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Chapter- 7

Impact of some selected organophosphate pesticides on non-target fish species

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Abstract:

Pesticides are important for protecting agricultural crops against harmful pests. However, pesticide usage has become a serious issue of concern as they cause severe damage to the ecosystem and environment. Organophosphates are one of the most extensively applied insecticides in agriculture. Agricultural runoff containing pesticide-laden effluent ultimately pollutes the aquatic environment. Pollutants introduced to aquatic ecosystems are absorbed by various flora and fauna, including fish, causing harmful effects and mortality. The LC50 values of a few organophosphate pesticides in several fish species and other investigations relevant to sublethal toxic effects such as haematological, biochemical, histopathological, and behavioural alterations were reviewed in this article.

Introduction:

Pesticides are harmful toxic substances used to kill, reduce, or restrict the growth of certain target organisms known as pests (Aktar, 2009). In the agricultural field, they are commonly used because of their quick and successful action and also to increase crop output (Popp et al., 2013). Apart from safeguarding crops, pesticides have become a serious issue of water pollution. Extensive use of pesticides causes severe damage to the ecosystem and environment (Murthy et al., 2013; Samal et al., 2017; Dey and Dey, 2022). Agricultural runoffs carrying a variety of pesticide residues damage the groundwater and neighbouring aquatic bodies. Regardless of where it occurs, pesticide contamination is expected to eventually end up in aquatic environments (Fırat et al., 2011; Mondal et al., 2022). The majority of pesticides have various half-lives and are difficult to break down. They persist for long periods in the soil, sediments, and aquatic environment (Ramaswamy et al., 2007). Due to the restriction on organochlorine pesticides, farmers in tropical nations like India are increasingly turning to organophosphate as a viable option. Organophosphate insecticides have become the most popular as they are cheaper and have little environmental persistence (Özcan Oruç et al., 2006; Banerjee et al., 2021). Soil samples collected from several agricultural farms in India revealed residues of various organophosphate pesticides such as chlorpyriphos, malathion and quinalphos (Kumari et al., 2008).

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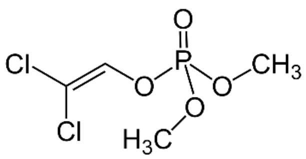
Organophosphates are a powerful neurotoxin and block synaptic transmission Imetaln cholinergic neurons by suppressing AChE activity. These pesticides are toxic to fish and other non-target aquatic animals and have been associated with various detrimental effects on development, physiology and behaviour (Saha et al., 2017). They can cause parasympathetic disorder and possibly death if taken directly (Van Cong et al., 2008). Some of the organophosphates are very dangerous and recognized as hazardous classes (1a and 1b) by World Health Organization (Kumar et al., 2016). According to recent studies, just 0.1% of pesticides applied on agricultural land are efficient, whereas the remaining 99.9% contaminate water, land, and the air, eventually being taken by non-target organisms (Zhang et al., 2011). When these toxicants enter water bodies at much higher concentrations than acceptable limits, the environment becomes hostile for all aquatic organisms, resulting in high mortality for all fish and shellfish. Lower concentrations of these toxicants cause sub-lethal effects on multiple organs and bioaccumulation, eventually reaching humans via the food chain (Xie et al., 1996; Morel et al., 1998; Abedi et al., 2013). In this article, selected organophosphates such as profenofos, dichlorvos, chlorpyrifos induced hazardous effects in different fish species are taken into consideration.

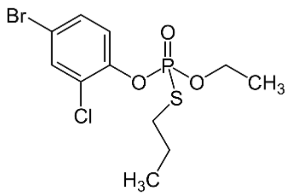
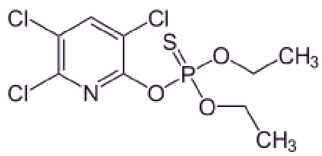
Direct impact:

Pesticides cause a variety of sublethal toxicity in fish, including changes in fish behaviour, changes in the architecture of tissues, blood parameters, changes in histopathological architectures (Das et al., 2000; Rani et al., 2014), changes in enzyme function and different biochemical components, genotoxicity, interruption in endocrine function (Gupta et al., 2015), and changes in the antioxidant enzyme (Rani et al., 2014). When a fish is exposed to a pesticide, various organs display distinct sorts of harmful effects. The gills, liver, and kidneys were shown to be the organs with the most impact in several investigations. Some of these changes have been detailed in the following under different subheadings to make it more comprehensive.

