**Acute toxicity of Mancozeb (fungicide) on juvenile Common carp (*Cyprinus carpio var. communis*)**

ABSTRACT

Pesticides, particularly fungicides like Mancozeb (MZ), are extensively used in agriculture for controlling fungal pathogens, but their persistence in environmental systems poses significant risks to aquatic ecosystems. This study investigates the acute toxicity of Mancozeb on juvenile common carp (*Cyprinus carpio var. communis*) by assessing its effects through a 96-hour static bioassay. The study determined the median lethal concentration (LC50) values of Mancozeb at 24, 48, 72, and 96 hours. Water quality parameters during the bioassay remained stable, ensuring the reliability of the toxicity test. Results indicated that the LC50 values for Mancozeb decreased with increased exposure time, with values of 16.940, 13.615, 9.886, and 8.764 ppm at 24, 48, 72, and 96 hours, respectively, highlighting a concentration-dependent increase in fish mortality. The findings suggest that Mancozeb exhibits moderate toxicity to juvenile common carp with increased mortality over prolonged exposure. The study bridges the knowledge gap regarding the ecological impact of Mancozeb, emphasizing the importance of safe pesticide practices to mitigate risks to aquatic biodiversity and human consumers reliant on fisheries.

Key words: *Cyprinus carpio* var *communis*, Mancozeb, Acute toxicity, LC50, Pesticide.

**Introduction**

Pesticides became the backbone of modern agriculture following the Green Revolution of the 1960s, providing effective protection against pests, weeds, and fungal infections (Srivastava and Singh, 2014). Their widespread use in agriculture, forestry, and public health stems from their effectiveness in increasing crop yields and reducing disease vectors (Gagnaire *et al.,* 2004; Jain et al., 2005). But the overuse has raised ecological and public health concerns. Pesticides enter soil and water bodies through agricultural runoff and bioaccumulate in organisms and disrupt ecosystems (Xie *et al.,* 1996; Morel *et al.,* 1998; Abedi *et al.,* 2013). According to FAO (2002) pesticides are substances intended to kill or repel pests, they include herbicides, insecticides, fungicides and rodenticides etc. Among these fungicides like mancozeb (MZ)-a manganese-zinc ethylene bisdithiocarbamate-are widely used for their broad-spectrum activity against fungal pathogens (Leader *et al.,* 2008). But its persistence in agricultural runoff poses risk to non-target aquatic organisms including fish (Rodríguez *et al.,* 2015). Mancozeb acts as a contact profungicide, it inhibits sulfhydryl-dependent enzymes in fungal cells (Ludwig and Thorn, 1960; Kaars, 1982). Once it enters into water bodies Mancozeb degrades into metabolites like ethylenethiourea (ETU) which disrupts thyroid function and is carcinogenic to vertebrates. Mancozeb is neurotoxic and endocrine disruptor in mammals and aquatic species (Srivastava and Singh, 2013b). It reduces acetylcholinesterase (AChE) activity leading to neuromuscular dysfunction (Sikka and Gurbuz, 2006) and induces oxidative stress, DNA damage and thyroid dysfunction (Bisson and Hontela, 2002; Kubrak *et al.,* 2012; Atamaniuk *et al.,* 2014). In fish sublethal exposure disrupts hematological parameters, immune responses and reproductive health (Marques *et al.,* 2016; Zizza *et al.,* 2017). MZ is one of the most used agrochemical Worldwide (Fitsanakis *et al.,* 2002) but its ecological impact is understudied especially in freshwater ecosystems. Fish as bioindicators of aquatic health are more vulnerable, pesticide exposure has been linked to oxidative stress, neurotoxicity and hematological alterations (Kubrak *et al.,* 2012; Bisson & Hontela, 2002). Common carp (*Cyprinus carpio* var. *communis*) a species of economic and ecological importance is highly sensitive to contaminants and is an ideal model to study pesticide toxicity (Marques *et al.,* 2016). This study investigates acute toxicity of Mancozeb exposure on juvenile common carp and bridges the knowledge gap and provides safer pesticide practices to conserve aquatic biodiversity and human consumers dependent on fishery.

