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A Review on Toxicity of Pesticides in Fish: Histological, Heamatological, Behavioural and Biochemical Alterations

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A B S T R A C T

In the integrated rice and fish farming, fish growing in paddy fields by using the same area without impacting of rice quality and yield. One of the main disadvantage of paddy cum fish culture is the extensive use of pesticide in paddy field for eradication of different pest and these are toxic to fish. The extensive use of pesticides has been criticized in recent years due to their persistence in the tissue of living organisms.Even though effects of pesticides on mortality of fishes have been studied by many workers.Fish are ideal sentinels for behavioural assays of various stressors and toxic chemical exposure due to theirconstant, direct contact with the aquatic environment.Observations made after pesticide exposure indicate unusual behaviour such as cough, yawn, fin flickering, threat, nudge and nip were also increased due to pesticide poisoning. The haematological response of fish to the exposure pesticide showed a significant decrease ($p<0.001$) of erythrocyte (RBC), haemoglobin (Hb), hematocrite (Ht), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC) values compared to the control group. The studiessuggests that exposure to pesticide at low concentration results in histological alterations in liver, gill and kidney. Pesticide exposure leads to changes in the liver tissue of the fish. The noticed changes include degeneration of cytoplasm in hepatocytes, formation of vacuoles and necrosis. Alterations in gill include hyperplasia of the epithelial lining of secondary lamellae, necrosis, shortening of the secondary lamella, aneurism and fusion of several secondary lamellae. In kidney, disintegration of the convoluted tubules, necrosis, shrinkage of the glomerulus and increased space within the Bowman's capsule were observed due to pesticide poisoning. The study suggests that exposure to pesticide at low concentration results in significant biochemical alterations. The biochemical response of fishes to the exposure to sub lethal concentrations of pesticide showed a significant decrease ($p<0.001$) of cholesterol, proteins and glycogen in muscle and liver tissues compared to the control group.

Keywords: Pesticides, Fish, Heamatological Behavioural,Toxicity

1. Introduction

Increased demand for food and fibre has lead to indiscriminate use of chemicals in agriculture and has reached on such a stage that modern agriculture is heavily dependent on high yielding varieties, which can only be grown under the influence of fertilizers and pesticides. There are many types of pests and not surprisingly, many types of pesticides developed to fight them. Three well known categories are; polychlorinated insecticides like lindane, aldrin and hepatochlor, Organophosphate insecticides like parathion, malathion, and the carbamate insecticides. Among these, organochlorines are very persistent in the environment and tend to accumulate in biological and non biological materials. Chronically the organochlorine insecticides have more toxic potential than organophosphorous and carbamate (Prestt, 1967).

Integrated farming is farming system with simultaneous activities involving crop and animals. The main purpose of integrated farming is farming components support one another; hence reducing external inputs. For instance, the crop can provide as animal feed, and the livestock as fertilizer for the crop. Some livestock can act as weed control by foraging on the weeds.

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Integrated farming provides the option for getting extra income along with main crop. Rice and fish farming widely practiced in South East countries when compared to Western countries. One of the main disadvantages of paddy cum fish culture is the extensive use of pesticide in paddy field for eradication of different pest and these are toxic to fish. The extensive use of insecticides has been criticized in recent years due to their persistence in the tissue of living organisms. Nowadays, more than 1400 different pesticide formulations are used, mainly in agriculture. Due to the persistency of pesticides, it enters in living organisms present in paddy field and negatively affect them. This may leads to bioaccumulation, bioconcentration and biomagnifications.

Numerous reviews have traced these advancements in the field of Behavioural toxicology (Gray, 1990; Little and Finger, 1990; Doving, 1991). However, the recognition of behavioural toxicology as an important tool in aquatic toxicology is most clearly seen in the acceptance of behavioural endpoints in federal regulations.Behaviour is the product of interaction of an organism with its external environment and represents the integration and underlying physiological process and mechanisms. Behavioural plasticity is the ability of a single genotype to produce more than one alternative, potentially adaptive behaviour in response to environmental conditions. An organisms ability to alter its behavioural response to contaminant induced changes, whether internal or environmental, may significantly affect its chances for survival or reproduction.

Behavioural toxicology is a tool for hazard assessment in water pollution (Hara *et al.,* 1976). Behavioural changes in animals are indicative of internal disturbances of body functions such as inhibition of enzyme functions (Cearley, 1971), impairment in neural transmission (Veena*et al*., 1997), and disturbances in metabolic pathways (Das and Banergee, 1980). The development of response criteria in animals varies from detailed physiological measurements to whole animal response, especially preference or avoidance behaviour (Hartwell *et al*., 1989).

