

Review Paper

Biochemical Toxicity of Organophosphate Compounds in Fishes

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ABSTRACT

Organophosphorus compounds have replaced organo-chlorines to a greater extent and are frequently sprayed on agricultural crops. These xenobiotics primarily find their way into aquatic ecosystems thereby induce toxicity to non-target species. Among the various harms caused to fishes by organophosphates, alteration in their biochemical indices is important as it induces severe damage to various vital metabolic processes in fish. The primary effect of organophosphate toxicity, which is their mechanism of action as well, is the inhibition of acetylcholinesterase enzyme in synapses which leads to severe morbidity and loss of balance and motion or even paralysis. Various other biochemical metabolisms like protein metabolism, lipid metabolism, carbohydrate metabolism and liver enzymes get severely impaired due to the toxicity induced by organophosphates. Therefore, it is necessary to protect the natural water bodies from getting contaminated with pesticides to allow fishes to grow healthier and harmless for human consumption. The present paper reviews the work done on biochemical toxicity of organophosphorus compounds in fish.

Key words: Aquatic ecosystems, biochemical alterations, fish, organophosphates, toxicity

Organophosphates constitute a large group of chemicals used over past 60 years to protect the crops, livestock and human as well as is used as warfare agents. Based on the structural characteristics, they have broadly been divided into 13 types, including phosphates, phosphonates, phosphinates, phosphorothioates (S^-), phosphorothioates (S^-), phosphoro-thioates (S substituted), phosphonothioates (S substituted), phosphorodithioates, phosphoro-trithioates and phosphoramidothioates (Gupta, 2006). These are probably the most pervasive compounds, many of which form important biochemical substances like DNA, RNA and various co-factors, essential for the sustenance of life. In addition, organophosphates represent a diverse class of insecticides or nerve agents acutely toxic to

bees, wild life and human populations. Organophosphates were developed in Germany during 1930's as a potential chemical warfare and are presently used to control insects on fruits, vegetables, grain crops and stored seeds. In household, these compounds are efficient in controlling cockroaches, houseflies, termites, etc. and effective in protecting the horticultural and field crops from insect pest attacks (Fulton and Key, 2001).

Use of organophosphate insecticides is on rise as they, unlike organochlorine compounds, are less persistent in the environment. They quickly are transformed into non-toxic compounds, despite the fact that of having high non-specific acute toxicity that may lead to frequent intoxication of non-target organisms (Nunes, 2011). However, under specific conditions some organophosphates remain active in soils upto six months after their application, thus increasing the duration which may be harmful to non-target species (Köprücü *et al.*, 2006). In natural waters, they have several mechanisms of degradation such as oxidation, photodegradation and hydrolysis (Pehkonen and Zhang, 2000). This is what actually determines the pattern of insecticide usage in India which is 76% against 44% total insecticide use globally (Mathur, 1999).

Organophosphorus compounds like other classes find their way into aquatic habitats such as rivers, lakes and ponds (Tabrizi, 2001). They have been found toxic to the organisms contributing substantially to the food chain and water bodies (Bagheri, 2007). Fishes which encounter with organophosphorous compounds develop various metabolic abnormalities (John, 2007). Pesticides move into environmental components either by spray drift, aerial spray or washing from the atmosphere, by precipitation, leaching, percolation and/or runoff from agricultural land (Honarpajouh, 2003; Vryzas *et al.*, 2009). Most insecticides ultimately find their way into rivers, lakes and ponds (Shayeghi *et al.*, 2007; Werimo *et al.*, 2009; Arjmandi *et al.*, 2010). The problems associated with organophosphates include their lack of specificity and high water solubility. They are able to enter aquatic environment where they prove toxic to both vertebrates and invertebrates (Tuduri *et al.*, 2006). This illegitimate entrance of organophosphate xenobiotics leads to bio-magnification when contaminant load increases significantly with each successive trophic level (Landrum and Fisher, 1998). Bio-accumulation and bio-magnification of these substances in aquatic organisms including fish leads to chronic toxicity and behavioural changes in extreme cases. In fish, the uptake of pesticides take place through absorption by gills and residue uptake through gills and is related to the metabolic rate and body size (Murphy, 1971). These xenobiotics not only lead to higher instances of toxicity but alter various biochemical indices including some important physiological processes and enzyme kinetics in fishes. There are various biochemical alterations caused by the toxicity of organophosphorus compounds in fishes.

Biochemical alterations in fishes due to organophosphorus toxicity

Teleosts exhibit severe biochemical alterations due to the toxicity of organophosphorus pesticides. These xenobiotics are potent to cause physiological dysfunction thereby affecting the organisms at organ, cellular and molecular levels which, in turn, is responsible for biochemical changes in fish. Various biochemical alterations in fish due to organophosphorus toxicity include inhibition of acetylcholinesterase enzyme, impaired metabolism of carbohydrate, protein and lipid metabolism and alterations in enzymes.

