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Biomarkers for the Assessment of Pesticide Toxicity in Fish

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Abstract: Pesticide (a type of biocide) is any substance or a mixture of several compounds produced to control or to kill various unwanted species of animals and plants. Even in modern days pesticide is the ultimate and easy choice for preventing a vast range of agricultural pests. But the indiscriminate use of these synthetic chemicals in agricultural field has created serious problems to the target as well as to the non-target organisms living in agricultural field as well in the nearby aquatic bodies like river, pond, stream etc. Fish, the important source of protein, is highly affected by the agricultural usage of different classes of pesticides. On pesticide exposure fish respond biochemically by altering the level of DNA, RNA, liver and muscle glycogen. Several enzymes like acetylcholine esterase, alanine aminotransferase, aspartate aminotransferase activity also changed by different classes of pesticides. Haematological parameters like RBC count, WBC count, mean cell volume, haemoglobin level, blood glucose level act as important biomarkers during the assessment of toxicity caused by pesticide. Several studies reported that major organs like liver, kidney, spleen, brain, intestine of fish are highly affected by pesticides. Hypertrophy, necrosis, pyknosis of hepatocytes, fusion of primary lamellae of gills and atrophy of epithelial cells of intestine are the most common histopathological symptoms caused by pesticides. The immune system of fish respond to pesticide entry into the biological system by altering the expression level of several parameters like immunoglobulin, complement protein, lysozyme activity and others. Besides these physiological parameters there are various behavioural parameters which are the primary responses of fish on pesticide encounter. So, these responses against pesticides can act as vital biomarkers for the assessment of toxicity of a pesticide in biological system. In this mini-review, the biological responses in terms of biomarkers of effects have been discussed due to the exposure to chemical pesticides in fish.

Keywords: Pesticide, Biochemical, Immunological, Histopathological, Behavioural biomarker

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Introduction

A huge number of synthetic chemical pesticides having diverged chemical structures and properties are frequently applied in agricultural fields to control the pest population. These pesticides are used to kill a target organism, particularly the pest, but in most cases directly or indirectly these also affect several non-target organisms (Bhatnagar *et al*., 1992; Tudi *et al*., 2021). A number of studies reported that only a small proportion of the applied chemical pesticides enter into the physiological system of the target pests, while a large amount either gets deposited in the soil or move some other places like rivers, ponds and other aquatic system through agricultural as well as urban run-off (Ullah *et al*., 2014). Several investigations documented the presence of residual pesticides in water bodies, soil sediment and fish tissue (Table 1) indicating that the aquatic systems are highly contaminated with chemical pesticides which may lead to a serious problem for the economically important fish species. Fish is an important source of dietary proteins and lipids, a vast range of organisms including humans ingest fish as their principal food. Pesticide-contaminated water triggers a cascade of physiological, biochemical, immunological and behavioural responses in fish after entering the fish body through the gills, intestine or alimentary canal (Schlenk, 2005; Banaee and Vayghan, 2011; Vani *et al*., 2012). These biological responses have been used as biomarkers to detect the adverse effects of pesticides on fish. Because, a single biomarker is not practically effective in the assessment of the toxicity of chemical pesticides on fish, several biomarkers are used (Joseph and Raj, 2011) and these biomarkers are commonly known as biomarkers of xenobiotics.

Biomarkers of xenobiotics can be classified into three types, biomarkers of susceptibility, biomarkers of exposure, and biomarkers of effect. Biomarkers of susceptibility indicate responses like changes in the expression of certain protein or receptor genes which affect the susceptibility of the organism to the toxicant. Biomarkers of effect detect biological responses like biochemical or physiological or behavioural changes in biological systems after exposure to compounds. Biomarkers of exposure are involved in the measurement of the quantity of compound or its metabolites inside a biological system (Van der Oost *et al*., 2003).

These biomarkers, particularly the biomarker of effects, play an important role in the assessment of the toxic effects of pesticides in economically important organisms like fish. In this mini-review, the biological responses in terms of biomarkers of effects have been discussed due to the exposure to chemical pesticides in fish.

Biomarkers of effects for pesticide toxicity:

The toxicity of chemical pesticide(s) in an organism can be determined by several mechanisms. In the exposed organisms the functional responses are elicited either as a result of the action of the pesticide(s) or as adaptive responses. These functional responses include different physiological, biochemical, histopathological, immunological and behavioural responses (Das and Mukherjee, 2003). All these responses serve as good biomarkers for the monitoring of the toxicity of a pesticide in an organism like fish and are known as biomarkers of effects.

