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**REVIEW ARTICLE** **OPEN ACCESS**

# Haematological, Biochemical and Genomic impacts of Pesticideson freshwater air breathing fishes: A Review

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**ABSTRACT**
*Use of synthetic pesticides to enhance crop production has increased tremendously in agricultural sector. Various pesticides such as insecticides, fungicides and herbicides are currently used based upon their mode of action. Residues of these pesticides can reach nearby water bodies through surface run off from agricultural areas. These residues can enter the body of aquatic life forms, especially fishes. The release of these pesticides into aquatic ecosystem may have profound toxic effect on aquatic organisms. The present review emphasized the haematological, biochemical and genomic impact of pesticides on certain air breathing fishes namely.Anabas testudineus, Clarias species and Channa species.Pesticides induced a significant reduction in haematological parameters such ashaemoglobin,red blood cell and packed cell volume, while white blood cellvalues showed a significant increase. Glucose,urea and creatinine valueswere increased in the experimental group while a opposite tendency was noted in the level of glycogen. Biochemical parameters such as total protein, alanine transaminase, aspartate transaminase andalkaline phosphatase exhibited a significant alterationwith pesticide under exposure. Induction of micronucleus frequency and other nuclear abnormalities were observed in the erythrocytes of exposed fishes. This finding revealed the genotoxicity induced by pesticides in fishes. The haematological, biochemical and genomic assessment revealed the severe situation exerted by pesticides on fishes. Application of safe and environment relevant concentration of pesticides in agriculture can lessen the impact on aquatic ecosystems. Proper guidance should be provided to minimize the usage of synthetic pesticides on crop production.*
*Keywords:Pesticides, Haematological, Biochemical, Genomic, Anabas testudineus, Clarias, Channa*

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## INTRODUCTION

Rapid industrialization and urbanization along with an increase in overall population paved the way for the utilization of natural resources. Increase in population resulted in contamination of our natural resources such as air, water, soil etc. Various anthropogenic activities, for example, agricultural,industrial and domestic activities resulted in contamination of our natural freshwater resources [1].Nowadays the prime objective of any country is to increase their agricultural production in order to meet the demands of their growing population. Agricultural production can be increased only through the use of various agrochemicals such as pesticides.

Pesticides are substances used to eradicate the growth of pests. Pesticides are categorized as insecticides, fungicides and herbicides based upon their mode of action. Use of pesticides showed a rapid rise in crop production and yield. These chemicals enter the environment through various routes such as spraying activities, soil seepage and water contamination [2] and found to be detrimental to human health and environment. They also have a chance to leach out into nearby water bodies from their point of action. Changes in the physicochemical parameters of aquatic sources influence the metabolism and homeostasis of aquatic life and in turn cause disturbance in food web [3].Residues of these pesticides can affect the aquatic ecosystem to a great extent. These residues enter the body of aquatic organisms through various mechanisms such as oral, dermal and inhalation.

Fishes serve as a major biomarker of aquatic ecosystem. Bioaccumulations of these pesticides threat the long-term survival of fishes by disrupting the ecological relationships between organisms and loss of biodiversity. Indiscriminate use of pesticides may have impacts on non-target organisms especially aquatic forms which can ultimately pose a serious threat to the health of human communities [3]. Long-term exposure of pesticides induces various abnormalities such as physiological, behavioral,

histopathological, haematological, biochemical, immunosuppression, hormone disruption, diminished intelligence, reproductive abnormalities and cancer [4]. In most cases, pesticide related fish death goes unreported and in documented cases, number often underestimated. Long term exposure of fish to pesticides in turn resulted in continuous health hazard to the human population[5].

Fish breathe air while either in water (aquatic air breathers) or on land (amphibious air breathers). Some fishes use air as an auxiliary respiratory mode to cope up with harsh environmental conditions.Air breathing helps them to survive in habitats where aquatic respiration is not possible [6]. The present review analyzed the haematological, biochemical, histopathological and genomic impact of pesticides on certain air breathing fishes, namely, *Anabas testudineus,* Clarias species and Channa species.

## HAEMATOLOGICAL PARAMETERS

Pesticides induced severe abnormalities in haematological parameters including haemoglobin, RBC, WBC and packed cell volume. Different studies conducted so far put forth a reduction in haemoglobin, red blood cell count and packed cell volume. Haematological parameters can be considered as diagnostic guides of the pathological conditions and also serve as a sensitive tool for evaluating the toxic effects of many pesticides [7].Dahegaonkar *et al*.,[8] stated that red blood cell count, haemoglobin content and packed cell volume exhibited significant decrease in *Clarias gariepinus* treated with Cypermethrin.Oluah *et al*.,[9]also reported a significant reduction in erythrocyte count, hemoglobin concentration and packed cell volume in *Clariasgariepinus* intoxicated with herbicide Ronster. Similar to this finding, a significant decrease in number of RBC, Hb and PCV values was noted in *Clariasgariepinus*[10]and *Heteropneusteus fossilis*[11]when exposed to Paraquat dichloride.