Behavioural changes in fish due to acetylcholinesterase enzyme are the prime indicator of organophosphorus toxicity in fish. Plasma enzymes levels in fish have also been proposed to be good indicators of extreme stress and provide information of organ dysfunction (Wells *et al.*, 1986). Enzymes are increasingly used not only as indicators of pathological processes but also as indicators of stress (Jiminez and Stegeman, 1990). Biochemical and physiological indicators such as enzymes could be used as biomarkers to identify possible environment contamination before the health of aquatic organisms is seriously affected (Barnhoorn and Van Vuren, 2004). Changes in plasma enzyme activity are used as indicators of tissue injury, environmental stress or a diseased condition. The rate of increase in plasma enzyme activity depends on the concentration of enzyme in cells, the rate of leakage caused by injury and the rate of clearance of enzyme from plasma (Boyd, 1983).

Inhibition of acetylcholinesterase

Due to their effect on wide class of organisms, organophosphorus insecticides are widely sprayed in orchards and agricultural fields. They depict cholinergic property (carbamates group of pesticide also act on the same enzyme but through a different mechanism), hence act as neurotoxins to a broad spectrum of pests. The inhibition of enzyme has been observed in different tissues of fishes including their brain (Rahman *et al.*, 2004; Mushigeri and David, 2005). Inhibition of this enzyme leads to the accumulation of acetylcholine in the synapse, resulting in increased firing of the postsynaptic neuron or increased neuro-effector activity. The consequences of increased cholinergic activity in parasympathetic autonomic nervous system (muscarinic receptors) may include increased mucous secretion, loss of balance, frenzied movements with alternate quiescent stages, hypoxia and erosion of gills. The effects of increased neuroeffector activity on skeletal muscles may include muscle fasciculation, cramps, muscle weakness and depolarization type paralysis (Binukumar and Gill, 2010). In addition to the acute effects, some organophosphorus compounds have been found associated with delayed neurotoxicity, known as organophosphorus-induced delayed neuropathy (OPIDN) (Hudgson, 2004). Inhibition of acetylcholinesterase due to organophosphorus compounds and a positive correlation between them in fishes has been studied by several workers (Siddiqui, *et al.*, 1989; Thangnipon, *et al.*, 1995; Sturm, *et al.*, 2000; Rao, *et al.*, 2005).

Protein metabolism

Proteins are primarily involved in cell architecture, which is a chief source of nitrogenous metabolism and source of energy during chronic periods of stress. Protein metabolism in fishes reflects decreased alterations in total protein content levels after an acute exposure of organophosphorus pesticides (Chebbi and David, 2011; Ahmad and Gautam, 2014). During stress conditions, fish needs more energy to detoxify toxicants; hence more protein is required to meet the increased energy demands to overcome stress conditions. Thus depletion of protein fraction in liver may have been due to their degradation and possible utilization for metabolic purposes. Medda *et al.* (1992) who studied the effects of sub-lethal doses of dichlorvas 76% on *Labeo rohita* and *Cirrhina mrigala* fingerlings drew similar conclusions. Tilak *et al.* (2009) reported decrease in total proteins, glycogen and even in DNA and RNA contents due to organophosphorus toxicity. Decrease in protein levels may also be due to their degradation or destruction of

hepatocytes or due to necrotic changes occurring in organophosphate toxicity and consequent impairment in protein synthesis machinery (Ogueji and Auta, 2007). Oxidative stress due to organophosphate toxicity has also been found responsible for protein degradation in fishes (Malkovich, 1995).

Ganeshwade (2011, 2012) while working on biochemical changes induced by dimethoate in the liver of fresh water fish *Puntius ticto* (Hamilton) reported a significant decrease in protein levels in testis, ovary and brain; slight decrease in intestines, muscles, liver and gills, and increase in protein levels in kidney. Chronic toxicity reflected a decrease in the levels of protein content in ovary, brain, intestine, muscles, gills and liver to 2.506 and 1.253 ppm exposure; whereas in testis protein level was increased to 1.253 ppm. Irshad and Gautam (2014) found a significant decrease in total protein levels, both in albumin and globulin content after acute exposure of *Heteropneustes fossilis* to an organophosphorus pesticide Nuvan. Thus, it may be concluded that exposure to organophosphates leads to severe hindrances in vital metabolic processes so induce severe alterations in lipid metabolism in fishes.

