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ASSESSING THE TOXIC IMPACT OF COBOX ON SALMO TRUTTA FARIO: LETHAL CONCENTRATION AND BEHAVIORAL RESPONSE ANALYSIS

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Abstract
Pesticides, whether biological or chemical in nature, are widely used to eliminate, suppress, or control pests such as weeds, insects, and other harmful organisms. However, beyond their intended targets, pesticides often exert adverse effects on non-target species. The present study aimed to determine the lethal concentrations and behavioral effects of Cobox (Copper oxychloride) on Salmo trutta fario (brown trout). Cobox is extensively used in various regions of District Swat, Pakistan. Experimental fish were selected based on body weight and acclimatized in aquaria under controlled laboratory conditions for four weeks, maintaining a 12:12-hour light–dark cycle. Different concentrations of Cobox were introduced to determine the lethal concentration values (LC_0, LC_{50}, and LC_{100}) at 24, 48, 72, and 96 hours. The LC_0 values were recorded as 40, 17.5, 10, and 7.5 mg/L; LC_{50} values as 50, 22.5, 17.5, and 10 mg/L; and LC_{100} values as 60, 30, 25, and 15 mg/L for the respective exposure periods. Exposure to Copper oxychloride induced significant behavioral alterations, including erratic swimming, jumping, loss of equilibrium, mucus secretion, skin lesions, and rapid gill movements. These findings indicate that Cobox exhibits acute toxicity to Salmo trutta fario, adversely affecting both survival and behavioral responses, thereby highlighting its potential ecological risk to aquatic life.

Introduction

Pest is any organisms such as insects, weeds, nematodes, microbes, mollusks, plants pathogens, which compete with humans for foods, destroy their properties or may act as a vector for transmitting different diseases (Rajani and Dave 2020). The most frequent method used to control pests and plant diseases in agriculture regions is through the use of pesticides (Sharifzadeh, Abdollahzadeh et al. 2018). An agent or chemical which is used to destroy pests is called a pesticide (Napit 2013).

The three main groups of pesticides are fungicides (which control fungal infections), insecticides (which control insects), and herbicides (which control weeds). It is well known that insecticides have the most acute toxicity of these. Based on composition, the organochlorines, carbamates, neonicotinoids, pyrethroids, and organophosphates are all common classes of insecticides (Banaee 2013).

Pesticides are not only used in agriculture but also used in homes to control mosquitoes, rats, fleas, ticks, cockroaches but overuse of these pesticides also kills non targeted organisms along with the targeted organisms and affect the terrestrial and aquatic organisms thus lead to the destruction of biodiversity (Mahmood, Imadi et al. 2016).

Pesticides can cause acute or long-term damage to fish when these pesticides are passively introduced to water bodies. Thus have consequences on the fish, including altered behaviour, stunted growth, impaired reproduction and cause huge mortalities

(Sabra and Mehana 2015). Fish are especially susceptible to environmental contaminants contaminating their water. Large-scale aquaculture losses and mass fish mortality events are two immediate consequences of pollutants, like in large quantities (Ullah, Ullah et al. 2021). Therefore, when pollutants like insecticides enter fish organs such as gills, liver, haematopoietic tissues (including the spleen, the kidneys marrow, endocrine tissues, as well as brain) are also affected histopathologically. They can interfere with vital physiological and biochemical processes (Banaee 2013).

By interfering with biochemical processes such as enzyme inhibition, oxidative stress induction, and disruption of hormone regulation, pesticides can cause physiologic disruption in fish (Sabra and Mehana 2015). The exposure of fingerlings of common carp to insecticide Dimethoate causes different behavioural abnormalities such as interrupted schooling behavior, low and irregular swimming, higher secretion of mucus, higher defecation, loss of equilibrium, change in color of the skin and decrease rate of oxygen utilization etc (Singh, Pandey et al. 2009). Chlorpyrifos can cause histopathological changes in the kidneys of fishes and causes necrosis of the renal tubules, and also cause hematological changes in fish, such as a decrease in erythrocytes and hemoglobin, thus leading to anemia in fish (Ismail, Ali et al. 2018). Long term exposure of fish to Chlorpyrifos can act as a mutagenic and induce DNA damage in tissues specifically higher DNA damage in the gill cells (Ali, Nagpure et al. 2009).

