Environment Conservation Journal 25 (4): 1113-1119, 2024

Journal homepage:https://www.environcj.in/

Environment Conservation Journal

ISSN 0972-3099 (Print) 2278-5124 (Online)

Acute toxicity of dithane M-45 in freshwater fish *Labeo rohita*: A laboratory study

Puspendra Kumar Maurya

P.G. Dept. of Zoology, IFTM University, Moradabad, U.P. India

Smita Jyoti ✉

P.G. Dept. of Zoology, IFTM University, Moradabad, U.P. India

Pradeep Kumar Maurya

Jal Jeevan Mission, Bageshwar, Uttarakhand, India

ARTICLE INFO

Received : 05 July 2024
Revised : 05 September 2024
Accepted : 15 October 2024

Available online: 05 November 2024

Key Words:
Environmental contamination
Ethological responses
Fish physiology
Fungicide toxicity
Pesticide exposure
Toxicological assessment

ABSTRACT

This study evaluated the toxicity of Dithane M-45, a common fungicide, in the freshwater fish species *Labeo rohita*. The lab-scale experiments were conducted to determine the concentration-dependent effects on fish mortality and behavioral changes. The median lethal concentration (LC50) values were identified as 190 mg/L at 48 hours, 170 mg/L at 72 hours, and 160 mg/L at 96 hours, indicating increased toxicity over time. Observations during the initial hour of exposure included pronounced behavioral responses such as jumping, increased movement, and frequent chelae scraping against body parts. These acute responses gradually subsided, with the fish returning to a more sedentary state within 36 hours. Subsequently, the fish settled at the bottom of the aquaria, displaying reduced activity. The gills and the inner linings of the branchiostegal began to produce mucus after 36 hours of exposure. This mucus production became more pronounced with prolonged exposure, particularly at the 96-hour mark. These findings suggest that Dithane M-45 has significant acute and sub-acute toxic effects on *L. rohita*, impacting both physiological and behavioral parameters. The findings showed the importance of monitoring and regulating the use of Dithane M-45 in aquatic environments to mitigate potential ecological and health risks.

Introduction

Pesticides play a critical role in modern agriculture by controlling pest populations (Popp *et al.*, 2013; Silva *et al.*, 2023). However, their widespread use has raised concerns about their potential environmental impact, particularly on non-target aquatic organisms. Among these, freshwater fish are often at risk due to their proximity to agricultural runoff and their sensitivity to chemical pollutants (Bukola *et al.*, 2015; Sudhakaran and Suja, 2024). Freshwater fish species are particularly sensitive to chemical pollutants, making them critical indicators of environmental health. A lot more pesticides, heavy metals, polycyclic aromatic hydrocarbons, polychlorinated biphenyls, and natural toxicants are ending up in waterways because of more agrochemicals and industrial compounds being used (Gürol *et al.*, 2020; Kumar *et al.*, 2021; Maurya *et al.*, 2019). Discharge of industrial effluents, often containing harmful substances, into water bodies causes abrupt and potentially lethal changes in the ecosystem (Bukola *et al.*, 2015; Lanjewar *et al.*, 2023). Therefore, understanding the effects of pesticides on this species is crucial for both environmental and public health.

Dithane M-45, a commonly used fungicide, contains the active ingredient mancozeb, a dithiocarbamate compound (Numan *et al.*, 2018). Dithane M-45 is a fungicide with the chemical formula $C_4H_6MnN_2S_4.Zn$. Mancozeb, a coordination complex of manganese, zinc, and ethylene bis (dithiocarbamate), makes up its composition (Dulama *et al.*, 2018; Rao and Jayabhaye, 2022). Plants are often protected from fungal diseases with this substance because it stops the activity of fungal enzymes like glutathione reductase, pyruvate dehydrogenase, and α-ketoglutarate dehydrogenase. It stops these enzymes from working by reacting with their sulfhydryl (-SH) groups and breaking up their active sites. This makes them less effective at catalyzing reactions. This interference disrupts cellular processes in fungi, such as energy production and detoxification, ultimately inhibiting fungal growth and proliferation. Agriculture widely applies it to control fungal diseases in crops (Szépvölgyi *et al.*, 1989). Despite its well-documented efficacy in pest control, its impact on aquatic ecosystems, particularly on fish species like *L. rohita*, continues to raise concerns. The active ingredient mancozeb degrades into ethylene thiourea (ETU), a compound known

Corresponding author E-mail: sjn.777@gmail.com
Doi:https://doi.org/10.36953/ECJ.28752902
This work is licensed under Attribution-Non Commercial 4.0 International (CC BY-NC 4.0)
© ASEA

for its potential toxicity. The level of acute toxicity and the ethological (behavioral) responses of fish to these chemicals are important signs of the health of the environment (Dutra Costa *et al.*, 2020). These indicators help in assessing the ecological risks associated with pesticide use and guide regulatory frameworks to ensure the safety of aquatic life (Scherer, 1992).

