PROTECTIVE EFFECTS OF MORINGA OLEIFERA AND ARTHROSPIRA PLATENSIS ON BLOOD BIOCHEMISTRY AND GILL TISSUE ALTERNATIONS IN NILE TILAPIA EXPOSED TO THIOPHENATE METHYL TOXICITY

Waqar Noor[1], Maliha Ghaffar[*2], Fatima Akhtari[3], Anam Abbas[4], Shafiq Ahmed[5], Serina[6], Sohair Khalid[7]

[1,*2,3]*Department of Biology, University of Okara, Okara 56130, Pakistan*

[1]wiki8845@gmail.com, [*2]malihaghaffar@uo.edu.pk, [3]fatimaakhtari022@gmail.com, [4]anamabbas473@gmail.com, [5]muhammadshafique.eu@gmail.com, [6]sarinakhan247@gmail.com

DOI: https://doi.org/10.5281/zenodo.18161807

Keywords

Article History
Received: 01 October 2025
Accepted: 10 November 2025
Published: 29 November 2025

Corresponding Author: *
Maliha Ghaffar

Abstract

This study evaluated the protective effects of Moringa oleifera and Arthrospira platensis (Spirulina) on biochemical parameters and gill histology of Nile tilapia (Oreochromis niloticus) exposed to thiophanate methyl toxicity. Nile tilapia fish samples were divided into four groups: control, thiophanate methyl-exposed (0.6 mg/L), thiophanate methyl + M. oleifera (5% of diet), and thiophanate methyl + Spirulina (150 mg/kg body weight/day) for a 28-day trial. Biochemical analyses revealed that thiophanate methyl exposure significantly elevated serum cholesterol, triglycerides, LDL, VLDL, AST, and ALT, while reducing HDL, total protein, albumin, and globulin levels. Urea, BUN, and creatinine levels were also increased, indicating hepatic and renal stress. Supplementation with M. oleifera and Spirulina ameliorated these alterations, with Spirulina showing slightly better improvements in protein profile and renal markers, and M. oleifera being more effective in moderating lipid and liver enzyme changes. Histological examination of gills from thiophanate methyl-exposed fish showed severe lamellar fusion, epithelial lifting, and hyperplasia, while supplemented groups exhibited improved gill architecture with reduced pathological alterations. The results demonstrate that both M. oleifera and Spirulina provide significant protection against pesticide-induced biochemical and histopathological damage, supporting their use as sustainable, natural feed additives in aquaculture to mitigate agrochemical toxicity.

INTRODUCTION

Pollution is a major problem that affects everyone and is getting worse as a result of global population expansion and industrialization. Aquatic species and all other levels of biological organization can be endangered by environmental contamination brought on by chemical usage.(Bhat et al., 2022; Zhao et al., 2023). Fungicides are a type of pesticide that is widely used to treat a range of fungal diseases in field crops and fruits (Savary et al., 2019). Chemical fungicides are predominantly employed in commercial agriculture to safeguard crops from fungal infections by exterminating and inhibiting their cells and spores. Nonetheless, due to their cost-effectiveness and user-friendliness, they are often utilized excessively (Youssef et al., 2019).

Methyl thiophanate [dimethyl ((1,2-phenylene) bis(iminocarbonothioyl)) bis(carbamate)]. is a benzimidazole fungicide that works systemically to stop the creation of fungal DNA, changing the way cells divide (Seiler, 1975). This fungicide is used both before and after harvest to stop diseases in vegetables brought on by the Ascomycota fungus (Ye et al., 2008). Reactive oxygen species generation and its interaction with human serum albumin are connected to the toxicity of this fungicide (Saquib et al., 2010). TM is under category IV, or low toxicity (Organization, 2020). However, it has been demonstrated that TM and its metabolite, methylbenzimidazole-2-yl carbamate (MBC), cause histological damage to the thyroid and adrenal glands of lizards (De Falco et al., 2007) as well as the rats' blood, liver, and kidneys (Ibtissem et al., 2017).

Oreochromis niloticus, often known as the Nile tilapia, is a vital fish species for aquaculture worldwide. Its rapid rate of development, significant resilience to environmental stress, ease of reproduction, and robust market demand make it the species of choice (El-Sayed, 2006). Its distinguishing traits are resilience to disease, quick growth, and simplicity in adjusting to commercial diets (Abdel-Latif & Khafaga, 2020). One of the most commonly utilized model fish in aquatic toxicology studies these days is tilapia (Yan et al., 2020).

M. *oleifera* is referred to as the "kelor tree" in some parts of the world and the "drumstick tree" or "horse radish tree" in others (Anwar & Bhanger, 2003). "Sohanjna" is the native name for M. *oleifera*, which is planted and grown all throughout Pakistan (Anwar et al., 2005). In addition to improving energy, memory, alertness, and healthy skin, moringa is used to treat diarrhea, fevers, UTIs, sore throats, and asthma. Additionally, it contains anti-inflammatory, anti-bacterial, tissue-protective, anti-diabetic, and anti-cancer properties (Bhattacharya et al., 2018; Dhakad et al., 2019; Leone et al., 2015). Essential vitamins, minerals, and phytochemicals can be found in abundance in the leaves of the moringa tree (Yuniati et al., 2022). It is drought-tolerant and possesses medicinal, nutritional, and water-cleaning properties. Its roots, leaves, flowers, and fruits are consumed locally. Eastern allopathic medicine uses this plant to treat a range of ailments and problems, including physiological issues, due to its therapeutic and medicinal properties (Akhtar et al., 2007).

Arthrospira, another name for the symbiotic microalga Spirulina, is a free-floating organism with spiral filaments (Deng & Chow, 2010; Hussein et al., 2018; Rout et al., 2015). This algae is also known as blue-green algae (Ross & Dominy, 1990). One nutraceutical food that has showed potential in healing the majority of ailments is spirulina. Spirulina has been used as a dietary supplement to prevent and treat excessive cholesterol, diabetes, cardiovascular disease, allergies, cancer, toxicities caused by drugs and environmental contaminants, and some inflammatory disorders (Karkos et al., 2011). Spirulina dramatically increased the Nile tilapia's growth rate, immunity, and resistance to disease, according to the majority of research (Abdel-Tawwab et al., 2008; Amer, 2016). Recently, the use of spirulina as a wastewater treatment method has grown in favor, particularly for fish production effluent (Kamilya et al., 2006).

Thiophanate-methyl is a widely used systemic fungicide employed in agriculture which are common in ecosystem monitoring. When present in aquatic ecosystems thiophanate-methyl subjects fish to oxidative stress, inhibits their metabolism, leads to liver, kidney, gills damage, and damage to internal organs can yield a mortality result. Natural and bioactive substances like the compounds offered by M. *oleifera* and A. *platensis*, may be an innovative and safe method of increasing fish tolerances to environmental toxicity of toxic chemicals. This market-driven research will quantitate certain biochemical and histopathological parameters in order to determine the impact of natural supplements that can avoid the damage caused by Thiophanate Methyl, and also promote fish physiologies upon stress caused by fungicides, and can offer assessment regarding the impact of fungicides on fish.

Material and Methods

Chemical

Methyl thiophanate is employed as a poison. An appropriate quantity of thiophanate methyl was dissolved in distilled water to create the stock solution. Additionally, spirulina powder and moringa leaf were bought from a nearby herbal store.

