UTTAR PRADESH JOURNAL OF ZOOLOGY

42(19): 137-145, 2021
ISSN: 0256-971X (P)

IMPACTS OF FUNGICIDES ON BIOCHEMICAL, HAEMATOLOGICAL, HISTOPATHOLOGICAL, DEVELOPMENT, REPRODUCTION, OXIDATIVE STRESS AND BEHAVIORAL ASPECTS: A REVIEW

SHALU SOMAN[1*] AND A. U. ARUN[2]

[1]PG and Research Department of Zoology, Nirmala College, Muvattupuzha, Kerala, India.
[2]Department of Zoology, St. Peter's College, Kolenchery, Kerala, India.

AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

Article Information

Editor(s):
(1) Dr. Sirigireddy Sivajothi, Sri Venkateswara Veterinary University, India.
Reviewers:
(1) Muresan Mihaela, Romania.
(2) Luciana Cristina Machado, University of São Paulo, Brazil.

Received: 12 July 2021
Accepted: 18 September 2021
Published: 23 September 2021

Review Article

ABSTRACT

Pesticides, insecticides, herbicides and fungicides are being used by farmers all over the world in order to increase their food production. Apart from agriculture, fungicides also used to treat fungal infections in case of animals. Release of these fungicides from agriculture and related areas can reach nearby water bodies, which in turn can affect aquatic fauna. The present review highlighted the ill effects of fungicides on biochemical, haematological, histopathological, development, reproduction and behavior on different life forms. Exposure to fungicides resulted in an elevated levels of plasma enzymes, serum transaminases and glucose with reduction in level of protein. Red blood cell count, white blood cell count, haemoglobin, packed cell volume, mean corpuscular volume, mean corpuscular haemoglobin concentration and mean corpuscular haemoglobin values were affected. Fungicides resulted in severe abnormalities on gills, liver and kidney of test species. It also affected reproduction and induced various malformations during development. It also led to the formation of reactive oxygen species, which resulted in oxidative stress. Alterations of these parameters can be used as potential biomarkers to evaluate the potential ecotoxicity of fungicide to organisms. Therefore, we should take stringent measures to minimize the use of these fungicides on agriculture and other purposes.

Keywords: Fungicides; azoxystrobin; carbendazim; edifenphos; emisan; propiconazole; tricyclazole.

**Corresponding author: Email: shalusoman33@gmail.com;*

1. INTRODUCTION

Pesticides are used in agriculture to protect crops from damages caused by pests. Due to their widespread use, they are released on a large scale into the environment and in turn contaminate aquatic ecosystems. The extensive use of pesticides, insecticides, herbicides and fungicides is being promoted by Government of India to enhance the crop production to meet the demand of the growing population. Only 0.1 % of the applied pesticides reach the target pests and the remaining 99.9 % find their way to different components of the ecosystem [1].

Fungicides are pesticides intended to inhibit the growth of fungi or fungal spores. Fungicides are used for numerous purposes. In addition to agriculture, fungicides are used in industry to protect products during shipment, suppress mildews that attack painted surfaces, preserve wood, control fungal growth in paper pulps, and protect household carpet and fabric. In veterinary medicine, fungicides are commonly used for antibacterial/antiseptic treatment. Due to these wide variety of applications, humans and other animals most often encounter with these agents in their day to day life [2].

Agricultural run-off near waterbodies lead to the deposition of these pesticides in aquatic ecosystem, which affect the survival of aquatic fauna by disrupting ecological relationship between organisms and loss of biodiversity [3]. Fishes are relatively sensitive to changes in their surrounding environment including an increase in pollution. Fish health may thus reflect, and give a good indication of the health of a specific aquatic ecosystem [4]. The current review emphasized the effect of some major fungicides on biochemical, haematological, histopathological, development, reproduction and behavior on aquatic fauna.

2. ACUTE TOXICITY STUDIES

Cyproconazole exhibited a relatively low toxicity to zebrafish, with a 96-h LC50 value of 90.6 µm (26.4 mg/l) [5]. Triazoles fungicides such as Difenoconazole, Prothioconazole, Propiconazole, Tricyclazole, Epoxiconazole, and Tebuconazole were reported to be toxic to adult zebrafish, with 96-h LC50 values of 1.45, 1.83, 4.9, 11.50, 12.56, and 26.8 mg/L, respectively[6-9].Acute toxicity of Mancozeb to freshwater fish, *Oreochromis mossambicus* (Tilapia) and LC50 value was found to be 11.68 mg/l [6], which corresponds with the findings of earlier workers to *Punctius ticto* (12.95 mg/l) and *Clarius batracus* adult (14.36 mg/l) and its fingerlings (14.04 mg/l) [10,11].