The common fungal infections in aquaculture include saprolegniasis, which is caused by species of saprolegnia and affects the skin, gills, and eggs of fish, making them appear white and cotton-like (Van Den Berg *et al.*, 2013). Branchiomyces sanguinis or B. demigrans, which affects gill tissues and causes necrotic changes and respiratory function impairment, causes Branchiomycosis (Khalil *et al.*, 2015). *Ichthyophonus hoferi* causes Ichthyophonus disease, which affects internal organs such as the liver, spleen, and heart (Hershberger *et al.*, 2002). Epizootic Ulcerative Syndrome, which is linked to the pathogen *Aphanomyces invadans*, makes fish vulnerable to getting deep sores in their muscles and infections that spread throughout their bodies. However, aquaculture has used fungicides such as potassium permanganate, formalin, and malachite green to treat fungal infections (Sutili and Gressler, 2021). More specifically, potassium permanganate increases oxygen levels but can be toxic at higher levels of concentration, while formalin is an active agent against fungal spores with associated toxicity to fish and handlers. Similarly, despite its effectiveness, several regions have banned malachite green due to its carcinogenic properties (Martins *et al.*, 2021). Aquaculture fungicides work by messing up the structures or metabolic processes of fungi, but they can also change the chemistry of the water, which could affect organisms that aren't intended to be killed (Maurya *et al.*, 2019; Sarkar *et al.*, 2022). As a result, careful administration and adherence to set guidelines are essential in avoiding risks (Maurya and Malik, 2016).

Labeo rohita (commonly known as Rohu) is a species of freshwater fish prevalent in rivers and ponds across various temperate and tropical regions, including Vietnam, Pakistan, Nepal, India, Myanmar, and Bangladesh (Ashaf-Ud-Doulah *et al.*, 2020). This species prefers habitats such as bund-type tanks and rivers, where it primarily breeds. However, it does not typically reproduce in confined waters, making it reliant on specific environmental conditions for its life cycle (Ahmed *et al.*, 2012). The region highly values Rohu not only for its ecological role but also for its economic significance. Fish is a staple food source, rich in proteins and essential minerals and thus playing a significant role in local diets and economies. According to McKenzie *et al.* (2007), toxicants can cause behavioral changes in fish that serve as sensitive indicators of sub-lethal stress. These changes include alterations in swimming patterns, feeding behavior, respiratory distress, and general activity levels (Little *et al.*, 1990). Monitoring these responses can provide early warning signals of environmental stress and help in identifying the underlying causes of fish mortality in polluted waters (Dahunsi *et al.*, 2011).

Previous studies have documented various toxic effects of pesticides on fish, including oxidative stress, enzymatic alterations, and histopathological changes (Xing *et al.*, 2012; Nataraj *et al.*, 2017; Farhan *et al.*, 2021). However, there is limited information on the specific effects of Dithane M-45 on *L. rohita* (Maurya *et al.*, 2023). Given the widespread use of this fungicide, it is essential to understand its impact on this economically and ecologically important species. The goal of this study is to fill in the gaps in our knowledge about how Dithane M-45 affects L. rohita by looking into its acute toxicity levels and animal responses. The findings will be crucial for developing appropriate guidelines for pesticide use in agriculture and for safeguarding the health of aquatic ecosystems. The outcomes of this research will not only contribute to the existing body of knowledge on pesticide toxicity in fish but also provide valuable data for risk assessment and management strategies in aquaculture and fisheries.

Materials and methods

Collection and acclimatization of fish

Freshwater fish, *L. rohita*, were collected from a Ramganga river through assistance of local fishermen (28°50'12.1" N and 78°47'45.6" E). The fish were transported to the laboratory and maintained in glass aquaria for a minimum period of one week to allow for acclimatization. Before experimental treatment, the fish were washed (dipped) with a 0.1% solution of potassium permanganate to eliminate any potential pathogens present on their bodies.