Organophosphate pesticides are very detrimental to vertebrates and may stop the activity of an enzyme called cholinesterase (Napit 2013). The broad-spectrum organophosphate fungicide called copper oxychloride ($3Cu(OH)_2 \cdot CuCl_2$) has been widely utilised to protect crops from pests. However, it has been stated that using fungicides based on copper is harmful for the environment (Ferreira, Scavroni et al. 2014).

Copper-containing pesticides can cause various kinds of cancer, allergic reactions. Eyes contact with these copper-based pesticides can cause conjunctivitis, clouding of the cornea, itching and destruction of blood corpuscles, liver and kidneys. Copper sulphate, when entering the body, can irritate the digestive system (Husak 2015).

The freshwater fish species known as brown trout (*Salmo trutta fario*) is valuable for its ecological, recreational, and commercial importance. It is native to North American cold-water lake and river systems and is well-known for its vibrant colouring, adaptability, and wonderful taste (Khaki 2018). Because of their sensitivity to pollution and water quality, rainbow trout are an essential indicator species for environmental health and are widely acquired for aquaculture (Nevoux, Finstad et al. 2019).

The present study is designed to determine the lethal doses of Cobox (Copper oxychloride) for *Salmo trutta fario* and also its impact on fish's behaviour. According to our best knowledge, no previous work is done on the present study at District Swat. The present study will open a new guidance for future research.

Materials and Methods Experimental Design

The present study was designed to find out the effects of Cobox (Copper oxychloride 100%) on *Salmo trutta fario.* Cobox (Copper oxychloride) is widely used against many pests by farmers at District Swat, Pakistan. The *Salmo trutta fario* were collected from Miandam Trout Fish Farm and were transported to the laboratory in oxygenated plastic bags where these fish were kept in aquaria. Ethical approval for this study was granted by the ethical committee of the Centre for Animal Sciences & Fisheries, University of Swat.

Acclimatization of Fish

The fish were acclimatized in aquaria with the capacity of 49 liters of water for four weeks. The temperature was maintained at 7 to 14 °C with pH 8.12 to 8.88 and dissolved oxygen level was maintained at 5mg/L with the help of aerator motor pump. The fish were fed twice based on their body weight with artificial fish pellet feed. The water of the aquaria was changed twice daily (Ullah, Said et al. 2021).

Pesticide concentrations used in experiments

The fish were divided into control and

experimental groups based on their sizes. The fish in the experimental groups were exposed to various concentrations of Cobox (Copper oxychloride 100%) to find out the LC0 (Safe lethal concentration), LC50 (Sub lethal concentration) & LC100 (Lethal concentration) for 24, 48, 72 and 96 hours (Ullah, Khan et al. 2024).

Monitoring of behavioural changes

After exposure to pesticides, the fish in the experimental groups were carefully monitored for behavioural changes. These abnormal behavioural changes included irregular swimming, loss of body equilibrium, loss of scales from the body, increased opercular movements, over secretion of mucous from the body, altered feeding behaviour, jerky type movements, coming towards water surface or becoming bottom dwellers. This data was quantified and analyzed by software (Ullah, Khan et al. 2024).

Monitoring of Mortality

After exposure to pesticides, careful monitoring was done to note down any mortality if occurred. The numbers of dead fish was counted and were then removed from the aquaria (Ullah, Khan et al. 2024). Data analysis The data were analyzed by using MS Excel Software.