**Material and Methodology**

Experiment was carried out as per OECD (2019) Guidelines. The test chemical Mancozeb used in the study was collected from the local commercial shop. Juveniles of common carp, *Cyprinus carpio* var. *communis* weighing of 10±2 g were collected from hatchery of Faculty of Fisheries SKUAST-K. They were brought to laboratory in plastic bags with adequate water to avoid any physical injury and disinfected by giving them a bath for two minutes in 0.05%KMnO4. Thereafter they were transferred to glass aquaria measuring 60×30×40 cm. Prior to the introduction of fishes, aquaria were also washed with KMnO4 to avoid infection. Fishes were acclimatized for two weeks and fed with artificial diet during that period. Leftover food in the tank was removed daily when water was changed.

Bioassay: A short-term static bioassay was performed with the standard ethical protocol. Static type of bioassay for 96 hours was carried out to calculate the 24, 48, 72 and 96-hour median lethal concentration (LC50) for Mancozeb during which no food was given to fishes. Feeding was stopped 24 hours before the start of experiment. During the experiment also, no food was given to fishes. Test organisms were introduced to the test chamber (aquaria) within one hour after the toxicant was added to the dilution water. The rough range-finding tests were performed initially to estimate concentrations for the definitive tests. Concentrations of 2, 4, 8, 16, 32, and 64 ppm were finalized for the experiment and each concentration was replicated thrice with 10 specimens per replicate. A control was run simultaneously for each test concentration. Dead test organisms were removed from aquaria as soon as observed to avoid any organic decomposition. Fishes were treated dead if any sign of immobilization, loss of equilibrium, lack of opercular movement, or morbidity was seen. This reflected an indication of pending death. After the experiment was over, test solution was disposed of and container scrubbed and washed thoroughly with 10% HCl.

Water Quality Parameters of Aquarium Waters during Bioassay: All the water quality parameters were analysed following standard methods as per APHA, 2017. Physico-chemical parameters of water such as water temperature, pH, dissolved oxygen, free carbon dioxide, total dissolved solids were calculated at 24, 48, 72 and 96 h post exposure.

Statistical Analysis: Fish mortality data with respect to time was analysed by probit regression analysis in SPSS (20.0 version), a statistical software, based on Finney Probit Method (Finney, 1971) for determination of LC50 values and 95% upper and lower confidence limits.

**Result**

Various water quality parameters of aquarium waters assessed during static bioassay are presented in Table 1. Water temperature fluctuated between 22.9 & 27.97oC. Values of carbon dioxide ranged between 2.09 to 2.11 mg/l showing very less variation throughout the experiment signifying non-hypoxic conditions in the aquarium throughout the assay. Dissolved oxygen varied from 5.33to 6.19 mg/l, pH from 7.2 to 7.51 and total dissolved solids ranged from 206.27 to 241.60 mg/l. The given water quality parameters revealed very less variation throughout the bioassay, ensuring the stable environmental conditions. This stability is critical for ensuring the reliability and validity of the toxicity test conducted on mancozeb.No mortality and 100% mortality of *Cyprinus carpio* var. *communis* was recorded at 2 and 64 mg/l of Mancozeb respectively. The 24, 48, 72 and 96 h LC50 values (with 95% confidence limit) have been summarized in table 2. No mortality of test organism was found in control during the bioassay. The LC50 values with 95% confidence limit of Mancozeb for *Cyprinus carpio* var. *communis* were estimated as 16.940 (11.940–25.298) ppm at 24 hours, 13.615 (9.281–20.274) ppm at 48 hours, 9.886 (6.736–14.373) ppm at 72 hours, and 8.764 (6.069–12.564) ppm at 96 hours. The toxicity of Mancozeb increases with prolonged exposure, as evidenced by the decreasing LC50 values from 24 hours to 96 hours**.** A significant, concentration-dependent increase in fish mortality rates (p<0.05) were observed as the percentage mortality and probit mortality has been got increased with increasing the concentration of the toxicant.