Experimental Animal

The Head Baloki fish pond was used to obtain 30 fingerlings of the freshwater fish Nile Tilapia, which had an average weight of 25 ± 1.9 grams and a length of 13.5 ± 1.2 centimeters. Fish were securely brought to the University of Okara's fish lab, where they were acclimated for seven days in a 100-liter glass tank with an aerator to preserve oxygen levels. Dechlorinated water with the following physicochemical characteristics was employed during the experiment period: pH 7.3 ± 0.07, dissolved oxygen 7.40 ± 0.94 mg/L, and temperature 24.31 ± 1.17 ◦ C. Commercial food was given to the fish twice a day.

Experimental Design

A technique called Completely Randomized Design (CRD) was used. The experiment lasted for 28 days, during which time the fish were kept in aquariums with forty liters of water each. The fish received commercial feed once every twenty-four hours for the duration of the trial. Based on their individual weights, the fish were placed together. There were four treatment groups in order to identify the negative effects of TM and attempt to examine the preventive qualities of natural supplements. Group 1 served as the control in order to establish a baseline for the data. Group 2 was administered thiophanate methyl concentration of 0.6 mg/L to assess the substance's toxicological effects. Sublethal quantity was selected by the previous studies, described by Jia et al. (2020). Group 3 was TM (0.6 mg/L) given moringa leaf powder with standard feed according to their body weight following the methods of the Association of Official Analytical Chemists (Reynolds, 1989), while Group 4 was given TM (0.6 mg/L) Spirulina at 150 mg/kg bodyweight/day following the feed formulation guidelines of the National Research Council (NRC, 1993). To make the powders stick, they were thoroughly mixed with regular feed with a small amount of water and fish oil. The mixture was then dried and fed to the fish once a day. Using procedures authorized by the University of Okara Ethical Committee, these dosages from past research were used to evaluate their possible protective effects against thiophanate methyl poisoning. On day 28, randomly ten fish were taken from group each group, and all fish were collected for the abovementioned assays.

Biochemical Analysis

A chemistry analyzer evaluated biochemical indicators. To separate the plasma, the remaining blood samples were placed in a cooling centrifuge set to 9400×g for 20 minutes. The plasma was then stored at 4 ◦ C. The protein contents were estimated using the (Waterborg, 2009) method. The technique outlined by was used to assess AST and ALT activity (Reitman & Frankel, 1957). Using kits supplied by Biomerieux (France), serum creatinine and urea levels were determined. Serum levels of HDL, triglycerides, and cholesterol were assessed using standard kits using the procedure of (Hassan et al., 1995). Levels of VLDL and LDL were determined using the usual procedure provided by (Zaahkouk et al., 1996).

Histological studies

Fish were dissected for histological analysis following blood collection. Gill soft tissues were extracted and treated as usual, which included sectioning at a thickness of 5 µm on a rotating microtome, fixing in a 10% neutral buffered formalin solution, standard processing, and staining with hematoxylin and eosin in accordance with the procedure of (Bancroft & Gamble, 2008). After that, stained slides were inspected using a microscope equipped with a camera (Olympus BX51, Tokyo, Japan).

Statistical analysis

The findings of the fully randomized experimental design are displayed using one-way analysis of variance (ANOVA) and mean ± standard deviation. Ordinary one-way ANOVA from GraphPad Prism (version 9.3.1) was the ANOVA version utilized in the analysis.

The Research of Medical Science Review
ISSN: 3007-1208 & 3007-1216 Volume 3, Issue 11, 2025

Results

Biochemical Analysis

During the course of this investigation, we noticed that fish which were treated to thiophanate methyl demonstrated much greater. In contrast to the control group, the blood of Nile tilapia exposed to pesticides showed significantly lower levels of HDL, total proteins, albumin, and globulin, while the levels of triglycerides, cholesterol, VLDL, VDL, AST, ALT, and BUN were significantly higher ($p \leq 0.05$). Following the administration of Nile tilapia to the Moringa and spirulina with TM groups, we observed a slight decrease in BUN and a slight increase in HDL, total proteins, albumin, and globulin levels, as well as a slight alteration in cholesterol, triglycerides, LDL, VLDL, ALT, and AST.

Table 1: Biochemical profile of Nile Tilapia exposed to thiophanate methyl, thiophanate methyl and moringa powder (TM+ moringa), and thiophanate methyl and spirulina powder (TM+ spirulina). The data are represented as mean ± SD. Asterisk (*) bearing values show significant differences (p < 0.05) as compared with the control group. NS = not significant, TM = Thiophanate Methyl.

Parameters	Control	TM	TM + Moringa	TM + Spirulina
Total Cholestrol (mg/dL)	90.67 ± 4.04	119.7 ± 3.51***	110.7 ± 5.13***	109.3 ± 2.51**
Triglycerides (mg/dL)	110.0 ± 2.00	136.7 ± 3.51***	123.3 ± 4.04**	125.0-±4.58**
HDL (mg/dL)	23.13 ± 0.61	14.33 ± 0.86***	19.33 ± 0.60***	18.57 ± 0.70***
LDL (mg/dL)	45.20 ± 1.11	63.03 ± 1.05***	53.03 ± 0.95***	52.00 ± 1.00***
VLDL (mg/dL)	22.00 ± 0.70	35.03 ± 0.85***	28.97 ± 0.75***	28.00 ± 1.00***
ALT (U/L)	274.7 ± 6.50	345.0 ± 10.15***	306.0 ± 12.53*	300.7 ± 9.50*
AST (U/L)	310.7 ± 7.02	353.7 ± 8.02***	345.3 ± 5.50***	339.3 ± 7.50**
Total Proteins (g/dL)	4.00 ± 0.08	3.43 ± 0.07***	3.65 ± 0.09**	3.59 ± 0.11**
Globulin (g/dL)	2.88 ± 0.07	2.43 ± 0.08***	2.66 ± 0.09*	2.63 ± 0.11*
Albumin (g/dL)	1.09 ± 0.07	0.80 ± 0.03***	0.96 ± 0.04*	1.00 ± 0.05 NS
Urea (mg/dL)	15.00 ± 1.00	19.37 ± 0.61***	17.67 ± 0.60**	18.40 ± 0.50***
BUN (mg/dL)	7.10 ± 0.45	12.03 ± 0.65***	10.03 ± 0.55***	9.16 ± 0.66**
Creatinine (mg/dL)	1.06 ± 0.15	1.51 ± 0.10**	1.40 ± 0.08*	1.35 ± 0.06*

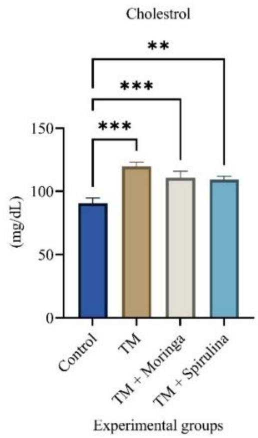

Figure 1: Showing variations in Cholesterol in Nile Tilapia among various groups. The data are represented as mean ± SD.

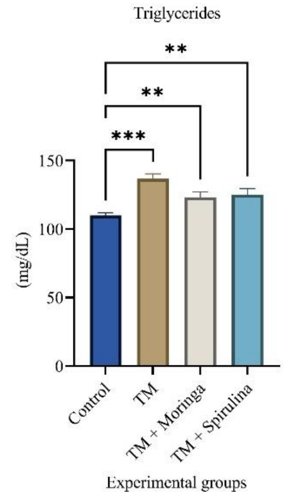

Figure 2: Showing variations in Triglycerides in Nile Tilapia among various groups. The data are represented as mean ± SD.