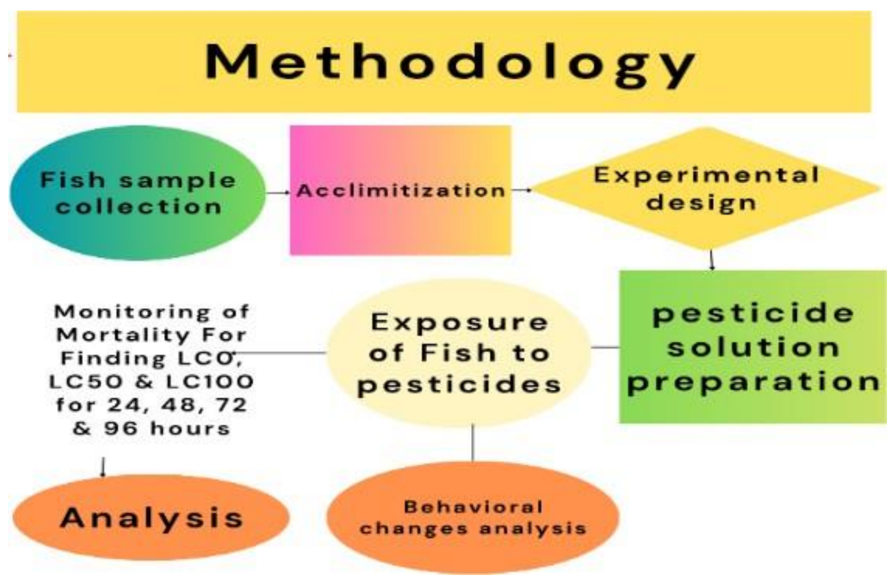

Figure 1. Methodology Results

The present study was designed to investigate the harmful effects of Cobox (Copper oxychloride) on *Salmo trutta fario*. In this study, the *Salmo trutta fario* were exposed to various concentrations of Cobox (Copper oxychloride 100%) for 24, 48, 72 and 96 hours to find out LC0, LC50 & LC100. The fish in the experimental group also showed abnormal behaviours as compared to the control group.

Effects of Cobox on the behaviour of Salmo trutta fario

The fish of the control group or pesticide-free group showed normal behaviors and no mortality was recorded. The fish of the exposed groups showed different abnormal behaviors. The behavioral changes showed by fishes as a result of exposure to Copper oxychloride included Swimming on backs, loss of body balance, increase opercular movements, irregular or disturbed swimming, higher secretion of mucous over all body, coming near to the surface of water, jerky type movements, jumping out of the water, marks on the skin and loss of some scales, altered feeding behaviour, open mouths in the bottom before death and eventually the fish died and fall to the bottom of the aquaria or remains at the surface of water deadly. These behavioural changes were increased as the amount of pesticide and time of exposure were increased (See Table no 1

Annexure).

S. No	Exposure time	LC0 value (mg/L)	LC50 value (mg/L)	LC100 value (mg//L)
1	24 hours	40	50	60
2	48 hours	17.5	22.5	30
3	72 hours	10	17.5	25
4	96 hours	7.5	12.5	15

Determination of LC0, LC50 and LC100 for 24, 48, 72 & 96 hours

The LC0 (Safe lethal concentration) for 24, 48, 72 and 96 hours were 40mg/l, 17.5mg/l, 10mg/l and 7.5mg/l. The LC50 (sub-lethal concentration) for 24, 48, 72 and 96 hours were 50mg/l, 22.5mg/l, 17.5mg/l and 12.5mg/l. The LC100 (Lethal concentration) values for 24, 48, 72 and 96 hours were 60mg/l, 30mg/l, 25mg/l and 15mg/l (Figure 2, Table 2).

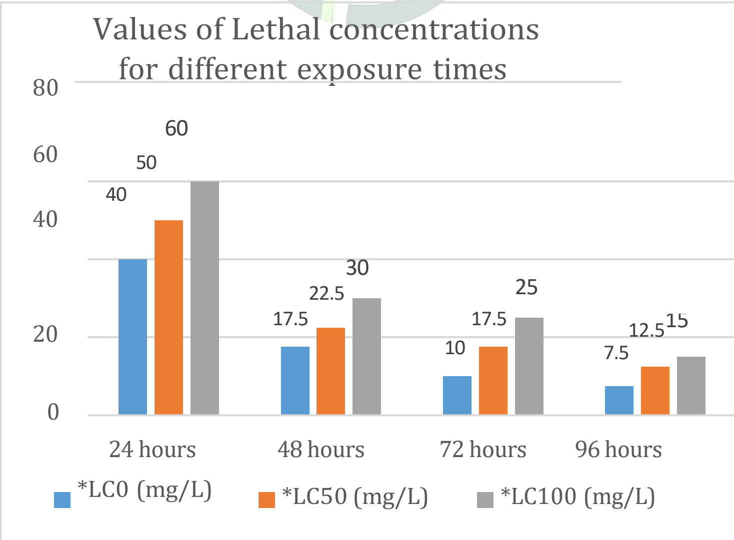

Figure 2. Lethal concentration values Table 2. LC levels of Cobox to Salmo trutta fario for different times

Discussion

In this study, the Salmo trutta fario were exposed to various concentrations of Cobox to find out LC0, LC50 & LC100 for 24, 48, 72 and 96 hours. The LC0 for 24, 48, 72 and 96 hours were 40mg/l, 17.5mg/l, 10mg/l and 7.5mg/l. The LC50 for 24, 48, 72 and 96 hours were 50mg/l, 22.5mg/l, 17.5mg/l and 12.5mg/l.