Carbohydrate metabolism

The blood glucose level serves as an indicator of biological stress caused by pollutants such as pesticides and metals (Silbergeld, 1974). Koudinya and Ramamurthi (1979) reported that a lethal concentration of 6 mg/L sumithion, an organophosphate pesticide, increased blood glucose level and phosphorylase activity in freshwater fish *Sarotherodon mossambicus*. A reduction in hepatic glycogen indicating impairment in the carbohydrate metabolism has been reported in *Anabas testudineus* treated with acute lethal and sublethal concentrations of Furadan (Bhakhavalsalam and Reddy, 1982). A reduction in the glycogen content was noted in whole body homogenate of fingerlings of *Labeo rohita* and *Cirrhina mrigala* exposed to different sublethal concentrations of nuvan (Medda *et al.*, 1992). Begum and Vijayaraghavan (1995) studied the acute effect of dimethoate on some aspects of carbohydrate metabolism of hepatic tissue in cat fish, *Clarias batrachus*, after 1, 2, 4 and 8 days of exposure to sublethal concentration. They reported depletion in glycogen content and increase in lactate level. The activity levels of glycogen phosphorylase in the hepatic tissue also increased. Ganeshwade (2012) observed that the fish exposed to dimethoate seemed to induce glycogenolysis, possibly by increasing the activity of glycogen phosphorylase to meet the energy demand under stress condition or the toxicant might have affected glycogenesis by inhibiting the activity of carbohydrate metabolism. These results suggest that carbohydrate metabolism was adversely affected in hepatic tissue by organophosphate pesticide dimethoate. Fall in the glycogen level in gills of *Punctius ticto* indicates its rapid utilization to meet the enhanced energy demands under dimethoate toxicity through glycolysis or hexosmonophosphate pathway (Cappon and Nicholas, 1975). It could also be due to the prevalence of hypoxic or anoxic conditions, which normally enhances glycogen utilization (Dezwaan and Zandee, 1973). Enhanced utilization of glycogen and its consequent depletion in tissues may be attributed to hypoxia as it increases carbohydrate consumption. Under hypoxic conditions, the animals derive their energy from anaerobic breakdown of glucose, available to the cell by the increased glycogenolysis (Chandravathy and Reddy, 1995). Further, the decline in glycogen might partly be due to its utilization in the formation of glycoprotein and glycol-lipids, which are the essential constituents of various cells and other membranes (Vutukuru, 2005).

The effect of fenitrothion on the energy metabolism of European eel, *Anguilla anguilla* was studied by Sancho *et al.* (1997) who reported no significant change in liver glycogen level but increase in blood glucose levels. Similarly freshwater cat fish, *Clarias batrachus*, when exposed to sublethal concentrations of an organophosphate and a carbamate pesticide reflected hyperglycaemic response to both the pesticides (Jyothi and Narayan, 1999). General hypoglycemia and reduction in hepatic glycogen content in fishes exposed to experimental concentrations of different pesticides has previously been reported (Gill *et al.*, 1991; Verma and Panigrahi, 1998; Sharma, 1999). Ramani *et al.* (2002) observed a decrease in carbohydrate content of fish *Labeo rohita* exposed to sublethal concentration of monocrotophos. Increased blood glucose level was found in the same fish in response to the toxicity of cypermethrin (Das and Mukherjee, 2003). Exposure of fishes to organophosphates elicits consistent hyperglycemia apart from liver and muscle glycogenesis (Singh *et al.*, 1996). Apart from decrease in total protein due to organophosphorus toxicity, various other alterations such as increase in plasma glucose (Das and Mukherjee, 2001; Ramesh and Saravanan, 2008; Bakhshwan *et al.*, 2009; Al-Ghanim *et al.*, 2008), amino acids (Magar and Afsar, 2012) and ammonia (Narra *et al.*, 2013) has also been reported.

Ganeshwade (2011, 2012) reported that glycogen, cholesterol and ascorbic acid contents in the gills decreased after organophosphorus exposure. Begum and Vijayaraghavan (1995) studied the acute effect of dimethoate on carbohydrate metabolism of hepatic tissue in cat fish, *Clarias batrachus*, after 1, 2, 4 and 8 days of exposure to sublethal concentration and found that the glycogen content was depleted and lactate level increased. The activity levels of glycogen phosphorylase in hepatic tissues also showed increase. These results conclude that carbohydrate metabolism is adversely affected in hepatic tissue by organophosphate pesticide toxicity.

Lipid metabolism

Lipid metabolism in fishes is seriously impaired due to organophosphate exposure. Several studies support the opinion that lipid metabolism is altered due to the acute organophosphorus toxicity. However, Singh and Singh (1980) reported that cythion and hexadrin caused no significant variations in liver and ovarian lipid content in freshwater fish, *Heteropneustes fossilis*. On the other hand, Rao *et al.* (1985) studied the combined and individual effects of carbaryl and fenthate on the levels of total lipids, free fatty acids, cholesterol and lipase activity in fish *Channa punctatus* and reported that free fatty acids, cholesterol and lipase activity were increased while total lipid was decreased. Lal and Singh (1987) studied the effect of two pesticides, an organophosphate and an organochlorine, on total lipid and its various fractions. Except for the elevated liver lipid in males in response to malathion, no significant change in total lipid was observed following pesticide exposure. The organophosphate methyl-parathion was found to reduce the efficiency of hormone sumaach to increase the ovarian activity in freshwater fish *Channa punctatus* and lipid content of the ovary under treatment was reduced with sumaach (Patil and Kulkarni, 1996). There was a decrease in liver cholesterol content (25%) in fish *Ctenopharyngodon idella* exposed to sublethal doses of fenvalerate at the end of 1st week of exposure and it became 48% at the end of 4th week (Shakoori *et al.*, 1996). Plasma cholesterol and triglyceride contents decreased during 96 hour treatment with sublethal concentration of diazinon in fish *Anguilla anguilla* (Ceron *et al.*, 1996). They reported that

similar to lipid content there was a reduction in cholesterol content as well, even under the effect of sumaach, whereas in control fishes the sumaach was able to increase the amount of ovarian cholesterol concentration. A decrease in total lipid content of liver, muscle and gill of two different size groups of *Tilapia mossambica* exposed to phosalone suggested that lipids might have been channeled for energy production under stress condition (Parvathy *et al.*, 2000). Zutshi (2003) reported an increase in cholesterol content of testes in fish *Glossogobius giuris* treated with sublethal concentration of fenthion. Venkataraman *et al.* (2006) reported malathion induced depletion of cholesterol in heart muscles of fish *Glossogobius giuris*. Therefore, it is evident that organophosphates induce toxicity in fish, thereby impair lipid metabolism in them.