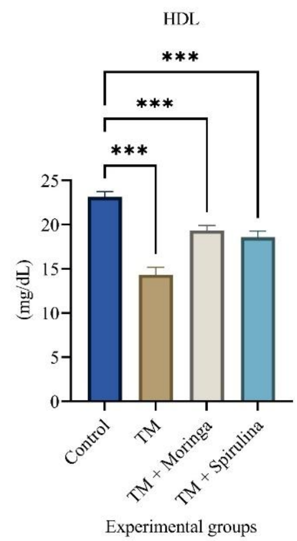

Figure 3: Showing variations in HDL in Nile Tilapia among various groups. The data are represented as mean ± SD.

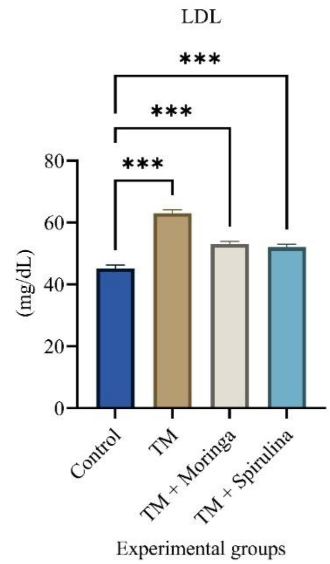

Figure 4: Showing variations in LDL in Nile Tilapia among various groups. The data are represented as mean ± SD.

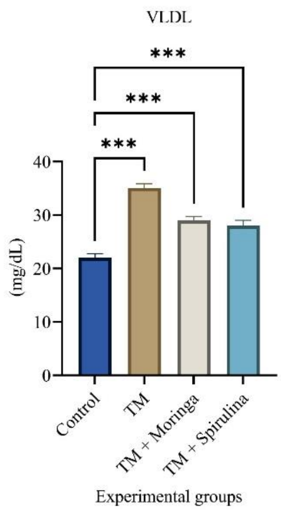

Figure 5: Showing variations in VLDL in Nile Tilapia among various groups. The data are represented as mean ± SD.

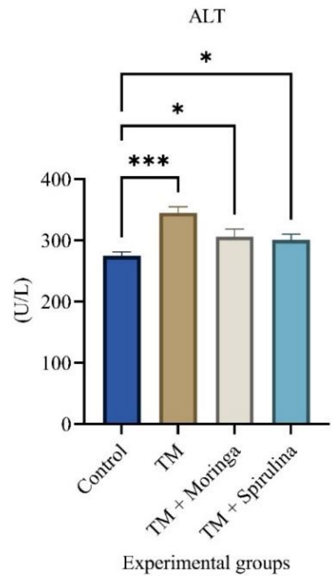

Figure 6: Showing variations in ALT in Nile Tilapia among various groups. The data are represented as mean ± SD.

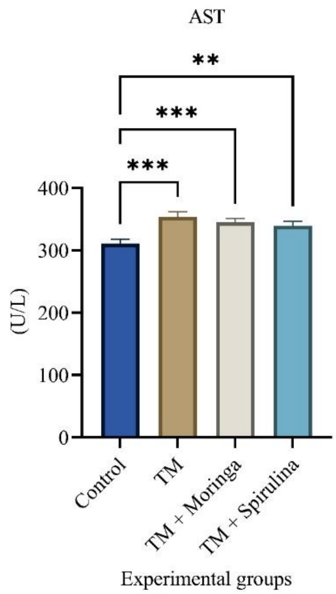

Figure 7: Showing variations in AST in Nile Tilapia among various groups. The data are represented as mean ± SD.

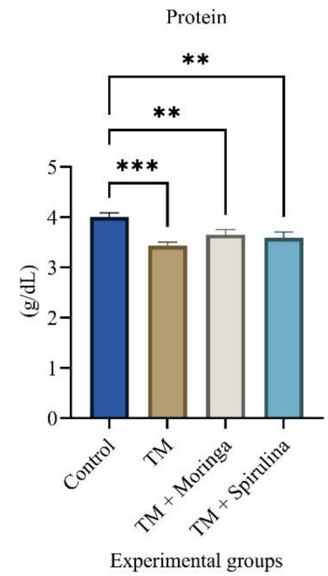

Figure 8: Showing variations in Protein in Nile Tilapia among various groups. The data are represented as mean ± SD.

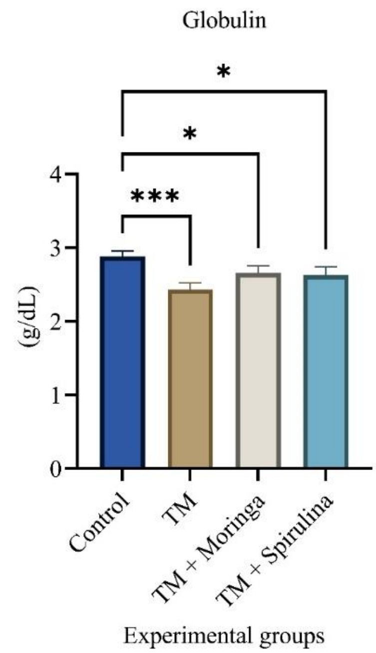

Figure 9: Showing variations in Globulin in Nile Tilapia among various groups. The data are represented as mean ± SD.

The Research of Medical Science Review
ISSN: 3007-1208 & 3007-1216 Volume 3, Issue 11, 2025

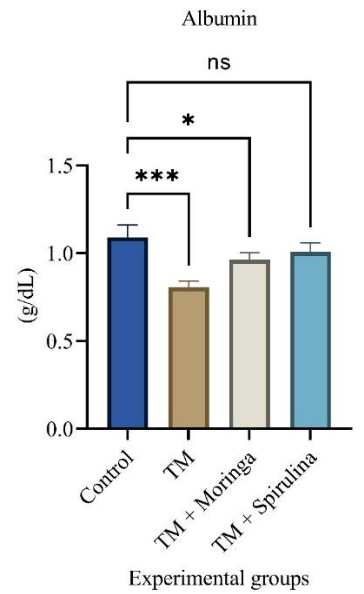

Figure 10: Showing variations in Albumin in Nile Tilapia among various groups. The data are represented as mean ± SD.

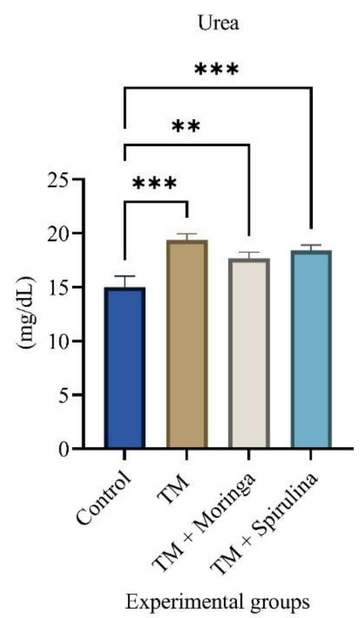

Figure 11: Showing variations in Urea in Nile Tilapia among various groups. The data are represented as mean ± SD.