Chlorpyrifos[12] and Monocrotophos [13] affected thehaemoglobin, RBC count and PCV value in blood of *Anabas testudineus*.Yadav [14] observed a reduction in haemoglobin content in organophosphate exposed *Channa punctatus*. Treatment of *Clarias*gariepinuswith Atrazine [15], Glyphosate based herbicide Glycot[16],Phostoxin[17], Oxyfluorfen[18], Chlorpyrifos [7] and insecticide Cyperdicot[19]also disturbed the RBC, haemoglobin and packed cell volume. Similar reduction was noted when *Channa striatus was* treated with Endosulphan[20] and *Channa punctatus* with Sumithion[21], Furadan [22] and Deltamethrin [23].Reduction in Hb in turn reduced the capacity of blood to transport oxygen to tissues. Hb reduction invariably contributes to the stress and anaemic state of organisms which further alters respiration, metabolism and triggers morbidity and death. Significant reduction in RBC may be due to the disruptive action of pesticides on the erythropoietic tissue. Destruction of RBC in turn reduced hemoglobin and packed cell volume in fishes.

Increase in WBC can be considered as an adaptation of the fish to cope up with toxicant stress. Oluahet al.,[9] reported leukocytosis in Ronster exposed *Clarias* species. Similar results were obtained with Paraquat dichloride in *Clarias* [10]and *Heteropneustes fossilis*[11]. An increase in WBC count was also noted in *Clarias* treated withPhostoxin[17], Glyphosate based herbicide Glycot[18] and Atrazine [15]. Velmurugan et al.,[12] and Ashafali et al., [13] also observed similar results when *Anabas testudineus* was intoxicated with Chlorpyrifos and Monocrotophos. The result of this study is in agreement with the effect of Chlorpyrifos [7]and Cyperdicotin *Clarias* [19]. Similar results were noted by Desmukh [20] when *Channa striatus* was treated with Endosulphan. Increase in WBC content with increase in concentrations may be due to the activation of the animal's defense mechanism and the immune system. Several chemical compounds including insecticides, generate antibodies which interference with the immune system and result in increased WBC count [24].

## BIOCHEMICAL PARAMETERS

Numerous laboratory experiments showed the biochemical effect of various pesticides in air breathing fishes. Sublethal effect of pesticides including Atazine, Clotrimazole, Almix, Dichlorvos, Glyphosate, Paraquat, Carbofuran, Malathion and Atachlor altered enzymatic activities such as alanine transaminase, aspartate transaminase and alkaline phosphatase.Opute and Oboh [25] noted a significant increase in serum alanine transaminase, aspartate transaminase and alkaline phosphatase in *Clariasgariepinus* exposed to Atrazine. Similarly, increase in activities of enzymes were also noted when administered with Clotrimazole [26].Samanta et al.,[27] arrived at similar opinion when *Anabas testudineus*and *Heteroneupsteus fossilis* treated with Almix herbicide. Giri et al.,[28] observed an elevation in alkaline phosphatase activity while studying the impact of Dichlorvos on *Anabas testudineus*. This result is in conformity when *Clariasgariepinus* treated with Glyphosate and Paraquat based herbicides [29]. This study contradicts the works of Rajani et al.,[30] and Harabawy et al., [31] who pointed out adeclinein activity of alkaline phosphatase.It could be considered as an adaptive mechanism of the fish to meet the energy demand aiding anaerobic breakdown of glycogen under pesticide toxicity.

Studies on *Clariasgariepinus* using pesticides such asAtrazine [32], Glyphosate and Paraquat [29]and Carbofuran [31] disturbed various enzymes such as aspartate transaminase, alanine transaminase and alkaline phosphatase. An increase in activity of alanine transaminase with Malathionwas demonstrated by Ahmad [33]. An increase in the activities of the enzyme may bedue to the induction of hepatotoxic effects. The increase in enzyme activity can also be due to damaged tissues and impairment of fish metabolism.Stress condition can also lead to the breakdown of free amino acids to meet the additional energy requirements, which in turn enhanced its activity.