Impact on enzymes

Insecticides may cause serious impairment to physiological and health status of fish. Biochemical tests are routine laboratory tests useful in recognizing acute or chronic toxicity of insecticides (Banaee *et al.*, 2008; Al-Kahtani, 2011) and serves as a practical tool to diagnose toxicity effects in target organs and to determine the physiological status in fish. Enzyme activities in fishes are severely disrupted due to pesticide exposure. Liver enzymes show alterations directly proportional to the time of exposure. Blood biochemistry test indicates the happening in fish body exposed to insecticides. When different tissues are injured, the damaged cells release specific enzymes into plasma and one can recognize their abnormality levels in blood. Then it helps to locate the lesions. Also, if certain organs are not eliminating certain waste products or are not synthesizing certain important materials, this suggests that they are not functioning properly. In some cases due to the severity of damage to the tissues, particularly liver, the synthesis of many biochemical parameters may reduce significantly in cells, which may decrease some biochemical factors in blood of fish exposed to insecticides. These changes were reported in *Heteropneustes fossilis* (Saha and Kaviraj, 2009), *Cirrhinus mrigala* (Prashanth and Neelagund, 2008) and *Oncorhynchus mykiss* (Banaee *et al.*, 2011) exposed to dimethoate, cypermethrin, and diazinon, respectively.

The activities of aspartate and alanine amino transferases (AAT and AAT) which serve as strategic links between protein and carbohydrate metabolisms are known to alter under several physiological and pathological conditions (Shivaknmar, 2005). Glutamate dehydrogenase (GDH), a mitochondrial enzyme, catalyses the oxidative deamination of glutamate and provides α -ketoglutarate to the Kerb's cycle (Reddy and Venugopal, 1990). This enzyme has metabolic functions with great physiological significance and is closely associated with the detoxification mechanisms of tissues. GDH in extra-hepatic tissues could be utilized for channeling of ammonia released during proteolysis for its detoxification into urea in liver. Hence, the activities of AAT, ALT and GDH are considered as sensitive indicators of stress (Gould *et al.*, 1976). Levels of these enzymes get elevated on account of acute organophosphate exposure indicating stress conditions in fishes due to toxicity (Joseph and Raj, 2011). Similar results were reported by Naveed *et al.* (2011) who worked on biochemical alterations induced in *Channa punctatus* by an organophosphate triazophos. prashanth and neelagund (2008) suggested that the steady rise in the activities of GDH, AAT and ALT in the organs of fish exposed to sublethal concentration of cypermethrin may be due to the synthesis of these enzymes under sub-acute cypermethrin stress. The increase in these enzyme activities could be helpful to the

fish for structural reorganization of proteins and incorporation of keto acids into TCA cycle to favour gluconeogenesis or energy production.

The activity of LDH reportedly decreased on account of organophosphorus exposure to fishes (Naveed *et al.*, 2011). Tilak *et al.* (2009) reported increase in the activity of enzymes AAT, ALT and LDH due to the toxic stress of alachlor in *Channa punctatus*. Increase in the activity of LDH has also been reported by Amanullah *et al.* (2010) in gills, hepato-pancreas and muscle. Susan *et al.* (2010) reported increased alkaline phosphatase, acid phosphatase and cholesterol while many workers reported decrease (Jaroli and Sharma, 2005; Banaee *et al.*, 2008; Bakhshwan *et al.*, 2009). Shamoushaki *et al.* (2012) studied the effects of organophosphate, diazinon, on some biochemical changes in *Rutilus frisii kutum* male brood stocks and reported that diazinon caused an increase in AST, ALP and adrenaline. Various enzymes like serum creatinine, bilirubin and serum urea and different ions like blood potassium, calcium, magnesium and liver chloride were elevated by altered levels while as blood sodium, chloride and liver sodium, potassium and calcium levels were significantly decreased (Logaswamy *et al.*, 2007; Ahmad and Gautam, 2014).

CONCLUSION

Organophosphorus insecticides induce serious biochemical alterations in fishes and, in extreme cases, paralyze them completely by inhibiting acetylcholinesterase enzyme in synapse. Hence, it is essential to prevent natural water bodies from pesticide contamination by having checks and balances at the point and non-point sources of pollution.