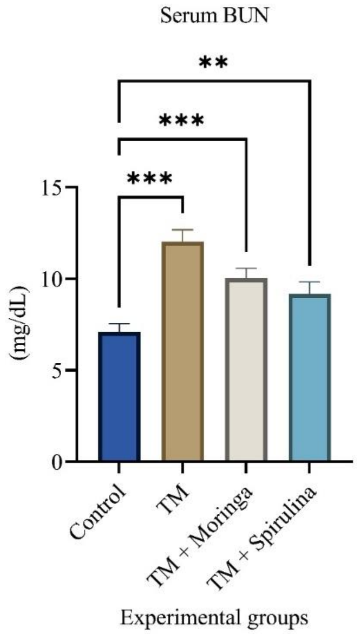

Figure 12: Showing variations in Serum BUN in Nile Tilapia among various groups. The data are represented as mean ± SD.

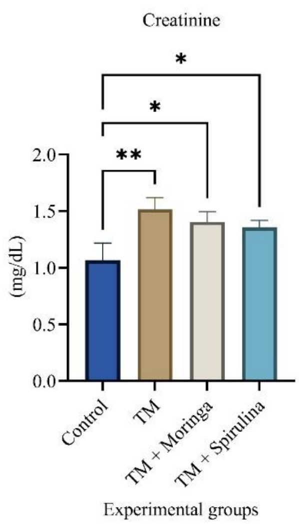

Figure 13: Showing variations in Creatinine in Nile Tilapia among various groups. The data are represented as mean ± SD.

Histology

Nile tilapia exposed to thiophanate methyl showed histological changes in their gills. All of the fish tissues under study showed no signs of injury in the chemical-free control group. The current study's findings revealed moderate to severe histological changes in groups B, C, and D, including degenerative primary gill lamellae, bone cell abnormalities, lamellar aneurysms, and hyperlexia. Histological observations of gill tissues showed bone cell deformities, lamellar aneurysm, hyperplasia, degenerative primary gill lamellae in Fig 14 (b). In moringa and spirulina group gill showed some recovery shown in in Fig 14(c) and (d) respectively.

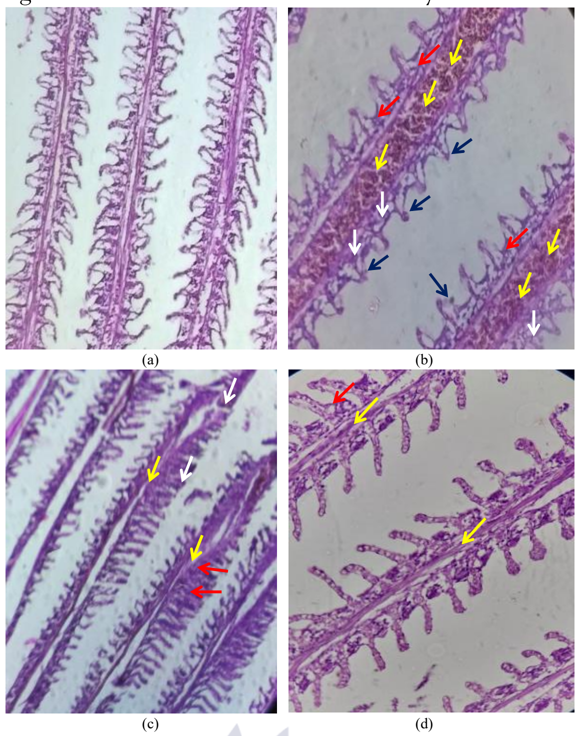

Figure 14: showing histological changes in gill tissues as (a) showing normal arrangement of gill filaments. (b), (c), and (d) showing variations in gill tissues as bone cell deformities (yellow arrow), lamellar aneurysm (red arrow), hyperlexia (black arrow), degenerative primary gill lamellae (white arrow).

Discussion

A broad-spectrum internal absorption fungicide, thiophanate-methyl effectively prevents and controls a range of crop diseases (Nagai & Fukazu, 1969), consequently, it is frequently employed as a bactericide in the agricultural industry. Because it can be converted into Methyl 2-benzim-idazolecarbamate (MBC), which has fungicidal properties in water and plant and animal tissues, TM has a bactericidal function (Cycoń et al., 2011). We evaluated the protective benefits of moringa and spirulina against the detrimental effects of thiophanate methyl on the lipid profile, liver enzymes, and organ damage of exposed fish using Nile tilapia in our toxicity studies.

Biochemical Analysis:

Understanding the alterations in aquatic species' bodies can be aided by biochemical measurements (Poopal et al., 2017). Serum biochemistry serves as an excellent indicator for evaluating a fish's ability to adapt to its environment, general health, and nutritional status (Sharma et al., 2021). In our research, fish exposed to deltamethrin insecticide showed increases in cholesterol, triglycerides, LDL, and VLDL, whereas HDL dropped in comparison to the control group. These results are similar to those of Ismail et al. (2012). Cholesterol is necessary for the synthesis of vitamin D, steroid hormones, and bile acids. Although the quantity produced varies according to the type of cell and the organ's function, cholesterol is produced by all mammalian cells. Between 20 and 25 percent of the daily total cholesterol production comes from the liver; greater amounts are also created by the intestines, adrenal glands, and reproductive systems (Espenshade & Hughes, 2007). Elevated blood cholesterol levels are strongly associated with the development of arteriosclerotic vascular disease, depending on how it is transported by lipoproteins; nevertheless, elevated blood cholesterol levels can also prevent the production of new cholesterol. Both the cytosolic and membrane domains of HMG CoA reductase contribute to its catalytic activity. The membrane domain detects signals for its breakdown. As cholesterol (and other sterol) concentrations rise, this domain's oligomerization state changes, increasing its susceptibility to proteosome destruction (Berg et al., 2007).

In the present study, we discovered that fish given thiophanate methyl exhibited noticeably increased AST and ALT activity. Ismail et al. (2012) and Ibtissem et al. (2017) showed similar results in rats. Their release into the bloodstream increases most during the acute phase of cellular necrosis, and this increase is related to the extent of cellular damage. According to other observers, the plasma of MT groups showed oxidative damage, hepatocellular necrosis, and increased plasma membrane permeability at the maximum concentration of each enzyme (Sharma et al., 2002). The blood proteins of a fish can reveal information about its physiological, metabolic, and ecological circumstances. They are essential for the movement of several foreign compounds and metabolites that shield fish against illness (Kovyrshina & Rudneva, 2012). Pesticide exposure caused a sharp decline in the blood levels of albumin and total proteins in Nile tilapia. Same results were found by Ismail et al. (2012) and Ibtissem et al. (2017). The decrease in albumin level had an effect on the plasmatic colloidal osmotic pressure. Since the liver produces albumin, which accounts for 60% of protein, a decrease in albumin was mirrored in a decrease in total protein. This drop in albumin and total protein supports the theory that damaged liver cells compromised the liver's ability to synthesize. Chronic liver illnesses such as cirrhosis or nephrotic syndrome cause decreases in albumin levels, which are then expelled through the urine (Singh et al., 1987). In the present investigation, the plasma levels of creatinine and urea were greater in the TM-treated group than in the controls, which is equivalent to the Feki et al. (2017) and Ibtissem et al. (2017) reported in their studies. While the rate of urea excretion is inversely correlated with the plasma amount of urea, protein absorption is directly correlated with it (Rahman et al., 2001). When factors that promote the production of urea, such as intestinal hemorrhage, corticosteroid treatment, and a high-protein diet, are not present, increases in urea levels may suggest a decrease in GFR, an increase in nitrogenous metabolism linked to decreased renal blood flow (Mizutani et al., 2000) and causes acute renal failure (Singh et al., 2001). Conversely, creatinine is a therapeutically important analyte that is used to measure renal glomerular filtration rate and identify the presence of kidney and muscle diseases. Creatinine is a byproduct of the metabolism of muscular energy that the kidneys remove from the blood and excrete into the urine (El-Sawi Mahmoud et al., 2001). Creatinine is a sensitive measure of renal function since liver function has little effect on it (Aronson et al., 2008).