Fishes are susceptible to any change that may occur in the environment, it is exposed that these changes would be reflected in the physiology of the fish, particularly in the values of haematological parameters and haematology has been used as an index of health status of a number of fish species (Blaxhall, 1972). Since blood forms a unique compart between the external and internal environments, agents including pesticides that cause stress in fish can alter the composition of the blood. Hence, the study of the haematological parameters of the fish responsive to pesticide stress is useful tools for monitoring the level of pollutants in the environment.

Blood takes part directly or indirectly in almost all activities of fish and that it can be a good indicator of stress conditions. The use of haematological parameters as indicators of sub-lethal stress can provide information on the physiological responses that the fish make to a changing environment. This is the result of close association of the circulatory system with the external environment and with the tissues. Since haematological tests have been an important diagnostic tool in medicine for many years, it is speculated that they may be an equally valuable indicator of stress or disease condition of fish (Larsson, 1975).

Haematological changes have been detected in responses to disease, pollutants, surgical procedures, hypoxia (Eisler and Edmunds, 1966). Blood alterations or damage to the haemopoietic organs in these organisms may also be associated with pathological conditions related to waterborne pollutants. One of the important functions of blood is the transportation of oxygen and carbon dioxide in the body. The immature RBC is uniquely concerned with the synthesis of haemoglobin when the maturation is complete. RBC functions in the transport of haemoglobin.

The haemoglobin present in the RBC enables the blood to carry adequate amount of gases to different tissues as the capacity of the haemoglobin to carry these gases is very high. Hence, an estimation of haemoglobin in the blood provides us information about the physiological state of the body. The increase or decrease of haemoglobin content and RBC, variations in the packed cell volume, or haematocrite, Mean Cell Haemoglobin Concentration etc, indirectly indicates the oxygen carrying capacity of the blood. Alternations in the haematological parameters can be due to factors like retention of metabolites, metabolic problems, and oxidation of haemoglobin, increased or decreased erythropoiesis, hemodilution or hemoconcentrations.

Histopathological alterations can be used as an indicator for the effects of various anthropogenic pollutants on organisms and are a reflection of the overall health of the entire population in the ecosystem. These histopathological biomarkers are closely related to other biomarkers of stress since may pollutants have to undergo metabolic activation in order to be able to provoke cellular change in the affected organism. The mechanism of action of several xenobiotics could initiate the formation of a specific enzyme in the kidney and causes changes in the metabolism, further leading to cellular intoxication and death, at cellular level, whereas this manifests as necrosis, ie. histopathological biomarkers on a tissue level.

The respiratory system of fish seems to be the prime target of many pollutants. When tissue of the animal do not receive sufficient oxygen they must either reduce the overall energy demand or respire anaerobically. Since glycogen is the ready source of energy even in aerobic condition, the depletion of glycogen from the tissue is exposed to be an immediate manifestation of hypoxia. During severe hypoxia, flounder reduces its oxygen consumption and partially compensates by increasing anaerobic energy metabolism based on fermentation of glycogen and glucose with lactic acid as the major anaerobic end product (Jorgensen and Mustafa, 1980).

2. Behavioural Alterations during Pesticide Exposure

Dembele*et al*. (2000) study of cabofuran and dizinon in *Cyprinuscarpio* shows significant depression in the level ofAChE in brain. Exposure of fish to different levels of pesticides caused severe abnormalities in their behaviour, such as disequilibrium in swimming and retardation in opercula movement The test animals exhibited abnormal behaviour earlier at higher than lower concentrations. With all insecticides tested, test animals died rapidly when exposed to the highest concentration. This level of depression induced by chronic exposure can be used as biomarker to predict agrochemical pollution.

Sarikaya*et al*. (2003) LC_{50} value of 2, 4-D herbicide on the common carp determined as 66 mg/l. The necropsy of carp subjected to different concentrations of 2, 4-D revealed that they had widespread hemorrage in their excretory and digestive systems and enlargement in the livers. The behavioural abnormalities in C.*carpio* subjected to various concentrations of 2,4-D are the anxiety, sudden jerks, loss of balance, upside down or vertical manner, respiratory difficulties, excessive mucosal secretions and lightening in body colour.