3. IMPACT ON BIOCHEMICAL PARAMETERS

Long-term exposure to Propiconazole resulted in higher concentration of ammonia, creatine kinase and lactate dehydrogenase on blood plasma profile of rainbow trout [12]. Elevated plasma ammonia indicated that detoxifying mechanisms were unable to convert the toxic ammonia to less harmful substances [13]. Creatine kinase catalyzes the conversion of creatine and consumes adenosine triphosphate (ATP) to create phosphocreatine and adenosine diphosphate (ADP), as a plasma marker of environmental stress [14]. Lactate dehydrogenase is a tetrameric enzyme recognized as a potential marker for assessing the toxicity of a chemical. The elevated levels of lactate dehydrogenase in the haemolymph might be due to the release of isozymes from the destroyed tissues [15]. Decrease in lactate dehydrogenase was noted while studying the biochemical effect of Tricyclazole on zebrafish. Rise in lactate dehydrogenase is due to the increase of anaerobic pathway as a result of hypoxic condition caused by the pesticide stress. Increase of the lactate dehydrogenase activity found in exposed zebrafish indicated the induction of anaerobic glycolysis to meet the required energy demands [16].

Glycogen content of muscle decreased very much due to the action of the Imidacloprid in freshwater fish, *Aplocheilus lineatus* [17]. Fishes exposed to Dimecron and Ziram were also reported decrease in glycogen to meet the energy requisites for muscular activity [18].

Exposure to even a small concentration of Tricyclazole resulted in hyperuremia and hypercholesterolemia indicating altered carbohydrate, lipid and protein metabolism in the fish *Channa punctatus* [19]. Lipid and carbohydrate metabolism as well as several enzymatic activities such as gamma-glutamil transpeptidase, alanine aminotransferase (AlAT) and alkaline phosphatase (AP) are affected in zebrafish as a consequence of the exposure to the fungicide Tebuconazole [20]. Levels of lactate, cholesterol and triglycerides also showed an increase during the exposure period. Effect of Emisan on *Channa punctatus* noticed a marked reduction in the levels of lipid, an elevation in cholesterol and acid and alkaline phosphatase. The elevated hepatic cholesterol levels are indicative of liver damage, and disruption of its normal functioning. [21]. Increased hepatic levels of acid and alkaline phosphatases in *Channa punctatus* may be due to degeneration of hepatocytes and rupture of lysosomes, resulting in their accumulation in the liver.

A significant increase in values of glucose was noted in *Clarias gariepinus* when treated with Clotrimazole [22]. Sattanathan et al. [23] revealed a significantly higher blood glucose level on exposed *Oreochromis niloticus* to Copper oxychloride. This is in agreement with Li et al. [13] while studying long term effect of Propiconazole on rainbow trout. Sancho et al. [20] also arrived at similar conclusion while studying effect of Tebuconazole on zebrafish. The hyperglycemia indicates disrupted carbohydrate metabolism probably after the elevated glycogenolysis. The hyperglycemia is a reliable indicator of environmental stress due to general secondary response of intoxicated fish.

Melefa et al. [22] observed a reduction in total serum protein in *Clarius gariepinus* after exposure to Clotrimazole. A significant decrease in total serum proteins after exposure may be due to liver damage where most of plasma protein synthesis usually occurs in the liver. This is in agreement with Palanikumar et al. [1] while studying the effect of Carbendazim on milkfish, *Chanos chanos*. Similar observations were reported by Ram et al. [21] and Gafar et al. [24] while determining the effect of Emisan on *Channa punctatus* and Edifenphos on *Oreochromis niloticus*. Similar studies were noted by Pandit and Rani [19] and Sattanathan et al. [23] on exposing *Channa* to Tricyclazole and *Oreochromis mossambicus* to Copper oxychloride.

There was decrease in muscle protein in *Aplocheilus lineatus* due to increased rate of proteolytic activity or repeated breakdown of proteins to yield energy due to stress [17]. A significant increase in the levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) were reported by Melefa et al. [22] on exposing *Clarias gariepinus* to Clotrimazole. Gafaar et al. [24] revealed a significant increase in serum ALT and AST and ALP at the beginning of the experiment, these findings supported the hypothesis that the increased serum transaminases (ALT and AST) may reflect hepatic toxicity which leads to extensive liberation of the enzymes into the blood circulation. Li et al. [13] also reported an increase in activity of AST, ALT and ALP in rainbow trout exposed to Propiconazole.