Contrary to the results of the current investigation, fish Nile Tilapia exposed to Moringa and TM simultaneously reported that Moringa leaf powder significantly decreased the harmful effects of thiophanate methyl. *Moringa oleifera* is widely utilized in alternative medicine due to its anti-oxidant and health-promoting qualities (Pandu et al., 2018). The high nutritional value of M. oleifera leaves makes them a superfood that can be utilized as a remedy for a number of chronic conditions (Gopalakrishnan et al., 2016). Because of the positive effects of Moringa, we noticed a small change in cholesterol in Nile tilapia exposed to a combination of thiophanate methyl and Moringa oleifera. Triglyceride and cholesterol levels may have dropped because M. oleifera includes substances that lower cholesterol, such as phytoconstituents and β-sitosterol, which prevent lipolysis and lessen the synthesis of fatty acids (Riry et al., 2018). *M. oleifera* is an intriguing source of fiber (7.98g/100g DW), which helps maintain a healthy digestive system and causes a noticeable drop in the peak level of serum glucose and a well-balanced level of testosterone (Kaline et al., 2007). In the present study, exposure to moringa leaf powder marginally increased the levels of albumin and total proteins. Moringa leaf powder increases serum albumin, total proteins, and globulin because it lowers oxidative stress, which may help stop oxidative protein degradation (Gupta et al., 2012). When M. *oleifera* was exposed to fish, the levels of ALT and AST trivially dropped. The hepatoprotective qualities of M. oleifera leaf extract may be one reason why it corrected elevated serum intracellular enzyme levels. Previous research indicated that the hepatoprotective effect was caused by the presence of a well-known flavonoid (quercetin and kaempferol) (Hamza, 2010). M. oleifera leaf extract considerably reduced hepatocyte necrosis, indicating a reduction in hepatocellular damage (Sharifudin et al., 2013). Urea and creatinine levels in fish treated with moringa also slightly changed. Urine production may be increased by moringa's diuretic effects. Blood urea and creatinine are reduced as a result of the body eliminating waste and toxins through increased urine output (Akter et al., 2021).

The lipid profile revealed a significant drop in the amount of total cholesterol in fish exposed to spirulina group, based on the findings of the current observations. Spirulina lowers cholesterol due to its function in lipid metabolism (Khani et al., 2017). It has been demonstrated that spirulina and other organic antioxidants can prevent hypercholesterolemia. Spirulina contains antioxidants such phycocyanin, β-carotene, and phenolic compounds that reduce serum cholesterol levels (Colla et al., 2008). Additionally, it has been shown that spirulina's polyunsaturated fatty acids have therapeutic advantages, such as the ability to reduce blood cholesterol (Yamamoto et al., 1989). The enzymes aspartate aminotransferase (AST) and alanine transaminase (ALT) are also found in lower concentrations in fish blood. It demonstrates the existence of a metabolic system that produces energy (Rehman et al., 2017). The liver biochemical markers (ALT and AST) in *Oreochromis niloticus* are impacted by this microalgae (Marzouk et al., 2013). It is indisputable that spirulina has antioxidant properties that prevent the generation of ion radicals (Mohammad & Bashi, 2020). The presence of β-carotene may be the reason for spirulina's beneficial effects. By decreasing cellular damage, particularly deoxyribonucleic acid (DNA), β-carotene, which is found in spirulina, may aid in the repair of injured liver cells (AlFadhly et al., 2022). Fish treated with spirulina also showed a small rise in the ratio of total protein to albumin. Spirulina offers preventative qualities that help keep the fish's kidney function in a healthy state (Mokhbatly et al., 2020).

Histology of fish gill:

Histological changes can be used to measure the degree of harm and damage caused by toxins, and they have proven to be very useful measurements (Raibeemol & Chitra, 2020). Several histological investigations reveal that the gills are the first organ to be attacked and the primary indicator of water quality in fish exposed to pesticides. In addition to respiration, the gills of fish are essential for a number of other critical processes, including the control of ions, the release of nitrogenous waste products, and the regulation of acid-base. The function of the fish's gills was significantly harmed by exposure to any toxicants or pollutants (Nagaraju Bantu et al., 2017). In the present study various histopathological changes were observed in fish gills when they exposed to Thiophanate methyl. In chemical free control group, no damage was observed in gill tissues of fish. Histological observations of gill tissues showed bone cell deformities, lamellar aneurysm, hyperplasia, degenerative primary gill lamellae in fig 14 (b). Jamil et al. (2024) showed similar finding when fish exposed to pesticide. A number of previously published studies reported similar degenerative alterations in the gills in response to pesticide exposure (Nilsson, 2007; Tabassum et al., 2016). Under extreme circumstances, a large number of erythrocytes that remain in capillaries develop aneurysms, which harm the epithelium (Melletti et al., 2004). Fig 14 (c) showed some recovery patterns when fish exposed to the moringa. Shahzadi et al. (2024) showed similar finding when fish exposed to moringa leaf powder. After exposed to spirulina the gills also show some repairing patterns as shown in fig14 (d). Similar studied by Satyantini et al. (2022) in fish gills.

Conclusion

The present investigation provides clear evidence that thiophanate methyl exerts profound toxic effects on *Oreochromis niloticus*, disrupting lipid metabolism, impairing liver and kidney function, reducing protein synthesis, and causing severe structural damage to gill tissues. Biochemical alterations, including significant elevations in cholesterol, triglycerides, LDL, VLDL, hepatic enzymes, and renal markers, alongside marked reductions in HDL, total protein, albumin, and globulin, reflect systemic physiological stress. Histopathological examination confirmed extensive gill damage, such as bone cell deformities, lamellar aneurysm, epithelial hyperplasia, and degeneration of primary lamellae. Dietary supplementation with *Moringa oleifera* and *Arthrospira platensis* markedly mitigated these alterations through antioxidant, hepatoprotective, and reno-protective mechanisms, with Spirulina showing slightly superior efficacy in restoring protein and renal profiles, while Moringa demonstrated strong liver-protective effects. These findings not only validate the protective potential of these natural feed additives but also underscore their relevance as sustainable, eco-friendly interventions for enhancing fish resilience and ensuring the long-term health and productivity of aquaculture systems in pesticide-contaminated environments.

REFERENCES:

Abdel-Tawwab, M., Ahmad, M. H., Abdel-Hadi, Y. M., & Seden, M. E. (2008). Use of Spirulina (Arthrospir platensis) as a growth and immunity promoter for nile tilapia, Oreochromis niloticus (L.) fry challenged with pathogenic Aeromonas hydrophila. 8th International Symposium on Tilapia in Aquaculture, Cairo, Egypt,

Abdel-Latif, H. M., & Khafaga, A. F. (2020). Natural co-infection of cultured Nile tilapia Oreochromis niloticus with Aeromonas hydrophila and Gyrodactylus cichlidarum experiencing high mortality during summer. *Aquaculture Research*, *51*(5), 1880-1892.

