (1-3), pp 33-47

Daniel, W. W. (1987) Biostatics: A foundation for analysis in the health science. pp 276-296 4th Wiley, New York

Das, B.K. and Mukherjee, S.C. (2003) Toxicity of cypermethrin in *Labeo rohita* fingerlings: biochemical, enzymatic and haematological consequences, *Comp. Biochem. Physiol. C.* **134**(1), pp 109-121

Dorval, J., Leblond, V., Deblois, C. and Hontela, A. (2005) Oxidative stress and endocrine endpoints in white sucker (*Catostomus commersoni*) from a river impacted by agricultural chemicals, *Environ. Toxicol. Chem.* **24**, pp 1273-1280

Elia, A. C., Galarini, R., Taticchi, M. I., Dorr, A. J. and Mantilacci, L. (2003) Antioxidant responses and bioaccumulation in *Ictalurus melas* under mercury exposure, *Ecotoxicol. Environ. Saf.* **55** (2), pp 162-167

Ellman, G. L. (1959) Tissue sulfhydryl groups, *Arch. Biochem. Biophys.* **82** (1), pp 70-77

Ellman G. L., Courtney K. D., Andres V. Jr. and Feather-stone, R. M. (1961) A new and rapid colorimetric determination of acetylcholinesterase activity, *Biochem. Pharmacol.* 7, pp 88-95

Ferrari A, Venturino A, Pechen de D'Angelo AM. (2004) Time course of brain cholinesterase inhibition and recovery following acute and subacute azinphosmethyl, parathion and carbaryl exposure in the goldfish (*Carassius auratus*), *Ecotoxicology and Environment Safety,* **57**: pp 420-425.

Fournier, D., Bride, J. M., Poirie, M., Berge, J. B. and Plapp, F. W. (1992) Insect glutathione S-transferases., biochemical characteristics of the major forms of houseflies susceptible and resistant to insecticides, *J. Biol. Chem.* **267**(3), pp 1840-1845

Glynn, P. (2006) A mechanism for organophosphate-induced delayed neuropathy, *Toxicol. Lett.* **162**(1), pp 94-97

Gupta, P. K. (2004) Pesticide exposure-Indian scene, *Toxicology,* **198** (1-3), pp 83-90

Habig, W. H., Pabst, M. J. and Jackoby, W. B. (1974) Glutathione S-transferases. The first enzymatic step in mercapturic acid formation, *J. Biol. Chem.* **249** (22), pp 7130-7139

Hermes-Lima, M., Willmore, W. G. and Storey, K. B. (1995) Quantification of lipid peroxidation in tissue extracts based on Fe (III) xylenol orange complex formation, *Free Radic. Biol. Med.* **643** (19), pp 271-280

Misra, H. P. and Fridovich, I. (1972) The role of superoxide anion in the auto-oxidation of epinephrine and a simple assay for superoxide dismutase, *J. Biol. Chem.* **247**(10), pp 3170 -3175

Monserrat, J. M., Geracitano, L. A., Pinho, C.L., Vinagre, T. M., Faleiros, M., Alciati, J. C., and Bianchini, A. (2003) Determination of lipid peroxides in invertebrate tissues using the Fe (III) xylenol orange complex formation, *Arch. Environ. Contam. Toxicol.* **45** (2), pp 177-183

Monterrio, D. A., Almeida, J. A., Rantin, F. T. and Kalinin, A.L. (2006) Oxidative stress biomarkers in the fresh water characid fish, *Brycon cephalus*, exposed to organophosphorus insecticide Folisuper 600 (methylparathion), *Comp. Biochem. Physio. C. Toxicol. Pharmacol.* **143** (2), pp 141-149

Nath, B. S. and Kumar, R. P. S. (1999) Toxic Impact of Organophosphorus Insecticides on Acetylcholinesterase Activity in the Silkwarm, *Bombyx mori L. Ecotoxico, Environ. Saft.* **42**(2), pp 157-162

Nair, V. P., nair, J. Mercy, T. V. A. (2007) The individual and combined acute letal toxicity of biocide combination on the juveniles of rohu (*Labeo rohita*) under tropical conditions, *Indian J. Fish.* **53** (3), 267-274

Ohkawa, H., Onishi, N and Yagi. (1979) Assay of lipid peroxides in animal tissues bt thiobarbituric acid reaction, *Anal. Biochem*, **95** (2), pp 351-358

Pagila, D.E. and Valentaine, W.N. (1967) Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase, *J. Lab. Clin. Med.* **70** (1), pp 158-169

Rady, A. A. (1993) Environmental temperature shift induced adaptive changes of carp (*Cyprinus carpio*. L) erythrocyte plasma membrane *in vivo, Comp. Biochem. Physiol.* **105**, pp 513-518

Rao, T. S., Dutt, S.and Mangaiah, K. (1967) TLM values of some modern pesticides to the fresh-water fish *Puntius puckelli, Environ. Health.* **9** (2), pp 103-109

Reish, D.L. and Oshida, P.S. (1987) Manual of methods in aquatic environment research part 10 Short Term Static. Bioasssy *FAO Fisheries Technical Paper* 247. FAO, Rome, 62 p

Remacle, J., Lambert, D., Raes, M., Pigeolet, E. and Michiels, C. (1992) Importance of various antioxidant enzymes for cell stability: conformation between theoretical and experimental data, *J. Biochem.* **286**, pp 41-46

Sayeed, I., Parvez, S., Pandey, S., Bin-Hafeez, B., Haque, R. and Raisuddin, S. (2003) Oxidative stress biomarkers of exposure to deltamethrin in freshwater fish, *Channa punctatus* Bloch. *Ecotoxicol. Environ. Saf.* **56** (2), pp 295-301

Storey, K. B. (1996). Oxidative stress: animal adaptations in nature, *Braz. J. Med. Biol. Res.* **29** (12), pp 1715-1733

Tabatabai, T. and Floyd, R. A. (1994) Susceptibility of glutathione peroxidase and glutathione reductase to oxidative damage and the protective effect of spin trapping agents, *Arch. Biochem. Biophys.* **314** (1), pp 112-119

Takahara, S., Hamilton, H. B., Neel, J. V., Kobara, T. Y., Ogura, Y. and Nishimura, E. T. (1960) Hypocatalasemia: a new genetic carrier state, *J. Clin. Invest.* **39**, pp 610-619

Venkateswara Rao, J. (2006). Sublethal effects of an organophosphorus insecticides (RPR-II) on biochemical paraments of tilapia, *Oreochromis mossambicus, Comp. Biochem. Phy.* **143** (4), pp 492-498

Weiss, C. M. (1961) Physiological effect of organic phosphorus insecticides in several species of fish, *Trans. Am. Fish. Soc.* **90**, pp 143-152

Zhang, J., Shen, H., Wang, X., Wu, J. and Xue, Y. (2004) Effects of chronic exposure of 2,4-dichlorophenol on the antioxidant system in liver of freshwater fish *Carassius auratus*, *Chemosphere*, **55**, pp 167-174

Zinkl, J. G., Lockhard, W. L., Kenny, S. A. and Ward. F. J. (1991). The effects of cholinesterase inhibiting insecticides on fish, In: *Cholinesterase-inhibiting insecticides*. (Mineau, P., Ed), pp 233-254, Elsevier, New York, USA