4. IMPACT ON HAEMATOLOGICAL PARAMETERS

Generally, a decrease in nonspecific immunity of the fish due to pesticide exposure leads to alterations in haematological parameters. Furthermore, the decrease in the haematological parameters might have resulted from disruptive action of the pesticides on the membranes and cell viability. Melefa et al. [22] showed significant decrease in red blood cell (RBC), haemoglobin (Hb) and packed cell volume (PCV) in the *Clarius gariepinus* when exposed to Clotrimazole. Ghane et al. [25] also determined the haematological impact of Paclobutrazol (PBZ) to fingerling of tilapia, *Oreochromis mossambicus* and observed similar decrease in RBC, Hb and PCV. Similar results were noted by Hemalatha et al. [26] while studying the effect of Propiconazole on *Labeo rohita*. Lysing or shrinkage of erythrocytes due to pesticide action on the erythropoietic tissue may lead to a reduction in haemoglobin and haematocrit [27].

Shahi and Singh [28] demonstrated decreases in haemoglobin (Hb), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), and red blood cell (RBC) levels in *Clarias batrachus* when exposed to sub lethal concentration of Mancozeb. This results were in agreement with [29] while studying the impact of Curzate on *Oreochromis niloticus*. Li *et al* [13] proved these changes in blood parameters in rainbow trout intoxicated with Propiconazole. The haemolytic and destructive effects of the pesticides on blood cells was supported by Gafaar et al. [24] after studying effects of Edifenphos on *Oreochromis niloticus*.

Pandit et al. [30] demonstrated that total erythrocyte number, haemoglobin, packed cell volume, basophils, neutrophils, monocytes and thrombocytes decreased significantly while erythrocyte sedimentation rate increased significantly on exposure of *Channa* to Tricyclazole. It seems that due to the toxicity of Tricyclazole, haemopoietic organs get affected and become unable to release normal RBCs in circulation and thus can be held responsible for a decline in erythrocytes. Reduction in haemoglobin is directly caused not only by the enhanced haemoglobin destruction but also by the reduced synthesis of haemoglobin.

Significant increase in WBC was noted by Hemalatha et al. [26] on *Labeo rohita* exposed to Propiconazole.Melefa et al. [22] showed a significant elevation in WBC number of Clarias intoxicated with Clotrimazole. Pandit et al. [30] also came to similar findings in case of *Channa punctatus* exposed to Tricyclazole. Similar observation was revealed by Sattanathan et al. [19] on exposing *Oreochromis mossambicus* to Copper oxychloride. This finding is in agreement with Shahi and Singh [28] on exposure of *Clarias* to Mancozeb. Observation on Propiconazole intoxicated rainbow trout showed a significant increase in leucocyte count [13]. The observed elevation of WBC count indicates a protective mechanism against fungicide toxicity or the activation of immune system to manage the stress caused by pesticides.

5. IMPACT ON HISTOPATHOLOGY

Jia et al. [31], Ibtissem et al. [32] and Ben Amara et al. [33] reported changes in liver tissue due to the hepatocyte damage. Oxidative stress and the activation of caspase-3 in apoptotic pathways are two characteristic reasons for agrochemicals-induced toxicity [34]. Abnormalities induced by Azoxystrobin and fungicide Captan were observed in zebrafish and rainbow trout liver such as cellular vacuolization, partial necrosis and congestion around the central vein [35]. Boran et al. [36] results are also supported by Gafaar et al. [24] following the exposure of *Oreochromis niloticus* on Edifenphos; Vivek et al. [37] on intoxicated *Ophiocephalus leucopunctatus* with tricyclazole; and Ram and Sathyanesan [21] on *Channa punctatus* with Emisan.

Boran et al. [36] found that Captan exposed gills of rainbow trout had hypertrophy and necrosis of epithelium, separation of epithelium from lamellae (epithelial lifting), lamellar fusion, hyperplasia and the space under the epithelium filled with eosinophilic material. Apart from above findings, Melanomacrophage centers, intercellular edema and splenic necrosis were also noted on Captan exposed fish. Similarly, necrosis of epithelium, lamellar fusion, hyperplasia and lamellar edema were also observed in *Oreochromis niloticus* exposed with Edifenphos [24].

Captan exposed rainbow trout had tubular necrosis, shrinkage of glomeruli, renal tubular degeneration and hyaline droplets degeneration [36]. Gafaar et al. [24] observed congestion, diffuse granular and vacuolar degenerative changes and focal hyaline droplet degeneration following exposure of *Oreochromis niloticus* to Edifenphos. Nephrocalcinosis, cellular rupture and decrease in Bowmann's space, illustrated the deleterious effect of Tricyclazole on *Ophiocephalus*. [37]. Similar observations were noted by Tabassum et al. [38] on exposing *Channa punctatus* with Propiconazole.

6. EFFECT ON DEVELOPMENT AND REPRODUCTION

Pericardial edema, yolk sac edema, tail deformation and spine deformation were observed in Cyproconazole-treated embryos [39]. Similar observations were noted by Souder et al. [40] when zebrafish was treated with Propiconazole. This is in agreement with Mu et al. [9] when dealing with developmental toxicity of Difenoconazole in zebrafish embryos after 96-h exposure. Similar observations were reported by studying developmental toxicity of Triadimefon [41] and Penconazole in zebrafish embryos [42]. Kim et al. [43] reported malformations such as pericardial edema, spinal lordosis, and head edema in zebrafish embryos/larvae treated with Benomyl fungicide.