Ahmed, M. U., Hasan, Q., Hossain, M. M., Saito, M., & Tamiya, E. (2010). Meat species identification based on the loop mediated isothermal amplification and electrochemical DNA sensor. *Food Control*, *21*(5), 599-605.

Akhtar, M., Hasany, S. M., Bhanger, M., & Iqbal, S. (2007). Sorption potential of Moringa oleifera pods for the removal of organic pollutants from aqueous solutions. *Journal of hazardous materials*, *141*(3), 546-556.

Akter, T., Rahman, M. A., Moni, A., Apu, M. A. I., Fariha, A., Hannan, M. A., & Uddin, M. J. (2021). Prospects for protective potential of Moringa oleifera against kidney diseases. *Plants*, *10*(12), 2818. https://doi.org/https://doi.org/10.3390/plants10122818

AlFadhly, N. K., Alhelfi, N., Altemimi, A. B., Verma, D. K., Cacciola, F., & Narayanankutty, A. (2022). Trends and technological advancements in the possible food applications of Spirulina and their health benefits: A Review. *Molecules*, *27*(17), 5584.

Amer, S. A. (2016). Effect of Spirulina platensis as feed supplement on growth performance, immune response and antioxidant status of mono-sex Nile Tilapia (Oreochromis niloticus). *Benha Veterinary Medical Journal*, *30*(1), 1-10.

Anwar, F., Ashraf, M., & Bhanger, M. I. (2005). Interprovenance variation in the composition of Moringa oleifera oilseeds from Pakistan. *Journal of the American Oil Chemists' Society*, *82*(1), 45-51.

Anwar, F., & Bhanger, M. (2003). Analytical characterization of Moringa oleifera seed oil grown in temperate regions of Pakistan. *Journal of Agricultural and food Chemistry*, *51*(22), 6558-6563.

Anwar, F., Latif, S., Ashraf, M., & Gilani, A. H. (2007). Moringa oleifera: a food plant with multiple medicinal uses. *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives*, *21*(1), 17-25.

Aronson, D., Hammerman, H., Beyar, R., Yalonetsky, S., Kapeliovich, M., Markiewicz, W., & Goldberg, A. (2008). Serum blood urea nitrogen and long-term mortality in acute ST-elevation myocardial infarction. *International journal of cardiology*, *127*(3), 380-385.

Bancroft, J. D., & Gamble, M. (2008). *Theory and practice of histological techniques*. Elsevier health sciences.

Berg, J. M., Tymoczko, J. L., & Stryer, L. (2007). *Biochemistry (loose-leaf)*. Macmillan.

Bhat, R. A., Beigh, B. A., Mir, S. A., Dar, S. A., Dervash, M. A., Rashid, A., & Lone, R. (2022). Biopesticide techniques to remediate pesticides in polluted ecosystems. In *Research Anthology on Emerging Techniques in Environmental Remediation* (pp. 336-356). IGI Global Scientific Publishing.

Bhattacharya, A., Tiwari, P., Sahu, P. K., & Kumar, S. (2018). A review of the phytochemical and pharmacological characteristics of Moringa oleifera. *Journal of Pharmacy and Bioallied Sciences*, *10*(4), 181-191.

Borges, A., Piassão, J., Albani, S., Albertoni, E., Martins, M., Cansian, R., Valduga, A., Hepp, L., & Mielniczki-Pereira, A. (2021). Multiple metals and agricultural use affects oxidative stress biomarkers in freshwater Aegla crabs. *Brazilian Journal of Biology*, *82*, e230147.

Colla, L. M., Muccillo-Baisch, A. L., & Costa, J. A. V. (2008). Spirulina platensis effects on the levels of total cholesterol, HDL and triacylglycerols in rabbits fed with a hypercholesterolemic diet. *Brazilian Archives of Biology and Technology*, *51*, 405-411.

Cosgrove, S., Jefferson, B., & Jarvis, P. (2019). Pesticide removal from drinking water sources by adsorption: a review. *Environmental Technology Reviews*, *8*(1), 1-24.

Cycoń, M., Wójcik, M., & Piotrowska-Seget, Z. (2011). Biodegradation kinetics of the benzimidazole fungicide thiophanate-methyl by bacteria isolated from loamy sand soil. *Biodegradation*, *22*, 573-583.

De Falco, M., Sciarrillo, R., Capaldo, A., Russo, T., Gay, F., Valiante, S., Varano, L., & Laforgia, V. (2007). The effects of the fungicide methyl thiophanate on adrenal gland morphophysiology of the lizard, Podarcis sicula. *Archives of Environmental Contamination and Toxicology*, *53*, 241-248.

Deng, R., & Chow, T. J. (2010). Hypolipidemic, antioxidant, and antiinflammatory activities of microalgae Spirulina. *Cardiovascular therapeutics*, *28*(4), e33-e45.

Dhakad, A. K., Ikram, M., Sharma, S., Khan, S., Pandey, V. V., & Singh, A. (2019). Biological, nutritional, and therapeutic significance of Moringa oleifera Lam. *Phytotherapy Research*, *33*(11), 2870-2903.

El-Sawi Mahmoud, N. M., El-Wassimy, M. M., & Youssef, O. A. (2001). Effect of verrucarin J toxin on liver and kidney tissue of male mice.

El-Sayed, A.-F. M. (2006). *Tilapia culture*. CABI publishing.

Espenshade, P. J., & Hughes, A. L. (2007). Regulation of sterol synthesis in eukaryotes. *Annu. Rev. Genet.*, *41*(1), 401-427.

Fan, S., Zhao, P., Zhang, F., Yu, C., & Pan, C. (2013). Spinach or amaranth may represent highest residue of thiophanate-methyl with open field application on six leaf vegetables. *Bulletin of environmental contamination and toxicology*, 90, 477-481.

Feki, A., Saad, H. B., Jaballi, I., Magne, C., Boudawara, O., Zeghal, K., Hakim, A., Ali, Y. B., & Amara, I. B. (2017). Methyl thiophanate-induced toxicity in liver and kidney of adult rats: a biochemical, molecular and histopathological approach. *Cellular and Molecular Biology*, *63*(2), 20-28.

Gopalakrishnan, L., Doriya, K., & Kumar, D. S. (2016). Moringa oleifera: A review on nutritive importance and its medicinal application. *Food science and human wellness*, *5*(2), 49-56.

Gupta, R., Mathur, M., Bajaj, V. K., Katariya, P., Yadav, S., Kamal, R., & Gupta, R. S. (2012). Evaluation of antidiabetic and antioxidant activity of Moringa oleifera in experimental diabetes. *Journal of diabetes*, *4*(2), 164-171.

Hamza, A. A. (2010). Ameliorative effects of Moringa oleifera Lam seed extract on liver fibrosis in rats. *Food and Chemical Toxicology*, *48*(1), 345-355.

Hassan, A., El-Khalili, M., Hussein, N., & Kido, R. (1995). Changes in serum lipid profile and esterases of rats after sublethal daily doses of dimethoate. *The Journal of the Egyptian Public Health Association*, 70(3-4), 431-447.