Omitoyin*et al.* (2006) behavioural changes reported in*Clariasgariepinus* such as uncoordinated movements, convulsion, excess secretion of mucus, erratic swimming and increase in operculum ventilation, respiratory distress, paralysis, darkening of body during the exposure to lindane. The liver of the control fish showed no significant lesion. In experimental fish, there was moderate change in the liver focal lymphocyte and infiltration seen in portal areas of the liver. As the concentration of the lindane increased, there was more degeneration of portal cells.

Marigoudar*et al*. (2009) study on the effect of cypermethrin in *Labeorohita*shows that they migrated immediately to the bottom of the tank. The schooling behaviour was observed to be disrupted in the first day itself and the fish occupied twice the area than that of the control group. Respiratory disruption was observed in the normal ventilating cycle with a more rapid, repeated opening and closing of the mouth and opercular coverings.

Parithabhanu (2013) different pesticides negatively affect the behaviour of *Oreochromismossambicus.* The mortality of the fish under the toxic stress of pesticide is dose and duration dependent. The fish were restlessness showing rapid movement and fast engulfment of air with faster opercular movement along with surfacing activity. Erratic swimming, difficulty in respiration, loss of balance and jerking movements also noticed.

Vel*et al*. (2013 a) The LC50 values obtained at 24, 48, 72 and 96 hours exposure in freshwater fish *Labeorohita*shows that lambda cyhalothrin showed higher toxicity than monocrotophos. The body colour changed from original silvery white to dark colour in pesticide treated fish. Erratic swimming, abnormal posture, disbalance are also observed in pesticide exposed fishes.

Siddiqa*et al*. (2016) the result of toxicity of organophosphorus insecticide on local fish species of Bangladesh showed physiological responses like rapid opercular movement, hyper secretion of mucus and frequent gulping of air. This unusual behavior may be due to obstructed functions of neurotransmitters.

3. Histological Alterations during Pesticide Exposure

Juan *et al*. (2003) contamination with lindane in *Mugil sp.* and *Cyprinuscarpio* produce some degenerative change in gills, dilation of blood capillaries ,hyperplasia of all epithelial lining of the secondary lamellae , shortening of the secondary lamella, abnormal raising or swelling of the epithelium and fusion of the secondary lamellae. In liver, the hepatic cells appeared compactly arranged with a strong cytoplasmic vacuolisation and an increased basophilia within the cytoplasm of some hepatocytes, which had an eccentric and pyknotic nucleus. Hepatocellular necrosis with parenchymal vacuolisation, hypertrophy of hepatocyts, haemorrhages and widening of blood sinusoids were also observed. In lindane polluted fish, the kidney showed a disintegration of the convoluted tubules, and large intra cytoplasmic vacuoles in the epithelial cells of these tubules. Shrinkage of the glomerulus and increased space within the Bowman's capsule were also observed.

Munteanu*et al*. (2005) specific activity of catalase after 96h exposure of lindane in *Carassiusauratusgibelio* was higher than the control in liver, kidney and gill. The specific activity of glucose-6-phosphate dehydrogenase increased following exposure to the pesticide for 96h in liver and kidney. The specific activity of glutathione reductaseand glutathione s-transferase after 96h exposure of fish to lindane decreased very significantly in liver, kidney and gill. Individuals treated with pesticide showed a significant decrease in liver and a significant increase in kidney and gill for the glutathione peroxidase specific activity.

Vel*et al.* (2013 b) Glycogen content was reduced in liver of lamdacyhalothrin exposed *Oreochromismossambicus* with a concurrent increase in blood glucose concentrations. Marked toxic effects were observed at the structural and cellular level in the liver. In 10 days exposed fish, disintegration of cell boundaries and slight dilation of blood sinusoid was observed. After 20 days, many hepatic cells were completely damaged. Intracellular vacuolation was also apparent. After 30 days, damage to the hepatocytes was prominent. In most of the hepatic cells the integrity of cell was completely lost.

Sachar*et al*. (2013) histopathological alterations induced by lindane in a minor carp, *Aspidopariamorar* showed that many alterations in liver tissue including widening of blood sinusoids, necrosis, vacuolation and degenerative process in cellular architechture. Necrosis of kidney, tubular vacuolation, and tubular degeneration was observed in kidney in exposed fishes. Experimental fishes shows deposition of haemosiderin pigment, necrosis, vacuolisation, inflammation and total degeneration in the splenic tissue.