Li et al. [44] confirmed that Fluquinconazole exposure interferes with the early embryonic development of zebrafish by reducing the expression of representative marker genes and increasing oxidative stress. Exposure to the Succinate dehydrogenase inhibitor (SDHI) fungicides such as Isopyrazam, Penthiopyrad and Sedaxane also induce developmental malformation in zebrafish [45,46]. Zhang et al. [47] observed that Zoxamide induced teratogenicity in the form of bent tails, curved spines, and shortening of the body in zebrafish. Similar to zoxamide-exposed fish, Carbendazim exposed fish also exhibited a reduction in body length and severe impairments to the cytoskeleton and skeletal system [48].

Sounders et al. [49] revealed that relatively high doses of Triticonazole induced deformities, impaired oxidative respiration and altered locomotor activity in early developmental stages of zebrafish. Exposure to Propiconazole significantly inhibited the development and reduced locomotor activity of zebrafish embryos and larvae [50]. Skolness et al. [51] found that Propiconazole inhibited steroidogenesis in female fathead minnow which inturn leads to decreased plasma concentration of 17β estradiol and vitellogen, affecting the development as well as release of oocyte.

Cao et al. [35] indicated that Azoxystrobin had adverse effects on egg production, the fertilization rate, the gonadosomatic index and hepatosomatic index, sex steroid hormones and vitellogin concentrations, gonadal and hepatic histology, and the relative mRNA levels of the tested genes in the HPG axis and liver of adult zebrafish. These results suggested that widespread application of Azoxystrobin might negatively affect fish reproduction. Similar results reported that Trifloxystrobin, one of the strobilurin fungicides, induced significant up-regulation of vitellogin genes in the larvae of medaka [52].

7. OXIDATIVE DAMAGE

Significant increase was observed in all antioxidant parameters (superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR), glutathione S-transferase (GST), reduced glutathione (GSH), malondialdehyde (MDA)) on treatment of crayfish with Penconazole[53].A decrease in GSH was detected when *Chaos chaos* was intoxicated with

Carbendazim[1]. Tricyclazole induced production of reactive oxygen species, apoptosis and metabolic disturbance in zebrafish liver was reported by Qiu et al. [54].

Propiconazole, which directly act on the cell membrane can induce lipid peroxidation in larval zebrafish as a primary effect [55]. It is reported that exposure to Prothioconazole also caused lipid peroxidation and induced oxidative damage in zebrafish [56]. Jiang et al. [57] reported the accumulation of reactive oxygen species in zebrafish on treating with Azoxystrobin. Accumulation of reactive oxygen species in zebrafish with Azoxystrobin was also noted by Hans et al. [58]. Similarly, Carbendazim also induced lipid peroxidation in *Chanos chanos* [1].

Kumar et al. [59] observed a reduction in mitochondrial bioenergetics which induced oxidative stress, decreased standard body length and a reduction in movement in Strobilurins-exposed zebrafish. Souders et al. [40] demonstrated that Propiconazole can induce hypopigmentation in zebrafish, disrupt mitochondrial bioenergetics, and can alter locomotor activity. This result is in agreement with Wang et al. [60] when zebrafish was intoxicated with Fluazinam. Azoxystrobin induced mitochondrial dysfunction in larval zebrafish and liver of adult zebrafish by significantly decreasing mitochondrial complex III activity, ATP concentration and transcription of cyt b [61]. Olsvik et al. [62] also arrived at similar conclusion in Atlantic salmon smolt with Azoxystrobin.

8. IMPACT ON BEHAVIOUR

Valadas et al. [63] reported that exposure to Propiconazole disrupted normal fish behavior by decreasing the number of crossings, entries, and time spent in the top, and increased the time spent in the bottom area of the tank. Hypo/hyper-activity in a dose specific manner were exhibited by Fluazinam treated zebrafish [60]. Larval zebrafish exhibited hypoactive swimming behavior upon exposure to 250 mg/L of Pyraclostrobin [64]. Sivastava and Singh [65] reported that Propiconazole showed significant behavioral changes such as hyperactive movement, hypomovement, vertical position and loss of equilibrium in *Clarias batrachus*.

There is a close relationship between agrochemicals and cardiovascular diseases [66]. Exposure to Penconazole [67] and Tebuconazole [68] affected normal functioning of myocardial cells in male adult rats. Triadimefon also induced cardiovascular toxicity in case of zebrafish [69]. Fenbuconazole exposure also affected the cardiac development and function in zebrafish larvae [70].