REFERENCES

- Ahmad, S.I. and Gautam R.K. 2014. Effect of organophosphate pesticide nuvan on serum biochemical parameters of fresh water catfish *Heteropneustes fossilis* (Bloch.). *International Research Journal of Environment Sciences*, **3**(10): 1-6.
- Al-Ghanim, K.A., Al-Balawi, H.F., Al-Kahem, A., Ali, S., Al-Misned, F., Zubair, A. and Annazri, H. 2008. Ethological response and haematological and biochemical profiles of carp (*Cyprinus carpio*) exposed to trichlorfon. *Journal of Food, Agriculture and Environment*, **6**: 473-479.
- Al-Kahtani, M.A. 2011. Effect of an insecticide abamectin on some biochemical characteristics of tilapia fish (*Oreochromis Niloticus*). *American Journal of Agricultural and Biological Sciences*, **6**: 62-68.
- Amanullah, B., Stalin, A., Prabu, P. and Dhanapal, S. 2010. Analysis of AChE and LDH in mollusc, *Lamellidens marginalis* after exposure to chlorpyrifos. *Journal of Environmental Biology*, **31**: 417-419.
- Arjmandi, R., Tavakol, M. and Shayeghi, M. 2010. Determination of organophosphorus insecticide residues in the rice paddies. *International Journal of Environmental Science Technology*, **7**: 175-182.

- Bagheri, F. 2007. *Study of Pesticide Residues (Diazinon, Azinphosmethyl) in the Rivers of Golestan Province (Gorgan, Roud and Gharehsou)*. M.Sc. thesis, Tehran University of Medical Science. Tehran, Iran.
- Bakhshwan, S.A., Marzouk, M.S., Hanna, M.I. and Hamed, H.S. 2009. Some investigations on the clinical and biochemical alterations associated with diazinon toxicity in *Clarias gariepinus*. *Egyptian Journal of Aquatic Biology and Fisheries*, **13**: 173-197.
- Bakthavathsalam, R., Reddy, Y. and Srinivasa. 1982. Changes in the content of glycogen and its metabolites during acute exposure of *Anabas testudineus* (Bloch) to furadan. *Journal of Bioscience*, **4**: 19-27.
- Banaee, M., Mirvaghefi, A.R., Rafei, G.R. and Majazi, A.B. 2008. Effect of sub-lethal diazinon concentrations on blood plasma biochemistry. *International Journal of Environmental Research*, **2**: 189-198.
- Banaee, M., Sureda, A., Mirvaghefi, A.R. and Ahmadi, K. 2011. Effects of diazinon on biochemical parameters of blood in rainbow trout (*Oncorhynchus mykiss*). *Pesticide Biochemistry and Physiology*, **99**: 1-6.
- Barnhoorn, I.E.J. and Van Vuren, J.H.J. 2004. The use of different enzymes in feral freshwater fish as a tool for the assessment of water pollution in South Africa. *Ecotoxicology and Environmental Safety*, **59**: 180-185.
- Begum, G. and Vijayaraghavan, S. 1995. Carbohydrate metabolism in hepatic tissue of freshwater cat fish, *Clarias batrachus* (Linn.) during dimethoate exposure. *Food and Chemical Toxicology*, **33**: 423-426.
- Binukumar, B.K. and Gill, K.D. 2010. Cellular and molecular mechanisms of dichlorvos neurotoxicity: Cholinergic, non-cholinergic, cell signaling, gene expression and therapeutic aspects. *Indian Journal of Experimental Biology*, **48**: 697-709.
- Boyd, J.W. 1983. The mechanism relating to increases in plasma enzymes and isoenzymes in diseases of animals. *Veterinary Clinical Pathology*, **12**: 9-24.
- Cappon, I.D. and Nicholas D.M. 1975. Factors involved in increased protein synthesis in liver microsome after administration of DDT. *Pesticide Biochemistry and Physiology*, **5**: 109-118.
- Ceron, J.J., Sancho, E., Ferando, M.D., Gutierrez, C. and Andreu, E. 1996. Metabolic effects of diazinon on the European eel *Anguilla anguilla*. *Journal of Environmental Science and Health, Part B. Pesticides, Food Contaminants and Agricultural Wastes*, **31**: 1029-1040.
- Chandrawathy, M. and Reddy, S.L.N. 1995. *In vivo* effects of lead acetate on dehydrogenase activities and metabolites in the freshwater fish, *Anabus scandens*. *Journal of Eco-Toxicology and Environmental Monitoring*, **5**: 107-111.
- Chebbi, S.G. and David, M. 2011. Modulation in the protein metabolism under sublethal concentration of quinalphos. Intoxication in the freshwater common carp, *Cyprinus carpio* (Linnaeus, 1758). *International Journal of Pharmaceutical and Biological Archives*, **2**: 1183-1189.
- Das, B.K. and Mukherjee, S.C. 2001. Effect of nuvan on hematology of rohu *Labeo rohita* (Ham.). *Indian Journal of Fisheries*, **48**: 85-92.
- Das, B.K. and Mukherjee, S.C. 2003. Toxicity of cypermethrin in *Labeo rohita* fingerlings: Biochemical, enzymatic and haematological consequences. *Comparative Biochemistry and Physiology*, **134C**: 109-121.