LC50 Value of some organophosphate pesticides:

Several researchers have studied the acute toxicity and other impacts of different organophosphates on various commercially important fish species. The following are a few of them:

Pesticides	Chemical Class	Fish Species	Duration	Measured LC50 value
Dichlorvos 	Phosphate	*Anabas testudineus*	96 hours	2.35mg/L (Patar et al., 2015)
		Aphanius iberus	96 hours	3.17mg/L (Varó et al., 2018)
		Clarias batrachus	96 hours	0.07ml/L (Gautam, 2013)
		Channa punctatus	96 hours	0.024ml/L (Wokoma, 2019)
		Cirrhinus mrigala	96 hours	20mg/L (Srivastava et al., 2014)
		Cyprinus carpio	96 hours	0.95mg/L (Tak et al., 2014)

		Labeo rohita	96 hours	0.11mg/ml (Rao et al., 2017)
		Heteropneustes fossilis	96 hours	6.4mg/L (Ahmad et al., 2013)
Profenofos 	Phosphorothiolate	*Channa punctatus*	96 hours	2.68 µg/L(Pandey et al., 2011)
		Labeo rohita	96 hours	0.1mg/L (Khan et al., 2018)
		Catla catla	96 hours	0.0079ppm (Maharajan et al., 2013)]
		Cyprinus carpio	96 hours	62.4 µg/L (Ismail et al., 2009)
		Oreochromis niloticus	96 hours	0.435 mg/L (Bhatnagar et al., 2015)
Chlorpyrifos 	Phosphorothionate	*Cirrhinus mrigala*	96 hours	0.44 mg/L (Bhatnagar et al., 2017)
		Labeo bata	96 hours	109.64µg/L (Samajdar et al., 2015)
		Cyprinus carpio	96 hours	0.160 mg/L (Halappa et al., 2009).
		Puntius chola	96 hours	0.219mg/L (Verma et al., 2013)
		Labeo rohita	96 hours	442.8µg/L (Ismail et al., 2009)

Behavioural Changes:

Indian major carp *Cyprinus carpio* treated with a sublethal concentration of chlorpyrifos showed loss of schooling behaviour, swimming independence, lack of co-ordination in movement and localised to the bottom of the test tank (Halappa et al., 2009). According to one study, rainbow fish subjected to chlorpyrifos displayed behavioural aggression, rapid water gulping, fast movement of the operculum, and unpredictable abnormal swim pattern. Fishes were increasingly agitated, stressed, lethargic, and showed hyperactivity before death (Sharbidre et al., 2011). Fish treated with profenofos exhibited hyperactivity, greying colouration, erratic swimming, and profuse mucous production on the body and gills before dying (Pandey et al., 2011). *Oreochromis niloticus* displayed frantic movement, loss of balance, and death following exposure to low concentrations of dichlorvos. At a higher dose of toxicant, a period of inactivity was seen, then a phase of air gulp and finally death (Mallum et al., 2016). *Clarias* *gariepinus* exposed to low concentrations of dichlorvos displayed unusual behavioural patterns, including restlessness, quick circular motions, flipping on the back, and increased mucous production on the body (Ogamba et al., 2014).