9. CONCLUSION

These review revealed the biochemical, haematological, histopathological, development and reproduction and oxidative damage caused by various fungicides on non-target organisms. Therefore, serious concern has to be taken towards the potential damage of fungicides to organisms and its use in agricultural practices. Use of biological fungicides instead of chemical ones can minimize the risk to non-target organisms.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Palanikumar L, Kumaraguru AK, Ramakritinan CM. Toxicity, biochemical and clastogenic response of chlorpyrifos and carbendazim in milkfish *Chanos chanos*. Int. J. Environ. Sci. Technol. 2013;11:765–774.
2. Oruc Hasan. Fungicides and Their Effects on Animals. Fungicides; 2010.
3. Srivastava P, Singh A, Pandey AK. Pesticides toxicity in fishes: Biochemical, physiological and genotoxic aspects. Biochem. Cell. Arch. 2016;16(2):199-218.
4. Capkin E, Terzi E, Boran H, Yandi I, Altinok I. Effects of some pesticides on the vital organs of juvenile rainbow trout (*Oncorhynchus mykiss*). Tissue and Cell. 2010;42:376-382.
5. Cao F, Souders CL, Li P, Pang S, Qiu L, & Martyniuk CJ. Developmental toxicity of the triazole fungicide Cyproconazole in embryo-larval stages of zebrafish (*Danio rerio*). Environmental Science and Pollution Research. 2019;26:4913–4923.
6. Andreu-Sanchez O, Paraiba LC, Jonsson CM, Carrasco JM. Acute toxicity and bioconcentration of fungicide Tebuconazole in zebrafish (*Danio rerio*). Environmental Toxicology.2012;27(2):109–116.
7. Coors A, Frische T. Predicting the aquatic toxicity of commercial pesticide mixtures. Environmental Sciences Europe. 2011;23(1): 1-18.
8. Guo J, Wenhua S, Feng D, Jinyang Z, Zhen L, Xiaoying C, Jinhua Z, Jie L. Acute toxicity study on zebrafish (*Danio rerio*) exposure to triazole fungicides. Journal of Southeast University.2010;29(4):402–406.