Certain other studies revealed the toxic effects of pesticides on enzymes in the organs and tissues of different fishes. Significant elevation of alanine amino transaminase was determined in liver tissue of Atachlor exposed *Clarias batrachus*[30] and Glyphosate exposed *Clariasgariepinus*[34].Similarly, significant elevation of aspartate amino transaminase was detected in gill of Atachlor treated *Clarias batrachus* [30] and Glyphosate treated *Clariasgariepinus*[34].The highest activity of transaminases observed in gill indicated cellular damage and this may be due to release of these enzymes across the damaged plasma membranes into the serum and/or blood stream [27].Decrease of alanine amino transaminase was noted in muscle tissue of *Clarias*species treated with Atachlor[30] and Deltamethrin [35]. Decreased activities of alanine amino transaminase suggested that inactive transamination and oxidative deamination in the tissues occurred thereby disrupting the metabolic process, which could affect physiological processes in the fish.

Fish use protein to cope up with the stress induced by exposure with various pesticides.Experiments with various pesticides demonstrated a decline in protein content in *Heteropneusteus fossilis, Clariasgariepinus, Clariasbatrachus and Anabas testudineus*. Decline in serum protein was reported in *Heteropneusteus fossilis* treated with Chlorpyrifos[36] and*Clariasgariepinus* with Atrazine [25],Clotrimazole [26],Deltamethrin [37] and Carbofuran [31].Narra*et al*., [38] also reported a reduction in total protein in *Clariasbatrachus* exposed to individual and combined effect of Endosulfan, Carbofuran, Methyl parathion and Cypermethrin. The decrease in total protein may be due to may be due to interruptionof protein synthesis and the interference of the toxicant with protein metabolism. The decrease in total protein could also result from a state of dehydration and utilization of proteins to meet the excess demand due to stress. A significant reduction in muscle protein was noted in *Clariasbatrachus* treated with Atachlor[30] and Deltamethrin treated *Anabas testudineus* [35].The reduction of protein content in muscle tissue reflects poor assimilation of food and low amino acid uptake resulting in reduction of protein synthesis. Depletion of protein fraction in various tissues of the experimental fish may be due to their degradation and possible utilization of degraded products for metabolic purposes [39].

Oghale*et al*.,[40]reported decreased glycogen content in *Clariasgariepinus* intoxicated with Dimethoate.This result is in agreement with that of glycogen content in *Heteropneustes fossilis* exposed to Chlorpyrifos[36], *Clariasbatrachus* to a combination ofEndosulfan, Carbofuran, Methyl parathion and Cypermethrin [38] and*Anabas testudineus* to Deltamethrin and Permethrin[35].Similar study was reported by [33] when *Clariasgariepinus* was treated with Malathion.Such a decrease in glycogen is due to the utilization of carbohydrates to overcome the stress. The stored glycogen is converted to glucose to meet the excess energy required during the stress condition.

Elevated blood glucose condition is an immediate response to overcome the stressful situation. An increase in blood sugar was noted in *Heteropneusteus fossilis* treated with Chlorpyrifos [36].This result is in agreement when *Anabas testudineus* was intoxicated with Dichlorvos [28] and Methyl parathion [41]. Yadav *et al*.,[14] reported an elevated blood sugar in *Channa punctatus*treated with Malathion.Elevated blood glucose condition was noted when *Clariasbatrachus*was exposed to individual and combined effect of Endosulfan, Carbofuran, Methyl parathion and Cypermethrin[38].Similar results were obtained when *Clariasgariepinus* was exposed with Deltamethrin[37], Carbofuran[31] and Lambda cyhalothrin [42]. The elevation in blood sugar may be due to the excess energy needed to overcome the stress caused by pesticides. The stress condition force fish brain to release excess amount of catecholamines and corticosteroid hormones, which induced the conversion of stored glycogen to glucose.

Pesticides induced significant changes in level of serum creatinine and urea content in fishes. Dichlorvos increased creatinine and urea content in serum of *Anabas testudineus*[28].Higher urea levels can occur in liver impairment, infections and impaired kidney functions. Higher urea contentmay be due to gill dysfunction which led to disturbance in diffusion ofurea between blood and water. Thoker *et al*.,[43]reported increased urea content in blood ofMalathion treated *Channa*, which resulted fromthe catabolisation of proteins and aminoacids. Similar findings were put forward by [31] when *Clarias gariepinus* was intoxicated with Carbofuran. Higher creatinine level was observed when *Clarias batrachus* was treated with Endosulfan, Carbofuran, Methyl parathion and Cypermethrin [38]. Elevated creatinine content resulted from glomerular insufficiency due to renal injury causing tubular cell necrosis and increased muscle tissue catabolism.

## GENOTOXIC ASSESSMENT

Genotoxic assay was performed by inspecting DNA damage in cells exposed to various toxic substances. DNA damage can be single and double strand breaks, cross-linking, loss of excision repair, alkalilabile sites, point mutations, and structural and numerical chromosomal aberrations.Many techniques including Ames Assay, *in vitro* and *in vivo* Toxicology Tests and Comet Assay, Micro-Nuclei test have been used to assess the genotoxic potential of chemicals[44]. DNA damage was determined using comet assay and nuclear abnormalities using micronucleus test.