Haematological Changes:

Organophosphate insecticides have been proven to induce deadly impact on different hematological parameter, cytological changes in blood corpuscle (RBC. WBC), changes in hemoglobin (Hb) levels, mean cell volume (MCV) and volume percentage of RBC or haematocrit (Hct). For chronic toxicity analysis, tilapia fish treated with a sublethal dose of profenofos (1/10 of LC50) exhibited a sufficient increase in total white blood cell count and a huge fall in red blood cell count, marked decline in haemoglobin content and hematocrit percentage (Sharafeldin et al., 2015). *Oreochromis niloticus* subjected to dichlorvos treatment for 96 hours revealed a significant decrease in packed cell volume, haemoglobin, erythrocyte, and leucocyte count, as well as the lowest haematocrit (Hct) values (Mallum et al., 2016). *Channa punctatus* was treated with two sublethal chlorpyrifos dosages and its mean erythrocyte, leukocyte, haemoglobin, and hematocrit values were lowered (Ali et al.. 2012). Changes in haematological indicators such ESR rate were seen in *Channa punctatus* treated with chlorpyrifos. Increasing chlorpyrifos dose and exposure increases ESR rate (Malla et al., 2009). When compared to control fish, *Cyprinus carpio* treated with chlorpyrifos for 24 hours had lower RBC (-72.43%) and haemoglobin (18.35%) and more WBC count (+57.94%). (Ramesh et al., 2008). Blood glucose rose and Total Count increased very high after sublethal exposure to nuvan (dichlorvos) for 45 days in *Labeo rohita* compared to the control value, whereas total Hb dropped (Das et al., 2001).

Effects in Acetyl cholinesterase (AChE):

Acetyl cholinesterase activity is a good biomarker for pesticide exposure, particularly organophosphates. 66% brain AChE inhibition was seen in *Poecila reticulata* treated with a sublethal chlorpyrifos concentration (Sharbidre et al., 2011). Dichlorvos treatment for 40 days with three sublethal doses (0.47, 0.047, 0.0047mg/L) on AChE activity in Anabas testudineus tissues revealed lower AChE action in the liver, kidney, gills and brain. After 20 days, when fishes were withdrawn to fresh water, restoration of AChE in the liver, kidney, brain and gill was found (Patar et al., 2015). In research involving different age groups of chlorpyrifos-treated tilapia, brain tissue exhibited a higher degree of enzyme inhibition than the liver. After being moved to clean water, most exposed fish recovered their AChE activity. The liver tissue restored its function faster than the brain. Recovery of AChE activity in tiny fish was much faster than in larger fish. The degree of recovery is inversely proportional to the length of exposure (Rath et al., 1981). AChE enzyme isolated from the Amazonian fish *Colossoma macropomum* at low dichlorvos treatment concentrations showed 18% enzyme inhibition. An exponential reduction in AChE function was recorded after incubation with high levels of dichlorvos dosage (Asis et al., 2007).

Biochemical Changes:

Three sub-acute concentration dosages of profenofos were taken to inspect biochemical alteration on *Catla catla* and a significant dose-dependent drop in total protein, carbohydrate, and cholesterol levels were observed (Jagadeesana et al., 2012). Significant decline in liver glycogen, protein, lipid, alkaline phosphatase, and acid phosphatase levels and elevation in serum glutamic oxaloacetic transaminase and serum glutamic pyruvic transaminase levels, were seen in *Channa punctatus* treated to pesticide nuvan at 0.024ml/L for 4 days (Kumar, 2014). *Heteropneustes fossilis* exposed to nuvan with varied sublethal doses for consecutive 60 days show a progressive reduction in total protein and albumin content but a rising bilirubin level was observed with a steep elevation on the last day of investigation. A dramatic increase in urea and creatinine levels was also observed (Ahmad et al., 2013). *Oreochromis mossambicus* was treated with different sublethal doses of dichlorvos for three weeks and decreased levels of tissue glycogen, protein, and albumin levels in gill and muscle were found (Lakshmanan et al., 2013). In an investigation, organophospahte nuvan exposed *Clarias batrachus* group compared to the control level of cholesterol declined exceedingly and a rise in glucose and urea level was observed with the substantial increase in AST and ALP enzyme levels (Gautam et al., 2013). *Heteropneustes fossilis* exposed to significant sublethal doses of Chlorpyrifos and hypocalcemia is observed in fish [58]. Sub-lethal dose of chlorpyrifos for 30 days revealed alterations in thyroid hormones in *Heteropneustes fossilis*. Significant decline in blood TSH, T3 and T4 hormone levels revealed diminished thyroid gland function (Khatun et al., 2014). Dichlorvos induced acute toxicity on *Danio rerio* was determined by providing various dosages during 24-h, 48-h period and the result revealed total protein content and lipid peroxide levels were elevated in brain tissue. In contrast, sodium dismutase and catalase enzyme levels became lower. The glutathione peroxidase activity reduced drastically in the different treatment groups (Usharani, 2013). *Oreochromis niloticus* chlorpyrifos exposure caused reduced levels of oestrogen and testosterone. The levels of estradiol also dropped after 15 days of exposure. Cortisol hormone levels reduced significantly when the concentration of chlorpyrifos was increased compared to the control (Oruç, 2010).