Biochemical and enzymatic biomarkers:

The evaluation of the toxic effects of pesticides in non-target organisms is determined by different biochemical and enzymatic responses. Enzymes like cytochrome p450 monooxygenases, esterases, and glutathione-s-transferases play a pivotal role in the biotransformation of xenobiotic compounds by phase I and phase II reactions. Pesticide entry into the fish body induces the expression level of these detoxifying enzymes (Winston and Di Giulio, 1991; Radice *et al*., 2001; Fent, 2001; Livingston, 2001; Peixoto *et al*., 2006; Rao, 2006). Therefore, these enzymes could be good indicators for the assessment of pesticide toxicity. Biotransformation of pesticides by cytochrome p450 monooxygenases results in the production of reactive oxygen species (ROS) in the cells which lead to lipid peroxidation.

The expression level of other enzymes like acetylcholine esterase, lactate dehydrogenase, pyruvate dehydrogenase and succinate dehydrogenase decreases due to bioaccumulation of pesticide in different tissues like the brain, liver, muscle, kidney and intestine, although alanine aminotransferase activity was reversed in most cases due to the disruption of hematopoiesis and

Table 1: Reports on the contamination of different water bodies along with sediment and fish tissue with pesticides in India (OCs=Organochlorines, OPs=Organophosphates)

Sites of Assessment	Class of pesticides	Source	Results	References
Different regions Along the east coast of India	OCs	Sediment	Total DDT ranged from 0.02 -0.49 ppm with a mean value of 0.02 ppm pp'-DDT (0.01- 0.02 ppm), op'-DDT (0.02-0.D4 ppm), pp'-DDE (0.01-0.34 ppm), op'-DDE (0.O2-O.21 ppm), pp'-DDD (0.02-0.30 ppm) and op'-DDD (0.04-0.35 ppm)	Sarkar and Gupta (1988)
Kolleru lake, India	OCs	Sediment	(α–BCH, γ-BCH, malathion, Chlorpyrifos and endosulfan) > (isodrin, dieldrin and p,p'-DDT)	Rao and Pillala (2001)
River: Ganges form different locations of Bihar, India	OCs	Fish	Contamination pattern DDT> HCHs> aldrin> endosulfan DDT = 13.6-1665.9 ng/gm HCHs = 115.8-1206.8 ng/gm Aldrin = 3.1-86.1 ng/gm Endosulfan = 2.9-74.5 ng/gm	Kumari and Sinha (2001)
River: Gomti, Uttar Pradesh, India	OCs	Water & sediment	Total organochlorine pesticide in river water 0.02-4997.0 ng/L in sediment 0.02-1765.4 ng/gm	Singh *et al.* (2005)
River: Gomti, Uttar Pradesh, India	OCs	Fish (*channa punctatus*): muscle	Fish muscles total OCPs ranged between 2.58–22.56 ng g–1 with a mean value: 9.66 ± 5.60 ng g–1. Aldrin was the predominant OCP HCB and methoxychlor could not be detected. α-HCH and β-HCH (the isomers of HCH) and pp-DDE (metabolites of DDT) were the most frequently detected OCPs.	Malik *et al.* (2007)
Nine freshwater bodies, Punjub, India	OCs	Fish Rohu, common carp, grass carp, catla, silver carp	DDTs were the predominant organochlorine ontaminants in all species with pp DDT and pp DDE as the main pollutants HCH isomers and dieldrin were also found at lower levels in fish species α -HCH was the dominant isomer of HCH in all fish species followed by γ-, β- and δ-HCH. aldrin, chlordane, heptachlor and endosulphan were not detected	Kaur *et al.* (2008)
River: Ganga around Kolkata	OCs	Fish	total-HCH concentration remains above the MRL values	Aktar *et al.* (2009)
River: Yamuna, Delhi, India	OCs (20 nos.)	Water	Endrin aldehyde, Endosulfan sulfate and DDT showed the highest percentage composition of OCP at all the sampling sites in all the three seasons The total or-ganochlorine pesticides level ranged from 157.71 - 307.66 ng/g in Pre-monsoon 195.86 - 577.74 ng/g in Monsoon 306.9 - 844.45 ng/g in the Post-monsoon season.	Pandey *et al.* (2011)