- Dezwaan, A. and Zandee, D.I. 1973. Body distribution and seasonal changes in glycogen content of the common sea mussel, *Mytilus edulis*. *Comparative Biochemistry and Physiology*, **43**: 53-55.
- Fulton, M.H. and Key, P.B. 2001. Acetylcholinesterase inhibition in estuarine fish and invertebrates as an indicator of organophosphorus insecticide exposure and effects. *Environmental Toxicology and Chemistry*, **20**: 37-45.
- Ganeshwade, R.M. 2012. Biochemical changes induced by dimethoate (Rogor 30%EC) in the gills of fresh water fish *Puntius ticto* (Hamilton). *Journal of Recent Trends in Bioscience*, **2**: 58-64.
- Ganeshwade, R.M. 2011. Biochemical changes induced by dimethoate in the liver of fresh water fish *Puntius ticto* (HAM). *Biological Forum - An International Journal*, **3**: 65-68.
- Gill, T.S., Pande, J. and Tewari, H. 1991. Effects of endosulfan on the blood and organ chemistry of freshwater fish, *Barbus conchoni* Hamilton. *Ecotoxicology and Environmental Safety*, **21**: 80-91.
- Gould, E., Collier, R.S., Karolous, J.J. and Givenus. 1976. Heart transaminase in the rock crab, *Cancer irroratus* exposed to cadmium salts. *Bulletin of Environmental Contamination and Toxicology*, **15**: 635-643.
- Gupta, R.C. 2006. *Toxicology of Organophosphate and Carbamate Compounds*. Elsevier Academic Press, New York, USA.
- Honarpojoh, K. 2003. *Study and Identification of Organophosphorus Pesticide Residues (Azinphosmethyl and Diazinon) in the Mahabad and Siminerood Rivers*. M.Sc. thesis. Tehran University of Medical Science. Tehran, Iran.
- Hudgson, E. 2004. *A Text Book of Modern Toxicology* (3rd edn.). Department of Environmental and Biochemical Toxicology, North California State University, California, USA.
- Irshad, S.A. and Gautam, R.K. 2014. Effect of organophosphate pesticide nuvan on serum biochemical parameters of fresh water catfish *Heteropneustes fossilis* (Bloch.). *International Research Journal of Environment Sciences*, **3**: 1-6.
- Jaroli, D.P. and Sharma, B.L. 2005. Effect of organophosphate insecticide on the organic constituents in liver of *Channa punctatus*. *Asian Journal of Experimental Sciences*, **19**: 121-129.
- Jimenez, B.D. and Stegeman, J.J. 1990. *American Fisheries Society Symposium*, **8**: 69-79.
- John, P.J. 2007. Alteration of certain blood parameters of freshwater teleost *Mystus vittatus* after chronic exposure to metasytox and sevin. *Fish Physiology and Biochemistry*, **33**: 15-20.
- Joseph, B. and Raj, S.J. 2011. Impact of pesticide toxicity on selected biomarkers in fishes. *International Journal of Zoology Research*, **7**: 212-222.
- Jyothi, B. and Narayan, G. 1999. Certain pesticide-induced carbohydrate metabolic disorders in the serum of freshwater fish *Clarias batrachus* (Linn.). *Food and Chemical Toxicology*, **37**: 417-421.
- Köprücü, S.Ş., Köprücü, K., Ural, M.Ş., İspir, Ü. and Pala, M. 2006. Acute toxicity of organophosphorus pesticide diazinon and its effects on behaviour and some hematological parameters of fingerling European catfish (*Silurus glanis* L.). *Pesticide Biochemistry and Physiology*, **86**: 99-105.

- Koundinya, P.R. and Ramamurthi, R. 1979. Effect of organophosphate pesticide sumithion (fenitrothion) on some aspects of carbohydrate metabolism in a fresh water fish *Tilapia mossambica* (Peters). *Experientia*, **35**: 1632-1639.
- Lal, B. and Singh, T.P. 1987. Impact of pesticides on lipid metabolism in the freshwater catfish, *Clarias batrachus*, during the vitellogenic phase of its annual reproductive cycle. *Ecotoxicology and Environmental Safety*, **13**: 13-23.
- Landrum, P.F. and Fisher, S.W. 1998. Influence of lipids on the bioaccumulation and trophic transfer of organic contaminants in aquatic organisms. pp. 203-234. **In:** *Lipids and Freshwater Ecosystems* (eds. M. Arts and B. Wainman). New York, USA.
- Logaswamy, S., Radha, G., Subhashini, S. and Logankumar, K. 2007. Alterations in the levels of ions in blood and liver of freshwater fish, *Cyprinus carpio* var. *communis* exposed to dimethoate. *Environmental Monitoring and Assessment*, **131**: 439-444.
- Magar, R.S. and Afsar, S. 2012. Biochemical changes in proteins and amino acids in *Channa punctatus* in responses to sub-lethal treatment with the insecticide malathion. *Trends in Life Sciences*, **1**: 2319-2331.
- Malkovich, B. 1995. Effect of organophosphates on the antioxidant systems of fish tissue. *Acta Biologica Hungarica*, **46**: 9-11.
- Mathur, S.C. 1999. Future of Indian pesticides industry in next millennium. *Pesticide Information*, **24**: 9-23.
- Medda, C., Sarkar, S.K., Ganguly, S. and Basu, T.K. 1992. Effects of dichlorvos, nuvan on some important bioindicators of two fresh water major carps *Labeo rohita* and *Cirrhinus mrigala*. *Journal of Inland Fish Society*, **24**: 36-39.
- Murphy, P.G. 1971. Effects of salinity of on uptake of DDT, DDE and DDD by fish *Bulletin of Environmental Contamination and Toxicology*, **5**: 404-407.
- Mushigeri, S.B. and David, M. 2005. Fenvalerate induced changes in the ACh and associated AChE activity in different tissues of fish, *Cirrhinus mrigala* (Hamilton) under lethal and sub-lethal exposure period. *Environmental Toxicology and Pharmacology*, **20**: 65-72.
- Narra, M.R., Regatte, R.R. and Kodimiyala, R. 2013. Influence of chlorpyrifos stress on protein metabolism of edible crab, *Barytelphusa guerini*, and its recovery. *Journal of Stress Physiology and Biochemistry*, **9**: 219-231.
- Naveed, A., Venkaeshwarlu, P. and Janaiah, C. 2011. Biochemical alteration induced by triazophos in the blood plasma of fish, *Channa punctatus* (Bloch). *Annals of Biological Research*, **2**(4): 31-37.
- Nunes, B. 2011. The use of cholinesterases in ecotoxicology. *Reviews of Environmental Contamination and Toxicology*, **22**: 1-59.
- Ogueji, E.O. and Auta, J. 2007. Investigation of biochemical effects of acute concentration of lambda-cyhalothrin on African catfish, *Clarias gariepinus* (Teguels). *Journal of Fisheries International*, **2**: 86-90.
- Parvathi, M.L.S., Reddy, C.S. and Chetty, N. 2000. *In vivo* recovery and long term effect of phosalone on total lipid and triglycerides in freshwater fish *Tilapia mossambica* (Peters). *Pollution Research*, **19**: 345-351.
- Patil, M. and Kulkarni, R.S. 1996. Ovarian lipid and cholesterol response to Sumaach (a crude form of HCG) under pesticide treatment in the freshwater fish, *Channa*