Action of any genotoxic chemical may give rise to an increase in micronucleus frequency. This finding was reported by Ayanda *et al*.,[45] when *Clariasgariepinus*was exposed to acute concentration of Glyphosate and Paraquat for 96 hours. Toxicant induced micronuclei formation and other cell abnormalities such as apoptosis, bean shaped cell and lobed nuclei in fish which was concentration dependent. Lobednucleus is a reflection of abnormal cell division and bean-shaped blood cells seen as alterations in which the outlines of these cells have undergone necrosis. Trivedi et al., [46] assessed the genotoxic potential of Phorate on *Channa punctatus* and also observed the formation of micronuclei along with notched nuclei, curved nuclei, blebed nuclei, kidney shaped nuclei, V shaped nuclei, nuclear buds, nucleoplasmic bridges, dumbbell nuclei and condensed /rounded nuclei in a concentration dependent manner.

An increase in micronuclei frequency was also noted in peripheral erythrocyte of *Channa punctatus* with increasing concentrations of carbamate insecticide, Carbaryl[47]. This is in accordance with the findings of Amaeze et al., [48] when *Clarias gariepinus* treated with Abamectin, Carbofuran, Chlorpyrifos, Cypermethrin, Deltamethrin, Dichlorvos, Dimethoate, Fipronil, Lambda-Cyhalothrin and Paraquat. Studies on sublethal concentration of Lambda Cyhalothrin in *Clarias gariepinus* for 28 days also revealed the formation of micronucleus, lobed, binucleated and blebed nucleus [49]. Erythrocyte nuclear abnormalities were demonstrated in *Anabas testudineus* and *Heteropneusteus fossilis* treated with Almix herbicide [50]. Changes in the morphology of the nucleus are a product of exposure to xenobiotic contaminants, originating from a genotoxic event.

Increased micronucleus frequency in Pendimethalin exposed *Clarias batrachus* was due to breaks in one or in both strand of DNA, DNA adducts formations and DNA–DNA and DNA–protein cross links[51].Induction of micronucleus frequency was noted when *Clarias batrachus* was exposed to Bispyribac Sodium [52] and *Clariasgariepinus* treated with Dichlorvos and Paraquat [53],Chlorpyrifos based Termicot[54] and Glyphosate based Herbicide Glycot[18].Exposure to Bioallethrin also induced the formation of roundish micronuclei in erythrocytes of *Channa punctatus*[55]. Erythrocytes of *Clariasgariepinus* exhibited elevated micronucleus frequency when exposed with different pesticides such as mixture of Endosulphan and Deltametrin[56],Fenthion [57]and Act Force Gold®, Butaforce®, and Atraforce [58].

Pandey *et al*., [59] demonstrated that Profenfos exposed erythrocyte cells of *Channa punctatus* exhibited significantly higher % tail DNA damage compared to normal erythrocytes. Exposure of *Clarias gariepinu*s to Roundup and Stomp pesticides induced significant DNA damage through increased tail length,tail moment and tail DNA % [60]. Chromosomal aberrations were noticed in Carbaryl exposed *Channa punctatus*[47] and Dichlorvos and Paraquat treated *Clariasgariepinus* [53].The disruptions of mitotic cell division might have led to the formation of different chromosomal aberrations in Chlorpyrifos treated *Clarias gariepinus*, reported by Iyiola*et al*., [61].Different types of aberrations like chromosomal gap, sticky plates, chromatid separation and breaks were observed in the cells of *Channa punctatus* exposed to Bifenthrin[62].

## CONCLUSIONS

The current review meant to provide an awareness about the impact of agrochemicals on certain air breathing fishes. Pesticides induced haematological, biochemical and genomic effects in bimodal breathers such as *Anabas testudineus*, *Clarias* and *Channa*species. Haematological parameters such as RBC, haemoglobin and packed cell volume decreased while WBC count increased in pesticide treated groups. Pesticide resulted in elevated level of glucose, urea and creatinine along with a fall in glycogen content in fishes. Various nuclear abnormalities and chromosomal aberrations were also noticed at the genetic level. So, it is obvious to conclude that usage of pesticides should be reduced for the protection of aquatic fauna. Biopesticides should take the place of those synthetic ones available in the market.More and more research should be carried out to reveal the impact of currently available synthetic pesticides on the environment. Large scale food production must be based on ecological principles and not through intensified use of pesticides.

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**CONFLICT OF INTEREST**

Authors declared there is no conflict of interest.

**AUTHOR'S CONTRIBUTION**

This work was carried out in collaboration between both authors. Shalu Soman, Research scholar collected the literature and drafted the manuscript and Dr. A.U. Arun, Research guide corrected the manuscript. Both authors read and approved the final manuscript.

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