Deomoni from the Terai region of West Bengal	OCs, OPs	River water, sediment & fish muscle	chlorpyrifos, dicofol and ethion in water sample as ~0.0091 ppm, ~0.0180 ppm and ~0.0892 ppm, respectively in sediments ~0.0513 ppm, ~0.0414 ppm and ~0.1271 ppm and in fish muscles (Puntius sp.) ~5.0371 ppm, ~3.7700 ppm and ~2.9599 ppm, respectively in fish muscles (Puntius sp.) ~5.0371 ppm, ~3.7700 ppm and ~2.9599 ppm, respectively	Singh, *et al.* (2015)
Thamirabarani river system of southern peninsular India	OCs , Pyrethroids	River water, Fish, sediment	Concentrations of Endosulfan, aldrin, dieldrin, endrin and heptachlor were found to be higher than the MRL value.	Arisekar *et al.* (2019)

proteosynthesis (Bani and Vayghan, 2011). Vani *et al*. (2012) reported that on the exposure of *Catla catla* to sub-lethal doses of cypermethrin the activities of membrane transport enzymes like the total adenosine triphosphatase, sodium–potassium adenosine triphosphatase and magnesium adenosine triphosphatase were decreased remarkably in the gill tissues.

Other parameters like DNA level, RNA level, hepatic glycogen, and muscle glycogen level decrease (Table 2) in response to pesticide encounter (Bani and Vayghan, 2011,). Several studies reported that in cypermethrin-exposed *Cyprinus carpio* the DNA and RNA content decreased significantly in brain, gills and liver after a certain period of exposure (Gowri *et al*., 2013; Neelima *et al*., 2015). The same results were also found in other fishes like *Channa punctatus*, *Labeo rohita* (common edible carp), *Cirrhinus mrigala* (Raksheskar, 2012; Tiwari *et al*., 2012; Vasantharaja *et al*., 2013).

Haematological parameters:

Haematological parameters like haemoglobin content, RBC and WBC count, hematocrit, mean cell volume (MCV), mean corpuscular haemoglobin, plasma protein, plasma glucose etc. have been used for several years as good bioindicators of toxicosis of fish after exposure to different classes of pesticides like organochlorines, organophosphates, pyrethroids, carbamates (Atamanalp *et al*., 2002; Adhikari *et al*., 2004; Banaee *et al*., 2008). On pesticide exposure haemoglobin content, RBC content and serum protein content decrease. Haemoglobin content decreases due to the oxidation of haemoglobin into methaemoglobin by the toxic action of the pesticide. As a result of toxicant induce haemolysis, RBC content also decreases. Anaemia is the primary sign of this haemolysis. Many arguments have been made as to the cause of the depletion of serum protein. Protein content is depleted due to the enhanced proteolysis to generate products which provide energy during stressful situations (Ravinder *et al*., 1988). Others opined that protein content is depleted due to the reduction in food intake and increased utilization of energy tissue repairing, and detoxification mechanisms (Neff, 1985). To cope with the stress induced by toxicant blood glucose level increases (Banaee *et al*., 2013). In stressful situations, the organisms need more energy which is supplied by glucose. Adrenal hormones (catecholamines and cortisol) play a significant role in the production of glucose by gluconeogenesis (Chan and Woo, 1978; Van Raaji *et al*., 1995). WBC content was reported to be increased rapidly for the production of antibodies against the pesticide(s).

Immunological parameter:

The immune system of fish is of two types: innate and adaptive. Both of these are divided into cell-

Table 2: List of different biomarkers of effect and their response in fishes exposed to different classes of pesticides (OC=Organochlorine, OP=Organophosphate, Py=Pyrethroids, Ca=Carbamate, Tr= Triazine)