- punctatus* (Bloch). *Proceedings of National Academy of Sciences India, Section B*, **66**: 135-137.
- Pehkonen, S.O. and Zhang, Q. 2002. The degradation of organophosphorus pesticides in natural waters: A critical review. *Critical Reviews in Environmental Science and Technology*, **32**: 17-72.
- Prashanth, M.S. and Neelagund, S.E. 2008. Impact of cypermethrin on enzyme activities in the freshwater fish *Cirrhinus mrigala* (Hamilton). *Caspian Journal of Environmental Sciences*, **6**: 91-95.
- Rahman, M.F., Mahboob, M. and Grover, P. 2004. *In vitro* acetylcholinesterase inhibition by novel op compounds in various tissues of the fish *Channa punctatus*. *Bulletin of Environmental Contamination and Toxicology*, **72**: 38-44.
- Ramani, M.B., Mercy, A.T.V., Nair, R.J. and Sherief, P.M. 2002. Changes in the proximate composition of *Labeo rohita* (Ham.) exposed to sublethal concentrations of monocrotophos. *Indian Journal of Fisheries*, **49**: 427-432.
- Ramesh, M. and Saravanan, M. 2008. Haematological and biochemical responses in fresh water fish *Cyprinus carpio* exposed to chlorpyrifos. *International Journal of Integrative Biology*, **3**: 80-83.
- Rao, J.V., Begum, G., Sridhar, V. and Reddy, N.C. 2005. Sublethal effects of monocrotophos on locomotor behaviour and gill architecture of the mosquito fish, *Gambusia affinis*. *Journal of Environmental Science and Health*, **40**: 813-825.
- Rao, K.S., Rao, K.R. and Sahib, I.K. 1985. Combined action of carbaryl and phenthoate on tissue lipid derivatives of murrel, *Channa punctatus* (Bloch). *Ecotoxicology and Environmental Safety*, **9**: 107-111.
- Reddy, S.L.N. and Venugopal, N.B.R.K. 1990. Fluoride-induced changes in protein metabolism in the tissue of freshwater crab, *Barytelphusa guerini*. *Environmental Pollution*, **67**: 97-108.
- Saha, S. and Kaviraj, A. 2009. Effects of cypermethrin on some biochemical parameters and its amelioration through dietary supplementation of ascorbic acid in freshwater catfish, *Heteropneustes fossilis*. *Chemosphere*, **74**: 1254-1259.
- Sancho, E., Ferrando, M.D. and Andreu, A., 1997. Sublethal effects of an organophosphate insecticide on the European eel, *Anguilla anguilla*. *Ecotoxicology and Environmental Safety*, **36**: 57-65.
- Shakoori, A.R., Mughal, A.L. and Iqbal, M.J. 1996. Effects of sublethal doses of fenvalerate (a synthetic pyrethroid) administered continuously for four weeks on the blood, liver and muscles of a fresh water fish, *Ctenopharyngodon idella*. *Bulletin of Environmental Contamination and Toxicology*, **57**: 487-494.
- Shamoushaki, M.M.N., Soltani, M., Kamali, A., Imanpoor, M.R., Sharifpour, I. and Khara, H. 2012. Effects of organophosphate, diazinon on some haematological and biochemical changes in *Rutilus frisii kutum* (Kamensky, 1901) male brood stocks. *Iranian Journal of Fisheries Sciences*, **11**: 105-117.
- Sharma, B. 1999. Effect of carbaryl on some biochemical constituents of the blood and liver of *Clarias batrachus*, a fresh water teleost. *Journal of Toxicological Sciences*, **24**: 157-164.
- Shayeghi, M., Darabi, H., Abtahi, H., Sadeghi, M., Pakbaz, F. and Golestaneh, S.R. 2007. Assessment of persistence and residue of diazinon and malathion in three rivers