9. Mu X, Pang S, Sun X, Gao J, Chen J, Chen X, Li X, Wang Evaluation of acute and developmental effects of Difenoconazole via multiple stage zebrafish assays. Environmental Pollution. 2013;175:147–157.
10. Saha NC, Giri SK, Chatterjee N, Biswas SJ, Bej S. Acute toxic effects of Mancozeb to fish *Oreochromis mossambicus* (W. K. H. Peters, 1852) and their behaviour. International Journal of Advanced Research in Biological Sciences. 2016;3(6):40–44.
11. Srivastava P, Singh A. In vivo study of effects of dithiocarbamates fungicide (Mancozeb) and its metabolite ethylenethiourea (ETU) on freshwater fish *Clarias batrachus*. Journal of Biology and Earth Sciences. 2013;3(2): B228–B235.
12. Sharma MR, Mushtaq SA, Allayie, Vardhan H. Assessment of lethal toxicity of mancozeb and its consequences on the behavior of fresh water fish, *puntius ticto*. Journal of International Academic research for Multidisciplinary. 2016;4(2):132–138.
13. Li ZH, Zlabek V, Velíšek J, Grabic R, Machova J, Kolarova J, Li P, Randak T. Antioxidant responses and plasma biochemical characteristics in the freshwater rainbow trout, *Oncorhynchus mykiss*, after acute exposure to the fungicide Propiconazole. Czech Journal of Animal Science. 2011a;56(2):61–69.
14. Li ZH, Velisek J, Grabic R, Li P, Kolarova J, Randak T. Use of hematological and plasma biochemical parameters to assess the chronic effects of a fungicide Propiconazole on a freshwater teleost. Chemosphere. 2011 b;83(4):572–578.
15. Li ZH, Velisek J, Zlabek V, Grabic R, Machova J, Kolarova J, Randak T. Hepatic antioxidant status and hematological parameters in rainbow trout,*Oncorhynchus mykiss*, after chronic exposure to Carbamazepine. Chemico-Biological Interactions. 2010;183(1): 98.
16. Sancho E, Fernández-Vega, Cristina, Villarroel Ma, Andreu E, Ferrando, Ma. Physiological Effects of Tricyclazole on Zebrafish (Danio rerio) and Post-Exposure Recovery. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology. 2009; 150:25-32.
17. Vijayan AS, Thomas G. Study on the effect of Imidacloprid on the biochemical parameters of freshwater fish,*Aplocheilus lineatus*. Journal of Applied Zoological Researches.2018;29(1):89–92.
18. Thangavel P, Niveditha H, Ramaswamy M. Comparative study on individual and combined effects of Dimecron and Ziram on carbohydrate metabolites in Liver, Muscle, Heart and Blood of fresh water teleost *Sarotherodon mossambicus* (Peters). Bulletin of Environmental Contamination and Toxicology. 2004;72(8):365–372.
19. Pandit DN, Rani U. Toxicity of Tricyclazole on certain serum biochemical markers of an Indian paddy-field fish, *Channa punctatus* (Bloch). International Journal of Fisheries and Aquatic Studies. 2019;7(6):246–250.
20. Sancho E, Villarroel MJ, Fernández C, Andreu E, Ferrando M.D. Short-term exposure to sublethal Tebuconazole induces physiological impairment in male zebrafish (*Danio rerio*). Ecotoxicology and Environmental Safety. 2010;73(3):370–376.
21. Ram RN, Sathyanesan AG. Histopathological and biochemical changes in the liver of a teleost fish, *Channa punctatus* (Bloch) induced by a mercurial fungicide. Environmental Pollution. 1987;47(2):135–145.
22. Melefa TD, Mgbenka BO, Aguzie IO, Andong FA, Nwakor U, Nwani CD. Morphological, haematological and biochemical changes in African catfish *Clarias gariepinus* (Burchell 1822) juveniles exposed to Clotrimazole. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology. 2020;236.
23. Sattanathan G, Amsath A, Senthilmurugan S, Tamizhazhagan V. Toxicity of Copper oxychloride(fungicide)in *Oreochromis mossambicus* on haemato-immunological and biochemical alterations and recovery assessment by marine algae Chaetomarpha aerea. International Journal of Zoology and Applied Biosciences. 2019;4(4):184–194.
24. Gaafar AY, El-Manakhly EM, Soliman MK, Soufy H, Zaki MS, Safinaz G. Mohamed SG, Hassan SM. Some pathological, biochemical and hematological investigations on Nile tilapia *(Oreochromis niloticus*) following chronic exposure to edifenphos pesticide.Journal of American Science.2010;6(10):542–551.
25. Ghane MN, Dhamagaye HB, Meshram SJ, Salunke AD. Toxicity Effect of Paclobutrazol Fungicide on Haematological Parameters in *Oreochromis mossambicus* Fingerlings.Trends in Biosciences. 2017;10(33):7146–7155.
26. Hemalatha D, Muthukumar A, Rangasamy B, Nataraj B, Ramesh M. Impact of sublethal concentration of a fungicide Propiconazole on certain health biomarkers of Indian major carp *Labeo rohita*. Biocatalysis and Agricultural Biotechnology. 2016;8:321–327.
27. Saravanan M, Prabhu KK, Ramesh M. Haematological and biochemical responses of

freshwater teleost fish *Cyprinus carpio* (Actinopterygii: Cypriniformes) during acute and chronic sublethal exposure to lindane. Pesticide Biochemistry and Physiology. 2011; 100(3):206–211.