Table 1: Means and SD of water quality parameters of aquarium water during dimethoate bioassay on different intervals.

|  |  |
| --- | --- |
| Water quality parameter | Day |
| **1** | **2** | **3** | **4** |
| Lab temperature (oC) | 29±0 | 27±0 | 25±0 | 29±0 |
| Water temperature(0C) | 27.97±0.10 | 25.09±0.21 | 22.97±0.05 | 26.99±0.07 |
| D.O (mg/l) | 5.33±0.5 | 6.01±0.72 | 6.19±0.54 | 5.45±0.74 |
| CO2 (mg/l) | 2.11±0.15 | 2.10±0.21 | 2.09±0.13 | 2.09±0.15 |
| pH | 7.32±0.32 | 7.46±0.24 | 7.51±0.30 | 7.2±0.26 |
| T.D.S (mg/l)  | 230.08±17.75 | 223.66±28.96 | 206.27±28.50 | 241.60±30.01 |

**Table 2: LC50 values with 95% confidence limits for Mancozeb based on dissolved concentrations estimated according to Probit method.**

|  |  |  |  |
| --- | --- | --- | --- |
| Exposure time (h) | LC50 value (mg/l) with 95% (Upper and lower) limit | Intercept± SE | Slope±SE |
| 24 | 16.940(25.298-11.726) | 3.568±0.789 | 2.904±0.633 |
| 48 | 13.615 (20.274-9.281) | 3.112±0.695 | 2.742±0.588 |
| 72 | 9.886 (14.374-6.376) | 2.876±0.656 | 2.891±0.615 |
| 96 | 8.764 (12.564-6.069) | 2.973±0.695 | 3.154 ±0.691 |

**Discussion**

Acute toxicity tests on fish are used to determine how chemicals, pollutants, and other substances affect aquatic ecosystems over a short period of time, usually 24 to 96 hours (EPA 1994). The findings help establish water quality regulations, set waste discharge limits, and develop strategies to safeguard aquatic ecosystems from dangerous contaminants (EPA 2021). Furthermore, acute toxicity testing serves as a foundation for the development of long-term toxicity studies and ecological risk assessments, both of which are critical to preserving biodiversity and sustaining ecosystems (Landis, 2009). Given the importance of fish in food webs and as a resource for human consumption, these studies are critical to both environmental conservation and public health protection. In aquatic toxicology, LC50 less than 1000 ppb indicates "very toxic" material, between 1000 and 10000 ppb indicates "moderately toxic" substance, and greater than 10000 ppb indicates "less toxic" substance (Siemering *et al.,* 2005). 96 h LC50 value determined in the present study was 8.764 mg/L, indicating a moderate level of toxicity. The resulted LC50 obtained in the present study was similar to that reported in common carp fingerling (Simkani *et al.*, 2018). The acute toxicity of mancozeb varies in different species. Hejduk and Svobodova (1980) stated that LC50 of *Cyprinus carpio* exposed to Novozir MN 80(80% mancozeb) was found to be 24 mg/L for 48 hours, while in case of more sensitive species, like rainbow trout *Oncorhynchus mykiss*, the LC50 value was much lower, at 1.85 mg/L, and in case of *Poecilia reticulata*, LC50 during the same time period was 2.2 mg/L. LC50 value of 11.68 mg/L was obtained for *Oreochromis mossambicus* (Saha *et al.,*2016) and 12.95 mg/L for *Punctius ticto* (Sharma *et al.,* 2016)*,* which is slightly higher to the results found in the current investigation for *common carp.* *Channa punctatus* exposed to Mancozeb had an LC50 value of 9.5 mg/l (Choudhury and Das, 2020) with slight variability in the sensitivities across different studies. Kuppuswamy and Seetharaman, (2020), reported that LC50 after 96 hours for an adult zebrafish was 2.76 mg/L, while in *Lophiosilurus alexandri,* Silva *et al.* (2023) cited its LC50 to be 2.29 mg/L. However, much greater median lethal concentration of 550 mg/L was reported for *Labeo rohita* (Maurya *et al.*, 2023) and 410.90 mg/L for *Clarias gariepinus* (Odo *et al.*, 2023) exposed to mancozeb. The 96h LC50 value in common carp exposed to chromium was much higher, about 40 mg/l (Deepak *et al.,*2021). The toxicity potential of pesticide changes with respect to age, size and exposure time in different fish species (Sousa *et al.,* 2019). The difference in the toxicity of mancozeb among different fish species can be attributed to the difference in susceptibility and tolerance regarding absorption, biotransformation and excretion of pesticide (Dutta *et al.,* 1992). The highest concentration of the toxicant resulted in the highest mortality rate which is in agreement with the study of FAO (1997) and Ayoola (2008), who reported that in all toxicants a threshold is researched, above which there is no drastic survival of animal and below the threshold, the animal is in a tolerance zone, while below the tolerance zone is the zone of resistance. The lower LC50 values observed in this study attributed to small size of fishes (10±2 g) which have potentially weak immune system to carry out the elimination of the toxicant from the body.