Hussein, S., El-hamid, A., El-tawil, O., Laz, E., & Taha, W. (2018). The potential protective effect of spirulina platensis against mycotoxin induced oxidative stress and liver damage in rats. *Benha Veterinary Medical Journal*, *35*(2), 375-383.

Ibtissem, B. A., Hajer, B. S., Ahmed, H., Awatef, E., Choumous, K., Ons, B., Mounir, Z. K., & Najiba, Z. (2017). Oxidative stress and histopathological changes induced by methylthiophanate, a systemic fungicide, in blood, liver and kidney of adult rats. *African health sciences*, *17*(1), 154-163.

Ismail, A., El-Rafai, E. M., Mahmoud, Y., Abd El–Aziz, M. F., & Moustafa, M. (2012). TOXICOLOGICAL STUDIES ON TOPSIN M70 USED TO CONTROL WHITE MOLD DISEASE ON CUCUMBER. *Journal of Plant Protection and Pathology*, *3*(5), 469-479.

Jamil, I., Shabbir, A., Hasnain, A., Iqra, A., Muqadas, S., Sajid, A., Sheraz, K., & Khurram, S. (2024). The potential ameliorative effects of Moringa oleifera in emamectin benzoate induced toxicity in freshwater Labeo rohita fish. *International Journal of Biology and Chemistry*, *17*(1), 60-69.

Jia, K., Cheng, B., Huang, L., Xiao, J., Bai, Z., Liao, X., Cao, Z., Shen, T., Zhang, C., & Hu, C. (2020). Thiophanate-methyl induces severe hepatotoxicity in zebrafish. *Chemosphere*, *248*, 125941.

Kaline, K., Bornstein, S., Bergmann, A., Hauner, H., & Schwarz, P. (2007). The importance and effect of dietary fiber in diabetes prevention with particular consideration of whole grain products. *Hormone and metabolic research*, *39*(09), 687-693.

Kamilya, D., Sarkar, S., Maiti, T. K., Bandyopadhyay, S., & Mal, B. C. (2006). Growth and nutrient removal rates of Spirulina platensis and Nostoc muscorum in fish culture effluent: a laboratory-scale study. *Aquaculture Research*, *37*(15).

Karkos, P., Leong, S., Karkos, C., Sivaji, N., & Assimakopoulos, D. (2011). Spirulina in clinical practice: evidence-based human applications. *Evidence-based complementary and alternative medicine*, *2011*(1), 531053.

Khani, M., Soltani, M., Shamsaie Mehrjan, M., Foroudi, F., & Ghaeni, M. (2017). The effects of Chlorella vulgaris supplementation on growth performance, blood characteristics, and digestive enzymes in Koi (Cyprinus carpio). *Iranian Journal of Fisheries Sciences*, *16*(2), 832-843.

Kovyrshina, T., & Rudneva, I. (2012). Seasonal dynamics of activity of oxidative modification of proteins and oxidation-inhibiting enzymes in the blood of goby Neogobius melanostomus inhabiting the Black Sea and the Sea of Azov. *Journal of Ichthyology*, *52*, 277-283.

Leone, A., Spada, A., Battezzati, A., Schiraldi, A., Aristil, J., & Bertoli, S. (2015). Cultivation, genetic, ethnopharmacology, phytochemistry and pharmacology of Moringa oleifera leaves: An overview. *International journal of molecular sciences*, *16*(6), 12791-12835.

Liu, Z., Chen, Y., Han, J., Chen, D., Yang, G., Lan, T., Li, J., & Zhang, K. (2021). Determination, dissipation dynamics, terminal residues and dietary risk assessment of thiophanate-methyl and its metabolite carbendazim in cowpeas collected from different locations in China under field conditions. *Journal of the Science of Food and Agriculture*, *101*(13), 5498-5507.

Marzouk, M., Mostafa, M., Ibrahim, N., & Sharaf, M. (2013). Effect of freshwater toxic and non toxic cyanobacteria,(Microcystis aeruginosa) strains on some biochemical parameters of Oreochromis niloticus. *Egyptian Journal of Aquatic Biology and Fisheries*, *17*(1), 55-68.

Melletti, P., Rocha, O., & Martinez, C. (2004). Avaliação da degradação ambiental na Bacia do Rio Mogi-Guaçu por meio de testes de toxicidade com sedimento e análises histopatológicas em peixes. *Brigante J, Espíndola ELG. Limnologia fluvial: um estudo no rio Mogi-Guaçu*, *1*, 249-280.

Mizutani, A., Okajima, K., Uchiba, M., & Noguchi, T. (2000). Activated protein C reduces ischemia/reperfusion-induced renal injury in rats by inhibiting leukocyte activation. *Blood, The Journal of the American Society of Hematology*, *95*(12), 3781-3787.

Mohammad, M., & Bashi, W. Q. (2020). Effect of partial Substitution spirulina instead of soybean meal in common carp Cyprinus carpio L. Diet on some blood picture and some biochemical criteria. *The Iraqi Journal of Agricultural Science*, *51*(6), 1740-1746.

Mokhbatly, A.-A. A., Assar, D. H., Ghazy, E. W., Elbialy, Z., Rizk, S. A., Omar, A. A., Gaafar, A. Y., & Dawood, M. A. (2020). The protective role of spirulina and β-glucan in African catfish (Clarias gariepinus) against chronic toxicity of chlorpyrifos: hemato-biochemistry, histopathology, and oxidative stress traits. *Environmental Science and Pollution Research*, *27*, 31636-31651.

Nagai, Y., & Fukazu, R. (1969). Effects of fungicides against Sclerotinia rot and gray mold cucumber. *Ann. Phytopathol. Sot. Jap*, *35*(2), 139-140.

Nagaraju Bantu, N. B., Karri, K., Krishnan, V., Nirmala Kumari, N. K., & Rathnamma Vakita, R. V. (2017). Histological alteration in different tissues of Indian major carp, Labeo rohita (Hamilton) exposed to profenofos 50% EC and carbosulfan 25% EC formulations.

Nilsson, G. r. E. (2007). Gill remodeling in fish–a new fashion or an ancient secret? *Journal of experimental Biology*, *210*(14), 2403-2409. https://doi.org/https://doi.org/10.1242/jeb.000281

NRC, N. R. C. (1993). *Nutrient requirements of fish*. Washington DC: National Acedemiy Press.

Organization, W. H. (2020). The WHO recommended classification of pesticides by hazard and guidelines to classification 2019. In *The WHO recommended classification of pesticides by hazard and guidelines to classification 2019*.

Pandu, S. M., Cherupanalli, R., & Muthukumar, S. P. (2018). Effects of bound phenolic from defatted Moringa oleifera seed flour on diet-induced hypercholesterolemic mice. *Journal of Food Biochemistry*, *42*(5), e12553.

Poopal, R., Ramesh, M., Maruthappan, V., & Babu Rajendran, R. J. T. r. (2017). Potential effects of low molecular weight phthalate esters ($C_{16}H_{22}O_4$ and $C_{12}H_{14}O_4$) on the freshwater fish Cyprinus carpio. 6(4), 505-520.

Prousalis, K. P., Polygenis, D. A., Syrokou, A., Lamari, F. N., & Tsegenidis, T. (2004). Determination of carbendazim, thiabendazole, and o-phenylphenol residues in lemons by HPLC following sample clean-up by ion-pairing. *Analytical and bioanalytical chemistry*, *379*, 458-463.