28. Shahi J, Singh A. Genotoxic and haematological effect of commonly used fungicide on fish *Clarias batracus*. Journal of Biology and Earth Sciences. 2014;4 (2): B137–B143.
29. Desai B, Parikh P. Impact of Curzate (fungicide) on Hematological Parameters of *Oreochromis mossambicus*. International Journal of Scientific & Engineering Research.2012;3(7).
30. Pandit DN, Rani U, Sardana M. Tricyclazole Induced Changes in Certain Haematological Indices of *Channa punctatus* (Bloch) as Diagnostic Biomarkers of Stress Response. International Journal of Pharma and Bio Sciences. 2020;12(1):97-103.
31. Jia K, Cheng B, Huang L, Xiao J, Bai Z, Liao X, Cao Z, Shen T, Zhang C, Hu C, Lu H. Thiophanate-methyl induces severe hepatotoxicity in zebrafish. Chemosphere. 2020; 248.
32. Ibtissem BA, Hajer BS, Ahmed H, Awatef E, Choumous K, Ons B, Mounir ZK, Najiba Z. Oxidative stress and histopathological changes induced by Methylthiophanate, a systemic fungicide, in blood, liver and kidney of adult rats. African Health Sciences. 2017;17(1):154–163.
33. Ben Amara I, Ben Saad H, Cherif B, Elwej A, Lassoued S, Kallel C, Zeghal N. Methyl-thiophanate increases reactive oxygen species production and induces genotoxicity in rat peripheral blood. Toxicology Mechanisms and Methods. 2014;24:679–687.
34. Jiang J, Wu S, Wu C, An X, Cai L, Zhao X. Embryonic exposure to Carbendazim induces the transcription of genes related to apoptosis, immunotoxicity and endocrine disruption in zebrafish (*Danio rerio*). Fish and Shellfish Immunology.2014;41(2):493–500.
35. Cao F, Zhu L, Li H, Yu S, Wang C, Qiu L. Reproductive toxicity of Azoxystrobin to adult zebrafish (*Danio rerio*). Environmental Pollution. 2016;219:1109–1121.
36. Boran H, Capkin E, Altinok I, Terzi, E. Assessment of acute toxicity and histopathology of the fungicide Captan in rainbow trout. Experimental and Toxicologic Pathology.2012;64:175–179.
37. Vivek S, Indumathi SP, Radha T. Ecological Risk *Assessment* of the Fungicide Tricyclazole (75%) on *Ophiocephalus leucopunctatus* (Sykes,1839) with Respect to Hepatic Enzymes and Pathological Anamolies. Research Journal of Agriculture and Biological Sciences. 2009; 5(4) :445–451.
38. Tabassum H, Dawood AQ, Sharma P, Khan J, Raisuddin S, Parvez S. Multi-organ toxicological impact of fungicide Propiconazole on biochemical and histological profile of freshwater fish *Channa punctata* Bloch. Ecological Indicators. 2016;63:359–365.
39. Cao F, Souders CL, Li P, Pang S, Qiu L, Martyniuk, CJ. Developmental toxicity of the triazole fungicide Cyproconazole in embryo-larval stages of zebrafish (*Danio rerio*). Environmental Science and Pollution Research. 2019a;26:4913–4923.
40. Souders CL, Xavier P, Perez-Rodriquez V, Ector N, Zhang JL, Martyniuk CJ. Sub-lethal effects of the triazole fungicide Propiconazole on zebrafish (*Danio rerio*) development, oxidative respiration, and larval locomotor activity. Neurotoxicology and Teratology. 2019a ;74.
41. Liu S, Chang J, Zhu G. Developmental toxicity of Triadimefon in embryo-larval stages of zebrafish. International Conference on Digital Manufacturing & Automation. 2010;1:552–555.
42. Aksakal FI, Ciltas A. Developmental toxicity of Penconazole in zebrafish (*Danio rerio*) embryos. Chemosphere. 2018;200: 8–15.
43. Kim DJ, Seok SH, Baek MW, Lee HY, Na YR, Park SH, Lee HK, Dutta NK., Kawakami K, Park JH. Benomyl induction of brain aromatase and toxic effects in the zebrafish embryo. Journal of Applied Toxicology. 2009;29(4): 289–294.
44. Li W, Wu Y, Yuan M, Liu X. Fluxapyroxad induces developmental delay in zebrafish (*Danio rerio*). Chemosphere. 2020;256.
45. Yao H, Xu X, Zhou Y, Xu C. Impacts of Isopyrazam exposure on the development of early-life zebrafish (*Danio rerio*). Environmental Science and Pollution Research. 2018;25(14): 23799–23808.
46. Qian L, Qi S, Cao F, Zhang J, Li C, Song M, Wang C. Effects of penthiopyrad on the development and behaviour of zebrafish in early-life stages. Chemosphere. 2019; 214:184–194.
47. Zhang X, Zhang P, Perez-Rodriguez V, Souders CL, Martyniuk CJ. Assessing the toxicity of the benzamide fungicide Zoxamide in zebrafish (*Danio rerio*): Towards an adverse outcome pathway for beta-tubulin inhibitors.

Environmental Toxicology and Pharmacology. 2020;78.