**Conclusion**

In conclusion mancozeb can be classified as moderately toxic substance for juvenile *Cyprinus carpio* Var. *communis* with 96-hour LC50 value of 8.764 mg/L. Toxicity increased with prolonged exposure, highlighting the risks of pesticide contamination to aquatic ecosystems. These studies are critical in understanding the fast impacts of pollutants on fish populations, which serve as crucial indicators of water quality and ecosystem health. The results emphasize the need for careful pesticide management to protect freshwater biodiversity.

**Disclaimer (Artificial intelligence)**

Option 1:

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

Option 2:

Author(s) hereby declare that generative AI technologies such as Large Language Models, etc. have been used during the writing or editing of manuscripts. This explanation will include the name, version, model, and source of the generative AI technology and as well as all input prompts provided to the generative AI technology

Details of the AI usage are given below:

1.

2.

3.

**References:**

Abedi, Z., Hasantabar, F., Khalesi, M. K. and Babaei, S. 2013. Enzymatic Activities in Common Carp, *Cyprinus carpio* Influenced by Sublethal Concentrations of Cadmium, Lead and Chromium*. World Journal of Fish and Marine Sciences* **5**:144-151.

APHA. 2017. Standard Methods for the Examination of Water and Wastewater, *23rd edition.* (Eds. E.W. Rice, R.B. Baird, A.D. Eaton). *American Public Health Association, American Water Works Association, Water Environment Federation.*

Atamaniuk, T. M., Kubrak, O. I., Husak, V. V., Storey, K. B. and Lushchak, V. I. 2014. The Mancozeb-Containing Carbamate Fungicide Tattoo Induces Mild Oxidative Stress in Goldfish Brain, Liver, and Kidney. *Environmental Toxicology* **29**:1227–1235.

Ayoola, S. O. 2008. Histopathological effects of glyphosate on juvenile African catfish (*Clarias gariepinus*). *American-Eurasian Journal of Agricultural and Environmental Sciences* **4**:362-367.

Bisson, M. and Hontela, A. 2002. Cytotoxic and Endocrine-Disrupting Potential of Atrazine, Diazinon, Endosulfan, and Mancozeb in Adrenocortical Steroidogenic Cells of Rainbow Trout Exposed in Vitro. *Toxicology and Applied Pharmacology* **180**(2):110–117

Choudhury, R. And Das, P. 2020. Histopathological Studies on Liver and Kidney of *Channa Punctatus* (Bloch) exposed To Mancozeb Containing Pesticide Dithane M-45. *Pollution Research Paper* **39**:(87-91).