Reddy *et al.* (2015 b).The total glycogen and protein levels decreased in all the tissues of confidor exposed *Labeorohita*. In liver, a vital organ of carbohydrate metabolism was drastically affected by confidor. Glycogen level decreased more in liver and protein level decreased more in gill and muscle. The reduced levels of protein can be due to reduction in protein synthesis and the liver cirrhosis. The results shows that the shortened and clubbing of the ends of the secondary gill lamellae, fusion of adjacent secondary gill lamellae and necrosis in the primary gill lamellae. Pathological changes observed in the liver include change in the shape and size of the hepatocytes, rupture and degeneration of hepatic cells, formation of more number of vacuoles. Changes in the kidney include vacuolization, degeneration of cell membrane, damage of haemopoietic tissue and renal tubules and hypertrophy of nuclei.

Patel *et al*. (2016) kidney of imidacloprid and cruzateexposed*Oreochromismossambicus*and *Labeorohita*shows necrosis of tubular epithelial cell, thickening of bowman'scapsule and shrinkage of the glomeruli along with severe degenerative and necrotic changes in the renal tubules with focal areas of necrosis and hemorrhage, haemolysis. The histological changes in gills include curling of secondary lamellae followed by disorganization, rupture in the secondary lamellae, hemorrhage at primary lamellae and bulging at the tip of primary filament.

4. Biochemical Alterations during Pesticide Exposure

Al-Kahtani (2011) Rate of oxygen consumption was declined during all the abamectin exposure in*Oreochromisniloticus*. And significant decrease in protein, carbohydrate, cholesterol can be observed.

Srivastav*et al*. (2012) chloropyrifosexposure in freshwater catfish *Heteropneustesfossilis*showshypocalcemia, which caused hyperactivity of prolactin cells. So exposed fish may have disturbed physiological functions including reproduction and sperm motility.

Xing *et al. (*2012) exposure to atrazine and chlorpyrifos in *Cyprinuscarpio*leads to decrease in catalase, glutathione peroxidise and superoxide dismutase activities in liver and gill tissues in dose dependent manner. Atrazine and chlorpyrifos exposure caused increase in the malondialdehyde content in liver and gill tissues. Liver tissue of common carp revealed different degree of hydropic degeneration, vacuolisation, pyknotic nuclei, and fatty infiltration. Gills of common carp displayed varied degrees of epithelial hypertrophy, telangiectasis, oedema with epithelial separation from basement membranes and general necrosis.

Priya*et al.* (2012) imidacloprid exposure in *Channapunctatus*shows significant increase in serum glucose, cholesterol, creatinine, and creatin. On the other hand the serum protein, albumin, and globulin have been decreased significantly after imidacloprid treatment with different concentrations.

Priya and Maruthi (2013) study on imidacloprid toxicity of biochemical constituents in liver tissue of fresh water teleost *Channapunctatus*revealedthat the liver tissue showed significant depletion of glycogen, protein and lipids and significant increase of cholesterol.

Binukumari and Sudha (2013) pesticide endosulfan 35% EC exposure in fresh water fish, *catlacatla* showed that the decreased in cholesterol content. The reduced cholesterol level may due to the inhibition of cholesterol biosynthesis in the liver or due to reduced absorption of dietary cholesterol.

Olaganathan and Patterson (2013) increase in protein and lipid contents during 7^{th} day with a marked decline on 18^{th} and 30^{th} day exposure of anthrequinon dyes in *Channapunctatus* and *Cyprinuscarpio*was reported. Carbohydrate content decreased significantly in both the fish species exposed to the sublethal concentration of the dyes.

Satyavardhan (2013) fenvalarate and malathionexposure of freshwater fish, *Ctenopharyngodonidella* shows the depletion on biochemical parameters like protein, glycogen and free amino acid. Fenvelarate is exhibiting more toxic than the melathion.

Kumar and Ali (2013) an experiment conducted to examine the toxic impacts of two organophosphorus pesticides on the acetylcholinesterase activity and biochemical composition of freshwater fairy shrimp *Streptocephalusdichotomus.* The AChE activity was found to decrease in shrimps exposed to sub lethal concentrations of malathion and glyphosate. The decrease was higher in malathion exposed test shrimps than that of the glyophosate. In this study, the reduction of protein recorded may be due to proteolysis and increased metabolism under toxicant stress. The decrease in carbohydrate content indicates the fact that it was extremely utilized due to energy demand occurred in test shrimp exposed to pesticides. The present investigation showed that the lipid content decreased significantly which indicates its necessity to meet out required high energy demand due to pesticides toxicity in test shrimp.