Preparation of test solutions

The dilution technique was employed to determine the required concentration of the toxicants. Measured quantities of Dithane M-45 were added separately to different glass aquaria and then diluted to the desired concentration. The physicochemical properties of the diluent water (pH 6.4-7.2, dissolved oxygen 5.3-7.1 mg/L, and total solids 27.6-33.3 mg/L) were analyzed according to the methods outlined by APHA (1985).

Bioassay setup

A series of test media with varying concentrations (toxic ranges determined by preliminary exploratory tests) were prepared in 20-liter glass aquaria, each containing 10 liters of diluent water. Twenty healthy fishes, each measuring 70-75 mm in length, were introduced into each aquarium containing the test medium. An additional aquarium containing 10 liters of diluent water served as the control.

Maintenance and observation

The quality of the water was monitored daily, and evaporated water was replenished as needed. Throughout the experiment, the behavior of the test animals was closely observed. Any dead specimens were promptly removed to prevent the depletion of dissolved oxygen in the aquaria, which could otherwise affect the results of the bioassay.

Determination of LC50

The median lethal concentration (LC50) was calculated for 48, 72, and 96 hours by interpolating the data on semi-logarithmic coordinates using OriginPro software. A straight line was drawn between two points representing survival at two concentrations: one that was lethal to more than half and another that was lethal to less than half of the *L. rohita* specimens. The concentration at which this line intersected the 50% survival line was recorded as the LC50 or median tolerance limit.

Data analysis

In this study, Microsoft Office Excel 2019 (Microsoft Corp., USA) and OriginPro (2023, OriginLab Corp., USA) software packages were used for data analysis.

Results and discussion

Lethal concentration (LC50) of *L. rohita* exposed to Dithane M-45

This study determined the median lethal concentration (LC50) for the freshwater bivalve *L. rohita* to be 195 mg/L, 180 mg/L, and 160 mg/L after 48, 72, and 96 hours of exposure, respectively (Table 1). The behavior of the test animals in response to Dithane M-45 exposure was also examined. Notable behavioral alterations were observed in *L. rohita* after exposure to the chemical. Immediately after entering the test medium, the fish initially show increased movement and heightened cheliped scraping of body parts. This increased surface activity was particularly pronounced during the first few hours of exposure but normalized within 24 hours. Subsequently, the fish settled at the bottom of the aquaria and became increasingly sluggish after 96 hours of exposure, with reduced cheliped scraping and a diminished response to gentle prodding. Mucus production on the gills and inner lining of the branchiostegite was normal until 36 hours of exposure, eventually leading to a nearly complete mucus covering of the body parts after 96 hours. Figure 1 shows a dose-response curve depicting the relationship between the concentration of a substance (in log mg/L) and the survival percentage of the test organism. As concentration increases, survival rate decreases, showing a typical sigmoidal curve. The LC_{50} is the concentration at which 50% of the test organisms survive.

These findings are in line with those observed in previous studies. Sarkar *et al.* (2014) conducted a study to investigate the impact of the pesticides Cypermethrin and Carbofuran on the ovarian cycle of the Indian major carp, *L. rohita.* The study found that exposure to these chemicals disrupted the normal ovarian cycle, leading to reproductive impair-

Table 1 Median lethal concentration (LC_{50}) *L. rohita* exposed to different concentrations of Dithane M-45

Concentration (mg/l)	Mortality (% in 48 h)	Mortality (% in 72 h)	Mortality (% in 96 h)
40	00	00	00
60	00	00	00
80	00	00	00
100	00	00	10
110	00	05	15
120	05	10	20
130	10	20	30
140	20	25	35
150	25	35	45
160	35	40	50
170	40	50	60
180	45	60	65
190	50	70	75
200	60	75	85
210	65	85	95
220	75	95	100
230	85	100	100
240	90	100	-
250	100	-	-
260	100	-	-

Note: ***L. rohita*** **(*n*= 20) were exposed at each of the above concentrations of Dithane M-45**