Deepak, T., Sanjay, G. B. and Shivakumar, C. S. 2021. Acute Toxicity of Hexavalent Chromium on Behavioural Parameters and Histopathology Damages in Freshwater Common Carp, *Cyprinus carpio*. *Asian Journal of Research in Zoology* **4**(3):1-8.

Dutta, H. M., Marcelino, J and Richmonds, C. 1992. Brain acetylcholinesterase activity and optomotor behavior in bluegills, Lepomis macrochirus, exposed to five different concentrations of diazinon. *Archives of International Physiology, Biochemistry, and Biophysics***100**:331–334.

FAO. 1977. Manual of methods in aquatic environment research, part 4. Bases for selecting biological test to evaluate marine pollution. *FAO fisheries technical paper***104**:31.

Finney, D. Statistical logic in the monitoring of reactions to therapeutic drugs. *Methods Inf. Med.* **1971**, *10*, 237–245.

Fitsanakis, V. A., Amarnath, V. and Moore, J. T. 2002. Catalysis of catechol oxidation by metal dithiocarbamate complexes in pesticides. *Free Radical Biology and Medicine* **33**: 1714-1723.

Food and Agriculture Organization of the United Nations. 2002. *International code of conduct on the distribution and use of pesticides (Revised version)*. FAO.

Gagnaire, B., Thomas-Guyon, H. and Renault, T. 2004. In vitro effects of cadmium and chromium on pacific oyster, *Crassostrea gigas* (Thunberg) haemocytes. *Fish and Shellfish Immunology* **10**: 502-512.

Hejduk, I. and Svobodova, Z. 1980. Acute Toxicity of Carbamate-based Pesticides for Fish*. Acta Veterinaria Brno* **49**:251- 257.

Jain, P., Sharma, J. D., Sohu, D. and Sharma, P. 2005. Chemical analysis of drinking water of villages of Sanganer Tehsil, Jaipur district. *International Journal of Environmental Science and Technology* **2**: 373-379.

Kaars, S. A. 1982. Mechanism of action of fungicides. Fungicide Resistance in Crop Plants. J. Dekker and S. G. Georgopoulos, eds. Center for Agricultural Publishing and Documentation, Wageningen, the Netherlands: 32-45.

Kubrak, O. I., Atamaniuk, T. M., Husak, V. V., Drohomyretska, I. Z., Storey, J. M., Storey, K. B. and Lushchak, V. I. 2012. Oxidative stress responses in blood and gills of *Carassius auratus* exposed to the mancozeb-containing carbamate fungicide tattoo. *Ecotoxicology and Environmental Saftey* **85**: 37–43.

Kuppuswamy, J. M. and Seetharaman, B. 2020. Mancozeb exposure at sublethal concentration alters the transcription of the genes related to apoptosis in the adult zebrafish *(Danio rerio*) brain. *Research Journal of Pharmacy and Technology* **13**(10): 4801-4804.

Landis, W. G. 2009. Why has ecological risk assessment found such limited application? *Human and Ecological Risk Assessment* **15**(5):849-857,

Leader, A., Kemmitt, G. and Orpin, C. 2008. Dithane keeping an old friend going! Proc. 2008 Euroblight Workshop, Hamar, Norway. [http://www.euroblight.net/Workshop/2008Hamar/PPT/26\_Leader\_Dithane\_keeping\_old\_friend\_going \_Hamar\_oct2008.pdf](http://www.euroblight.net/Workshop/2008Hamar/PPT/26_Leader_Dithane_keeping_old_friend_going%20_Hamar_oct2008.pdf).

Ludwig, R. D. and Thorn, G. D. 1960. Chemistry and mode of action of Dithiocarbamate fungicides. *Advances in Pest Control Research* **30**: 219-252.