48. Andrade TS, Henriques JF, Almeida AR, Machado A, Koba O, Giang PT, Soares A, Domingues I. Carbendazim exposure induces developmental, biochemical and behavioural disturbance in zebrafish embryos. Aquatic Toxicology. 2016;170:390–399.
49. Souders CL, Perez –Rodriguez V, Ahmadie NE, Zhang X, Tischuk C, Martyniuk CJ. Investigation into the sub-lethal effects of the triazole fungicide Triticonazole in zebrafish (*Danio rerio*) embryos/larvae. Environmental Toxicology. 2019b;35(1):1–15.
50. Teng M, Zhao F, Zhou Y, Yan S, Tian S, Yan J, Meng , Bi S, Wang. Effect of Propiconazole on the Lipid Metabolism of Zebrafish Embryos (*Danio rerio*). Journal of Agricultural and Food Chemistry. 2019;67:4623−4631.
51. Skolness SY, Blanksma CA, Cavallin JE, Churchill JJ, Durhan EJ, Jensen KM, … Ankley GT. Propiconazole Inhibits Steroidogenesis and Reproduction in the Fathead Minnow (*Pimephales promelas*). Toxicological Sciences.2013;132(2):284–297.
52. Zhu L, Wang H, Liu H, Li W. Effect of Trifloxystrobin on hatching, survival, and gene expression of endocrine biomarkers in early life stages of medaka (*Oryzias latipes*). Environmental Toxicology. 2015;30(6):648–655.
53. Alkan Uckun, Aysel and Barim Ozden. Acute exposure to the fungicide Penconazole affects some biochemical parameters in the crayfish (*Astacus leptodactylus Eschscholtz*, 1823). Environmental Science and Pollution Research. 2020;27:5626 – 5637.
54. Qiu L, Jia K, Huang L, Liao X, Guo X, Lu H. Hepatotoxicity of Tricyclazole in zebrafish (*Danio rerio*). Chemosphere. 2019; 232(6):171–179.
55. Kumar N, Awoyemi O, Willis A, Schmit C, Ramalingam L, Moustaid-Moussa N, Crago J. Comparative lipid peroxidation and apoptosis in embryo-larval zebrafish exposed to 3 azole fungicides, Tebuconazole, Propiconazole,and Myclobutanil, at environmentally relevant concentrations. Environmental Toxicology and Chemistry. 2019;38:1455–1466.
56. Tian S, Teng M, Meng Z, Yan S, Jia M, Li R. Toxicity effects in zebrafish embryos (*Danio rerio*) induced by Prothioconazole. Environmental Pollution. 2019;255: 113269.
57. Jiang J, Shi Y, Yu R, Chen L, Zhao X. Biological response of zebrafish after short-term exposure to Azoxystrobin. Chemosphere. 2018;202:56–64.
58. Han Y, Liu T, Wang J, Wang J, Zhang C, Zhu L. Genotoxicity and oxidative stress induced by the fungicide Azoxystrobin in zebrafish (*Danio rerio*) livers. Pesticide Biochemistry and Physiology. 2016;133:3–19.
59. Kumar N, Willis A, Satbhai K, Ramalingam L, Schmitt, Moustaid-Moussa N, Crago J. Developmental toxicity in embryo-larval zebrafish (*Danio rerio*) exposed to strobilurin fungicides (Azoxystrobin and Pyraclostrobin). Chemosphere. 2020;241.
60. Wang XH, Zheng SS, Huang T, Su LM, Zhao YH, Souders CL, Martyniuk CJ. Fluazinam impairs oxidative phosphorylation and induces hyper/hypo-activity in a dose specific manner in zebrafish larvae. Chemosphere. 2018;210:633–644.
61. Cao F, Wu P, Huang L, Li H, Qian L, Pang S, Qiu L. Short-term developmental effects and potential mechanisms of Azoxystrobin in larval and adult zebrafish (*Danio rerio*) . Aquatic Toxicology. 2018;198:129–140.
62. Olsvik PA, Kroglund F, Finstad B, Kristensen T. Effects of the fungicide Azoxystrobin on Atlantic salmon (*Salmo salar L.*) smolt. Ecotoxicology and Environmental Safety. 2010;73(8):1852–1861.
63. Valadas J, Mocelin R, Sachett A, Marcon M, Zanette RA, Dallegrave E, Herrmann AP, Piato A. Propiconazole induces abnormal behavior and oxidative stress in zebrafish. Environmental Science and Pollution Research. 2019; 26:27808–27815.
64. Reif DM, Truong L, Mandrell D, Marve S, Zhang G, Tanguay RL. High throughput characterization of chemical-associated embryonic behavioral changes predicts teratogenic outcomes. Archieves of Toxicology. 2016;90:1459–1470.
65. Srivasthava P, Singh A. Behavioral changes by inhibition of acetylcholinesterase induced by trizole(Propiconazole) fungicide on freshwater fish *Clarias batrachus*.World Journal of Fish and Marine Sciences. 2014;6(1):82–86.
66. Matome S, Kotsedi M, Masezi S. Exposure to agrochemicals and cardiovascular disease: a review. International Journal of Environmental Research and Public Health.2016;13(2): 229.
67. Chaabane M, Tir M, Hamdi S, Boudawara O, Jamoussi K, Boudawara T. Improvement of heart redox states contributes to the beneficial effects of selenium against Penconazole-induced cardiotoxicity in adult rats. Biological Trace Element Research. 2016;169 (2): 261–270.

68. Ben Othmene Y, Hamdi H, Annabi E, Amara I, Ben Salem I, Neffati F. Tebuconazole induced cardiotoxicity in male adult rat. Food and Chemical Toxicology. 2020;137:111134.
69. Liu H, Chu T, Chen L, Gui W, Zhu G. The cardiovascular toxicity of Triadimefon in c early life stage of zebrafish and potential implications to human health. Environmental Pollution. 2017;23(Pt 1):1093–1103.
70. Wu Y, Yang Q, Chen M, Zhang Y, Zuo Z, Wang C. Fenbuconazole exposure impacts the development of zebrafish embryos. Ecotoxicology and Environmental Safety. 2018;158:293–299.