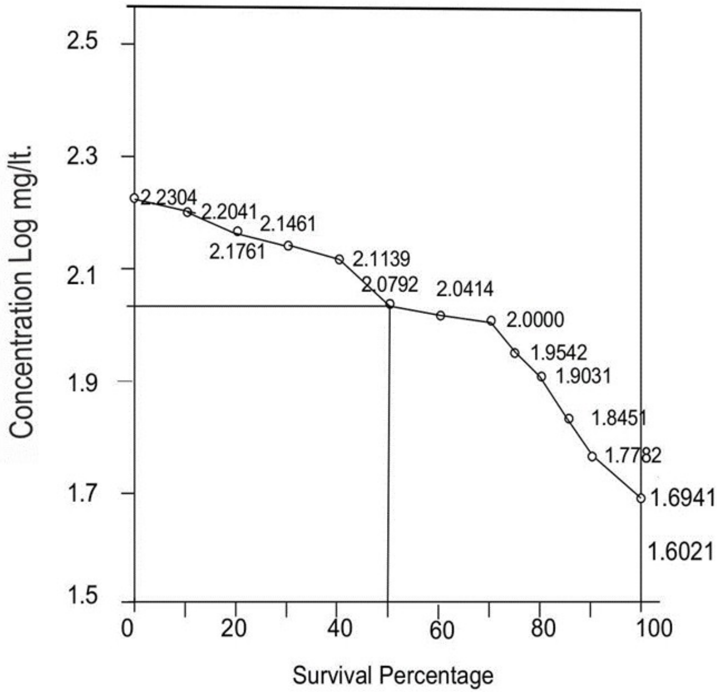

Figure 1: LC50 for Diathane M-45 of *L. rohita*

ments, which could have significant implications for fish population dynamics and aquaculture productivity. A study by Tripathi and Yadav (2015) investigated the impact of organophosphorus pesticides on the fish *L. rohita.* They studied how exposure to these pesticides affects the fish's physiology, behavior, and survival. Results indicate significant adverse effects, including impaired growth, altered behavior, and increased mortality, highlighting the toxicological risks of organophosphorus pesticides to aquatic life. Plonka and Neff (1969) suggested that excessive mucus secretion in response to toxic levels of heavy metals could lead to suffocation and death due to mucus coagulation and metal precipitation on nervous tissues. Bello-Olusoji and Abebola (2006) observed significant effects, such as loss of reflex, increased hyperactivity, discoloration, and hyper-reticulation in *L. rohita* exposed to aldrin and copper sulfate. Under copper sulfate stress, Lodhi *et al.* (2006) also found that *L. rohita* moved faster, scraped its cheliped surfaces more, and made more mucus on its gills and the inside of its branchiostegal. Majumder (2024) also found that chlorpyrifos had significantly reduced the morphological traits of two freshwater fishes, i.e., *L. rohita* and *Mystus vittatus*. Qayoom *et al.* (2024) also found that organophosphate insecticide had strong retention in the muscles of common carp fish. Similarly, Rathnamma and Nagaraju (2013) determined the median LC_{50} of the pesticide Chlorantraniliprole for the freshwater fish *L. rohita.* Their study revealed that exposure led to significant behavioral changes, including erratic swimming and respiratory distress, underscoring the pesticide's toxic effects on aquatic organisms.

The behavioral responses of *L. rohita* exposed to Dithane M-45 were observed.

Table 2 provides the behavioral responses of *L. rohita* exposed to Dithane M-45. During the experiments, the behavior of *L. rohita* was observed under varying concentrations of Dithane M-45, ranging from 5 mg/L to 80 mg/L, over exposure durations of 12, 24, 48, 72, and 96 hours. The fish exhibited several altered behavioral responses. Initially, there was increased operculum movement and reduced bottom-dwelling activity. After 48 hours, eye functioning slowed noticeably, and the fish began crawling along the aquarium floor. They also adhered to the aquarium's sidewalls, which may suggest avoiding contact with the toxic liquid medium. The fish attempted to mitigate stress by increasing mucus production, which intensified with higher toxicant concentrations and prolonged exposure. After initially increasing their operculum movement, all fish died at a concentration of 140 mg/L for 1 day. Similarly, at 130 mg/L over 2 days, the exposure proved lethal, with fish losing motor control and dying within 48 hrs. At 80 mg/L for 4 days, half the fish survived, displaying stress responses such as operculum closure, crowding at the sidewalls, and increased mucus secretion, indicating an attempt to cope with the toxic environment. At 40 mg/L over 15 days, a lower mortality rate (5 out of 20 fish) was observed, with the fish initially showing increased operculum movement but eventually returning to normal behavior. At the lowest concentration of 10 mg/L over 30 days, all fish survived with normal movements throughout the experiment. The results suggest that higher concen-