Lakshmanan*et al*. (2013) experiment on impact of dichlorvos on tissue glycogen and protein content in freshwater fingerlings, *Oreochromismossambicus* shows decreasing trend of glycogen, protein, and albumin content in liver, kidney and muscle. Magar and Dubi (2013) study on the fresh water fish *Channapunctatus* exposed to sublethal concentration of malathion showed decrease in the level of protein, glycogen and lipid content in cardiac muscle of treated group. In treated fishes cardiac muscle showed congestion and atrophy. In some area cardiac muscle showed haemorrhage and haemolysis along with aggregation of inflammatory cells.

Verma*et al*. (2014) studies on influence of endocel and rogor on serum free amino acid and total protein level in *Clariasbatrachus* shows the presence of 5 amino acids (glutamic acid, serine, tyrosine, methionine and glycine) and decline of protein content.

Wani*et al*. (2014) Depletion of protein occurs after pesticidal exposure shows greater tendency for accumulation of pesticide polo in the body of fresh water fish *Labeorohita.*

5. Haematological Alterations During Pesticide Exposure

Tilak*et al*. (2005) phenol exposedIndian major carps *Catlacatla, Labeorohita*and*Cirrhinusmrigala* and shows that the decrease in total erythrocyte count, haemoglobin concentration, packed cell volume and an increase in blood sugar concentration. Velisek*et al*. (2009) the values recorded for RBC, Hb, leukocyte count is high in common carp with bifenthrin exposure when compared to control group. Histopathological examination revealed severe telangiectasia in the secondary lamellae of gills, with the rupture of pillar cells. Degeneration of hepatocytes especially in the periportal zones was observed. Affected hepatocytes showed pycnotic nuclei and many small or single large vacuoles in the cytoplasm.

Binukumari and Vasanthi (2013) an experiment conducted to examine the effect ofdimethoate on the haematological parameters of fresh water fish, *Labeorohita.* All haematological parameters except WBC and differential count of eosinophil and lymphocyte were decreased from the control. The WBC and lymphocytes were increased in all the dimethoate exposures.

Jeyapriya*et al*. (2013) studies on acute and subchronic effect of monocrotophos on *Catlacatla* shows significant decrease in the total erythrocyte count, packed cell volume, haemoglobin, mean corpuscular haemoglobin, mean corpuscular haemoglobinconcentration counts and significant increase in mean corpuscular value count. In differential cell count, the percentage of neutrophil and lymphocytes increases and the percentage of eosinophil and basophil decreased. No changes were observed in monocytes.

Haider and Rauf (2014) fish *Cirrhinusmrigala* exposed to diazinon showed a significant decrease in RBCs, haemoglobin, hematocrite, mean corpuscular value and mean corpuscular haemoglobin. The total WBC count of exposed fish showed a delayed decrease. Result of plasma biochemical profile indicated a significant decrease in plasma levels of protein, albumin, globulin, and plasma activity of AChE in diazinon exposed fish.

Sachar and Raina (2014) Haematological parameters related to oxygen transport (RBC, Hb and Hct), defence mechanisms (WBC) and the calculated indices (MCV, MCH and MCHC) all exhibited marked differences between control and experimental groups in response to insecticide lindane in *Aspidopariamorar*.

6. Conclusion

The behavioural changes in fishesunderpesticide exposure shows behavioral changes include cough, yawn, fin-flickering, partial jerk, S-jerk, burst swimming, nudge, threat and nip. Histology serves as an important tool in studying the pesticide toxicity and the changes observed in tissues of the organ. Pesticide exposure effect on gills, liver and kidney of the fish. Gills of the treated fishes showed proliferation of lamellar epithelium. There occurred a reduction in the total respiratory surface of gills. Liver also showed loss of architecture as the exposure progressed. During earlier phases of exposure, mild necrosis was observed in the hepatocyte tissues, as exposure progressed, vacuolization of hepatocytes and constrictions of blood sinusoids were seen. All these changes resulted in the total loss of normal architecture of liver tissue. The changes in the kidney were more evident in the renal tubules than in Bowmann's capsule. There occurred a constriction of tubular lumen. Tubular epithelial cells were found to be detached from the basement membrane.Tissue level protein and glycogen showed a significant reduction when the fishes were treated with sub lethal concentrations of pesticide. Reduction was more evident in muscle tissues.Haematology serves as an important tool in studying the pesticide toxicity and the changes observed in haematological parameters. Decline in RBC and WBC was followed by a reduction in the Haemoglobin concentration (Hb%). Reduction as also noticed for the haematocrite value. The Erythrocyte indices such as MCV, MCH and MCHC were also showed a reduction in their values. These changes were found to be time dependent.

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