Biomarkers of effect		***Effects/ Response***	***Class of pesticides***	***References***
	DNA level	Decreased	OC, OP, Py	Adhikari *et al.* (2004); Ahmad and Gautam, (2014); Al-Otaibi *et al.* (2018); Bani and Vayghan (2011); Bojarski and Witeska (2020); Das and Mukherjee, (2003); Ghasemzadeh *et al*. (2015); Ghayyur *et al.* (2021); Gholami-Seyedkolaei *et al.* (2013); Khan *et al*. (2019); Kumar *et al*. (2007); Ramesh and Saravanan (2008); Rao (2006); Rathod *et al*. (2010); Sayeed *et al*. (2003); Suvetha *et al*. (2015); Tripathi and Verma (2004); Vani *et al*. (2012); Velmurugan *et al*. (2022)
	RNA level	Decreased	OC, OP, Py	
Biochemical & enzymatic	Hepatic glycogen	Decreased	OC, OP, Ca	
	Muscle glycogen	Decreased	OC, OP, Ca	
	Lactate level in liver	Increased	OP, Ca	
	Lactate level in white muscle	Increased	OP, Ca	
	Total protein in liver	Decreased	OP, Ca	
	Albumin content	Decreased	Py	
	Globulin content	Decreased	OP, Py	
	Catalase activity	Decreased	OP, Py	
	Acetylcholine esterase activity in brain	Decreased in brain, liver & muscle	OP, Py, Ca	
	Alanine amino transferase activity	Increased	OP	
	Aspartate amino transferase activity	Increased	OP	
	Lactate dehydrogenase activity	Decreased in brain (OC), Increased in brain, (OP, Ca, Py); Decreased in liver, muscle, kidney & intestine (OP, OC, Ca, Py)	OC, OP, Py, Ca	
	Pyruvate dehydrogenase activity	Decreased in all tissue	OP, Ca	
	Succinate dehydrogenase activity	Decreased in brain, kidney & liver	Py, Ca	
	Alkaline phosphatase activity	Increased (Py) in liver & kidney, Decreased (OP)	OP, Py	
	ATPase activity	Decreased in brain, kidney and liver	Py	
	Lipid peroxidation	induced in all the tissues, gill showing the highest	Py	
	Ascorbic acid level	increased in kidney and liver, but decreased in gills	Py	
	Glutathione	Increased	Py	
	Glutamic oxalo acetate trans-aminase	Increased in blood	Py	
	Glutamic pyruvate trans-aminase	Increased in blood	Py	
Haematologica l	Haemoglobin level	Decreased	OC, OP, Py, Ca, Tr	Adhikari *et al*. (2004); Ahmad *et al*. (2020); Das and Mukherjee (2003); Dorucu and Girgin (2001);
	Haematocrit value	Decreased	OC, OP, Ca	
	RBC count	Decreased	OC, OP, Py, Ca, Tr	

	WBC count	Increased up to first 20-25 days of exposure then recovered (OC, OP, Py, Ca, Tr), Decreased (Py)	OC, OP, Py, Ca, Tr	Far *et al*. (2012); George *et al*. (2017); Gholami-Seyedkolaei *et al*. (2013); Hedayati and Niazie (2015); Nayak *et al*. (2004); Ramesh and Saravanan (2008); Sinha *et al*. (2022); Vani *et al*. (2012)
	Plasma/serum protein concentration	Increased (OC) [acute treatment], Decreased (OC, OP, Py, Tr)	OC, OP, Py, Tr	
	Mean cell volume (mcv)	Increased (OP, Ca), Decreased (OC)	OC, OP, Ca	
	Mean cell haemoglobin (mch)	Increased (OP, Ca) Decreased (OC)	OC, OP, Ca	
	Blood glucose level	Increased (OP, OC, Py), Decreased (Tr)	OC, OP, Py, Tr	
	Peripheral blood leukocytes viability	Decreased	Py, Tr	
Immunological	Non specific immune response	Reduced	Py	Dawood *et al*. (2020); Li *et al*. (2013); Nayak *et al*. (2004); Oluah,*et al*. (2020); Yang *et al*. (2021)
	Serum enzymes	Reduced	Py	
	Serum immunoglobulin IgM	Reduced in serum and spleen	OP, Py	
	Bactericidal activity/lysozyme activity	Decreased in plasma	OP, Py	
	C3 complement protein	Increased in serum & Kidney	OP	
	Phagocytic index	Reduced	Py	
Histopathological	Hepatocytes	Showing hypertrophy, Diffuse necrosis, pyknosis, cordal disarrangement, Individualization	OC, OP, Py, Ca	Bharti and Rasool (2021); Maurya *et al*. (2019); Sarkar *et al*. (2005); Ullah *et al*. (2015); Xing *et al*. (2012)
	Tubular cell of kidney	Necrosis	OC, OP, Py, Ca	
	Intestine	Lesions, infiltration of eosinophils into the lamina propria & atrophy of epithelial cells	OC, OP, Py, Ca	
	Gill tissue	fusion of primary lamellae, congestion of blood vessels and hyperplasia of branchial plates	OC, OP, Py, Ca, Tr	
Behavioural	Swimming behaviour during the exposure	Irregular, erratic and darting swimming movements; Restlessness; arena movements; loss of equilibrium; sinking to the bottom; increased opercular activities strong spasm; paralysis and sudden quick movements	OC, OP, Py, Ca, Tr	Marigoudar *et al*. (2009); Mutshekwa *et al*. (2022); Nwani *et al*. (2013); Patil and David, (2008); Sharma (2019)

mediated and adaptive branches. In fish, all these branches of the immune system work together to protect non-self matter. Neutrophils and macrophages are important cells involved in the first line of defence in fish (Ellis *et al*., 1976).