Table 2: Behavioral changes in *L. rohita* exposed to Dithane M-45

No. of Fishes	Weight (g)	Temp. (°C)	Conc. (mg/l)	Duration of Treatment	No. of Fish died	No. Fish alive	Behavior
20	24±5	28±3	140	1 day	All	Nil	From the beginning of emersion till the opening of the operculum and increase in its movement after 24 hours died
20	25±4	26±4	130	2 days	All	Nil	The dose proves to be lethal. After some time, the fish lose the ability to swim and settle down at the bottom and die after 48 hours
20	26±4	28±4	80	4 days	10	10	Primarily the fish least opened their operculum and tried to avoid stress conditions through movement on the side wall of glass aquaria and secreted mucus.
20	28±3	29±4	40	15 days	05	15	Initially, the fish opens their operculum and increases its movement after some time normal movements are regained.
20	28±4	30±4	10	30 days	Nil	20	Normal Movements were observed

trations of the toxicant induce rapid mortality and significant behavioral changes, while lower concentrations allow for some level of recovery or adaptation, with survival rates improving as the concentration decreases and exposure duration increases.
Previously, Singh and Saxena (2021) investigated behavioral changes in *L. rohita* following acute heavy metal exposure. They found significant alterations in fish behavior, contributing to understanding the impact of pollutants on aquatic life and informing environmental management practices. Similarly, Karmakar *et al.* (2021) looked at how nonylphenol changed the behavior, histology, hematology, and enzymes of *L. rohita* and discovered that this chemical has major toxicological effects on freshwater fish. Patil and David (2010) also found that *L. rohita* changed in shape and behavior after being exposed to malathion at levels that were not lethal. This showed early signs of chemical toxicity in freshwater fish. Bhat *et al.* (2016) assessed the acute toxicity and behavioral responses of *L. rohita* to the organophosphate dichlorvos, demonstrating the effects of this pesticide on freshwater fish. Walia *et al.* (2013) documented behavioral and morphological changes in *L. rohita* exposed to tannery effluent, emphasizing the environmental effects of industrial waste on aquatic organisms. Also, Marigoudar *et al.* (2009) investigated the respiratory and behavioral responses of *L. rohita* to cypermethrin exposure, contributing to our understanding of the effects of pesticide pollution on fish. Raymundo *et al.* (2024) also found that chlorpyrifos and cadmium metal showed significant changes in the behavior of *Ceriodaphnia rigaudi* and *C. silvestrii*. Similarly, Kumar and Singh (2024) found that *L. rohita* fish showed abnormal behavior in pesticide-contaminated river water within Jaunpur district, Uttar Pradesh, India. Therefore, these studies corroborate our findings regarding the impact of pesticide pollution on freshwater fishes and their potential consequences on the ecosystem.

Conclusion

The study found that Dithane M-45 is very harmful to *L. rohita*, and that the median LC50 value decreased over time, which means that longer exposure makes the poison more deadly. Acute behavioral responses, such as movement and chelae scraping, were observed initially, followed by a return to sedentary behavior and increased mucus production on the gills and other body parts. These results also showed that Dithane M-45 was bad for the fish's health and behavior. This shows how important it is to carefully control and keep an eye on this fungicide when it is used in water. The study talks about the possible health and environmental problems that Dithane M-45 could cause and suggests ways to lessen its effects on aquatic life, especially in places where *L. rohita* and other similar species live.

Acknowledgment

The authors are highly thankful to the Director, School of Science, Head, Department of Zoology, IFTM University, Moradabad, U.P., India for providing the necessary facilities.

Ethical approval

This study was approved by the ethical committee on human and animal experiments of IFTM University, Moradabad, U.P. India.

Conflict of interest

The authors declare that they have no conflicts of interest.

References

Ahmed, M. S., Shafiq, K., & Kiani, M. S. (2012). Growth performance of major carp, *Labeo rohita* fingerlings on commercial feeds. *Journal of Animal and Plant Sciences*, *22*(1), 93-96.