Rahman, M., Siddiqui, M. K., & Jamil, K. (2001). Effects of Vepacide (Azadirachta indica) on asp artate and al anine aminotransferase profiles in a subchronic study with rats. *Human & experimental toxicology*, *20*(5), 243-249.

Raibeemol, K., & Chitra, K. (2020). Induction of immunological, hormonal and histological alterations after sublethal exposure of chlorpyrifos in the freshwater fish, Pseudetroplus maculatus (Bloch, 1795). *Fish & Shellfish Immunology*, *102*, 1-12.

Rehman, H., Aziz, A. T., Saggu, S., VanWert, A. L., Zidan, N., & Saggu, S. (2017). Additive toxic effect of deltamethrin and cadmium on hepatic, hematological, and immunological parameters in mice. *Toxicology and Industrial Health*, *33*(6), 495-502.

Reitman, S., & Frankel, S. (1957). A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. *American journal of clinical pathology*, *28*(1), 56-63.

Reynolds, H. L. (1989). Association of official analytical chemists: 1964–1988. *Reviews of Environmental Contamination and Toxicology: Continuation of Residue Reviews*, 109-136.

Riry, F., Elkloub, K., Moustafa, M., MAM, M., Hanan, A., & Youssef, S. (2018). Effect of partial replacement of soybean meal by Moringa oleifera seed meal on Japanese quail performance during laying period. *Egypt. Poult. Sci. J*, *38*, 255-267.

Ross, E., & Dominy, W. (1990). The nutritional value of dehydrated, blue-green algae (Spirulina plantensis) for poultry. *Poultry science*, *69*(5), 794-800.

Rout, N. P., Khandual, S., Gutierrez-Mora, A., Ibarra-Montoya, J. L., & Vega-Valero, G. (2015). Divergence in three newly identified Arthrospira species from Mexico. *World Journal of Microbiology and Biotechnology*, *31*, 1157-1165.

Saquib, Q., Al-Khedhairy, A. A., Alarifi, S. A., Dwivedi, S., Mustafa, J., & Musarrat, J. (2010). Fungicide methyl thiophanate binding at sub-domain IIA of human serum albumin triggers conformational change and protein damage. *International journal of biological macromolecules*, *47*(1), 60-67.

Satyantini, W., Rahmawati, W., & Masithah, E. D. (2022). The addition of Spirulina platensis extract in feed on gill histopathology and survival rate of Osphronemus gouramy after infected with Aeromonas hydrophila. IOP Conference Series: Earth and Environmental Science,

Savary, S., Willocquet, L., Pethybridge, S. J., Esker, P., McRoberts, N., & Nelson, A. (2019). The global burden of pathogens and pests on major food crops. *Nature ecology & evolution*, *3*(3), 430-439.

Seiler, J. (1975). Toxicology and genetic effects of benzimidazole compounds. *Mutation Research/Reviews in Genetic Toxicology*, *32*(2), 151-167.

Shahzadi, M., Ahmad, S., Rafique, H., Akmal, H., Ditta, A., Ali, S., Akram, I., & Shahzad, K. (2024). Protective effects of Moringa oleifera against carbofuran induced toxicity in fish (Labeo rohita): Insight into hematobiochemical, histology, oxidative and antioxidant biomarkers. *Kuwait Journal of Science*, *51*(3), 100249.

Sharifudin, S. A., Fakurazi, S., Hidayat, M. T., Hairuszah, I., Aris Mohd Moklas, M., & Arulselvan, P. (2013). Therapeutic potential of Moringa oleifera extracts against acetaminophen-induced hepatotoxicity in rats. *Pharmaceutical biology*, *51*(3), 279-288.

Sharma, M., CHADHA, P., & SHARMA, P. (2021). Environmental biomonitoring using different biomarkers and their contribution in aquatic environmental research. *Inter. J. Fish. Aquat. Studies*, *9*(5), 205-214. https://doi.org/https://doi.org/10.22271/fish.2021.v9.i5c.2570

Sharma, M. K., Kumar, M., & Kumar, A. (2002). Ocimum sanctum aqueous leaf extract provides protection against mercury induced toxicity in Swiss albino mice.

Singh, S. N., Vats, P., Suri, S., Shyam, R., Kumria, M., Ranganathan, S., & Sridharan, K. (2001). Effect of an antidiabetic extract of Catharanthus roseus on enzymic activities in streptozotocin induced diabetic rats. *Journal of Ethnopharmacology*, *76*(3), 269-277.

Singh, T., Garg, B., & Verma, P. (1987). Thiophanate methyl acute, subacute and chronic toxicity in rats. *Indian Journal of Pharmacology*, *19*(2), 159-161.

Tabassum, H., Ashafaq, M., Khan, J., Shah, M. Z., Raisuddin, S., & Parvez, S. (2016). Short term exposure of pendimethalin induces biochemical and histological perturbations in liver, kidney and gill of freshwater fish. *Ecological Indicators*, *63*, 29-36. https://doi.org/https://doi.org/10.1016/j.ecolind.2015.11.044

Waterborg, J. H. (2009). The Lowry method for protein quantitation. *The protein protocols handbook*, 7-10.

Yamamoto, A., Kamiya, T., Yamamura, T., Yokoyama, S., Horiguchi, Y., Funahashi, T., Kawaguchi, A., Miyake, Y., Beppu, S., & Ishikawa, K. (1989). Clinical features of familial hypercholesterolemia. *Arteriosclerosis (Dallas, Tex.)*, *9*(1 Suppl), I66-74.

Yan, W., Hamid, N., Deng, S., Jia, P.-P., & Pei, D.-S. (2020). Individual and combined toxicogenetic effects of microplastics and heavy metals (Cd, Pb, and Zn) perturb gut microbiota homeostasis and gonadal development in marine medaka (Oryzias melastigma). *Journal of hazardous materials*, *397*, 122795.

Ye, C., Zhou, Q., & Wang, X. (2008). Determination of thiophanate-methyl and chlorotoluron in water samples by improved single-drop microextraction coupled with high-performance liquid chromatography. *International Journal of Environmental and Analytical Chemistry*, *88*(7), 461-471.

Youssef, K., de Oliveira, A. G., Tischer, C. A., Hussain, I., & Roberto, S. R. (2019). Synergistic effect of a novel chitosan/silica nanocomposites-based formulation against gray mold of table grapes and its possible mode of action. *International journal of biological macromolecules*, *141*, 247-258.

Yuniati, N., Kusumiyati, K., Mubarok, S., & Nurhadi, B. (2022). The role of moringa leaf extract as a plant biostimulant in improving the quality of agricultural products. *Plants*, *11*(17), 2186.

Zaahkouk, S., Helal, E., & Hassan, A. (1996). Changes in some hematological and biochemical parameters of adult male rats in response to 8-hydroxy quinalidine N, N-dimethyl carbamate dimethyl sulphate. *Al-Azhar Bull Sci*, *7*(2), 1401-1411.

Zhao, H., Zhang, Y., Hou, L., Lu, H., Zhang, Y., & Xing, M. (2023). Effects of environmentally relevant cypermethrin and sulfamethoxazole on intestinal health, microbiome, and liver metabolism in grass carp. *Aquatic toxicology*, *265*, 106760.