Pesticides released into the aquatic system modulate the immune system of fish in different ways. Several studies reported that pesticide exposure in fish leads to immunotoxicity which is in most cases represented by the change in phagocytic and lysozyme activity. Immunotoxicity in fish can also be monitored by other parameters like immunoglobulin concentration, complement protein C3 level and cytokine production (Li *et al*., 2013; Yang *et al*., 2021). Pesticides like organophosphates act on the nervous system by inhibiting acetylcholine esterase as a result of which the level of acetylcholine increases in the synaptic cleft. This acetylcholine can regulate the immune function of fish. Organophosphates can also inhibit serine hydrolase enzymes of the complement system, which directly modulate the immune function of fish (Díaz-Resendiz *et al*., 2015).

Histopathological parameter:

Histological investigation of different tissues of pesticide-exposed fish is a useful tool in monitoring pesticide effects. Organs which are highly affected by pesticide exposure are the gills, liver, kidney, intestine, spleen and gonads. ROS produced during the biotransformation process is the main causative agent of tissue damage.

Gills are important organs of fish because they are involved in many biological mechanisms of fish. These are respiration, ion exchange, and excretion of waste products (Zayed and Mohamed, 2004). Epithelial and mucosa cell hyperplasia and fusion of secondary lamellae are the major alterations in fish gills caused by pesticides. When gill is damaged by pesticide encounter, it is easily detected by simple histological techniques.

Concerning unexposed fish, the liver of exposed fish shows different signs of damage. The major ones are dilation of sinusoids, hypertrophied hepatocytes, vacuolization in cytoplasmic content of liver cells, liver swelling with pyknotic nuclei, liver necrosis, congestion of RBCs into the hepatic portal vein (Ortiz *et al*., 2002; Maurya *et al*., 2019).

Several reports documented that fish encountered with different pesticides show some common damage signs in their affected kidney. These include necrosis, degeneration of glomerulus, dialataion of glomerular capillaries, vacuolated cytoplasm, hypertrophied epithelial cells of renal tubules and narrowing of the tubular lumen (Banaee, 2013; Sharma, 2019).

Behavioural parameter:

The behaviour indicates a unified response of fish following complex physiological and biochemical changes. Pesticide-induced behavioural changes point out their effects on internal homeostasis. The behavioural terminus may thus be used as a sensitive indicator of the toxic effects of the pesticide. The common behavioural responses of fish which have drawn the attention of researchers are locomotion and swimming pattern, feeding behaviour, body colour change, foraging behaviour, prey-predator relationship, aggression, territoriality, attraction avoidance, mucous secretion and learning (Sharma, 2019).

Several studies reported that in response to pesticide exposure food intake of fish decreases (Parveen and Javed, 2010). Decreased food intake ultimately affects the growth and reproduction of the fish. Food intake is influenced by swimming and the avoidance behaviour of fish.

Erratic, irregular swimming patterns are observed in fishes after a certain period of exposure to pesticides. Swimming pattern change resulted from the accumulation of acetylcholine at the synaptic cleft due to the inhibition of acetylcholine esterase enzyme by a certain class of pesticides (Rao *et al*., 2005). Increased mucous secretion is an adaptive response of fish to reduce the irritation caused by pesticides. Opercular movement decreases to prevent poison entry through the gills. Slowly the fish becomes lethargic, hyperexcited, and restless. Most of the time the final response is the sinking of the fish at the bottom of the water and death with mouth wide open.

Conclusion

Chemical pesticides are often considered a quick, easy and inexpensive solution for controlling pests in urban landscapes. However, the use of chemical pesticides comes at a significant cost. In most of the cases pesticide not only damages a target organism but also affects several non-target organisms and causes environmental pollution. The best way to reduce pesticide contamination in our environment is for all of us to do our part to use safer, non-chemical pest control (including weed control) methods.

Assessment of pesticide effects on different biological parameters of fish provides fruitful information about the toxic levels of the pesticide used in the agricultural field. This information could use to ban the further application of that specific synthetic chemical pesticide(s) in the agricultural fields and encourage the production of less toxic, eco-friendly, species-specific and less persistent environmental pesticides for future use in pest control.

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