Ashaf-Ud-Doulah, M., Al Mamun, A., Rahman, M. L., Islam, S. M., Jannat, R., Hossain, M. A. R., & Shahjahan, M. (2020). High temperature acclimation alters upper thermal limits and growth performance of Indian major carp, rohu, *Labeo rohita* (Hamilton, 1822). *Journal of Thermal Biology*, *93*, 102738.

Bello-Olusoji, O. A., & Adebola, B. O. (2006). Toxic-effect of aldrin and copper sulphate on freshwater prawn-*Caridina africana*. *Journal of Fisheries International*, *1*(1), 12-16.

Bhat, B. A., Bhat, I. A., Vishwakarma, S., Verma, A., & Saxena, G. (2016). Acute Toxicity and Behavioral Responses of Labeo rohita (Hamilton) to an Organophosphate (Dichlorvos). *Biosciences Biotechnology Research Asia*, *9* (1), 447-450.

Bukola, D., Zaid, A., Olalekan, E. I., & Falilu, A. (2015). Consequences of anthropogenic activities on fish and the aquatic environment. *Poultry, Fisheries & Wildlife Sciences*, *3* (2), 1-12.

Dahunsi, S. O., Oranusi, S. U., & Ishola, R. O. (2011). Biochemical profile of *Clarias gariepinus* exposed to sub-lethal concentrations of chemical additives effluent. *International Journal of Research in Environmental Science and Technology*, *1*(4), 52-58.

Dulama, I. D., Radulescu, C., Bucurica, I. A., Teodorescu, S., Stirbescu, R. M., Cimpoca, G. V., & Gurgu, I. V. (2018). Quartz crystal microbalance used as sensor for pesticides detection. *Journal of Science and Arts*, *18*, 445-452.

Dutra Costa, B. P., Aquino Moura, L., Gomes Pinto, S. A., Lima-Maximino, M., & Maximino, C. (2020). Zebrafish models in neural and behavioral toxicology across the life stages. *Fishes*, *5*(3), 23.

Farhan, M., Wajid, A., Hussain, T., Jabeen, F., Ishaque, U., Iftikhar, M., ... & Noureen, A. (2021). Investigation of oxidative stress enzymes and histological alterations in tilapia exposed to chlorpyrifos. *Environmental Science and Pollution Research*, *28*, 13105-13111.

Ghate, H. V., & Mulherkar, L. (1979). Histological changes in the gills of two freshwater prawn species exposed to copper sulphate. *Indian Journal of Experimental Biology*, *17*(8), 838-840.

Gürol, M. A., Arman, S., & Yön, N. D. (2020). Effects of mancozeb on the testicular histology of the zebrafish (*Danio rerio*). In Annales de Limnologie-International Journal of Limnology (Vol. 56, p. 10). EDP Sciences.

Hershberger, P. K., Stick, K., Bui, B., Carroll, C., Fall, B., Mork, C., & Kocan, R. (2002). Incidence of *Ichthyophonus hoferi* in Puget Sound fishes and its increase with age of Pacific herring. *Journal of Aquatic Animal Health*, *14*(1), 50-56.

Karmakar, S., Karmakar, S., Jana, P., Chhaba, B., Das, S. A., & Rout, S. K. (2021). Nonylphenol exposure in *Labeo rohita* (Ham.): Evaluation of behavioural response, histological, haematological and enzymatic alterations. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, *247*, 109058.

Khalil, R. H., Saad, T. T., Selema, T. A. A., & Abdel-Latif, H. M. (2015). *Branchiomyces demigrans* infection in farm-reared common carp (*Cyprinus carpio* L.) and Nile tilapia (*Oreochromis niloticus*) at different localities in Egypt, with special emphasis to the role of environmental stress factors. *International Journal of Innovative Studies in Aquatic Biology and Fisheries*, *1*(1), 15-23.

Kumar, M., & Singh, S. K. (2024). Studies on Morphological Effect of Some Pesticides on Fresh Water Fish, *Labeo rohita* in Sai River of Jaunpur District, Uttar Pradesh, India. *Uttar Pradesh Journal of Zoology*, *45*(12), 189-195.

Kumar, R., Sankhla, M. S., Kumar, R., & Sonone, S. S. (2021). Impact of pesticide toxicity in aquatic environment. Biointerface *Research in Applied Chemistry*, *11*(3), 10131-10140.