- (Mond, Shahpour and Dalaky) of Bushehr province in 2004-2005 years. *Iranian South Medical Journals*, **10**: 54-60.
- Shivakumar, R. 2005. *Endosulfan Induced Metabolic Alternation in Freshwater Fish, Catla catla*. Ph.D. thesis, Karnataka University, Dharwad, Karnataka, India.
- Siddiqui, M.K.J., Rahman, M.F., Anjum, F. and Mustafa, M. 1989. Species selectivity in acetylcholinesterase inhibition by parathion and its oxygen analogue, paraoxon. *Advances in Bioscience*, **8**(Suppl.): 33-40.
- Silbergeld, E.K. 1974. Blood glucose: A sensitive indicator of environmental stress in fish. *Bulletin of Environmental Contamination and Toxicology*, **11**: 20-25.
- Singh, H. and Singh, T.P. 1980. Effect of two pesticides on testicular ³²P uptake, gonadotrophic potency, lipid and cholesterol content of the testes, liver and blood serum during spawning phase in *Heteropneustes fossilis* (Bloch). *Endokrinologie*, **76**: 288-296.
- Singh, N.N., Das, V.K. and Singh, S. 1996. Effect of aldrin on carbohydrate, protein and ionic metabolism of a freshwater cat fish *Heteropneustes fossilis*. *Bulletin of Environmental Contamination and Toxicology*, **57**: 204-210.
- Sturm, A., Wogram, J., Segner, H. and Liess, M. 2000. Different sensitivity to organophosphates of acetylcholinesterase and butyrylcholinesterase from three-spined stickleback (*Gasterosteus aculeatus*): Application on biomonitoring. *Environmental Toxicology and Chemistry*, **19**: 1607-1617.
- Susan, T.A., Sobha, K., Veeraiyah, K. and Tilak, K.S. 2010. Studies on biochemical changes in the tissues of *Labeo rohita* and *Cirrhinus mrigala* exposed to fenvalerate technical grade. *Journal of Toxicology and Environmental Health Sciences*, **2**(5): 53-62.
- Tabrizi, T.S. 2001. *Study of Pesticide Residues (Diazinon, Malathion, Metasystox) in the Tabriz Nahand River*. M.Sc. thesis, Tehran University of Medical Science, Tehran, Iran.
- Thangnipon, W., Thangnipon, W. Luangpaiboon, P. and Chinabut, S. 1995. Effects of the organophosphate insecticide, Monocrotophos, on acetylcholinesterase activity in the Nile tilapia fish (*Oreochromis niloticus*). *Brain Neurochemistry Research*, **20**: 587-591.
- Tilak, K.S., Wilson Raju, P. and Butchiram, M.S. 2009. Effects of alachlor on biochemical parameters of the freshwater fish, *Channa punctatus* (Bloch). *Journal of Environmental Biology*, **30**: 421-426.
- Tuduri, L. Harner, T., Blanchard, P., Li, Y.F., Poissant, L., Waite, D.T., Murphy, C. and Belzer, W. 2006. A review of currently used pesticides (CUPs) in Canadian air and precipitation: Part 2: Regional information and perspectives. *Atmospheric Environment*, **40**: 1563-1578.
- Venkataraman, G.V., Rani, P.N. and Murthy, P.S. 2006. Impact of malathion on the biochemical parameters of gobiid fish, *Glossogobius giuris* (Ham). *Journal of Environmental Biology*, **27**: 199-212.
- Verma, G.P. and Panigrahi, P. 1998. Effect of agrofens on blood parameters of *Oreochromis mossambica* (Peters). *Proceedings of National Academy, India. B (Biological Sciences)*, **68**: 29-36.
- Vryzas, Z., Vassiliou, G., Alexoudis, C. and Papadopoulou-Mourkidou, E. 2009. Spatial and temporal distribution of pesticide residues in surface waters in north eastern Greece. *Water Research*, **43**: 1-10.

- Vutukuru, S.S. 2005. Acute effects of hexavalent chromium on survival, oxygen consumption, hematological parameters and some biochemical profiles of the Indian major carp, *Labeo rohita*. *International Journal of Environmental Research and Public Health*, **2**: 456-462.
- Wells, R.M.G., McIntyre, R.H., Morgan, A.K. and Davie, P.S. 1986. Physiological stress responses in big gamefish after capture: Observation on plasma chemistry and blood factors. *Comparative Biochemistry and Physiology*, **84A**: 565-571.
- Werimo, K., Bergwerff, A.A. and Seinen, W. 2009. Residue levels of organochlorines and organophosphates in water, fish and sediments from lake Victoria-Kenyan portion. *Aquatic Ecosystem Health and Management*, **12**: 337-341.
- Zutshi, B. 2003. Effect of fenthion on the testis of freshwater fish *Glossogobius giuris* (histochemical and biochemical). *Pollution Research*, **22**: 231-236.