Histopathological Changes:

The effects of toxicants, particularly chronic ones, can be quickly detected by changes in histological architecture in various tissues and organs. *Channa punctatus* subjected to a sublethal dose of nuvan, after 24 hours the liver showed increasing sinusoid space, cirrhosis, mild necrosis, fat accumulation, cytoplasmic granule deposition, and shrinkage. 48 hrs. later, tissue necrosis, sinusoid inflammation, and ischemic symptoms were observed (Kumar et al., 2016). After two sublethal doses of nuvan in *Cirrhinus mrigala*, degenerative alterations in the epithelium of the gill filament and secondary gill lamellae were observed. It was discovered that the secondary gill lamellae showed thickening and the epithelium of gill filaments was associated with adjoining secondary lamellae. In epithelium, there was also a significant decrease in the density and area of mucous-producing goblet cells (Srivastava et al., 2014). Rainbow trout exposed to chlorpyrifos to investigate histopathological damage in gill and liver tissues hyperaemia and severe liver damage were also found hyperaemia of gill lamellae, oedemas, clump formation, cellular damage, overgrowth, and decaying of all gill tissues observed (Topal et al., 2003). *Oreochromis mossambicus* subjected to a sublethal dose of chlorpyrifos displayed terminal bulge formation on secondary lamellae, lesions, and demolished base of lamellae. A dense layer of mucous coat on the gill filament was also observed (Rao et al., 2003)

Genotoxicity:

Phenmenon of nuclear abnormalities in the erythrocytes of the fish *Cirrhinus mrigala* subjected sublethal dose of chlorpyrifos was evaluated using the micronucleus (MN) test and the formation of micronuclei was observed. The existence of nuclear abnormalities was marked by changes in cell shape, broken eggs and large micronuclei (Bhatnagar et al., 2016). A substantial dose-dependent increase in the creation of micronuclei inside the erythrocytes was found in *Clarias gariepinus* treated with sublethal concentrations of dichlorvos for for 28 days (Oladokun et al., 2020). Comet assay was used to study the toxicity of dichlorvos in *Mystus vittatus* and considerable DNA damage was found (Shukla et al., 2010). Comet assay and RAPD analysis was done on profenofos exposed *Channa punctatus* in a semi-static system, revealing a sub-lethal dose of profenofos can induce considerable DNA damage compared to the control (Pandey et al., 2018).

Conclusion:

Fishes are a crucial biotic component of aquatic ecosystems and the most nutritious vertebrate food to humans, rich in protein, fatty acids, and vitamins. They are extremely valuable for economic, nutritional, medicinal, and industrial purposes. Organophosphate pesticides in aquatic environments create many sublethal impacts on fishes, such as in behaviour, histopathology, haematology, biochemical alteration, inhibition of AChE enzyme activity, endocrine system, genotoxicity etc. Research on various natural plant extracts that can reduce these pesticide-induced adverse effects in fish should also be encouraged. Furthermore, there is a possibility that these pesticides can contaminate the food chain and end up in humans. So, it might be concluded that extreme caution must be taken while using organophosphate pesticides in farming activities, particularly those conducted close to aquatic habitats, to reduce their negative impacts on aquatic biota and the environment.

Conflict of interest:

None

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