Lanjewar, K. H., Zade, S. B., Bhaisare, L. Y., & Chaudhary, D. D. (2023). Impact of hexaconazole on histo-architecture of liver and intestine of freshwater African catfish, *Clarias gariepinus* (Burchell, 1822). *Biochemical & Cellular Archives*, *23*(1), 1-15.

Little, E. E., Archeski, R. D., Flerov, B. A., & Kozlovskaya, V. I. (1990). Behavioral indicators of sublethal toxicity in rainbow trout. *Archives of Environmental Contamination and Toxicology*, *19*, 380-385.

Lodhi, H. S., Khan, M. A., Verma, R. S., & Sharma, U. D. (2006). Acute toxicity of copper sulphate to fresh water prawns. *Journal of Environmental Biology*, *27*(3), 585-588.

Majumder, R. (2024). Comparative Acute Toxicity Studies of Chlorpyrifos Technical Grade with its Emulsifiable Concentrate (20% EC) on *Labeo rohita*, a Freshwater Major Carp, and Mystus vittatus, a Freshwater Catfish. *Bulletin of Environmental Contamination and Toxicology*, *113*(2), 1-8.

Marigoudar, S. R., Ahmed, R. N., & David, M. (2009). Cypermethrin induced respiratory and behavioural responses of the freshwater teleost, *Labeo rohita* (Hamilton). *Veterinarski arhiv*, *79*(6), 583-590.

Martins, M. L., Jerônimo, G. T., Figueredo, A. B., Tancredo, K. R., Bertaglia, E. A., Furtado, W. E., & Mouriño, J. L. (2021). Antiparasitic agents. In *Aquaculture Pharmacology* (pp. 169-217). Academic Press.

Maurya, P. K., & Malik, D. S. (2016). Accumulation and distribution of organochlorine and organophosphorus pesticide residues in water, sediments and fishes, *Heteropneustis fossilis* and *Puntius ticto* from Kali River, India. *Journal of Toxicology and Environmental Health Sciences*, *8*(5), 30-40.

Maurya, P. K., Malik, D. S., Yadav, K. K., Kumar, A., Kumar, S., & Kamyab, H. (2019). Bioaccumulation and potential sources of heavy metal contamination in fish species in River Ganga basin: Possible human health risks evaluation. *Toxicology Reports*, *6*, 472-481.

Maurya, P. K., Meenu, K., & Maddheshiya, R. (2023). Evaluation of the effect of a fungicide, dithane m-45 upon the protein content in gills, muscle, kidney and hepatopancreas of the fresh water teleost, *Labeo rohita. Journal of Experimental Zoology India*, *26*(2), 1-11.

McKenzie, D. J., Garofalo, E., Winter, M. J., Ceradini, S., Verweij, F., Day, N., & Taylor, E. W. (2007). Complex physiological traits as biomarkers of the sub-lethal toxicological effects of pollutant exposure in fishes. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *362* (1487), 2043-2059.

Nataraj, B., Hemalatha, D., Rangasamy, B., Maharajan, K., & Ramesh, M. (2017). Hepatic oxidative stress, genotoxicity and histopathological alteration in fresh water fish *Labeo rohita* exposed to organophosphorus pesticide profenofos. *Biocatalysis and Agricultural Biotechnology*, *12*, 185-190.

Numan, A., Khan, M., Uddin, R., Rahman, M., Bhuiyan, M., & Akter, N. (2018). Risk assessment of commonly used major pesticides for tomato (*Solanum lycopersicum* L.) cultivation in Bangladesh. *Advances in Nutrition and Food Science*, *1* (20), 109.

Patil, V. K., & David, M. (2010). Behavioral and morphological endpoints: as an early response to sublethal malathion intoxication in the freshwater fish, Labeo rohita. *Drug and Chemical Toxicology*, *33*(2), 160-165.

Plonka, A. C., & Neff, W. H. (1969). Mucopolysaccharide histochemistry of gill epithelial secretions in brook trout exposed to acid pH. In Proceedings of the Pennsylvania Academy of Science (pp. 53-55). Pennsylvania Academy of Science.

Popp, J., Pető, K., & Nagy, J. (2013). Pesticide productivity and food security. A review. *Agronomy for Sustainable Development*, *33*, 243-255.