Marques, A., Rego, A., Guilherme, S., Gaivao, I., Santos, M. A. and Pacheco, M. 2016. Evidences of DNA and chromosomal damage induced by the mancozeb-based fungicide Mancozan in fish (*Anguilla anguilla* L.) *Pesticide Biochemistry and Physiology* **133**: 52-58.

Maurya, P. K., Jyoti, S and Maurya, P. K. 2024. Acute toxicity of dithane M-45 in freshwater fish *Labeo rohita*: A laboratory study*. Environment Conservation Journal* **25**(4):1113-1119,

Morel, F. M. M., Kraepiel, A. M. L. and Amyot, M. 1998. The chemical cycle and bioaccumulation of mercury. *Annual Review* of *Ecology* *and Systematics* **29**: 543-566.

Odo, U., Ebuka, I., Chijioke, F. and Odo, G, E. 2023. Morphometric indices and Oxidative Stress Biomarkers of African Catfish *Clarias gariepinus* juveniles Exposed to Agricultural Fungicide Mancozeb. *Toxi App Pharma Insights 6*(1):54-64.

OECD. 2019. Test Guideline No. 203. Fish acute toxicity testing. OECD Guidelines for Testing of Chemicals. OECD Publishing.

Saha, N. C., Giri, S. K., Chatterjee, N., Biswas, S. J. and Bej, S. 2016. Acute toxic effects of mancozeb to fish *Oreochromis mossambicus* (WKH Peters, 1852) and their behaviour. *International Journal of Advance Research in Biological Sciences* **3**: 40-44.

Sharma, M. R., Mushtaq, R., Allayie, S. A. and Vardhan, H. 2016. 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* **4**(2): 132-138.

Siemering, G., David, N., Hayworth, J., Franz, A., and Malamud-Roam, K. (2005). *Aquatic pesticides monitoring program literature review* (Contribution No. 71). San Francisco Estuary Institute, Oakland, CA.

Sikka, S. C. and Gurbuz, N. 2006. Reproductive Toxicity of Carbamate Pesticides. Toxicology of Carbamate Compounds pp 447-462.

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

Simakani, P., Abolhasani, M. H. and Hoseini, S. M. 2018. Determination of mancozeb toxicity and biochemical affects in common carp (*Cyprinus carpio*). *International Journal* *of Aquatic Biology* **6**(3): 157-161.

Sousa, N. C., Santos, A. C. G., Silva, K. F., Lima, R. C. D., Rosa, R. A., Santos, S. L., Silva Junior, W. L., Paixão, P. E. G. and Couto, M.V. S., 2019. Effect of the acute toxicity of gasoline on fingerlings of spider (*Pterophyllum scalare*). *Biota Amazônia* 9(1):48-50.

Srivastava, P. and Singh, A. 2013. Study on Some Neural and Behavioral Changes Induced by Carbamate (Mancozeb) Fungicide on Freshwater Fish *Clarias batrachus*. *World Journal of Zoology* **8**(4): 376-380.

Srivastava, P. and Singh, A. 2014. Fate of fungicides on fish, Clarias batrachus- a complete study. LAP LAMBERT Academic Publishing, Germany pp 134.

U.S. Environmental Protection Agency. 2021. Permit limits - Whole effluent toxicity (WET). National Pollutant Discharge Elimination System (NPDES). *U.S. Environmental Protection Agency.*

United States Environmental Protection Agency. (1994). *Intermittent Bulletin:*(2)1 (Publication No. 9345.0-05I). Office of Solid Waste and Emergency Response.

Xie, P., Zhuge, Y. and Dai, M. 1996. Impacts of eutrophication on biodiversity of plankton community. Acta Hydrobiol. Sinica 20 (Suppl.), 30-37.

Zizza, M., Lorenzo, M. D., Laforgia, V., Furia, E., Sindona, G., Canonaco, M. and Facciolo, R. M. 2017. HSP90 and pCREB alterations are linked to mancozeb-dependent behavioral and neurodegenerative effects in a marine teleost. *Toxicology and Applied Pharmacology* **323**: 26-35.