The LC100 values for 24, 48, 72 and 96 hours were 60mg/l, 30mg/l, 25mg/l and 15mg/l. The abnormal behavioural changes included irregular swimming, loss of body equillibrium, increased opercular movements, over secretion of mucous from the body, altered feeding behaviour, jerky type movements.

A work was also done by (Sattanathan, Amsath et al. 2019), to evaluate the impact of copper oxychloride on the Oreochromis mossambicu. The Oreochromis mossambicu were exposed to copper oxychloride for 96 hours to find out LC50. The LC50 for 96 hours was recorded as 30.53 mg/L, while in current study the LC50 for 96 hours was recorded as 12.5mg/l. It is concluded that brown trout (Salmo trutta fario) is much more sensitive to environmental stressors and pollution than Oreochromis mossambicu. A work was done to determine the LC50 value of an insecticide, Chlorpyrifos, on the fingerlings of Common carp (Cyprinus carpio) for 96 hours, which was 0.160 mg/L. In the current study, the Salmo trutta fario wereexposedto different pesticides, Copper oxychloride, for which the LC50 value for 96 hours was 12.5 mg/l. The behavioural changes in their work were similar to the present work, they also identified some of the morphological changes such as caudal bending which is also observed in the current study. But the LC50 values were different in both studies.

A work was done by (Ullah, Khan et al. 2024) to study the effects of Cobox (Copper oxychloride 50%) on common carp. For 24, 48, 72, and 96 hours, the LC0 levels were 110, 75, 50, and 30 mg/l. The LC50 levels were 258, 175, 129, and 90 mg/L. The LC100 levels were 386, 322, 258 and 175 mg/l. Their work supports the cuurent study. The current study reported the safe, sub and lethal concentrations of Cobox (Copper oxychloride 100%) to Salmo trutta fario, the LC0 for 24, 48, 72 and 96 hours were 40, 17.5, 10 and 7.5. The LC50 were 50, 22.5, 17.5 and 12.5. The LC100 were 60, 30, 25 and 15mg/l. The behavioural changes in their study such as coming to the water's surface, impaired body balance, increased gill movement, disturbed swimming, mucus secretion throughout the body, open mouth at the bottom before death, and finally the fish's death, were all also observed in the current study.

A work was also done by (Alkobaby, Abd El-Wahed et al. 2017), to determine the LC50 of pesticide Copper sulphate on the fingerlings of Nile tilapia fishes for 96 hours of exposure. The LC50 value determined in their research study was 31.2 mg/l. The LC50 value of copper oxychloride for 96 hours to Salmo trutta fario was 12.5 mg/l. It is concluded that the toxicity of copper oxychloride is greater than that of Copper sulphate. The behavioural changes caused by copper sulphate in the previous study and Copper oxychloride in the present study were similar. They reported that exposed fish exhibited erratic swimming, fatigue, and a tendency to float straight up near the water's surface along with their mouths open before sinking to the bottom and remaining still until they died which was similar to the present study.

A study was also done by (Singh, Pandey et al. 2009), in which they exposed the fingerlings of common carps to an organophosphate pesticide i.e., Dimethoate to determine the LC50 values for 24, 48, 72 and 96 hours and behavioral abnormalities causes by the pesticide Dimethoate. The LC50 value for 24, 48, 72 and 96 hours obtained were 1.84, 1.78, 1.68 and 1.61 mg/L respectively. As compared to the present study, as the time of exposure to pesticides was increased, the value of LC50 decreased, which is similar in both studies. In the current study, the LC50 for 24, 48, 72 and 96 hours were 50mg/l, 22.5mg/l, 17.5mg/l and 12.5mg/l. The behavioral changes induced by the pesticides in both the studies were similar, which supports the current work. In their study, increasing exposure duration caused erratic swimming, increased surfacing, excessive mucus secretion, decreased agility, and an inability to maintain proper posture and balance, which is totally to the present study. While they reported decreased opercular movement rate was not observed in the current work but instead the opercular movements were increased in the current study.

Conclusion

From the present study it is concluded that Cobox (Copper oxychloride) fungicide is toxic to Salmo trutta fario and its high conncentrations caused mortality. It also adversely affected their behaviours such as irregular swimming, jerky

movements, altered feeding and social behaviours, loss of equilibrium and loss of body scales, etc. Acknowledgement
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