Qayoom, I., Balkhi, M., Mukhtar, M., Abubakr, A., Siddiqui, U., Khan, S., & Mastinu, A. (2024). Assessing organophosphate insecticide retention in muscle tissues of juvenile common carp fish under acute toxicity tests. *Toxicology Reports*, *12*, 253-259.

Rao, K. C. S., & Jayabhaye, U. M. (2022). Study on acute toxicity and haematological alterations induced by the exposure of diclofenac to common carp (*Cyprinus carpio*). *Journal of Research in Agriculture and Animal Science*, *9*(1), 28-33.

Rathnamma, V. V., & Nagaraju, B. (2013). Median lethal concentrations (LC50) of Chlorantraniliprole and its effects on behavioral changes in freshwater fish L*abeo rohita. International Journal of Public Health Science*, *2*, 137-142.

Raymundo, L. B., Gomes, D. F., Miguel, M., Moreira, R. A., & Rocha, O. (2024). Effects of acute toxicity of the pesticide Chlorpyrifos and the metal Cadmium, both individually and in mixtures, on two species of native neotropical cladocerans. *Ecotoxicology*, 1-11.

Sarkar, B., Mahanty, A., Saha, A., Pal, A., Bandyapadhyay, P., Sarkar, S. K., & Ayyappan, S. (2014). Impact of Cypermethrin and Carbofuran on the Ovarian Cycle of the Indian Major Carp, *Labeo rohita* (Hamilton). *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences*, *84*, 989-996.

Sarkar, P., Raju, V. S., Kuppusamy, G., Rahman, M. A., Elumalai, P., Harikrishnan, R., & Arockiaraj, J. (2022). Pathogenic fungi affecting fishes through their virulence molecules. *Aquaculture*, *548*, 737553.

Scherer, E. (1992). Behavioural responses as indicators of environmental alterations: approaches, results, developments. *Journal of Applied Ichthyology*, *8*(1-4), 122-131.

Silva, A. L., Albinati, A. C. L., Souza, S. A., Marques, J. V. S., Andrade, I. B. M., Souza, Y. R. C., & Amorim, A. G. (2023). Evaluation of the acute and sublethal toxicity of Mancozeb in Pacamã (*Lophiosilurus alexandri*). *Brazilian Journal of Biology*, *83*, e274393.

Singh, N., & Saxena, B. (2021). Assessment of Behavioral Alterations in Labeo rohita Due to Acute Exposure of Heavy Metals. *G-Journal of Environmental Science and Technology*, *9*(1&2), 13-18.

Sudhakaran, B. V., & Suja, S. (2024). Ovarian Histological Analysis of *Anabas testudineus* as a Marker for Pesticide Residue Analysis in Freshwater Ecosystems. *Toxicology International*, *31*(1), 93-99.

Sutili, F. J., & Gressler, L. T. (2021). Antimicrobial agents. In *Aquaculture Pharmacology* (pp. 131-168). Academic Press.

Szépvölgyi, J., Nagy, K., SajgónéVukán, K., Regöly-Mérei, A., Soos, K., Toth, K., & Antal, M. (1989). Subacute toxicological examination of Dithane M-45. *Food and Chemical Toxicology*, *27*(8), 531-538.

Tripathi, V. K., & Yadav, R. K. (2015). Effect of pesticide (organophosphorus) on aquatic fish *Labeo rohita. International Journal of Chemical Sciences*, *13*(2), 625-640.

Van Den Berg, A. H., McLaggan, D., Diéguez-Uribeondo, J., & Van West, P. (2013). The impact of the water moulds *Saprolegnia diclina and Saprolegnia parasitica* on natural ecosystems and the aquaculture industry. *Fungal Biology Reviews*, *27*(2), 33-42.

Walia, G. K., Handa, D., Kaur, H., & Kalotra, R. (2013). Behavioral and morphological changes in a freshwater fish, *Labeo rohita* exposed to tannery industry effluent. *Zoology*, 2(8), 1-10.

Xing, H., Li, S., Wang, Z., Gao, X., Xu, S., & Wang, X. (2012). Oxidative stress response and histopathological changes due to atrazine and chlorpyrifos exposure in common carp. *Pesticide Biochemistry and Physiology*, *103*(1), 74-80.

Publisher's Note: The ASEA remains neutral with regard to jurisdictional claims in published maps and figures.